Genotype and host microbiome alter competitive interactions between *Microcystis aeruginosa* and *Chlorella sorokiniana*

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ABSTRACT

Cyanobacterial harmful algal blooms (cyanoHABs) continue to increase in frequency and magnitude, threatening global freshwater ecosystems and services. In north-temperate lakes cyanobacteria appear in early summer, succeeding green algae as the dominant phytoplankton group, a pattern thought to be mediated by changes in temperature and bioavailable nutrients. To understand additional drivers of this successional pattern our study used reciprocal invasion experiments to examine the competitive interaction between *Microcystis aeruginosa*, a dominant contributor to cyanoHABs, and the green alga *Chlorella sorokiniana*. We considered two factors that may impact these interactions: (1) strain variation, with a specific emphasis on the presence or absence of the gene for the hepatotoxin microcystin, and (2) host-associated bacteria. We used toxic *M. aeruginosa* PCC 7806 (microcystin producing strain), a non-toxic mutant of PCC 7806, non-toxic *M. aeruginosa* PCC 9701 (non-microcystin producing strain), and *C. sorokiniana*. Each organism was available free of all bacteria (i.e., axenic) and with a re-introduced defined bacterial community to generate their xenic counterparts. Competitive interactions were assessed with reciprocal invasion experiments between paired xenic and paired axenic populations of *C. sorokiniana* and one of the two *Microcystis* strains, each assessed separately. Flow cytometry and random forest models were used to rapidly discriminate and quantify phytoplankton population densities with 99% accuracy. We found that *M. aeruginosa* PCC 7806, but not strain PCC 9701, could proliferate from low abundance in a steady-state population of *C. sorokiniana*. Further, the presence of bacteria allowed *M. aeruginosa* PCC 7806 to grow to a higher population density into an established *C. sorokiniana* population than when grown axenic. Conversely, when *M. aeruginosa* was dominant, *C. sorokiniana* was only able to proliferate from low density into the PCC 9701 strain, and only when axenic. The mutant of PCC 7806 lacking the ability to produce microcystin behaved similarly to the toxic wild-type, implying microcystin is not responsible for the difference in competitive abilities observed between the two wild-type strains. Quantification of microcystins (MCs) when PCC 7806 *M. aeruginosa* was introduced into the *C. sorokiniana* culture showed two-fold more MCs per cell when host-associated bacteria were absent compared to present in both species cultures. Our results show that the ability of *M. aeruginosa* to compete with *C. sorokiniana* is determined by genomic differences beyond genes involved in microcystin toxin generation and indicate an important role of host-associated bacteria in mediating phytoplankton interspecies interactions. These results expand our understanding of the key drivers of phytoplankton succession and the establishment and persistence of freshwater harmful cyanobacterial blooms.

1. Introduction

Cyanobacterial harmful algal blooms (cyanoHABs) in freshwater systems are increasing globally in frequency and magnitude due to human-caused eutrophication, rising temperatures, and changing weather and phenological patterns, including regional ice coverage, duration of stratification, and hydrology (Huisman et al., 2004; Paerl and Huisman 2008 and 2009; Paerl et al., 2016; Lürling et al., 2018). CyanoHABs impact ecosystem function by depleting bioavailable nutrients, causing localized hypoxia after their demise (Watson et al., 2016; Zilius et al., 2015), and by altering the physicochemical environment through the release of secondary metabolites (Cirri and

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Pohnert, 2019). These impacts of cyanoHABs can generate cascading effects through trophic levels (Huisman et al., 2018). Freshwater cyanoHABs are often dominated by *Microcystis aeruginosa* (Harke et al., 2016), which can release an array of secondary metabolites, including the hepatotoxin microcystin, that can negatively affect water quality essential for animal and human health.

Cyanobacteria are globally distributed in freshwater environments and naturally co-occur with eukaryotic phytoplankton (Zhou et al., 2018). In north temperate lakes, cyanobacteria are a normal component of phytoplankton succession, typically following early summer dominance by green algae (Sommer, 1989). This pattern has historically been driven by changes in temperature and the bioavailability of nitrogen and phosphorus (Sommer, 1989; Domis et al., 2007). However, anthropogenic changes in climate and land-use have led to increased temperatures and nutrient loading that have altered the environment and conditions that favour the proliferation of cyanoHABs (Paerl and Otten, 2013a). Recent marker gene and genomic studies have revealed that cyanoHABs, particularly *Microcystis aeruginosa*, contain significant within-species genetic diversity between freshwater systems, and across space and time within a single long-term bloom. This includes variation in strain toxicity (Berry et al., 2017; Jankowiak et al., 2019; Kardinaal et al., 2007a, 2007b; Pérez-Carrascal et al., 2019), mechanisms to overcome nutrient limitation including carbon-concentrating mechanisms and phosphate transporters (Jackrel et al., 2019; Sandrini et al., 2014; Sandrini et al., 2016a, 2016b; Visser et al., 2016), and variation in associated heterotrophic communities or microbiomes (Chun et al., 2020; Cook et al., 2020; Kim et al., 2019; Jackrel et al., 2019). Succession from toxic to non-toxic variants of *M. aeruginosa* has been shown in several thermally stratified lakes across the globe (Chun et al., 2020; Dong et al., 2019; Fastner et al., 2001; Kardinaal et al., 2007b; Welker et al., 2007). Various explanations have been offered to explain this succession pattern, including physicochemical factors that might benefit certain variants over others, such as light levels (Kardinaal et al., 2007a; Phelan and Downing, 2011), phosphonate and nitrate concentrations (Harke and Golber, 2013; Vézie et al., 2002; Wang et al., 2015), reactive oxygen species concentrations (Dziallas and Grossart, 2011b; Paerl and Otten, 2013a), and temperature (Davis et al., 2009; Dziallas and Grossart, 2011a, 2011b). Explanations for these successional patterns have also included biological factors, such as cyanophage lysis-mediated strain succession (Yoshida et al., 2008). However, in complex, natural environments a combination of these factors likely drives strain heterogeneity across time and space (Chun et al., 2020; Li and Li 2012).

Competitive interactions, determined by species and strain optimization to the above listed abiotic and biotic factors, are well understood to be essential in the limitation and control of nuisance phytoplankton (Tilman, 1982; Tilman et al., 1997; Shea and Chesson, 2002). In this study we focus on competitive interactions between *Microcystis* variants and a robust green algal competitor, *Chlorella sorokiniana*. While many species of green algae co-occur with *Microcystis*, we use a model genus for green algae, *Chlorella* that has been used in studies examining competitive interactions with *M. aeruginosa* (Ji et al., 2017; Ma et al., 2015; Song et al., 2017; Wang et al., 2015; Wang et al., 2017; Zhang et al., 2007). In addition to resource competition, we also have to consider interference competition. Multiple studies have examined allelopathy in intraspecific interactions between toxic and non-toxic *Microcystis* variants (Kardinaal et al., 2007a), and interspecific interactions between *Microcystis* variants and other phytoplankton, including green algae (Bittencourt-Oliveira et al., 2014; Dong et al., 2019; Ji et al., 2017; Ma et al., 2015; Song et al., 2017; Yang et al., 2018; Zang et al. 2007), and diatoms (Wang et al., 2017). The biological function of microcystin in these interactions, and its function in general, has been widely debated. Proposed theories for the role of microcystin include acting as a primary and secondary metabolite (Schatz et al., 2007), an allelochemical (Li and Li, 2012; Waal et al. 2011), protection against oxidative stress (Hernando et al., 2016; Paerl and Otten, 2013b; Phelan and Downing, 2011; Zilliges et al., 2011), and as a carbon

allocation mechanism (Jähnichen et al., 2007).

The outcomes of competitive interactions often depend on the environmental constraints imposed, including the biotic environment. In this study, we focus on the role of host-associated bacteria, or microbiomes, which are known to alter numerous physicochemical factors and therefore may have similar impacts as physicochemical environmental conditions on the interactions between their algal hosts. Heterotrophic bacteria are known to influence phytoplankton host fitness, by altering the availability of micronutrients, remineralizing macronutrients, and aiding in the assimilation of vitamins such as B12 (Cho et al., 2015; reviewed in Seymour et al., 2017; Samo et al., 2018; Cirri and Pohnert, 2019). Metagenomic studies of cyanobacteria microbiomes show divergence of associated taxa or functional genes based on nutrient gradients, and to a lesser extent based on host genotype (Jackrel et al., 2019; Frischkorn et al., 2017). Further, Cook et al. (2020) provided evidence of a co-evolved interactome of associated bacteria and *Microcystis* that is reinforced by complementary biochemical pathways. Therefore, assessing the microbiome of *Microcystis* and other phytoplankton is critical in understanding the physiological constraints on phytoplankton host fitness, and thus predicting the outcome of interspecific interactions that lead to the proliferation of cyanoHABs.

Impacts of microbiomes on competitive interactions between their hosts have been shown for green algae and plants (Siefert et al., 2018 and 2019; Jackrel et al., 2020), and here we address whether they also impact interactions between the cyanoHAB forming species *Microcystis* and a model green alga species *C. sorokiniana*. To understand the interplay between genetic diversity within *Microcystis aeruginosa*, specifically the role of microcystin, and phytoplankton microbiomes in driving cyanobacterial bloom successional patterns, we performed competition experiments. During these experiments, we tested two main hypotheses: (i) *M. aeruginosa* strain variation, including the occurrence of the microcystin **biosynthesis** gene, alters the competitive outcome with *C. sorokiniana* due to the relative fitness advantage of microcystin production, and (ii) the presence of host-associated bacteria alters the competitive outcome between *C. sorokiniana* and the two *M. aeruginosa* populations by affecting host fitness. Throughout the study, we use the term microbiome to refer to an engineered bacterial community that associated with our phytoplankton hosts. During the competition experiments, we monitored phytoplankton population growth using flow cytometry to rapidly discriminate and quantify population densities. We also quantified total microcystin content during competition experiments to determine the relative effects of toxin production and host-associated bacteria in mediating phytoplankton successional dynamics.

2. Materials & methods

2.1. Phytoplankton cultures

We obtained three strains of *Microcystis aeruginosa* from the Pasteur Culture Collection of Cyanobacteria (Pasteur Institute, Paris, France). PCC 9701 is a non-microcystin producing strain, PCC 7806 is a microcystin-producing strain, and the *mcyB*- PCC 7806 mutant strain cannot synthesize microcystin due to a mutation of the microcystin synthetase gene (Dittmann et al., 1997). We obtained a strain of the green alga, *Chlorella sorokiniana* (UTEX 2805), from the University of Texas Culture Collection of Algae (UTEX; Austin, Texas, USA). We grew cultures of all strains in COMBO medium, a defined freshwater media for algae and zooplankton (Kilham et al., 1998). All incubations were performed on shaker tables set to a continuous 80 RPM under a light intensity of 30 µmol photons m^{-2} s^{-1} set to a 16:8 h light:dark cycle and 20 °C. This temperature represents an intermediate temperature optimum between favourable conditions for green algae and *Microcystis* that ensures that neither phytoplankton group is favored competitively (addressed in Nolan et al. 2019).

Our strain of *C. sorokiniana* had been stored long-term in a laboratory

collection at the University of Michigan, where heterotrophic bacteria were observed within the algal phycosphere. All *M. aeruginosa* strains were purchased axenic and required no manipulation. *Chlorella sorokiniana* was rendered axenic following the protocol outlined in Jackrel et al. (2020). Briefly, this approach combines ultrasonication of phytoplankton to liberate host-associated bacteria and single-cell fluorescence-activated cell sorting of algae onto plated solid media. Plates were then sealed with breathe-easy films (Diversified Biotech), which allow for gas exchange and maintain sterility, and incubated until visible colonies were examined under a dissecting scope for heterotroph contamination (Pereira et al., 2011). Colonies of *C. sorokiniana* that appeared heterotroph-free were streaked on COMBO-agar. Single colonies of *C. sorokiniana* were then inoculated into sterile COMBO media to generate axenic stock cultures.

2.2. Confirming axenic state

Samples taken from each phytoplankton culture were stained with DAPI (4′−6-diamidino-2-phenylindole) and visualized for heterotroph contamination on an Axio Imager 2 Zeiss fluorescent microscope under 1000X magnification with an oil immersion lens and DAPI filter (bandpass, 470/20 nm excitation; long pass, 515 nm emission). We confirmed the absence of culturable bacteria in axenic *M. aeruginosa* and *C. sorokiniana* cultures by streaking phytoplankton cultures on R2A agar plates. Plates were incubated in the dark at room temperature and visualized for heterotroph growth after 5 days. For *C. sorokiniana,* we confirmed the absence of heterotrophic bacteria, including those that could not grow on R2A medium through colony PCR. We detail this protocol in Jackrel et al. (2020). We confirmed the absence of phytoplankton heterotrophs in stock and experimental cultures periodically using microscopy and DAPI staining.

2.3. Bacterial isolation and identification

We isolated host-associated bacteria from phytoplankton cultures using a culture-based approach to later generate host-specific defined bacterial communities. For *M. aeruginosa,* bacteria were isolated from the freshwater strain LE3 (Brittain et al., 2000) because the toxin-producing strain (PCC 7806) and non-toxin producing (PCC 9701) strains were purchased axenic, meaning the culture was unialgal and had no bacteria present in the culture. We chose to isolate bacteria from *M. aeruginosa* strain LE3 because this is an environmentally relevant strain isolated from Lake Erie, USA that has been previously used (Rinta-Kanto et al., 2009; Saxton et al., 2012). For *C. sorokiniana,* we isolated bacterial heterotrophs that were present in the xenic *C. sorokiniana* culture. Using aseptic technique, both phytoplankton cultures were streaked on R2A agar, which is a medium optimized for slow-growing bacteria in potable freshwater (Reasoner and Geldreich, 1985). Plates were incubated in the dark at room temperature for 2–5 days and streaked for colony isolation.

We extracted DNA from morphologically distinct colonies by dissolving a single colony in 10 μL nuclease-free water and incubating for 10 min at 100 °C using the PCR Mastercycler (Nexus gradient). We amplified the 16S rRNA gene from the extracted DNA using the product supernatant as the PCR template with the universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTAC-GACTT-3′). Each 25 μL PCR reaction contained 13 μL nuclease-free water, 10 μL NEBNext High-Fidelity 2X PCR Master Mix, 1 μL template, 0.5 μL 10 μM forward primer, and 0.5 μL 10 μM reverse primer. PCR conditions were as follows: 94 °C for 3 min, 35 cycles of denaturation (94 °C for 45 s.), annealing (46 °C for 60 s.), and extension (72 °C for 90 s.), and a final extension at 72 °C for 10 min. Amplified PCR products were analysed by electrophoresis on a 1% agarose gel, purified using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany), and sequenced with an Applied Biosystems 3730xl DNA analyzer (University of Michigan DNA Sequencing Core, MI, USA). Sequences were compared to the NCBI Genbank database using default BLASTn parameters to identify the bacterial isolate with the highest nucleotide pairwise identity.

2.4. Engineering defined xenic cultures

Isolated bacteria were introduced to axenic phytoplankton to generate host-specific defined bacterial communities. Bacterial colonies were inoculated into sterile R2A broth and incubated at room temperature on shaker tables overnight, or until growth was visible. Bacterial densities were approximated based on optical density measurements at 600 nm (OD_{600}). Thirty microliters of each bacterial culture, with OD_{600} measurements ranging from 0.10–0.34, were spiked into 100 mL of axenic phytoplankton at a density of 10,000 cells mL^{-1}. Based on the number of bacteria we could culture, two bacterial heterotrophs isolated from the long-term culture of *C. sorokiniana* were added to axenic *C. sorokiniana* (isolates #1 and #2, Table 2), and three bacterial heterotrophs isolated from LE3 were added to axenic strains of toxic and non-toxic *M. aeruginosa* (isolates #3–5, Table 2). After bacteria were spiked into the axenic phytoplankton cultures, the cultures were incubated for two-weeks prior to starting experiments to allow bacteria to associate with their algal host. During the two-week incubation period, fresh COMBO media was added to phytoplankton cultures in order to maintain viable phytoplankton growth. Prior to introducing the second phytoplankton population, we confirmed the retention of the bacterial community via microscopy. As there was no evidence of density-dependant effects of the bacterial inoculum size on phytoplankton host fitness (Supp. Figure 1), we did not continue to monitor bacterial densities in the phytoplankton cultures.

2.5. Competition experiments

After establishing axenic phytoplankton monocultures and their engineered xenic counterparts, we inoculated all cultures at 10,000 phytoplankton cells mL^{-1} in fresh COMBO media and incubated as described previously in Section 2.1. We evaluated pairwise species interactions between *C. sorokiniana* and the toxic and non-toxic strain of *M. aeruginosa* in the presence versus absence of host-associated bacteria (i.e. a xenic culture interacting with xenic culture, and axenic culture interacting with axenic culture) using the mutual invasibility criterion (Chesson 2000). This approach has been frequently used to examine interspecific interactions that mediate species coexistence, and can be more broadly applied to understand competitive interactions that mediate natural successional turnover in phytoplankton communities (Jackrel et al., 2020; Siefert et al., 2019). This criterion uses an experimental approach in which a species pair, A and B, are grown in monoculture until reaching steady-state, which we define as the measurement at which chlorophyll-a fluorescence did not change significantly for two days. Subsequently, the second species is introduced at low density to determine whether it can exhibit growth in presence of the established species (i.e., whether it can invade the established culture). Phytoplankton monoculture growth was tracked by chlorophyll-a fluorescence using a plate reader (Biotek Multi Plate Reader) until steady-state growth was achieved (Supp. Figure 2). From initial inoculation until steady-state, we assessed the effects of the phytoplankton microbiome on monoculture host fitness by estimating maximum growth rate and final carrying capacity. We tested pairwise species combinations of each of our phytoplankton pairs (PCC7806 and Chlorella, PCC9701 and Chlorella), but assessed only xenic phytoplankton against other xenic phytoplankton and only axenic phytoplankton against other axenic phytoplankton. Each treatment was examined in triplicate, with all replicates spatially randomized in a Percival incubator. Once steady state was reached for the resident species, the second phytoplankton population was introduced at a density of 10,000 phytoplankton cells mL^{-1}. To be able to track growth of each phytoplankton species when grown together, we differentiated and quantified

cells of *C. sorokiniana* and *M. aeruginosa* using flow cytometry (Attune NxT Acoustic Focusing Cytometer, Invitrogen, USA). Due to the results from this first experiment, we then conducted a second competition experiment between axenic *C. sorokiniana* and the *mcyB-* mutant strain of *M. aeruginosa*, following the methods described above, to test explicitly the effect of the presence versus absence of the microcystin **biosynthesis** gene in the competitive outcome. To control for variation in competition outcome that may result due to time, phytoplankton monocultures and the pairwise interaction of PCC7806 and Chlorella were also assessed in triplicate and physically randomized in the Percival incubator.

2.6. Flow cytometry quantification

We used flow cytometry to distinguish between phytoplankton populations using multiple intrinsic cellular properties including morphology, internal complexity, and autofluorescence. Following flow cytometric procedures described extensively in Props et al. (2016), we excluded laser noise and characterized single-cells by cell size (forward scatter), granularity (side scatter), chlorophyll-b fluorescence (blue laser excitation 488 nm, emission 695/40 nm), and chlorophyll-a fluorescence (violet laser excitation 405 nm, emission 660/20 nm). All flow cytometric data was visualized in the R studio using the R package "Phenoflow" (Props et al., 2016). We tracked phytoplankton population densities over 19 days, and discriminated between populations using the Random Forest classifier for supervised demarcation (RandomF_FCS function in Phenoflow), described further in Section 2.8.

2.7. Microcystin quantification

We also monitored cultures used in the first competition experiment for total microcystin content (combined intracellular and extracellular) using the Abraxis Microcystins/ Nodularins (ADDA) ELISA Kit (Warminster, PA, USA). This kit allows for a congener-independent detection of various microcystins with the LR-microcystin variant being the most toxic and common (Omidi et al., 2018). Cultures were sampled 15 days after the second phytoplankton population was added, based on our findings that our phytoplankton populations reach steady-state growth at approximately two-weeks. For microcystin sampling, each treatment flask was gently mixed before pipetting 1 mL culture into a cryovial containing 5 μL of 25% glutaraldehyde. The cryovials were then snap

frozen in liquid nitrogen and stored at −80 °C until processed. To obtain total microcystin concentrations, samples were lysed using 3 freeze-thaw cycles and an Abraxis QuikLyse™ Kit. Samples were then immediately processed following the manufacturer's instructions for the ELISA assay.

2.8. Statistical analyses

For surveys on the effect of bacterial communities on host growth, we fit logistic growth models to estimates of phytoplankton population growth based on chlorophyll-a fluorescence measurements in monocultures with and without their associated microbiomes. We fit the model to each replicate to obtain multiple, individual calculations of phytoplankton maximum growth rate (μ) using the 'growthrates' package in R software (Petzoldt 2018). Estimates of carrying capacity for each monoculture replicate were estimated based on the average chlorophyll-a fluorescence during the last two days of growth (Fig. 1). We ran a linear mixed effects model with day interacting with either axenic/xenic state or phytoplankton population identity as the fixed effects, and biological replicate as a random effect. P-values were calculated using the analysis of variance function in the "nlme" package (Pinheiro and Bates, 2000; Supp. Table 1).

To rapidly and accurately estimate phytoplankton population densities based on flow cytometry characterization, we trained Random Forest models for each species interaction pair (i.e. *C. sorokiniana* and toxic *M. aeruginosa*). We used flow cytometric data from each monoculture population to train the Random Forest classifier. For each model we randomly downsampled to 10,000 cells per sample and used 75% of our training data to train the model and generated a new model for every three day increment to account for phenotypic shifts in the population over time. Samples were cross-validated 10 fold and repeated 3 times. The model was tested on the remaining 25% of the training data. In the competition experiments, we compared the ability of a population to grow when rare in an established population in the axenic/xenic state using the linear mixed effect model and analysis of variance as described above for monoculture growth. We also compared monoculture growth of the established population versus growth in the presence of another population to account for potential false-positive cell counts as a result of Random Forest model error.
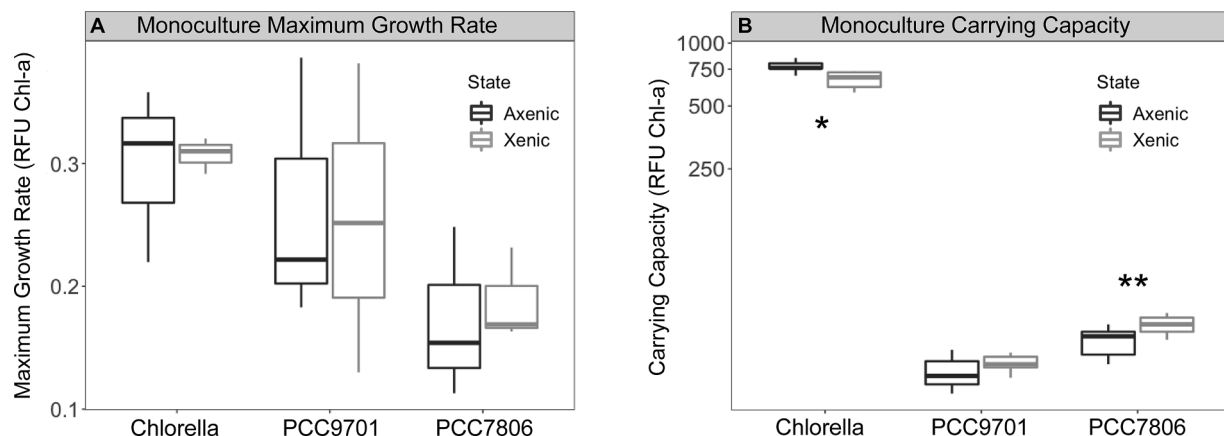

Fig. 1. The presence of host associated-heterotrophs had a mixed effect on phytoplankton fitness as determined by maximum growth rate (μ_{max}) and carrying capacity (K) of the monocultures. (**A**) The microbiome had no effect on the (μ_{max}) of their phytoplankton host in monoculture. The (μ_{max}) of each of the three replicates were calculated from a parametric growth model fit to chlorophyll-a raw fluorescence unit (RFU) measures using the "growthrates" package in R. (**B**) The presence of the host-microbiome decreased K for *C. sorokiniana* (Chlorella), increased K for toxic *M. aeruginosa* (PCC7806), and had no effect on the non-toxic *M. aeruginosa* (PCC9701) strain. We calculated K by taking the average chlorophyll-a RFU for each of the three replicates on day 18 and 19, when cultures were at steady-state growth. For both **A** and **B**, axenic monocultures (black boxes) are compared to a xenic monoculture (light grey boxes) containing a defined bacterial community. Asterisks indicate for which phytoplankton cultures' axenic/xenic state significantly affected growth, determined by performing an analysis of variance on a linear mixed model on the data. A single and double black asterisk indicates P value of ≤ 0.05 and ≤ 0.01 respectively.

3. Results

We isolated five bacteria from our xenic phytoplankton cultures, two derived from *C. sorokiniana* and three from *M. aeruginosa* strain LE3 (Table 1). Based on the NCBI blast database, the closest matches to the 16S rRNA gene sequence of our two *C. sorokiniana*-associated isolates were *Variovorax paradoxus* (96% identity) and *Pedobacter glacialis* (96.5%), and to our three *M. aeruginosa*-associated isolates were *Aeromicrobium ponti* (97%), *Rhizobium* sp. (96.8%), and *Blastomonas fulva* (97%), respectively. We engineered defined bacterial communities instead of using undefined bacterial communities to facilitate interpretations of the potential role of the bacteria taxa and host genotype in determining competitive interactions. We first examined the effect of host-associated bacteria on phytoplankton fitness by comparing growth of axenic phytoplankton cultures and defined xenic phytoplankton monocultures. From the logistic models, we found that our bacterial communities had no significant effect on maximum growth rate for either *C. sorokiniana* or *M. aeruginosa* strains in comparison to the axenic monoculture (Fig. 1, p-values in Supp. Table 1). Final carrying capacity for each monoculture was calculated on day 19 of growth and determined based on chlorophyll-a fluorescence. The carrying capacity of *C. sorokiniana* decreased when in the presence of its microbiome when compared to its axenic monoculture (p-value: 0.0141). Inversely, the xenic population of toxic *M. aeruginosa* reached a higher carrying capacity than the axenic population (p-value: 0.006). Host-associated bacteria had no effect on the final carrying capacity of the non-toxic *M. aeruginosa* strain (p-value: 0.29).

Random forest models were trained on each phytoplankton interaction pair (e.g., *C. sorokiniana* and PCC7806 *M. aeruginosa*) and compared multiple cytometric features, including forward scatter (proxy for cell size), side scatter (granularity), chlorophyll-b fluorescence (blue laser excitation 488 nm, emission 695/40 nm), and chlorophyll-a fluorescence (violet laser excitation 405 nm, emission 660/20 nm) (Fig. 2). When all cytometric features were taken together, our models consistently performed with 99% accuracy (Fig. 2, panel C) across interaction pair and over the course of the experiment.

During the competition experiments we found that strain variation and host-associated bacteria altered the strength and in some cases the outcome of competition. When comparing the effect of strain variation amongst axenic phytoplankton cultures, we found that *C. sorokiniana* was only able to invade and proliferate in a steady-state population of the non-toxic *M. aeruginosa* strain PCC 9701 (Fig. 3a). In the reverse interaction, when *C. sorokiniana* was the dominant population, only the toxic strain of *M. aeruginosa* (PCC 7806) was able to invade and proliferate, whereas the non-toxic *M. aeruginosa* strain (PCC 9701) was excluded (Fig. 3a, *strain:day* interaction p-value: <0.0001, other significant interactions in Supp. Table 1). We found this trend in two out of the three biological replicate competitive interactions of toxic *M. aeruginosa* (PCC 7806) invading *C. sorokiniana*. In the replicate that did not follow this trend, the population was able to grow in an established culture of *C. sorokiniana*, however, did not reach as high of carrying capacity as the other two replicates. Additionally, one replicate, in the axenic state where *C. sorokiniana* is rare in an established culture of *M. aeruginosa* PCC 9701 was removed from our analysis due to bacterial

contamination determined by microscopy.

Based on the differential competitive abilities of the toxic PCC 7806 and non-toxic PCC 9701 *M. aeruginosa* strains with *C. sorokiniana*, we ran a follow-up experiment to test whether this result was dependant on the occurrence of the microcystin toxin gene, or on other phenotypic factors related to strain variation. To answer this question, we used a *mcyB*-mutant of the toxic *M. aeruginosa* strain PCC 7806, which is deficient in microcystin production. We found that, similar to our observation for the wild-type strain, the *mcyB*- mutant was able to invade and proliferate into a steady-state population of *C. sorokiniana* and in the reverse interaction, *C. sorokiniana* was unable to invade into a steady-state population of the *mcyB*- mutant (p-value > 0.5, Fig. 3b).

When examining the effect of host-microbiomes on phytoplankton competitive interactions, we found that the presence of host-associated bacteria altered the growth of the toxic *M. aeruginosa* strain when toxic *M. aeruginosa* was rare (nlme p-value: 0.006; lme4 Pr(>Chisq): 0.004), namely allowing toxic *M. aeruginosa* to reach a marginally higher final population density (p-value: 0.056) than when grown in the absence of host-associated bacteria (Fig. 4). Similar to the axenic state, we found this trend in the xenic state in two out of the three biological replicate competitive interactions. However, in the axenic and xenic outlier replicates, the xenic culture remained the stronger competitor (Fig. 4). These trends were also confirmed through monitoring phycocyanin fluorescence over the course of the experiment, in which an increase in phycocyanin fluorescence indicates an increase in *M. aeruginosa* population density (Supp. Figure 3). In the axenic/xenic outlier replicate there was no significant difference in phycocyanin fluorescence between *C. sorokiniana* growing in monoculture and growing in competition, further indicating it as an outlier. For the inverse interaction where *M. aeruginosa* was dominant, *C. sorokiniana* was unable to invade the toxic *M. aeruginosa* strain population in either the presence or absence of bacteria. However, *C. sorokiniana* was able to invade and persist in non-toxic *M. aeruginosa,* PCC 9701 in the axenic state (*state:day* interaction p-value: <0.0001), while xenic *M. aeruginosa* PCC 9701 resisted the invasion of xenic *C. sorokiniana*.

In the majority of our competition experiments, the introduction of a second population inhibited the population density of the established species. We found that when comparing the steady-state population of phytoplankton in monoculture versus competition, inhibition of the resident population was particularly evident in the combinations where the invader successfully established. For example, the population density of the established species *C. sorokiniana* decreased over the course of the experiment in the xenic state, and maintained a lower population density in the axenic state when grown with the toxic *M. aeruginosa* strain in comparison to *C. sorokiniana* growing alone (i.e. monoculture) (Supp. Figure 4). Further, in the axenic state, the growth of PCC9701 was strongly inhibited by the invasion of *C. sorokiniana* relative to PCC9701 monoculture growth (Supp. Figure 4).

We quantified the total microcystin content (combined intracellular and extracellular) 15 days after the introduction of the invader. We chose this timeframe based on the observation that phytoplankton cultures reached steady-state growth after two weeks. Microcystin concentration per cell (µg/cell) was calculated by dividing microcystin content by toxic *M. aeruginosa* population density determined by flow

Table 1

Bacteria isolated from xenic populations of the corresponding phytoplankton species were characterized by colony morphology and Sanger sequencing of the 16S rRNA gene. Sequences were compared to the NCBI Genbank database using BLASTn and the bacterial isolates with the highest percent identity are listed. Xenic phytoplankton populations used in our study were engineered by adding isolates 1 and 2 to *C. sorokiniana* UTEX 2805 and isolates 3–5 to toxic *M. aeruginosa* PCC 7806 and non-toxic *M. aeruginosa* PCC 9701.

| Isolate #	Algal Host	Morphological Description	Family ID	Spp. ID (Perc. Ident.)
1	*C. sorokiniana*	Shiny, slimy, yellow	*Comamonadaceae*	*Variovorax paradoxus* (96%)
2	*C. sorokiniana*	Smooth, round, yellow	*Sphingobacteriaceae*	*Pedobacter glacialis* (96.5%)
3	LE3 *M. aeruginosa*	Semi-translucent, yellow, non-distinct edges	*Nocardioidaceae*	*Aeromicrobium ponti* (97%)
4	LE3 *M. aeruginosa*	White, cloudy	*Rhizobiaceae*	*Rhizobium* sp. (96.8%)
5	LE3 *M. aeruginosa*	Bright orange	*Sphingomonadaceae*	*Blastomonas fulva* (97%)
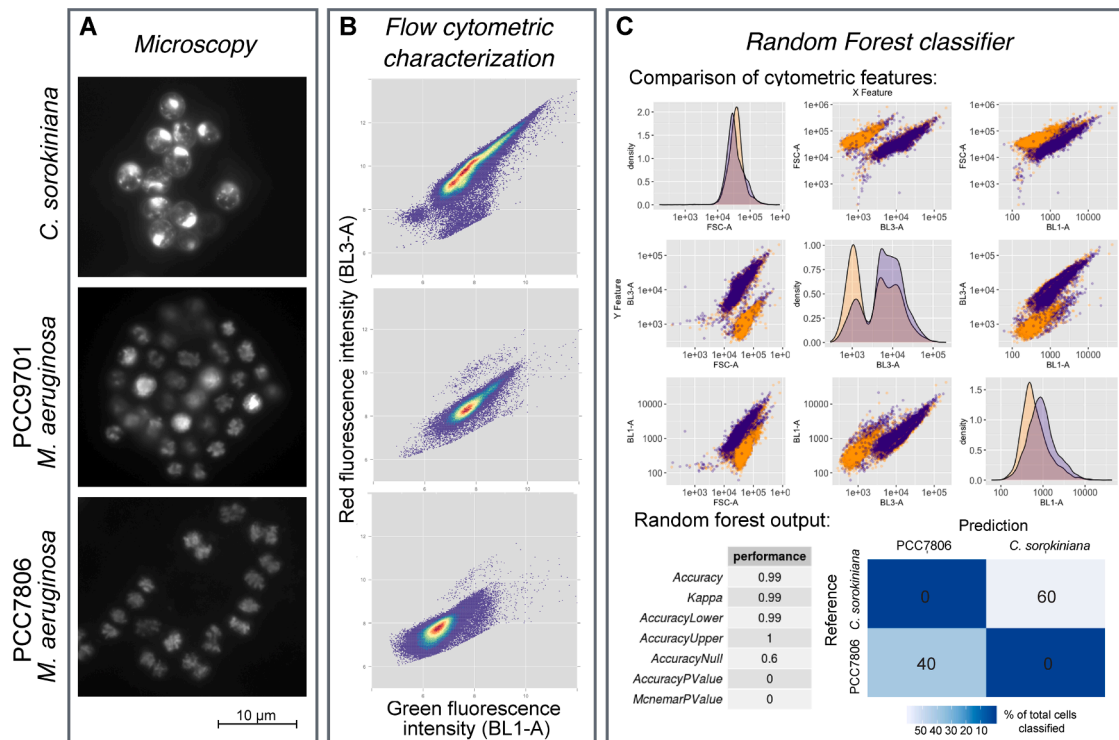
Fig. 2. Phytoplankton populations were rapidly differentiated in competition experiments using flow cytometry and a machine learning algorithm based on monoculture data. (**A**) Absence of bacterial heterotrophs confirmed with microscopy of phytoplankton cultures. Micrographs were taken at 1000 X oil immersion magnification stained with DAPI ((4′−6-diamidino-2-phenylindole) nucleic-acid stain and visualized under the DAPI filter (Axio Imager 2 Zeiss fluorescent microscope, bandpass, 470/20 nm excitation; long pass, 515 nm emission). As these phytoplankton cultures are morphologically indistinguishable without nucleic acid staining, populations of *C. sorokiniana* and toxic, PCC7806 or non-toxic, PCC9701 *M. aeruginosa* were differentiated based on their intracellular fluorescence signals. (**B**) Flow cytometric characterization of monoculture populations based on multiple cellular intrinsic properties, two of which are presented in the panel (green fluorescence intensity vs. red fluorescence intensity). In the flow cytometry (FCM) plots, points represent cells and warm to cool colors represent high to low density based on the number of detected cells with those properties. Monoculture populations have overlapping spectra patterns, and thus several cellular properties were recorded for discrimination in bicultures. (**C**) At the top, select cellular properties derived from FCM are compared against one another with the colors representing data from *C. sorokiniana* (orange) and toxic *M. aeruginosa*, PCC7806 (purple) populations. Below, FCM data is used to train a Random Forest classifier that is able to differentiate between populations with 99% accuracy, results of the model predictions are shown in the table.

cytometry. We found that when toxic *M. aeruginosa* invaded *C. sorokiniana* two-fold more microcystin per *M. aeruginosa* cell was present in the absence of host-associated bacteria (1.7×10^{-3} μg/cell) than in the presence of host-associated bacteria (8.5×10^{-4} μg/cell) (p-value: 0.02, Fig. 5).

4. Discussion

The present study revealed that host-associated bacteria can alter the competitive interaction between *M. aeruginosa* and *C. sorokiniana*. The magnitude of these effects were dependant on strain variation within *M. aeruginosa*, as well as the direction of succession (i.e. cyanobacteria succeeding green algae, versus green algae succeeding cyanobacteria). While numerous studies have assessed the environmental and biotic factors contributing to *Microcystis* dominated cyanoHABs, our study provides experimental evidence that heterotrophic bacteria influence competitive interactions between phytoplankton, using an approach rooted in coexistence theory. These results support recent findings from in situ sampling and isolate collections derived from the environment that heterotrophic bacteria play significant roles in cyanobacterial metabolism and intraspecific niche divergence (Jackrel et al., 2019; Cook et al., 2020) that likely govern interspecific interactions amongst phytoplankton.

Stable coexistence of two species requires capability for mutual invasion, i.e., that either species can grow from low density in the presence of an established population of the other species (Chesson, 2000). Results from our competition experiments, independent of the strain used, never showed mutual invasion capabilities, suggesting that *M. aeruginosa* strains and *C. sorokiniana* are unable to stably coexist within a homogenous/mixed, nutrient replete environment. This result supports findings by Ma et al. (2015) that axenic strains of toxic *M. aeruginosa* FACHB-905 and non-toxic *M. aeruginosa* FACHB-469 are unable to stably coexist with *Chlorella vulgaris* in mesocosm conditions in temperatures ranging 20 to 25 °C. The mutual invasibility experimental criterion has been widely used to study the theory of phytoplankton coexistence, but it can be more broadly applied to assess interspecific interactions that underlie environmental phenomena. Specifically, the ability of a species to proliferate in the presence of an established population mirrors phytoplankton successional patterns in some environments, such as in north-temperate lakes where green algae reach peak abundance in early spring followed by cyanobacteria (Sommer, 1989). According to stochastic niche theory, the invading species, such as *M. aeruginosa*, can become established only if the population can survive stochastic mortality while growing on the resources unused by the established species, such as *C. sorokiniana* (Tilman, 2004). In our competition experiments, we mimicked eutrophic environmental conditions by growing *M. aeruginosa* and *C. sorokiniana* cultures in nutrient rich COMBO media without media replenishment. In this setup, we can conclude that if a second population can establish when rare, where amongst species intraspecific competition is limited, this must be due either to (1) relative fitness differences between the two species, which give way to competitive hierarchies (i.e. resource uptake efficiency), or (2) niche differences between the two species that offset competitive differences in population growth. While we found no evidence for
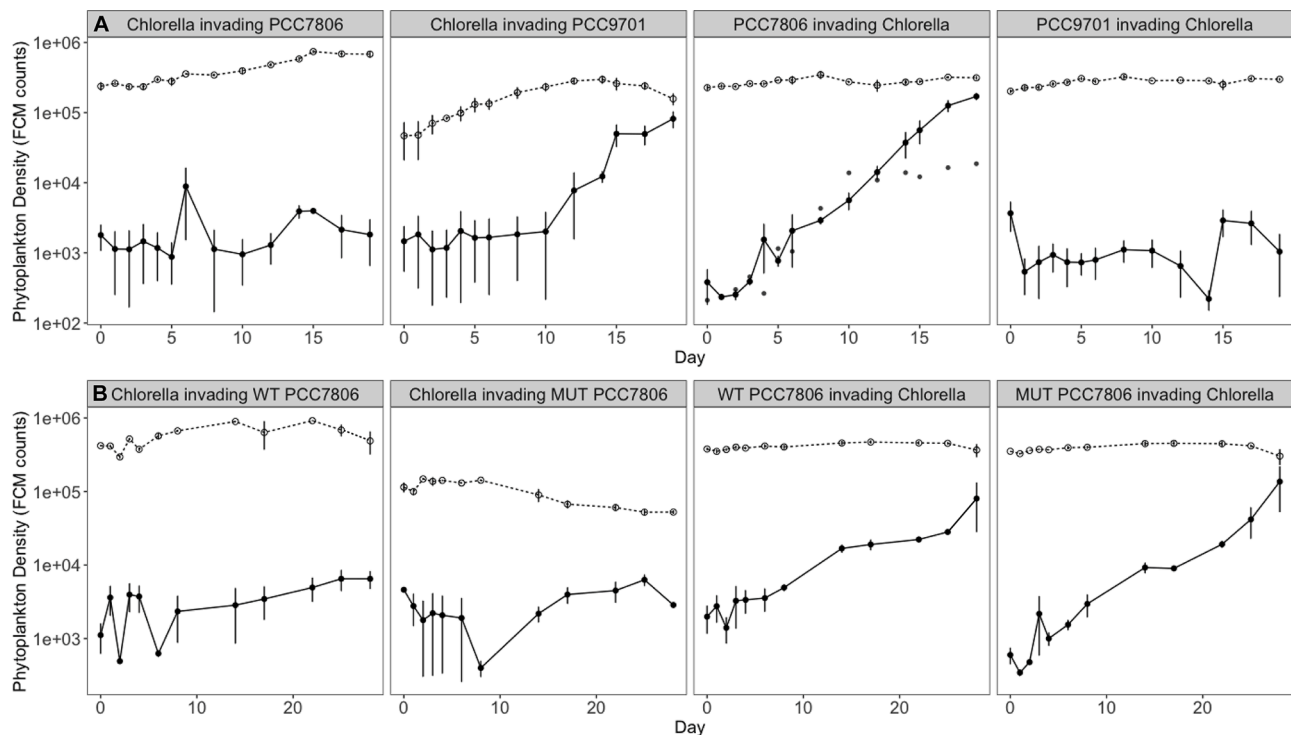
Fig. 3. Strain variation in the cyanobacterium *M. aeruginosa* alters the competitive interaction with the green alga *C. sorokiniana* under axenic conditions. Two sets of competition experiments were conducted under the same laboratory conditions, but at two separate time-points tracking both the resident phytoplankton population (open circles) and the invading population (filled in circles). (**A**) In the first experiment, the toxic *M. aeruginosa* strain, PCC7806, capable of producing the microcystin toxin, but not the non-toxic strain, PCC9701, was able to invade and proliferate into a steady-state population of *C. sorokiniana*. In the reverse interaction, *C. sorokiniana* was not able to establish in PCC7806, however *C. sorokiniana* was able to invade PCC9701. (**B**) In the second experiment, the *mcyB*- mutant ("MUT PCC7806") of the toxic *M. aeruginosa* strain, which is deficient in microcystin production, was able to invade and establish in a steady-state population of *C. sorokiniana*, however *C. sorokiniana* was not able to establish in the MUT PCC7806 population. There is no statistical difference between the competitive interactions between the WT and MUT PCC7806 *M. aeruginosa* strains and *C. sorokiniana*. Circles on the graph represent the mean phytoplankton density estimated by flow cytometry of three biological replicates and error bars represent the standard error of the mean, with exception of the PCC7806 invading *Chlorella*, in the first experiment, were one outlier is not included in the average, but is represented on the graph as individual measurements across time.

bi-directional invasibility amongst our phytoplankton interactions, unidirectional invisibility suggests relative fitness differences between our phytoplankton populations, with the most likely explanation being differential ability to access light or nutrients, although we could not explicitly quantify these factors based on our experimental design.

Host microbiomes are known to alter the physiology and response of their phytoplankton hosts to their environment (Cirri and Pohnert, 2019; Frischkorn et al., 2017; Ramanan et al., 2016), and are therefore suspected to play key roles in mediating phytoplankton between species interspecific interactions during harmful algal blooms (Seymour et al., 2017). These interactions between phytoplankton and bacteria, although occurring on a scale of microns, can exert ecosystem-scale effects on biogeochemical cycling, nutrient cycling, primary productivity, and toxin production (Buchan et al., 2014; reviewed in Seymour et al., 2017; Frischkorn et al., 2017). Specifically, heterotrophic bacteria have been shown to both augment and buffer the effects of harmful algal blooms by enhancing the growth of HAB forming species, increasing toxin production, and terminating HABs through the algicidal lysing of toxic phytoplankton (Bates et al., 1995; Kodama et al., 2006; Sison-Mangus et al., 2013; Zhang et al., 2019). Similar to these previous observations, our results show that host response to their biotic environment can be modified by microbiomes. Specifically, bacteria provide a fitness advantage in competitive interactions between *M. aeruginosa* and *C. sorokiniana*, as well as impact the concentration of total microcystin.

The functional role of the microbiome in altering the ability of *Microcystis* strains and *C. sorokiniana* to grow from low abundance in each other's presence was not explicitly tested. In general, heterotrophs

may directly and indirectly benefit their host in competitive interspecific interactions. Associated heterotrophs remineralize macronutrients (Buchan et al., 2014), provide vitamins and micronutrients (Amin et al., 2012), and compete for limited inorganic nutrients with their phytoplankton host (Cook et al., 2020; Ramanan et al., 2016). Additionally, heterotrophs can degrade toxic metabolic byproducts (Amin et al., 2012), produce algicidal compounds (Buchan et al., 2014; Kodama et al., 2006), provide defence against pathogens through the interference of microbial signalling pathways (Satola et al., 2013), and could potentially use resources that may limit the proliferation of a competing species. Two bacterial families of which isolates were included in our study, *Sphingobacteriaceae* and *Comamonadaceae*, are commonly associated with green-algal cultures and their genes have been found to be highly transcribed within algal microbiomes sampled from the environment (Krohn-Molt et al., 2017). *Variovorax paradoxus* (family *Comamonadaceae*), the 16S rRNA gene sequence of which was the most closely related match to the sequence of a bacterium we isolated from *C. sorokiniana,* is a common plant mutualist, thought to provide plant host protection against pathogens (Satola et al., 2013). Based on culture-dependant analyses, *Rhizobium* species are highly abundant in *M. aeruginosa* microbiomes, and have been found to promote host growth of axenic toxic *M. aeruginosa* PCC 7806 through catalase activity and the provision of fixed nitrogen resources (Kim et al., 2019). Additionally, *Blastomonas fulva* (family *Sphingobacteriaceae*) has been previously isolated from a *Microcystis* culture (Lee et al., 2017). The marginal decrease of the carrying capacity for *C. sorokiniana* relative to the axenic population may help explain why *C. sorokiniana* could no longer establish itself in the presence of the non-toxic *M. aeruginosa* strain PCC
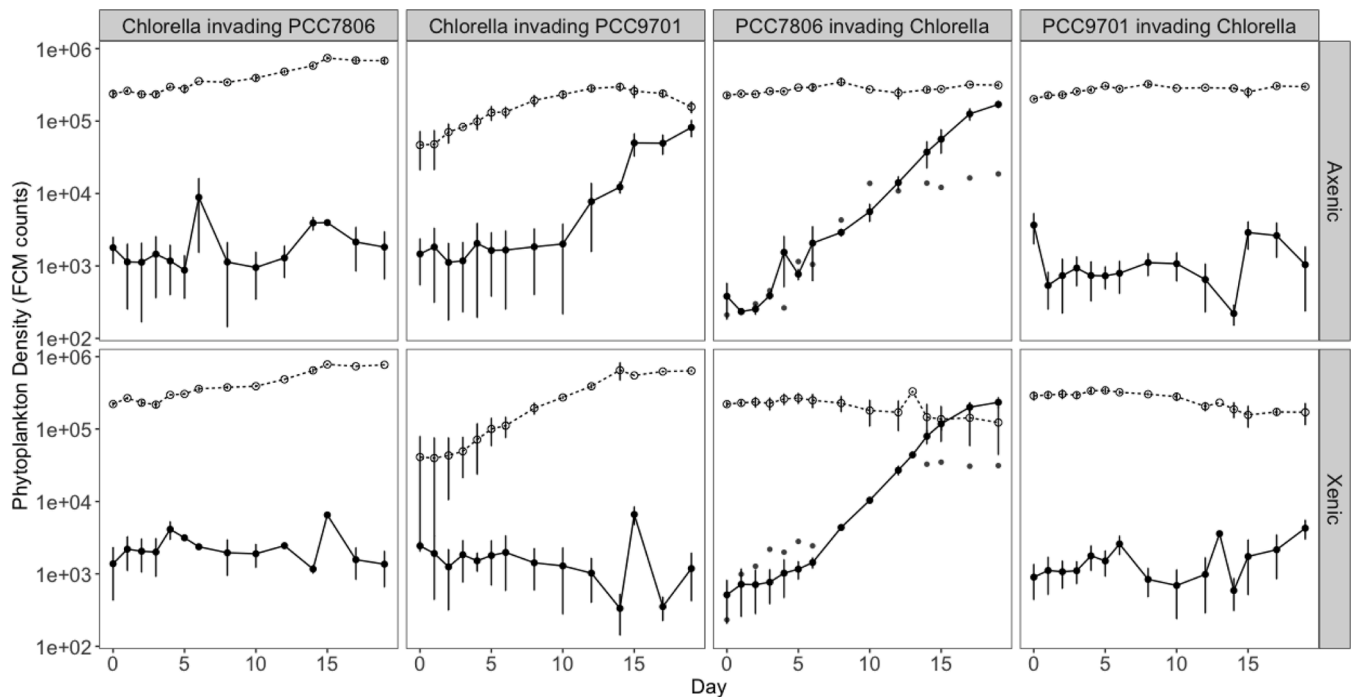
Fig. 4. The presence of host-associated bacteria altered the competitive interaction between variants of the cyanobacterium *M. aeruginosa* and the green alga *C. sorokiniana*. During the competition experiments, *C. sorokiniana* was not able to establish in PCC7806 in either axenic/xenic state and *C. sorokiniana* was only able to invade and persist in non-toxic *M. aeruginosa* PCC 9701 in the axenic state (p-value: <0.0001), while in the xenic state *M. aeruginosa* PCC 9701 resisted the invasion of xenic *C. sorokiniana*. In the reverse interaction, when *C. sorokiniana* was dominant, the toxic *M. aeruginosa* strain PCC7806, but not the non-toxic strain, PCC9701, was able to invade and proliferate in both the axenic and xenic states. Circles on the graph represent the mean phytoplankton density of the steady-state phytoplankton population (open circled) and the invading population (filled in circles), estimated by flow cytometry of each biological replicate. Error bars indicate the standard error of the mean. In the case of PCC7806 invading *C. sorokiniana*, one outlier replicate in both the axenic and xenic state was not included in the mean phytoplankton density, but the phytoplankton population densities are plotted in the graph. For the remaining two replicates, PCC7806 reached a higher final population density when invading *C. sorokiniana* in the xenic state, in comparison to the axenic state ($p \leq 0.05$).

9701 once bacteria were present, in addition to why establishment of the toxic *M. aeruginosa* strain into a *C. sorokiniana* culture was facilitated by the presence of host microbiomes. Hence, some of the more indirect benefits or emergent properties that arise from bacteria-bacteria interactions between the microbiomes of two hosts may help explain our observations. Additionally, the effect of the microbiome on host fitness may not be host specific (Jackrel et al., 2020). Host microbiomes could affect competitive interactions not only by altering their host, but also by becoming associated over time with and altering the fitness of the second phytoplankton population.

Strain level variation within *M. aeruginosa* is extensive, with large variation in gene content between strains beyond the occurrence of microcystin biosynthesis genes (Humbert et al., 2013; Jackrel et al., 2019; Meyer et al., 2017; Pérez-Carrascal et al., 2019). Our results suggest the toxic *M. aeruginosa* strain PCC 7806 is a more robust competitor relative to the non-toxic *M. aeruginosa* strain PCC 9701, however this competitive edge is independent of the ability to produce microcystin. The two specific strains we used vary from each other in the ability to produce a series of secondary metabolites beyond microcystin. Specifically, the toxic *M. aeruginosa* strain PCC 7806 contains genes related to the biosynthesis of aeruginosin and the cytotoxic cyclic hexapeptide microcyclamide that are absent or incomplete in the non-toxic *M. aeruginosa* strain PCC 9701 (Pérez-Carrascal et al., 2019). Conversely, the PCC 9701 strain contains the complete biosynthetic gene cluster for the protease inhibitor Anabaenopeptin that is absent in the PCC 7806 strain (Pérez-Carrascal et al., 2019). Our results support other research that the competitive advantage of toxic *Microcystis* strains is not necessarily due to the production of microcystin or microcystin acting as an allelopathic chemical (Briand et al., 2012; Chia et al., 2018; Dong et al., 2019).

The concentration of cyanotoxins produced during cyanoHABs, specifically the hepatotoxin microcystin, has direct implications for human and environmental health. We found that the presence of host-microbiomes greatly reduced the concentration of microcystin per cell relative to axenic competitive interactions. This could be either due to reduced production or due to more rapid decay of microcystin due to bacterial biodegradation. Based on the data available, we can only reasonably speculate regarding the potential of the heterotrophs in our engineered microbiomes contributing to microcystin decay. Degradation of extracellular microcystin by heterotrophic bacteria has been well supported both in the environment and laboratory settings (Dziallas and Grossart, 2011a, Kormas and Lymperopoulou, 2013; Thees et al., 2019; Zhu et al., 2016). Specifically, the bacterium *Variovorax paradoxus*, which is related to an isolate from *C. sorokiniana*, is a known degrader of biogenic and anthropogenic compounds (Satola et al., 2013). Further, a bacterial community from Lake Erie containing *V. paradoxus* was found capable of degrading microcystin (Thees et al., 2019). Additionally, the bacterium *Blastomonas fulva*, which is related to an isolate from *M. aeruginosa*, belongs to the family *Sphingomonadaceae*, which includes strains that are capable of degrading cyanobacterial toxins, including LR-microcystin, and organic compounds (Dziallas and Grossart, 2011a).

Through a combined approach of direct competition experiments and flow cytometry for rapid cell quantification, we were able to readily assess the competitive interactions between two phytoplankton populations under conditions that reflect population dynamics during cyanoHABs. Previously, quantifying the competitive interaction between phytoplankton groups has often relied on various, interdependent proxies of phytoplankton fitness, including the correlation between chlorophyll-a biovolume and cell density (Briand et al., 2012; Nolan and Cardinale, 2019). These approximations often lack accuracy at low
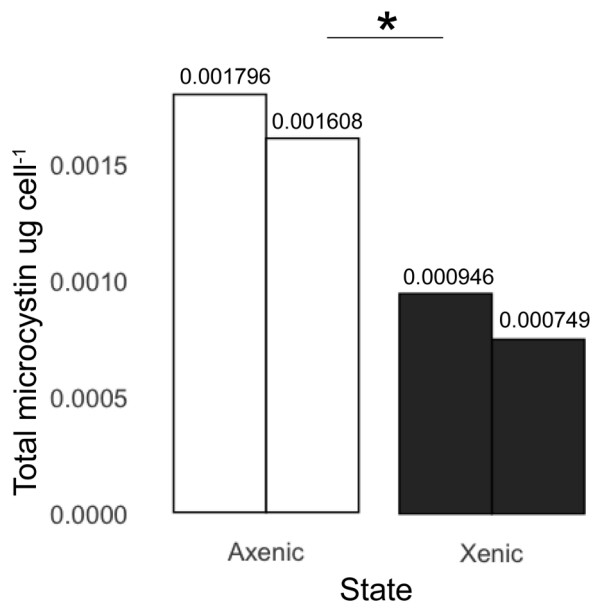
Fig. 5. The absence of host-microbiomes resulted in two-fold increase in the concentration of total microcystin (μg) per cell when toxic *M. aeruginosa* was introduced into a steady-state population of *C. sorokiniana*. Samples for total microcystin content (combined intracellular and extracellular) were quantified 15 days after the introduction of the invader using the Abraxis Microcystins/ Nodularins (ADDA) ELISA Kit. The total microcystin concentration per cell (μg/cell) populations in the axenic/xenic state was calculated by dividing microcystin content by the population density of toxic *M. aeruginosa* PCC7806 determined by flow cytometry. Bars on the graph each represent a biological replicate of the axenic or xenic competitive interaction;, single outlier replicates for each the axenic and xenic state observed in the growth dynamics in Fig. 4 were not included in this analysis. The single asterisk represents a p-value of 0.02 and indicates the phytoplankton cultures' axenic/xenic state significantly affected microcystin concentrations, determined by performing an analysis of variance on a linear mixed model on the data.

cellular densities, due to overlapping fluorescent spectra and difficulty decoupling chlorophyll/phycocyanin levels and cell densities. The use of flow cytometry combined with machine learning approaches to deconvolute the mixed signal from two co-occurring populations allowed us to rapidly quantify population densities at the high temporal resolution needed to quantitatively assess the impact of genotypic variation and the microbiome on phytoplankton competitive interactions (Rubbens et al., 2019). In contrast to time-consuming manual counting, or less reliable fluorescence-based proxy measurements, the flow cytometry approach increases throughput and likely increases accuracy and reproducibility, especially in quantifying low population densities. While we did not apply it here, flow cytometric population tracking also allows for the rapid enumeration of the associated heterotrophic community. This method of accessing interspecific phytoplankton interactions also has other promising applications, e.g., tracking of population-level phenotypic shifts over time that has been previously applied to bacterioplankton communities (Props et al., 2018). Assessing competition through co-culturing methods rather than independent measurements of fitness differences is also important as recent studies have shown stronger inhibition in green algae growth when directly co-cultured with cyanobacteria (Song et al., 2017; Wang et al., 2017).

We found that host microbiomes influence the competitive interaction of two phytoplankton species, however, there are several limitations and future directions that need to be addressed to understand the role of phytoplankton microbiomes in the proliferation and succession of *Microcystis* dominated cyanoHABs. Our study examined only a few bacterial taxa that we isolated from long-term laboratory cultures of phytoplankton, which generally harbour less diverse bacterial

communities (Kim et al., 2019; Krohn-Molt et al., 2017) than phytoplankton-associated communities in the environment (Frischkorn et al., 2017). Similarly, community richness differed between *M. aeruginosa* (3 taxa) and *Chlorella* (2 taxa) microbiomes. Evenness of the bacterial community may also have fluctuated over time, as bacterial population densities were not tracked over the course of the experiment, limiting our understanding of the relative influence of bacterial community dynamics on phytoplankton host fitness and competitive interactions. Additionally, using a culture-based approach for bacterial isolation may represent a limited range of interactions between phytoplankton and their associated heterotrophs. We chose to use defined bacterial microbiomes during our fitness and competition assays to be better able to interpret our observations. Future studies are needed to gain a mechanistic understanding of how the microbiome influences competitive outcomes between phytoplankton species, and how these relationships differ across the many diverse genotypes relevant to *Microcystis* dominated cyanoHABs, and what the influence of microbiome composition variation is on these interactions. Additionally, the lab-based approaches used in our study are not representative of the full complexities of natural systems, where competitive outcomes are often attributed to a combination of bottom-up and top-down controls. Outcomes of our experiments and the extent of microbiome effects on phytoplankton fitness are dependant on the nutrient and experimental conditions provided, and outcomes may vary given different environmental conditions known to impact phytoplankton fitness, including temperature, light, and growth media. Further analyses of environmental conditions are necessary to determine the generality of these outcomes in nature. Our study suggests that host microbiomes may alter bottom-up controls, i.e. interspecific competition for limited resources, or interference competition through production of allelochemicals, such as those seen in Song et al. (2017) in which *M. aeruginosa* inhibited growth of *Chlorella vulgaris* via positive feedback inhibition of linoleic acid. Yet, it does not take into account a number of top-down controls known to variably affect population dynamics amongst and within phytoplankton species in nature, including grazing by zooplankton (e.g., *Daphnia* spp. (Ekvall et al., 2014; Lemaire et al., 2012) and eukaryotic protists (Fuhrman and Noble, 1995), bivalves (reviewed in Harke et al., 2016), as well as viral mediated lysis (Yoshida et al., 2008).

Overall, understanding all the mechanisms that control phytoplankton interspecific interactions is critical in understanding the proliferation of nuisance phytoplankton species, and thus how community composition and ecosystem services will change in a world rapidly changed by anthropogenic disturbances (Jankowiak et al., 2019; Paerl and Huisman, 2008; Paerl and Otten, 2013a). Our study shows that the ability of *M. aeruginosa* to compete with *C. sorokiniana* is determined by the presence of host-associated microbiomes, as well as intraspecific genetic differences with *M. aeruginosa* beyond those genes specifically involved in microcystin toxin generation. Furthermore, our study describes the application of a promising method for examining population interactions, capable of including multiple phytoplankton species. These results using controlled lab-based experiments further support environmental observations and laboratory-based studies regarding the role of microbiomes in regulating host growth (Buchan et al., 2014; Cook et al., 2020) and provide new insights to the role of host microbiomes in mediating interspecific competitive interactions, and thus the succession of phytoplankton species during cyanoHABs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2020.101939.

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