



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

Justyna J. Hampel¹, Mark J. McCarthy^{1,2}, Wayne S. Gardner², Lu Zhang³, Hai Xu³, Guangwei Zhu³, and Silvia E. Newell¹

¹Department of Earth & Environmental Sciences, Wright State University, Dayton, OH, USA

²The University of Texas at Austin, Marine Science Institute, Port Aransas, TX, USA

³Taihu Laboratory for Lake Ecosystem Research, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing, China

Correspondence: Justyna J. Hampel (hampel.4@wright.edu)

Received: 8 September 2017 – Discussion started: 15 September 2017

Revised: 18 December 2017 – Accepted: 20 December 2017 – Published: 6 February 2018

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 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 to 6.80 $\mu\text{mol L}^{-1} \text{h}^{-1}$ in the light and from 0.05 to 3.33 $\mu\text{mol L}^{-1} \text{h}^{-1}$ in the dark, and NH_4^+ regeneration rates ranged from 0.03 to 2.37 $\mu\text{mol L}^{-1} \text{h}^{-1}$. Nitrification rates exceeded previously reported rates in most freshwater systems. Total nitrification often exceeded 200 $\text{nmol L}^{-1} \text{d}^{-1}$ and was > 1000 $\text{nmol L}^{-1} \text{d}^{-1}$ at one station near a river discharge. 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 ($p < 0.005$). Nitrification rates in Taihu Lake 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 were poor competitors for NH_4^+ during the bloom.

Regeneration results suggested that cyanobacteria relied extensively on regenerated NH_4^+ to sustain the bloom. Internal NH_4^+ regeneration exceeded external N loading to the lake by a factor of 2 but was ultimately fueled by external N loads. Our results thus support the growing literature calling for watershed N loading reductions in concert with existing management of P loads.

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_2 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 has correlated with 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 are 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 eutrophic systems (Gardner et al., 1998, 2017; McCarthy et al., 2013). For example, water column regeneration was up to 6 times higher than sediment regeneration in Taihu Lake, 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 Lake, the abundance of ammonia-oxidizing organisms (AOOs) was investigated in sediments where AOA outnumbered AOB, often by an order of magnitude (Wu et al., 2010; Zeng et al., 2012; Zhao et al., 2013). Another sediment study revealed that, while AOOs 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 re-

moval 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 AOOs and cyanobacteria, such as *Microcystis*, have a strong affinity for NH_4^+ (Martens-Habbena, 2009; Baldia et al., 2009), we are unaware of measurements made when AOOs and cyanobacteria were in direct competition. At some point in the bloom progression, cyanobacteria must outcompete AOOs for available NH_4^+ .

The overall objective of this study was to investigate seasonal NH_4^+ dynamics and the degree of competition between AOOs and cyanobacteria in hypereutrophic Taihu Lake. We measured community NH_4^+ uptake and regeneration rates, and nitrification 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 within the system via water column regeneration/remineralization; and (4) characterize the community composition of AOOs. We hypothesized that (1) lower nitrification rates occur during cyanoHABs due to increased competition for NH_4^+ ; (2) rates of nitrification are higher in Taihu Lake 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.

2 Methods

2.1 Site description and time frame

Taihu Lake (from the Chinese for “Great Lake”) is China’s third-largest freshwater lake. Due to industrial development and urbanization in the watershed, Taihu Lake has shifted from a diatom-dominated, mesotrophic lake to a hypereutrophic lake experiencing cyanoHABs (Paerl et al., 2014; Qin et al., 2007). Historically, these blooms have been 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 (Chen et al., 2003; Duan et al., 2009; Ma et al., 2016; Otten and Paerl 2011). 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 Lake is a large (2338 km²), 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

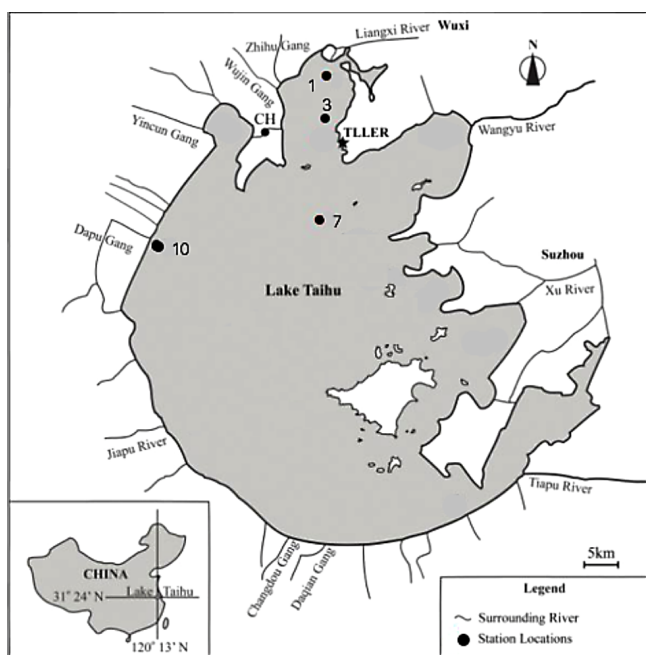


Figure 1. Map of sampling stations in Taihu Lake (modified from Paerl et al., 2011).

important source of freshwater and resources for the ~40 million people within the watershed. Taihu Lake 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. Taihu Lake has a relatively long residence time of approximately 280–300 days (Paerl et al., 2014; Xu et al., 2010).

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 Lake 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 – DO; pH; total dissolved solids – TDSs; and chlorophyll *a*) were measured in situ at each site using a YSI 6600 multi-sensor sonde.

Water samples were collected in August 2013, June 2014, March 2015, and July 2016. Each of these sampling events corresponded with a pronounced *Microcystis* bloom at all sites (Ma et al., 2016; Deng et al., 2014; Li et al., 2017; Su et al., 2017; Qian et al., 2017), except stations 7 and 10 in March 2015 (visual observation). Our sampling dates were representative of seasonal conditions in the region, specific to this subtropical climate zone, and did not correspond with any extreme weather patterns (e.g., typhoons, droughts). Temperature and precipitation patterns were average for this climate region. Water was collected into 4 L carboys at the surface (top 20 cm) and near the 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; August 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 isotope retention time shift (AIRTS) high-performance liquid chromatography (HPLC) at UTMSI (Gardner et al., 1995). Briefly, the atom % $^{15}\text{N-NH}_4^+$ 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. Five hundred milliliters from each site/depth was amended with 98 % $^{15}\text{NH}_4\text{Cl}$ (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). The goal of the substrate additions in these uptake/regeneration experiments was to add more-than-trace levels to ensure that all of the label was not taken up during the incubations; our goal was to add the label concentration at an equivalent value to the most recent monitoring data we could obtain for NH_4^+ concentrations, or at least 8 μM (even when concentrations are low, recycling rates can be quite high). 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 levels 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; regeneration is an actual rate that 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

Nitrification rates were measured directly using the $^{15}\text{NH}_4^+$ tracer addition method. Five hundred milliliters 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 98% $^{15}\text{NH}_4\text{Cl}$ (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 levels. Samples were returned frozen to WSU for analysis.

Accumulation of $^{15}\text{NO}_2^-$ was measured 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.

Accumulation of $^{15}\text{NO}_3^-$ was measured 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 $1000\times g$ 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 were sent inverted to the University of California Davis Stable Isotope Facility for isotopic analysis of $^{45}/^{44}\text{N}_2\text{O}$ using a Thermo Finnigan GasBench + PreCon trace gas concentration system interfaced to a Thermo Scientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany). Nitrification rates were corrected for NaN_3 reduction efficiency, and $^{15}\text{NO}_2^-$ production was calculated as

$$\text{NH}_3 \text{ Ox (in nM day}^{-1}\text{)} = \left(\left(\left[\frac{^{15}\text{N}}{^{14}\text{N}} \cdot [\text{NO}_2^-] \right]_{24\text{h}} - \left[\frac{^{15}\text{N}}{^{14}\text{N}} \cdot [\text{NO}_2^-] \right]_{0\text{h}} \right) / \alpha \cdot t, \right.$$

where $\alpha = [^{15}\text{NH}_4^+] / ([^{15}\text{NH}_4^+] + [^{14}\text{NH}_4^+])$.

$^{15}\text{NO}_3^-$ production was calculated as

$$\text{NO}_2^- \text{ Ox (in nM day}^{-1}\text{)} = \left(\left(\left[\frac{^{15}\text{N}}{^{14}\text{N}} \cdot [\text{NO}_3^-] \right]_{24\text{h}} - \left[\frac{^{15}\text{N}}{^{14}\text{N}} \cdot [\text{NO}_3^-] \right]_{0\text{h}} \right) / \alpha \cdot t, \right.$$

where $\alpha = [^{15}\text{NO}_2^-] / ([^{15}\text{NO}_2^-] + [^{14}\text{NO}_2^-])$.

Total nitrification rates were calculated from the sum of $^{15}\text{NO}_2^-$ and $^{15}\text{NO}_3^-$ accumulation.

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-buffered 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, Thermo Scientific). 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 10 μM primer, and 2–15 ng of template DNA.

All PCR work was performed in a PCR fume hood after cleaning the surface with DNA AWAY (Thermo Scientific, 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 s, 53 °C annealing for 45 s, and 72 °C extension for 1 min; 45 cycles) and AOB (95 °C initial denaturation for 5 min, 95 °C denaturation for 30 s, 56 °C annealing for 45 s, 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 $(\text{ng} \times \text{number mol}^{-1}) / (\text{bp} \times \text{ng g}^{-1} \times \text{g mol}^{-1} \text{ of bp})$ and is reported in gene copies per milliliter of sample water. The detection limit was 980 copies mL^{-1} for AOB and 4807 copies mL^{-1} for AOA. These calculated detection limits do not represent the greatest sensitivity possible with our method, as the standard concentrations were selected to bracket the expected environmental concentrations. Indeed, our reported values are above the detection limit for both AOA (by 2 orders of magnitude) and AOB.

2.5 Statistical analysis

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 TDSs. 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 to running the model.

3 Results

3.1 Lake ambient conditions

Physicochemical parameters in Taihu Lake varied seasonally and spatially (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 ($p < 0.001$). Chlorophyll *a* concentrations were lowest in March 2015 (mean = 11.1 $\mu\text{g L}^{-1}$), but bloom conditions ($> 20 \mu\text{g L}^{-1}$; Xu et al., 2015) were observed at some locations (e.g., 20.3 $\mu\text{g L}^{-1}$ at station 3, and visual confirmation at stations 1 and 3 and several other areas of the lake). Bloom conditions were also present and observed at all sites in June 2014 (mean = 36.6 $\mu\text{g L}^{-1}$), July 2016 (mean = 58.1 $\mu\text{g L}^{-1}$), and August 2013 (43.7 $\mu\text{g L}^{-1}$).

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 = $0.40 \pm 0.04 \mu\text{mol L}^{-1} \text{h}^{-1}$) and did not vary between surface and bottom waters (Fig. 2a). In June 2014, light uptake rates in surface waters at stations 1, 7, and 10 (mean = $0.80 \pm 0.06 \mu\text{mol L}^{-1} \text{h}^{-1}$) were significantly higher than deep rates (mean = $0.31 \pm 0.08 \mu\text{mol L}^{-1} \text{h}^{-1}$; $p < 0.001$). 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 ($0.25 \pm 0.01 \mu\text{mol L}^{-1} \text{h}^{-1}$) and June 2014 ($0.13 \pm 0.05 \mu\text{mol L}^{-1} \text{h}^{-1}$) were significantly lower than light uptake rates (Fig. 2b; $p < 0.05$). In March 2015, light uptake rates at stations 1–7 (mean = $0.12 \pm 0.04 \mu\text{mol L}^{-1} \text{h}^{-1}$) were lower than those during August 2013 and June 2014 (mean = $0.43 \pm 0.41 \mu\text{mol L}^{-1} \text{h}^{-1}$) except for station 10, where the rates were significantly higher (mean = $1.36 \pm 0.20 \mu\text{mol L}^{-1} \text{h}^{-1}$; $p < 0.001$). In contrast to summer, dark uptake rates in March 2015 were not significantly different than light rates (Fig. 2b). In July 2016,

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

Year/ month	Station	T ($^{\circ}\text{C}$)	DO (mgL^{-1})	pH	Chl a (μgL^{-1})	TDSs	$[\text{NH}_4^+]$ (μM)	$[\text{NO}_2^-]$ (μM)	$[\text{NO}_3^-]$ (μM)	$[\text{PO}_4^{3-}]$ (μM)
2013/August	1S	30.9	3.53	8.11	53.9	377	1.37	0.28	2.09	2.51
	1D	30.8	4.24	8.05		377	1.79	0.23	2.17	2.96
	3S	32.5	9.07	9.02	57.6	390	0.51	0.23	1.84	1.64
	3D	31.9	7.40	8.97		390	0.56	0.25	0.60	1.62
	7S	30.4	3.40	8.05	22.2	357	0.26	0.21	2.20	0.41
	7D	30.4	3.40	8.18		357	0.32	0.14	0.90	2.73
	10S	32.1	8.60	9.33	40.8	375	0.61	1.90	7.74	4.83
	10D	32.0	8.00	9.43		375	0.29	1.04	3.76	5.69
2014/June	1S	23.9	8.50	8.11	13.7	436	6.16	3.33	87.5	1.75
	1D	22.7	5.10	8.07		437	8.34	3.36	87.1	0.69
	3S	27.2	8.60	8.73	11.1	419	1.09	1.72	58.3	0.24
	3D	25.4	7.30	8.71		411	1.20	2.61	57.4	0.35
	7S	22.8	9.70	7.85	42.4	383	1.55	0.83	66.3	0.39
	7D	22.5	8.60	7.69		384	1.59	0.74	61.6	2.13
	10S	26.3	5.60	8.89	79.5	424	35.4	14.9	70.0	2.43
	10D	26.4	5.50	8.60		424	35.7	15.1	68.9	2.52
2015/March	1S	11.6	10.1	8.34	7.5	393	2.49	0.55	53.9	0.20
	1D	11.7	3.40	6.67		393	2.49	0.58	54.7	0.04
	3S	9.4	12.8	7.74	20.4	414	< 0.04	0.82	119.4	0.03
	3D	8.2	12.9	7.52		414	0.83	0.86	117.6	0.05
	7S	10.8	11.3	8.40	10.5	416	5.93	1.95	172.2	0.02
	7D	10.7	10.7	8.01		416	5.93	1.44	136.2	0.12
	10S	9.6	8.90	7.94	6.0	422	131	7.05	270.6	1.41
	10D	9.4	8.71	7.73		421	132	6.97	269.5	1.36
2016/July	1S	26.7	11.3	7.89	96.8	445	43.3	8.86	79.7	1.95
	1D	25.5	7.55	7.67		458	20.0	6.71	58.8	1.31
	3S	26.1	7.00	8.50	101.0	410	17.6	0.86	3.81	1.05
	3D	26.3	7.30	8.50		410	21.1	0.72	3.87	1.16
	7S	25.8	10.0	7.95	13.2	465	0.33	0.08	16.4	0.03
	7D	25.1	8.88	7.88		466	0.25	0.11	16.5	0.05
	10S	25.6	4.10	7.75	21.3	470	13.4	9.66	94.0	2.43
	10D	23.4	4.10	7.62		470	65.3	8.45	66.8	3.18

* Nutrient analysis detection limits: $\text{NH}_4^+ = 0.04 \mu\text{M}$; $\text{NO}_x = 0.04 \mu\text{M}$; $\text{OP} = 0.008 \mu\text{M}$.

light uptake rates were highest at stations 1, 7, and 10 ($1.31\text{--}6.82 \mu\text{molL}^{-1} \text{h}^{-1}$). Stations 3 and 7 rates were highest in bottom waters (0.80 ± 0.16 and $2.55 \pm 0.14 \mu\text{molL}^{-1} \text{h}^{-1}$, 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 ($3.33 \pm 0.67 \mu\text{molL}^{-1} \text{h}^{-1}$). Light uptake rates, across all stations and seasons, correlated positively with TDSs and $\text{NH}_4^+ : \text{NO}_3^-$ and negatively with pH, while dark uptake rates correlated positively with TDSs, NH_4^+ , and $\text{NH}_4^+ : \text{NO}_3^-$, 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 = $0.22 \pm 0.03 \mu\text{molL}^{-1} \text{h}^{-1}$), but July 2016 regeneration rates (mean = $0.75 \pm 0.16 \mu\text{molL}^{-1} \text{h}^{-1}$) 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 = $2.37 \pm 0.16 \mu\text{molL}^{-1} \text{h}^{-1}$). In March 2015, mean surface and deep

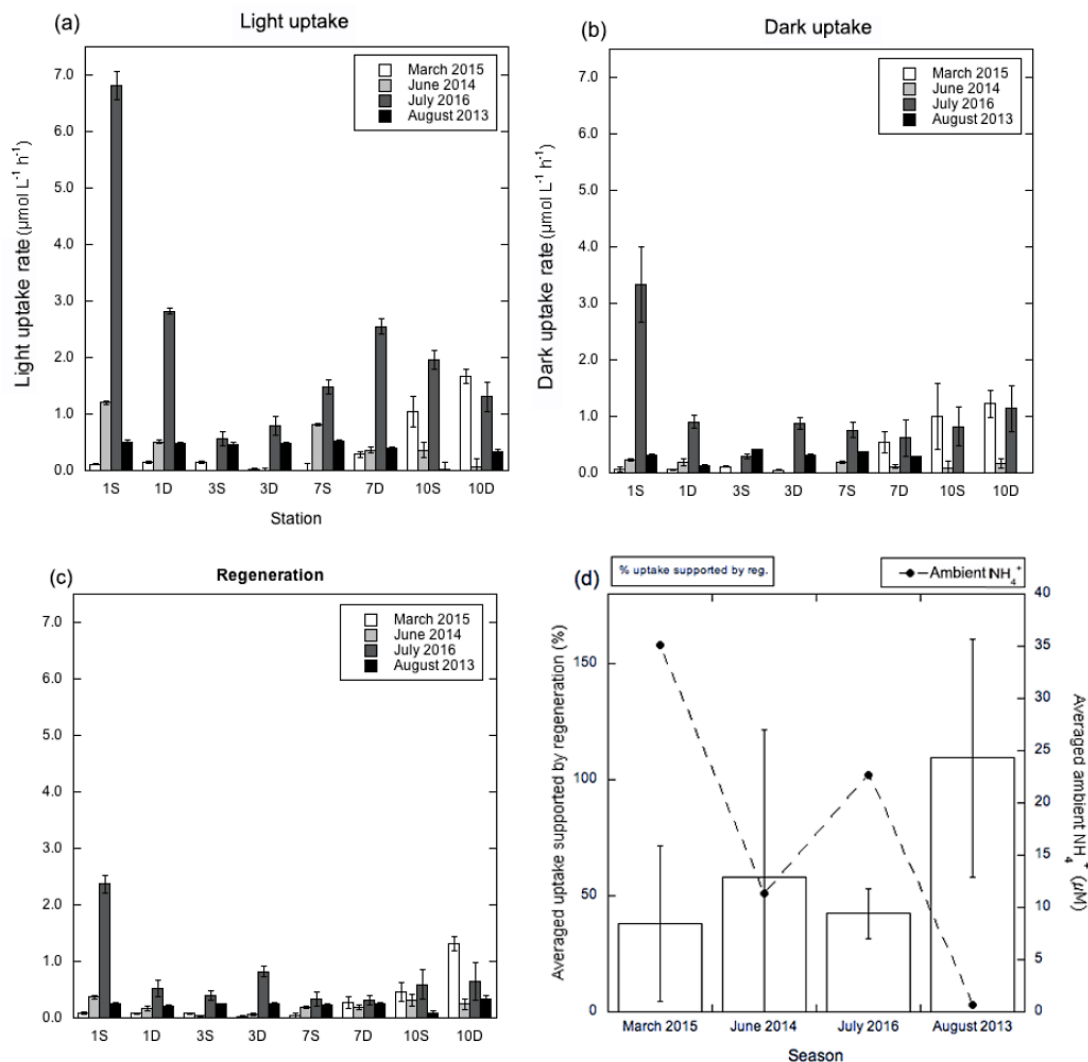


Figure 2. Ammonium dynamics in Taihu Lake. (a) Potential light uptake rates ± 1 standard error. (b) Potential dark uptake rates ± 1 standard error. (c) Mean light and dark regeneration rates ± 1 standard error. (d) Seasonal averaged percent of light uptake supported by regeneration ± 1 standard error and averaged in situ NH_4^+ concentrations.

regeneration rates decreased from the river mouth (station 10; $0.88 \pm 0.15 \mu\text{mol L}^{-1} \text{h}^{-1}$) towards the center of the lake, with significantly higher regeneration rates at station 10 than stations 1–7 (mean = $0.10 \pm 0.03 \mu\text{mol L}^{-1} \text{h}^{-1}$; $p < 0.01$). Regeneration rates were positively correlated with TDSs, NH_4^+ , and o-PO_4^{3-} concentrations, and $\text{NH}_4^+ : \text{NO}_3^-$ (Table 2).

3.4 Nitrification (2014–2016)

Note that nitrification rates are presented in units of nanomoles per liter per day ($\text{nmol L}^{-1} \text{d}^{-1}$) for consistency with literature-reported values (Fig. 3). At stations 1, 3, and 7 $^{15}\text{NH}_4^+$ additions, 91.8% of the label was detected as $^{15}\text{NO}_3^-$ and only 8.2% as $^{15}\text{NO}_2^-$ (Fig. 3a). 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 2015 (mean = $663 \pm 69.4 \text{ nmol L}^{-1} \text{d}^{-1}$) in both surface and deep waters compared to the lowest rates in July 2016 (mean = $1.58 \pm 0.78 \text{ nmol L}^{-1} \text{d}^{-1}$). At station 1, the highest rates were measured in surface waters in July 2016 (mean = $773 \pm 50.7 \text{ nmol L}^{-1} \text{d}^{-1}$), but the rates at depth followed a seasonal pattern from high in the spring (mean = $646 \pm 158 \text{ nmol L}^{-1} \text{d}^{-1}$) to an order of magnitude lower in the summer (mean = $9.86 \pm 3.28 \text{ nmol L}^{-1} \text{d}^{-1}$).

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 $148\text{--}3750 \text{ nmol L}^{-1} \text{d}^{-1}$ (mean = $1590 \pm 1390 \text{ nmol L}^{-1} \text{d}^{-1}$), compared to stations 1–7 ranging $2.00\text{--}771 \text{ nmol L}^{-1} \text{d}^{-1}$ (mean = $270 \pm 277 \text{ nmol L}^{-1} \text{d}^{-1}$). At sta-

Table 2. Details of non-parametric Kendall's correlation analysis. Statistically significant ($p < 0.05$) Kendall's Tau (T) coefficients are bold.

		Temp	DO	pH	Chl <i>a</i>	TDSs	NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻	PO ₄ ³⁻	NH ₄ ⁺ : NO ₃ ⁻
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
	<i>p</i> 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
	<i>p</i> 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
	<i>p</i> 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
	<i>p</i> value	0.346	0.385	0.143	0.273	0.691	0.009	0.020	0.010	0.020	0.063
AOA	Kendall's T	0.109	0.179	0.083	0.273	0.161	0.015	-0.014	-0.051	0.043	-0.004
	<i>p</i> 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
	<i>p</i> value	0.234	0.286	0.309	0.217	0.233	0.002	0.020	0.372	0.001	0.004

tion 10 in July 2016, 80 % of the ¹⁵NH₄⁻ addition was detected as ¹⁵NO₂⁻.

3.5 Ammonia oxidizer abundance

Abundance of the bacterial *amoA* gene for all years (2014–2016) varied from undetectable to $2.85 \times 10^5 \pm 5.20 \times 10^4$ copies mL⁻¹. Archaeal *amoA* abundance ranged from undetectable to $1.03 \times 10^7 \pm 3.37 \times 10^6$ 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 (1.81) was reported at station 3 in Meiliang Bay (Fig. 4b), and the lowest ratio (0.01) was observed at station 7. AOB gene abundance was positively correlated with NH₄⁺, NO₂⁻, and o-PO₄³⁻ concentrations, and NH₄⁺ : NO₃⁻, while AOA gene abundance was not significantly correlated with any environmental variable (Table 2).

4 Discussion

4.1 Ammonium regeneration and potential uptake

Ammonium uptake rates (0.02–6.82 μmol L⁻¹ h⁻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 h⁻¹) and the central lake (0.03–0.32 μmol L⁻¹ h⁻¹) but within the range of rates reported in the Liangxihe River (0.70–4.19 μmol L⁻¹ h⁻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, and eutrophic New Zealand lakes Rotorua and Rotoiti (Table 3 and references therein). Higher light uptake rates were reported only in hypereutrophic Lake Maracaibo,

Venezuela (Table 3), and in Maumee Bay, Lake Erie, during a summer cyanoHAB bloom (Gardner et al., 2017). Potential NH₄⁺ uptake rates in these systems, evaluated using the same methods, increase with chlorophyll *a* ($p < 0.05$), but the proportion of community uptake that can be supported by regeneration remains relatively consistent (Table 3).

Light uptake rates in Taihu Lake were marginally higher ($p = 0.08$) than dark uptake rates, presumably due to reduced photosynthetic phytoplankton activity. Photoautotrophs may continue to assimilate nutrients in the dark under nutrient limitation (Cochlan et al., 1991), but Taihu Lake is generally nutrient replete, so we assume that dark uptake rates can be attributed mostly to heterotrophic or chemolithoautotrophic organisms. 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 (<https://www.worldweatheronline.com/>; accessed on 8 February 2017); however, it is within the range of typical summer rainfall (185–320 mm; <https://www.worldweatheronline.com/>). Dark uptake rates in Taihu Lake exceeded dark rates reported in Lake Okeechobee (0.02–0.04 μmol L⁻¹ h⁻¹; James et al., 2011), Missisquoi Bay, Lake Champlain (0.10 μmol L⁻¹ h⁻¹; McCarthy et al., 2013), and Lake Michigan (7 nmol L⁻¹ h⁻¹; Gardner et al., 2004), suggesting high activity of both heterotrophs and chemolithoautotrophs in Taihu Lake. A previous metagenomics study of the bloom composition in Taihu Lake 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 NH₄⁺ cycling via regeneration is important in Taihu Lake and varies seasonally (McCarthy et al., 2007;

Table 3. Comparison of ammonium dynamics (in $\mu\text{mol L}^{-1} \text{h}^{-1}$) and chlorophyll *a* concentrations among different freshwater studies.

	Uptake (Light)	Uptake (Dark)	Regeneration	Chl <i>a</i> ($\mu\text{g L}^{-1}$)	Reference
Lake Lugano	0.017 ± 0.001	0.008 ± 0.003	0.010 ± 0.002	< 2.00	McCarthy (unpublished)
Lake Michigan	0.019 ± 0.004	0.01 ± 0.002	0.008 ± 0.001	2.44	Gardner et al. (2004)
Lake Rotorua	0.114 ± 0.008	0.021 ± 0.005	0.047 ± 0.007	23.3	Gardner et al. (2017)
Lake Rotoiti	0.132 ± 0.033	0.08 ± 0.019	0.063 ± 0.018	7.66	Gardner et al. (2017)
Missisquoi Bay	0.205 ± 0.022	0.104 ± 0.015	0.085 ± 0.013	16.2	McCarthy et al. (2013)
Lake Erie	0.258 ± 0.128	0.036 ± 0.009	0.124 ± 0.052	19.9	McCarthy (unpublished)
Lake Okeechobee	0.577 ± 0.006	0.029 ± 0.01	0.160 ± 0.021	16.8	James et al. (2011)
Taihu Lake	0.655 ± 0.285	0.271 ± 0.111	0.325 ± 0.144	11.5	McCarthy et al. (2007)
Taihu Lake	0.886 ± 0.09	0.399 ± 0.121	0.368 ± 0.071	37.4	This study
Lake Maracaibo	3.35 ± 0.795	2.73 ± 0.643	0.389 ± 0.175	22.0	Gardner et al. (1998)

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 (109 %). The importance of regeneration corresponded to decreasing in situ NH_4^+ concentrations (Fig. 2d). These results suggest that, in March and June, regeneration supplemented ambient NH_4^+ in the water column to support algal production, whereas cyanobacteria relied more heavily on NH_4^+ from regeneration to sustain blooms in July and August. Water column regeneration may supply more NH_4^+ for blooms than sediment NH_4^+ regeneration in Taihu Lake due to combined spatial, temperature, and biogeochemical factors (McCarthy et al., 2007; Gardner et al., 2017). Rapid decomposition of cyanoHAB biomass may provide NH_4^+ for nitrification, which provides substrate for denitrification. High rates of sediment denitrification (McCarthy et al., 2007) also may drive N limitation in late summer and fall (Paerl et al., 2011; Xu et al., 2010)

To calculate whole-lake water column NH_4^+ regeneration and uptake rates, we divided the lake (2338 km²; Qin et al., 2007) into four different sections based on geochemical and ecological properties (Qin, 2008): (1) three northern bays (361.8 km²; depth = 1.9 m) most affected by the blooms; (2) the main lake (1523.9 km²; depth = 1.9 m); (3) the east Taihu Lake region, dominated by rooted and floating macrophytes (357.5 km²; depth = 1.4 m); and (4) shorelines < 1 m deep (94.8 km²). We considered regeneration and uptake 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 Lake (McCarthy et al., 2007; Paerl et al., 2011). When extrapolated to the volume of these four zones in Taihu Lake, regeneration returned about 3.04×10^7 kg of NH_4^+ annually in the three northern bays, 6.71×10^7 kg of NH_4^+ in the main lake, 8.87×10^6 kg of NH_4^+ along the shorelines, and 2.88×10^6 kg of NH_4^+ in east Taihu Lake. These values sum to 1.09×10^8 kg of NH_4^+ recycled in the water column, approximately 2 times

higher than reported external N loadings, which range from 5.11×10^7 to 7.00×10^7 kg annually (Chen et al., 2012; Yan et al., 2011). The same procedure for extrapolation of whole-lake uptake rates yields 3.5×10^8 kg of NH_4^+ , which is 4–6 times higher than external N loads. The combination of external loads and regeneration cannot support the demand for NH_4^+ , suggesting that the remaining NH_4^+ demand must be satisfied by internal loads from sediments or some other unknown source, or that reported total nitrogen (TN) loads are underestimated. These rough estimates of lake-wide regeneration and uptake are based on rates measured at specific stations at discreet times; improved spatial and temporal resolution of measurements is needed to improve these estimates. Additionally, these calculated values are probably an overestimate given that most of the rates measured and reported in this study are during spring and summer months, not fall and winter, when we might expect lower rates. Taihu Lake 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 Lake are likely an underestimate. However, our results show that these external N loads lead to higher biomass and fuel high regeneration rates. Combined with high ambient nutrient concentrations, these data suggest that microbial denitrification cannot remove N fast enough to keep pace with external N loading. Increasing nutrient loads 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

Total 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, USA ($60\text{--}480 \text{ nmol L}^{-1} \text{ d}^{-1}$; Carini and Joye, 2008), and Lake Superior, USA ($0\text{--}51 \text{ nmol L}^{-1} \text{ d}^{-1}$; Small et al., 2013),

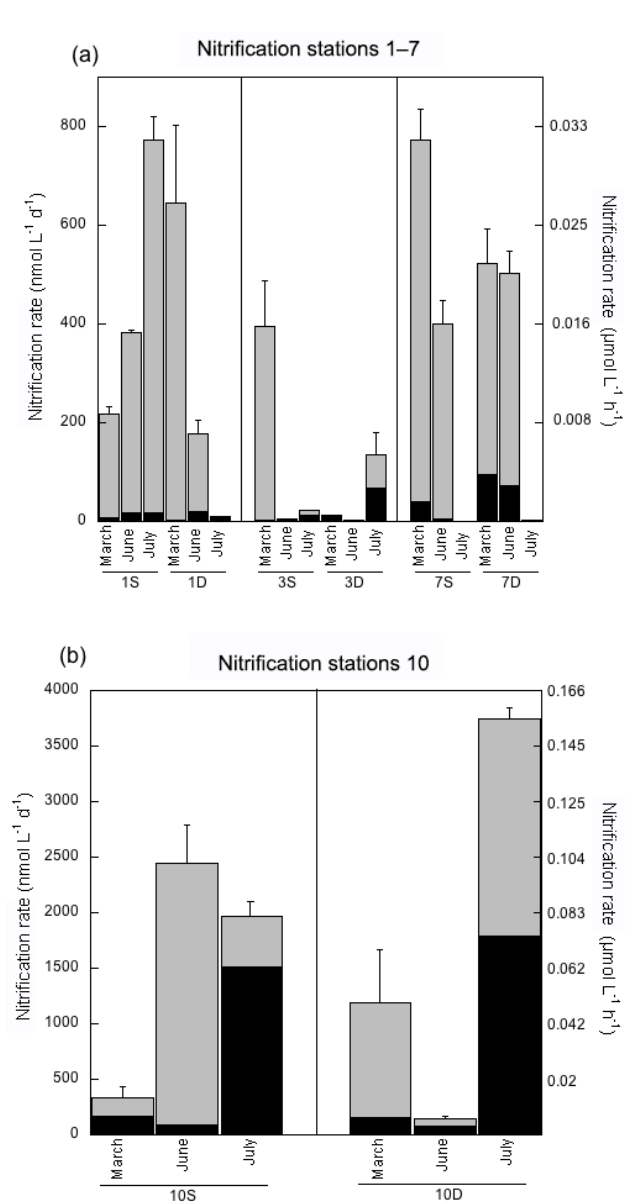


Figure 3. Total nitrification rates calculated from accumulation of $^{15}\text{NO}_2^-$ (grey) and $^{15}\text{NO}_3^-$ (black) ± 1 standard deviation. (a) Stations 1–7. (b) Station 10. The two axes show different units for total nitrification rates: $\text{nmol L}^{-1} \text{d}^{-1}$ and $\mu\text{mol L}^{-1} \text{h}^{-1}$.

both measured via $^{15}\text{NH}_4^+$ tracer additions, and Lake Okechobee, FL, USA ($67\text{--}97 \text{ nmol L}^{-1} \text{h}^{-1}$; James et al., 2011), measured via the $^{15}\text{NO}_3^-$ pool dilution method (Carini et al., 2010). Rates on this scale were previously reported only in eutrophic Lake Mendota (WI, USA; $1700\text{--}26\,000 \text{ nmol L}^{-1} \text{h}^{-1}$; Hall, 1986) and the Pearl River estuary (China; $2100\text{--}65\,100 \text{ nmol L}^{-1} \text{d}^{-1}$; Dai et al., 2008). However, these rates were measured from accumulation of NO_2^- and NO_3^- , not stable-isotope additions. High total nitrification rates in Taihu Lake can be attributed to high am-

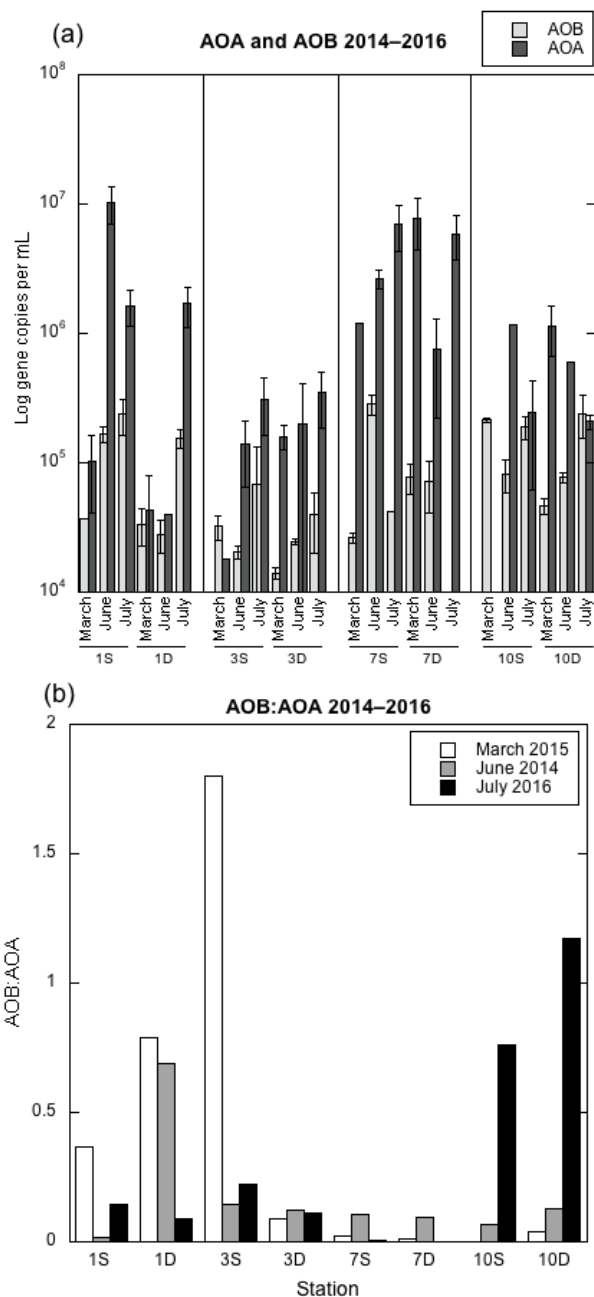


Figure 4. Ammonia-oxidizing organism population characteristics. (a) Ammonia oxidizer abundance (DNA) ± 1 standard deviation. (b) Ratio of abundance of AOB to AOA.

bient NH_4^+ concentrations, up to $40 \mu\text{M}$ at station 1 in 2016 and $135 \mu\text{M}$ 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 $\sim 1.32 \times 10^7 \text{ kg}$ was loaded as NH_4^+ in 2009 (Yan et al., 2011). The significant relationships between nitrification and NH_4^+ , NO_2^- , and NO_3^- concentrations ($p < 0.05$; Table 2) support these observations.

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). Accumulation of $^{15}\text{NO}_3^-$ exceeded accumulation of $^{15}\text{NO}_2^-$ by a factor of 9 at stations 1, 3, and 7 across all sampling events (Fig. 3a), indicating that NO_2^- oxidation is keeping pace with or exceeding NH_4^+ oxidation. Higher accumulation of $^{15}\text{NO}_3^-$ was expected, since NO_3^- is the final product of total nitrification.

At station 10, accumulation of $^{15}\text{NO}_3^-$ exceeded $^{15}\text{NO}_2^-$ in March 2015 and June 2014. In July 2016, however, accumulation of $^{15}\text{NO}_2^-$ was 3 times higher in surface water and comparable at depth (Fig. 3b). Ambient NO_2^- concentration at station 10 in July 2016 was $9.6\ \mu\text{M}$ in surface water and $8.4\ \mu\text{M}$ at depth (Table 1). This accumulation of NO_2^- suggests that NO_2^- oxidizers were saturated, consistent with K_m values reported for NO_2^- oxidation in the oligotrophic open ocean of $0.25 \pm 0.16\ \mu\text{M}$ (Sun et al., 2017). However, culture experiments report K_m values ranging from 6 to $544\ \mu\text{M}$ 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 Lake 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. As stated above, chemolithoautotrophs (including nitrifiers) do not rely on light for energy and continue to assimilate NH_4^+ in dark conditions, while photoautotrophic cyanobacteria generally assimilate NH_4^+ in the dark only when nutrient limited (Cochlan et al., 1991). 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). Although not statistically linked to changes in temperature, the contribution of nitrification to total uptake rates decreased in summer months, 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^+ in high-nutrient environments (Blomqvist et al., 1994). With a high saturation threshold and reported K_m values from 26.5 to $37\ \mu\text{M}$ (Baldia et al., 2007; Nicklisch and Kohl 1983) in culture, and up to $112.9\ \mu\text{M}$ in Taihu Lake populations (Yang

et al., 2017), *Microcystis* should be able to outcompete nitrifiers at the high ambient NH_4^+ concentrations in Taihu Lake as nitrifiers may become saturated at much lower concentrations. 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 station 10 differed dramatically from other stations. Total nitrification 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. 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 ($K_m = 0.132\ \mu\text{M}$; 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 – $1000\ \mu\text{M}$) 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).

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	SD estimate	P	Adj. R^2	F	P
Uptake light	T	1.048	0.216	0.0001	0.643	10.3	9.14×10^{-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	1.66×10^{-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	1.42×10^{-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	0.498	4.80	0.004

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

The differences in abundance of AOs 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 = $3.69 \mu\text{M}$). In contrast, AOB, with higher K_m , thrive at higher NH_4^+ concentrations at nearshore locations (mean nearshore NH_4^+ concentration = $31.3 \mu\text{M}$). These results agree with previous research in Taihu Lake, where AOA outnumbered AOB in sediments at mesotrophic sites and AOB were more abundant at hypereutrophic locations (Hou et al., 2013). Another study on Taihu Lake sediments also reported that both AOA abundance and AOA : AOB were negatively correlated with ambient NH_4^+ concentration (Wu et al., 2010). However, the data reported in this study show no

significant relationship between AOA abundance and NH_4^+ , NO_2^- , and NO_3^- concentrations (Table 2).

Despite AOA outnumbering AOB, AOB abundance was correlated with total nitrification rates for all stations and all seasons ($p < 0.005$), but AOA abundance was not. This result agrees with a previous study on Taihu Lake sediments, where AOA were negatively correlated ($r = 0.53$, $p < 0.05$) with potential nitrification rates (0 – $3.0 \mu\text{gNO}_3^- \text{Ng}^{-1}$ dry sediment; Hou et al., 2013). We speculate that AOA oxidized NH_4^+ at lower rates due to oversaturation and inhibition and may not have contributed as much as AOB to nitrification rates in our study. This conclusion was also reached in Plum Island Sound (MA, USA), where abundance of archaeal *amoA* was higher than bacterial *amoA*, but potential nitrification rates did not correlate with AOA (Bernhard et al., 2010). The authors hypothesized various scenarios, including inhibition of AOA due to high substrate concentrations, competition for NH_4^+ with AOB, or AOA using an alternative energy source (Bernhard et al., 2010). Our results support the interpretation that AOA are at a disadvantage when competing with AOB for NH_4^+ in a hypereutrophic system and most likely did not play a major role in observed nitrification in Taihu Lake. Recent studies show that AOA can oxidize cyanate (Palatinszky et al., 2015) and urea (Tolar et al., 2016), although growth and oxidation rates may be slow. Therefore, AOA may play an expanded role in Taihu Lake, beyond just NH_4^+ oxidation.

4.4 Multiple-regression model

The best-fitting multiple-regression models for N dynamics in Taihu Lake (Table 4) supported the Kendall non-parametric analysis (Table 2). Ammonium uptake and regeneration rates and nitrification were correlated with ambient NH_4^+ , NO_2^- , and NO_3^- concentrations. Additionally, the best-fitting models revealed that variables changing 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, the model for nitrification 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 in providing a large proportion of the substrate necessary to sustain cyanoHABs. The results also show that nitrification does not account for a large proportion of NH_4^+ demand during cyanoHABs in Taihu Lake. 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 AOOs 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.

Data availability. All relevant environmental data are included as graphics and tables in the paper. All raw data will be made available on request.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgements. We thank Guang Gao for laboratory space at NIGLAS and Kaijun Lu and other graduate students at NIGLAS and TLLER for help in the field and in the lab. We also thank Richard Doucett at the UC Davis Stable Isotope Facility for ^{15}N sample analysis and Justin Myers, Megan Reed, and Ashlynn Boedecker at WSU for help with nutrient analysis. We also thank Daniel Hoffman at WSU for valuable help with nitrification experiments and Elise Heiss for her input on statistical analysis. This work was jointly supported by the International Science & Technology Cooperation Program of China (2015DFG91980) and the National Natural Science Foundation of China (41573076, 41771519).

Edited by: Jack Middelburg

Reviewed by: two anonymous referees

References

- Akaike, H.: A new look at the statistical model identification, *IEEE T. Automat. Contr.*, 19, 716–723, 1974.
- An, S. and Joye, S. B.: Enhancement of coupled nitrification-denitrification by benthic photosynthesis in shallow estuarine sediments, *Limnol. Oceanogr.*, 46, 62–74, <https://doi.org/10.4319/lo.2001.46.1.0062>, 2001.
- Baldia, S. F., Evangelista, A. D., Aralar, E. V., and Santiago, A. E.: Nitrogen and phosphorus utilization in the cyanobacterium *Microcystis aeruginosa* isolated from Laguna de Bay, Philippines, *J. Appl. Phycol.*, 19, 607–613, <https://doi.org/10.1007/s10811-007-9209-0>, 2007.
- Beman, J. M., Popp, B. N., and Francis, C. A.: Molecular and biogeochemical evidence for ammonia oxidation by marine Crenarchaeota in the Gulf of California, *ISME J.*, 2, 429–441, <https://doi.org/10.1038/ismej.2008.33>, 2008.
- Bernhard, A. E., Landry, Z. C., Blevins, A., De La Torre, J. R., Giblin, A. E., and Stahl, D. A.: Abundance of ammonia-oxidizing archaea and bacteria along an estuarine salinity gradient in relation to potential nitrification rates, *Appl. Environ. Microbiol.*, 76, 1285–1289, <https://doi.org/10.1128/AEM.02018-09>, 2010.
- Blackburn, T. H.: Method for Measuring Rates of NH_4^+ Turnover in Anoxic Marine Sediments, Using a ^{15}N - NH_4^+ Dilution Technique, *Appl. Environ. Microbiol.*, 37, 760–765, 1979.
- Blackburne, R., Vadivelu, V. M., Yuan, Z., and Keller, J.: Kinetic characterisation of an enriched *Nitrospira* culture with comparison to *Nitrobacter*, *Water Res.*, 41, 3033–3042, <https://doi.org/10.1016/j.watres.2007.01.043>, 2007.
- Blomqvist, P., Petterson, A., and Hyenstrand, P.: Ammonium-nitrogen: a key regulatory factor causing dominance of non-nitrogen-fixing cyanobacteria in aquatic systems, *Arch. Hydrobiol.*, 132, 141–164, 1994.
- Bollmann, A., Bullerjahn, G. and McKay, R. M.: Abundance and diversity of ammonia-oxidizing archaea and bacteria in sediments of trophic end members of the Laurentian Great Lakes, Erie and Superior, *PLoS ONE*, 9, e97068, <https://doi.org/10.1371/journal.pone.0097068>, 2014.

- Bristow, L. A., Sarode, N., Cartee, J., Caro-Quintero, A., Thamdrup, B., and Stewart, F. J.: Biogeochemical and metagenomic analysis of nitrite accumulation in the Gulf of Mexico hypoxic zone, *Limnol. Oceanogr.*, 60, 1733–1750, 2015.
- Brookes, J. D. and Ganf, G. G.: Variations in the buoyancy response of *Microcystis aeruginosa* to nitrogen, phosphorus and light, *J. Plankton Res.*, 23, 1399–1411, 2001.
- Bruesewitz, D. A., Gardner, W. S., Mooney, R. F., and Buskey, E. J.: Seasonal water column NH_4^+ cycling along a semi-arid sub-tropical river/estuary continuum: Responses to episodic events and drought conditions, *Ecosystems*, 18, 792–812, <https://doi.org/10.1007/s10021-015-9863-z>, 2015.
- Caperon, J., Schell, D., Hirota, J., and Laws, E.: Ammonium excretion rates in Kaneohe Bay, Hawaii, measured by a ^{15}N isotope dilution technique, *Mar. Biol.*, 54, 33–40, <https://doi.org/10.1007/BF00387049>, 1979.
- Carini, S. A. and Joye, S. B.: Nitrification in Mono Lake, California: Activity and community composition during contrasting hydrological regimes, *Limnol. Oceanogr.*, 53, 2546–2557, <https://doi.org/10.4319/lo.2008.53.6.2546>, 2008.
- Carini, S. A., McCarthy, M. J., and Gardner, W. S.: An isotope dilution method to measure nitrification rates in the northern Gulf of Mexico and other eutrophic waters, *Cont. Shelf Res.*, 30, 1795–1801, <https://doi.org/10.1016/j.csr.2010.08.001>, 2010.
- Chen, Y., Qin, B., Teubner, K., and Dokulil, M.: Long-term dynamics of phytoplankton assemblages: *Microcystis*-domination in Lake Taihu, a large shallow lake in China, *J. Plankton Res.*, 25, 445–453, 2003.
- Chen, X. F., Chuai, X. M., Zeng, J., Liu, T., and Yang, L. Y.: Nitrogenous fluxes and its self-purification capacity in Lake Taihu, *Huan Jing Ke Xue*, 33, 2309–2314, 2012.
- Cochlan, W. P., Harrison, P. J., and Denman, K. L.: Diel periodicity of nitrogen uptake by marine phytoplankton in nitrate-rich environments, *Limnol. Oceanogr.*, 36, 1689–1700, 1991.
- Dai, M., Wang, L., Guo, X., Zhai, W., Li, Q., He, B., and Kao, S.-J.: Nitrification and inorganic nitrogen distribution in a large perturbed river/estuarine system: the Pearl River Estuary, China, *Biogeosciences*, 5, 1227–1244, <https://doi.org/10.5194/bg-5-1227-2008>, 2008.
- Daims, H., Lebedeva, E. V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R. H., Bergen, M. von, Rattei, T., Bendinger, B., Nielsen, P. H., and Wagner, M.: Complete nitrification by *Nitrospira* bacteria, *Nature*, 528, 504–509, <https://doi.org/10.1038/nature16461>, 2015.
- Deng, J., Qin, B., Paerl, H. W., Zhang, Y., Ma, J., and Chen, Y.: Earlier and warmer springs increase cyanobacterial (*Microcystis* spp.) blooms in subtropical Lake Taihu, China, *Freshwater Biol.*, 59, 1076–1085, <https://doi.org/10.1111/fwb.12330>, 2014.
- Duan, H., Ma, R., Xu, X., Kong, F., Zhang, S., Kong, W., Hao, J., and Shang, L.: Two-decade reconstruction of algal blooms in China's Lake Taihu, *Environ. Sci. Technol.*, 43, 3522–3528, 2009.
- Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., and Smith, J. E.: Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems, *Ecol. Lett.*, 10, 1135–1142, <https://doi.org/10.1111/j.1461-0248.2007.01113.x>, 2007.
- Francis, C. A., Roberts, K. J., Beman, J. M., Santoro, A. E., and Oakley, B. B.: Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean, *P. Natl. Acad. Sci. USA*, 102, 14683–14688, <https://doi.org/10.1073/pnas.0506625102>, 2005.
- Füssel, J., Lam, P., Lavik, G., Jensen, M. M., Holtappels, M., Günter, M., and Kuypers, M. M. M.: Nitrite oxidation in the Namibian oxygen minimum zone, *ISME J.*, 6, 1200–1209, <https://doi.org/10.1038/ismej.2011.178>, 2012.
- Gardner, W. S. and McCarthy, M. J.: Nitrogen dynamics at the sediment-water interface in shallow, sub-tropical Florida Bay: Why denitrification efficiency may decrease with increased eutrophication, *Biogeochemistry*, 95, 185–198, <https://doi.org/10.1007/s10533-009-9329-5>, 2009.
- Gardner, W. S., Bootsma, H. A., Evans, C., and John, P. A. S.: Improved chromatographic analysis of ^{15}N : ^{14}N ratios in ammonium or nitrate for isotope addition experiments, *Mar. Chem.*, 48, 271–282, [https://doi.org/10.1016/0304-4203\(94\)00060-Q](https://doi.org/10.1016/0304-4203(94)00060-Q), 1995.
- Gardner, W. S., St. John, P. A., Evans, C., and Cavaletto, J.: HPLC retention-time-shift determination of nitrogen isotope ratios in enriched water, *American Laboratory (Distinguished Authors' Issue)* 28, 17C–17H, 1996.
- Gardner, W. S., Cavaletto, J. F., Bootsma, H. A., Lavrentyev, P. J., and Troncone, F.: Nitrogen cycling rates and light effects in tropical Lake Maracaibo, Venezuela, *Limnol. Oceanogr.*, 43, 1814–1825, 1998.
- Gardner, W. S., Lavrentyev, P. J., Cavaletto, J. F., McCarthy, M. J., Eadie, B. J., Johengen, T. H., and Cotner, J. B.: Distribution and dynamics of nitrogen and microbial plankton in southern Lake Michigan during spring transition 1999–2000, *J. Geophys. Res.*, 109, C03007, <https://doi.org/10.1029/2002JC001588>, 2004.
- 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., and Paerl, H. W.: Community Biological Ammonium Demand (CBAD): A Conceptual Model for Cyanobacteria Blooms in Eutrophic Lakes, *Environ. Sci. Technol.*, 51, 7785–7793, <https://doi.org/10.1021/acs.est.6b06296>, 2017.
- Glibert, P. M., Maranger, R., Sobota, D. J., and Bowman, L.: The Haber-Bosch-harmful algal bloom (HB-HAB) link, *Environ. Res. Lett.*, 9, 105001, <https://doi.org/10.1088/1748-9326/9/10/105001>, 2014.
- Glibert, P. M., Wilkerson, F. P., Dugdale, R. C., Raven, J. A., Dupont, C. L., Leavitt, P. R., Parker, A. E., Burkholder, J. M., and Kana, T. M.: 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, <https://doi.org/10.1002/lno.10203>, 2016.
- Granger, J. and Sigman, D. M.: Removal of nitrite with sulfamic acid for nitrate N and O isotope analysis with the denitrifier method, *Rapid Commun. Mass Sp.*, 23, 3753–3762, <https://doi.org/10.1002/rcm.4307>, 2009.
- Hall, G. H.: Nitrification in lakes, in: *Nitrification*, 1st Edn., edited by: Prosser, J. I., IRL Press, Washington, DC, 127–156, 1986.
- Heiss, E. M. and Fulweiler, R. W.: Coastal water column ammonium and nitrite oxidation are decoupled in summer, *Estuar. Coast. Shelf S.*, 178, 110–119, <https://doi.org/10.1016/j.ecss.2016.06.002>, 2016.

- Hou, J., Song, C., Cao, X., and Zhou, Y.: Shifts between ammonia-oxidizing bacteria and archaea in relation to nitrification potential across trophic gradients in two large Chinese lakes (Lake Taihu and Lake Chaohu), *Water Res.*, 47, 2285–2296, <https://doi.org/10.1016/j.watres.2013.01.042>, 2013.
- James, R. T., Gardner, W. S., McCarthy, M. J., and Carini, S. A.: Nitrogen dynamics in Lake Okeechobee: Forms, functions, and changes, *Hydrobiologia*, 669, 199–212, <https://doi.org/10.1007/s10750-011-0683-7>, 2011.
- Jenkins, M. C. and Kemp, W. M.: The coupling of nitrification and denitrification in two estuarine sediments, *Limnol. Oceanogr.*, 29, 609–619, 1984.
- Jia, Z. and Conrad, R.: Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil, *Environ. Microbiol.*, 11, 1658–1671, <https://doi.org/10.1111/j.1462-2920.2009.01891.x>, 2009.
- Jung, M. Y., Park, S. J., Min, D., Kim, J. S., Rijnstra, W. I. C., Damsté, J. S. S., Kim, G. J., Madsen, E. L., and Rhee, S. K.: Enrichment and characterization of an autotrophic ammonia-oxidizing archaeon of mesophilic crenarchaeal group I.1a from an agricultural soil, *Appl. Environ. Microb.*, 77, 8635–8647, <https://doi.org/10.1128/AEM.05787-11>, 2011.
- Könneke, M., Bernhard, A. E., De La Torre, J. R., Walker, C. B., Waterbury, J. B., and Stahl, D. A.: Isolation of an autotrophic ammonia-oxidizing marine archaeon, *Nature*, 437, 543–6, <https://doi.org/10.1038/nature03911>, 2005.
- Kowalchuk, G. A. and Stephen, J. R.: Ammonia-oxidizing bacteria: a model for molecular microbial ecology, *Annu. Rev. Microbiol.*, 55, 485–529, <https://doi.org/10.1146/annurev.micro.55.1.485>, 2001.
- Krausfeldt, L. E., Tang, X., van de Kamp, J., Gao, G., Boddrossy, L., Boyer, G. L., and Wilhelm, S. W.: Spatial and temporal variability in the nitrogen cyclers of hyper-eutrophic Lake Taihu, *FEMS Microbiol. Ecol.*, 93, 1–11, <https://doi.org/10.1093/femsec/fix024>, 2017.
- Li, D.-M., Zheng, H.-Y., Pan, J.-L., Zhang, T.-Q., Tang, S.-K., Lu, J.-M., Zhong, L., Liu, Y.-S., and Liu, X.-W.: Seasonal dynamics of photosynthetic activity, *Microcystis* genotypes and microcystin production in Lake Taihu, China, *J. Great Lakes Res.*, 43, 710–716, <https://doi.org/10.1016/j.jglr.2017.04.005>, 2017.
- Ma, J., Qin, B., Paerl, H. W., Brookes, J. D., Hall, N. S., Shi, K., Zhou, Y., Guo, J., Li, Z., Xu, H., Wu, T., and Long, S.: The persistence of cyanobacterial (*Microcystis* spp.) blooms throughout winter in Lake Taihu, China, *Limnol. Oceanogr.*, 61, 711–722, <https://doi.org/10.1002/lno.10246>, 2016.
- Martens-Habben, W., Berube, P. M., Urakawa, H., De La Torre, J. R., Stahl, D. A., and Torre, J.: Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria, *Nature*, 461, 976–979, <https://doi.org/10.1038/nature08465>, 2009.
- McCarthy, M. J., Lavrentyev, P. J., Yang, L., Zhang, L., Chen, Y., Qin, B., and Gardner, W. S.: Nitrogen dynamics and microbial food web structure during a summer cyanobacterial bloom in a subtropical, shallow, well-mixed, eutrophic lake (Lake Taihu, China), *Hydrobiologia*, 581, 195–207, <https://doi.org/10.1007/s10750-006-0496-2>, 2007.
- McCarthy, M. J., James, R. T., Chen, Y., East, T. L., and Gardner, W. S.: Nutrient ratios and phytoplankton community structure in the large, shallow, eutrophic, subtropical Lakes Okeechobee (Florida, USA) and Taihu (China), *Limnology*, 10, 215–227, <https://doi.org/10.1007/s10201-009-0277-5>, 2009.
- McCarthy, M. J., Gardner, W. S., Lehmann, M. F., and Bird, D. F.: 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, <https://doi.org/10.1007/s10750-013-1614-6>, 2013.
- McIlvin, M. R. and Altabet, M. A.: Chemical conversion of nitrate and nitrite to nitrous oxide for nitrogen and oxygen isotopic analysis in freshwater and seawater, *Anal. Chem.*, 77, 5589–5595, <https://doi.org/10.1021/ac050528s>, 2005.
- Merbt, S. N., Stahl, D. A., Casamayor, E. O., Marti, E., Nicol, G. W., and Prosser, J. I.: Differential photoinhibition of bacterial and archaeal ammonia oxidation, *FEMS Microbiol. Lett.*, 327, 41–46, <https://doi.org/10.1111/j.1574-6968.2011.02457.x>, 2012.
- Mulholland, P. J., Helton, A. M., Poole, G. C., Hall, R. O., Hamilton, S. K., Peterson, B. J., Tank, J. L., Ashkenas, L. R., Cooper, L. W., Dahm, C. N., Dodds, W. K., Findlay, S. E. G., Gregory, S. V., Grimm, N. B., Johnson, S. L., McDowell, W. H., Meyer, J. L., Valett, H. M., Webster, J. R., Arango, C. P., Beaulieu, J. J., Bernot, M. J., Burgin, A. J., Crenshaw, C. L., Johnson, L. T., Niederlehner, B. R., O'Brien, J. M., Potter, J. D., Sheibley, R. W., Sobota, D. J., and Thomas, S. M.: Stream denitrification across biomes and its response to anthropogenic nitrate loading, *Nature*, 452, 202–205, <https://doi.org/10.1038/nature06686>, 2008.
- Newell, S. E., Babbín, A. R., Jayakumar, D. A., and Ward, B. B.: Ammonia oxidation rates and nitrification in the Arabian Sea, *Global Biogeochem. Cy.*, 25, GB4016, <https://doi.org/10.1029/2010GB003940>, 2011.
- Nicklisch, A. and Kohl, J. G.: 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, 1983.
- Nowka, B., Daims, H., and Spieck, E.: Comparison of oxidation kinetics of nitrite-oxidizing bacteria: Nitrite availability as a key factor in niche differentiation, *Appl. Environ. Microb.*, 81, 745–753, <https://doi.org/10.1128/AEM.02734-14>, 2015.
- Otten, T. G. and Paerl, H. W.: Phylogenetic Inference of Colony Isolates Comprising Seasonal *Microcystis* Blooms in Lake Taihu, China, *Microb. Ecol.*, 62, 907–918, <https://doi.org/10.1007/s00248-011-9884-x>, 2011.
- Paerl, H. W. and Paul, V. J.: Climate change: Links to global expansion of harmful cyanobacteria, *Water Res.*, 46, 1349–1363, <https://doi.org/10.1016/j.watres.2011.08.002>, 2012.
- Paerl, H. W., Xu, H., McCarthy, M. J., Zhu, G., Qin, B., Li, Y., and Gardner, W. S.: 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, <https://doi.org/10.1016/j.watres.2010.09.018>, 2011.
- Paerl, H. W., Xu, H., Hall, N. S., Zhu, G., Qin, B., Wu, Y., Rossignol, K. L., Dong, L., McCarthy, M. J., and Joyner, A. R.: Controlling cyanobacterial blooms in hypertrophic Lake Taihu, China: Will nitrogen reductions cause replacement of non-N₂ Fixing by N₂ fixing taxa?, *PLoS ONE*, 9, e113123, <https://doi.org/10.1371/journal.pone.0113123>, 2014.
- Paerl, H. W., Gardner, W. S., Havens, K. E., Joyner, A. R., McCarthy, M. J., Newell, S. E., Qin, B., and Scott, J. T.: Mitigating cyanobacterial harmful algal blooms in aquatic ecosystems impacted by climate change and anthropogenic nutrients, *Harmful*

- Algae, 54, 213–222, <https://doi.org/10.1016/j.hal.2015.09.009>, 2016.
- Palatinszky, M., Herbold, C., Jehmlich, N., Pogoda, M., Han, P., von Bergen, M., Lagkouvardos, I., Karst, S. M., Galushko, A., Koch, H., Berry, D., Daims, H., and Wagner, M.: Cyanate as an energy source for nitrifiers, *Nature*, 524, 105–108, <https://doi.org/10.1038/nature14856>, 2015.
- Qian, H., Lu, T., Song, H., Lavoie, M., Xu, J., Fan, X., and Pan, X.: Spatial Variability of Cyanobacteria and Heterotrophic Bacteria in Lake Taihu (China), *B. Environ. Contam. Tox.*, 99, 380–384, <https://doi.org/10.1007/s00128-017-2149-8>, 2017.
- Qin, B., Xu, P., Wu, Q. L., Luo, L., and Zhang, Y.: Environmental issues of Lake Taihu, China, *Hydrobiologia*, 581, 3–14, <https://doi.org/10.1007/s10750-006-0521-5>, 2007.
- Qin, B., Zhu, G., Gao, G., Zhang, Y., Li, W., Paerl, H. W., and Carmichael, W. W.: A drinking water crisis in Lake Taihu, China: Linkage to climatic variability and lake management, *Environ. Manage.*, 45, 105–112, <https://doi.org/10.1007/s00267-009-9393-6>, 2010.
- Qin, B. Q.: Lake Taihu, China, in: *Dynamics and Environmental Change*, Springer, Netherlands, 2008.
- Rotthauwe, J.-H., Witzel, K.-P., and Liesack, W.: The Ammonia Monooxygenase Structural Gene *amoA* as a Functional Marker: Molecular Fine-Scale Analysis of Natural Ammonia-Oxidizing Populations, *Appl. Environ. Microb.*, 63, 4704–4712, 1997.
- Saba, G. K., Steinberg, D. K., and Bronk, D. A.: The relative importance of sloppy feeding, excretion, and fecal pellet leaching in the release of dissolved carbon and nitrogen by *Acartia tonsa* copepods, *J. Exp. Mar. Biol. Ecol.*, 404, 47–56, <https://doi.org/10.1016/j.jembe.2011.04.013>, 2011.
- Small, G. E., Bullerjahn, G., Sterner, R. W., Beall, B. F. N., Brovold, S., Finlay, J. C., McKay, R. M., and Mukherjee, M.: Rates and controls of nitrification in a large oligotrophic lake, *Limnol. Oceanogr.*, 58, 276–286, <https://doi.org/10.4319/lo.2013.58.1.0276>, 2013.
- Steffen, M. M., Li, Z., Effler, T. C., Hauser, L. J., Boyer, G. L., and Wilhelm, S. W.: Comparative Metagenomics of Toxic Freshwater Cyanobacteria Bloom Communities on Two Continents, *PLoS ONE*, 7, e44002, <https://doi.org/10.1371/journal.pone.0044002>, 2012.
- Steffen, M. M., Davis, T. W., McKay, R. M., Bullerjahn, G. S., Krausfeldt, L. E., Stough, J. M. A., Neitzey, M. L., Gilbert, N. E., Boyer, G. L., Johengen, T. H., Gossiaux, D. C., Burtner, A. M., Palladino, D., Rowe, M., Dick, G. J., Meyer, K., Levy, S., Boone, B., Stumpf, R., Wynne, T., Zimba, P. V., Gutierrez, D. B., and Wilhelm, S. W.: Ecophysiological examination of the Lake Erie *Microcystis* bloom in 2014: Linkages between biology and the water supply shutdown of Toledo, Ohio, *Environ. Sci. Technol.*, <https://doi.org/10.1021/acs.est.7b00856>, 2017.
- Su, X., Steinman, A. D., Xue, Q., Zhao, Y., Tang, X., and Xie, L.: Temporal patterns of phyto- and bacterioplankton and their relationships with environmental factors in Lake Taihu, China, *Chemosphere*, 184, 299–308, <https://doi.org/10.1016/j.chemosphere.2017.06.003>, 2017.
- Sun, X., Ji, Q., Jayakumar, A., and Ward, B. B.: Dependence of nitrite oxidation on nitrite and oxygen in low oxygen seawater, *Geophys. Res. Lett.*, 44, 7883–7891, <https://doi.org/10.1002/2017GL074355>, 2017.
- Tolar, B. B., Wallsgrove, N. J., Popp, B. N., and Hollibaugh, J. T.: Oxidation of urea-derived nitrogen by thaumarchaeota-dominated marine nitrifying communities, *Environ. Microbiol.*, 19, 4838–4850, <https://doi.org/10.1111/1462-2920.13457>, 2016.
- Tourna, M., Stieglmeier, M., Spang, A., Könneke, M., Schintlmeister, A., and Urich, T.: *Nitrososphaera viennensis*, an ammonia oxidizing archaeon from soil, *P. Natl. Acad. Sci. USA*, 108, 8420–8425, <https://doi.org/10.1073/pnas.1013488108>, 2011.
- Ushiki, N., Jinno, M., Fujitani, H., Suenaga, T., Terada, A., and Tsuneda, S.: Nitrite oxidation kinetics of two *Nitrospira* strains: The quest for competition and ecological niche differentiation, *J. Biosci. Bioeng.*, 123, 581–589, 2017.
- Verhamme, D. T., Prosser, J. I., and Nicol, G. W.: Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms, *ISME J.*, 5, 1067–1071, <https://doi.org/10.1038/ismej.2010.191>, 2011.
- Vitousek, P. M., Menge, D. N., Reed, S. C., and Clevinger, C. C.: Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems, *Philos. T. Roy. Soc. B*, 368, 20130119, <https://doi.org/10.1098/rstb.2013.0119>, 2013.
- Ward, B. B.: Nitrification in marine systems, in: *Nitrogen in the Marine Environment*, 2nd Edn., edited by: Capone, D., Bronk, D., Mulholland, M., and Carpenter, E., Elsevier, Amsterdam, 2008.
- Wu, Y., Xiang, Y., Wang, J., Zhong, J., He, J., and Wu, Q. L.: Heterogeneity of archaeal and bacterial ammonia-oxidizing communities in Lake Taihu, China, *Env. Microbiol. Rep.*, 2, 569–576, <https://doi.org/10.1111/j.1758-2229.2010.00146.x>, 2010.
- Xu, H., Paerl, H. W., Qin, B., Zhu, G., and Gao, G.: Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China, *Limnol. Oceanogr.*, 55, 420–432, 2010.
- Xu, H., Paerl, H. W., Qin, B., Zhu, G., Hall, N. S., and Wu, Y.: Determining critical nutrient thresholds needed to control harmful cyanobacterial blooms in eutrophic Lake Taihu, China, *Environ. Sci. Technol.*, 49, 1051–1059, <https://doi.org/10.1021/es503744q>, 2015.
- Yao, X., Zhang, L., Zhang, Y., Xu, H., and Jiang, X.: Denitrification occurring on suspended sediment in a large, shallow, subtropical lake (Poyang Lake, China), *Environ. Pollut.*, 219, 501–511, 2016.
- Yan, S., Yu, H., Zhang, L., and Xu, J.: Water quantity and pollutant fluxes of inflow and outflow rivers of Lake Taihu, 2009, *J. Lake Sci.* 6, 855–862, 2011 (in Chinese).
- Yang, J., Gao, H., Glibert, P. M., Wang, Y., and Tong, M.: Rates of nitrogen uptake by cyanobacterially-dominated assemblages in Lake Taihu, China, during late summer, *Harmful Algae*, 65, 71–84, <https://doi.org/10.1016/j.hal.2017.04.001>, 2017.
- Zeng, J., Zhao, D., Huang, R., and Wu, Q. L.: Abundance and community composition of ammonia-oxidizing archaea and bacteria in two different zones of Lake Taihu, *Can. J. Microbiol.*, 58, 1018–1026, <https://doi.org/10.1139/w2012-078>, 2012.
- Zhao, D., Zeng, J., Wan, W., Liang, H., Huang, R., and Wu, Q. L.: Vertical distribution of ammonia-oxidizing archaea and bacteria in sediments of a eutrophic lake, *Curr. Microbiol.*, 67, 327–332, <https://doi.org/10.1007/s00284-013-0369-7>, 2013.