



## Nitrogen fixation contribution to nitrogen cycling during cyanobacterial blooms in Utah Lake



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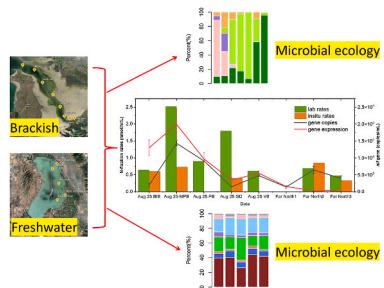
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### HIGHLIGHTS

- N export through denitrification exceeded N-inputs through N-fixation in freshwater and brackish eutrophic lakes.
- The N-fixation rates were positively correlated with nitrogenase gene expressions.
- *Dolichospermum* and *Nodularia* were potential N<sub>2</sub>-fixers for freshwater and brackish lakes, respectively.
- Significantly positive correlations were found among *amoA*, *nirS* and *nirK* genes.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Nitrogen (N) cycling is an essential process in lake systems and N-fixation is an important component of it. Recent studies have also found that nitrate reduction through heterotrophic denitrification in lake systems did not prevent harmful cyanobacterial blooms, but instead, may have favored the dominance of N<sub>2</sub>-fixing cyanobacteria. The overall objective of this study was to estimate nitrogen fixation rates and the expressions of associated nitrogenase (*nif* gene) functional gene at several sites at different occasions in freshwater Utah Lake. For comparison purposes, one time sampling was also conducted in the brackish Farmington Bay of Great Salt Lake (GSL). The microbial ecology of the top 20-cm of surface water was investigated to assess the dominant cyanobacterial communities and N-related metabolisms. Our study revealed that *Dolichospermum* and *Nodularia* were potential N<sub>2</sub>-fixers for Utah Lake and brackish Farmington Bay, respectively. The *in situ* N<sub>2</sub>-fixation rates were 0–0.73 nmol N hr<sup>-1</sup> L<sup>-1</sup> for Utah Lake and 0–0.85 nmol N hr<sup>-1</sup> L<sup>-1</sup> for Farmington Bay, and these rates positively correlated with the abundance and expressions of the *nif* gene. In addition, nitrate reduction was measured in sediment (0.002–0.094 mg N VSS<sup>-1</sup> hr<sup>-1</sup>). Significantly positive correlations were found among *amoA*, *nirS* and *nirK* abundance ( $R = 0.56\text{--}0.87$ ,  $p < 0.05$ , Spearman) in both lakes. An exception was the lower *nirK* gene abundance detected at one site in Farmington Bay where high ammonium retentions were also detected. Based on a mass balance approach, we concluded that the amount of inorganic N loss through denitrification still exceeded the N input by N<sub>2</sub>-fixation, much like in most lakes, rivers, and marine ecosystems. This

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indicates that N cycling processes such as denitrification mediated by heterotrophic bacteria contributes to N-export from the lakes resulting in N limitations.

## 1. Introduction

The occurrence of cyanobacterial blooms (cyanoblooms) is a global issue as it greatly affects water quality and human health (Gobler, 2020). Nitrogen (N) and phosphorus (P) are two most critical nutrients of concern, although their relative contribution to eutrophication is debatable (Kolzau et al., 2014; Paerl, 2017). Traditionally, primary production of phytoplankton in freshwater systems has been viewed as long-term P limited, while N has been considered a temporary limiting nutrient (Stumpf et al., 2012; Schindler et al., 2016). The potential significance of N increased in the last decade due to the co-occurrence and dominance of non-diazotrophs (e.g., *Microcystis* and *Planktothrix*) with the enhancement of N loadings (Paerl et al., 2011; Harke et al., 2016). By contrast, N is commonly the limiting macronutrient in temperate coastal seas and brackish water with greater phosphorus availability (Blomqvist et al., 2004; Rolff and Elfwing, 2015).

In contrast to the P cycle, which is primarily driven by physicochemical processes, N-related biogeochemical processes are mainly mediated by microbial activities. N cycling is an essential process in lake systems. In the overall N cycle, denitrification, either mediated by heterotrophic bacterial population or autotrophic anaerobic ammonium oxidation (Anammox), exports the net dissolved inorganic N to the atmosphere (Du et al., 2019). On the other hand, N fixation, which is an enzymatic conversion of N<sub>2</sub> to dissolved ammonium by N-fixing bacteria convert gaseous N to ammonium (Vicente and Dean, 2017; Moutinho et al., 2021), imports atmospheric N to the aquatic environment. N<sub>2</sub>-fixation by certain cyanobacteria has been shown to be one of the important ecological processes supporting cyanobacterial abundance in aquatic ecosystems (Beversdorf et al., 2013; Singh et al., 2016; Tanvir et al., 2021; Wang et al., 2021). In fact, dense blooms of heterocystous N-fixing bacteria could occur when phosphorus is not limiting. Although, efforts have measured N<sub>2</sub> fixation and denitrification separately in lake environments, these processes have rarely been measured simultaneously in lake environment. Hence, understanding the ecology and relative rates of denitrification and N-fixation in aquatic ecosystems is imperative for understanding the ecosystem response to exogenous N.

In general, N<sub>2</sub>-fixation regulates the enzymatic conversion of atmospheric N<sub>2</sub> to ammonium predominantly in surface water, while denitrification is an anaerobic process, occurring mostly in lake sediments and oxygen minimum zones of the water column to release N<sub>2</sub> gas from inorganic nitrate ions. A reliable competition between the two processes could decide whether the aquatic system in question is tending to be N limited or N sufficient. In general, sufficient degrees of denitrification, either through heterotrophic or autotrophic pathway could lead to N-limited conditions forcing N-fixer cyanobacteria to flourish (El-Khaled et al., 2021). As photosynthetic organisms, cyanobacteria drive nitrate uptake and reduction to nitrite and ammonium by photosynthetically generated assimilatory power, which is referred to as assimilatory nitrate reduction (Flores et al., 2005). However, according to previous reports, N-limitation could result from nitrate loss to heterotrophs (e.g., denitrifiers) via assimilation, denitrification, and other biochemical processes, rather than nutrient consumption by cyanobacteria (Chen et al., 2012; Holmroos et al., 2012). It was found that diazotrophic cyanobacteria can compensate for the resulting N-deficit in the long term through N<sub>2</sub> fixation by developing special types of cells-heterocysts (Schindler et al., 2008). This activity is mediated by nitrogenase, which is encoded by *nif HDK* clusters (Passaglia et al., 1991). This new N source can thus be brought into the ecosystem and fuel the phytoplankton community, mitigating N depletion problems (Beversdorf et al., 2013; Scott and Grantz, 2013). During cyanoblooms, N fixation activities have been detected in the surface water communities of various water bodies

(Schindler et al., 2008; Beversdorf et al., 2013). Unlike those diazotrophic cyanobacteria like *Aphanizomenon* and *Dolichospermum* that commonly dominated eutrophic freshwater lakes (Li et al., 2020a), *Nodularia* were primarily found in eutrophic brackish water bodies with high salinity, such as Farmington Bay of the Great Salt Lake (GSL) (Wurtsbaugh and Marcarelli, 2006). Despite the role of N-fixation in supporting algal blooms, the environmental factors regulating this N-cycling process have not been characterized. Additionally, the relative contribution of N-fixation and heterotrophic denitrification during blooming periods are not well understood.

Sediment can also contribute to lake eutrophication because it contains forms of inorganic N that are readily used for algal growth. Denitrification is the only significant N-cycling process that reduces nitrate and regenerates N<sub>2</sub> into the atmosphere (Jaffe, 2000; Thamdrup and Dalsgaard, 2002). The copper (*nirK*) and cytochrome cd<sub>1</sub> (*nirS*)-containing nitrite reductase are commonly used as functional markers of denitrification (Braker et al., 2000; Kandeler et al., 2006). Investigating these biological N transformations in sediment will also be necessary to understand the N-cycle of ecosystems during cyanoblooms.

The overall objective of the current study was to investigate the microbially mediated N cycle in freshwater Utah lake with sampling spread both spatially and temporally during blooming period. Since N-fixation is also an important N-cycling process in marine environments and brackish lakes, we also sampled the brackish water Farmington Bay of GSL during blooming period one time. Although, previous studies have evaluated the role of N-fixation and denitrification on N dynamics in lakes, studies are lacking incorporating gene expressions with rates and a close comparison between freshwater and brackish water systems. Hence, the Sampling GSL also provided us the opportunity to compare freshwater and brackish water lakes experiencing nearly similar climatic conditions primarily dry arid conditions with extreme temperatures. Although N-cycle has been studied in many eutrophic aquatic ecosystems (Tuomainen et al., 2003; Beversdorf et al., 2013; Yao et al., 2018; Hamilton et al., 2020), comparison and contrast of N dynamics for fresh and brackish water within the same climate zone is still lacking. Additionally, we monitored N-fixation over a nearly complete bloom period (start to end) through three sampling events. On the other hand, one time sampling during peak of the bloom in the brackish Great Salt Lake Farmington Bay was conducted for comparison purposes. Utah Lake and the Great Salt Lake are the two biggest fresh and brackish lake ecosystems, respectively, located in Utah, USA. Apart from detecting physicochemical parameters, high-throughput sequencing and real-time polymerase chain reaction (qPCR) were utilized to analyze microbial communities and target environmentally relevant functional genes. Thus, the correlations among environmental parameters and the surface water/sediment microbial community, and the N dynamics of water and sediment in Utah Lake were studied in reference to that in GSL within the same climate zone and this analysis allowed us to evaluate the dependency of N-fixation and denitrification on other environmental factors.

## 2. Methods

### 2.1. Sites selection and sample collection

The samplings were performed in Utah Lake and Farmington Bay of The Great Salt Lake (GSL) (Fig. 1). The lakes are located nearly 65 miles apart in the vicinity of Salt Lake City, UT. 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 an urban-influenced waterbody, Utah Lake has been experiencing frequent cyanoblooms in

recent years and is impaired by excess total P ( $>0.025$  mg/L) (PSOMAS and SWCA, 2007; Li et al., 2019; Li et al., 2020a). Potential N<sub>2</sub>-fixing cyanobacteria (e.g., *Aphanizomenon* and *Dolichospermum*) dominated the summer bloom seasons (Li et al., 2019, 2020a). GSL is the largest saltwater lake in the Western Hemisphere, although the surface area has been largely reduced in recent years due to extreme temperatures. GSL sampling was conducted in Farmington Bay, which covers 52 mi<sup>2</sup> (135 km<sup>2</sup>) in the SW corner of the lake (Fig. 1). Farmington Bay only represents 5.7% area of the total GSL area but has critical importance in nutrient (N and P) cycling and processing (Wurtsbaugh et al., 2009). Specifically, the northern part of the bay is connected to the main lake and blooms of the heterocystous cyanobacteria *Nodularia spumigena* have been detected in the past (Wurtsbaugh and Marcarelli, 2006; Marden et al., 2013; Goel and Myers, 2009; Wurtsbaugh et al., 2012; Waddell et al., 2009).

In Utah Lake, sampling was conducted before (June: beginning of bloom), during (August: peak of bloom), and after (September: decline of bloom) the peak cyanobloom in 2020. For comparison purposes, a one-time sampling event was conducted on August 19th during the cyanoblooms in the Farmington Bay. Due to boat accessibility and COVID restrictions, the sampling sites for Utah lake were clustered at the east side of the lake, and the sites chosen for Farmington Bay were situated north and south of, and approximately equidistant from the historical Oil Drain (Fig. 1). Depth-integrated surface water samples were collected from the top 20 cm in Utah Lake at sites with water depths ranging from 0.5 to 4.3 m. Due to shallow depth under recent drought conditions (the last 15 years, Farmington Bay has had a maximum depth of 1.3 m and an average depth of 20–35 cm), water samples in the Farmington Bay wetlands were taken from the top 10 cm to make sure the sediments were not disturbed. Water samples were collected in HDPE sampling bottles and collection procedures followed the standard operating procedure for the collection of phytoplankton to detect harmful algal blooms (UDWQ, 2016). Similarly, the top 10 cm of sediments were collected from all sites except for the North sites 1, 2 and 3, where the sediment texture is mostly gravel rendering it difficult to collect sediments. Sediment was collected using the coring method following the standard operating procedure for collection of sediment

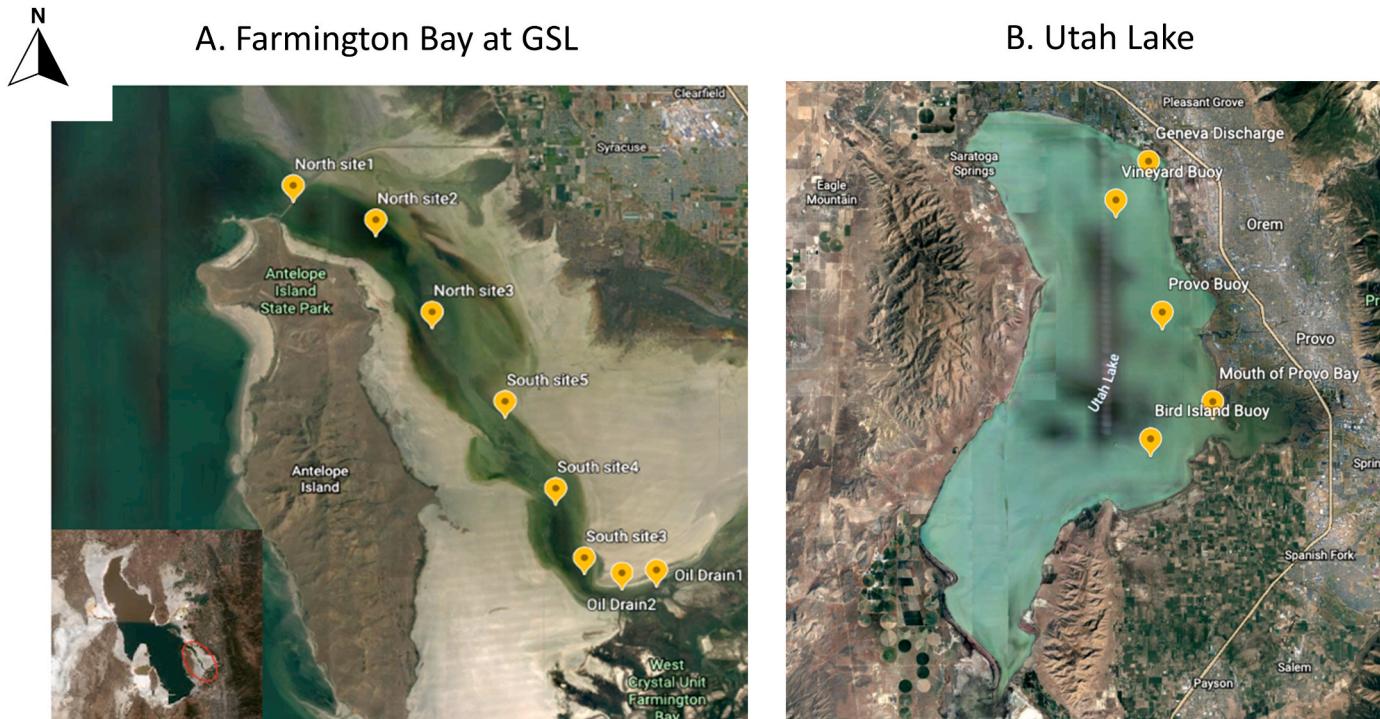
samples in wetlands (UDWQ, 2011).

## 2.2. Physicochemical parameters

Temperature, pH, and conductivity were measured in-situ using a YSI multiparameter sonde (YSI, USA). Water samples (100–200 mL depending on the sites' eutrophication conditions) were directly filtered through 0.22 µm filters in duplicate (HPLV 4700, Fisher Scientific) before measuring soluble nutrients. One set of filters with planktonic biomass were stored in Invitrogen® RNALater stabilization solution for RNA extraction and another set was stored at  $-20^{\circ}\text{C}$  for genomic DNA extraction (section 2.5). Dissolved nutrient anions (nitrate-N, nitrite-N, and orthophosphate-P) in the filtrate were analyzed using Ion Chromatography (IC) (883 Basic IC plus, Metrohm USA) following EPA method 300 (Pfaff, 1993). The ammonia-N, total N (TN), and total P (TP) were measured using the Low Range Ammonia TNTplus Vial Test Kit (TNT830, Hach, USA), Total Nitrogen TNT Reagent Set (LR, Hach), and Total Phosphorus TNT Reagent Set (LR, Hach) respectively. Chlorophyll *a* (Chl *a*) was measured spectrophotometrically and corrected for pheophytin following the standard methods for water and wastewater (Apha, 1999). Additionally, pore water (trapped in sediments) was filtered through 0.22 µm filters and subjected to dissolved nutrients analysis using methods described previously and total organic carbon (TOC) analysis with a SHIMADZU® TOC analyzer (SHIMADZU, Japan).

## 2.3. N<sub>2</sub>-fixation measurement by acetylene reduction assay

An N<sub>2</sub>-fixation test was conducted following the acetylene reduction assay with some modifications of the method from Stewart et al. (1967). Although the ideal way would be to use N<sup>15</sup> N<sub>2</sub> gas to accurately determine N-fixation rates, we could not use N<sup>15</sup> because the stable isotope facility was closed due to COVID restrictions at our institute at the time of sampling in 2021 summer. Nevertheless, we conducted serum bottle tests with most quality control and we believe that the data obtained is of sufficient intellectual merit. Additionally, acetylene reduction method to determine N<sub>2</sub> fixation rates is a widely used method (Jensen and Cox, 1983). Briefly, acetylene gas was injected into the



**Fig. 1.** Sampling sites. (A) Farmington Bay at the Great Salt Lake. (B) Utah Lake.

headspace of 160 mL serum bottles containing 100 mL lake water to make a 10% atmosphere. Incubation time was 3 to 4-hrs in-situ and measured immediately back in the lab. To estimate the maximum capacity of N<sub>2</sub>-fixers, serum bottles were also incubated in the lab for 2 h with slow shaking and under a SunBlaster®T5 High Output Fluorescent Strip Light (SunBlaster Lighting, Canada) to simulate optimal conditions. Ethylene gas formed was measured by an Agilent® gas chromatograph (7890A GC System, Agilent Technologies, USA) equipped with a flame ionization detector (FID), capillary carbon column (J&W GS-CarbonPLOT GC Column, 30 m, 0.32 mm, 3.00 µm, 7-inch cage) with air (400 mL/min) and H<sub>2</sub> (30 mL/min) as the carrier gas. The theoretical converting factor between ethylene and N<sub>2</sub> gas is 4:1 and is used for N-fixation rates estimation (Jensen and Cox, 1983). All N<sub>2</sub>-fixation values were expressed as nmol N hr<sup>-1</sup>L<sup>-1</sup>.

#### 2.4. Inorganic N dynamics of surface sediment

A serum bottle test for nitrate reduction (to estimate denitrification rates) was conducted to estimate denitrification potentials for sediments collected from each site. The test was conducted with the slurry collected from each site (30 g of sediment + 100 mL 3–3.5 mg/L sodium nitrate solution). N<sub>2</sub> gas with 5% CO<sub>2</sub> was purged into serum bottles initially to create oxygen free conditions. The sediment slurry was mixed by gently shaking on a shaker (100 RPM, Thermolyne® AROs 160) during the incubation. Samples were taken every 2 h to monitor changes in nutrient concentrations. Additionally, sediments collected from sites of Farmington Bay were subjected to nitrification tests. These sites were known for the high amount of ammonium-N in sediment pore water (Wurtsbaugh and Marcarelli, 2006). Therefore, no additional nutrients were added as supplements. For the nitrification serum bottle test, 30 g of sediment was mixed by manually shaking with 100 mL of lake water collected near the sediment layer. Air was pumped into the slurry in the serum bottles throughout the 6-h procedure, with samples taken every 2 h. Samples taken for both nitrification and nitrate reduction tests were filtered through a 0.22 µm filter and used for the dissolved nutrient analysis (see section 2.2). To estimate the percentages of organic matter in the sediment, total suspended solids (TSS) and volatile suspended solids (VSS) concentrations were measured immediately after dispersion following USEPA method 1684. The organic content was expressed as g/L VSS of the slurry. The percentage of organic matter of sediment was expressed as VSS:TSS%. The nitrification and nitrate reduction rates were calculated as a result of 6-h incubation (mg N L<sup>-1</sup>hr<sup>-1</sup>) and normalized based on the VSS of slurry (mg N g VSS<sup>-1</sup>hr<sup>-1</sup>).

#### 2.5. Microbial community analysis and metabolic potential

##### 2.5.1. DNA and RNA extraction

To analyze the bacterial functions and community ecology, whole community genomic DNA was extracted from the surface water phytoplanktonic biomass and surface sediments. Genomic DNA was extracted from the filtered biomass (0.22 µm) using a PowerWater® DNA isolation kit (Qiagen, Germany) according to the manufacturer's instructions. DNA from 1 g sediment was extracted using a PowerSoil® DNA Isolation Kit (Qiagen, Germany). To further analyze the gene expression by N<sub>2</sub>-fixers in the surface water, the RNA of phytoplanktonic biomass was extracted using a PureLink® RNA mini kit (Life Technology, USA). Following RNA extraction, residual genomic DNA was removed from total RNA using an on-column PureLink® DNase set (Life Technologies, USA).

The concentrations and quality of DNA and RNA were measured using a Nanodrop® 2000c (ThermoFisher, USA) and stored at -80 °C. Specifically, samples with a 260/280 ratio of around 1.8 (pure DNA) and 2.0 (pure RNA) were selected for downstream analysis. After RNA extraction, reverse transcription was immediately conducted to convert 0.4 µg RNA to cDNA using the SuperScript® VILO™ cDNA synthesis kit (Life Technology, USA). The cDNA templates were further used for gene

expression analysis.

##### 2.5.2. High-throughput amplicon sequencing and analysis

Depth integrated surface water DNA samples with 260/280 ratios higher than 1.80 were selected for high-throughput sequencing. An amplicon library of the V4 region of the bacterial 16S rRNA gene was prepared by the RTSF Genomics Core at Michigan State University using primer set 515F/806R, following the protocol described by Kozich et al. (2013). Samples were 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 as described in our previous study (Li et al., 2020a). The raw data were demultiplexed and converted to Fastq format for downstream bioinformatics analyses.

Bacterial community composition and function prediction analyses were conducted sequentially. The community analysis was conducted according to QIIME 2 "moving picture" tutorials (<https://docs.qiime2.org/2019.10/tutorials/moving-pictures/>). Samples were imported as QIIME2 artifacts, quality filtered using DADA2 (Callahan et al., 2016), and clustered into features (operational taxonomic units) according to the QIIME2 protocol (Bolyen et al., 2019). After picking the most representative sequence for each feature, QIIME 2 used the q2-feature-classifier plugin to assign taxonomy against a classifier trained on the Greengenes 13\_8 99% OTUs (DeSantis et al., 2006). The bacterial community composition and the relative taxonomic abundance at the phylum and genus level were calculated. Species identifications and relative abundances of eukaryotic algae were estimated by their chloroplast gene traits. Cyanobacteria phylum sequences were filtered from all bacteria communities for function prediction analysis using the q2-picrust2 plugin v2019.10 (Douglas et al., 2020). PICRUSt2 was proven to be more robust and accurate than PICRUSt1 with the increased diversity of the 16S database and allowed the addition of custom reference databases (Douglas et al., 2020). A maximum nearest sequenced taxon index (NSTI) cut-off of 2 was implemented by default in PICRUSt2, which exhibited significantly higher precision against most PICRUSt1 predictions. For function prediction, features (OTUs or sequence variants) were closed-reference picked using "qiime vsearch cluster-features-closed-reference" against the Greengenes 13\_5 99% classifier at 99 percent identity. To compare differences in cyanobacterial N metabolism between the two ecosystems, KEGG Orthology (KO) counts of N-related pathways were plotted using the heatmap option with a log scale using STAMP v2.1.3 (Parks et al., 2014).

##### 2.5.3. Using qPCR and reverse transcript (RT)-qPCR to detect the functional gene quantity and gene expressions of microbial community

We conducted qPCR to quantify the functional ammonia-oxidizing gene (ammonium monooxygenase *amoA*) and nitrite reductase genes (*nirK* and *nirS*) of the sediment bacterial community and N<sub>2</sub>-fixation gene (*nif*) in the surface water community. A copper and a cytochrome cd<sub>1</sub>-containing nitrite reductase, encoded by the *nirK* and *nirS* genes respectively, are two structurally different but functionally equivalent enzymes that catalyze nitrite reduction. Additionally, reverse transcript (RT)-qPCR was performed for the *nif* gene to quantify the N<sub>2</sub>-fixing gene expressions. The primers were used for both gene and transcript

**Table 1**  
Primers used in gene detection and gene expressions.

Target	Primers	Sequences	Tm (°C)	References
<i>nif</i>	nif_nostF3 nif_nostR3	ATCGTTCAACACGCAGAATTG TCATCCATTTCGATAGGTGTGG	60	Lu et al. (2019)
<i>amoA</i>	amoA_1F amoA_2R	GGGGTTCTACTGGTGGT CCCCCTCKGSAAAGCCTCTTC	56	Rotthauwe et al. (1997)
<i>nirK</i>	nirK876 nirK1040	ATYGGGGVAYGGCGA GCCTCGATCAGRTTRTGGTT	58	Henry et al. (2004)
<i>nirS</i>	nirScd3aF nirSR3cd	AACGYSAAAGGARACSGG GASTTCGGRTGSGTTSAYGAA	60	Kandeler et al. (2006)

quantifications and listed in Table 1.

To prepare for qPCR and RT-qPCR, positive controls of each gene (plasmids) were cloned using TOPO® TA cloning kit/TOP10 (ThermoFisher, USA) followed by *E. coli* incubation until all plasmids could be extracted using Zyppy® Plasmid Miniprep Kit (Zymo Research, USA). The cloned plasmids were Sanger sequenced at the Health Science Center Cores, the University of Utah, to confirm target genes. All reactions were carried out in a total volume of 20  $\mu$ L, containing 10  $\mu$ L AB Power SYBR® Green Master Mix (ThermoFisher, USA), 1  $\mu$ L of each primer (10  $\mu$ M), 2  $\mu$ L of DNA or cDNA template and 6  $\mu$ L of nuclease-free water. Plasmids were serially tenfold diluted using ultrapure water from initial concentrations of  $10^9$  copies to  $10^1$  copies per 20  $\mu$ L of reaction volume. The qPCR reaction was conducted with a QuantStudio® 3 Real-Time PCR System (Applied Biosystems, USA). The cycling reaction for *amoA* gene consisted of 2 min at 50 °C, 5 min at 94 °C; then 40 cycles consisting of 60 s at 94 °C (denaturation), 90 s at 56 °C (annealing), 90 s at 72 °C (elongation), and a final cycle of 10 min at 72 °C. For the other three functional genes, the qPCR reaction consisted of 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 temperatures (Table 1), and 30 s at 72 °C. All samples, including plasmids (positive controls) and nuclease free water (negative control), were conducted in triplicates. No amplification was detected in nuclease free water. The qPCR amplification efficiencies were 86.28%, 72.27%, 80.08% and 74.72% for the *nif*, *amoA*, *nirK*, *nirS* genes with  $R^2$  of 0.995, 0.973, 0.965 and 0.996. For the estimation of gene abundance and transcriptcopy numbers were multiplied by the diluting factors to convert results to copies/mL of lake water and copies/g VSS of sediment.

## 2.6. Statistical analysis

Spearman correlation tests and PCA plots were conducted among the physicochemical parameters (temperature, pH, nutrients), community composition, and functional gene quantity/gene expressions. Spearman correlation analysis was conducted using the ggscatter function in the “ggpupr” package at a 95% confidence interval (R Development Core team, 2013). Principal Component Analysis (PCA) was performed on surface water and sediment communities respectively using the “FactoMineR” and “Factoextra” R packages. OriginPro v.2021b was also applied for drawing line and bar plots.

## 3. Results

### 3.1. Eutrophication conditions in Utah Lake and Farmington Bay

At the time of sampling, many sites in both water bodies were characterized as near the eutrophic-hypereutrophic boundary based on their TN, TP and Chl a concentrations (Li et al., 2020a, 2020b; Dodds et al., 2006). In Farmington Bay, the northern sites were similar to seawater in terms of conductivity values (27.7–52.85 mS/cm), while the southern sites (2.77–3.96 mS/cm) were more comparable to freshwater systems (Supplementary Table 1). Additionally, the pH was relatively lower in the southern part of the bay (<8.0 for Oil Drain 1, 2 and the South site 3) than the northern part (8.13–8.77 for rest of the five sites). Compared to the northern part of the bay (0.42–0.50 ± 0.01 mg/L), the southern region also had more TP (1.24–3.93 ± 0.01 mg/L) and more inorganic N and P. Moreover, the highest Chl a concentrations were detected at North site 2 (75.65  $\mu$ g/L) and South site 4 (74.76  $\mu$ g/L), indicating massive bloom conditions. Nutrient uptake at an optimum N:P ratio of 16:1 may promote phytoplankton growth, but the succession and proliferation of each phytoplankton type may occur differently (Paerl et al., 2019). Recently lake eutrophication has been accompanied by a shift of stoichiometry (atomic ratio of C: N:P), resulting in algal nutrient limitation. The fundamentals behind such changes are not fully understood. It remains unclear how certain cyanobacteria flourish over other bacterioplankton and phytoplankton under nutrient-limited conditions (Zhou et al., 2021). Sediment from the northern sites was not

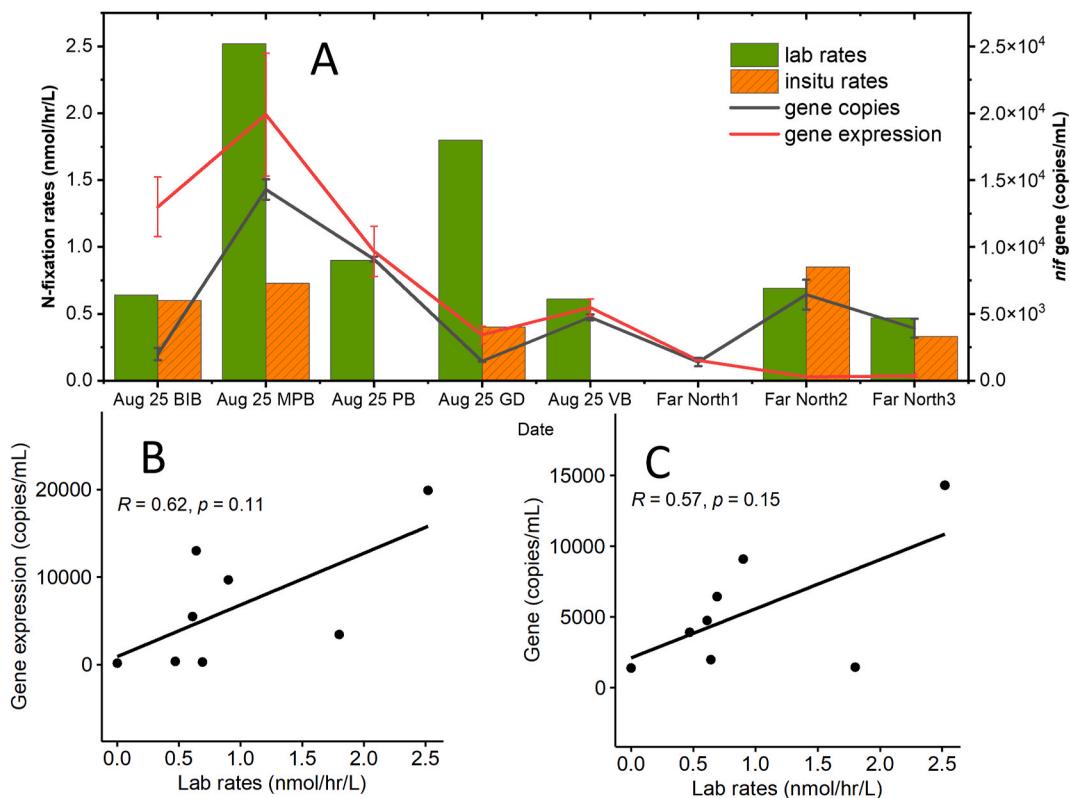
collected due to its gravelly characteristics. Notably, pore water of southern sites detected elevated ortho-P (0.865–5.489 mg/L) and ammonium-N (1.49–13.10 mg/L) concentrations, especially for the Oil Drain 1 and South site 3.

As a freshwater lake, Utah Lake has relatively lower dissolved nutrients compared to Farmington Bay (Supplementary Table 2). The average pH was slightly above 8.3 at all sites. The pH increased slightly during the month of the heavy algal bloom (8.56–8.77, August), indicating bicarbonate consumption during robust phytoplanktonic photosynthesis. Similarly, Chl a concentrations reached their highest levels in August (33.82–119.26  $\mu$ g/L) as compared to June (0–12.21  $\mu$ g/L) or September (0–30.26  $\mu$ g/L) concentrations. The conductivity range was 1.67–2.08 mS/cm, which was higher than those in typical freshwater lakes but much lower than the conductivity measured at Farmington Bay sites (2.77–52.85 mS/cm). Apart from the low ammonium concentrations detected (0.012–0.546 mg N/L), nitrate, nitrite, and ortho-P were almost non-detectable, especially during the bloom period. The TP and TN concentration ranges were  $0.23 \pm 0.01$  to  $0.59 \pm 0.02$  mg/L and  $0.23 \pm 0.06$  to  $4.20 \pm 0.10$  mg/L with TN: TP molar ratios of 36–94, respectively. The ratios were also similar to ratios in the northern part of Farmington Bay (43–51), in which a noticeable massive bloom of N<sub>2</sub>-fixing cyanobacteria occurred, yet P-deficient growth conditions were indicated, with TN: TP molar ratios above 20 (Guildford and Hecky, 2000).

### 3.2. N fixation activity of the surface water community

To further look into the N<sub>2</sub>-fixation activities of the surface water community, N<sub>2</sub>-fixation rates were measured both *in-situ* and in the laboratory. Generally, N<sub>2</sub>-fixation rates detected *in-situ* were lower than laboratory measurements (Fig. 2A). *In-situ* N<sub>2</sub>-fixation rates detected ranged between 0 and 0.85 nmol N hr<sup>-1</sup>L<sup>-1</sup>, consistent with previous observations that cyanobacterial N<sub>2</sub>-fixation rates were mainly below 1 nmol N hr<sup>-1</sup>L<sup>-1</sup> (Loick-Wilde et al., 2017). Recently, Natwora and Sheik (2021) measured N-fixation rates in Great Lakes and other lakes and reported 0.024, 0.020, 0.069, 0.145, and 0.078 nmol N<sub>2</sub>/L/hr for lakes Superior, Michigan, Huron, Erie and Ontario respectively. Our estimated N-fixation rates very well aligns with these reported values and confirms the fact that significant N-fixation could contribute to total N imports to inland lakes. When comparing the N<sub>2</sub>-fixation rates at different locations, the sites at Farmington Bay (0–0.85 nmol N hr<sup>-1</sup>L<sup>-1</sup>) showed slightly higher *in-situ* N<sub>2</sub>-fixation rates and capacities than Utah Lake (0–0.73 nmol N hr<sup>-1</sup>L<sup>-1</sup>). Nevertheless, laboratory-measured N<sub>2</sub>-fixation rates were higher for Utah Lake overall (0–2.56 nmol N hr<sup>-1</sup>L<sup>-1</sup>) compared to Farmington Bay (0–0.69 nmol N hr<sup>-1</sup>L<sup>-1</sup>). Among all sites, the highest N<sub>2</sub>-fixation rates were detected at the Mouth of Provo Bay for Utah Lake (*in-situ*: 0.73 nmol N hr<sup>-1</sup>L<sup>-1</sup>; lab: 2.52 nmol N hr<sup>-1</sup>L<sup>-1</sup>) and North site 2 of Farmington Bay (*in-situ*: 0.85 nmol N hr<sup>-1</sup>L<sup>-1</sup>; lab: 0.69 nmol N hr<sup>-1</sup>L<sup>-1</sup>), which correlated with the high eutrophic status of the shallow Provo Bay and northern Farmington Bay.

Similarly, higher *nif* gene copies and gene expressions were detected in Utah Lake than in Farmington Bay. Specifically, the highest *nif* gene quantity ( $1.43E+04 \pm 7.57E+02$  copy/mL) and gene expressions ( $1.99E+04 \pm 4.95E+03$  copy/mL) were detected at the entrance to Provo Bay (MPB), which corresponded with the in-laboratory N<sub>2</sub>-fixation rates at this site (Fig. 2A). Gene expressions closely corresponded to absolute gene copies at Utah Lake sites, suggesting notable N<sub>2</sub>-fixation activities during the sampling events in August. There were positive correlations between the N<sub>2</sub>-fixation rates and *nif* gene expressions (Fig. 2B) and, between N<sub>2</sub>-fixation rates and absolute *nif* gene copy numbers (Fig. 2C). Fig. 2 also reflects that better correlations were found between the gene copies/gene expressions and lab rates than with the *in-situ* rates (not shown), suggesting that laboratory-measured rates could be good indicators for biological N<sub>2</sub>-fixation activities during a bloom.



**Fig. 2.** Quantification of N<sub>2</sub>-fixation rates of the surface community and *nif* gene/gene expression quantification. (A) Comparison of N<sub>2</sub>-fixation rates, gene copies and gene expressions for two sampling locations. (B) Correlations between *nif* gene expression and lab-measured N<sub>2</sub>-fixation rates. (C) Correlations between *nif* gene copies and lab-measured N<sub>2</sub>-fixation rates.

### 3.3. Nitrification and denitrification potentials of surface sediment

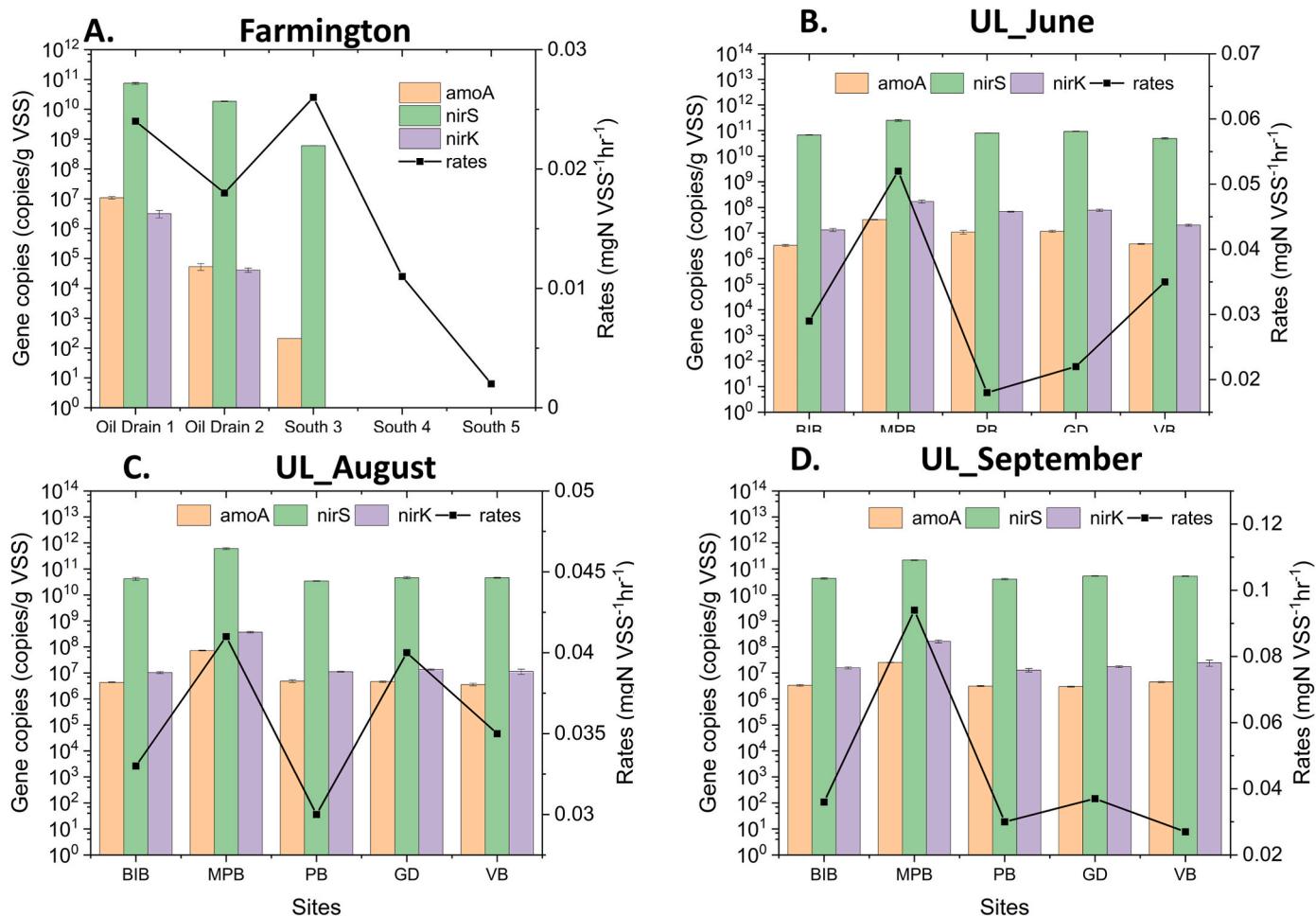
With high concentrations of ammonium detected, sediment collected from Farmington Bay was subjected to an ammonia oxidation test. No significant ammonium oxidation was recorded in water samples collected from any of the sampled sites (Supplementary Table 3). Instead, increasing trends of ammonium were observed at three of five sites (i.e., Oil Drain 1, Oil Drain 2 and South site 5) located in Farmington Bay, suggesting the possibility of nutrient flux from sediment or dissimilatory nitrate reduction activities (Supplementary Table 3). No nitrification test was conducted for Utah Lake due to the anaerobic conditions of the lake sediment and negligible ammonium concentrations (Austin and Lee, 1973).

In contrast, denitrification tests showed a linear decreasing trend in nitrate concentrations for all sediment samples in Farmington Bay (Supplementary Table 4). Nitrate reduction rates of 0.009–0.289 mg N L<sup>-1</sup>hr<sup>-1</sup> were detected during the 6-h incubation period. The nitrate reduction rates corresponded well with the sediment's texture and organic matter content, which were represented by VSS, VSS:TSS% ratios and TOC values (Supplementary Table 4). Specifically, the nitrate reduction rates decreased from Oil Drain 1 site downstream with the increased sandy and gravel sediment at Farmington Bay. Specifically, the percent of VSS decreased from 7.87% (Oil Drain 1) to 3.24% (South site 5). As a natural wetland, sediment collected from Farmington Bay had higher organic content (13.1 ± 2.1 to 18.3 ± 7 g/L) than Utah Lake (0.71 ± 0.01 to 6.79 ± 0.01 g/L). The rockiest site was the Mouth of Provo Bay, which had the lowest VSS:TSS% (0.98 ± 0.44%). It is notable that the TOC content slightly increased in August and September, especially at the Mouth of Provo Bay site. This phenomenon could be explained by the intensive photosynthesis and decay of phytoplankton during cyanoblooms, which added new dissolved organic matter to sediment via the sedimentation of dead cells (Gao et al., 2014).

Nitrate reduction rates were normalized to VSS (Fig. 3) to compare among different locations. The *amoA*, *nirK* and *nirS* genes were detected at most of the sampling sites except for South sites 4 and 5 of Farmington Bay. After normalization, nitrate reduction rates and gene quantities were 0.002–0.094 mg N g VSS<sup>-1</sup>hr<sup>-1</sup>, 0 to 7.26E+07 ± 2.51E+06 copies/g VSS (*amoA*), 0 to 3.77E+06 ± 3.67E+05 copies/g VSS (*nirK*), and 0 to 5.84E+09 ± 4.49E+08 copies/g VSS (*nirS*), respectively. A general tendency for nitrate reduction rates and gene quantities for different sites is shown in Fig. 3. For example, both gene quantities and nitrate reduction rates had decreasing tendencies from the Oil Drain 1 to South site 5 in Farmington Bay. Similarly, the highest gene copies and nitrate reduction rates were detected at the Mouth of Provo Bay of Utah Lake. The maximum number of *amoA* gene, responsible for aerobic ammonium oxidation, copies detected was 10<sup>6</sup> copies g<sup>-1</sup> VSS for both locations, while nitrite reductase (*nirK* and *nirS*, respectively) gene representative of denitrification numbers were one or two-fold higher than those for Farmington Bay samples. Additionally, a closer relationship was found between *nirK* gene and nitrate reduction rates (Spearman correlation test, r = 0.47, p < 0.05). However, an outlier for the *nirK* gene was observed at Oil Drain 1. The *nirK* gene copies detected at this site were significantly lower than the corresponding *amoA* or *nirS* gene counts (Fig. 3A). No *nirK* gene copies were detected at the South site 3. Similar to our findings of lower *nirK* gene copies at the metal-polluted Oil Drain 1, previous studies have found reduced *nirK* and nitrous oxide reductase (*nosZ*) genes in metal-polluted paddy soils compared to non-polluted soils (Liu et al., 2016). Significant positive correlations were found among these three genes in sediment (p < 0.05), with the most significant correlation between *nirK* and *nirS* (Fig. 4).

### 3.4. Microbial community diversities

Overall, high-throughput sequencing of 22 samples (15 from Utah



**Fig. 3.** Nitrate reduction rates of sediment and *amoA*, *nirS* and *nirK* gene quantification. (A) Southern sites of Farmington Bay. (B) June sampling at Utah Lake. (C) August sampling at Utah Lake. (D) September sampling at Utah Lake.

Lake and 7 from Farmington Bay) yielded a total of 2,046,593 sequences. After quality control, 1,607,099 reads (70.96–81.94% of initial sequences of each sample) were retained and clustered into 7632 features (Supplementary Table 5). Rarefaction curves (Shannon diversity index and observed\_OTUs) tended to approach the saturation plateau, implying sufficient sampling depth and coverage (Supplemental Figure 1). The alpha diversity analyses generally indicated higher taxonomic diversity and evenness for Farmington Bay sites compared to alpha diversity for sites in Utah Lake (Supplementary Table 6). Among sites in Farmington Bay, South sites 3 and 4 had the highest and lowest species abundance, respectively, as indicated by the chao1 index. No significant variations were found among Utah Lake samples except for the relatively lower evenness in August. Beta diversity analyses grouped samples by northern (alkaline) and southern sites (freshwater) for Farmington Bay and monthly variations for Utah Lake samples (Supplemental Figure 2). In addition, no significant clustering was observed with Weighted Unifrac, which puts more weight on higher-abundance features.

Bacterial community composition at the phylum and genus levels is shown in Fig. 5 for both Farmington Bay and Utah Lake sites. Actinobacteria, Bacteroidetes, Proteobacteria, and Cyanobacteria were the dominant phyla at all sites in Farmington Bay and Utah Lake (Fig. 5A). One major difference in community composition between Farmington Bay and Utah Lake was the presence of Tenericutes at some sites in Farmington Bay and Chlorobi at Utah Lake sites. Tenericutes is a phylum of bacteria that lacks a peptidoglycan cell wall. It contains the class Mollicutes, which can be pathogens and/or mutualistic symbionts in the

gut of their host species (Wang et al., 2020). For phytoplankton, we found the highest relative abundances of Cyanobacteria in Farmington Bay at North site 2 (35.17%) and South site 4 (70.57%). For Utah Lake, the greatest relative abundance of Cyanobacteria was observed in August (29.52–58.32%), corresponding with the massive algal bloom.

At the genus level, all sequences assigned to chloroplasts were grouped into eukaryotic “algae”, including Chlorophyta (commonly known as “green algae”), Stramenopiles, Streptophyta, and others (Fig. 5B). For Farmington Bay, the relative abundance of algae was significantly higher at the southern sites (i.e., Oil drain 2 and S3) with excessive nutrients compared to northern sites of the bay. Similarly, algae had higher relative abundances at Utah Lake sites in June before the occurrence of the cyanobloom. *Nodularia* and *Halomicronema* dominated the northern alkaline sites while *Synechococcus* was relatively more abundant in the southern areas of Farmington Bay. *Halomicronema* is a non-heterocystous filamentous cyanobacterium that is moderately halophilic and halotolerant (Abed et al., 2002). For Utah Lake, *Dolichospermum* dominated the peak bloom period, but *Planktothrix* became dominant when cyanoblooms waned towards the end of September. *Cylindrospermum*, a potential toxin-producing filamentous cyanobacterium, was also detected at one of the most eutrophic sites—Provo Buoy. *Microcystis*, a potential microcystin-producer, was detected at high relative abundance mainly in June (1.06–15.66%). Overall, cyanobacteria were the primary phytoplanktonic populations for both ecosystems.

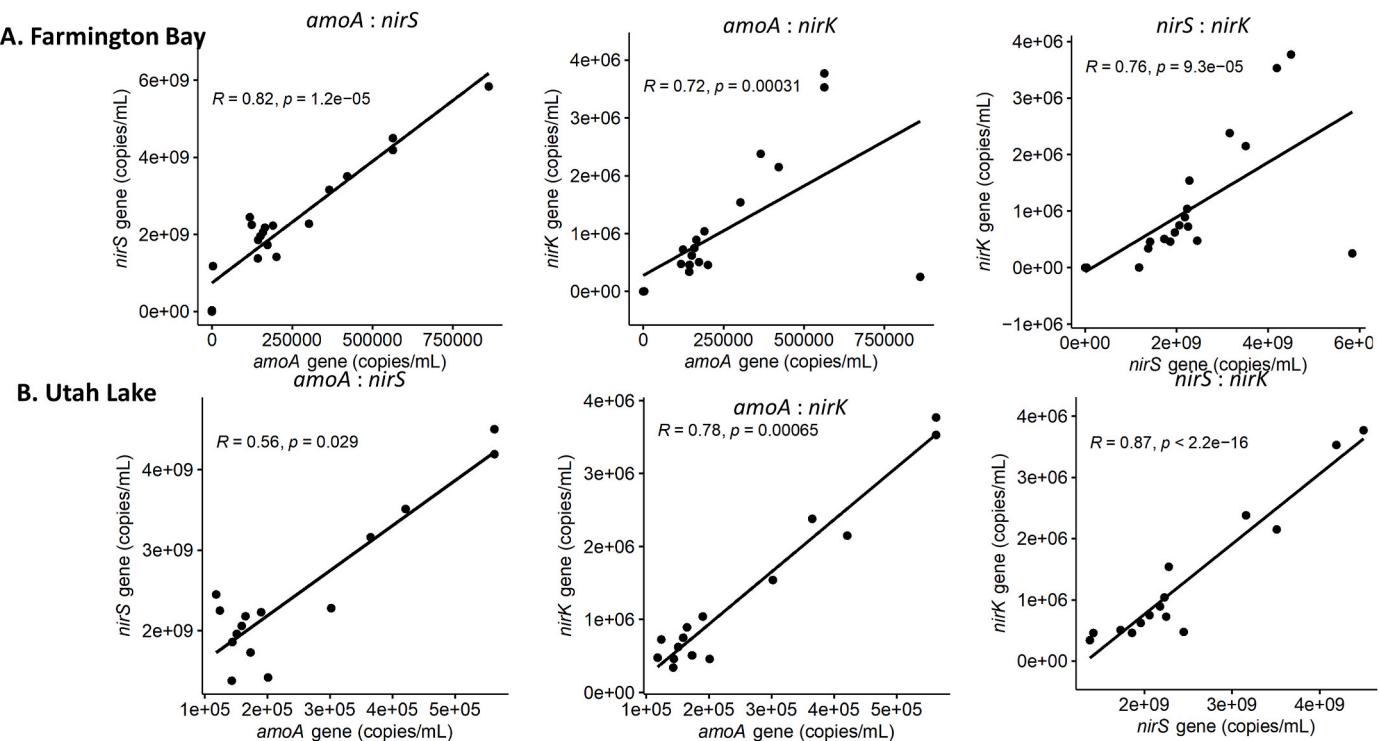


Fig. 4. Pairwise correlations among *amoA*, *nirS* and *nirK* genes. (A) The ratios for all samples. (B) The ratios for Utah Lake only.

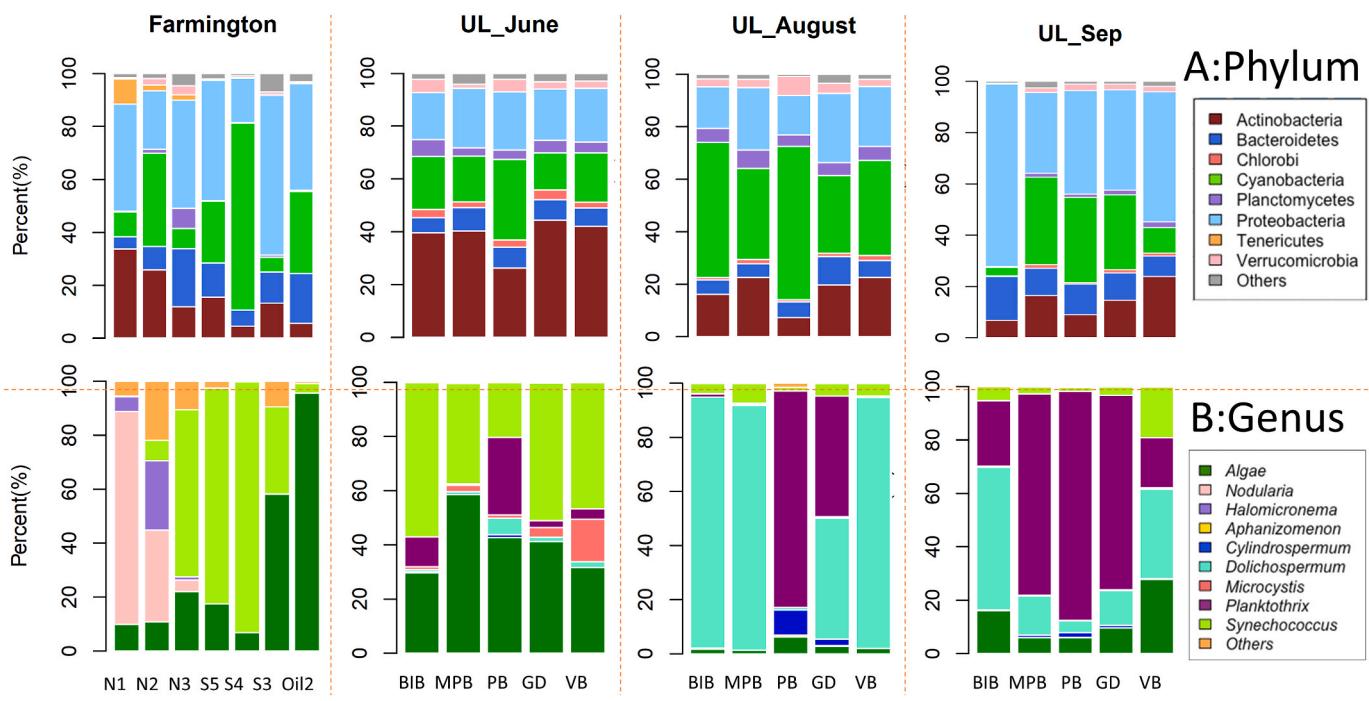


Fig. 5. Surface water bacterial community analysis at phylum and genus level. (A) Bacterial community composition at the phylum level. (B) Bacterial community composition at the genus level. Site names are abbreviated as North site 1 (N1), North site 2 (N2), North site 3 (N3), South site 5 (S5), South site 4 (S4), South site 3 (S3) and Oil Drain 2 (Oil2) for Farmington Bay, and Bird Island Buoy (BIB), Mouth of Provo Bay (MPB), Provo Buoy (PB), Geneva Discharge (GD) and Vineyard Buoy (VB) for Utah Lake.

### 3.5. Prediction of N metabolism within the surface water cyanobacterial community

With an NSTI cut-off of 2, the PICRUSt2 mapped 410,054 sequences to the cyanobacterial community and generated 138 features.

Specifically, ammonium-N is the inorganic N source preferentially used by most cyanobacteria (Boussiba and Gibson, 1991) and a central component of their N cycling (Fig. 6). Ammonium can be imported into cyanobacterial cells by processes of ammonium assimilation, N<sub>2</sub>-fixation, nitrate uptake and assimilatory nitrate reduction, while N sources

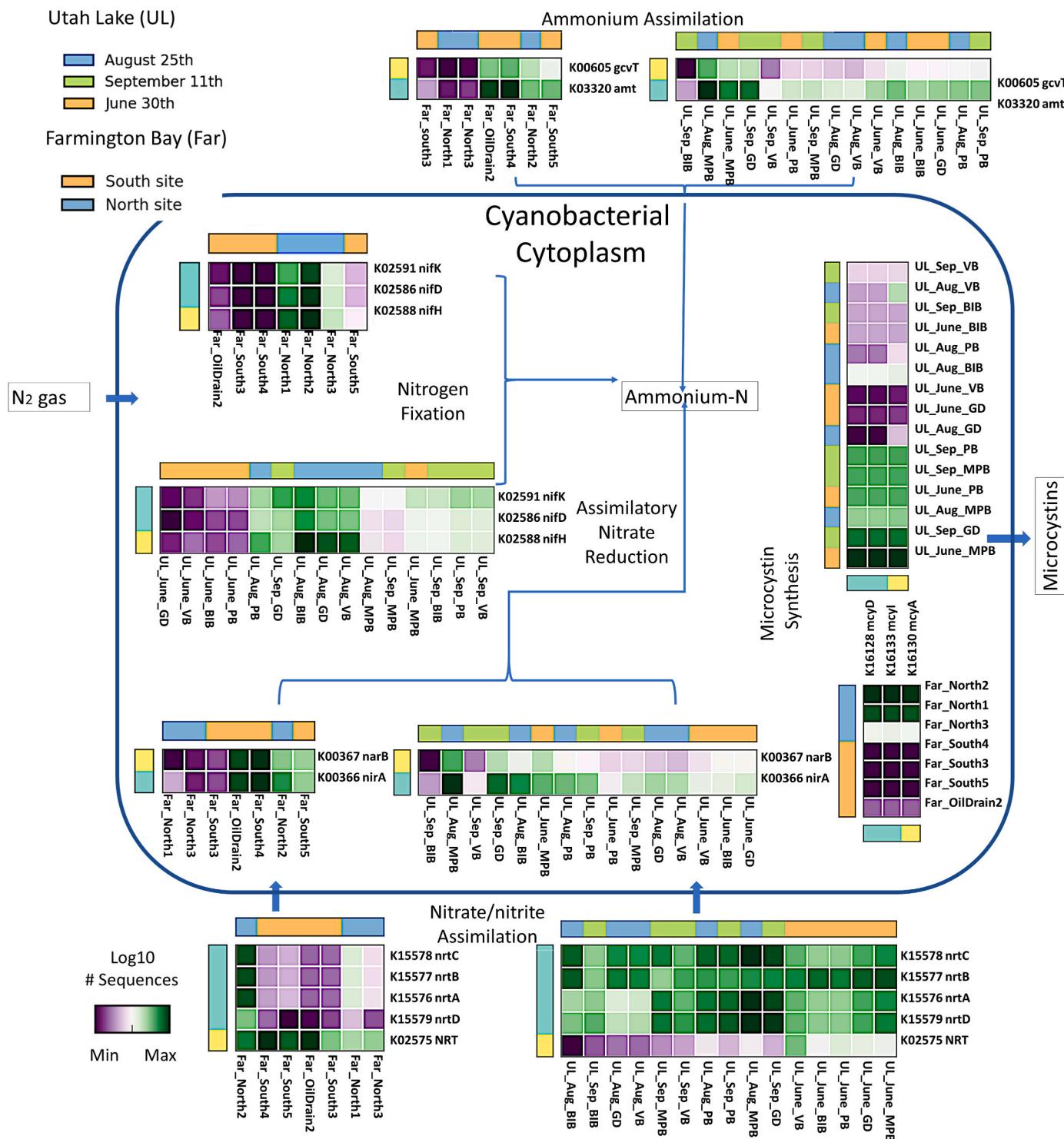


Fig. 6. Nitrogen related metabolism prediction for the surface water cyanobacterial community at both sampling lakes.

can be exported through exuding *N*-enrich cyanotoxins (e.g., microcystins). Nitrate uptake and reduction to nitrite and ammonium are driven in cyanobacteria by photosynthetically generated assimilatory power (Flores et al., 2005). Ammonium acts as an intermediate product and affects several key enzymes in N metabolism, such as glutamine synthesis (Boussiba and Gibson, 1991). The counts for key KOs of N pathways were predicted using PICRUSt at both Farmington Bay and Utah Lake sites. They are nitrogenase (*nifHDK*) for the N fixation process, nitrate/nitrite transport system (*nrtA-D* and *NRT*) for nitrate uptake, and ferredoxin-nitrate reductase (*narB*) and ferredoxin-nitrite

reductase (*nirA*) for assimilatory nitrate reduction, ammonium transporter (*amt*) for ammonia assimilation and microcystin synthesis (*mcy*). Among these N metabolisms, *nifHDK* counts were relatively higher for the northern sites of Farmington Bay and some Utah Lake sites sampled in August, suggesting that heterocystous cyanobacteria actively brought new N to the ecosystem. Additionally, lower nitrate transporter (*nrtA-D*) operons were detected for all sites of Farmington Bay except for North site 2. In contrast, no significant differences were observed for most nitrate/nitrite assimilation genes of Utah Lake samples, except for the low counts of *nrtD* observed in August and September. Nevertheless,

only a slight variation was observed for assimilatory nitrate reduction (*narB* and *nirA*), which regulates reductions from nitrate to ammonium for microbial biomass incorporation. Similarly, no significant variation was detected for the ammonium transporter (*amt*) and amino-methyltransferase (*gcvT*) at most Utah Lake sites except for relatively higher counts found at the eutrophic sites, such as the Mouth of Provo Bay and North site 2.

Cellular N compounds (ammonium or organic N) could also be released into the ambient environment through leaking or mineralization of cell debris. These N sources can feed the surrounding community and even induce a subsequent bloom of non-heterocystous cyanobacteria and toxin-producers (Agawin et al., 2007; Lu et al., 2019). Additionally, the release of microcystin, an N-rich cyanotoxin, could be an N output pathway of toxic cyanobacteria. PICRUSt 2 further predicted microcystins synthesis (*mcyA-E, G, I-J*) clusters within samples (Fig. 6). Specifically, the *mcy* operon was highly predicted at northern sites of Farmington Bay and eutrophic sites (e.g., the Mouth of Provo Bay and Provo Buoy) of Utah Lake. Some studies have found microcystin production to be directly correlated with nitrate uptake but possibly suppressed by ambient ammonium concentrations (Downing et al., 2005; Chen et al., 2019). Unlike most bioavailable compounds, microcystin degradation is unpredictable in aquatic ecosystem and could affect N cycles by inhibiting ammonia-oxidizers (Schmidt et al., 2014; Li et al., 2020b).

#### 4. Discussion

##### 4.1. Correlations between microbial community and environmental parameters

Environmental parameters shape the *in situ* microbial community for both water and sediment niches; resultant microbiological activity is a crucial parameter mediating aquatic ecosystems functions. For Utah Lake, a peak bloom event in August was observed together with the highest Chl a concentrations (33.82–119.26 µg/L), lower ambient nutrient concentrations, and the greatest relative abundance of cyanobacteria and N<sub>2</sub>-fixation capacities. The availability of inorganic N sources (e.g., ammonium and nitrate) may control cyanobacterial community successions, with the dominant genus shifting from eukaryotic algae and picocyanobacteria towards heterocystous, then to non-heterocystous cyanobacteria (Fig. 5). For Farmington Bay, a salinity gradient was observed from the northern to the southern region of the bay, with the salinity dropping from the range of 1–6‰ to almost freshwater characteristics (Marden et al., 2013). The bacterial communities were remarkably differentiated by salinity and eutrophic status, with a *Nodularia* bloom prevalent at the northern sites with high salinity (Fig. 5). Regarding the nutrient-limiting status, a previous study found that all bays of the GSL had TN: TP ratios of 25 or higher—indicating N would be adequate while P would in fact be the limiting nutrient (Wurtsbaugh et al., 2012). Despite high TN: TP ratios (43–51) and P-deficiency measurements at the northern sites, the detection of N<sub>2</sub>-fixation activities from these locations may actually indicate N availability limitations.

Correlations with surface water microbiota were also revealed by PCA plots (Supplementary Figure 3). Notably, N<sub>2</sub>-fixation related parameters (e.g., *nif*\_gene abundance and *nif*\_transcript) highly correlated with the August cyanobacterial community (*Dolichospermum* and *Aphanizomenon*) and Chl a for Utah Lake. The N<sub>2</sub> fixation microbiota were more prevalent in the northern part of the bay, although both regions (northern and southern) had abundant phytoplankton communities. The PCA plot showed clustering of samples by sediment parameters and microbiota for both lakes (Supplementary Figure 4). Nitrification and denitrification gene copies were positively correlated with nitrate reduction rates. Although sediment of Farmington Bay was richer in organic content (highly correlated with VSS and TOC), it had relatively lower nitrate reduction rates and gene copies after

normalization. Pollution by various point and nonpoint discharges could have affected nitrogen fate in the lake. Northwest Oil Drain is the sewage canal that transports Salt Lake City Publicly Owned Treatment Works (POTW) effluent to Oil Drain 1 at the southern part of the bay. This site, along with the several downstream sites, was reported to be impacted by substantial inputs of N and P as well as heavy metals (e.g., detection of mercury and selenium from ambient zooplankton or bird eggs) (Marden et al., 2013; Wurtsbaugh et al., 2012; Waddell et al., 2009). This may also explain the retention of high amount of ammonium in the sediments of Oil Drain 1 and other southern sites. Although the *amoA* gene was detected in sediment of Farmington Bay sites, no significant ammonia-oxidizing activity was observed with the serum bottle test (Supplementary Table 3). Nitrate reduction was detected at all sites, which could be partially due to incomplete denitrification and alternative reaction pathways (e.g., dissimilatory nitrate reduction) in addition to complete denitrification (Laverman et al., 2007; Schmidt et al., 2011). Salinity could also affect denitrification more than the dissimilatory nitrate reduction process (Laverman et al., 2007). This is the first time *amoA*, *nirK* and *nirS* genes were detected in sediment of Farmington Bay and Utah Lake. Gene copy numbers were within ranges detected in previous lake sediment studies, although the *amoA* gene copies were less abundant than the denitrifying gene clusters (e.g., combined *nirK* and *nirS*) (Vila-Costa et al., 2016; Wan et al., 2019). The significant correlations found among these three genes is similar to results of a root function diversity study of submerged vegetation in lake sediments (Vila-Costa et al., 2016). An exception was the significantly lower copies of *nirK* at Oil Drain 1 compared to the fitted curve and no detection of the gene at South site 3 (Figs. 3 and 4). The *nirK* gene is a copper-containing reductase and addition of trace copper (26 µM Cu) can lead to its abundance/enhanced expression (Giannopoulos et al., 2020). However, *nirK* was also found to negatively correlate with numerous heavy metals (e.g., Ag and methyl-mercury) in the San Francisco Bay estuary (Mosier and Francis, 2010). As a discharging port of heavy metals, it can be hypothesized that the Oil Drain site 1 was severely heavy metal-polluted and the decline of *nirK* could thus result from heavy metal (e.g., mercury) pollution.

##### 4.2. Implications of internal N mass balance of the two ecosystems

From the N<sub>2</sub>-fixation and nitrate reduction activities measured, we can roughly estimate the internal N mass balance. Biological N fixation is the only natural means to convert N<sub>2</sub>, the dominant gas in the atmosphere, into a bioavailable N source. It has been postulated that the impact of N on cyanoblooms may not depend on overall loading; rather, newly added N (e.g., by N<sub>2</sub>-fixation) prior to blooms could be a controlling factor (Lu et al., 2019). Generally, N fixation activities correspond well with the eutrophic (especially high P) status of aquatic systems. Planktonic N fixation tends to be low in oligotrophic and mesotrophic lakes (generally <0.1 g N m<sup>-2</sup> yr<sup>-1</sup>) but is often high in eutrophic lakes (0.2–9.2 g N m<sup>-2</sup> yr<sup>-1</sup>) (Howarth et al., 1988). In this study, N fixation rates were 0–0.10 g N m<sup>-2</sup> yr<sup>-1</sup> *in situ* and 0–0.31 g N m<sup>-2</sup> yr<sup>-1</sup> for in-laboratory measurements, revealing some eutrophic potential. Earlier studies found that N fixation generally offsets potential N loading reductions in eutrophic lakes (Higgins et al., 2018; Schindler et al., 2008). Instead, a cyanobacterial community shift towards N<sub>2</sub>-fixers was observed for lakes subjected to reduced N-loading management strategies without decreasing P (Schindler et al., 2008). In total, N<sub>2</sub>-fixation accounted for 69–86% of total N loading to the epilimnion during the period of rapid bloom development, and 72–86% of total N loading during the May–October period (Higgins et al., 2018). With abundant P at both locations (Marden et al., 2013; Randall et al., 2019), it can be inferred that the N<sub>2</sub> fixation community would likely continue to adaptively provide N to the lake systems. Additionally, the enhanced N<sub>2</sub>-fixation rates in lab tests could be a result of increased light intensity compared to *in situ* measurements. A previous study of the light response curve with photon flux densities (PFDs) found that the

nitrogenase activity of heterocystous cells can keep increasing until they are saturated at certain PFDs ( $0\text{--}300 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) (Severin and Stal, 2008).

In contrast, denitrification is a primary process for losing N from aquatic systems (Ward et al., 2009). In a eutrophic lake, the maximum denitrification rate of water-sediment interactions measured was  $29 \text{ g m}^{-2} \text{ yr}^{-1}$  (Liu et al., 2018). The high nitrate removal rates ( $31.54\text{--}1012.66 \text{ g m}^{-2} \text{ yr}^{-1}$ ) measured in this study were likely due to microbially mediated reduction of sufficiently high soluble nitrate and organically rich sediments, as detected using in-laboratory serum bottle tests. Also, incomplete denitrification and dissimilatory nitrate reduction may account for higher percentages of nitrate removal from sediment (Laverman et al., 2007). Denitrification and dissimilatory nitrate reduction are two processes occurring simultaneously under oxygen-limited or anaerobic conditions that compete for nitrate and organic carbon. Assuming half of the nitrate reduction measured was due to denitrification (Seitzinger, 1988), complete denitrification still accounted for  $15.77\text{--}506.33 \text{ g m}^{-2} \text{ yr}^{-1}$ . Denitrification also occurred in the water column with accumulating cyanobacteria biomass as a source of organic carbon (Chen et al., 2012; Peng et al., 2017). We did not evaluate the denitrification potential of surface water in our study, although bloom impacts on the increase of dissolved organic matter can be inferred from the slightly enhanced TOC content in the sediment (Supplementary Table 4). Taken together, the amount of N loss through denitrification could still exceed the N input by  $\text{N}_2$ -fixation, similar to most lakes, rivers, and marine ecosystems (Seitzinger, 1988; Shatwell and Köhler, 2019). A net N loss was estimated based on  $N$ -fixation and denitrification rates, despite any N deposition and other N loading processes. Therefore, a long-term N reduction strategy may still be one of the primary solutions for reducing cyanoblooms as blooms of diazotrophic cyanobacteria cannot compensate for the resulting N-deficit (Shatwell and Köhler, 2019).

## 5. Conclusions

Eutrophication and cyanoblooms have been two prevailing concerns in fresh and alkaline aquatic ecosystems with droughts and anthropogenic activities being major contributors. This study investigated and compared the N dynamics for a freshwater lake and a brackish wetland within the same climate zone in Utah. Specifically,  $\text{N}_2$ -fixation rates and cyanobacterial communities were detected in the surface water from both locations during the blooms. *Dolichospermum* and *Nodularia* were potential  $\text{N}_2$ -fixers for Utah Lake and Farmington Bay, respectively. Their presence corresponded well with the detection of *nif* gene abundance/gene expression.  $\text{N}_2$ -fixation, along with other N processes (e.g., assimilatory nitrate reduction), contributed to the synthesis of ammonium, a central component of N cycling in surface water. Additionally, nitrate reductions were mainly detected within sediment, which could be attributed to denitrification, dissimilatory nitrate reduction, and other processes. Significant correlations were found among *amoA*, *nirS* and *nirK* genes at both locations. One exception was the lower *nirK* gene detected at Oil Drain site 1 and South site 3, where N-cycling was inferred to be hampered by heavy metals. Moreover, no ammonium-oxidizing activity was observed even where ambient ammonium concentrations were high at Farmington Bay. Overall, the amount of N loss through denitrification could still exceed the N input by  $\text{N}_2$ -fixation, but the latter process played an important role in enabling non-heterocystous cyanoblooms via providing new N to the lake ecosystem. In order to control  $\text{N}_2$  fixation as a mitigation strategy for cyanoblooms, reduction of P level should be considered although other sources of N inputs other than  $N$ -fixation should not be ignored.

## Disclaimer

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## Author contribution

HL conducted all the fieldwork, analysis, and genomic work. HL also prepared the first draft of the manuscript. TM provided helpful comments on the manuscript helped fund the study. JL and RKG designed the study, revised the manuscript, and prepared it for the final submission.

## 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.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2022.134784>.

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