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Hampel, Justyna J.; McCarthy, Mark J.; Neudeck, Michelle; Bullerjahn, George S.; McKay, Robert Michael L.; and Newell, Silvia E.. (2019). Ammonium recycling supports toxic Planktothrix blooms in Sandusky Bay, Lake Erie: Evidence from stable isotope and metatranscriptome data. *Harmful Algae*, 81, 42-52.
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Harmful Algae 81 (2019) 42–52

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Ammonium recycling supports toxic *Planktothrix* blooms in Sandusky Bay, Lake Erie: Evidence from stable isotope and metatranscriptome data

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ARTICLE INFO

Keywords:
Nitrogen
Cyanobacteria
Planktothrix
Nutrient management
Lake Erie
Sandusky Bay

ABSTRACT

Sandusky Bay, Lake Erie, receives high nutrient loadings (nitrogen and phosphorus) from the Sandusky River, which drains an agricultural watershed. Eutrophication and cyanobacterial harmful algal blooms (cyanoHABs) persist throughout summer. *Planktothrix agardhii* is the dominant bloom-forming species and the main producer of microcystins in Sandusky Bay. Non-N_2 fixing cyanobacteria, such as *Planktothrix* and *Microcystis*, thrive on chemically reduced forms of nitrogen, such as ammonium (NH_4^+) and urea. Ammonium regeneration and potential uptake rates and total microbial community demand for NH_4^+ were quantified in Sandusky Bay. Potential NH_4^+ uptake rates in the light increased from June to August at all stations. Dark uptake rates also increased seasonally and, by the end of August, were on par with light uptake rates. Regeneration rates followed a similar pattern and were significantly higher in August than June. Ammonium uptake kinetics during a *Planktothrix*-dominated bloom in Sandusky Bay and a *Microcystis*-dominated bloom in Maumee Bay were also compared. The highest half saturation constant (K_m) in Sandusky Bay was measured in June and decreased throughout the season. In contrast, K_m values in Maumee Bay were lowest at the beginning of summer and increased in October. A significant increase in V_{max} in Sandusky Bay was observed between July and the end of August, reflective of intense competition for depleted NH_4^+. Metatranscriptome results from Sandusky Bay show a shift from cyanophycin synthetase (luxury NH_4^+ uptake; *cphA1*) expression in early summer to cyanophycinase (intracellular N mobilization; *cphB/cphA2*) expression in August, supporting the interpretation that the microbial community is nitrogen-starved in late summer. Combined, our results show that, in late summer, when nitrogen concentrations are low, cyanoHABs in Sandusky Bay rely on regenerated NH_4^+ to support growth and toxin production. Increased dark NH_4^+ uptake late in summer suggests an important heterotrophic contribution to NH_4^+ depletion in the phycosphere. Kinetic experiments in the two bays suggest a competitive advantage for *Planktothrix* over *Microcystis* in Sandusky Bay due to its higher affinity for NH_4^+ at low concentrations.

1. Introduction

Lake Erie, the shallowest and most productive of the Laurentian Great Lakes, provides key ecosystem services and supports an annual US$50 billion tourism, fisheries, and boating industry (Watson et al., 2016). However, Lake Erie has been subjected to eutrophication, habitat loss, impoundments, and introduction of invasive species. The western basin of Lake Erie is particularly susceptible to eutrophication and cyanobacterial harmful algal blooms (cyanoHABs), which have increased since the mid-1990's, threatening its ability to provide ecosystem services (Watson et al., 2016). In the 1960's and 1970's, cyanoHABs in Lake Erie consisted mostly of nitrogen (N) fixing taxa (*e.g.*, *Dolichospermum,* [*formerly Anabaena*], and *Aphanizomenon*). However, upon re-eutrophication in the 1990's, cyanoHABs shifted to mostly non-N_2 fixing taxa (Steffen et al., 2014; Watson et al., 2016; Chaffin et al., 2018). CyanoHABs in the western basin are related to increased N and phosphorus (P) loadings from the Maumee River, which carries runoff from a primarily agricultural watershed (Richards et al., 2010). In Maumee Bay, non-diazotrophic *Microcystis aeruginosa* is the dominant bloom organism, a common cyanoHAB species found globally (Havens et al., 2001; McCarthy et al., 2009; Kurmayer et al., 2015). However, blooms in Sandusky Bay, east of the western basin, are almost entirely attributed to the filamentous, non-N_2 fixing *Planktothrix agardhii* (Davis et al., 2015; Salk et al., 2018). *P. agardhii* has a wide distribution and is

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https://doi.org/10.1016/j.hal.2018.11.011
Received 10 May 2018; Received in revised form 10 August 2018; Accepted 16 November 2018
Available online 01 December 2018
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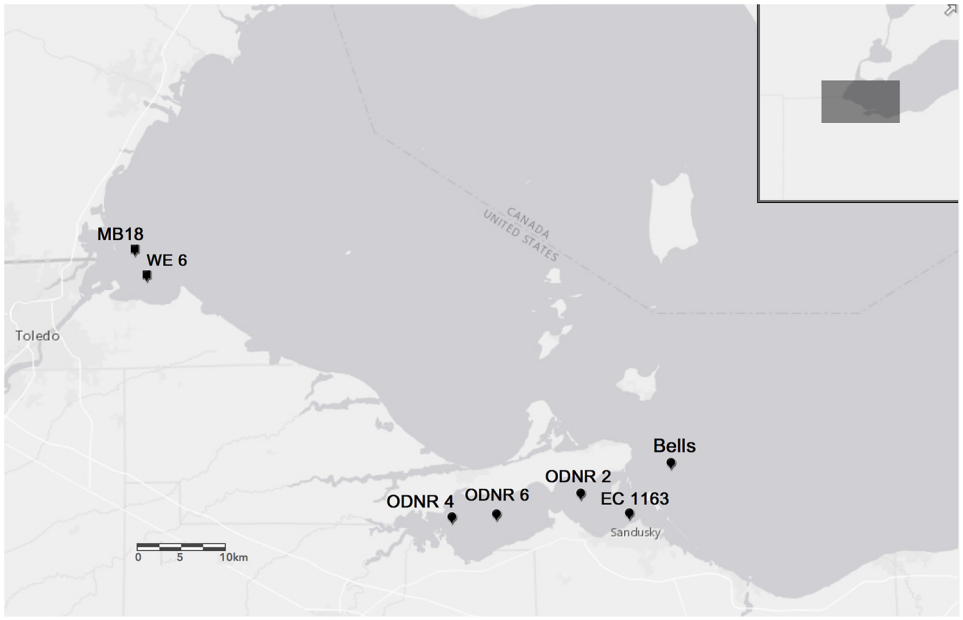

Fig. 1. Map of sampling locations in Sandusky Bay (41.46883; -82.85299) and Maumee Bay (41.71516; -83.39496). The inset shows the location of the western basin relative to the rest of Lake Erie.

ubiquitous in eutrophic lakes globally (Suda et al., 2002; Steffen et al., 2014; Kurmayer et al., 2015).

Sandusky Bay is a shallow basin, formed from a drowned river mouth (mean depth = 2.6 m; area = 162 km^2) in the southern part of Lake Erie (Fig. 1; Conroy et al., 2007). Sandusky Bay receives high N and P loadings from the Sandusky River, which also flows through primarily agricultural areas (Conroy et al., 2007; Richards et al., 2010). The residence time in Sandusky Bay can vary from 8 to 81 days (Salk et al., 2018) and is similar to the residence time in Maumee Bay and the western basin (51 days; Millie et al., 2009). Total N concentrations in the bay decrease as the summer bloom progresses, starting with high concentrations of dissolved inorganic nitrogen (DIN) in June and July (50–600 μM), followed by low (< 5 μM) to undetectable DIN concentrations in August–October, mainly due to a decrease in NO_3^- (Davis et al., 2015; Salk et al., 2018). These low N concentrations by the end of summer, and elevated, albeit variable concentrations of soluble reactive phosphorus (SRP; Davis et al., 2015; Salk et al., 2018), suggest seasonal N limitation in Sandusky Bay. Nutrient addition experiments showed that both bloom growth and microcystins (MC) production were stimulated by additions of dissolved N, but not P, and that additions of both NH_4^+ + PO_4^{3-} and urea + PO_4^{3-} yielded highest MC concentrations (Davis et al., 2015). High ambient N concentrations are required for the production of microcystins, which contain 10 N atoms per microcystin molecule (Davis et al., 2015; Gobler et al., 2016). Another study from Sandusky Bay also showed growth stimulation by NH_4^+, NO_3^-, and urea, consistent with N limitation in the system (Chaffin and Bridgeman, 2014). These results emphasize the importance of chemically reduced N species during cyanoHABs (Glibert et al., 2016).

Comprehensive phytoplankton community studies in Sandusky Bay show that *P. agardhii* is the dominant species during the bloom season and the main producer of MC (Rinta-Kanto and Wilhelm, 2006; Conroy et al., 2007; Davis et al., 2015; Steffen et al., 2015; Salk et al., 2018). *P. agardhii* may proliferate in these waters due to its tolerance to a broad temperature range and acclimation to growth at low light intensity (Oberhaus et al., 2007). The shallow depth of Sandusky Bay leads to suspended sediment particles that create turbidity and low light conditions, where *Planktothrix* thrives (Scheffer et al., 1997). Additionally, *Planktothrix* is common in lakes with low bioavailable N and low N:P (Rücker et al., 1997), conditions that prevail in Sandusky Bay in late summer. However, these low N:P conditions are often caused by the cyanoHABs (*e.g.*, Xie et al., 2003), and this pattern of DIN depletion occurring after bloom initiation has been observed in Sandusky Bay (Chaffin and Bridgeman, 2014; Davis et al., 2015; Salk et al., 2018). Once low N:P conditions are established, *P. agardhii* has a low half-saturation constant (K_m) for NH_4^+ (Zevenboom and Mur, 1981), and thus high substrate affinity, compared to other non-diazotrophic cyanobacteria, *e.g.*, *Microcystis* (Nicklisch and Kohl, 1983). This high affinity, along with high maximum uptake rates (V_{max}; Zevenboom et al., 1980), makes *Planktothrix* an excellent competitor for N substrate in low N conditions.

Non-diazotrophs, such as *Microcystis* and *Planktothrix*, are highly competitive for chemically reduced N forms, such as NH_4^+ and urea (Blomqvist et al., 1994; Glibert et al., 2016; Gobler et al., 2016). NH_4^+ transport across the cell membrane, *via* ammonia transporters (*amt* genes), and assimilation into biomass, *via* the glutamine synthetase pathway (*gln* genes), are less energy intensive than for NO_3^- (Glibert et al., 2016). During high *in situ* DIN conditions, cyanobacteria can assimilate and store N intracellularly (luxury uptake) to use when DIN is depleted. Cyanobacteria including, *Planktothrix* spp., are capable of synthesizing cyanophycin granules as an N storage polymer (Van de Waal et al., 2010) when N is bioavailable, and synthesis of cyanophycin is dependent on cyanophycin synthetase, encoded by *cphA1*. Degradation of cyanophycin is a function encoded by *cphB,* cyanophycinase, and is co-transcribed with another cyanophycinase gene, *cphA2*, in the *cphBA2* operon. Cyanophycinase mobilizes stored N when DIN in the water column is depleted.

Due to high biological demand and fast turnover rates, NH_4^+ rarely accumulates in the water column, resulting in low *in situ* concentrations. Thus, NH_4^+ dynamics and turnover rates are important components of the aquatic N cycle and productivity in eutrophic lakes affected by cyanoHABs. Regeneration of NH_4^+ contributes to internal cycling and availability of NH_4^+ for assimilation (James et al., 2011; Paerl et al., 2011; McCarthy et al., 2013). For example, rapid NH_4^+ turnover can fuel and sustain blooms, despite low *in situ* NH_4^+ concentrations (Paerl et al., 2011; McCarthy et al., 2013; Hampel et al., 2018). On the other hand, cyanobacteria must compete with other organisms for NH_4^+; for example, nitrifiers are an important link between reduced N in the water column and its subsequent removal through denitrification (An and Joye, 2001). Studies that focus solely on monitoring static nutrient concentrations can miss important aspects of nutrient and cyanoHAB dynamics. Therefore, spatio-temporal NH_4^+ cycling, rather than *in situ* NH_4^+ concentration, can provide better insights into

understanding the dominance of non-N_2 fixing cyanoHABs (Hampel et al., 2018).

Little is known about NH_4^+ uptake and regeneration and the kinetics of NH_4^+ uptake during *Planktothrix* blooms. Light availability is likely not the only factor shaping phytoplankton community structure in Sandusky Bay, since other shallow, turbid lakes are dominated by *Microcystis* (*e.g.*, Taihu Lake; Paerl et al., 2011) instead of *Planktothrix*. The ability to compete for nutrients, or substrate affinity, likely plays an important role in distinguishing between *Microcystis* blooms in western Lake Erie and *Planktothrix* blooms in Sandusky Bay. The goals of this study were to: (1) quantify NH_4^+ regeneration and potential uptake dynamics and total microbial community demand for NH_4^+ in Sandusky Bay during the summer bloom (June – August); and (2) compare the kinetics of NH_4^+ uptake during a *Planktothrix*-dominated bloom in Sandusky Bay and a *Microcystis*-dominated bloom in Maumee Bay. We hypothesized that NH_4^+ regeneration and potential uptake rates would increase through the summer as *in situ* DIN is depleted and the *Planktothrix* bloom becomes more N stressed. Based on previous literature on NH_4^+ uptake kinetics for *Microcystis* (Nicklisch and Kohl, 1983) and *Planktothrix* (Zevenboom and Mur, 1981), we hypothesized that the *Planktothrix*-dominated bloom in Sandusky Bay would have higher affinity for NH_4^+, representing a competitive advantage at low NH_4^+ concentrations, than the *Microcystis*-dominated bloom in Maumee Bay.

2. Methods

2.1. Sample collection

Water samples from Sandusky Bay were collected on five occasions during summer 2017: June 5, June 26, July 31, August 14, and August 28. Surface water (top 20 cm) for NH_4^+ dynamics experiments was collected in 3 L Nalgene bottles and stored in a dark cooler until processing. All experiments were commenced within three hours of sampling. Samples were collected from four stations: Ohio Department of Natural Resources (ODNR) 4 and 6 in the inner part of the bay, ODNR 2 in the outer bay, and Bells, located just outside the bay in Lake Erie (Fig. 1). Samples for *in situ* nutrient analyses were filtered, with a 60 mL syringe, immediately in the field using 0.2 µm Sterivex cartridge filters (Millipore) into 60 mL acid-washed polyethylene bottles, stored in the dark on ice, and processed on the same day in the lab. Physico-chemical parameters, including temperature and pH, were measured using a multi-parameter sonde (YSI model 600 QS). Due to a malfunction in the dissolved oxygen (DO) probe on the YSI, daily DO measurements, starting 28 June 2017, were generated using a Great Lakes Observing System (GLOS) buoy located near ODNR 2 in Sandusky Bay. Water column DO values from June 5 and 26 were measured with a sonde deployed in the eastern outer bay (east of EC 1163; Fig. 1). Turbidity was measured using Secchi depth as a proxy. Water for chlorophyll a (Chl a) analysis was collected in amber bottles and stored on ice until return to the lab. Biomass was collected on the same day onto 0.2 µm polycarbonate filters and stored at -20 °C until treated with 10 mL of 90% acetone for 24 h. Chl *a* samples were analyzed with a Turner Designs Fluorometer (TD-700) using manufacturer's standards (Welschmeyer, 1994). Ambient nutrient analyses included NH_4^+, NO_2^-, NO_3^-, soluble reactive phosphorus (SRP), and total phosphorus (TP) and was performed at the National Center for Water Quality Research (NCWQR) at Heidelberg University.

Water samples for kinetic experiments in Maumee Bay were collected on three occasions from site WE6 (July 17, August 14, and October 10), and once from site MB18 (August 9; Fig.1), which is across the shipping channel from, and in close proximity to, WE6 (Fig. 1). Sampling at WE6 occurred in conjunction with NOAA Great Lakes Environmental Research Laboratory (GLERL) weekly sampling, whereas sampling at MB18 was conducted with Ohio State University Stone Laboratory personnel. Surface water (top 1 m) was collected using a 5 L Niskin bottle into a 10 L polyethylene cubitainer and stored in a dark cooler for transport to the lab. Samples for nutrient analyses were immediately filtered in the field into 15 mL clear, polypropylene tubes using 0.2 µm syringe filters (Nylon; Millipore), stored on ice in a dark cooler, and then frozen at -20 °C until analysis. Nutrient analyses (NH_4^+, NO_2^-, NO_3^-, and orthophosphate (OP)) were performed using a Lachat 8500 Quikchem nutrient analyzer (Hach). Note that Sandusky Bay data, analyzed by the NCWQR at Heidelberg university, is reported as SRP, whereas the Lachat used for Maumee Bay data measures OP. Chl *a* and geochemical data (DO and temperature) from WE6 in Maumee Bay were accessed using the NOAA GLERL annual data share and are single measurements for this station.

2.2. NH_4^+ dynamics

NH_4^+ uptake and regeneration experiments followed the protocol described in Hampel et al., (2018). Briefly, 1 L of water from each station was amended with 98% $^{15}NH_4Cl$ (Isotec; final concentration added 8–32 µM based on bloom status; *i.e.*, higher concentrations during heavier blooms to prevent total substrate depletion during incubation), mixed thoroughly, and decanted into six 125 mL, clear Nalgene incubation bottles (three light incubations and three dark). Initial samples were filtered through a 0.2 µm syringe filter, 12.5 mL into 15 mL clear, polypropylene tubes (for total NH_4^+ analysis) and 12 mL with no headspace into Exetainers (for $^{15}NH_4^+$ analysis). Dark bottles were wrapped with aluminum foil and submerged with unwrapped light bottles outside, submerged in water at near-ambient light and temperature for 18 h. After the incubation period, final samples were collected as described for initial samples. Total ($^{14}N + ^{15}N$) NH_4^+ concentrations were analyzed using the Lachat nutrient analyzer. $^{15}NH_4^+$ concentrations were measured using membrane inlet mass spectrometry (MIMS; Kana et al., 1994) combined with oxidation to N_2 gas (OXMIMS; Yin et al., 2014). Samples for $^{15}NH_4^+$ analysis were treated with 200 µl of hypobromite iodine solution (oxidizes $^{15}NH_4^+$ to $^{29/30}N_2$) and immediately measured on the MIMS. $^{15}NH_4^+$ standards (from 0.1 to 100 µM) were analyzed at the beginning and the end of each sample run. $^{15}NH_4^+$ concentrations were determined using the line equation from the standard curve and total $^{15}N_2$ production ($^{29}N_2 + 2 * {}^{30}N_2$; Yin et al., 2014).

Potential NH_4^+ uptake rates and actual regeneration rates were calculated using the Blackburn/Caperon isotope dilution model (Blackburn, 1979; Caperon et al., 1979; McCarthy et al., 2013). In this model, potential uptake is calculated from the depletion of both $^{14}NH_4^+$ and $^{15}NH_4^+$ pools, and regeneration is measured from dilution of the total NH_4^+ pool by regenerated $^{14}NH_4^+$ and is considered an actual rate (Gardner et al., 2017).

2.3. Community biological ammonium demand (CBAD)

CBAD is a conceptual model of internal NH_4^+ cycling proposed by Gardner et al. (2017). CBAD is represented by the difference between measured potential NH_4^+ uptake rates and actual regeneration rates during a bloom and reflects the net microbial community demand for NH_4^+. Average values for CBAD in light and dark incubations were calculated as:

$$CBAD = ([NH_4^+]_0 - [NH_4^+]_t) / t$$

where $[NH_4^+]_0$ is total ($^{14}N + ^{15}N$) NH_4^+ concentration at the initial time of incubation, $[NH_4^+]_t$ is the total NH_4^+ concentration at the end of the incubation, and t is elapsed time in hours.

2.4. Kinetic experiment

The Michaelis–Menten model (Caperon and Meyer, 1972; Martens-Habbena et al., 2009) was used to explore the kinetics of NH_4^+ uptake during *Planktothrix* and *Microcystis* blooms and investigate the

dependence of uptake rate on substrate concentrations. This model relates the reaction rate and substrate concentration following the formula:

$$V_0 = (V_{max}\,[S]) / K_m + [S]$$

Where V_{max} is the maximum velocity of the reaction, S is the substrate concentration, and K_m is the substrate concentration at ½ V_{max}.

To investigate the competitive abilities of different cyanobacteria in different parts of Lake Erie, water from Sandusky Bay and Maumee Bay was collected as described above. Unfiltered surface water collected in the field was transported to the lab and decanted into seven 125 mL, clear Nalgene bottles. One bottle was designated as a control and received no ^{15}N additions. The remaining bottles were amended with increasing $^{15}NH_4^+$ concentrations ranging from 0.25 μM to 16 μM at 5–6 substrate levels in Sandusky Bay and 4–5 in Maumee Bay. This range of concentrations was chosen based on preliminary trials. The kinetic experiment followed the protocol for NH_4^+ dynamics described above. Bottles were incubated at *in situ* temperature and light for 5 h on June 5, June 26, July 17, July 31, August 28, and October 10, 2017. On August 14, incubation time was limited to 15 min due to rapid NH_4^+ depletion. Uptake rates were calculated as above and plotted against spike concentrations using Kaleidagraph software (version 4.5.3) to create the Michaelis–Menten curve. Additionally, the Michaelis–Menten model was run in RStudio (version 1.1.383) with the drm package (version 0.5–8) for combined regression and association models.

2.5. Metatranscriptomic analyses

Biomass (500 mL) from several dates (June 8, June 22, July 6, July 13, August 3, and August 31) during the summer 2015 bloom was collected in the field onto 0.2 μm Sterivex cartridges, stored on dry ice, and placed in the −80 °C freezer until extractions. RNA was extracted using the Mo Bio PowerWater Sterivex™ DNA Isolation Kit (now available as Qiagen DNeasy® PowerWater® Sterivex™ Kit), with the alternate RNA Isolation protocol. Extracted RNA (3–5 μg per sample) was stored at −80 °C until it was sent to HudsonAlpha Institute for Biotechnology (Huntsville, AL) for RNA sequencing, where they were treated to reduce rRNA. The reads were single-end reads of 50 base pairs. All samples were from outer Sandusky Bay site ODNR 1, except for the June 8 sample taken at outer bay station EC 1163.

The metatranscriptome reads were trimmed, underwent quality control (QC) analysis, and then were assembled using the CLC Workbench software version 9.5.3 (Qiagen). The CLC Workbench 9.5.3 program removed failed sequences that did not pass QC according to the default parameters. The remaining reads were assembled *de novo* into contigs, then mapped back to assembled contigs using the reference genomes. Aligned RNA transcripts (RNAseq) files were aligned to the following reference genomes: *Sulfurimonas denitrificans* DSM 1251, *Microcystis aeruginosa*NIES-843, *Desulfovibrio magneticus*RS-1, *Desulfovibrio desulfuricans*ND132 (Final JGI assembly), *Anabaena cylindrica*PCC 7122, *Aphanizomenon flos-aquae*NIES-81, *Klebsiella pneumoniae* 1158, and *Burkholderia pseudomallei* K96243. The reference genomes were concatenated into a single reference library. An annotated genome of *Planktothrix agardhii* from Sandusky Bay was obtained from Dr. Greg Dick at the University of Michigan. These were selected to represent the different cyanobacteria and N fixers present in metagenomic datasets obtained previously from Sandusky Bay.

The reference genomes were confirmed for the presence of *cphA, cphB, amt, glnA,* and *nifH* genes by implementing a gene search on JGI IMG/M using the aforementioned sequences plus *Planktothrix agardhii* NIVA CYA 126/8. The BLAST tool of the CLC Workbench program was used to search for the gene sequence from each species of the reference genomes. Hits were obtained with outputs of % identity, Greatest Hit Lengths, and E values for assessing relatedness of the genes. Each of these sequences were then reconfirmed using the BLASTn and/or BLASTx function using the NCBI database with the "dissimilar sequence" setting. The RNAseq files for each date and site were filtered to find the corresponding Reads per Kilobase transcript per Million mapped reads (RPKM) value for the gene in question.

2.6. Statistical analysis

All statistical analyses were performed using RStudio software (version 1.1.383). Environmental data were checked for normality using the Shapiro–Wilk normality test. Temperature and TP were the only normally distributed variables. To investigate potential environmental drivers of NH_4^+ dynamics, a multivariate correlation analysis was performed using the Spearman correlation method for nonparametric data. A p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Environmental variables in Sandusky and Maumee Bays

Water temperature in Sandusky Bay ranged from 20.4 °C to 24.5 °C (Table 1). DO concentrations ranged from 9.18 to 9.71 mg L^{-1} between June and August 14 and decreased at the end of August (8.67 mg L^{-1}). Chl *a* concentrations showed seasonal variability, with greatest values at the end of June (mean = 75.2 ± 27.7 μg L^{-1}) and in July (mean = 122 ± 74.5 μg L^{-1}), and lower concentrations in August (mean = 44.0 ± 21.4 μg L^{-1}; $p < 0.05$). Chl *a* concentrations also varied spatially, with the Bells station in Lake Erie ranging from 5.80 to 44.8 μg L^{-1}, significantly lower than the ODNR stations (31.3–172 μg L^{-1}; $p < 0.05$). Lowest Chl *a* concentrations at Bells corresponded with greatest Secchi depths at this station (80–132 cm). At ODNR stations, within Sandusky Bay, Secchi depths were 32–43 cm throughout the summer (not measured in July).

NH_4^+ concentrations in Sandusky Bay showed slight variation between June and August 14 (mean = 2.63 ± 0.49 μM; Table 1) but decreased significantly by August 28 (mean = 1.02 ± 0.32 μM; $p < 0.001$). NO_2^- concentrations were below the detection limit at all times except July 31 at all stations and August 14 at Bells (Table 1). NO_3^- concentrations gradually decreased from 62.0 to 251 μM at the beginning of June to 0–1.43 μM in August. SRP concentrations were lowest in June (mean = 0.20 ± 0.09 μM) and greater in August (0.50 ± 0.20 μM; Table 1).

Water temperature in Maumee Bay decreased from 23.9 °C – 25.1 °C in August to 20.7 °C in October (Table 2). DO peaked in mid-August (9.59 mg L^{-1}) and was lower in July (5.87 mg L^{-1}) and October (5.72 mg L^{-1}). Chl *a* increased from June (3.5 μg L^{-1}) to August (532 μg L^{-1}) and decreased to 40.9 μg L^{-1} in October. Secchi depth was 50 cm in October and 20 cm in August and June. Ambient NH_4^+ concentrations in Maumee Bay were highest in July (6.29 ± 0.03 μM) and decreased to undetectable by October. NO_2- concentrations were highest in July (5.13 ± 0.02 μM) and decreased to 0.02 ± 0.001 μM in October. A similar pattern was observed for NO_3- concentrations, with highest values in July (400 ± 0.9 μM) and lowest in October (0.40 ± 0.001 μM). OP was also highest in July (4.32 ± 0.04 μM) and decreased to 0.17 ± 0.01 μM in October.

3.2. Potential NH_4^+ uptake rates in Sandusky Bay

Potential NH_4^+ uptake rates in light incubations ranged from 0.06 to 3.12 μmol L^{-1} h^{-1} (Fig. 2A). Lower rates were consistently observed at Bells (mean = 0.16 ± 0.01 μmol L^{-1} h^{-1}) *versus* ODNR stations (mean = 1.78 ± 0.18 μmol L^{-1} h^{-1}; $p < 0.005$). Light uptake rates at ODNR 4, 6, and 2 were not different from each other ($p > 0.05$). At all stations, potential uptake rates in light incubations increased through the summer bloom, with lowest rates in June (mean = 0.53 ± 0.08 μmol L^{-1} h^{-1}), higher rates in July (mean = 1.00 ± 0.07 μmol L^{-1} h^{-1}), and highest rates in August (mean = 1.99 ± 0.20 μmol

Table 1
Environmental parameters and nutrient concentrations in Sandusky Bay. DO values from June were measured with a sonde deployed in the eastern outer bay (east of EC 1163). Nutrient concentrations were measured in triplicate within ± 10% error margin.

Date	Station	Temp (°C)	Dissolved Oxygen (mg L^{-1})	Chl *a* (μg L^{-1})	NH_4^+ (μM)	NO_2^- (μM)	NO_3^- (μM)	SRP (μM)	TP (μM)	Secchi (cm)
June 5	ODNR 4	22.2		44.2	2.57	BDL**	243	0.28	3.44	38
	ODNR 6	21.9		58.3	2.64	BDL	251	0.28	3.10	48
	ODNR 2	21.7	9.18†	48.1	2.43	BDL	101	0.13	3.03	38
	Bells	ND*		5.8	2.57	BDL	62.1	0.13	2.50	ND
June 26	ODNR 4	21.5		107	2.57	BDL	33.6	0.25	9.69	32
	ODNR 6	21.7		88.8	2.71	BDL	96.4	0.28	6.47	40
	ODNR 2	21.5	9.29†	60.7	2.57	BDL	35.7	0.16	4.78	32
	Bells	20.4		44.8	2.93	BDL	41.4	0.19	1.38	106
July 31	ODNR 4	23.5		167	3.36	2.14	58.6	0.50	4.91	ND
	ODNR 6	23.6		172	2.14	4.29	77.1	0.25	4.09	ND
	ODNR 2	24.2	9.71	136	2.57	2.86	69.3	0.31	3.47	ND
	Bells	24.5		13.1	1.29	0.71	15.7	0.28	1.00	ND
August 14	ODNR 4	23.6		61.0	3.64	BDL	BDL	0.59	6.22	34
	ODNR 6	23.5		59.4	2.71	BDL	BDL	0.47	6.22	44
	ODNR 2	23.7	9.57	57.9	1.71	BDL	BDL	0.43	4.25	54
	Bells	24.4		11.2	2.79	0.71	1.43	0.38	1.16	80
August 28	ODNR 4	22.3		56.7	0.71	BDL	BDL	0.88	7.81	30
	ODNR 6	22.2		59.5	1.00	BDL	BDL	0.31	5.56	34
	ODNR 2	22.7	8.67	31.3	1.36	BDL	1.43	0.31	4.78	38
	Bells	23.1		14.9	0.86	BDL	0.71	0.31	1.53	132

* ND – not determined.
** BDL – below the detection limit.
† Measured east of EC 1163.

$L^{-1} h^{-1}$). However, differences were only significant between June and August ($p < 0.05$). Light NH_4^+ uptake rates were positively correlated to ambient SRP and TP concentrations and Chl *a* (Spearman $p < 0.005$) and negatively correlated to ambient NO_3- concentrations and Secchi depth (Spearman $p < 0.05$; Table 3).

Potential NH_4^+ uptake rates in dark incubations ranged from 0.02 to 3.00 μmol $L^{-1} h^{-1}$ (Fig. 2B). Lowest dark uptake rates were observed at Bells (mean = 0.09 ± 0.01 μmol $L^{-1} h^{-1}$); however, the statistical significance of this difference from the ODNR stations was marginal ($p = 0.08$). The three ODNR stations did not exhibit significant differences in dark uptake (mean = 1.22 ± 0.04 μmol $L^{-1} h^{-1}$; $p > 0.5$). Dark rates also increased throughout the summer, with lowest rates in June (mean = 0.09 ± 0.01 μmol $L^{-1} h^{-1}$), higher rates in July (mean = 0.22 ± 0.01 μmol $L^{-1} h^{-1}$), and highest rates in August (mean = 1.72 ± 0.06 μmol $L^{-1} h^{-1}$). Dark rates in August were statistically different from those in June and July ($p < 0.05$). Dark uptake rates were positively correlated to SRP and TP concentrations (Spearman $p < 0.05$) and negatively correlated to NO_3- concentration (Spearman $p < 0.05$; Table 3). Light and dark NH_4^+ uptake rates were statistically different from each other only in July ($p = 0.05$), and neither were correlated to ambient NH_4^+ concentrations.

3.3. Actual NH_4^+ regeneration rates in Sandusky Bay

NH_4^+ regeneration rates in light and dark incubations were not statistically different, so they were averaged together (Fig. 2C). Averaged NH_4^+ regeneration rates ranged from 0 to 1.54 μmol $L^{-1} h^{-1}$. Regeneration rates at Bells (mean = 0.05 ± 0.01 μmol $L^{-1} h^{-1}$) were an order of magnitude lower than at ONDR stations (mean = 0.75 ± 0.10 μmol $L^{-1} h^{-1}$; $p < 0.05$). NH_4^+ regeneration rates increased from June (mean = 0.23 ± 0.04 μmol $L^{-1} h^{-1}$) to July (mean = 0.34 ± 0.04 μmol $L^{-1} h^{-1}$) and August (0.87 ± 0.12 μmol $L^{-1} h^{-1}$), and a statistical difference was observed between June and August ($p = 0.05$). Regeneration rates were positively correlated to TP and Chl *a* concentrations (Spearman $p < 0.05$) and negatively correlated to NO_3^- concentration and Secchi depth (Spearman $p < 0.05$).

3.4. CBAD

CBAD followed a similar pattern as NH_4^+ uptake rates (Fig. 3A), increasing from June (mean = 0.23 ± 0.01 μmol $L^{-1} h^{-1}$) to August (mean = 1.07 ± 0.03 μmol $L^{-1} h^{-1}$) across all stations. Light CBAD values during the two sampling trips in August were twice as high as the average of the other months (mean = 0.33 ± 0.01 μmol $L^{-1} h^{-1}$).

Dark CBAD also increased over the summer (Fig. 3B), starting with negative values (reflecting net NH_4^+ regeneration) in June. By the end of August, dark CBAD (1.05 ± 0.02 μmol $L^{-1} h^{-1}$) was as high as light CBAD (1.12 ± 0.03 μmol $L^{-1} h^{-1}$). Light and dark CBAD were lowest at Bells (0.10 ± 0.01 and 0.03 ± 0.01 μmol $L^{-1} h^{-1}$, respectively).

3.5. NH_4^+ uptake kinetics

K_m values in Sandusky Bay were highest in June (K_m = 8.7 μM; Fig. 4A) and ranged from 1.4 to 1.8 μM in subsequent experiments. However, V_{max} increased from July to the end of August (1.52 to 27.1 μmol $L^{-1} h^{-1}$, respectively).

Table 2
Environmental parameters and nutrient concentrations in Maumee Bay. Nutrient concentrations were measured in triplicate within ± 10% error margin.

Date	Station	Temp (°C)	Dissolved Oxygen (mg L^{-1})	Chl *a* (μg L^{-1})	NH_4^+ (μM)	NO_2^- (μM)	NO_3^- (μM)	OP (μM)	Secchi (cm)
July 17	WE6	24.6	5.21	3.50	6.29	5.13	400	4.32	20
August 9	MB18	23.9	9.18	NM	0.99	1.51	96.5	0.16	NM
August 14	WE6	25.1	10.7	532	0.33	1.46	122	0.53	20
October 10	WE6	20.7	6.00	40.9	BDL	0.02	0.40	0.17	50

*ND – not determined.

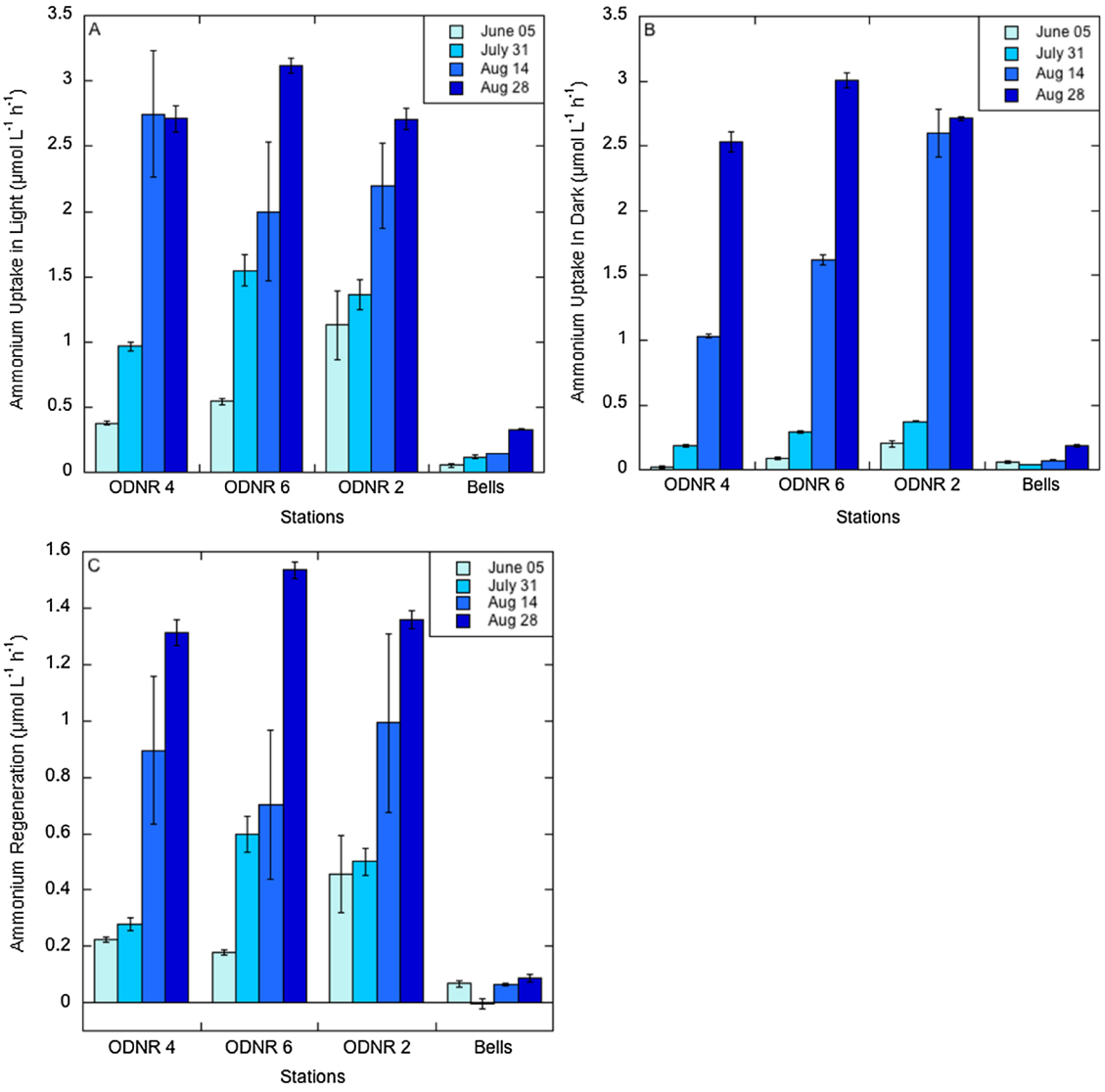

Fig. 2. Ammonium regeneration and potential uptake rates in Sandusky Bay in 2017. Potential light uptake rates (A), potential dark uptake rates (B), and averaged regeneration rates (C). Values are averaged (three replicates) with error bars showing ± one standard error.

Table 3
Spearman correlation for nonparametric data in Sandusky Bay.

		NH_4^+	NO_2^-	NO_3^-	SRP	TP	Temp	DO	Chl *a*	Secchi
Uptake Light	Spearman's Rho	−0.162	−0.252	**−0.561**	**0.524**	**0.874**	−0.2	−0.4	**0.597**	**−0.812**
	p value	0.5	0.3	**0.02**	**0.0**	**< 0.001**	0.5	0.2	**0.01**	**0.002**
Uptake Dark	Spearman's Rho	−0.283	−0.232	**−0.64**	**0.493**	**0.766**	−0.092	−0.132	0.464	−0.493
	p value	0.3	0.4	**0.007**	**0.05**	**0.001**	0.7	0.6	0.07	0.1
Regeneration	Spearman's Rho	−0.256	−0.298	**−0.521**	0.438	**0.857**	−0.228	−0.271	**0.517**	**−0.735**
	p value	0.3	0.3	**0.04**	0.09	**< 0.001**	0.4	0.6	**0.04**	**0.009**

K_m values in Maumee Bay showed the opposite pattern than observed in Sandusky Bay (Fig. 4B). K_m values increased from July (K_m = 0.32 µM) to the highest value in October (K_m = 8.52 µM). However, V_{max} in Maumee Bay remained in a tight range from 0.20 to 0.53 µmol L^{-1} h^{-1} for all experiments.

3.6. Metatranscriptomic analysis of N metabolism

During 2015, a series of Sandusky Bay metatranscriptomes obtained from June 8 to August 31 examined *cphA, cphB, amt, glnA,* and *nifH* gene expression in *P. agardhii* during the onset of N limitation (Fig. 5). *Planktothrix* expressed two distinct *cphA* genes, but at different times in the season corresponding to N availability. *Planktothrix* has the *cphBA* operon and an independent *cphA* (Forchhammer and Björn, 2016). The independent *cphA* (*cphA1*) was expressed when N was replete. *cphA2* was expressed, along with *cphB*, when N levels were low. No *Microcystis cphA* expressions were detected.

In early summer, when NH_4^+ and NO_3^- concentrations were high (Fig. 5A, B) due to riverine discharge following spring rains (Salk et al., 2018), *cphA1* was highly transcribed, suggesting luxury N storage *via* cyanophycin synthesis (Gupta and Carr, 1981; Allen, 1984; Forchhammer and Björn, 2016). When NH_4^+ and NO_3^- were depleted in late summer, no *cphA1* expression was detected, and *cphBA2* operon transcription was activated (Fig. 5A), suggesting cyanophycin

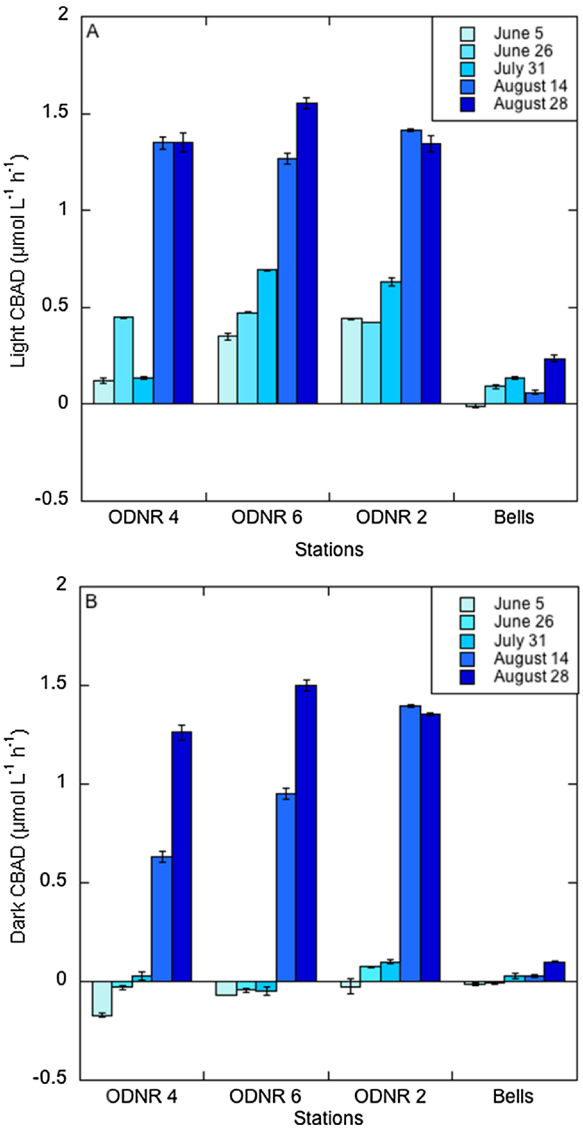

Fig. 3. Community Biological Ammonium Demand (CBAD) in Sandusky Bay in light (A) and dark (B). Values are averaged (three replicates) with error bars showing ± one standard error.

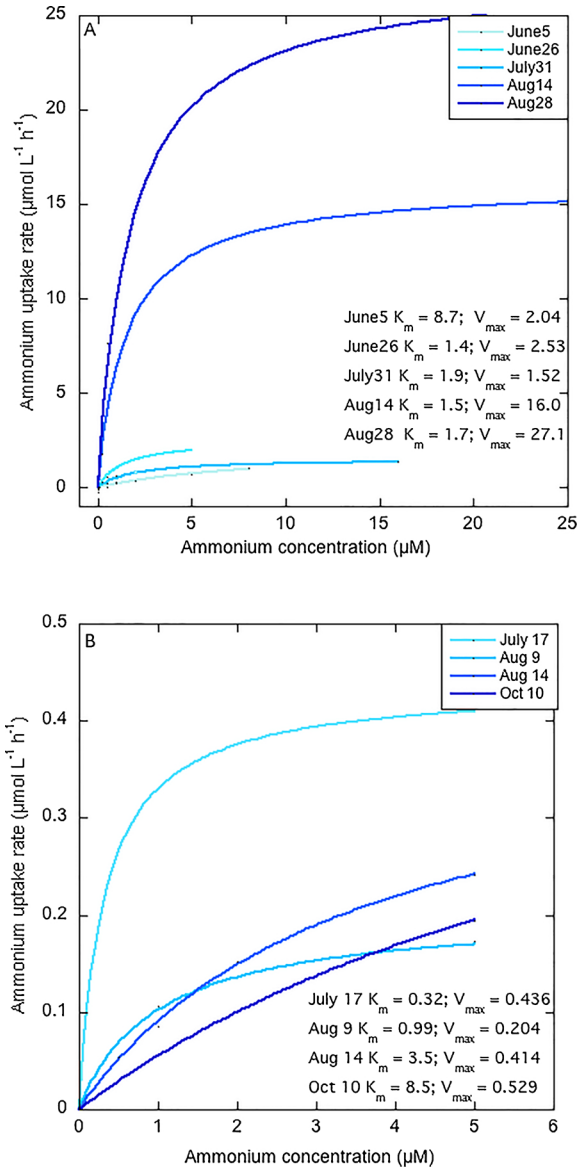

Fig. 4. Michaelis-Menten NH_4^+ uptake kinetics in Sandusky Bay (A) and Maumee Bay (B) in 2017. The Michaelis–Menten model fits for Sandusky Bay were: June 5 (r = 0.98), June 26 (r = 0.75), July 31 (r = 0.92), Aug 14 (r = 0.73), Aug 28 (r = 0.97), and Maumee Bay model fits were: July 17 (r = 0.38), Aug 9 (r = 0.98), Aug 14 (r = 0.96), Oct 10 (r = 0.98).

degradation (Richter et al., 1999; Ponndorf et al., 2017) as an adaptation to N limitation. Reflecting the increased competition for N was the expression of *glnA* in late summer, encoding glutamine synthetase, the high affinity assimilation pathway for NH_4^+ (Reyes et al., 1997). Genes for NH_4^+ transporters *amt1* and *amt3* were transcribed constitutively throughout the summer (Fig. 5B).

Earlier work documented the presence of a minor community of N-fixing cyanobacteria during the *Planktothrix*-dominated bloom (Salk et al., 2018). 16S rRNA reads assigned to *Nostocales* (predominantly *Aphanizomenon* spp. and *Dolichospermum* spp.) reached up to 25% of total cyanobacterial reads on a few occasions in 2015 before complete N depletion (Fig. 5B), but *Nostocales* reads were usually very low.

4. Discussion

4.1. Potential NH_4^+ uptake and CBAD

Nutrient concentrations and NH_4^+ dynamics exhibited expected patterns during the 2017 *Planktothrix* bloom in Sandusky Bay. After bloom initiation, DIN concentrations in the bay decreased to low or undetectable levels (Table 1), with NO_3^- often below detection, and detectable but low NH_4^+ concentrations. This pattern is consistent with previous work in Sandusky Bay (Chaffin et al., 2018; Salk et al., 2018) and suggests a high demand and competition for N in late summer. NH_4^+ uptake rates in light incubations increased throughout the summer at all stations, with late August rates approximately four times higher than those in June in the bay (ODNR 2, 4, and 6) and five times greater outside of the bay at Bells (Fig. 2A). As expected, these light uptake rates were correlated positively with Chl *a* ($p < 0.005$; Table 3), suggesting an increase in photoautotrophic activity. At Bells, where Chl *a* was consistently below bloom thresholds (< 20 µg L^{-1}; Xu et al., 2015), NH_4^+ uptake rates (and CBAD) were predictably lower than those at sites within Sandusky Bay. The NH_4^+ uptake rates reported in this study for Sandusky Bay are consistent with those reported in other freshwater, eutrophic, cyanoHAB-impacted lakes (Gu et al., 1997; Présing et al., 2001; James et al., 2011; McCarthy et al., 2013; Hampel et al., 2018).

Light uptake rates reported in this study are an order of magnitude

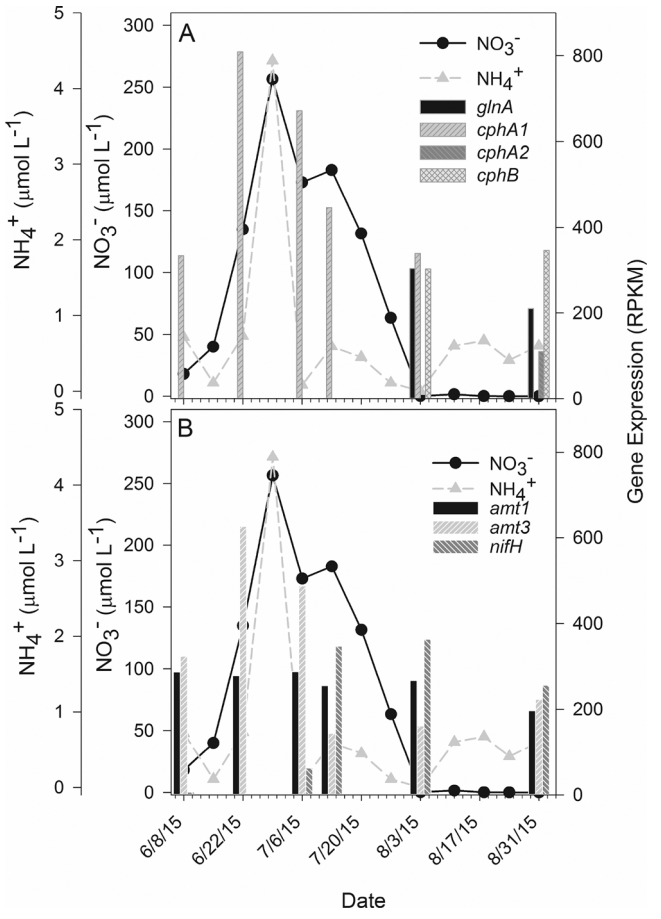

Fig. 5. Metatranscriptome data and ambient NH_4^+ and NO_3^- concentrations in Sandusky Bay in 2015. *cphA1* and *cphA2* are *Planktothrix agardhii* paralogs encoding cyanophycin synthetase, *cphB* encodes cyanophycinase, and *glnA* and *amt* encode glutamine synthetase and NH_4^+ transporters. Relative transcript abundance is presented as reads per kilobase of transcript per million mapped reads (RPKM).

greater that those reported recently in Sandusky Bay (Salk et al., 2018). In comparison with similarly eutrophic systems, those rates were exceptionally low, indeed more comparable to those measured in oligotrophic lakes (*e.g.*, Suttle and Harrison, 1988), including Lake Michigan in late winter and spring (Gardner et al., 2004). Stable isotope additions used in Salk et al., (2018) were tracer-level (*i.e.*, < 10% of the ambient DIN pool) and may have underestimated NH_4^+ cycling rates due to complete substrate depletion before incubations were ended (Paasche, 1988). Substrate depletion is especially problematic in highly productive systems, like Sandusky Bay; thus, we applied saturating-level stable isotope amendments, which are better suited for highly dynamic, eutrophic systems with high cyanobacterial biomass. Saturating additions of substrate can alter steady-state conditions (Glibert, 1988); therefore, NH_4^+ uptake rates reported in this study are qualified as potential rates, but results from saturating- and tracer-level isotope amendments tend to converge in eutrophic systems with high ambient NH_4^+ concentrations (Glibert, 1988).

Dark NH_4^+ uptake rates also increased with time at the bay stations and, to a lesser extent, at Bells (Fig. 2B). By late August, dark NH_4^+ uptake rates were not distinguishable from light uptake rates. Dark uptake rates in Sandusky Bay were higher than those observed in other eutrophic lakes affected by cyanoHABs (James et al., 2011; McCarthy et al., 2013; Hampel et al., 2018), suggesting an important role for heterotrophic organisms. Some photoautotrophs can assimilate NH_4^+ in the dark under N limiting conditions (*e.g.*, Cochlan et al., 1991); however, our saturating-level $^{15}NH_4^+$ additions likely minimized this effect. Dark uptake rates were not correlated with Chl *a* concentration (Table 3); thus, when NH_4^+ is scarce and competition for NH_4^+ is extreme in late summer, heterotrophic partnerships with cyanobacteria in the phycosphere may become important (Steffen et al., 2012).

The phycosphere concept was introduced by Bell and Mitchell, (1972) and is analogous to the rhizosphere concept in soils. In mixed microbial assemblages, heterotrophic bacteria can simultaneously regenerate and assimilate NH_4^+ (Tupas and Koike, 1991). These bidirectional interactions have been studied in diatoms, dinoflagellates, and other cyanobacteria (Amin et al., 2015; Lupette et al., 2016), yet little is known about phycosphere interactions during cyanobacterial blooms. Phycosphere interactions might play a key role in dynamic, eutrophic ecosystems, where competition for nutrients is high, and microbial interactions in the water column are complex. For example, NH_4^+ uptake by heterotrophic bacteria has been previously studied, mostly in marine environments (Kirchman et al., 1990; Tupas and Koike, 1991). Heterotrophic uptake of NH_4^+ in the light has been shown to increase with decreasing ambient concentrations (Kirchman et al., 1990), suggesting that heterotrophic bacteria can outcompete phytoplankton at low NH_4^+ concentrations. Our NH_4^+ cycling patterns support these findings and suggest that Sandusky Bay exhibits similarly complex microbial interactions between cyanobacteria and heterotrophic partners.

The CBAD model represents NH_4^+ dynamics and microbial productivity in N depleted systems (Gardner et al., 2017), and thus is a useful metric to investigate NH_4^+ cycling in Sandusky Bay. CBAD reflects the demand of the entire microbial community, including (light CBAD) and excluding photoautotrophs (dark CBAD; Gardner et al., 2017), assuming that photoautotrophs were not active in dark incubations. Within the bay, light CBAD followed the pattern of light uptake rates, with the largest increase observed between July 31 and August 14. Dark CBAD was negative, reflecting net NH_4^+ regeneration by the microbial community, or low in June and July (Fig. 3), indicating that demand for NH_4^+ in the dark was largely met by the supply from regeneration (Gardner et al., 2017). However, dark CBAD was not distinguishable from light CBAD in August, concomitant with decreased chlorophyll, suggesting that the increased dark CBAD reflects increased demand by non-photoautotrophs.

4.2. NH_4^+ regeneration

NH_4^+ regeneration rates at ODNR 6, 4, and 2 followed the same general pattern as uptake rates, with lowest values in June and highest in August. At these stations, regeneration rates at the end of August were almost twice as high as those in June, suggesting that N depletion by the bloom caused photoautotrophs to rely on regenerated NH_4^+ from increased heterotrophic activity and bloom biomass remineralization to support growth. While regeneration can supply substantial amount of NH_4^+, high biomass creates a great demand for N in August. The proportion of uptake supported by regeneration increased throughout the summer (Fig. 2C). In outer Sandusky Bay (ODNR 2), regeneration could support 36–40% of potential light NH_4^+ uptake. This proportion increased to 50% by the end of August, a pattern that is magnified in potential importance considering the large increase in uptake rates from June to August. Increasing dependence on regeneration corresponded with low ambient N concentrations in the bay, further highlighting the important role of recycled NH_4^+ in supporting cyanoHAB growth and bloom maintenance. Other cyanoHAB-impacted lakes exhibit similar patterns of dependence on NH_4^+ regenerated in the water column, including Lake Taihu (Paerl et al., 2011; Hampel et al., 2018), Lake Balaton (Présing et al., 2001), Lake Biwa (Haga et al., 1995; Takahashi et al., 1995), and Missisquoi Bay, Lake Champlain (McCarthy et al., 2013).

To compare regenerated NH_4^+ rates in the water column to external N loading, we extrapolated average NH_4^+ regeneration rates from ODNR 6, 4, and 2 to the whole-bay volume (0.423 km^3; Conroy et al., 2007). Daily Sandusky River flow data and total N (TN) and total

Kjeldahl N (TKN; TN = TKN + NO_3^- + NO_2^-) concentrations from 2017 were obtained from the NCWQR (https://ncwqr.org) and used to calculate daily and annual external N loading. Annual TN loading from the Sandusky River (October 2016 – September 2017; the NCWQR database was not updated beyond September 2017 as of manuscript preparation) introduced 8.58×10^3 metric tons of N into the bay during this 12 month period. Average summer regeneration from our incubations (June–August 2017) recycled 6.6×10^3 metric tons of N as NH_4^+. In just the three summer months evaluated, regeneration in the water column provided bioavailable N for primary production at the level equivalent to ~77 ± 7% of the annual N load.

When extrapolated to the whole bay volume, daily NH_4^+ regeneration exceeded daily TN loading at all sampling events (Table 4). During the week of June 5, regeneration contributed 2–5 times more N than the Sandusky River. During the week of July 31, regeneration provided 25–53 times more N than the river and, by the end of August, over 1000 times more N than the river (Table 4). While the contribution of regeneration increased throughout the summer, TN and TKN loading from the river decreased along with discharge. However, the proportion of TKN to TN in Sandusky River loading increased from 13.2% at the beginning of June to 91.9% by the end of August (Table 4), highlighting the importance of considering N forms and potential bioavailability in external loading.

This exercise exemplifies the critical role of internally recycled NH_4^+ during summer in sustaining the *Planktothrix* bloom, especially when ambient N was depleted. The large mass of internally recycled NH_4^+, driven by high external N loads from the watershed, is critical information for resource managers and regulators, who often base management decisions on ecosystem models that do not sufficiently consider the effects of internal N dynamics on eutrophication issues. Monitoring nutrient concentrations in eutrophic systems, while valuable, does not provide a sufficient characterization of these nutrient dynamics. High microbial demand and turnover rates can cause highly bioavailable nutrients, such as NH_4^+, to be undetectable or measured at low concentrations, even though their recycling rates are largely supporting system productivity at critical times (McCarthy et al., 2013).

4.3. NH_4^+ uptake kinetics

Kinetic NH_4^+ uptake experiments in Sandusky and Maumee Bays exhibited opposite patterns, suggesting that these microbial communities were distinctive (Fig. 4). K_m is often used to represent the affinity of a microbe for a substrate. Microbes with a low K_m have a competitive advantage at low nutrient concentrations and are excellent scavengers (Martens-Habbena et al., 2009). However, microbes with a high K_m thrive at high substrate concentrations and can assimilate more substrate before reaching saturation. With different K_m values, microbes can fill different niches in the environment to maximize their competitive abilities.

In Sandusky Bay (*Planktothrix*-dominated community; Davis et al., 2015; Salk et al., 2018), the highest K_m was observed in June, and it then decreased and stabilized throughout July and August. While K_m remained relatively constant in July and August, we observed a dramatic, significant increase in V_{max} from the end of July (V_{max} = 1.52 μmol L^{-1} h^{-1}) through August (V_{max} = 27.1 μmol L^{-1} h^{-1}), which reflects strong competition for depleted NH_4^+. The increase in V_{max} corresponded to significant increases in dark NH_4^+ uptake rates between the end of July and through August (Fig. 2). When NH_4^+ availability in the water column is low, dead and dying cells may be rapidly remineralized in the phycosphere, which may help explain increased heterotrophic activity (*e.g.*, Gardner and Lee, 1975). The constant and relatively low K_m of the late summer, *Planktothrix*–dominated community illustrates the strong affinity for NH_4^+ during N limited conditions. K_m values reported for NH_4^+ in *Planktothrix* are lacking both in culture and natural environments. One study investigated NH_4^+ uptake kinetics in chemostats under NO_3- limitation and reported K_m values of 8 ± 3 μM (Zevenboom and Mur, 1981), which are comparable to our values from June, but higher than those from late summer.

In contrast, K_m values in Maumee Bay increased from July (0.32 μM) to August (3.53 μM) and October (8.52 μM). This pattern may reflect the rapid increase in *Microcystis*-dominated cyanoHABs in Maumee Bay commonly observed in August and lasting into October (Steffen et al., 2014). Unlike Sandusky Bay, V_{max} in Maumee Bay was consistent (0.20–0.53 μmol L^{-1} h^{-1}), perhaps suggesting that competition for NH_4^+ in Maumee Bay was less intense than in Sandusky Bay. The Maumee River watershed (21,540 km^2) is 4.5 times larger than the Sandusky River watershed (4727 km^2), and, accordingly, the Maumee River supplied 66.8 metric tons of TN to western Lake Erie in August 2017, while the Sandusky River supplied 13.2 metric tons of TN to Sandusky Bay (NCWQR). DIN concentrations in 2017 were very low in Sandusky Bay from August through late summer (Table 1), but DIN concentrations were still high (> 100 μM, mostly comprised of NO_3-) in Maumee Bay in August (Table 2). Thus, while DIN in Sandusky Bay was scarce, *Microcystis* was not as substrate limited, perhaps affecting measured K_m and V_{max} values. Additionally, light and dark NH_4^+ uptake (0.125 and 0.058 μmol L^{-1} h^{-1}, respectively) and regeneration rates (0.162 μmol L^{-1} h^{-1}) at MB18 in August were 10–20 times lower than those in Sandusky Bay (Hampel et al., unpublished data). These results support the hypothesis that substrate competition was not as extreme in Maumee Bay as in Sandusky Bay.

The reported range of *Microcystis* K_m values is broad, including values up to 37 μM in culture (Nicklisch and Kohl, 1983) and 113 μM in hypereutrophic Lake Taihu (Yang et al., 2017). Thus, *Microcystis* can assimilate substantial amounts of NH_4^+ before becoming saturated. Overall, the results of this kinetic comparison suggest that, during the peak of a summer bloom when NH_4^+ was depleted, *Planktothrix* had a competitive advantage in its high affinity for NH_4^+, while N conditions in larger Maumee Bay allowed for less competition for substrate in the *Microcystis*-dominated community.

Table 4
Comparison of Sandusky River N loading (weekly ranges and weekly averages; mT = metric ton) to NH_4^+ regeneration (station range and average for ODNR 2, 4, 6) in the summer of 2017.

Sampling Date	TN $load_w$ (mT d^{-1})	TN $load_a$ (mT d^{-1})	TKN $load_w$ (mT d^{-1})	TKN $load_a$ (mT d^{-1})	TKN/TN_a	Reg. NH_{4d}^+ (mT)	Reg. NH_{4s}^+ (mT)	$Reg./TN_w$	$Reg./TKN_w$
June 5th	7.8–55.6	21.1	0.3–2.46	1.23	0.132	25.3–64.9	40.7	1.9	33.0
July 31st	0.9–3.1	1.73	0.29–1.05	0.54	0.318	39.7–84.9	65.2	37.7	121
August 14th	0.17–0.40	0.257	0.16–0.23	0.194	0.840	99.8–141	123	478	633
August 28th	0.11–0.19	0.167	0.11–0.17	0.145	0.919	186–218	199	1190	1375

w –weekly range.
a – weekly average.
d – station range for sampling on date.
s – station average for sampling on date.

4.4. Metatranscriptome

The transcriptional data in this paper address gene expression for N assimilation and storage functions within an active bloom. The data differ from what is observed in pure cultures of model cyanobacteria. Studies with *Dolichospermum (Anabaena)* and *Synechocystis* reveal cyanophycin synthesis during decreases in N, and amendment of N-starved cyanobacteria with exogenous NH_4^+ resulted in accumulation of cyanophycin (Mackerras et al., 1990). Cyanophycin is also responsive to shifts in light and nutrients (Allen, 1984; Van de Waal et al., 2010). It is unclear how these other factors may influence *cphA1* and *cphBA2* expression, but the pattern observed in Sandusky Bay suggests that cyanophycin synthesis and degradation is a strategy for *Planktothrix* success in a system prone to strong shifts in N availability. The expression of *glnA*, under the control of *ntcA*, a transcriptional activator and sensor of intracellular C:N ratios (Zhao et al., 2010), is also consistent with the observed declines in N concentrations. The *amt* expression is present across the sampling season and consistent with N supply from regeneration.

Patterns of N depletion in the summer and sustained *Planktothrix* blooms are well-documented in Sandusky Bay (Davis et al., 2015; Chaffin et al., 2018; Salk et al., 2018). The community composition in the present study resembled that of all prior years sampled: *Planktothrix*-dominated, with a minor fraction (5–20% of 16S rRNA reads) of N fixing cyanobacteria (Salk et al., 2018). While the field study described here was completed in 2017, metatranscriptomic data from 2015 can help elucidate genetic mechanisms for physiological processes underlying *Planktothrix* success in an N limited system. Early in the summer, when ambient bioavailable N concentrations were greater, genes for synthesis of the N-rich compound cyanophycin were transcribed, suggesting luxury uptake and N storage, as demonstrated in laboratory studies (Van de Waal et al., 2010). As ambient N was depleted late in the summer, the cyanophycinase gene was transcribed, mobilizing stored N from an intracellular pool to help meet metabolic N requirements. Concurrently, *glnA,* transcribed following N depletion, and constitutive *amt* transcription reveal active mechanisms to acquire extracellular NH_4^+. Together, these mechanisms of luxury uptake during N replete conditions, and high affinity NH_4^+ transport throughout the bloom, contribute to *Planktothrix* dominance as N is depleted in summer.

Despite the dominance of *Planktothrix* in Sandusky Bay for most of the bloom season, both cyanobacterial N-fixation (Salk et al., 2018) and *nif* transcription were detected as N concentrations decreased, supporting the interpretation from NH_4^+ dynamics and transcriptomic results that the cyanobacterial community evolved to N limitation over the course of the bloom season.

5. Conclusions

The results presented in this study highlight the dynamic nature of eutrophic Sandusky Bay during the *Planktothrix* bloom. Specifically, we emphasize the importance of internal NH_4^+ regeneration in sustaining summer non-N_2 fixing CyanoHABs, and likely influencing their toxicity as well (Davis et al., 2015). Internal NH_4^+ cycling and rapid NH_4^+ turnover rates should be considered in ecosystem models used to inform nutrient management strategies, which should incorporate dual nutrient management (N and P) efforts to prevent and mitigate non-N_2 fixing cyanoHABs in eutrophic lakes. Monitoring NH_4^+ turnover rates, rather than focusing solely on ambient nutrient monitoring, can improve our understanding of the aquatic N cycle in eutrophic lakes affected by cyanoHABs and how regeneration contributes to sustaining cyanoHABs.

Acknowledgments

We thank the Ohio Department of Natural Resources for providing access to boat time for sampling in Sandusky Bay and NOAA GLERL and the Ohio State University Stone Laboratory (Dr. Justin Chaffin) for boat access for sampling in Maumee Bay. We thank Megan Reed, Allison Savoie, and Ashlynn Boedecker for help in the lab. We also thank Taylor Tuttle, Daniel Hoffman, and NOAA GLERL for collecting water samples, and Ashley Burtner from NOAA GLERL for access to the data share. Project support was provided by Ohio Sea Grant awards to SEN (#60053689) and MJM (#R/ER-114) and NSF Molecular and Cell Biology (#1715909) to SEN. Partial support was provided by awards R/ER 114 from the Ohio Sea Grant College Program and R/HAB-2-BOR from the Ohio Department of Higher Education (to GSB and RMLM). [CG]

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.hal.2018.11.011.

References

Allen, M.M., 1984. Cyanobacterial cell inclusions. Annu. Rev. Microbiol. 38, 1–25.
Amin, S.A., Hmelo, L.R., van Tol, H.M., Durham, B.P., Carlson, L.T., Heal, K.R., Morales, R.L., Berthiaume, C.T., Parker, M.S., Djunaedi, B., Ingalls, A.E., Parsek, M.R., Moran, M.A., Armbrust, E.V., 2015. Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. Nature 522, 98–101.
An, S., Joye, S.B., 2001. Enhancement of coupled nitrification-denitrification by benthic photosynthesis in shallow estuarine sediments. Limnol. Oceanogr. 46, 62–74.
Bell, W., Mitchell, R., 1972. Chemotactic and growth responses of marine bacteria to algal extracellular products. The Biological Bulletin 143, 265–277.
Blackburn, T.H., 1979. Method for measuring rates of NH(4) turnover in anoxic marine sediments, using a N-NH(4) dilution technique. Appl. Environ. Microbiol. 37, 760–765.
Blomqvist, P., Pettersson, A., Hyenstrand, P., 1994. Ammonium-nitrogen: A key regulatory factor causing dominance of non-nitrogen-fixing cyanobacteria in aquatic systems. Arch. Hydrobiol. 132, 141–164.
Caperon, J., Meyer, J., 1972. Nitrogen-limited growth of marine phytoplankton—2. Uptake kinetics and their role in nutrient limited growth of phytoplankton. Deep-Sea Res. 19, 619–632.
Caperon, J., Schell, D., Hirota, J., Laws, E., 1979. Ammonium excretion rates in Kaneohe Bay, Hawaii, measured by a 15N isotope dilution technique. Mar. Biol. 54, 33–40.
Chaffin, J.D., Bridgeman, T.B., 2014. Organic and inorganic nitrogen utilization by nitrogen-stressed cyanobacteria during bloom conditions. J. Appl. Phycol. 26, 299–309.
Chaffin, J.D., Davis, T.W., Smith, D.J., Baer, M.M., Dick, G.J., 2018. Interactions between nitrogen form, loading rate, and light intensity on Microcystis and Planktothrix growth and microcystin production. Harmful Algae 73, 84–97.
Cochlan, W.P., Harrison, P.J., Denman, K.L., 1991. Diel periodicity of nitrogen uptake by marine phytoplankton in nitrate- rich environments. Limnol. Oceanogr. 36, 1689–1700.
Conroy, J.D., Quinlan, E.L., Kane, D.D., Culver, D.A., 2007. Cylindrospermopsis in Lake Erie: testing its association with other cyanobacterial genera and major limnological parameters. J. Gt. Lakes Res. 33, 519–535.
Davis, T.W., Bullerjahn, G.S., Tuttle, T., McKay, R.M., Watson, S.B., 2015. Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during planktothrix blooms in Sandusky Bay, Lake Erie. Environ. Sci. Technol. 49, 7197–7207.
Forchhammer, K., Björn, W., 2016. Closing a gap in cyanophycin metabolism. Microbiology 162, 727–729.
Gardner, W.S., Lee, G.F., 1975. The role of amino acids in the nitrogen cycle of Lake Mendota. Limnol. Oceanogr. 20, 379–388.
Gardner, W.S., Lavrentyev, P.J., Cavaletto, J.F., McCarthy, M.J., Eadie, B.J., Johengen, T.H., Cotner, J.B., 2004. Distribution and dynamics of nitrogen and microbial plankton in southern Lake Michigan during spring transition 1999-2000. J. Geophys. Res. 109.
Gardner, W.S., Newell, S.E., McCarthy, M.J., Hoffman, D.K., Lu, K., Lavrentyev, P.J., Hellweger, F.L., Wilhelm, S.W., Liu, Z., Bruesewitz, D.A., Paerl, H.W., 2017. Community biological ammonium demand: A conceptual model for cyanobacteria blooms in eutrophic lakes. Environ. Sci. Technol. 51, 7785–7793.
Glibert, P.M., 1988. Primary productivity and pelagic nitrogen cycling. In: Blackburn, T.H., Sorensen, J. (Eds.), Nitrogen Cycling in Coastal Marine Environments. John Wiley & Sons, Chichester, pp. 3–31.
Glibert, P.M., Wilkerson, F.P., Dugdale, R.C., Raven, J.A., Dupont, C.L., Leavitt, P.R., Parker, A.E., Burkholder, J.M., Kana, T.M., 2016. Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions. Limnol. Oceanogr. 61, 165–197.
Gobler, C.J., Burkholder, J.M., Davis, T.W., Harke, M.J., Johengen, T., Stow, C.A., Van de Waal, D.B., 2016. The dual role of nitrogen supply in controlling the growth and toxicity of cyanobacterial blooms. Harmful Algae 54, 87–97.
Gu, B., Havens, K.E., Schelske, C.L., Rosen, B.H., 1997. Uptake of dissolved nitrogen by phytoplankton in a eutrophic subtropical lake. J. Plank. Res. 19, 759–770.

Gupta, M., Carr, N.G., 1981. Enzyme activities related to cyanophycin metabolism in heterocysts and vegetative cells of Anabaena spp. Microbiology 125, 17–23.
Haga, H., Nagata, T., Sakamoto, M., 1995. Size-fractionated NH4+ regeneration in the pelagic environments of two mesotrophic lakes. Limnol. Oceanogr. 40, 1091–1099.
Hampel, J.J., McCarthy, M.J., Gardner, W.S., Zhang, L., Xu, H., Zhu, G., Newell, S.E., 2018. Nitrification and ammonium dynamics in Taihu Lake, China: seasonal competition for ammonium between nitrifiers and cyanobacteria. Biogeosciences 15, 733–748.
Havens, K.E., Fukushima, T., Xie, P., Iwakuma, T., James, R.T., Takamura, N., Hanazato, T., Yamamoto, T., 2001. Nutrient dynamics and the eutrophication of shallow lakes Kasumigaura (Japan), Donghu (PR China), and Okeechobee (USA). Environ. Pollut. 111, 263–272.
James, R.T., Gardner, W.S., McCarthy, M.J., Carini, S.A., 2011. Nitrogen dynamics in Lake Okeechobee: forms, functions, and changes. Hydrobiologia 669, 199–212.
Kana, T.M., Darkangelo, C., Hunt, M.D., Oldham, J.B., Bennett, G.E., Cornwell, J.C., 1994. Membrane inlet mass spectrometer for rapid high-precision determination of N2, O2, and Ar in environmental water samples. Anal. Chem. 66, 4166–4170.
Kirchman, D.L., Keil, R.G., Wheeler, P.A., 1990. Carbon limitation of ammonium uptake by heterotrophic bacteria in the subarctic Pacific. Limnol. Oceanogr. 35, 1258–1266.
Kurmayer, R., Blom, J.F., Deng, L., Pernthaler, J., 2015. Integrating phylogeny, geographic niche partitioning and secondary metabolite synthesis in bloom-forming planktothrix. ISME J. 9, 909–921.
Lupette, J., Lami, R., Krasovec, M., Grimsley, N., Moreau, H., Piganeau, G., Sanchez-Ferandin, S., 2016. Marinobacter dominates the bacterial community of the Ostreococcus Tauri phycosphere in culture. Front. Microbiol. 7, 1–14.
Mackerras, A.H., de Chazal, N.M., Smith, G.D., 1990. Transient accumulations of cyanophycin in Anabaena cylindrica and Synechocystis 6308. Microbiology 136, 2057–2065.
Martens-Habbena, W., Berube, P.M., Urakawa, H., De La Torre, J.R., Stahl, D.A., Torre, J., 2009. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. Nature 461, 976–979.
McCarthy, M.J., James, R.T., Chen, Y., East, T.L., Gardner, W.S., 2009. Nutrient ratios and phytoplankton community structure in the large, shallow, eutrophic, subtropical Lakes Okeechobee (Florida, USA) and Taihu (China). Limnology 10, 215–227.
McCarthy, M.J., Gardner, W.S., Lehmann, M.F., Bird, D.F., 2013. Implications of water column ammonium uptake and regeneration for the nitrogen budget in temperate, eutrophic Missisquoi Bay, Lake Champlain (Canada/USA). Hydrobiologia 718, 173–188.
Millie, D.F., Fahnenstiel, G.L., Bressie, J.D., Pigg, R.J., Rediske, R.R., Klarer, D.M., Tester, P.A., Litaker, R.W., 2009. Late-summer phytoplankton in western Lake Erie (Laurentian Great Lakes): bloom distributions, toxicity, and environmental influences. Aquat. Ecol. 43, 915–934.
Nicklisch, A., Kohl, J.G., 1983. Growth kinetics of Microcystis aeruginosa (Kütz) Kütz as a basis for modelling its population dynamics. Internationale Revue der gesamten Hydrobiologie und Hydrographie 68, 317–326.
Oberhaus, L., Briand, J.F., Leboulanger, C., Jacquet, S., Humbert, J.F., 2007. Comparative effects of the quality and quantity of light and temperature on the growth of planktothrix agardhii and P. rubescens. J. Phycol. 43, 1191–1199.
Paasche, E., 1988. Pelagic primary production in nearshore waters. In: Blackburn, T.H., Sorensen, J. (Eds.), Nitrogen Cycling in Coastal Marine Environments. John Wiley & Sons, Chichester, pp. 33–57.
Paerl, H.W., Xu, H., McCarthy, M.J., Zhu, G., Qin, B., Li, Y., Gardner, W.S., 2011. Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): The need for a dual nutrient (N & P) management strategy. Water Res. 45, 1973–1983.
Ponndorf, D., Ehmke, S., Walliser, B., Thoss, K., Unger, C., Görs, S., Daş, G., Metges, C.C., Broer, I., Nausch, H., 2017. Stable production of cyanophycinase in Nicotiana benthamiana and its functionality to hydrolyse cyanophycin in the murine intestine. Plant Biotechnol. J. 15, 605–613.
Présing, M., Herodek, S., Preston, T., Vörös, L., 2001. Nitrogen uptake and the importance of internal nitrogen loading in Lake Balaton. Freshw. Biol. 46, 125–139.
Reyes, J.C., Muro-Pastor, M.I., Florencio, F.J., 1997. Transcription of glutamine synthetase genes (glnA and glnN) from the cyanobacterium Synechocystis sp. strain PCC 6803 is differently regulated in response to nitrogen availability. J. Bacteriol. 179, 2678–2689.
Richards, R.P., Baker, D.B., Crumrine, J.P., Stearns, A.M., Erie, L., 2010. Unusually large loads in 2007 from the Maumee and Sandusky Rivers, tributaries to Lake Erie. J. Soil Water Conserv. 65, 450–462.
Richter, R., Hejazi, M., Kraft, R., Ziegler, K., Lockau, W., 1999. Cyanophycinase, a peptidase degrading the cyanobacterial reserve material multi-L-arginyl-poly-L-aspartic acid (cyanophycin). FEBS J. 263, 163–169.
Rinta-Kanto, J.M., Wilhelm, S.W., 2006. Diversity of microcystin-producing cyanobacteria in spatially isolated regions of Lake Erie. Appl. Environ. Microbiol. 72, 5083–5085.
Rücker, J., Wiedner, C., Zippel, P., 1997. Factors controlling the dominance of Planktothrix agardhii and Limnothrix redekei in eutrophic shallow lakes. Hydrobiologia 342, 107–115.
Salk, K.R., Bullerjahn, G.S., Mckay, R.M.L., Chaffin, J.D., Nathaniel, E., 2018. Nitrogen cycling in Sandusky Bay, Lake Erie: oscillations between strong and weak export and implications for harmful algal blooms. Biogeosciences 15, 2891–2907.
Scheffer, M., Rinaldi, S., Gragnani, A., Mur, L.R., van Nes, E.H., 1997. On the dominance of filamentous cyanobacteria in shallow, turbid lakes. Ecology 78, 272–282.
Steffen, M.M., Li, Z., Effler, T.C., Hauser, L.J., Boyer, G.L., Wilhelm, S.W., 2012. Comparative metagenomics of toxic freshwater cyanobacteria bloom communities on two continents. PloS One 7, e44002.
Steffen, M.M., Belisle, B.S., Watson, S.B., Boyer, G.L., Wilhelm, S.W., 2014. Status, causes and controls of cyanobacterial blooms in Lake Erie. J. Gt. Lakes Res. 40, 215–225.
Steffen, M.M., Belisle, B.S., Watson, S.B., Boyer, G.L., Bourbonniere, R.A., Wilhelm, S.W., 2015. Metatranscriptomic evidence for co-occurring top-down and bottom-up controls on toxic cyanobacterial communities. Appl. Environ. Microbiol. 81, 3268–3276.
Suda, S., Watanabe, M.M., Otsuka, S., Mahakahant, A., Yongmanitchai, W., Nopartnaraporn, N., Liu, Y., Day, J.G., 2002. Taxonomic revision of water-bloom-forming species of oscillatorioid cyanobacteria. Int. J. Syst. Evol. Microbiol. 52, 1577–1595.
Suttle, C.A., Harrison, P.J., 1988. Ammonium and phosphate uptake rates, N: P supply ratios, and evidence for N and P limitation in some oligotrophic lakes. Limnol. Oceanogr. 33, 186–202.
Takahashi, M., Hama, T., Matsunaga, K., Handa, N., 1995. Nitrogenous nutrient uptake by phytoplankton and ammonium regeneration by microbial assemblage in Lake Biwa. J. Plank. Res. 17, 1027–1037.
Tupas, L., Koike, I., 1991. Simultaneous uptake and regeneration of ammonium by mixed assemblages of heterotrophic marine bacteria. Mar. Ecol. Prog. Ser. 70, 273–282.
Van de Waal, D.B., Ferreruela, G., Tonk, L., Van Donk, E., Huisman, J., Visser, P.M., Matthijs, H.C.P., 2010. Pulsed nitrogen supply induces dynamic changes in the amino acid compositionand microcystin production of the harmful cyanobacterium Planktothrix agardhii. FEMS Microbiol. Ecol. 74, 430–438.
Watson, S.B., Miller, C., Arhonditsis, G., Boyer, G.L., Carmichael, W., Charlton, M.N., Confesor, R., Depew, D.C., Höök, T.O., Ludsin, S.A., Matisoff, G., McElmurry, S.P., Murray, M.W., Peter Richards, R., Rao, Y.R., Steffen, M.M., Wilhelm, S.W., 2016. The re-eutrophication of Lake Erie: harmful algal blooms and hypoxia. Harmful Algae 56, 44–66.
Welschmeyer, N.A., 1994. Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. Limnol. Oceanogr. 39, 1985–1992.
Xie, L., Xie, P., Li, S., Tang, H., Liu, H., 2003. The low TN: TP ratio, a cause or a result of Microcystis blooms? Water Res. 37, 2073–2080.
Xu, H., Paerl, H.W., Qin, B., Zhu, G., Hall, N.S., Wu, Y., 2015. Determining critical nutrient thresholds needed to control harmful cyanobacterial blooms in eutrophic Lake Taihu, China. Environ. Sci. Technol. 49, 1051–1059.
Yang, J., Gao, H., Glibert, P.M., Wang, Y., Tong, M., 2017. Rates of nitrogen uptake by cyanobacterially-dominated assemblages in Lake Taihu, China, during late summer. Harmful Algae 65, 71–84.
Yin, G., Hou, L., Liu, M., Liu, Z., Gardner, W.S., 2014. A novel membrane inlet mass spectrometer method to measure $^{15}NH4_4^+$ for isotope-enrichment experiments in aquatic ecosystems. Environ. Sci. Technol. 48, 9555–9562.
Zevenboom, W., Mur, L.R., 1981. Simultaneous short-term uptake of nitrate and ammonium by Oscillatoria agardhii grown in nitrate-or light-limited continuous culture. Microbiology 126, 355–363.
Zevenboom, W., de Groot, G.J., Mur, L.R., 1980. Effects of light on nitrate-limited Oscillatoria agardhii in chemostat cultures. Arch. Microbiol. 125, 59–65.
Zhao, M.X., Jiang, Y.L., He, Y.X., Chen, Y.F., Teng, Y.B., Chen, Y., Zhang, C.C., Zhou, C.Z., 2010. Structural basis for the allosteric control of the global transcription factor NtcA by the nitrogen starvation signal 2-oxoglutarate. Proc. Natl. Acad. Sci. U. S. A. 107, 12487–12492.