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Title: Nitrification and ammonium dynamics in Lake Taihu, China: seasonal competition for ammonium between nitrifiers and cyanobacteria.

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Abstract

Taihu Lake is hypereutrophic and experiences seasonal, cyanobacterial harmful algal blooms. These *Microcystis* blooms produce microcystin, a potent liver toxin, and are linked to anthropogenic nitrogen (N) and phosphorus (P) loads to lakes. *Microcystis spp.* cannot fix atmospheric N and must compete with ammonia-oxidizing and other organisms for ammonium (NH_4^+). We measured NH_4^+ regeneration and potential uptake rates and total nitrification using stable isotope techniques. Nitrification studies included separate NH_4^+ and nitrite (NO_2^-) oxidation rates and abundance of the functional gene for NH_4^+ oxidation, *amoA*, for ammonia-oxidizing archaea (AOA) and bacteria (AOB). Potential NH_4^+ uptake rates ranged from 0.02–6.80 µmol L^{-1} hr^{-1} in the light and 0.05–3.33 µmol L^{-1} hr^{-1} in the dark, and NH_4^+ regeneration rates ranged from 0.03–2.37 µmol L^{-1} hr^{-1}. Nitrification rates exceeded previously reported rates in most freshwater systems. Total nitrification often exceeded 200 nmol L^{-1} d^{-1} and exceeded 1000 nmol L^{-1} d^{-1} at one station near a river discharge. In Meiliang Bay and the open lake, average NO_2^- oxidation rates (248 ± 39.0 nmol L^{-1} d^{-1}) exceeded NH_4^+ oxidation rates (22.0 ± 6.00 nmol L^{-1} d^{-1}; $p < 0.001$) by an order of magnitude across all sampling events. AOA *amoA* gene copies were more abundant than AOB gene copies ($p < 0.005$) at all times; however, only abundance of AOB *amoA* (not AOA) was correlated with nitrification rates for all stations and all seasons ($p < 0.005$). Regeneration results suggested that cyanobacteria relied extensively on regenerated NH_4^+ to sustain the bloom in late summer. Nitrification rates in Taihu varied seasonally; at most stations, rates were highest in March, lower in June, and lowest in July, corresponding with cyanobacterial bloom progression, suggesting that nitrifiers are poor competitors for NH_4^+ during the bloom. Internal NH_4^+ regeneration exceeded external N loading to the lake by a factor of two and is ultimately fueled by external N loads. Our results thus

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37 support the growing literature calling for watershed N loading reductions in concert with existing
38 management of P loads.
39

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1. Introduction

Nitrogen (N) and phosphorus (P) are important nutrients in aquatic ecosystems, often co-limiting primary production (Elser et al., 2007). Biologically unavailable (except to diazotrophs) atmospheric N can be fixed to readily assimilable ammonium (NH_4^+) and biomass via N fixation (Vitousek et al., 2013). However, fertilizer production from anthropogenic N fixation (the Haber-Bosch process) has changed N cycling and the global N budget over the last century. Non-point source N loads from agriculture are a main driver of eutrophication in aquatic systems, which is often manifested as hypoxia, loss of biodiversity, cyanobacterial harmful algal blooms (cyanoHABs; Paerl et al., 2016; Paerl and Paul, 2012), and other detrimental characteristics. CyanoHABs are particularly problematic because they often produce toxins, compete for nutrients with other microbes and primary producers, and indicate unhealthy aquatic systems.

The increase in extent and frequency of cyanoHABs correlates to increased application of NH_4^+ and urea fertilizers, both globally and in China (Glibert et al., 2014). Diatoms are competitive for oxidized forms of N (e.g., NO_3^-), but non-N_2 fixing cyanobacteria, such as *Microcystis*, thrive on chemically reduced N forms, such as NH_4^+ and urea (Blomqvist et al. 1994; Glibert et al., 2016; McCarthy et al., 2009). NH_4^+ transport across the cell membrane and assimilation into biomass is less energy intensive than for NO_3^- (Glibert et al., 2016). Due to high biological demand and fast turnover rates, NH_4^+ often does not accumulate in the water column, resulting in low *in situ* concentrations. Ammonium regeneration is especially important to phytoplankton productivity in productive eutrophic systems (Gardner et al. 1998, 2017; McCarthy et al., 2013). For example, water column regeneration was up to six times higher than sediment regeneration in Lake Taihu, China (McCarthy et al., 2007; Paerl et al., 2011).

Nitrification is the link between chemically reduced and oxidized N forms. Most nitrification pathways are a two-step process; NH_4^+ is oxidized to nitrite (NO_2^-) via ammonia oxidation, and NO_2^- is then oxidized to NO_3^- via NO_2^- oxidation. Ammonia oxidation is a rate limiting step (Ward, 2008) carried out by chemolithoautotrophic, ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA; Könneke et al., 2005). NO_2^- oxidation is carried out by NO_2^- oxidizing bacteria (NOB). Recently, a species of NOB was described that is capable of one step, complete nitrification ("comammox"); however, comammox bacteria have yet to be well documented in the environment (Daims et al., 2015). The ammonia and NO_2^- oxidation steps are often tightly coupled, where the product of the first step serves as a substrate for the second step (Ward, 2008). However, some studies in marine environments suggest that the process can be decoupled, with one step outpacing the other (Füssel et al., 2012; Heiss and Fulweiler, 2016).

In Taihu, the abundance of ammonia oxidizing organisms (AOO) was investigated in sediments, where AOA outnumbered AOB, often by an order of magnitude (Wu et al., 2013; Zeng et al., 2012; Zhao et al., 2013). Another sediment study revealed that, while AOO were present at all sites, the distribution of AOA and AOB depended on lake trophic status (Hou et al., 2013). Abundance of AOA decreased, while AOB increased, with increasing trophic status, following the substrate concentration hypothesis presented in kinetic experiments (Martens-Habbena et al., 2009). A suite of environmental variables (substrate concentration, oxygen concentration, light intensity, pH, etc.) influences nitrification rates and AOO community composition, including AOA and AOB relative abundances (Bristow et al., 2015; Merbt et al., 2012; Ward, 2008)

Nitrification can be closely coupled in time and space to N removal via denitrification, particularly in shallow systems with tightly coupled benthic-pelagic interactions (An and Joye, 2001; Jenkins and Kemp, 1984). Microbial removal of excess N in eutrophic systems is a crucial process to mitigate excessive N loads, and substrate availability for denitrification can depend on nitrification. However, nitrifiers must compete with phytoplankton and other primary producers for NH_4^+. In eutrophic systems, this competition could help determine microbial community structure and cyanoHAB severity. Although both AOO and cyanoHABs, such as *Microcystis*, have a strong affinity for NH_4^+, we are unaware of measurements made when AOO and cyanoHABs were in direct competition. At some point in the bloom progression, cyanoHABs must outcompete AOO for available NH_4^+.

The overall objective of this study was to investigate seasonal NH_4^+ dynamics and the degree of competition between AOO and cyanoHABs in hypereutrophic Taihu. We measured NH_4^+ regeneration, nitrification, and potential uptake rates under different bloom conditions to help determine how cyanoHABs influence NH_4^+ fluxes. We compare these rates to: (1) investigate the competition for NH_4^+ between phytoplankton/cyanobacteria and nitrifying bacteria and archaea; (2) quantify the oxidation of NH_4^+ to NO_3^-, which is in turn available for removal via denitrification or assimilation by other organisms; (3) determine the fraction of NH_4^+ that is supplied internally via water column regeneration/remineralization; and (4) characterize the community composition of AOO. We hypothesized that: (1) lower nitrification rates occur during cyanoHABs due to increased competition for NH_4^+; (2) rates of nitrification are greater in Taihu than in most coastal and marine systems due to high *in situ* substrate concentrations; (3) rapid NH_4^+ turnover increases with phytoplankton biomass; and (4) AOB outnumber AOA due to higher saturation concentrations.

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2. Methods

2.1 Site description and time frame

Lake Tai (Taihu; from the Chinese for "Great Lake") is China's third largest freshwater lake. Due to industrial development and urbanization in the watershed, Taihu has shifted from a diatom-dominated, mesotrophic lake to a hypereutrophic lake experiencing cyanoHABs (Paerl et al., 2014; Qin et al., 2007). These blooms are associated with toxin producing, non-N_2 fixing *Microcystis spp.*, which can form surface scums on the lake for up to 10 months per year (Duan et al., 2009; Ma et al., 2016). The surface blooms have a well-documented negative impact on fisheries, tourism, and local economies, including a drinking water shutdown in 2007 (Qin et al., 2007; Steffen et al., 2017; Xu et al., 2010).

Taihu is a large (2,338 km^2), shallow (mean depth = 1.9 m) lake in southeast China, situated in the Yangtze river delta about 150 km west of Shanghai. The lake is an important source of freshwater and resources for the ~40 million people within the watershed. Taihu has a complicated hydrology, with 172 rivers and channels connected to the lake (Qin et al., 2007). This network of rivers carries nutrient loads from agricultural runoff, factories, and household wastewater.

Water samples were collected from four locations: Stations 1 and 3 in Meiliang Bay, Station 7 in the north-central part of the lake, and Station 10 on the western side of the lake basin (Fig. 1). In previous studies (e.g., McCarthy et al., 2007), sampling Stations 1, 3, and 7 followed a discharge gradient from the Liangxihe River in the northeast part of Meiliang Bay to the central lake, and Station 0 ("river") was located at the Liangxihe River discharge. However, in 2007, the Yangtze River was diverted into Taihu in an effort to decrease the lake residence time and flush *Microcystis spp.* and nutrients out of the lake (Qin et al., 2010). Diverted water from the Yangtze

River now flows into Gonghu Bay, the easternmost of the three northern bays. This diversion resulted in intermittent flow reversals through Meiliang Bay, where the Liangxihe River now mainly serves as an outflow. Since the discharge gradient from Station 1 to 7 was no longer consistent in Meiliang Bay, Station 0 was replaced with a new river input (Station 10) on the western side of the lake near the Dapugang River mouth. Environmental variables (temperature, dissolved oxygen, pH, total dissolved solids (TDS), and chlorophyll a) were measured in situ at each site using a YSI 6600 multi-sensor sonde.

Water samples were collected in August 2013 (late summer bloom), June 2014 (early summer bloom), March 2015 (no bloom/early spring bloom), and July 2016 (mid-summer bloom). This sampling pattern was chosen to investigate seasonal changes of nutrients and cyanobacteria in the lake. Water was collected into 4 l carboys at the surface (top 20 cm) and near-bottom (approximately 2 m depth) to investigate any changes in nutrient dynamics associated with depth. Samples for nutrient analyses (NO_3^-, NO_2^-, o-PO_4^{3-}, and urea) were filtered immediately in the field using 0.2 µm nylon syringe filters (GE Millipore) into 15 ml snap-cap tubes (Falcon) and stored frozen at -20°C. Nutrient samples were analyzed on a Lachat QuikChem 8000 nutrient analyzer at the University of Texas Marine Science Institute (UTMSI; Aug 2013, June 2014) or a Lachat 8500 nutrient analyzer at Wright State University (WSU; March 2015, July 2016) according to manufacturer directions. Ambient NH_4^+ concentrations were determined by ammonium retention time shift (AIRTS) high performance liquid chromatography (HPLC) at UTMSI (Gardner et al., 1995). Briefly, the atom % ^{15}N and total NH_4^+ concentration are determined by comparing the retention time shift of the sample relative to the natural abundance NH_4^+ standard (Gardner et al., 1996)

2.2 Water column NH_4^+ uptake and regeneration

NH_4^+ uptake and regeneration rates were determined following the protocol of McCarthy et al. (2013). Water collected in 4 l carboys was returned to the Taihu Laboratory for Lake Ecosystem Research (TLLER) for isotope amendments and incubations. 500 ml from each site/depth was amended with 99.8% $^{15}NH_4Cl$ (Isotec; concentration added 8–96 µM) and distributed into six (triplicates for light and dark) 70 ml, clear tissue culture bottles (Corning; McCarthy et al., 2007). Dark bottles were wrapped with thick aluminum foil. Initial samples (T_0) were withdrawn from each bottle with a rinsed syringe, filtered (0.2 µm filters) immediately into 8 ml glass vials (Wheaton), and frozen until analysis at UTMSI. Light and dark bottles were then submerged (approximate depth 0.2 m) in a mesh bag at in situ light and temperature in the lake. After ~24 h, final samples (T_f) were filtered in the same manner as the T_0 samples. Total NH_4^+ concentrations and atom % ^{15}N for all samples were determined by AIRTS/HPLC (Bruesewitz et al., 2015; Gardner et al., 1995). Potential uptake and actual regeneration rates were calculated using the Blackburn/Caperon isotope dilution model (Blackburn, 1979; Caperon et al., 1979; McCarthy et al., 2013). The uptake rate is considered a potential rate, which includes nitrification, assimilation, and other consumption processes, and regeneration encompasses remineralization, decomposition of dead organic matter, heterotrophic excretion, respiration, biodegradation, and sloppy feeding by zooplankton (Saba et al., 2011).

2.3 Ammonia and nitrite oxidation rates

Ammonia and NO_2^- oxidation rates were measured directly using the $^{15}NH_4^+$ tracer addition method. 500 ml of water from each station and depth was distributed into 750 ml polycarbonate bottles, enriched with a tracer amount (approximately 20% of the total pool) of 99.8% $^{15}NH_4Cl$ (Isotec), mixed thoroughly by inverting 10 times, and distributed into three 125 ml polycarbonate incubation bottles. Unenriched samples for each station and depth were

distributed into 125 ml incubation bottles. Initial samples (T_0) were filtered using 0.22 µm syringe filters into 30 ml polycarbonate bottles and frozen until analysis. Final samples were collected as described after incubating for 24 h at in situ light and temperature. Samples were returned frozen to WSU for analysis.

Ammonia oxidation rates were measured from accumulation of $^{15}NO_2^-$ using the sodium azide (NaN_3) reduction method (Heiss and Fulweiler, 2016; McIlvin and Altabet, 2005; Newell et al., 2011). Briefly, 7.5 ml from each sample was distributed into a 12 ml Exetainer vial (Labco, UK) and capped tightly. Each sample was then injected (with gastight syringe) with 0.25 ml of 1:1 (v:v) 2 M NaN_3 :20% CH_3COOH solution (previously purged with Ar for 30 min), followed by incubation for 1 h at 30 °C (McIlvin and Altabet, 2005). All NO_2^- accumulated in the sample from NH_3 oxidation was transformed chemically to N_2O. After 1 h, the reaction was stopped by injection of 0.15 ml of 10 M NaOH.

Nitrite oxidation rates were measured from accumulation of $^{15}NO_3^-$ using the Cd reduction/NaN_3 reduction method (Heiss and Fulweiler, 2016). Approximately 25 ml from each sample was transferred into 50 ml centrifuge tubes. First, in situ NO_2^- was removed with 0.25 ml of 0.4 M sulfamic acid (H_3NSO_3). After 10 min, the reaction was neutralized with 0.125 ml of 2 M NaOH (Granger and Sigman, 2009). NO_3^- was reduced to NO_2^- by addition of 100 mg of MgO, 6.6 g of NaCl, and 0.75–1 g of acidified Cd powder to each sample, followed by 17 h incubation on a shaker table (McIlvin and Altabet, 2005). Samples were centrifuged at 2000 rpm for 15 min, and 7.5 ml of supernatant was carefully transferred into 12 ml Exetainers. Cadmium-reduced NO_2^- was further reduced to N_2O with the previously described NaN_3 method.

Samples for NH_3 and NO_2^- oxidation were sent inverted to the University of California Davis Stable Isotope Facility for isotopic analysis of $^{45/44}N_2O$ using a ThermoFinnigan GasBench

+ PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany). Ammonia and NO_2^- oxidation rates were corrected for NaN_3 reduction efficiency, and NH_3 oxidation was calculated as:

$$NH_3\ Ox\ (in\ nM\ day^{-1}) = (({}^{15}N/{}^{14}N * [NO_2^-])_{24h} - ({}^{15}N/{}^{14}N * [NO_2^-])_{0h})/ \alpha * t$$

Where $\alpha = [{}^{15}NH_4^+] / ([{}^{15}NH_4^+] + [{}^{14}NH_4^+])$

For NO_2^- oxidation:

$$NO_2^-\ Ox\ (in\ nM\ day^{-1}) = (({}^{15}N/{}^{14}N * [NO_3^-])_{24h} - ({}^{15}N/{}^{14}N * [NO_3^-])_{0h})/ \alpha * t$$

Where $\alpha = [{}^{15}NO_2^-] / ([{}^{15}NO_2^-] + [{}^{14}NO_2^-])$

2.4 Quantitative Polymerase Chain Reaction (qPCR)

During the 2014–2016 sampling events, environmental DNA for AOO abundance was collected using 0.2 µm Sterivex filters (EMD Millipore, MA, USA) and preserved with Ambion RNAlater (Invitrogen, Carlsbad, CA, USA). Approximately 60–120 ml of site water was pushed through the filter for each station and depth and then stored filled with 5 mL RNAlater. Preserved filters were frozen at -80 ˚C and transported to WSU. DNA was extracted using the Gentra PureGene kit (Qiagen Inc., USA) extraction protocol with slight modifications (Newell et al., 2011). Sterivex filters were first washed with Phosphate Buffer Saline 1X Solution (Fisher BioReagents, USA) to remove any residual RNAlater. Lysis buffer (0.9 ml) and Proteinase K (4 µl) were added to the filters, followed by 1 h incubation at 55 °C and 1 h incubation at 65 °C. The solution was removed to a 1.5 ml tube, and the incubation was repeated with fresh lysis buffer and Proteinase K.

Concentration and purity of the DNA were measured spectrophotometrically (Nanodrop 2000, ThermoScientific). AOA were targeted with Arch-amoAF and Arch-amoAR primers targeting the 635 base pair (bp) region of the *amoA* gene, subunit A of the ammonia

monooxygenase enzyme (AMO; Francis et al. 2005). Bacterial *amoA* was quantified using amoAF and amoA2R primers (Rotthauwe et al., 1997) to target the 491 bp region of *amoA*. qPCR standards were prepared by cloning the fragment of interest for AOA and AOB with the TOPO TA Cloning Kit (Invitrogen, USA), inserting it into a competent cell plasmid (One Shot E. coli cells, Invitrogen, USA), and isolating the plasmid containing the *amoA* gene using the UltraClean Standard Mini Plasmid Prep Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA).

AOA and AOB qPCR assays were conducted within a single 96 well plate for each year (2014, 2015, and 2016). Each run included three negative controls (no template), five standards from serial dilution in triplicates, and the environmental DNA samples in triplicate. Each sample and standard received 12.5 µl of SYBR green Fast Mastermix (Qiagen Inc., USA), 0.5 µl of each 100 µM primer, and 2–15 ng of template DNA.

All PCR work was performed in a PCR fume hood after cleaning the surface with DNAaway (ThermoScientific, USA) and engaging the UV light (20 min) to prevent contamination. qPCR protocol followed the method of Bollmann et al. (2014) for AOA (95 °C initial denaturation for 5 min, 95 °C denaturation for 30 sec, 53 °C annealing for 45 sec, and 72 °C extension for 1 min; 45 cycles) and AOB (95 °C initial denaturation for 5 min, 95 °C denaturation for 30 sec, 56 °C annealing for 45 sec, 72 °C extension for 1 min; 45 cycles), followed by the melting curve. Automatic settings for the thermocycler (Realplex, Eppendorf) were used to determine threshold cycle (Ct values), efficiency (85–95%), and a standard curve with R^2 values above 0.9. Gene copy number was calculated as (ng * number mol $^{-1}$)/ (bp * ng g^{-1} * g mol^{-1} of bp) and is reported in gene copies/ml of sample water.

2.5 Statistical analysis

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All statistical analyses were performed using RStudio software (R Version 3.3.1). Prior to statistical analysis, data were checked for normality using the Shapiro–Wilk normality test. The only variables that were normally distributed were DO, pH, and TDS. To explore potential environmental drivers of the rates, a multivariate correlation analysis was performed using the Kendall correlation method for nonparametric data. A p-value of <0.05 was considered statistically significant. Additionally, stepwise multiple regression models were run using the MASS package (R Version 7.3). The best fitting model was selected based on the minimum Akaike's Information Criteria (AIC; Akaike 1974). To normalize data for parametric analysis, all non-normally distributed variables were log(x+1) transformed prior running the model.

3. Results

3.1 Lake ambient conditions

Physicochemical parameters in Taihu varied seasonally (Table 1). The most pronounced seasonal variations were observed in temperature and DO, with highest water temperature recorded in August. DO varied significantly, with highest values in March and lowest in August ($p < 0.01$). pH varied significantly with season, with lowest values in March and highest in August ($p < 0.01$). TDS values were highest in July 2016 and lowest in August 2013 ($p < 0.001$).

Ammonium concentrations remained high throughout all sampling events, with highest values in March 2015 and lowest values in August 2013, but differences were not statistically significant ($p = 0.125$). Nitrite concentrations were not different between seasons, although they were significantly higher at Station 10 than other stations ($p < 0.001$). Nitrate concentrations followed the pattern of NH_4^+ concentrations and were highest in March 2015 and lowest in August 2013 ($p < 0.001$). Orthophosphate concentrations followed a seasonal pattern with lowest

concentrations in March and highest in August ($p < 0.005$), and o-PO_4^{3-} concentrations at Station 10 were significantly higher than at any other station ($p < 0.001$).

3.2 Potential NH_4^+ uptake

In August 2013, light uptake rates (all NH_4^+ uptake are potential rates) were uniform across sites (mean = 0.40 ± 0.04 µmol L^{-1} hr^{-1}) and did not vary between surface and bottom waters (Fig. 2a). During the early summer bloom in June 2014, light uptake rates in surface waters at Stations 1, 7, and 10 (mean = 0.80 ± 0.06 µmol L^{-1} hr^{-1}) were significantly higher than deep rates (mean = 0.31 ± 0.08 µM hr^{-1}; $p < 0.001$). However, light uptake rates at Station 3 did not differ from zero at either depth (Fig. 2a). Mean surface and deep uptake rates in the dark in August 2013 (0.25 ± 0.01 µmol L^{-1} hr^{-1}) and June 2014 (0.13 ± 0.05 µmol L^{-1} hr^{-1}) were significantly lower than light uptake rates (Fig. 2b; $p < 0.05$). In the early spring bloom of March 2015, light uptake rates at Stations 1–7 (mean = 0.12 ± 0.04 µmol L^{-1} hr^{-1})were lower than those during the August and June summer bloom (mean = 0.43 ± 0.41 µmol L^{-1} hr^{-1}) except for Station 10, where the rates were significantly higher (mean = 1.36 ± 0.20 µmol L^{-1} hr^{-1}; $p < 0.001$). In contrast to summer, dark uptake rates in March 2015 were not significantly different than light rates (Fig. 2b). During the July 2016 bloom, light uptake rates were highest at Stations 1, 7, and 10 (1.31 – 6.82 µmol L^{-1} hr^{-1}). Stations 3 and 7 rates were highest in bottom waters (0.80 ± 0.16 µmol L^{-1} hr^{-1} and 2.55 ± 0.14 µmol L^{-1} hr^{-1}, respectively). In July 2016, light and dark uptake rates did not differ significantly ($p = 0.15$); highest dark uptake rates were observed at Station 1 in surface water (3.33 ± 0.67 µmol L^{-1} hr^{-1}). Light uptake rates, across all stations and seasons, correlated positively with TDS and NH_4^+:NO_3^- and negatively with pH, while dark uptake rates correlated positively with TDS, NH_4^+, and NH_4^+:NO_3^-, and negatively with pH (Table 2).

3.3 Regeneration of NH_4^+

Regeneration rates in the light and dark (all NH_4^+ regeneration rates are actual rates, not potential) were not significantly different from each other across all years and seasons; therefore, light and dark rates were averaged together (Fig. 2c). Regeneration rates did not differ significantly between the summer bloom sampling events in August 2013 and June 2014 (mean = 0.22 ± 0.03 µmol L^{-1} hr^{-1}), but July 2016 regeneration rates (mean = 0.75 ± 0.16 µmol L^{-1} hr^{-1}) were significantly higher than in August and June ($p = 0.004$), with exceptionally high regeneration rates occurring in surface waters in July at Station 1 (mean = 2.37 ± 0.16 µmol L^{-1} hr^{-1}). In March 2015, mean surface and deep regeneration rates decreased from the river mouth (Station 10; 0.88 ± 0.15 µmol L^{-1} hr^{-1}) towards the center of the lake, with significantly higher regeneration rates at 10 than Stations 1–7 (mean = 0.10 ± 0.03 µmol L^{-1} hr^{-1}; $p < 0.01$). Regeneration rates were positively correlated with TDS, NH_4^+, and o-PO_4^{3-} concentrations, and NH_4^+:NO_3^- (Table 2).

3.4 Nitrification (2014-2016)

Note that nitrification rates are presented in nmol L^{-1} d^{-1} for consistency with literature reported values. At Stations 1, 3, and 7, NO_2^- oxidation rates (mean = 248 ± 39.0 nmol L^{-1} d^{-1}) exceeded NH_4^+ oxidation rates (mean = 21.9 ± 6.34 nmol L^{-1} d^{-1}; $p < 0.001$) by an order of magnitude across all sampling events (Fig. 3a). The total nitrification rates at Station 3 did not vary across seasons. At Station 7 in the central lake, highest total nitrification rates were observed in March (mean = 663 ± 69.4 nmol L^{-1} d^{-1}) in both surface and deep waters compared to the lowest rates in July 2016 (mean = 1.58 ± 0.78 nmol L^{-1} d^{-1}). At Station 1, the highest rates were measured in surface waters in July (mean = 773 ± 50.7 nmol L^{-1} d^{-1}), but the rates at depth followed a seasonal pattern from high in the spring (mean = 646 ± 158 nmol L^{-1} d^{-1}) to an order of magnitude lower in the summer (mean = 9.86 ± 3.28 nmol L^{-1} d^{-1}).

Total nitrification rates at Station 10 were significantly higher than other stations (Fig. 3b; $p < 0.001$). Rates were, at times, orders of magnitude higher, and total nitrification ranged from 148 – 3750 nmol L^{-1} d^{-1} (mean = 1590 ± 1390 nmol L^{-1} d^{-1}), compared to Stations 1–7 ranging from 2.00 – 771 nmol L^{-1} d^{-1} (mean = 270 ± 277 nmol L^{-1} d^{-1}). While NO_2^- oxidation rates exceeded NH_4^+ oxidation rates in 2014 and 2015, NH_4^+ oxidation rates in July 2016 were significantly higher than other years (1650 ± 55.0 nmol L^{-1} d^{-1}; $p < 0.001$).

3.5 Ammonia oxidizer abundance

Abundance of the bacterial *amoA* gene for all years (2014–2016) varied between undetectable to 2.85 x 10^5 ± 5.20 x 10^4 copies ml^{-1}. Archaeal *amoA* abundance ranged from undetectable to 1.03 x 10^7 ± 3.37 x 10^6 copies ml^{-1} (Fig. 4a). Neither AOB nor AOA *amoA* gene copy abundances were statistically different between the three seasons. The highest ratio of AOB:AOA gene abundance was reported at Station 3 in Meiliang Bay (1.81; Fig. 4b) and lowest in the open lake (0.01; Station 7). AOB gene abundance was positively correlated with NH_4^+, NO_2^-, and o-PO_4^{3-} concentrations, and NH_4^+:NO_3^-, while AOA gene abundance was not significantly correlated to any environmental variable (Table 2).

4. Discussion

4.1 Ammonium regeneration and potential uptake

Ammonium uptake rates (0.02 – 6.82 µmol L^{-1} hr^{-1}) reported here were within the range of or slightly higher than rates reported in other studies (Table 3). Rates were higher than uptake rates reported previously in Meiliang Bay (0.11 – 1.54 µmol L^{-1} hr^{-1}) and the central lake (0.03 – 0.32 µmol L^{-1} hr^{-1}) but within the range of rates reported in the Liangxihe River (0.70 – 4.19 µmol L^{-1} hr^{-1}; McCarthy et al., 2007). Light uptake rates in March, June, and August resembled rates in eutrophic Lake Okeechobee but were higher than rates in Missisquoi Bay, Lake Champlain,

Lake Michigan, eutrophic New Zealand lakes Rotorua and Rotoiti, and the Mississippi River plume (Table 3 and references therein). Higher light uptake rates were reported in hypereutrophic Lake Maracaibo, Venezuela, and a Lake Erie coastal wetland, Old Woman Creek (Table 3). Light uptake rates were marginally higher ($p = 0.08$) than dark uptake rates, presumably due to photosynthetic phytoplankton activity. Photoautotrophic uptake is greatly reduced in the dark, so dark uptake rates can be attributed mostly to heterotrophic or chemolithoautotrophic organisms. However, photoautotrophs may assimilate nutrients in the dark under nutrient limitation (Cochlan et al., 1991). Uptake rates were significantly higher in July 2016 than at other times, which may have been due to higher precipitation and subsequent runoff; during summer 2016, average rainfall in June and July was about 305 mm compared to 106 mm in June 2014, 105 mm in August 2013, and 54 mm in March 2015 (WorldWeatherOnline.com; accessed on <08/02/2017>). Dark uptake rates in Taihu exceeded dark rates reported in Lake Okeechobee (0.02 – 0.04 µmol L^{-1} hr^{-1}; James et al. 2011), Missisquoi Bay, Lake Champlain (0.10 µmol L^{-1} hr^{-1}; McCarthy et al., 2013), and Lake Michigan (7 nmol L^{-1} hr^{-1}; Gardner et al., 2004) suggesting increased activity of both heterotrophs and chemolithoautotrophs in Taihu. A previous metagenomics study of the bloom composition in Taihu revealed an overlooked contribution of heterotrophic bacteria to N assimilation processes by *Microcystis*, which could be important in driving toxic blooms (Steffen et al., 2012).

Internal NH_4^+ cycling via regeneration is important in Taihu and varies seasonally (McCarthy et al., 2007; Paerl et al., 2011). In March 2015, about 38% of light uptake for all sites and depths was supported by regeneration (Fig. 2d). This proportion increased in June 2014 and July 2016 to 58% and 42%, respectively, and was highest in August 2013, when regeneration could

account for 109% of uptake. The importance of regeneration corresponded to decreasing in situ NH_4^+ concentrations (Fig. 2D). These results suggest that, in spring and early summer, regeneration supplemented the ambient NH_4^+ in the water column to support algal production, whereas in the late summer, cyanoHABs relied heavily on NH_4^+ from regeneration to sustain blooms. Water column regeneration may supply more NH_4^+ for blooms than sediment NH_4^+ regeneration in Taihu due to combined spatial, temperature, and biogeochemical factors (McCarthy et al., 2007; Gardner et al., 2017). Rapid decomposition of cyanoHABs biomass may provide NH_4^+ for nitrification, which provides substrate for denitrification. High rates of sediment denitrification (McCarthy et al., 2007) may lead to increased N limitation at the end of the bloom season in late summer and fall (Paerl et al., 2011; Xu et al., 2010)

To calculate whole-lake, water column NH_4^+ regeneration rates, we divided the lake (2,338 km^2; Qin et al., 2007) into four different sections based on geochemical and ecological properties (Qin, 2008): (1) three northern bays (361.8 km^2; depth = 1.9 m) most affected by the blooms; (2) the main lake (1,523.9 km^2; depth = 1.9 m); (3) the East Taihu region, dominated by rooted and floating macrophytes (357.5 km^2; depth = 1.4 m); and (4) shorelines <1 m deep (94.8 km^2). We considered regeneration rates from Stations 1 and 3 to represent the northern bays area, Station 7 as the main lake, Station 10 as shoreline, and regeneration rates previously reported for East Taihu (McCarthy et al., 2007; Paerl et al., 2011). When extrapolated to the volume of these four zones in Taihu, regeneration returned about 3.04 x 10^7 kg of NH_4^+ annually in the three northern bays, 6.71 x 10^7 kg of NH_4^+ in the main lake, 8.87 x 10^6 kg of NH_4^+ along the shorelines, and 2.88 x 10^6 kg of NH_4^+ in East Taihu Lake. These values sum to 1.09 x 10^8 kg of NH_4^+ recycled in the water column, approximately two times higher than reported external N loadings, which range from 5.11 x 10^7 and 7.00 x 10^7 kg annually (Chen et al., 2012; Yan et al., 2011). This

rough estimate of lake-wide regeneration is based on rates measured at specific stations at discreet times; improved spatial and temporal resolution of measurements are needed to improve these estimates. Additionally, these calculated values are an overestimate given that most of the rates measured and reported in this study are during spring and summer months, not fall and winter. Taihu is a complex ecosystem with 172 rivers and channels connected to the lake (Qin et al., 2007), making any estimations of total N loadings challenging. As such, we believe that the reported total N loads to Taihu are likely an underestimate. However, our results show that these external N loads are fueling high regeneration rates and suggest that microbial denitrification cannot keep pace with external N loads. Increasing nutrient loadings can result in decreasing efficiency of denitrification (Gardner and McCarthy, 2009; Mulholland et al., 2008), which will limit the ability of a system to self-mitigate excess N loads.

4.2 Nitrification

Nitrification rates reported in this study exceeded previously reported rates in most oligotrophic and mesotrophic freshwater systems. Published nitrification rates in lakes include the water columns of saline Lake Mono, CA (60–480 nmol L^{-1} d^{-1}; Carini and Joye, 2008), Lake Okeechobee, FL (67–97 nmol L^{-1} hr^{-1}; James et al., 2011), Lake Superior, USA (0–51 nmol L^{-1} d^{-1}; Small et al., 2013), and in sediments of Lake Onondaga (0.37 g N m^{-2} d^{-1}; Pauer and Auer, 2000). Similarly, only a few studies in freshwater systems report rates of ammonia oxidation (Lake Superior (18–34 nmol N L^{-1} d^{-1}); Small et al., 2013). Rates on this scale were previously reported only in eutrophic Lake Mendota (WI; 1700 – 26000 nmol L^{-1} hr^{-1}; Hall, 1986) and the Paerl River Estuary (China; 2100 – 65100 µmol L^{-1} d^{-1}; Dai et al., 2008). High nitrification rates in Taihu can be attributed to high ambient NH_4^+ concentrations, up to 40 µM at Station 1 in 2016 and 135 µM at Station 10 in 2014. These high concentrations of NH_4^+ are due to high external N

loadings, including N in organic matter, into the lake, of which ~1.32 x 10^7 kg were loaded as NH_4^+ in 2009 (Yan et al., 2011). The significant relationships between nitrification and NH_4^+ oxidation with NH_4^+, NO_2^-, and NO_3^- concentrations and between NH_4^+ oxidation and NH_4^+:NO_3^- (Table 2) support these observations.

Ammonia oxidation rates were positively correlated with ambient NH_4^+, NO_2^-, and NO_3^- concentrations ($p < 0.005$; Table 2), as expected. Substrate concentrations drive NH_4^+ oxidation rates and therefore end-product pools, since it is the rate limiting step of nitrification (i.e., completion of nitrification is dependent on the first step). Nitrite oxidation rates, however, were an order of magnitude higher than NH_4^+ oxidation rates and were correlated with ambient NH_4^+ and NO_3^- concentrations. Higher NO_2^- oxidation rates were expected, since NO_3^- is the product of NO_2^- oxidation, and NO_2^- oxidation relies on the product of NH_4^+ oxidation. Nitrite oxidation rates were not related to NO_2^- concentrations, perhaps due to the standing pool of ambient NO_2^-. However, at some stations, ambient NO_2^- was substantial (e.g., Station 10, June 2014; 14 – 15 µM). This accumulation of NO_2^- could indicate that NO_2^- oxidizers were saturated, as reported K_m values for NO_2^- oxidation in an oligotrophic, oxygen deficient region in the ocean were 0.25 ± 0.16 µM (Sun et al., 2017). However, culture experiments report K_m values ranging from 6–544 µM for *Nitrospira*, *Nitrobacter*, and *Nitrotoga spp.* (Blackburne et al., 2007; Nowka et al., 2015; Ushiki et al., 2017).

At most stations, nitrification rates in Taihu were highest in March, lower in June, and lowest in July. During the spring sampling, nitrification accounted for about 8% of light uptake and 15% of dark uptake at Stations 1 – 7. In June, nitrification accounted for 2.6% of light uptake and 9.6% of dark uptake, and in July only 0.2% and 0.3% of light and dark uptake, respectively. These results show a seasonal trend of decreasing contribution of nitrification to total uptake

rates and higher contribution of nitrifiers to dark uptake. Chemolithoautotrophs (including nitrifiers) do not rely on light for energy and continue to assimilate NH_4^+ in dark conditions. Phytoplankton, including cyanobacteria, can also assimilate NH_4^+ in the dark, especially when nutrients are limiting (Cochlan et al., 1991), and N has been shown to limit primary production in Lake Taihu, especially in summer (e.g., Paerl et al., 2011). However, the presence of high dissolved inorganic N concentrations in ambient water samples suggests that the observed dark uptake was likely performed primarily by non-photoautotrophs, including nitrifiers.

We observed no significant seasonal change in nitrification across all stations and no consistent pattern between temperature and nitrification. While the lack of relationship of nitrification with temperature agrees with nitrification studies in the ocean (Ward, 2008), other studies have reported temperature as a potential driver of nitrification in coastal waters (Heiss and Fulweiler, 2016). While not statistically linked to changes in temperature, the contribution of nitrification to total uptake rates decreased in summer, likely as a result of competition with the *Microcystis* bloom and associated heterotrophic bacteria. Non-N_2 fixing cyanobacteria, including *Microcystis*, are exceptional competitors for NH_4^+ (Blomqvist et al., 1994). With a high saturation threshold and reported K_m values of 26.5 µM (Nicklisch and Kohl 1983) and 37 µM (Baldia et al., 2007), it can outcompete nitrifiers at the high ambient NH_4^+ concentrations in Taihu. Additionally, *Microcystis* can regulate its buoyancy and scavenge nutrients throughout the water column to effectively compete for light with other phytoplankton (Brookes and Ganf, 2001).

Nitrification at the river mouth Station (10) differed dramatically from other stations. Total nitrification rates and NH_4^+ oxidation rates were, at times, orders of magnitude higher than at other stations. Also, Station 10 did not follow the trend of decreasing nitrification contribution

with the bloom. Nitrification accounted for 19% of light uptake and 64.8% of dark uptake in June and only 1.7% and 2%, respectively, in March. These very high NH_4^+ oxidation rates, along with high ambient NH_4^+ and NO_2^- concentrations, suggest that NH_4^+ and NO_2^- oxidation could be uncoupled at this station. We speculate that Station 10 differs from other stations because of the large nutrient and suspended particle loads from the Dapugang River, the second largest inflow into the lake (Yan et al., 2011). Suspended particles from sediments could trigger heterotrophic and anaerobic processes at Station 10, including reduction of NO_3^- to NO_2^- (Krausfeldt et al., 2017; Yao et al. 2016). In fact, denitrification and anammox gene transcripts were observed recently in the water column at Station 10 (Krausfeldt et al., 2017). These authors also speculated that the discharge of suspended sediments from the river might play a role in coupling anaerobic and aerobic processes in the turbid water column, resulting in rapid cycling of reduced and oxidized forms of N. Nitrification is the link between introduction of reduced N into the system and the removal of N through denitrification. Therefore, the efficiency of nitrification is crucial to the removal of N from this hypereutrophic lake.

4.3 Ammonia oxidizer abundance

AOB and AOA coexist in the environment, and environmental variables shape the community structure. AOA often dominate in environments with low substrate concentrations, such as the open ocean or oligotrophic lakes (Beman et al., 2008; Bollmann et al., 2014; Newell et al., 2011), while AOB are often more abundant in nutrient rich waters and soils (Hou et al., 2013; Jia and Conrad, 2009; Kowalchuk and Stephen, 2001; Verhamme et al., 2011). This substrate concentration adaptation is dictated by different physiological abilities to assimilate NH_4^+. Culture studies show that AOA have a very high affinity (low half saturation constant; K_m) for NH_4^+, and in general are saturated faster than AOB (Martens-Habbena et al., 2009). The

low half saturation constant (K_m = 0.132 µM; Martens-Habbena et al., 2009) of AOA gives them a competitive advantage in low NH_4^+ conditions. In contrast, the high K_m of AOB (10–1000 µM) allows them to assimilate more NH_4^+ before becoming fully saturated, an advantage for higher NH_4^+ concentration conditions. Although oligotrophic AOA appear to proliferate in the environment (Francis et al., 2005), some species adapt to higher substrate concentrations (Jung et al., 2011; Tourna et al., 2011).

Results from the *amoA* gene copy abundance analysis show that AOA were more abundant than AOB across all stations and seasons in Taihu. Although this result does not support our original hypothesis, the results agree with previous studies in the water column and sediments in Taihu (Zeng et al., 2012), which reported higher AOA abundance ($4.91 \times 10^5 – 8.65 \times 10^6$ copies g^{-1} sediment) than AOB ($3.74 \times 10^4 – 3.86 \times 10^5$ copies g^{-1} sediment) in Meiliang Bay. Similarly, another Taihu sediment study showed more AOA than AOB in sediments at all 20 investigated stations (Wu et al., 2010).

The differences in abundance of AOO between stations, represented as AOB:AOA, show spatial variability between the more nearshore and central lake stations (Fig. 4b). In this study, AOA were more abundant in the central lake (Station 7), whereas AOB were more abundant closer to shore. Due to a higher affinity for substrate (lower K_m), AOA are likely more competitive when nutrient concentrations are lower, such as in the open lake (mean offshore NH_4^+ concentration = 3.69 µM). In contrast, AOB, with higher K_m, thrive at higher NH_4^+ concentrations at nearshore locations (mean nearshore NH_4^+ concentration = 31.3 µM). These results agree with previous research in Taihu, where AOA outnumbered AOB in sediments at mesotrophic sites, and AOB were more abundant at hypereutrophic locations (Hou et al., 2013). Another study in Taihu sediments also reported that both AOA abundance and AOA:AOB were

497 negatively correlated with ambient NH_4^+ concentration (Wu et al., 2010). However, the data
498 reported in this study show no significant relationship between AOA and NH_4^+, NO_2^-, and NO_3^-
499 (Table 2).

500 Despite AOA outnumbering AOB, AOB abundance was correlated with total nitrification
501 rates for all stations and all seasons ($p < 0.005$), but AOA abundance was not. This result agrees
502 with a previous study in Taihu sediments, where AOA were negatively correlated ($r = 0.53$, $p <$
503 0.05) with potential nitrification rates (0 – 3.0 μg NO_3—N g^{-1} dry sediment; Hou et al., 2013). We
504 speculate that AOA oxidized NH_4^+ at lower rates due to oversaturation and inhibition and may
505 not have contributed as much as AOB to total nitrification rates in our study. This conclusion
506 was also reached in Plum Island Sound (MA, USA), where abundance of archaeal *amoA* was
507 higher than bacterial, but potential nitrification rates did not correlate with AOA (Bernhard et al.,
508 2010). The authors hypothesized various scenarios, including inhibition of AOA due to high
509 substrate concentrations, competition for NH_4^+ with AOB, or AOA using an alternative energy
510 source (Bernhard et al., 2010). Our results support the interpretation that AOA are at a
511 disadvantage when competing with AOB for NH_4^+ in a hypereutrophic system and most likely
512 did not play a major role in observed nitrification in Taihu. Recently studies show that AOA can
513 oxidize cyanate (Palatinszky et al., 2015) and urea (Tolar et al., 2016). Therefore, we speculate
514 that AOA might be playing a different role in Taihu.

515 **4.4 Multiple regression model**

516 The best-fitting multiple regression models for N dynamics in Taihu (Table 4) supported
517 the Kendall non-parametric analysis (Table 2). Ammonium uptake and regeneration rates and
518 nitrification were driven by ambient NH_4^+, NO_2^-, and NO_3^- concentrations. Additionally, the
519 best-fitting models revealed that variables that changed with season had major influences on the

models (Table 4). For example, uptake in the light and dark and regeneration rates were positively influenced by temperature and DO and negatively by pH. However, models for nitrification rates, and NH_4^+ and NO_2^- oxidation rates, did not reveal that the seasonal variables, such as temperature, played a major role in the model.

5. Conclusions

This study highlights the importance of water column NH_4^+ regeneration and nitrification in bloom formation and maintenance in Taihu. We showed that nitrification rates were detectable during the bloom but decreased as the bloom progressed, suggesting that nitrifiers are weaker competitors for substrate than *Microcystis*. Also, seasonal changes in light and dark NH_4^+ uptake and nitrification rates showed that AOO are outcompeted by *Microcystis*. Extremely high nitrification rates at the river mouth (Station 10) differed from rates at other stations, suggesting that other processes, such as coupled nitrification/denitrification, might be important in suspended sediments. Previous studies reported coupled denitrification with nitrification in sediments (McCarthy et al., 2007). Functional gene analysis suggested that gene abundance does not necessarily reflect performance of the function in eutrophic lakes. We speculate that AOA are present in the lake but do not contribute proportionately to nitrification, suggesting that AOA might play another role in the lake.

Ammonium inflow into the lake is a large source of reduced N, but external inputs are not the sole source. Extrapolated whole-lake regeneration rates in the water column were twice as high as external N loadings into the lake. To mitigate harmful algal blooms, N loadings into the lake must be reduced so that N can be efficiently removed through denitrification, instead of being recycled in the water column. Our results support the recent calls for dual nutrient (N + P)

management strategies (Paerl et al., 2011) and highlight the importance of (chemically) reduced N removal through nitrification and denitrification.

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Figure list

Figure 1. Map of sampling stations in Taihu (modified from Paerl et al. 2011).

Figure 2. Ammonium dynamics in Taihu. (a) potential light uptake rates ± one standard error. (b) potential dark uptake rates ± one standard error. (c) Mean light and dark regeneration rates ± one standard error. (d) Seasonal averaged percent of light uptake supported by regeneration ± one standard error and averaged in situ NH_4^+ concentrations.

Figure 3. Ammonia and nitrite oxidation rates (total nitrification) ± one standard deviation. (a) Stations 1–7. (b) Station 10. Note the difference in y-axis scale.

Figure 4. Ammonia oxidizing organism population characteristics. (a) Ammonia oxidizer abundance (DNA) ± one standard deviation. (b) Ratio of abundance of AOB to AOA.

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914 Table 1.
915 Environmental characteristics during sampling events for each station/depth: temperature,
916 dissolved oxygen (DO), pH, chlorophyll a (chl a; surface only), total dissolved solids (TDS), and
917 in situ nutrient concentrations. S in station name = surface water (0.2 m), and D = deep, near-
918 bottom water (~2 m).

Year/ Month	Station	Temp (°C)	DO (mg L^{-1})	pH	Chl a (µg L^{-1})	TDS	[NH_4^+] (µM)	[NO_2^-] (µM)	[NO_3^-] (µM)	[PO_4^{3-}] (µM)
2013	1S	30.9	3.53	8.11	53.99	377	1.37	0.28	2.09	2.51
August	1D	30.8	4.24	8.05		377	1.79	0.23	2.17	2.96
	3S	32.5	9.07	9.02	57.62	390	0.51	0.23	1.84	1.64
	3D	31.9	7.4	8.97		390	0.56	0.25	0.60	1.62
	7S	30.4	3.4	8.05	22.16	357	0.26	0.21	2.20	0.41
	7D	30.4	3.4	8.18		357	0.32	0.14	0.90	2.73
	10S	32.1	8.6	9.33	40.82	375	0.61	1.90	7.74	4.83
	10D	32.0	8.0	9.43		375	0.29	1.04	3.76	5.69
2014	1S	23.9	8.5	8.11	13.66	436	6.16	3.33	87.49	1.75
June	1D	22.7	5.1	8.07		437	8.34	3.36	87.09	0.69
	3S	27.2	8.6	8.73	11.05	419	1.09	1.72	58.29	0.24
	3D	25.4	7.3	8.71		411	1.20	2.61	57.41	0.35
	7S	22.8	9.7	7.85	42.41	383	1.55	0.83	66.32	0.39
	7D	22.5	8.6	7.69		384	1.59	0.74	61.59	2.13
	10S	26.3	5.6	8.89	79.52	424	35.39	14.93	69.98	2.43
	10D	26.4	5.5	8.6		424	35.75	15.13	68.93	2.52
2015	1S	11.6	10.05	8.34	7.48	393	2.49	0.55	53.89	0.20
March	1D	11.7	3.4	6.67		393	2.49	0.58	54.74	0.04
	3S	9.4	12.8	7.74	20.37	414	0.00	0.82	119.44	0.03
	3D	8.2	12.9	7.52		414	0.83	0.86	117.61	0.05
	7S	10.8	11.32	8.4	10.52	416	5.93	1.95	172.19	0.02
	7D	10.7	10.75	8.01		416	5.93	1.44	136.2	0.12
	10S	9.6	8.87	7.94	6.00	422	131.48	7.05	270.59	1.41
	10D	9.4	8.71	7.73		421	131.84	6.97	269.47	1.36
2016	1S	26.7	11.30	7.89	96.79	445	43.34	8.86	79.72	1.95
July	1D	25.5	7.55	7.67		458	20.03	6.71	58.78	1.31
	3S	26.1	7.0	8.50	100.99	410	17.59	0.86	3.81	1.05
	3D	26.3	7.3	8.50		410	21.08	0.72	3.87	1.16
	7S	25.8	10.03	7.95	13.22	465	0.33	0.08	16.39	0.03
	7D	25.1	8.88	7.88		466	0.25	0.11	16.52	0.05
	10S	25.6	4.10	7.75	21.31	470	13.41	9.66	93.96	2.43
	10D	23.4	4.10	7.62		470	65.31	8.45	66.77	3.18

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Table 2.
Details of non-parametric Kendall's correlation analysis. Statistically significant ($p < 0.05$) Kendall's Tau coefficients are bold.

		Temp	DO	pH	Chla	TDS	NH4	NO2	NO3	P	NH4:NO3
Uptake L	Kendall's T	-0.010	-0.061	**-0.326**	0.133	**0.321**	0.230	0.020	0.048	0.081	**0.301**
	p value	0.935	0.626	**0.009**	0.471	**0.010**	**0.064**	0.871	0.697	0.517	**0.016**
Uptake D	Kendall's T	-0.014	-0.041	**-0.293**	0.117	**0.337**	**0.295**	0.000	0.069	0.069	**0.369**
	p value	0.910	0.745	**0.019**	0.529	**0.007**	**0.018**	1.000	0.581	0.581	**0.003**
Regeneration	Kendall's T	0.095	-0.110	-0.103	0.300	**0.301**	**0.344**	0.149	0.012	**0.259**	**0.487**
	p value	0.446	0.381	0.408	0.105	**0.016**	**0.006**	0.230	0.923	**0.038**	**<0.001**
Nitrification	Kendall's T	-0.138	-0.128	-0.214	0.242	-0.058	**0.385**	**0.341**	**0.377**	**0.341**	0.272
	p value	0.346	0.385	0.143	0.273	0.691	**0.009**	**0.020**	**0.010**	**0.020**	0.063
NH_4^+ Ox.	Kendall's T	-0.080	-0.200	-0.011	-0.030	0.139	**0.575**	**0.514**	**0.406**	**0.543**	**0.461**
	p value	0.585	0.172	0.941	0.891	0.345	**<0.001**	**<0.001**	**0.005**	**<0.001**	**0.002**
NO_2^- Ox.	Kendall's T	-0.106	-0.081	-0.197	0.333	-0.077	**0.325**	0.266	**0.281**	0.266	0.277
	p value	0.471	0.585	0.180	0.131	0.602	**0.027**	0.070	**0.056**	0.070	0.059
AOA	Kendall's T	0.109	0.179	0.083	0.273	0.161	0.015	-0.014	-0.051	0.043	-0.004
	p value	0.457	0.224	0.568	0.217	0.275	0.921	0.921	0.728	0.766	0.980
AOB	Kendall's T	0.175	-0.157	-0.149	0.273	0.175	**0.458**	**0.341**	0.130	**0.500**	**0.425**
	p value	0.234	0.286	0.309	0.217	0.233	**0.002**	**0.020**	0.372	**0.001**	**0.004**

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Table 3.
Light (L) and dark (D) ammonium uptake and regeneration rates among different freshwater studies ± standard error.

	Uptake (L)	Uptake (D)	Reg Avg	Reference
Lake Lugano	0.017 ± 0.001	0.008 ± 0.003	0.010 ± 0.002	McCarthy unpublished
Lake Michigan	0.019 ± 0.004	0.01 ± 0.002	0.008 ± 0.001	Gardner et al. 2004
Lake Rotorua	0.114 ± 0.008	0.021 ± 0.005	0.047 ± 0.007	McCarthy unpublished
Lake Rotoiti	0.132 ± 0.033	0.08 ± 0.019	0.063 ± 0.018	McCarthy unpublished
Missisquoi Bay	0.205 ± 0.022	0.104 ± 0.015	0.085 ± 0.013	McCarthy et al. 2013
Lake Erie	0.258 ± 0.128	0.036 ± 0.009	0.124 ± 0.052	McCarthy unpublished
Lake Okeechobee	0.577 ± 0.006	0.029 ± 0.01	0.160 ± 0.021	James et al. 2011
Taihu Lake	0.655 ± 0.285	0.271 ± 0.111	0.325 ± 0.144	McCarthy et al. 2007
Taihu Lake	0.886 ± 0.09	0.399 ± 0.121	0.368 ± 0.071	This study
Old Woman Creek	0.925 ± 0.223	0.223 ± 0.043	0.320 ± 0.074	McCarthy et al. 2007

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Table 4.
Details of best-fitting multiple regression models determined by stepwise regression. All rates, temperature, and ambient nutrient concentrations were log-transformed prior to analysis.

Process	Variable	Parameter			Model		
		Estimate	Std. estimate	P	Adj. R^2	F	P
Uptake Light	T	1.048	0.216	0.0001	0.643	10.3	$9.14x10^{-6}$
	DO	0.053	0.012	0.0002			
	pH	-0.320	0.054	0.0000			
	NH_4^+	0.669	0.272	0.0213			
Uptake Dark	T	0.488	0.121	0.0005	0.745	16.1	$1.66x10^{-7}$
	DO	0.034	0.007	0.0000			
	pH	-0.187	0.031	0.0000			
	NH_4^+	0.579	0.153	0.0008			
	NO_2^-	-1.619	0.660	0.0215			
	NO_3^-	-0.098	0.034	0.0086			
Regeneration	T	0.321	0.098	0.0031	0.695	12.8	$1.42x10^{-6}$
	DO	0.025	0.005	0.0003			
	pH	-0.092	0.024	0.0008			
	NH_4^+	0.386	0.126	0.0053			
	NO_3^-	-0.061	0.027	0.0340			
Nitrification	NO_2^-	3.262	1.226	0.0165			
NH_4^+ Ox.	NO_2^-	3.917	0.806	0.0001	0.496	23.6	$7.35x10^{-5}$
NO_2^- Ox.	Chl a	0.655	0.275	0.0301	0.422	3.4	0.0202
	NH_4^+	4.227	1.529	0.0138			

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Figure 1

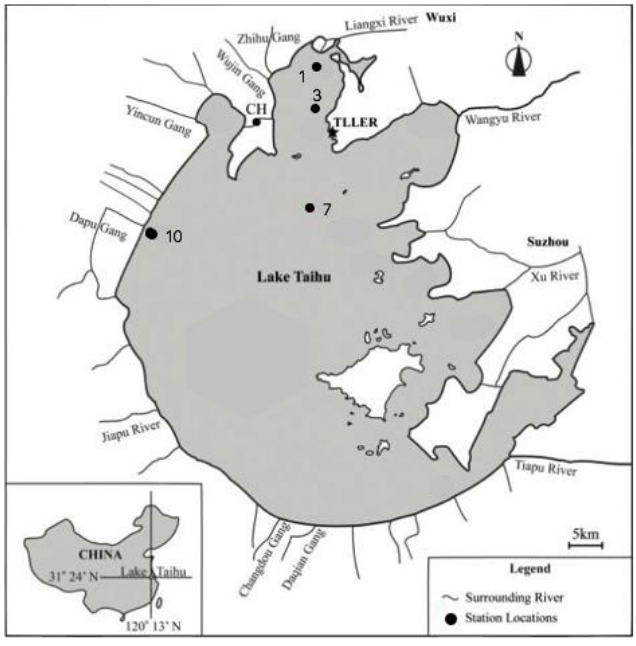

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Figure 2

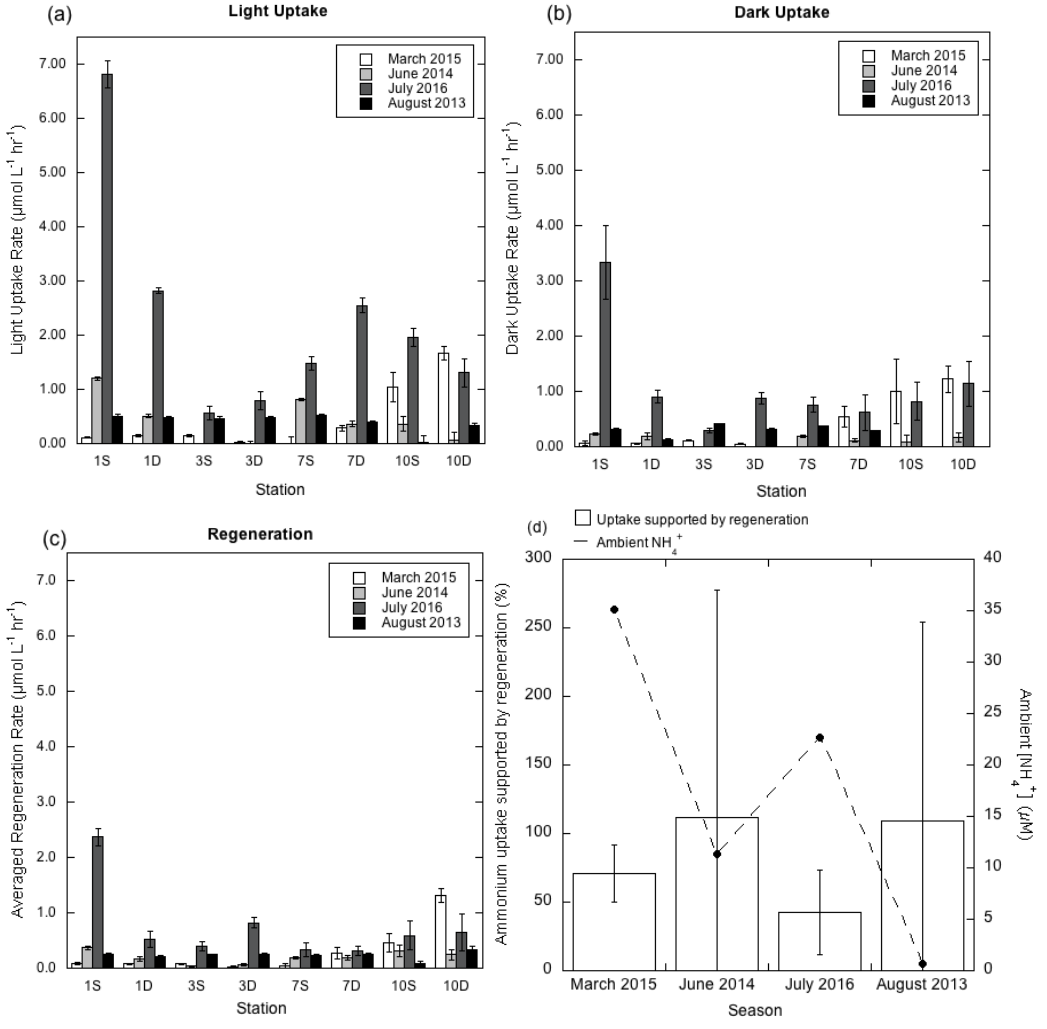

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Figure 3

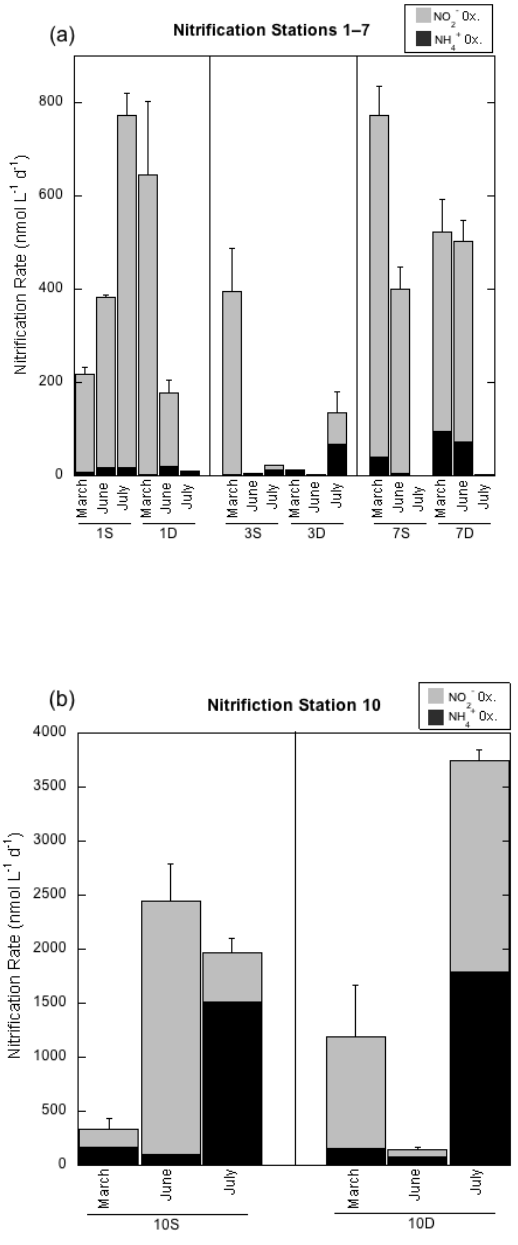

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Figure 4