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Critical Review

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Fresh Ideas Bloom in Gut Healthcare to Cross-Fertilize Lake Management

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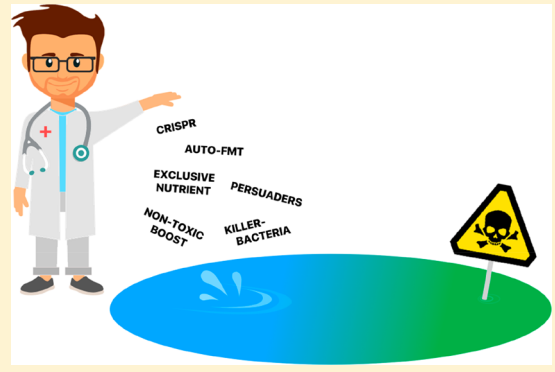
Supporting Information

ABSTRACT: Harmful bacteria may be the most significant threat to human gut and lake ecosystem health, and they are often managed using similar tools, like poisoning with antibiotics or algicides. Out-of-the-box thinking in human microbiome engineering is leading to novel methods, like engineering bacteria to kill pathogens, "persuade" them not to produce toxins, or "mop up" their toxins. The bacterial agent can be given a competitive edge via an exclusive nutrient, and they can be engineered to commit suicide once their work is done. Viruses can kill pathogens with specific DNA sequences or knock out their antibiotic resistance genes using CRISPR technology. Some of these ideas may work for lakes. We critically review novel methods for managing harmful bacteria in the gut from the perspective of managing toxic cyanobacteria in lakes, and discuss practical aspects such as modifying bacteria using genetic engineering or directed evolution, mass culturing and controlling the agents. A key knowledge gap is in the ecology of strains, like toxigenic vs nontoxigenic *Microcystis*, including allelopathic and Black Queen interactions. Some of the "gut methods" may have future potential for lakes, but there presently is no substitute for established management approaches, including reducing N and P nutrient inputs, and mitigating climate change.



INTRODUCTION

Harmful cyanobacteria are a significant threat to lake health.[1,2] Blooms increase turbidity and shade submerged plants, lead to oxygen depletion and fish kills, cause taste and odor problems, and often produce toxins that interfere with recreation and public water supply. Toxic cyanobacteria presently affect many lakes, the trend is increasing and expected to get worse with climate change and population growth. Sediment core data from lakes in North America and Europe suggest cyanobacteria have increased substantially in the Anthropocene, and more rapidly since 1945.[3] A model predicts that for an average lake in the U.S., the number of days with harmful cyanobacterial blooms will increase from 7 to 18−39 days per year by 2090.[4] Prominent examples include Lake Erie (U.S.) and Lake Taihu (China), which are plagued by toxic *Microcystis* that caused disruption of drinking water supply. Several management approaches for controlling cyanobacteria are available. However, the large scale, increasing trend and dim future projections, and the cost and resilience of some systems to these methods (e.g., re-eutrophication of Lake Erie after P load reduction) suggest we need to continue to look for new tools to manage this problem.

The human gastrointestinal (GI) tract system is also frequently threatened by harmful bacteria, including food and waterborne pathogens, like the Shiga toxin-producing *Escherichia coli* O157:H7. Worldwide, these infections cause about one million deaths per year, and this number is expected to increase with population growth and climate change.[5−7] Exasperating this problem is the emergence of antibiotic resistant strains, as well as problems caused by antibiotic treatment.[8,9] When the normal gut flora is disrupted by antimicrobials (i.e., during surgery), the pathogen *Clostridium difficile* can proliferate and produce toxins. Each year, *C. difficile* infection (CDI) leads to over 250 000 hospitalizations and 14 000 deaths in the U.S., and the trend is increasing due to the emergence of strain NAP1/BI/ribotype 027 with increased antibiotic resistance and toxin production.[9]

Controlling harmful microbes is a problem shared by gut and lake health managers, and historically similar tools have been applied. The importance of diet in human health is well recognized, and it also affects harmful bacteria.[10,11] For example, the western-style high-fat, low-fiber diet has been shown to promote microbes that increase cancer risk.[11] Lake eutrophication is, by definition, overenrichment with nutrients and consequently the most common and successful management strategy is reduction of nitrogen and phosphorus loading. In this context, a major difference between gut and lake

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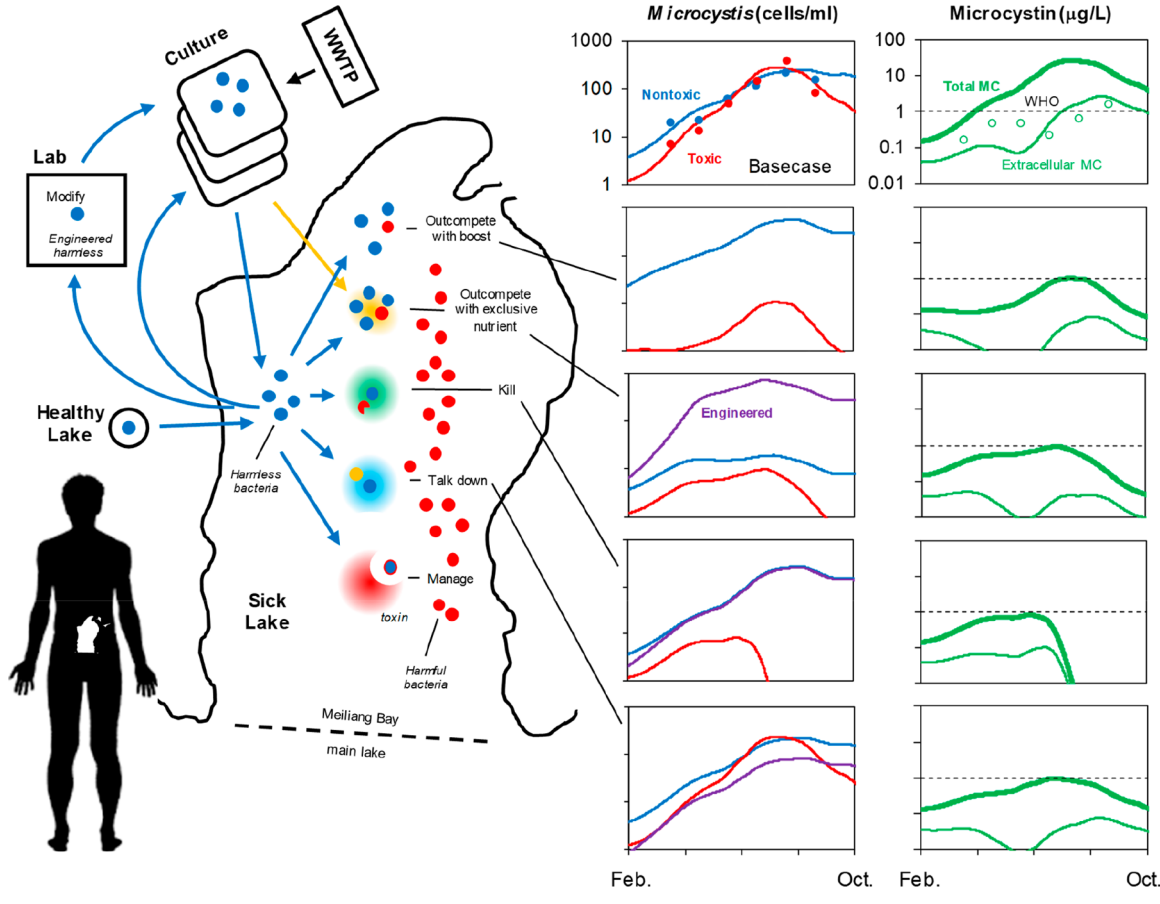

Figure 1. New ideas for managing harmful bacteria in the gut, applied to lakes. *Microcystis* and microcystin (MC) concentrations for Meiliang Bay, Lake Taihu, China. The left part of the figure conceptually illustrates some of the management approaches discussed in the text. The panels on the right side show observations (symbols, data from ref 19) and model predictions for the base case and various management approaches (lines, see SI for model details).

environments is the sediment bed, which may serve as a nutrient storage reservoir and significantly delay the recovery of a lake.[2] The quality of the "diet" (i.e., nutrient ratio) also matters. For example, an analysis of a number of lakes suggested that cyanobacteria are rare when the N:P ratio exceeds a threshold.[12] Poisoning, antibiotics in the gut or algicides in the lake, is a common control method. In both areas it is recognized that it is advantageous to keep the normal microbiota unaffected. Broad spectrum antibiotics, those that kill many different bacteria, also kill the harmless bacteria, thus rendering the system vulnerable to overgrowth or infection by pathogens, like *C. difficile*.[13] The collateral damage of broad-spectrum antibiotics motivates narrow spectrum antibiotics that are designed to only target the pathogen species, like fidaxomicin for *C. difficile*.[14] Similar considerations motivate the use of cyanocides (e.g., H_2O_2) over algicides for controlling cyanobacteria,[15−17] which spare eukaryotic phytoplankton and zooplankton.

Recently, tremendous progress has been made in the area of gut research, driven by increased recognition and understanding of the role of microbes in health, a large influx of funding and the availability of novel experimental and genetic tools. This is leading to new ideas for managing harmful bacteria and raises the question, if and how any of them can help manage harmful cyanobacteria in lakes. Environmental scientists and engineers have traditionally been reluctant to embrace novel technologies, maybe because much of the work in this area deals with their consequences (e.g., polychlorinated biphenyls, PCBs), and a guiding principle is to reduce the anthropogenic impact on the environment. These are important concerns, but we should also keep an eye on developments in other areas because "rapid advances in biotechnology [···] mean that ideas that once seemed like science fiction are becoming reality in the blink of a bionic eye".[18]

Here we critically review new methods gut scientists are exploring to control/engineer the microbiome and discuss their potential for lake management, specifically the control of toxin-producing cyanobacteria by example of microcystin (MC)-producing *Microcystis*. The technologies can be roughly categorized by their mode of action, including agents that outcompete, kill, talk down or manage harmful bacteria (Figure 1, the figure summarizes approaches discussed throughout the paper). This follows a discussion of some practical issues, including modifying the bacterial or viral agent using genetic engineering or directed evolution, producing (i.e., mass culturing) and controlling it.

FMT AND PROBIOTICS

Using a foreign, wild type, harmless bacteria to grow and outcompete a native harmful bacteria has been successful in human health management. A microbial population from a healthy donor (fecal microbiota transplantation, FMT) can effectively reduce overgrowth of *C. difficile* with a success rate

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of 80−90%.[9,20,21] Probiotic treatment using one or multiple strains can also be effective, where mechanisms may include production of an inhibitory substance (e.g., secondary bile acids,[22]) or resource competition (e.g., by a nontoxigenic *C. difficile* strain that occupies the same ecological niche,[23]).

For lakes, healthy donors and nontoxic strains are also readily available. A lake microbiota transplantation (LMT) could utilize biomass from a lake that has only nontoxic strains of *Microcystis*.[24] Alternatively, nontoxic versions of harmful strains, like nontoxigenic *Microcystis*,[25−27] which may compete for resources against the harmful strain in their niche, could be cultured and inoculated. The use of a commercial consortia, Effective Microorganisms (EM), has also been discussed.[28]

The ecology of gut and lake bacteria are governed by the same fundamental principles, including resource competition and chemical warfare, but there are a number of important differences between the gut and lake environments that control the chances of a foreign strain to grow and outcompete a resident strain (referred to as engraftment), including (1) closedness, (2) host association/priority (founder) effects, and (3) variability.

1. Closedness. The gut system is comparatively closed. Although relative import rates can be similar to lakes, the stomach acid barrier substantially reduces the number of bacteria entering the intestine (Figure 2A). Entry is a prerequisite to a successful invasion, but here this barrier can be easily overcome by administering a "healthy" dose. However, the closedness has another, indirect effect on the success of a foreign strain, because it results in lower diversity, suboptimal resource allocation, and more open niches[29] (Figure 3). Mice with high commensal *E. coli* concentrations are more susceptible to invasion by the closely related, but pathogenic, *Salmonella enterica*,[30] which may be a result of suboptimal resource utilization by the large *E. coli* population. In addition, diversity is also often further reduced during disease, an overgrowth of *C. difficile* means it occupies many niches and it can not possibly be an effective competitor in all of them.

In contrast, lakes are more open (Figure 2A). In the ambient environment, it is often assumed that microbes are highly dispersed and any biogeographic patterns are due to environmental factors: "everything is everywhere, but, the environment selects",[31,32] although some dispersal limitation has been quantified for lakes (ref 33 and references therein). Consequently, lakes are populated by bacteria that have been selected from a constant inflow of potential colonizers, and there are few open niches for a foreign strain (Figure 3). If a foreign strain would be competitive, it would be there already. In phytoplankton ecology, the presence of toxic bacteria is generally believed to be due to environmental conditions, and not an infection per se like in the gut.[31,32] This challenge is also recognized for other open systems, like wastewater treatment plants and groundwater aquifers.[34]

2. Host Association/Priority (Founder) Effects. Gut bacteria have a host-associated lifestyle, which includes interaction with the host immune system and physical niche occupation.[39,41] These mechanisms support priority effects and colonization resistance. *Bacteroides* cells colonize and physically occupy colonic crypts, which prevents other cells from invading them[39] (Figure 2B). The cells inside the crypts serve as a stable seed bank to support the outside population, a benefit only the first colonizer of the gut enjoys. Priority effects can decrease or increase the chances of a foreign strain (Figure 3). On the one hand, it helps a resident bacteria protect its niche against the foreign strain. On the other hand, it may enable a relatively uncompetitive strain to occupy a niche, which makes it vulnerable to invasion by a superior competitor.

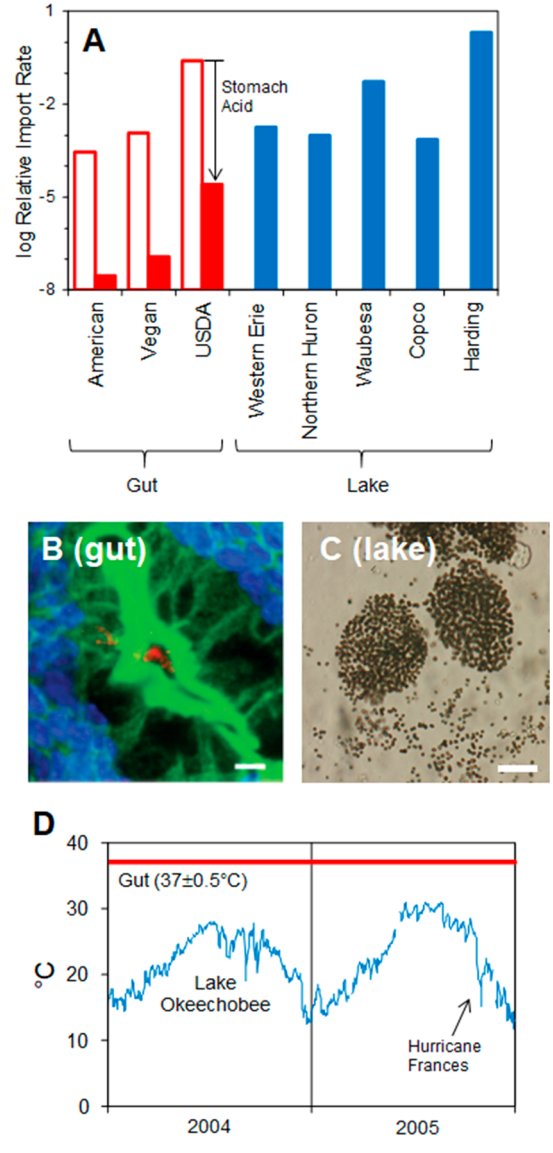

Figure 2. Ecological considerations: (A) Closedness, (B,C) host-associated vs free-living lifestyles, and (D) variability. (A) Relative inflow of bacteria into gut and lake ecosystems. The relative import rate (RI) is the ratio of cell import rate (cells/day) to growth rate (cells/day).[33] It quantifies how many of the new cells in the ecosystem come from outside relative to those that are born inside. Gut numbers are based on measured ingestion rates,[35] a total bacteria population of 1e14 cells with a specific growth rate of 0.5 per day.[36,37] Stomach removal of 99.99% is assumed.[38] Open and closed bars for gut are before and after stomach. Lake numbers are for *Microcystis*.[33] (B,C) Host-associated and free-living lifestyles in the gut and lake. (B) *B. fragilis* cells (red) physically occupy colonic crypts in the mouse gut. Scale bar = 5 μm.[39] (C) *Microcystis* cells form planktonic colonies in Lake Taihu. Scale bar = 20 μm.[40] (D) Temperature of gut and lake ecosystems.

In contrast to the gut, lake cyanobacteria have a predominantly free-living, suspended/planktonic lifestyle (Figure 2C). They can form resting stages and temporarily reside in the sediment bed, and there can be associations with heterotrophic bacteria and higher organisms (e.g., macro-

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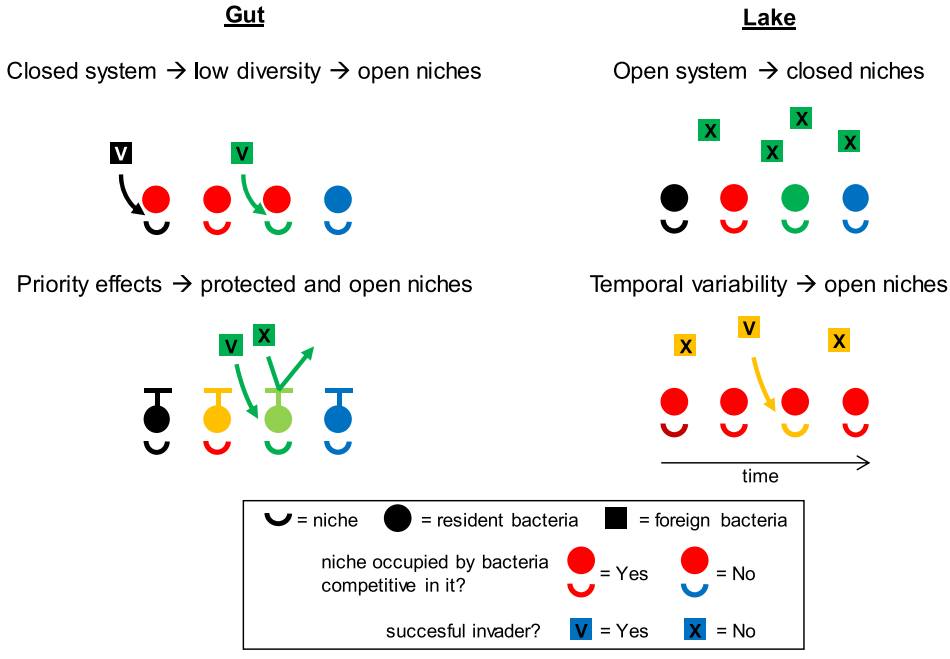

Figure 3. The role of closedness, priority (founder) effects and variability in the success of foreign bacteria in outcompeting a resident bacteria. Each part illustrates how an ecological mechanism or scenario affects the success of foreign bacteria. For example, an open system will have few open niches and consequently foreign bacteria are less likely to be successful (top right). See text for full discussion.

phytes).[42−44] However, these interactions are unlikely to result in substantial priority effects, and they are generally not included in our conceptual and mathematical phytoplankton ecology models.[45,46] Therefore, the resident bacteria cannot rely on priority effects to fend off potential invaders, but at the same time they are a competitive bunch that leave few open niches. The fact that the niches are not protected using priority effects is of little help to a foreign bacteria that is not competitive.

3. Variability. The gut of warm-blooded animals is an environmentally controlled growth chamber with constant conditions (Figure 2D), although there can be changes in diet and medication. A consequence of this stability, is that the human gut microbiome is also comparatively stable: most strains in an individual's intestine reside there for decades.[47] This stability allows absolute niche occupation,- a bacteria with a temperature optimum (T_{opt}) of 37 °C will not be outcompeted by a foreign strain on the basis of temperature adaptation.

Lakes are constantly exposed to strong changes in temperature, sunlight, and nutrient input at seasonal and weather time scales (Figure 2D), and this drives continuous succession in the phytoplankton community.[46,48] Fluctuation in resources can open up niches for invaders,[49] and temperature can have a similar effect (Figure 3). However, finding a strain that is able to exploit one of these temporary niches and also be as competitive as the resident strains with respect to all other factors would be difficult.

On balance, these three factors: closedness, host association/priority (founder) effects, and variability, suggest that lakes are highly competitive environments with few open niches, and that FMT or probiotics approaches are not feasible.

AUTO-FMT

As an alternative to bacteria from a healthy donor, those from the same person at an earlier, healthy time may be used, which is referred to as autologous FMT. Surgery is often associated with extensive antibiotic use to avoid infection at the site of surgery, which depletes the gut commensal microbiota and makes the system more vulnerable to infection. To reconstitute it, the feces can be collected and frozen (banked) prior to surgery, and then reintroduced afterward.[50]

In lakes, a population or strain from another, healthy time or location is unlikely to be competitive against the harmful strain for the same reasons that limit heterologous FMT or probiotics. However, recent molecular (i.e., genotype) field observations reveal that nontoxic bacteria often bloom at the same time and location as toxic bacteria (Figure 1).[19,51] Interestingly, this pattern is different from our traditional view of phytoplankton succession. Such a native, nontoxic cobloomer (NNC) may be a good candidate to outcompete the native toxic bacteria. This may be achieved by isolating an NNC strain, mass culturing it, and then inoculating to boost the lake population. If this is done at the onset of a bloom, (a) the optimal growth conditions and relaxed resource competition at the onset of the bloom[49] and (b) the similar growth properties of the NNC strain,[19,51] could work together to suppress the harmful bacteria (Figure 1). A deeper understanding of the ecology of these strains, including any interactions beyond simple competition for nutrient and light resources, and more fundamentally, why they coexist and cobloom in the first place, is an important knowledge gap here.

NNC strains are also excellent candidates for further modification, like genetic engineering to kill harmful bacteria. An agent that grows where and when the harmful strain grows, that is, shadows it, is in a good position to interact with it.

EXCLUSIVE NUTRIENT

Despite the success of FMT and probiotics in gut health management, the competitiveness of an introduced strain remains a challenge. If the agent is designed to produce a toxin or perform some other function (see below), that will come at

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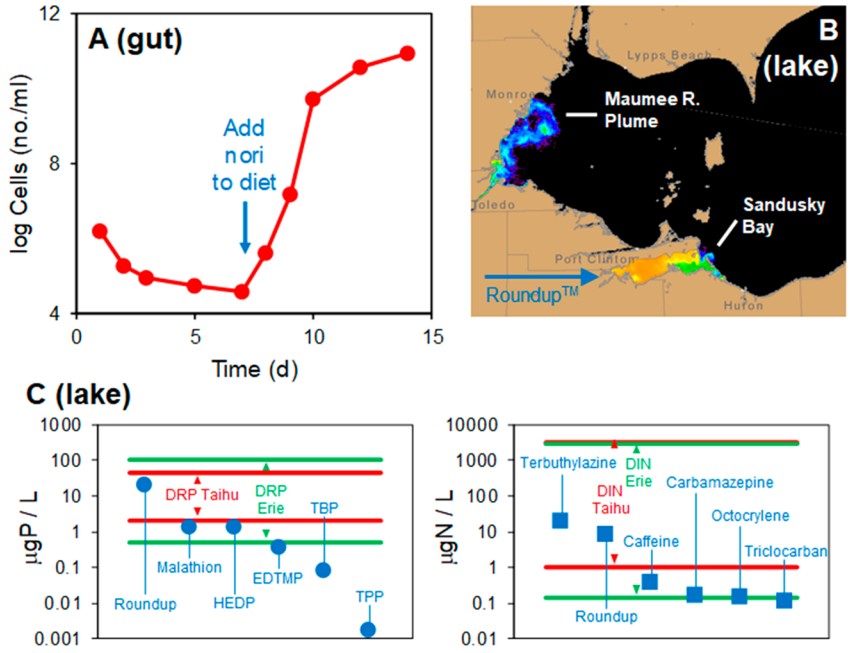

Figure 4. Support of specific bacteria using an exclusive nutrient. (A) A foreign strain capable of using a component of seaweed does not survive in a gut with a U.S. microbiome, but its growth can be supported by adding nori to the diet.[52] (B) The cyanobacterium *Planktothrix* is tolerant to the herbicide Roundup and can utilize it as a source for N and P, which affects its success in Sandusky Bay, Lake Erie, U.S.[53] Image source: NOAA. (C) Range of dissolved reactive phosphorus (DRP) and dissolved inorganic nitrogen (DIN) in two eutrophic lakes in comparison with observed concentrations of selected anthropogenic pollutants in surface waters. References for data: Lake Taihu;[54,55] Lake Erie;[56,57] Roundup, glyphosate, and its degradation product aminomethylphosphonic acid (AMPA), herbicide;[58] Malathion, insecticide;[59] HEDP, 1-hydroxyethane1,1-diphosphonicacid, bisphosphonate used in detergents, cosmetics and pharmaceuticals;[60] EDTMP, [bis(phosphonomethyl)amino]-methylphosphonic acid, chelating agent;[60] TBP, tributyl phosphate, defoamer and plasticizer;[59] TPP, triphenyl phosphate, flame retardant;[59] Terbuthylazine, herbicide;[59] caffeine, central nervous system stimulant;[61] carbamazepine, antiepileptic drug;[62] octocrylene, sunscreen (UV−B filter);[63] triclocarban, disinfection,.[63]

a metabolic cost that will slow its growth and reduce its chances of establishment. A new idea in this area is to genetically engineer bacteria to utilize a rare chemical as an "exclusive nutrient" and then add it to the diet to support the bacteria. For example, in a recent study a strain of the gut bacteria *Bacteroides* was genetically engineered to utilize the marine polysaccharide porphyran, a component of seaweed, which is uncommon in the U.S. diet (vs Japan, nori)[52] (Figure 4A).

To provide an exclusive nutrient for lake bacteria, we would have to find a substance that is abundant and nutritious enough to provide a significant advantage, and new enough so that the resident population is not already adapted to use it. When screening for chemicals, it is important to realize that gut bacteria are heterotrophs limited by organic C, whereas lake phytoplankton are autotrophs typically limited by N and/or P. However, some phytoplankton, like cyanobacterium *Synechocystis*,[64] have the ability to grow heterotrophically or mixotrophically, so organic carbon compounds should not be ruled out completely. Fortunately (for this purpose), there are many chemicals, including pesticides, pharmaceutical, and personal care products that we discharge into the environment, are present at substantial concentrations (on a P or N basis, Figure 4C), and can be utilized by bacteria.

For example, Roundup, the most widely used chemical herbicide, has been implicated in the success of *Planktothrix* (unfortunately also a toxin-producer) over *Microcystis* in Sandusky Bay, Lake Erie (ref 53, Figure 4B). *Microcystis* from Lake Erie do not grow on Roundup, but those from Greifensee (Switzerland) do.[53,65] The corresponding *phn* gene cluster has been subject to frequent horizontal gene transfer,[53,66] suggesting it could be introduced into the nontoxic Lake Erie strain. Of course, the nontoxic strain could then pass it on to the toxic strain, or the toxic strain may eventually acquire it from another donor, but that should take some time. With the increased use of Roundup, many microbes may evolve to use it, but giving the nontoxic strain an evolutionary head start on this still somewhat exclusive nutrient is an intriguing idea.

Thinking further outside the box or into the future, it is conceivable that we would design chemicals used for another purpose (e.g., pharmaceuticals) to also serve as exclusive nutrient. A new concept in chemical design is to make compounds that are easily degraded in the aquatic environment.[67] Going beyond that, and designing them to be beneficial in multiple compartments of the water cycle (e.g., cure a disease in people, help degrade a pollutant in wastewater treatment plants, promote beneficial bacteria in lakes, enhance filter performance of drinking water plants, etc.) would be an exciting new challenge for the chemical industry.

KILLER AND INHIBITOR BACTERIA

Beyond resource competition, an introduced agent can also control harmful bacteria via direct interaction mechanisms, like killing. Bacteria naturally produce antibiotics to kill or inhibit their competitors, bacterial warfare, which also plays an important role in gut health management. For example, the common probiotic *E. coli* NISSLE 1917 (sold as Mutaflor) produces microcins that inhibit other *E. coli* and *Salmonella enterica* serovar Typhimurium.[68] New approaches employ

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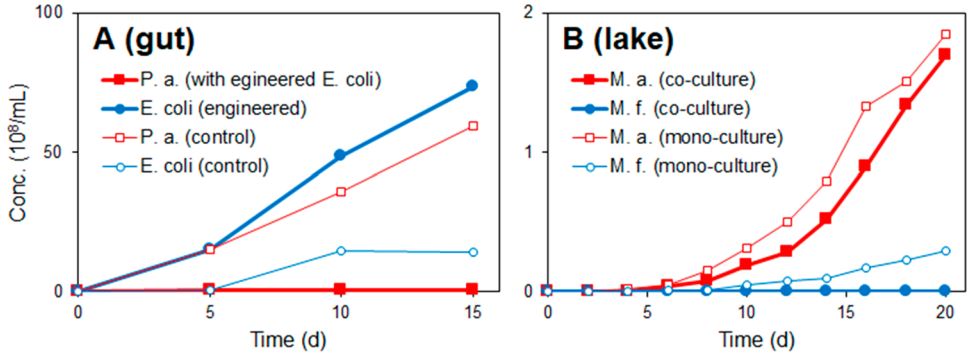

Figure 5. Killer and inhibitor bacteria. (A) Engineered *E. coli* kills pathogenic *Pseudomonas aeruginosa* in coculture.[70] (B) *Microcystis aeruginosa* inhibits *Microcystis flos-aquae* in coculture.[76]

genetic engineering and combine the killing function with sensing of the pathogen.[69] *E. coli* was engineered to sense the pathogenic *Pseudomonas aeruginosa* via its quorum sensing (QS) system, produce a toxin specific to the pathogen and then lyse (break open the cell) to release the toxin[70] (Figure 5A). This kamikaze strategy has clear implications for the fitness of the engineered strain and would affect its establishment in the lake. As an alternative to lysing the engineered bacteria, secretion of the toxin has been implemented.[71] Efficient secretion of folded proteins (i.e., the toxin) is a major problem in bioengineering, and in this case only 5% of the toxin was released and 1e5 killer cells were needed to kill one pathogen cell. Since culturing sufficient quantities is a challenge in the lake environment, a requirement to have 1e5 times more of the introduced strain is a problem. In another example, *E. coli* was engineered to (1) swim toward pathogens, (2) degrade their biofilm matrix, and (3) kill them.[72]

In lakes, bacteria concentrations are typically orders of magnitude lower, which makes interaction via chemicals harder. For example, at the WHO moderate risk level, the lake bacteria would have to produce 1000 times more to achieve the same concentration (Figure 6A). Nonetheless, chemical warfare (i.e., allelopathy) also plays an important role in lakes and there are many substances that kill or inhibit the growth of cyanobacteria via a number of mechanisms, like cell wall degradation or photosystem inhibition.[15,73] For *Microcystis*, 65 lysing bacteria and 21 cyanobacterial antagonists have been identified.[73] There are also a number of indirect mechanisms. For example, *Brevibacillus* induces *Microcystis* to produce the lytic compound β-cyclocitral, in a way causing it to commit suicide (autolysis).[74] Planktopeptin BL produced by *Planktothrix* induced lysis via induction of a lysogenic virus in some *Microcystis* strains.[75] Importantly, allelopathic interactions are also observed between species or strains of *Microcystis*.[73] For example, *Microcystis aeruginosa* inhibits *Microcystis flos-aquae*[76] (Figure 5B). A non-MC-producing *Microcystis* mutant, which evolved spontaneously from an MC-producing wild type in the lab, did not grow in the presence of extracellular products from the MC-producing strain or in the field.[77] Interestingly, in neither of these examples MC was implicated as the active compound (it is important to distinguish here what is toxic to humans and cyanobacteria). These cases suggest there may already be NNC strains that produce toxins or inhibitors targeted at the harmful strain, and we just need to figure out how to weaponize the mechanism.

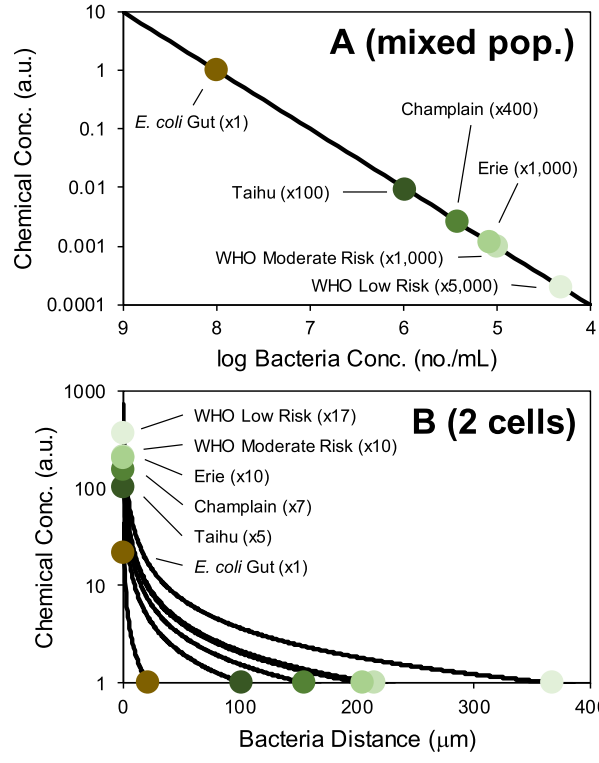

Figure 6. Cell-to-cell interaction via a chemical depends on population density and corresponding distance between neighboring cells. (A) A well mixed population. Steady-state concentration from a model with constant production and decay. (B) Diffusion between two cells. Calculations based on a 3D diffusion model.[78] *E. coli* density 1×10^8/mL,[79] lake cell concentrations are max. values from ref 80 and WHO are guidelines for moderate and low adverse health effects. Values in parentheses indicate how much more a lake producer (shown as symbols on the *y* axis) has to synthesize to result in the same concentration at the receiver (shown as symbols on the *x* axis).

For that, we need to have a better understanding of the chemicals produced by each strain, who is affected and how.

PHAGES AND PHAGEMIDS THAT KILL OR DISARM

The introduction of viruses, bacteriophage therapy, is an old idea in gut health management and there have been successful applications. For example, in one study, phage therapy was able to cure mice infected with *C. difficile*.[81] Recognized advantages over antibiotics include high specificity and self-replication (grows until the target pathogen is eliminated and then stops). Disadvantages include limited control over the host range of

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viruses, resistance via host mutation, and lysing of the host, which may result in the release of intracellular toxins. Some of these limitations are being addressed with genetic engineering. Novel molecular engineering technology can be used control the host range of viruses.[82] Also, the function of viruses can be modified. For example, a phagemid (combination of phage and plasmid) was engineered to deliver genes coding for antimicrobial peptides and protein toxins, which kills the pathogenic target bacteria without lysing it.[83] Since these phagemids do not replicate in the gut, a large number needs to be produced and introduced, here each mice was injected with 6e12 phagemid particles. In another example a phagemid was engineered using CRISPR-Cas to kill only bacteria with a specific DNA sequence or destroy the (plasmid-borne) antibiotic resistance gene in a bacteria without killing it.[84]

Virus contact rates are proportional to host and virus concentrations, which suggests this process may be less important in the relatively dilute lake environment. However, in Plussee (Germany), viruses were found to outnumber bacteria 40:1,[85] which is of similar magnitude as the gut (~10:1,[86]). Field observations suggest that the total *Microcystis* population and composition (i.e., toxic vs nontoxic) is affected by viruses.[87] The use of viruses to control cyanobacteria blooms has been discussed,[73] and similar concerns exist, like release of endotoxins. Microcystins (MCs) are mostly intracellular (~94%,[28]), so whether a management action is cyanostatic (i.e., bacteriostatic) vs cyanocidal (i.e., bacteriocidal),[17] is an important general consideration. On the one hand, lysing cells increases human health risk, because the extracellular form is harder to remove in drinking water plants.[88] On the other hand, lysis stops toxin production and makes available the existing mass for biodegradation.[16,89] For lytic approaches, the short-term increase in extracellular toxin has to be weighed against the longer-term reduction in biomass and toxin production. CRISPR-Cas-engineered phagemids could be used to target the toxin production gene(s) in lake cyanobacteria (e.g., *mcy* gene cluster in *Microcystis*) and thus selectively kill all toxin producers or render them nontoxic. A *Microcystis* strain where the toxin production gene was knocked out grows just as well under a range of light intensities,[27] suggesting a nontoxified population may continue to occupy its niche.

TALKING DOWN THE HARMFUL BACTERIA

As an alternative to killing the harmful bacteria, they may be "persuaded" to act harmlessly. For example, a small-molecule compound (called M21) was shown to change *Staphylococcus aureus* from a virulent to a nonvirulent state.[90] The signaling chemical can also be produced by engineered bacteria. For example, *E. coli* was modified to produce a chemical (cholera autoinducer I), which prevented virulence gene expression in *Vibrio cholerae* and increased survival of infected mice.[91]

Similar allelopathic interactions have also been observed for phytoplankton in lakes. For example, the cyanobacterium *Aphanizomenon ovalisporum* produces cylindrospermopsin that causes other phytoplankton to make alkaline phosphatase (APase, an extracellular enzyme used to break down unavailable esters to available inorganic phosphate), even though they are not phosphorus-limited.[92] Extracellular MC (as well as other compounds) increases MC production in *Microcystis*.[93] Importantly, this example shows that MC production can be controlled by extracellular chemical cues. As an alternative to "taming" the harmful bacteria, adding a small amount of a signaling chemical that "provokes" them to kill each other has been suggested.[94]

CLEANING UP AFTER HARMFUL BACTERIA

If we can not control the harmful bacteria, then we may manage their effect. For the gut, bacteria are being engineered that have surface proteins that mimic host receptors and bind the toxins produced by pathogens, such as *Vibrio cholerae*.[95]

In lakes this would only apply to extracellular toxins and then still would not eliminate the toxin from the system, and it may render it unavailable to biodegrading bacteria. Another potential management approach is to increase toxin biodegradation. Bacteria that degrade MC and the responsible genes (*mlrA*) have been identified,[96] which could form the basis for a toxin-degrading agent. However, it seems those bacteria are already naturally present and active in lakes. In one case, total MC concentration decreased by 99% in a few days following treatment with H_2O_2.[16] A rise in MC-degrading bacteria was observed during the decomposition of a *Microcystis* bloom in Lake Taihu.[89] Considering that MC-degrading bacteria are already there and (presumably) optimized and generally fast-growing, addition of bacteria to do the same job does not seem sensible.

MODIFYING THE AGENT

Genetic Engineering. Genetic engineering of bacteria is increasingly common, but an important constraint is that not all strains are amenable to genetic manipulation. *E. coli* is the most common prokaryotic model organism, biotechnology and synthetic biology workhorse, and since it is also a natural inhabitant of the gut it is often used in this area. However, *Bacteroidetes* and *Firmicutes* make up a larger fraction of the gut population, and genetic engineering is also starting to be applied to those.[52,97] Genetic engineering of *Clostridia* remains a challenge, but progress is also being made in this area using CRISPR technology.[98]

A number of conventional and CRISPR-based technologies for genetic/metabolic engineering have been applied to cyanobacteria, mostly to synthesize biofuels and chemical feedstocks.[99] CRISPR-Cas9 was used for gene knockout (*glgC*) and knock-in (*ppc*, *gltA*) in *Synechococcus* to produce succinate,[100] and CRISPR/Cpf1 was used to introduce three gene variants into a *Synechococcus* strain which tripled its growth rate.[101] Genetic engineering of cyanobacteria has focused mostly on harmless species, like *Synechococcus* and *Synechocystis*, but the range is expanding, including recent modification of *Anabaena* using CRISPR.[102] More directly relevant to harmful lake bacteria, homologous recombination was applied over 20 years ago to *Microcystis* to replace the toxin-producing with an antibiotic resistance gene.[103] These applications suggest that genetic engineering of an NNC strain to perform some of the functions discussed above is feasible.

Directed Evolution. Another approach to modifying the bacteria is to use targeted evolution. Laboratory evolution experiments are now common.[104] *E. coli*, can adapt to new temperatures relatively rapidly, in less than a year.[105] Evolution of new functions is slower: it took *E. coli* 31 000 generations to evolve the ability to grow on citrate.[104] Those past experiments were done mostly for the sake of science, but the approach is now also being applied to gut healthcare. The pathogen *Candida albicans* was evolved in vivo in mice, which resulted in

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a strain that is nonvirulent and protective against a number of infections.[106]

Evolution experiments are also increasingly done with phytoplankton. For example, experimental evolution showed that *Emiliania huxleyi* can adapt genetically to higher CO_2 levels in ~1 year.[107] The stress-free laboratory environment may be well suited to eliminate unwanted stress resistance functions (see below). However, there are many differences between the field and laboratory environments and experimental evolution experiments often include mutations to adapt to the "culture conditions",[105,107] and those would be expected to reduce fitness in the lake. For the gut, this problem can be overcome by performing evolution experiments in full ecosystems.[106] This is not an option for lakes, but it is conceivable that Biosphere 2 (an artificial, materially closed ecological system) could be repurposed for this.

A Dynamic Cocktail and a Collection of Strains. The overall management strategy does not have to be limited to one static strain, but can be dynamic and involve several strains. We may continually adjust and optimize a strain on a yearly basis, following the same annual reformulation approach used for the flu vaccine. A strain that successfully establishes itself in the lake may be reisolated at the end of the growing season to take advantage of any (presumably) beneficial mutations that may have occurred during in situ growth. We may also consider producing and inoculating multiple strains. This cocktail approach is used for probiotics,[108,109] as well as for antibiotics and the flu vaccine (WHO recommendations feature four strains). If the agents do not interfere with each other this would allow us to trial multiple solutions every year.

We can envision a collection of strains with different properties, which can be subject to continuous study and refinement. Over time specific strains may prove themselves to be especially effective, like *E. coli* NISSLE 1917 in the gut. Those could then be further studied, which may lead to understanding of the mechanisms responsible for their success.[68]

Exploiting the Variability of the Lake Environment. The seasonal variability of the lake environment (vs the gut) increases the chances of establishment of a foreign strain (see above), and it may be exploited further. The native strains are expected to be resistant to a number of conditions and stresses encountered during a typical year, and this comes at cost to the population growth rate during favorable conditions. For example, resistance is often achieved by phenotypic differentiation of a resistant and slow- or nongrowing fraction of the population (bet hedging), as in antibiotic persisters in the gut[110] or resting stages in cyanobacteria.[111] However, an agent cultured externally and introduced at the onset of the bloom only needs to grow during the favorable bloom conditions and eliminating a resistance mechanism should help it then. An additional benefit to removing resistance is that it may help constrain the growth of the agent to one season (see Controlling the Agent section below).

Interannual variability may also be exploited. It is fair to assume the native harmful strain is adapted optimally to the present conditions, and less optimally to other future conditions. If the change is predictable we may use this information, for example, by engineering a strain to have a higher temperature optimum. Observed long-term temperature trends for lakes are relatively small (e.g., <0.01 °C/year for Lake Erie,[112]) and will be overshadowed by interannual variability at the time scale relevant here (i.e., next year). However, seasonal forecasts are getting more accurate (e.g., up to 7 months[113]) and those could be used. This shorter time frame limits engineering and may require having a readily available collection of strains with different temperature optima. This idea does not only apply to temperature but also building or upgrading wastewater treatment plants, new lawn fertilizer regulations, etc. If we can predict future conditions we can always stay one step ahead of the native harmful strain.

MASS CULTURING

The relatively large size of lakes translates into a large inoculum biomass, which is a challenge. How much is needed will depend on the fitness of the introduced bacteria. A highly competitive (fast-growing or killer function) strain may establish itself from a few cells, whereas a slow-growing strain may need to be added at much larger biomass to be effective.

Large-scale algae cultivation is used for biofuel production and nutrient removal in wastewater treatment (i.e., high-rate algal ponds), and those uses can be combined in a dual-purpose system.[114] For these applications, systems are typically open ponds, which are subject to contamination and therefore not suitable for culturing a specific strain. Closed photobioreactors are more costly, but large-scale applications are being implemented, and they can produce biomass at a rate of ~25 gDW/m^2/d.[115] A closed system could also be attached to a WWTP if the effluent is disinfected, which is increasingly practiced in the wastewater industry as part of a fourth treatment stage. This could then be a triple-purpose system for the removal of nutrients, production of lake inoculum (at specific times) and production of biofuels or chemicals (at other times).

In the Meiliang Bay "boost" scenario (Figure 1) a mass of 1200 kgDW/d was added. This corresponds to a relative import rate of 0.21 (0.0082−15), which is substantially above those typically used for probiotics (2×10^{-7}−0.002).[108] Using the above production rate, this loading would require a culturing system with a size of 4.8 ha (220 × 220 m). An area of five football fields is not trivial, but certainly feasible and comparable to other large-scale environmental infrastructure projects. The culturing area can be larger or smaller depending on the size of the lake (e.g., Western Lake Erie: 140, West Point Lake: 3.9, Lake Kegonsa: 0.48, Müggelsee: 0.27, Copco Reservoir: 0.15, Lake Tegel: 0.15, ha, estimated using culturing area/lake area from Meiliang Bay: 3.7×10^{-4}). The required mass may also be further reduced considerably if the application can be targeted to the area where the bloom is expected to initiate (e.g., see Figure 4B).

It may also be possible to adjust the culture conditions to give the bacteria an advantage in the ecosystem. For probiotics, preadaption approaches that induce stress response are used to increase survival through the stomach acid barrier.[116] For phytoplankton, the ability to take up and store an order of magnitude more P than they need to grow ("luxury uptake") may be exploited.[117] Bacteria from a P-replete culture may have a significant advantage when introduced into a P-limited lake. This mechanisms has also been suggested to give *Microcystis* from the Maumee River an advantage in Lake Erie[118] (Figure 4B).

Using wastewater treatment plants to culture and discharge bacteria goes against the traditional civil engineering approach to purify and disinfect wastewater. However, in the drinking water industry, adding beneficial bacteria or manipulating plant

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operation to select for them (vs trying to kill all bacteria) so that those may control harmful bacteria, like *Legionella*, in the distribution system is presently also being contemplated.[119,120] For wastewater, this approach may be considered a new paradigm or "fifth treatment stage" (Figure 7).

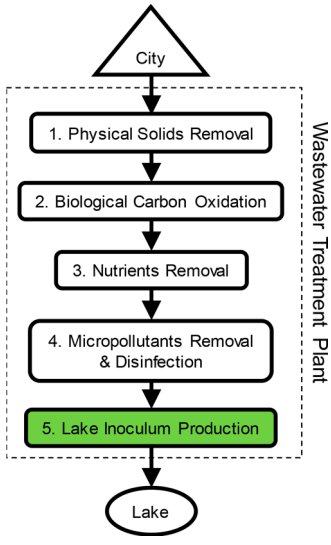

Figure 7. Mass culturing harmless bacteria to control harmful bacteria in lakes. Incorporation into wastewater treatment plant.

CONTROLLING THE AGENT

A common concern with introducing genetically engineered organisms into the environment is that they may have unintended effects, and that motivates control strategies. For example, to prevent transmission outside of the gut, *E. coli* was engineered to commit suicide (autolysis) once the temperature drops below body temperature.[121]

Control is also a concern in the lake environment. One possible and desirable outcome is that the engineered strain outcompetes or eradicates the harmful strain and then stays in the lake at low concentrations, providing it with immunity against the harmful strain. However, ecology is unpredictable and another possible and much less desirable outcome is that the introduced strain blooms heavily year-round, throughout the lake and spreads to all other lakes. This is not likely if we are using an NNC strain, because it should be naturally constrained to the niche of the harmful bacteria. Nonetheless explicit control of the engineered bacteria would be desirable.

For temperate environments, the most pragmatic approach may be to ensure that the introduced strain does not survive past the current growing season. For example, a model study suggested that knocking out akinete (resting stage) formation in *Anabaena* would significantly reduce the overwintering capability in a lake and lead to extinction.[111] An added benefit is that this engineered strain grows faster, because less biomass is invested into overwintering. Similar secondary benefits may come out of eliminating stress resistance functions that are not beneficial during normal growing conditions (see above). Of course, a temperature-activated "kill switch" would also serve this purpose.

For the exclusive nutrient concept, control could be achieved by adjusting the input of the nutrient.[52] Managing the use of a chemical at the scale of a watershed would be a challenge, but for many purposes there are often alternative chemicals that can be and are substituted at large scales (e.g., rotation of antibiotics in hospitals to control antibiotic resistance).[67,122]

PERSPECTIVES AND OUTLOOK

Gut health management is advancing at an unprecedented rate resulting in many exciting new principles and concepts (e.g., killer bacteria, exclusive nutrient, microbiota transplantation), and several may help manage harmful bacteria in lakes. Although there are a number of challenges (e.g., mass culturing, controlling the agent), there are also opportunities and these technologies have the potential to cross-fertilize (no pun intended) the field of lake management.

Maybe the most promising concept to be pursued initially is the idea of an autologous lake microbiota transplantation (auto-LMT), where a nontoxic, native cobloomer (NNC) strain is isolated, cultured and then reintroduced, boosting the NNC population and helping it outcompete the toxic strain. The authors and collaborators are presently planning a mesocosm-scale field test of this idea.

Scientists and engineers in other areas often explore futuristic ideas (e.g., bionic eye), because they may spur breakthroughs and inspire politicians, investors and the public, but in the environmental science and engineering profession there is more reluctance toward embracing new technologies.[18] For lake management, the notion that the problem is excessive nutrient input and thus the best solution is nutrient input reduction, is sensible. However, some lakes have problems even after extensive nutrient reduction (e.g., Lake Erie, Lake Tegel[43,123,124]). Further, a lake manager may have control of nutrient input, but there are other drivers (e.g., climate change, invasive species) that may prevent restoration to past conditions using nutrient reduction. The present status, trend and future projections for harmful cyanobacteria in lakes should motivate us to "boldly go where no man(ager) has gone before" (Star Trek, 1966).

For many of the strategies discussed here, success will hinge on understanding the ecology of toxic and nontoxic strains. For coarser phytoplankton categories, like species or functional groups, many spatial and temporal patterns can be explained using environmental factors, like nutrients, temperature, or light.[46,48] However, identifying environmental drivers for the ecology at a higher resolution (strains, genotypes) has been difficult. For example, some laboratory experiments show that nontoxic *Microcystis* strains are better competitors for light, and others show the opposite.[25,26] It is likely that allelopathic interactions are important at this resolution.[73,76,77] Also, interaction via "leaky" cell functions (Black Queen), like benefiting from the reactive oxygen species (ROS) scavenging function of another species,[125,126] may play a role.[16,127,128] Strain ecology is a key knowledge gap worthwhile of pursuit, and advances will benefit applied lake management and basic microbial ecology.

As our understanding of lake ecosystems becomes more mechanistically resolved, models (and modelers), which have a long and productive history in lake management, will have to evolve as well. Early models (e.g., Vollenweider,[129]) were based on simple P loading. Today's management models typically also include N and sometimes a few other processes, like vertical migration or surface scum formation,[46,130] but they are still generally based on very simple Monod-level kinetics. The progress in our models pales in comparison to the evolution of observations (e.g., genes, transcripts, proteins, metabolites)

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and associated biological and ecological understanding.[131,132] There is an urgent need and opportunity for developing/educating the next generation of models/modelers.

The solutions discussed here are potential future methods, that have not been tested, may never be tried and if implemented may fail or have detrimental effects. There presently is no substitute for established long-term control methods, like reducing N and P nutrient loadings and curbing climate change, and short-term methods, like H_2O_2 or vertical mixing, for controlling harmful bacteria in lakes. At the same time, the persistence of the harmful cyanobacteria problem dictates us to continue to look for new solutions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.9b04218.

Details of the model (e.g., equations), parametrization and application to Meiliang Bay of Lake Taihu, China (Figure 1) (PDF)

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Notes

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