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Microbial community successions and their dynamic functions during harmful cyanobacterial blooms in a freshwater lake

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Abstract

The current study reports the community succession of different toxin and non-toxin producing cyanobacteria at different stages of cyanobacterial harmful algal blooms (CyanoHABs) and their connectivity with nitrogen and phosphorus cycles in a freshwater lake using an ecogenomics framework. Comprehensive high throughput DNA sequencing, water quality parameter measurements, and functional gene expressions over temporal and spatial scales were employed. Among the cyanobacterial community, the lake was initially dominated by *Cyanobium* during the months of May, June, and early July, and later primarily by *Aphanizomenon* and *Dolichospermum* depicting functional redundancy. Finally, *Planktothrix* appeared in late August and then the dominance switched to *Planktothrix* in September. *Microcystis aeruginosa* and *Microcystis panniformis*; two species responsible for cyanotoxin production, were also present in August and September, but in significantly smaller relative abundance. MC-LR (0.06–1.32 μg/L) and MC-RR (0.01–0.26 μg/L) were two major types of cyanotoxins detected. The presence of MC-LR and MC-RR were significantly correlated with the *Microcystis*-related genes (*16SMic*/*mcyA*/*mcyG*) and their expressions ($r = 0.33$ to 0.8, $p < 0.05$). The metabolic analyses further linked the presence of different cyanobacterial groups with distinct functions. The nitrogen metabolisms detected a relatively higher abundance of nitrite/nitrate reductase in early summer, indicating significant denitrification activity and the activation of N-fixation in the blooms dominated by *Aphanizomenon*/*Dolichospermum* (community richness) during nutrient-limited conditions. The phosphorus and carbohydrate metabolisms detected a trend to initiate a nutrient starvation alert and store nutrients from early summer, while utilizing the stored polyphosphate and carbohydrate (PPX and F6PPK) during the extreme ortho-P scarcity period, mostly in August or September. Specifically, the abundance of *Aphanizomenon* and *Dolichospermum* was positively correlated

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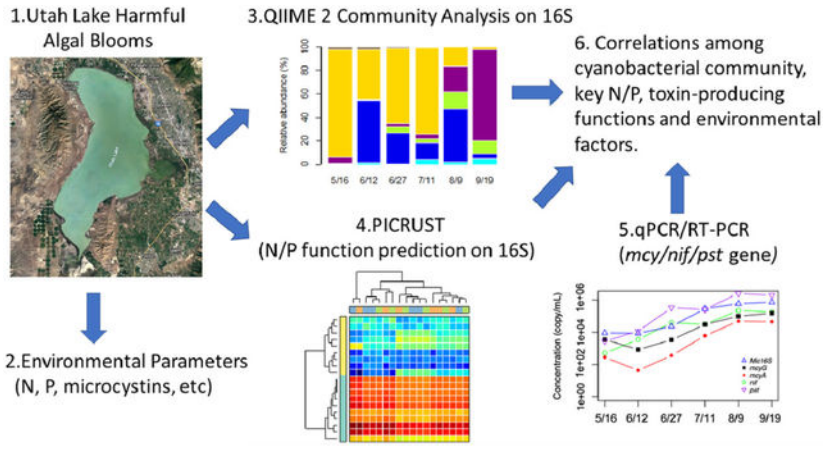
Declaration of Competing Interest
No conflict of interest declared.

with the nitrogen-fixing *nif* gene and ($p < 0.001$) and the PPX enzyme for the stored polyphosphate utilization ($r = 0.77$, $p < 0.001$). Interestingly, the lake experienced a longer N-fixing period (2–3 months) before non-fixing cyanobacteria (*Planktothrix*) dominated the entire lake in late summer. The Provo Bay site, which is known to be nutrient-rich historically, had early episodes of filamentous cyanobacteria blooms compared to the rest of the lake.

Graphical Abstract



Keywords

Harmful algal blooms; *Cyanobium*; *Aphanizomenon*; *Dolichospermum*; Nitrogen fixation; P Scavenging genes; Cyanotoxins

1. Introduction

The input of excess nutrients, primarily nitrogen and phosphorus, causes eutrophication in surface water bodies, leading to harmful algal blooms (HABs) in many freshwater lakes (Heisler et al., 2008; Dodds et al., 2009; Keck and Lepori, 2012; Drobac et al., 2013). Nitrogen (N) and phosphorus (P) are two of the most important nutrients of concern, although their relative contribution to eutrophication is always debatable (Carpenter, 2005; Conley et al., 2009; Kolzau et al., 2014; Paerl et al., 2017). Early studies recognized P as the primary limiting nutrient in most lakes based on the stoichiometry of N and P in phytoplankton (Schindler, 1977; Hecky and Kilham, 1988; Lewis and Wurtsbaugh, 2008). P addition-based bioassays have shown that P addition enhanced the growth of toxin-producing *Microcystis* (Davis et al., 2009). However, subsequent studies also found that N was often the limiting nutrient in shallow eutrophic lakes, while the oligotrophic deep lake was mostly P limited (Downing and McCauley, 1992; Reynolds, 2006). A switch from spring P to summer N limitation has also been demonstrated in some locations (Conley, 1999). Recent studies also recognized the dominance of cyanobacteria under low N/P ratios (Søndergaard et al., 2017; Isles et al., 2017). Generally, an N-limitation condition could result from nitrate lost to heterotrophs (e.g., denitrifiers) via assimilation, denitrification and other biochemical processes (Allen et al., 2005; Chen et al., 2012; Holmroos et al., 2012), while the levels of P were determined by interactions between sediment and water column of

seasonal hydrological processes (Armon and Starosvetsky, 2015; Hogsett et al., 2019; Ma et al., 2019). Nevertheless, none of the past efforts or recent literature have denied the importance of nitrogen and phosphorus in supporting surface water eutrophication (Downing et al., 2001; Håkanson et al., 2007).

With new species of cyanobacteria being identified, the paradigm that surface water is either N limited or P limited is fast changing because nutrient limitations also depend on which cyanobacterial species dominate the bloom (Cottingham et al., 2015). Under N stress conditions, many filamentous cyanobacteria (e.g., *Aphanizomenon, Dolichospermum*) can conduct both nitrogen fixation and photosynthesis by cell differentiation. It is well-known that vegetative cells conduct primary productivity, whereas the specialized cells, heterocysts, perform nitrogen fixation by utilizing nitrogenases (encoded by *nif* genes; Schindler et al., 2008; Paerl, 2017). Additionally, N regulatory genes (e.g., *ntrA, ntrC*) and PII signal transduction proteins are widely spread in bacteria that regulate the N assimilations under N starvations (Hirschman et al., 1985; Herrero et al., 2004; Huergo et al., 2013).

Similar to N systems, one of the commonly recognized strategies for bacteria to enhance phosphate assimilation is inducing the high-affinity inorganic phosphate (Pi) scavenging system-*Pho* regulon (Adams et al., 2008; Santos-Beneit, 2015), which includes members having the high-affinity Pi transport systems (encoded by *pst* genes; Makino et al., 1988; Pitt et al., 2010), enzymes polyphosphate kinase (PPK; Brown and Kornberg, 2004), exopolyphosphatase (PPX; Gomez-Garcia et al., 2003), and others. The P correlated metabolisms are even more complex to study, as many P-containing compounds in cells are tightly linked with carbohydrates assimilations (Harke et al., 2012; Harke and Gobler, 2013) or the stringent conditions alert (Abranches et al., 2009; Santos-Beneit, 2015). It is reported that phosphate bioavailability for diazotrophs was one of the constraint factors for nitrogen fixation rates as an interaction between N and P (Ward et al., 2013; Wu et al., 2018).

Recent studies have suggested that cyanobacterial N_2-fixation and Pi-scavenging also play important roles in promoting and sustaining cyanobacterial harmful algal blooms (CyanoHABs) (Beversdorf et al., 2013; Harke et al., 2015). A very recent meta-transcriptomic based study by Lu et al. (2019) revealed that expressions of genes involved in N_2-fixation (*nifDKH*) and high-affinity Pi transporter (*pstSABC*) were significantly upregulated during the bloom compared to pre-bloom in Harsha Lake. In this study, these researchers found that the temporal action of N_2-fixation (*nifDKH*) and high-affinity Pi transporter genes (*pstSABC*) controlled the ecology of cyanobacterial populations in Harsha Lake. Interestingly, many studies have observed the co-presence or succession of N-fixers (or *nif* genes) and toxin-producing strains at different stages of blooms (Elser et al., 2000; Beversdorf et al., 2013; Chia et al., 2018; Lu et al., 2019).

Eutrophication is a dynamic process where harmful toxin-producing and nontoxic blooms coexist, although their relative abundance may vary. Additionally, the transition of a lake ecosystem from being N limited to P limited or vice versa would not only depend on the exogenous input of nutrients but the relative expressions of N-fixing and P-affinity genes. Lastly, the presence of toxic cyanobacteria identified taxonomically does not necessarily mean that they are expressing their toxin-producing functional genes. Recent studies

successfully linked the dynamics of certain cyanobacterial species with their metabolic activities (Beversdorf et al., 2013; Harke et al., 2015; Lu et al., 2019). However, studies are still scarce for a whole-picture investigation into nutrient utilization pathways and toxin-producing functional genes at the entire bacterial community level during HABs.

The overall objective of this research was to take a holistic approach to illustrate the interdependency of CyanoHABs with several factors, including water quality parameters and genomic contents in a freshwater peri-urban lake. This study fills an important gap elated to the dynamics of P and N cycles during cyanoHABs. Unlike a previous publication from this group on the ecology of cyanobacteria in Utah Lake (Li et al., 2019), the objective also included studying N-fixing, P-regulating, and toxin-producing functional genes before the onset, during and after cyanoHABs in addition to spatial and temporal variations in the abundances of different cyanobacteria. The general N and P metabolic pathways and functions were predicted by Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (Langille et al., 2013); the functional gene/gene expressions for nutrient scavenging (*nif/pst*) and microcystin-producing (*mcy*) were precisely targeted by qPCR and Reverse-transcript qPCR (Freeman et al., 1999). The main objectives of the study were to: (1) detect the microbial community shift and predict key functional dynamics related to N and P; (2) study the dynamic behavior of *nif* genes, *pst* genes, and *mcy* genes during the CyanoHABs; (3) investigate the correlations among bacterial community presence, functions, and environmental factors.

2. Materials and methods

2.1. Sampling sites

Freshwater Utah Lake, which is located nearly 50 miles south of Salt Lake City, was considered as the model freshwater lake. Utah Lake is the largest natural freshwater lake in the western United States, with a maximum length of 38.6 km and a maximum width of 20.9 km. As a shallow alkaline lake, it has an average depth of 3.0–3.4 m in open water during standard reservoir operating conditions and has calcium-rich sediments. Utah Lake has experienced frequent CyanoHABs in recent years, with the cyanobacteria cell numbers up to 36 million cells per mL in 2016 and *Aphanizomenon flos-aquae* as the primary species (UDWQ, 2016a). Utah Lake was also shut down for recreational and irrigation purposes as MCs concentrations were detected to be much higher at some locations than the recreational exposure guideline of 10 μg/L by the World Health Organization (Bartram and Chorus, 1999). Site selections were based on the Utah Division of Water Quality's (UDWQ's) regular sampling. All of the sites are in open water and are located near major tributaries with varying depths (Fig. 1 and Table S1). Deepwater sites (depth> 1 m) included Saratoga Springs, Geneva Discharge, Vineyard Buoy, Provo Buoy, and Bird Island Buoy, while Entrance to Provo Bay and Goshen Bay were the shallow sites, with water levels less than 0.5 m during the regular summertime (e.g., early May to September). Sampling was conducted on a monthly basis from early May to late September in 2018, except for sampling twice in June when CyanoHABs typically occurred, based on historical observation updated warnings issued by UDWQ. During sampling, water samples were collected at three depths at each site and combined onsite into one composite sample. For

most in-lab analysis, samples were collected in HDPE sampling bottles following the Standard Operating Procedure for the collection of phytoplankton to detect harmful algal blooms (UDWQ, 2016b). Two exceptions were cyanotoxin and chlorophyll a, measurements of which required the use of glass amber bottles to prevent sunlight exposure. The containers were immediately stored in coolers and transferred to the Environmental Engineering and Microbiology Lab at the University of Utah for further physicochemical and biological analysis.

2.2. Measurement of physicochemical parameters

Temperature, pH, dissolved oxygen, conductivity, and depth were measured in-situ. Samples were directly filtered through 0.22 μm filters (HPLV 4700, Fisher Scientific) before the measurement of soluble nutrients. The filters with planktonic biomass were kept at −20 °C before genomic (Sections 2.3 and 2.4) analysis and in Invitrogen® RNALater stabilization solution before gene expression (Section 2.4) analysis. Dissolved nutrient anions (nitrate-N, nitrite-N, and orthophosphate-P) in the filtrate were analyzed using Ion Chromatography (IC) (Metrohm 883 Basic IC plus) following EPA method 300 (Pfaff, 1993). The ammonia-N, total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP) were measured using Low Range Ammonia TNTplus Vial Test (TNT830, Hach, USA), Total Nitrogen TNT Reagent Set (LR, Hach), and Total Phosphorus TNT Regent Set (LR, Hach), respectively. Chl a was measured spectrophotometrically and corrected for pheophytin following the standard methods of water and wastewater (APHA, 1999). The dissolved organic carbon was measured by the standard carbonaceous biochemical oxygen demand (cBOD5) bottle test following EPA 450.1. The microcystin concentrations (MCs) were measured starting in June according to EPA method 544. More precisely, microcystins were extracted from both filtrate (lake water) and filter (algal biomass), concentrated by solid-phase extraction, and detected on a Waters® ACQUITY UPLC with TQD mass spectrometry.

2.3. Genomic analysis of blooms

2.3.1. Genomic DNA extraction and high-throughput sequencing—Genomic DNA was extracted from the filtered biomass using a PowerWater® DNA isolation kit (Qiagen Inc, Valencia, California) according to the manufacturer's instructions. Concentrations were determined on a Thermo® NanoDrop 2000c, and samples with 260/280 ratios higher than 1.80 were selected for further analysis. Before being sent for Illumina® MiSeq sequencing, samples were diluted to 10 ng/μL for a total volume of 10 μL. The amplicon library preparation of the bacterial 16S rRNA gene V4 gene region was conducted in the RTSF Genomics Core at Michigan State University using primer set 515F/806R, following the protocol described by Kozich et al., 2013. The final PCR products obtained from the protocol were batch normalized using Invitrogen® SequalPrep DNA Normalization plates and pooled into each well. The pool was cleaned up using Ampure XP beads, quantified using a combination of Qubit® dsDNA HS, Agilent® Bioanalyzer DNA 1000, and Illumina® Kapa Library Quantification qPCR assays. It was then loaded onto an Illumina® MiSeq Standard v2 flow cell and sequenced in a 2 × 250 bp paired-end format using a v2 Standard 500 cycle MiSeq reagent cartridge. Custom sequencing and index primers were added to appropriate wells of the reagent cartridge as described. The Base

calling was done by Illumina® RealTime Analysis (RTA) v1.18.54, and the output of RTA was demultiplexed and converted to Fastq format with Illumina® Bcl2fastq v2.19.1.

2.3.2. The analysis of microbial community and the prediction of metabolic pathways and functional groups—The amplicon sequencing results were analyzed according to QIIME 2 "moving picture" tutorials (https://docs.qiime2.org/2018.11/tutorials/moving-pictures/) (Vázquez-Baeza et al., 2013; Bolyen et al., 2018). The overall analysis with read quality filtering demultiplexing, truncating of paired- end reads, operational taxonomic units (OTUs) formation, alpha diversity analysis, and other analyses were conducted similar to our earlier protocol (Li et al., 2019). The predicted metabolic functions of the microbial community were determined by PICRUSt v1.1.3 (Langille et al., 2013). To do so, features (OTUs or sequence variants) were closed-reference picked using "qiime vsearch cluster-features-closed-reference" against the database that was trained on the Greengenes version 13_5 with 99% identity cluster OTUs from the 515F/806R region of sequences (McDonald et al., 2012). The percent identity at which clustering should be performed (–p-perc-identity) was set at 1 to prevent any mismatches. The generated feature table was exported as a biom format file, which was then used as the input for PICRUSt to predict metabolic function counts by referencing the Kyoto Encyclopedia of Genes and Genome (KEGG) Orthology (KO) Database (Kanehisa and Goto, 2000; Kanehisa et al., 2014). The function prediction was achieved by processing through scripts of "normalize_by_copy_number.py" and "predict_metagenomes.py." The accuracy of metagenomic prediction was estimated by the Nearest Sequenced Taxon Index (NSTI) value, which is considered the standard method for validation of KEGG functional groups (Langille et al., 2013; Koo et al., 2017). A lower NSTI value (< 0.15) implies that samples are phylogenetically close in relationship and suitable for PICRUSt analysis. In our analysis, the weighted NSTI values ranged from 0.105 to 0.169 with a mean value of 0.127 ± 0.014. Thousands of predicted functions were further collapsed into KEGG pathways by "categorize_by_function.py." The KEGG Orthology (KO) counts of N and P/carbohydrate metabolisms related pathways were plotted using the heatmap option at the log scale in STAMP (Parks et al., 2014).

2.4. The quantitative PCR (qPCR) and functional gene expressions using reverse transcript (RT)-qPCR

Cloning and sequencing was conducted using mcyAcya/mcyEcya primers (Table 1) to identify possible *mcy* gene clusters within the entire bacterial community. Cloning was conducted from the lake biomass using TOPO® TA cloning kit (Invitrogen, USA), and plasmids were extracted by Zyppy® Plasmid Miniprep Kit (Zymo Research, USA). The cloned plasmids were sent for Sanger Sequencing at the Health Science Center Cores, University of Utah. The species or target genes were identified by blasting against the National Center for Biotechnology Information (NCBI) database.

Based on the cloning sequencing results, real-time quantitative polymerase chain reaction (qPCR) was further performed to quantify total gene copy numbers and reverse transcript qPCR (RT-qPCR) was conducted to estimate expressions of key functional genes responsible for nitrogen fixation, high-affinity Pi-transporter for *Nostocales*, and MCs

related genes for *Microcystis*. Primers used to quantify absolute gene copy numbers and genes targeted for expression are listed in Table 1. The genomic DNA templates were diluted to 20 ng/μL to prevent inhibition at high concentrations. Total RNA was extracted using PureLink® RNA Mini Kit (Thermal Fisher, USA) and immediately stored at −80 °C until used. Following RNA extraction, residual genomic DNA was removed from total RNA using an on-column PureLink® DNase set (Life Technologies, NY, USA). It was then converted to cDNA by SuperScript® VILO mastermix (Thermal Fisher,USA). The qPCR and RT-qPCR reaction mixture are 20 μL in total, containing 10 μL 2×Power SYBR® Green Master Mix (Applied Biosystems, Foster City, CA), 0.5 μM of each primer, 2 μL of templates (blank), and 6 μL nuclease-free water. The quantification cycling was conducted with a QuantStudio® 3 Real-Time PCR System (Applied Biosystems) following the process of an initial 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 30 s at respective annealing temperature, and 30 s at 72 °C. Before running samples, cloned standards for each gene were serially diluted to a range of 10^1 to 10^6 copies per 20 μL reaction using 10% purified plasmid DNA and 90% nuclease-free water. After the qPCR process, the template gene copies were directly calculated based on the linear regressions of standards versus the cycle threshold. The copy numbers were multiplied by the diluting factors to gain results of copies/mL of lake water.

2.5. Statistical analysis

Except for the default graph tools in bioinformatics software, all of the figures were created using R studio 1.1.419 (R Development Core Team, 2013). Specifically, the boxplots for predicted metabolic pathways were generated using the R package “ggpubr.” The associations of ambient water parameters, bacterial communities, metabolism prediction results and real-time gene/gene expression quantifications were estimated by Spearman correlation analysis using the R package “corrplot” at a 95% confidence interval.

3. Results

3.1. Water quality parameters

Water parameters sampled from May to September are listed in Table S2 and cyanotoxin concentrations in supplementary Table S3. Briefly, water temperatures increased from May (16 – 20 °C) to July (25 – 28 °C) and decreased to around 20 °C in September’s. The overall pH increased from May (8.23 – 8.38) to August (8.64 – 8.98) and leveled off in September. The only exception was the Entrance to Provo Bay site when the bloom and pH peaked on June 27th. Chl a and DO results were consistent with pH results. The Entrance to Provo Bay had earlier indicating of bloom in June (Chl a 100 μg/L and DO 10.6 −14.6 mg/L) than the entire lake. The sudden rise in cBOD in the lake was detected in August (5.2 – 22.4 mg/L) and September (6.6 −14.3 mg/L). As for cyanotoxins, MC-LR and MC-RR were two main variants of microcystins detected in the lake water, which peaked on June 12th at the sites Mouth of Goshen Bay and Entrance to Provo Bay and another peak on Sep 19th at all sites. The highest concentrations were 0.69 – 1.16 μg/L for MC-LR and 0.15 – 0.26 μg/L for MC-RR in September (Table S3).

The nutrient concentrations varied at locations and sampling dates. Generally, ammonium-N, nitrate-N, and ortho-P were measured in the range of 0 – 0.97 mg N/L, 0 – 0.09 mg N/L, 0 – 0.07 mg P/L respectively. Nitrite-N was mostly non-detectable. The south part of the lake showed high concentrations of ammonium-N, as high ammonium concentrations were measured in the Mouth of Goshen Bay (0.97 mg N/L) and Bird Island Buoy (0.63 mg N/L). Nutrients observed a decreasing trend overall except for some sudden increases in ammonium-nitrogen concentrations in July and August. Nitrate-N and ortho-P were non-detectable in August and September's sampling. Because of higher TDN concentrations (0.17 −7.07 mg N/L) compared with TDP (0.23 – 1.04 mg PO_4^{3-}/L), the atomic N:P ratios were generally higher than 16:1, except for some sites in September. Overall, Provo Bay experienced early blooming period in June followed by blooms in other parts of the lake in July, August and September.

3.2. Sequencing depth and diversity analysis

To identify the microbial community composition, a total of 54 samples were collected during different months, from different locations, and were then sequenced. High-throughput sequencing yielded a total of 8,099,216 sequences. A total 6,482,848 sequences remained after quality control and were clustered into 10,163 features. Table S4 summarizes the sequencing details and number of sequences obtained after quality filtering. The refraction curves tended to approach the saturation plateau, implying adequate sampling depth and coverages (Supplemental Fig, 1 A). Shannon's diversity index indicated a relatively lower abundance or evenness of species present in August's sampling (Supplemental Fig, 1 B). The observed OTU counts were detected moderately lower on May 16th than the other months (Supplemental Fig, 1A).

3.3. Microbial community classification at phylum and genus levels

The taxonomy classification and calculations of relative abundance were based on bacterial 16S sequencing. The microbial community at the phylum level is shown in panel A of Fig. 2. Actinobacteria, Bacteroidetes, Proteobacteria, and Cyanobacteria were the most observed phyla (Fig. 2). The relative abundance of cyanobacteria generally increased starting May 16th (7.88–22.7%) onward for subsequent months to 28.2–38.1% in September (depending on the site). However, different sites exhibited varying trends in the relative abundances of Cyanobacteria. For example, the Mouth of Goshen Bay, Bird Island Buoy, and Entrance to Provo Bay sites, which are mostly located toward the south part of the lake, reached the highest relative abundance, varying from 28.6 to 48.3% for cyanobacteria on June 27th when the lake was declared bloom dominated by the UDWQ based on cell counts. On the other hand, sites inside the Provo Buoy, Geneva Discharge, Vineyard Buoy, and Saratoga Springs, which are located toward the north part of the lake, had the highest relative abundance of cyanobacteria varying from 42.6 to 46.3% as observed in the August 9th sampling. Overall, among all sites and sampling occasions, the highest relative abundance for cyanobacteria (48.3%) was detected on June 27th at the Entrance to Provo Bay. Cyanobacteria was the highest phylum in terms of relative abundance during the occurrence of CyanoHABs, while Proteobacteria, Bacteroidetes, and Actinobacteria generally occupied higher relative abundance before or after the blooms (Fig. 2). A trend of decrease in the relative abundance

of heterotrophic bacterioplankton was also observed with increases of cyanobacterial relative abundance.

Due to the focus of this work on lake eutrophication and to further classify the taxonomic composition of cyanobacteria, we considered the bacterial assignments at the genus level and compared them within and among sites (Panel B in Fig. 2). *Cyanobium, Aphanizomenon, Dolichospermum, Microcystis,* and *Planktothrix* were the dominant genera present at all sites (Fig. 2). Initially, picocyanobacteria *Cyanobium* (72.2–99.8%) dominated the lake from May until July. The relative abundances of this genera varied from 91.7% at the Mouth of Goshen Bay to a high percentage of 98.2% Bird Island Buoy and Provo Buoy in May, thus representing itself the dominant cyanobacterial genera. In June and July sampling events, the relative abundances of *Cyanobium* varied between 22.1% and 81.8%, depending upon the site. There is not much information available about the genus *Cyanobium*. One paper by Komárek et al. (1999) suggests a closer relationship between the picocyanobacteria *Synechococcus* and *Cyanobium* with the identical thylakoid arrangement.

As for the bloom-forming genera, *Aphanizomenon* and *Dolichospermum* belonging to Nostocales appeared and gradually occupied higher percentages from May end until mid-August. In fact, *Aphanizomenon* was the dominant genera or equally abundant in late June, July, and August samplings at Bird Island Buoy, Provo Buoy, and Vineyard Buoy sites. *Aphanizomenon* and *Dolichospermum* appeared and gradually occupied higher percentages (a maximum of 68.9- for *Aphanizomenon* and 64.6% for *Dolichospermum*) until August's sampling. *Microcystis* was detected at all sites from June, although its relative abundance varied from 0.27–22.3% depending upon the sampling month (e.g., 22.3% at Vineyard Buoy on June 12th). Except for the first peak detected on June 12th, the relative abundance of this genus was generally high in September, varying from 3.66 to 11.4%. *Planktothrix*, mostly a non-diazotroph and common toxin producer in temperate lakes, started to appear in August and dominated in September with relative abundances as high as 58.3–87.9% at all sites. The Entrance to Provo Bay was unique as it formed early filamentous cyanobacteria blooms (mostly *Dolichospermum*) in June, while other sites had a peaked bloom mostly in August.

3.4. Function predictions by PICRUSt

PICRUSt matched 2,961,431 sequences to the Greengenes 13_5 and finally generated 655 features. The weighted NSTI values ranged from 0.105 to 0.169 with a mean value of 0.127 ± 0.014, suggesting the high reliability of predictions (Koo et al., 2017). Zhu et al., 2018 mentioned the NSTI scores of each sample to be varied from 0.11 to 0.17 (mean=0.14), which were mostly lower than the mean NSTI score (0.17) in soil samples reported by Langille et al., 2013, suggesting that the predictions were accurate and reliable. Hence, an average NSTI score of 0.127 ± 0.014 seems reasonably reliable. KEGG pathways of cellular process, environmental information processing, genetic information processing, microbial metabolism, and organismal systems at the highest hierarchy were predicted from the sequencing results. The main metabolisms predicted were amino acid (10.3% – 11.7%), carbohydrate (9.6% – 11.0%), energy (5.9% – 7.5%), membrane transport (10.3% – 11.4%), and replication and repair (7.1% – 7.6%). However, we primarily focused on nutrient-related pathways as these directly reflect the bacterioplankton assimilations and metabolic

dynamics. Fig. 3 shows these predictions for nitrogen, phosphatase, phosphotransferase, and photosynthesis-related pathways in different panels. Generally, the “nitrogen metabolism” contains genes that regulate the N-fixation (*nif*) and inorganic N reductions (Fig. 3A). All of them, as well as some N transporters and regulation genes, regulated N cycles in the lake. As for phosphate and carbohydrate assimilations, two of the important pathways for bacterioplankton are “Phosphonate (*phn*) and phosphinate metabolism” and “Phosphotransferase system (PTS)” (Fig. 3B, C) (Kotrba et al., 2001; Gomez-Garcia et al., 2011). Both pathways are highly correlated with the transferase and utilization of P and carbohydrate in phytoplankton cells. The PTS system enables bacteria to import sugars by forming P derivatives. However, no pathways were reported for the “Inorganic phosphorus scavenging system (*pst*)” due to the limitations of the database whereas our functional gene expressions show high abundances of pst between samples (see results later). Photosynthesis was counted relatively higher in August (Fig. 3D). PICRUSt can only predict gene families that are already known and included in the orthology reference used (KEGG KOs) by default. This could be considered as one of the limitations of PICTRUSt. Another limitation of PICRUSt includes any gaps or inaccuracies in pathway annotation or assignments of gene function. For example, many KEGG Orthology groups listed as participating in pathways not found in bacteria or otherwise not reflective of true function. This is simply due to bacteria containing homologs of enzymes showing important roles. Therefore, it is worth carefully checking KEGG pathway annotations to ensure that they are reasonable for any system studied. This result together with higher chl a and cBOD content detected in August, potentially implying higher carbon-fixation activities.

The KOs for N, P, and some carbohydrate-related pathways were further investigated. As for nitrogen cycles, the predicted functions were mainly divided into three groups, namely N-fixation genes (e.g., *nif*), N reduction-related genes (e.g., *nap, nir, nor*), and N regulatory proteins (e.g., PII) (Fig. 4). The most abundant counts were nitrate/nitrite reductase subunits, followed by the *nif* related genes and some specific N transporter or reductase proteins. It is noted that some *nif* or N-regulatory proteins were elevated at the end of June, early July, and August (e.g., *nifX, nifH, nifV*), while some are more abundant in May (e.g., *nifH*, N regulatory protein). By contrast, the main nitrate and nitrite reductase subunits were relatively higher in May and early June and less detectable in August and September, suggesting the availability of inorganic N at the beginning of the summer months.

As for P and carbohydrate-related functions, the KOs in response to assimilation or coregulated as part of the *Pho* regulon were mostly studied (Fig. 5). Specifically, polyphosphate kinase (PPK), inorganic pyrophosphatase (PPA), pyrophosphatase (PpaX), guanosine 3′-diphosphate 5′-diphosphate ((p)ppGpp), the inorganic phosphorus transporter (PiT) family, polyphosphate glucokinase (PPGK), and glucose-6-phosphate dehydrogenase (G6PD) were more abundant in May. It is suggested that phytoplankton responded to early summer nutrient stress and started to assimilate P and maybe created storage from the early summer. The exopolyphosphatase (PPX) and fructose-6-phosphate phosphoketolase (F6PPK) counts were elevated in August, indicating the possibility of utilizing stored nutrients during the peak bloom and extreme nutrient limitation conditions. The phosphonate and phosphinate-related proteins were increased in May (*phnB, phnP*) or August (*phnM, phnJ*), which could be most active in heterotrophic bacteria and some

cyanobacteria for organic P utilization (Dyhrman et al., 2009). Similarly, PTS-related functions were highly abundant, mostly during May or August, for the transportation and phosphorylation of a variety of sugars and sugar derivatives (Deutscher et al., 2014).

3.5. Genomic DNA-based gene quantification and mRNA-based gene expressions for nutrient starvation and MC related genes

The Sanger sequencing of cloned plasmids for the *mcy* gene suggested the presence of *Microcystis panniformis FACHB-1757* (14 out of 24 cloned) and *Microcystis aeruginosa strain UV027* (7 out of 24 cloned) in the lake. Nevertheless, no direct evidence was found for common *mcy* gene possessors, such as *Planktothrix* (Christiansen et al., 2003), *Anabaena* (Halinen et al., 2007), or *Dolichospermum* (Teikari et al., 2019). The change of functional genes (e.g., their absolute presence) and their corresponding expressions (based on mRNA) for *Microcystis* 16S (*Mic16S*), microcystin-producing (*mcyA* and *mcyG* in *Microcystis*), N-fixing (*nif*), and high-affinity Pi transport genes (*pst*) along with time are plotted in Figs. 6 and 7. All standards were amplified at efficiencies of nearly 80%. As for the absolute gene copies based on qPCR of genomic DNA, most sites had increased copies for all five genes with time and peaked in either early June or Aug/Sep (Fig. 6). The trend of the *nif* gene was consistent with the *pst* gene, while *Mic16S, mcyA*, and *mcyG* could be considered as a group. The copies of *nif* were mostly close to *pst*, except for the sites Mouth of Goshen Bay and Bird Island Buoy, where *pst* gene copies were 1–2 folds more than *nif* copies. Apart from that, the *nif/pst* gene copies were significantly higher than *Mic16S*/*mcyA/mcyG* at the Entrance to Provo Bay on June 12th (beginning of bloom) and June 27th (peak of bloom). Overall, the copies for *nif/pst* mostly peaked in the period of June 12th to Aug 9th; however, the highest copy numbers for *Mic16S*/*mcyA/mcyG* were found in September.

Fig. 7 shows the actual expressions of four functional genes based on mRNA and RT-qPCR. The expression of *nif/pst* genes (0 to 10^5 copies/mL) were relatively higher than *Microcystis's mcyA/mcyG* (0 to 10^3 copies/mL) groups at all the sites. Apart from the sites Mouth of Goshen Bay and Entrance to Provo Bay that had early significant expressions of *nif/pst* on May 16th and June 12th, most other sites experienced an increased expression after June 27th. The expressions of toxin-related genes kept increasing until September. Additionally, there was a small peak on June 12th at sites Mouth of Goshen Bay, Entrance to Provo Bay, Geneva Discharge, and Vineyard Buoy. The gene expressions were at the plateau from the end of June to September for the two deeper sites (Provo Buoy and Saratoga Springs). The *Mic16S* was presented at 4–8 folds and not shown in the graphs.

4. Discussion

4.1. Community successions in the microbial community during CyanoHABs

The analysis of bacterioplankton at the phylum level demonstrated the dominance of Actinobacteria, Bacteroidetes, and Proteobacteria in the summer season (panel A in Fig. 2). The most significant change at the phylum level was the increased relative abundance of Cyanobacteria during the bloom period, which is similar to the findings in 2017 and some other eutrophic lakes (Parulekar et al., 2017; Scherer et al., 2017; Li et al., 2019). Algal

blooms generally had a greater effect on community evenness rather than richness, according to the alpha diversity analysis (Berry et al., 2017), which was also detected by the pielou_e test in this study (Figure S1). For the phylum interactions, Cyanobacteria was significantly negatively correlated with Actinobacteria ($r = -0.84$, $p < 0.001$), Bacteroidetes ($r = -0.67$, $p < 0.001$), and Proteobacteria ($r = -0.68$, $p < 0.001$), based on the Spearman correlations.

In terms of the cyanobacterial community, the summer CyanoHABs in the shallow alkaline lake experienced three distinct stages based on cyanobacterial composition (Panel B in Fig. 2). Initially, in May, the lake was mainly composed of the genera *Cyanobium* belonging to the phylum Cyanobacteria and to the order *Synechococcales*. Cyanobium morphotypes are among the most abundant cyanobacteria in marine environments. As a consequence, their ability to produce toxins can represent a health risk worldwide (Das and Dash, 2019). Among all cyanobacteria, *Cyanobium* had a negative correlation with all the other genera, and the most significant correlation was with *Planktothrix* ($r = -0.81$, $p < 0.001$). Compared with larger phytoplanktonic cells, picocyanobacteria have a relatively smaller volume and larger surface-to-volume ratios, which enables faster nutrient uptake and growth rates (Suttle and Harrison, 1986). However, special N-fixation capabilities were not reported in either *Cyanobium* or *Synechococcus* (Zehr, 2011), showing them to be outcompeted by other, larger phytoplankton under N starvation.

The second stage was detected in early June when *Aphanizomenon* and *Dolichospermum* appeared in Provo Bay and gradually accounted for a higher percentage of the cyanobacterial community (Fig. 2). Toxin-producing *Microcystis* also appeared at this stage. The active presence *Microcystis* was further confirmed by absolute *mcy* gene quantification (Fig. 6) and *mcy* gene expression (Fig. 7). The bloom of *Aphanizomenon* and *Dolichospermum* in Provo Bay continued until the end of June when it gradually spread to the south and north parts of the lake. The early formation of *Aphanizomenon* and *Dolichospermum* community in Provo Bay could be attributed to the richer nutrients, which were rapidly consumed by earlier-stage algae and piccocyanobacteria Cyanobium and further triggered nitrogen-limitation conditions promoting the dominance of *Aphanizomenon* and *Dolichospermum*. Similar to other eutrophic lakes, this stage could be highly correlated with the N-fixation nature of diazotrophs (Beversdorf et al., 2013; Harke et al., 2015; Lu et al., 2019), which helped dominate the nutrient-limitation conditions. However, different from the lakes that observed cyanobacteria shift to toxic non-diazotrophs soon after the bloom of N-fixers, the relative abundance of *Cyanobium* again increased at some sites. As a result, the co-occurrence of *Cyanobium* and *Aphanizomenon*/*Dolichospermum* on the entire lake scale lasted for 2–3 months until *Aphanizomenon*/*Dolichospermum* became the dominant genus at most sites in August. The success of *Aphanizomenon* and *Dolichospermum* could be attributed to their having *nif*/*pst* gene clusters (Figs. 6 and 7) when available nitrate and ortho-P were extremely scarce in the lake (Beversdorf et al., 2013; Komárek, 2013; Lu et al., 2019). This was confirmed by our water quality sampling, where we found nitrate-N and ortho-P were non-detectable in August's and September's samplings. Further, the coexist of these two genera for a long period of time could be attributed to their similar genomic features, nutrient acquisition systems, and environmental niche (Driscoll et al., 2018).

In the third stage, *Planktothrix*, a genus typically without heterocyst, appeared in August's sampling and became the dominant group in September. A significant correlation was found between *Planktothrix* and *Aphanizomenon* ($r = -0.14$, $P < 0.01$), because *Planktothrix* dominated after *Aphanizomenon* in the lake. Along with them, the MCs-producing population was also enlarged and more MCs/MCs-producing genes were detected at this stage (Figs. 6 and 7). The previous sampling generally neglected the late summer *Planktothrix* blooms (Li et al., 2019) and attributed the presence of MCs mostly to the lysis of cell debris. The successional stages of CyanoHABs were also identified by alpha diversity (Figure S1). The unique nature of Provo Bay and its relatively higher nutrient conditions made it an easy target for blooms.

4.2. Bacterial dynamics in relation to different environmental factors

At the bacterial community level, Proteobacteria, Actinobacteria, and Bacteroidetes were observed to be negatively correlated with the bloom indicators (e.g., pH, DO, Chla, and cBDO), but mostly positively correlated with nutrients ($r = 0.03$ to 0.55). The varying r values indicated differences in nutrient requirements among organisms (Schauer et al., 2005; Allgaier et al., 2007; Jezbera et al., 2011), but could generally result in N or P deprivation conditions. Cyanobacteria was significantly negatively correlated with Actinobacteria ($r = -0.84$, $p < 0.001$), Bacteroidetes ($r = -0.67$, $p < 0.001$), and Proteobacteria ($r = -0.68$, $p < 0.001$), based on the Spearman correlations. By contrast, Cyanobacteria responded less to the surrounding nutrient conditions. Some cyanobacteria are affected less by the surrounding nutrient conditions and can trap into nutrient pools that are not typically accessible to other phytoplankton (Cottingham et al., 2015). It could have resulted from their possession of high-phosphorus system that activated under low P conditions (Dignum et al., 2005), as well as hydrolysis enzymes (e.g., PPA and PPX) that can release ortho-P from pyro- or poly-phosphates (Gómez-García et al., 2003). The N-fixation strategy is another tool for cyanobacteria to bring new "N" source into the ecosystem and fuel the phytoplankton community (Schindler et al., 2008; Beversdorf et al., 2013; Scott and Grantz, 2013). Among all cyanobacteria, *Cyanobium* had a negative correlation with all the other genera and the most significant negative correlation was with *Planktothrix* ($r = -0.81$, $p < 0.001$). *Cyanobium* and *Microcystis* were positively correlated with nitrate-N ($r = 0.23$–0.39, $p < 0.05$) and ortho-P ($r = 0.09$–0.36, $p < 0.05$). Compared with larger phytoplanktonic cells, picocyanobacteria have a relatively smaller volume and larger surface-to-volume ratios, which enables faster nutrient uptake and growth rates (Suttle and Harrison, 1986). However, only a few of them have special nutrient management strategies (e.g., N-fixation), causing them to be outcompeted by other, larger phytoplankton under nutrient starving conditions.

Another significant correlation was found between *Planktothrix* and *Aphanizomenon* ($r = -0.14$, $P < 0.01$), because *Planktothrix* dominated after *Aphanizomenon*/*Dolichospemum* in the lake. As for their response to nutrients, *Dolichospemum* and *Planktothrix* were negatively related to all the nutrients measured ($r = -0.1$ to 0.44). The increased temperature effect was positive for *Aphanizomenon* ($r = 0.53$, $p < 0.001$) but negative for *Planktothrix* ($r = -0.14$, $p < 0.01$). It is reported that *Aphanizomenon flos-aquae* could grow above 8 °C with an optimum temperature ranging from 23 to 29 °C (Tsujimura et al., 2001). Although *Planktothrix* favored higher temperatures (> 25 °C) (Lürling et al., 2013; Gomes et al.,

2015), our study did observe a dominance of *Planktothrix* when the temperature fell to around 20 °C.

4.3. The N metabolisms of bacterial community and successions

The N metabolisms mostly correlated the bacterial activities with the N-fixation, nitrite/nitrate reduction, and nitrogen genes regulation during different periods of bloom (Figs. 4, 6, and 7). Cyanobacteria bring new "N" sources into the ecosystem with strategies such as N-fixation under N-limiting conditions (Schindler et al., 2008; Beversdorf et al., 2013; Scott and Grantz, 2013). Although the lake was not N-limited most of the time, except for some occasions in September based on the TDN:TDP ratios (> 16:1, Table S2), potential N-fixers (e.g., *Aphanizomenon* and *Dolichospermum*) were some of the most dominant genera found in nitrate and ortho-P depleted conditions in August. Interestingly, *Aphanizomenon* was positively related to all the *nif* genes (*nifZ, nifW, nifV, nifT, nifK, nifN, nifX, nifE,* and *nifU*) reported in this study ($r = 0.60$ to 0.79, $p < 0.001$). *Aphanizomenon* is long found as a genus dominating summer assemblages and tends to enhance the growth of non-fixing cyanobacteria afterward (Elser et al., 2000; Beversdorf et al., 2013; Lu et al., 2019). Additionally, *Dolichospermum* is positively linked with *nif/nif* expressions ($r = 0.79$ to 0.89, $p < 0.001$), as it is known for the possession of heterocysts at regular intervals across the filament (Komárek, 2013) and blooms with/without N limitation (Yema et al., 2016; Scherer et al., 2017).

Different from the N-fixation period, nitrate and nitrite reductase subunits were relatively higher in the early summer (Fig. 4). The correlation analysis found that heterotrophic bacterioplankton (Actinobacteria, Bacteroidetes, and Proteobacteria) were positively linked with many nitrate reductase subunits ($r = 0.27$ to 0.84, $p < 0.05$) but negatively linked with *nif* gene and gene expressions ($r = -0.21$ to -0.73, $p < 0.05$). Among cyanobacteria, *Cyanobium* was negatively linked with *nif* gene/gene expressions ($r = -0.65$ to -0.70, $p < 0.01$), but positively correlated with the presence of some nitrate and nitrite reductase subunits ($r = 0.34$ to 0.49, $p < 0.05$) (Fig. 4). These cyanobacteria are known for not having heterocysts for N-fixation. This phenomenon could result from the significant composition of nitrate reducers in Proteobacteria, Actinobacteria, Firmicutes, and Cyanobacteria groups (Bru et al., 2007; Palmer and Horn, 2012; Zhao et al., 2015). In fact, except for a few N-fixers, most cyanobacteria rely on ammonia assimilation followed by glutamine synthesis and photosynthetic nitrate assimilations for biosynthesis in CyanoHABs (Andriesse et al., 1990; Flores et al., 2005; Muro-Pastor et al., 2005). Apart from that, cyanobacteria can act as a carbon source for predominant denitrifiers to remove N from the lake (Chen et al., 2012), creating an N-limitation environment. This condition may also favor the long-term stay of N-fixers in the lake.

As for N gene regulation systems, the N regulatory system PII has a higher abundance in May or early June, while the *nif*-specific regulation protein was also detected to be relatively high during other periods (Fig. 4). The N regulatory system PII did not significantly affect or correlate with any communities, which is surprising as it contains a broad group of signal transduction proteins present in Bacteria, Archaea, chloroplasts in Algae and plants (Herrero et al., 2001; Huergo et al., 2013). By contrast, the *nif*-specific regulatory protein was

negatively correlated with *Aphanizomenon* and *nif* gene ($r = -0.34$, $p < 0.05$), which is contrary to the finding that detected upregulation of *nif*- specific proteins under N-fixation conditions (Yan et al., 2010).

4.4. The P and some carbohydrate metabolisms of bacterial community and successions

The P and carbohydrate metabolisms detected a trend to report and respond to nutrient starvation (PPK, (p)ppGpp, PiT, Pho, and G6PD) during early summer, while utilizing the stored polyphosphate (PPX and F6PPK) during the extreme ortho-P scarce period, mostly in August or September (Fig. 5). The dominance of cyanobacteria during blooms could have resulted from their possession of a high-affinity Pi transport system that activated under low Pi conditions (Dignum et al., 2005), as well as hydrolysis enzymes (e.g., PPA and PPX) that can release ortho-P from pyro- or poly-phosphates (Gómez-García et al., 2003). Specifically, the PPX gene is activated at P starvation conditions to degrade poly-P (Adams et al., 2008), which is highly correlated with the presence of *Aphanizomenon* ($r = 0.77$, $p < 0.001$). Similar to PPX, PPA and PPaX are commonly upregulated under Pi-limitation conditions that convert pyrophosphates into two phosphate ions (Gómez-García et al., 2003; Harke and Gobler, 2013). In this study, the PPA and PPaX were activated early in May, when *Cyanobium*/heterotrophic bacterioplankton were the dominant groups and more pyrophosphate could be present (Fuszard et al., 2013). Apart from that, F6PPK was also highly elevated in August and significantly positively correlated with the presence of *Aphanizomenon* ($r = 0.79$, $p < 0.001$), which was a crucial enzyme involved in the central carbohydrate metabolism in heterofermentative bacteria and recently characterized in *Anabaena* sp. PCC 7120 (Moriyama et al., 2015). Cyanobacteria (especially *Aphanizomenon*) was also found positively correlated with “Phosphonate and phosphinate metabolism” related KOs ($r = 0.82$, $p < 0.001$), such as PhnM and PhnJ proteins, which are responsible for the hydrolysis of the C-P bond (Metcalf and Wanner, et al., 1993). It is not surprising that cyanobacteria may utilize organic matter (e.g., phosphonates), as it may give them more competition over autotrophs at Pi-limiting conditions (Gilbert et al., 2004; Vahtera et al., 2007; Harke et al., 2012; Harke and Gobler, 2013; Teikari et al., 2018).

By contrast, more functions related to nutrient storage and acquisition into cells were elevated during the early summer, including some stress or starvation-induction functions (Fig. 5). For example, the presence of PPK was slightly correlated with *Aphanizomenon* ($r = 0.08$) but highly correlated with heterotrophic bacterioplankton ($r = 0.28$ to 0.61, $p < 0.001$), which is a highly conserved region in prokaryotes and responsible for the reversible polymerization of ATP to make polyphosphate for Pi storage in cells (Brown and Kornberg, 2004). PPGK is a polyphosphate-dependent glucokinase found in many organisms, such as diazotrophic cyanobacteria (Klemke et al., 2014; Albi Rodríguez and Serrano, 2015), and was positively correlated with *Aphanizomenon* ($r = 0.33$, $p < 0.01$). Moreover, the G6PD was activated in May, not significantly correlated with *Aphanizomenon or Dolichospermum,* but it was reported to be reluctant for N-fixation in heterocysts and respiration for vegetative cells under dark conditions (Summers et al., 1995). Furthermore, Pi transporters represented by *pst* clusters were upregulated starting in May (Dyhrman and Haley, 2006; Pitt et al., 2010). Specifically, the PiT family was only positively correlated with *Aphanizomenon* ($r = 0.33$, $p < 0.01$), while the *pst* gene/gene expression quantified by qPCR and RT-qPCR were

positively correlated with *Aphanizomenon, Dolichospermum*, and *Planktothrix*. It is reported that *Nostocales* (e.g., *Aphanizomenon* and *Dolichospermum*) are selectively promoted by high P/low N conditions (Suikkanen et al., 2013; Andersson et al., 2015). Their associations with *pst* clusters may provide new thoughts into the P assimilation and explain their dominance in the lake's summer nutrient-limiting conditions (Lu et al., 2019).

The stress and starvation-inducible functions were induced in May (Fig. 5). Specifically, the phosphate starvation-inducible protein PhoH was upregulated during May and faded slightly after (Santos-Beneit, 2015). Although it's not clear evidence in the study, the activation of *pho*-like genes would cause an increase in both Pi uptake and polyphosphate accumulation rates (Morohoshi et al., 2002). Moreover, stringent response signaling (p)ppGpp, a stress response alarmone in response to amino acid starvations and mediate polyphosphate accumulation under nutritional stress (Kuroda, 2006; Abranches et al., 2009), was found activated in early May. It is commonly accumulated at the initial stage of heterocyst formation and triggered by darkness in cyanobacteria (Zhang et al., 2013; Hood et al., 2016).

4.5. The potential MCs- producing cyanobacteria and associations with Nostocales

Similar to other studies that observed an interaction of N-fixers and toxin-producing strains (Elser et al., 2000; Beversdorf et al., 2013; Chia et al., 2018; Lu et al., 2019), the lake also experienced a pre-toxic period in June and a post-toxic bloom in September, together with the bloom of *Nostocales*. The microcystin concentrations (Table S3) increased near the Provo Bay area in June, together with the observation of the first significant evidence of N-fixing genes/gene expressions or N-fixers. It was also correlated with the time when *Microcystis* initially appeared in the lake. However, the relative abundance of *Microcystis* decreased during June and again increased in September with enhanced total MCs concentrations at the sites Provo Buoy, Saratoga Springs, Geneva Discharge, and Vineyard Buoy. The post-toxic bloom was observed in September when CyanoHABs were considered to retreat due to temperature effects. The UDWQ also reported the detection of microcystins above 0.1 μg/L in the open water area until November (UDWQ, 2018). Before September, the entire lake experienced 2–3 months' dominance of *Aphanizomenon*/*Dolichospermum* from June to August. Above all, two peaks of *Microcystis* (up to 10^6 copies/mL 16S rRNA gene) were observed on June 12th (at some sites) and Sep 9th (the entire lake), which is right after or coexistent with the presence of filamentous cyanobacteria. Since *Microcystis* coexists or blooms after N-fixing cyanobacteria, it is safe to hypothesize that N-fixation would be one of the main N-providing pathways (Beversdorf et al., 2013).

The correlation analysis showed a significant negative correlation between toxin-producing genes/gene expressions (Figs. 6 and 7) and *Cyanobium* ($r = -0.61$ to -0.78, $p < 0.005$), while showing positive correlations with *Planktothrix* ($r = 0.62$ to 0.73, $p < 0.05$) and *Microcystis* ($r = 0.39$ to 0.62, $p < 0.1$). Moreover, the presence of MC-LR and MC-RR are significantly correlated with the *16SMic*/*mcyA*/*mcyG* gene and gene expressions ($r = 0.33$ to 0.8, $p < 0.05$). As for the detected MCs producers, *Microcystis aeruginosa* is commonly presented in many eutrophic lakes, while *Microcystis panniformis* is a species originated from tropical lakes (Bittencourt-Oliveira et al., 2007). *Microcystis panniformis* was first reported in Lake Taihu, a temperate lake, implying global warming has driven its distribution

from tropical zones to subtropical zones (Zhang et al., 2012). Typically, the proliferation of *Microcystis* is a phenomenon in many eutrophic lakes and was affected by N availability in the lake (Xu et al., 2010). Under P deficient conditions, the increases of MCs production were observed due to the activation of *Pho* regulon (Oh et al., 2000; Harke and Gobler, 2013). However, the N-limitation typically decreased the net microcystin-production by decreasing the specific cell division rate (Orr and Jones, 1998; Harke and Gobler, 2013). *Microcystis* and *Planktothrix* have distinct morphologies and functions (Guellati et al., 2017), however, they are both bloom-forming, potential MCs-producing cyanobacteria, and their co-habitation was seen in some freshwater lakes (Nixdorf et al., 2003; Paerl et al., 2011; Davis et al., 2014; Francy et al., 2016). Typically, *Planktothrix* dominated lakes with high TDP and low light conditions (Bonnilla et al., 2012). Their proliferation may be complemented with N sources produced by N-fixers. In this study, lower temperatures in late summer could be one of the main factors favoring *Planktothrix* (27.5 °C, Lürling et al., 2013) rather than *Microcystis* or *Aphanizomenon*, which require relatively higher growth temperatures (Reynolds, 2006).

5. Conclusions

By screening bacterial communities, specific functions, and monitoring water quality changes, we successfully found linkages among these parameters. Our data the suggested long-term dominance of N-fixing cyanobacteria in the eutrophic Utah Lake. The shift of the cyanobacterial community was driven by both environmental factors and metabolism dynamics, especially N and P metabolisms in the lake. The results suggested that the long-term dominance of *Aphanizomenon* and *Dolichospermum* in the lake could have been attributed to their activation of the nitrogen fixation (*nif)* and P-affinity (*pst)* genes under nutrient stress conditions. Additionally, the cop-presence of *Aphanizomenon* and *Dolichospermum* in the lake at different sampling events depicted the functional redundancy of in CyanoHABs. The results suggested how genomic contents can influence cyanobacterial diversity and richness, along with environmental factors such as nutrients. Additionally, cyanobacteria may function in early summer to initiate a starvation alert and store nutrients, while utilizing the stored nutrients or turn on N-fixation during heavy CyanoHABs and nutrient-limiting conditions. Excess N, fixed by diazotrophic filamentous cyanobacteria, could also be the food supplying the succeeding growth of *Microcystis* and *Planktothrix*. The detection of *Microcystis panniformis*, an MCs-producing species originated from the tropical zone, may imply potential climate change in subtropical areas. The correlations with different functions (e.g., organic matter utilization) suggested that cyanobacteria may develop varieties of metabolites to acquire energy. For future suggestions, sampling frequencies could be increased to closely monitor the community successions during CyanoHABs. More attention needs to be paid to late summer community succession and the post-toxic period (August and September). It is suggested that the reduction of nutrient input into the lake alone may not prevent CyanoHABs, but could reduce the toxicity levels by supplying fewer nutrients to non-fixing communities, thus preventing possible community shifting from nontoxic to toxic species in the future. Results acquired from this study could be helpful in identifying mechanisms in freshwater lakes that

observed a co-existence or succession of various members of a cyanobacterial community with distinct functions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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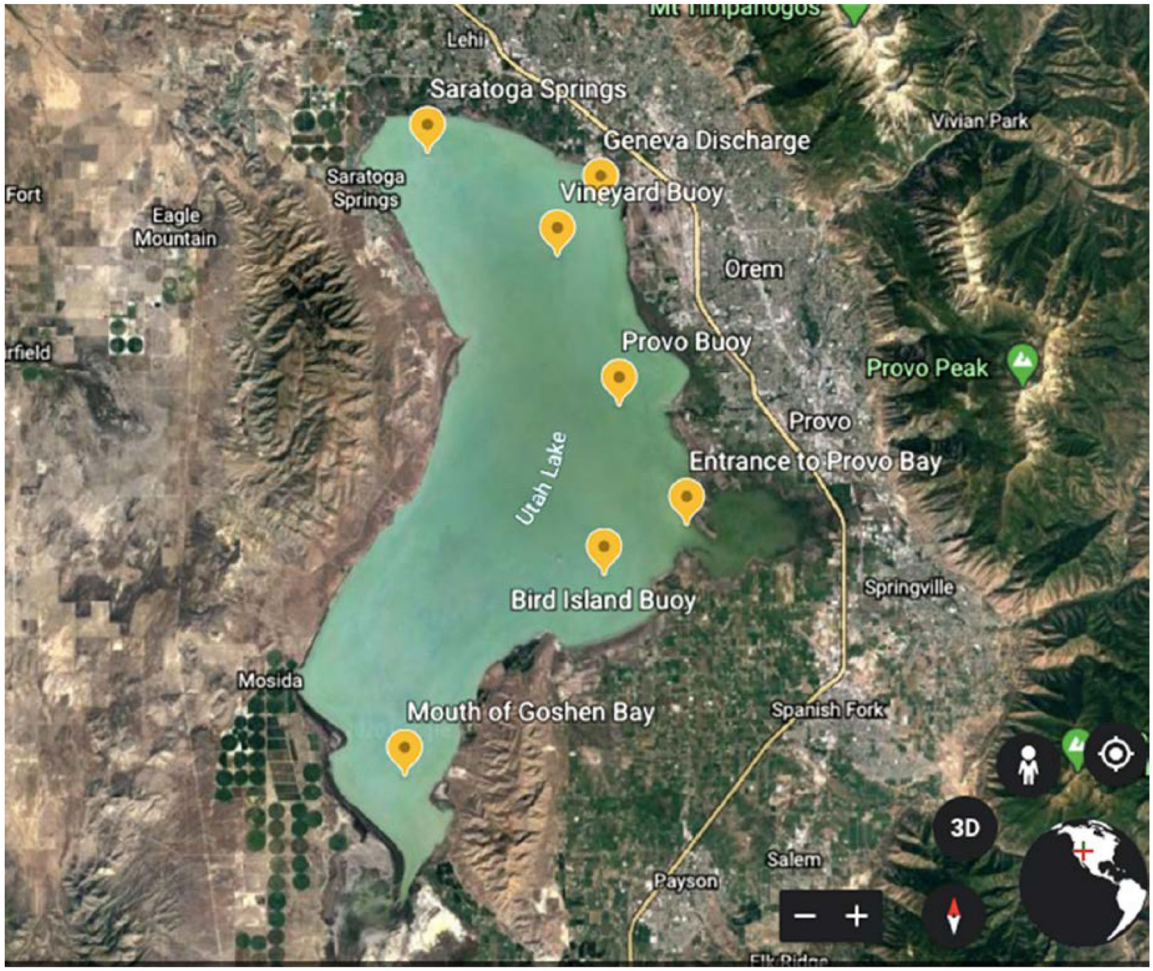

Figure 1:
Map of Utah Lake showing site locations and surroundings

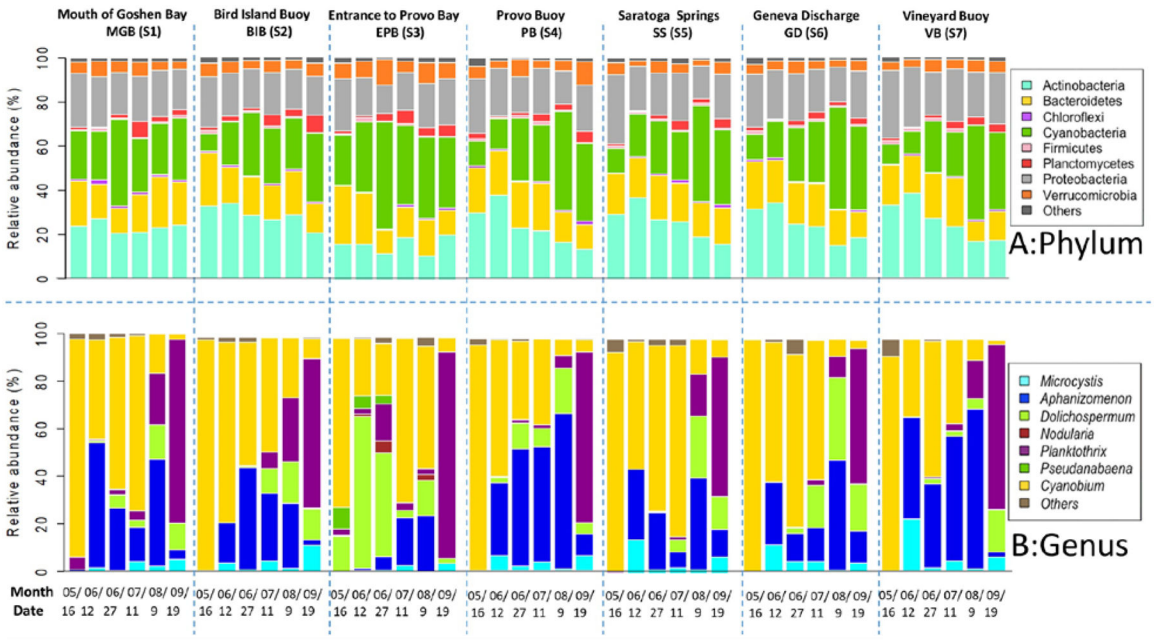

Figure 2.

Microbial community composition at the phylum (top panel) and cyanobacterial genus level (bottom panel) from different sampling sites. “Others” contains “unknown,” “unclassified,” and taxa with small relative abundance.

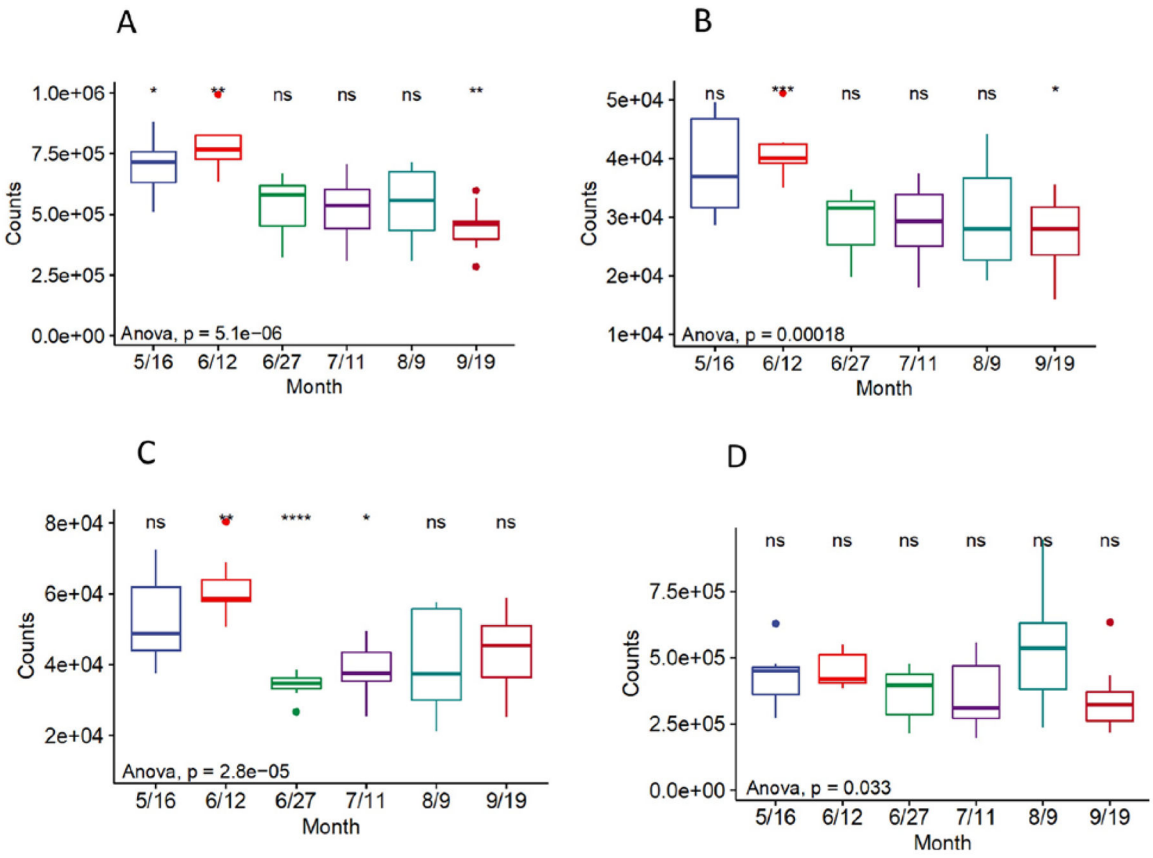

Figure 3.

Predicted metabolic pathways at a 99% sequence similarity. (A) Nitrogen Metabolism. (B, C) Carbohydrate/Phosphorus related metabolism. (D) Photosynthesis. Note the differences of y-axis scales. Reference group is “all”. The significance levels were symbolled as: ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001 ****: p <= 0.0001.

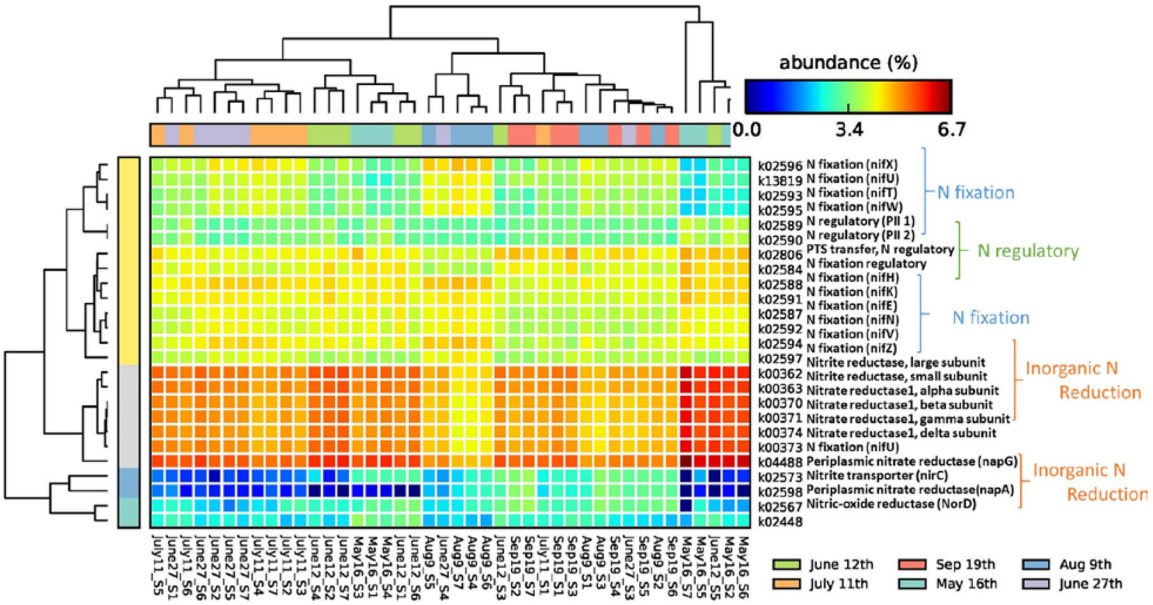

Figure 4.

KOs correlated with nitrogen metabolisms at log scale (samples were clustered by date).

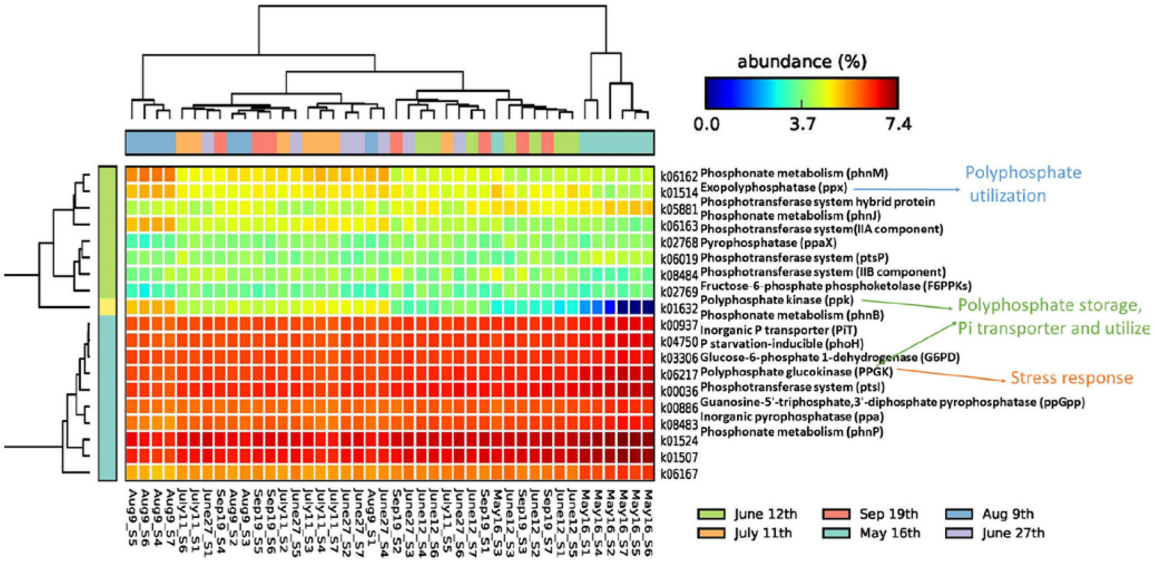

Figure 5.

KOs correlated with phosphorus and carbohydrate at log scale (samples were clustered by date).

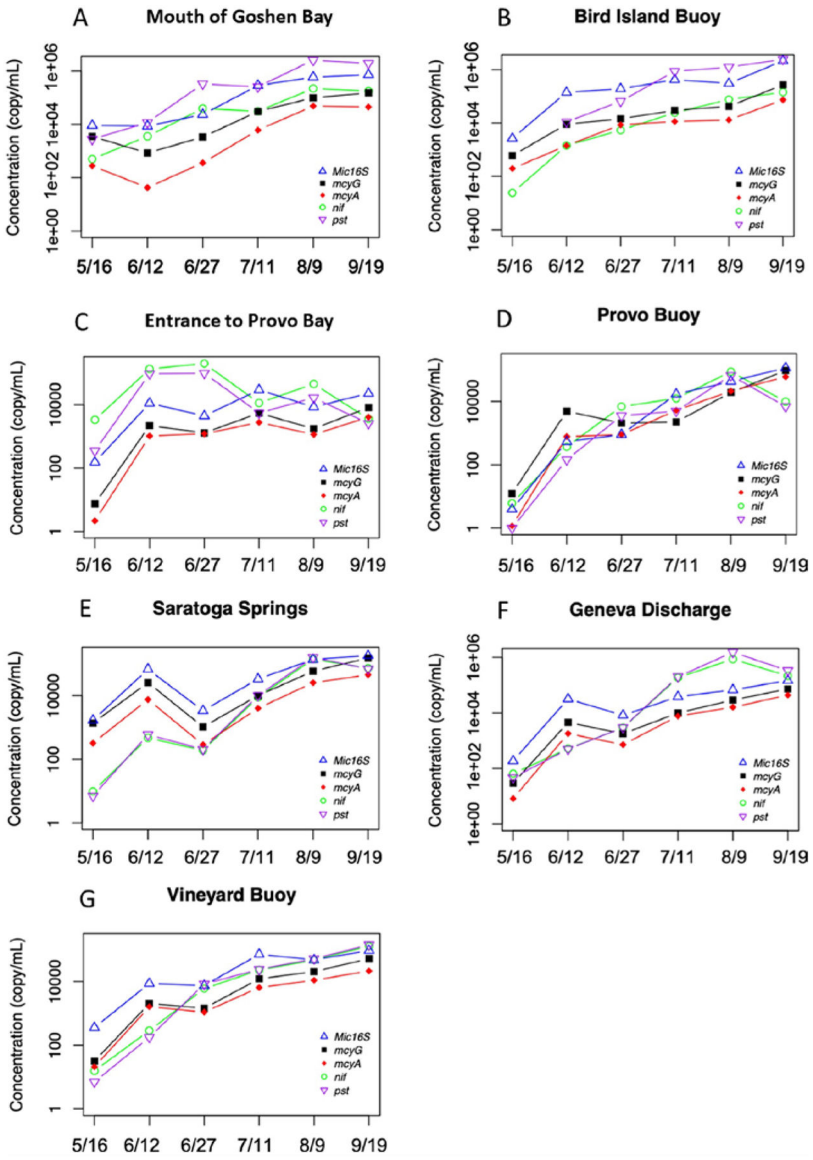

Figure 6.
qPCR for the quantification of gene distributions.

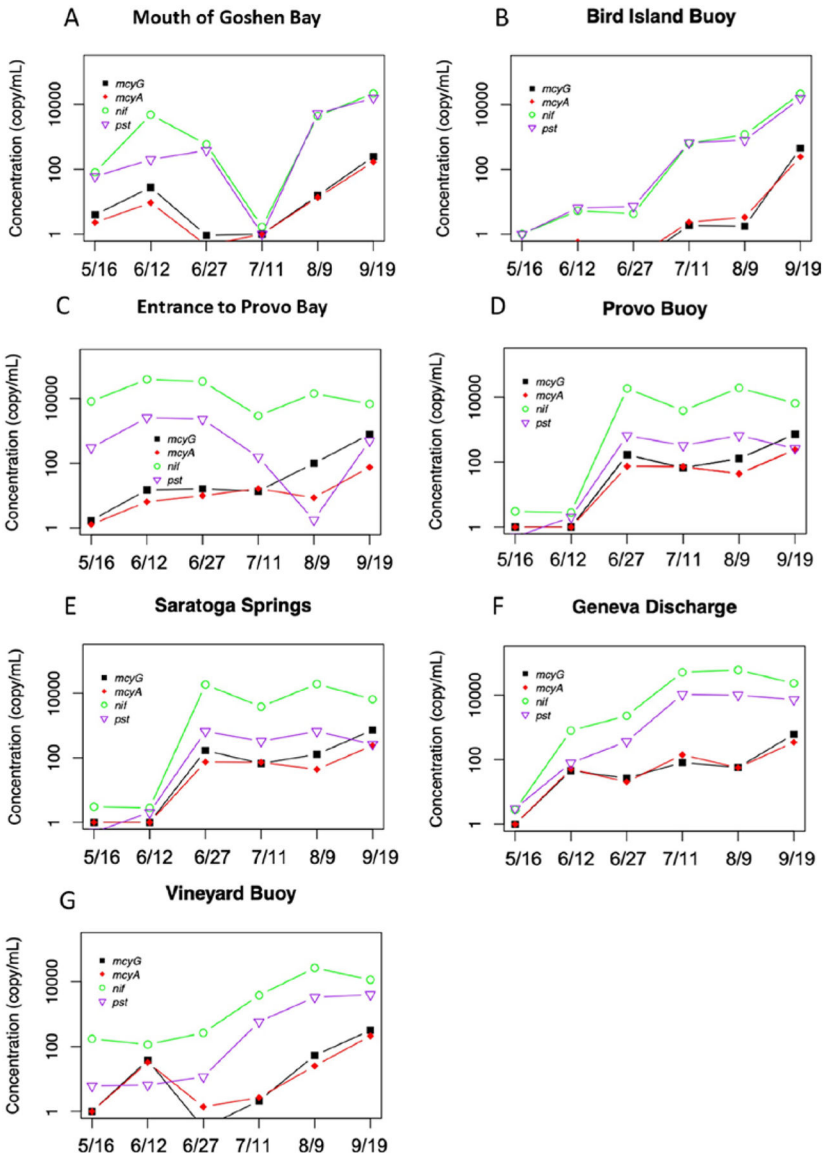

Figure 7.
RT-qPCR for the quantification of gene expressions.

Table 1.

List of Primers applied in the study

Oligo name	Sequences (5'−3')	Tm (°C)	Target	bp	Limit of detection (gn rx^{-1})	Reference
MICf	GCCGCRAGGTGAAAMCT	60	16S rRNA in*Microcystis*	248	1	Neilan et al., 1997
MICr	AATCCAAARACCTTCCTCCC					
mcyEcyaf	TTTGGGGTTAACTTTTTTGGGCATAGTC	56	*mcyE* or *ndaF*in Cyanobacteria	470	10	Jungblut and Neilan, 2006
mcyEcyar	AATTCTTGAGGCTGTAAATCGGGTTT					
mcyAcyaf	AAAAGTGTTTTATTAGCGGCTCAT	56	*mcyA* in Cyanobacteria	302	10	Hisbergues, 2003
mcyAcyar	ATCCAGCAGTTGAGCAAGC					
mcyAmsf	ATCCAGCAGTTGAGCAA	60	*mcyA* in*Microcystis*	171	10	Furukawa et al., 2006
mcyAmsr	GCCGATGTTTGGCTGTAAAT					
mcyGmicf	CAACCCAACAGGTTCTTAAAGC	60	*mcyG* in*Microcystis*	244	10	Ngwa, 2012
mcyGmicr	TGAGGCAAGGTTTCCTCTTG					
nif_nostF3	ATCGTTCAACACGCAGAATTG	60	*Ana, Nos, Cyl*	90	10	Lu et al., 2019
nif_nostR3	TCATCCATTTCGATAGGTGTGG					
pstSf3	TGGAATGTTACCAGCAGGAATAA	60	*A Flos Aq, AFA*	110	10	Lu et al., 2019
pstSr3	AGTGCTGCTTGACGTAAACT					