Response to Referee # 1

1. Throughout the presentation, the authors indicate the Lake Taihu bloom is comprised of Microcystis spp.. However, blooms can be highly dynamic and vary in population structure. The study does not provide supporting data that bloom studied was indeed Microcystis spp.. Furthermore, Microcystis spp. can have highly plastic genomes leading to genomic rearrangements and perhaps altered physiology. As such, evidence that the Lake Taihu Microcystis populations resemble those for which Km values have been measured (i.e. Nicklisch and Kohl 1983; Baldia et al., 2007) should be provided.

Thank you for pointing this out. Bloom composition was not part of our study, but published literature on cyanobacterial blooms in Taihu is extensive, and these blooms have always been associated with Microcystis (Chen et al., 2003; Otten and Paerl 2011; Tang et al., 2013 and 2014, and citations in the manuscript). Additionally, the years sampled in this study correspond to Microcystis identification in the following studies (Ma et al., 2016; Tang et al., 2017; Deng et al., 2014; Li et al., 2017; Su et al., 2017; Qian et al., 2017). We will add this information to our site description section of the methods:

Lines 116–118: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).

Lines 143–146: 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).

The K_m values from Nicklish and Kohl (1983) and Baldia et al., (2007) provided in the Discussion are meant to serve only as a reference to Microcystis studies done in culture. We did not perform any kinetic experiments in our study, but a kinetic study during a Microcystis bloom in Taihu showed extremely high K_m values ranging from 76.9 to 112.9 µM (Yang et al., 2017). We will add the following text to the Discussion section:

Line 505–510: With a high saturation threshold and reported K_m values from 26.5 µM to 37 µM (Baldia et al., 2007; Nicklisch and Kohl 1983) in culture and up to 112.9 µM in Taihu populations (Yang et al., 2017), Microcystis should be able to outcompete nitrifiers at the high ambient NH_4^+ concentrations in Taihu as nitrifiers may become saturated at much lower concentrations.

References not included in manuscript already:
Chen et al., 2003 https://doi.org/10.1093/plankt/25.4.445
Deng et al., 2014 doi: 10.1111/fwb.12330
Li et al., 2017 http://dx.doi.org/10.1016/j.jglr.2017.04.005
Otten and Paerl 2011 doi:10.1007/s00248-011-9884-x
Su et al., 2017 http://dx.doi.org/10.1016/j.hal.2017.08.007
Qian et al., 2017 doi: 10.1007/s00128-017-2149-8
Yang et al., 2017 doi:10.1016/j.hal.2017.04.001

2. The study aims to capture seasonal dynamics but the samples were collected from nonconsecutive time points (August 2013, June 2014, March 2015, July 2016). I realize that planning and coordinating field sampling campaigns can be challenging, but

are there any supporting information that the conditions for these years are comparable, blooms are of similar magnitude and timing etc. There is also no evidence that the samples captured bloom samples. For instance, what is the evidence for an early summer bloom in June 2014 vs. the mid-summer bloom in July 2016. This relates to my comment above - can you provide evidence that, at least during your samples, the blooms were comprised of Microcystis spp. to support the conclusions about competition for NH4 between Microcystis and AOO.

Historically, blooms in Taihu have occurred 8–10 months of the year (Ma et al., 2016). A year long study in 2014 showed that blooms usually start in March/April and last until December (Li et al. 2017). Our chlorophyll data (Table 1) shows the seasonal bloom characteristics, with lowest values in March and highest in June/August.

We will add a paragraph to the results section discussing the chlorophyll data in more detail:

Lines: 288–292 Chlorophyll a concentrations were lowest in March 2015 (mean = 11.1 μg L^{-1}), but bloom conditions (> 20 μg L^{-1}; Xu et al., 2015) were observed at some locations (e.g., 20.3 μg L^{-1} at Station 3, and visual confirmation at Stations 1, 3, and several other areas of the lake). Bloom conditions were also present and observed at all sites in June 2014 (mean = 36.6 μg L^{-1}), July 2016 (mean = 58.1 μg L^{-1}), and August 2013 (43.7 μg L^{-1}).

Reference:

Xu et al., 2015 doi:10.1021/es503744q

3. The multiple regression model seems to convey what one would expect - N cycling dynamics are driven by N availability. While I appreciate the care taken to calculate the model, I'm not convinced it adds to the study as presented.

The purpose of the multiple regression model was to show that NH_4^+ dynamics correlate with seasonally variable temperature and DO. While this relationship is not present in the Kendall's correlation table, a more complex model in Table 4 shows the importance of multiple variables at the same time. We thought it was important, for a complex environment like Taihu, to show that temperature plays a role in NH_4^+ dynamics. We pointed this out in lines 518–523 in the original version. The model also highlights the lack of temperature relationship with nitrification rates, which was an unexpected result.

4. In the discussion, there is no consideration of other features of the lake, i.e. hydraulic residence, that may contribute to different rates of N cycling in the different zones. Because the lake is large with multiple inputs, including multiple inputs that presumably vary in levels of nutrient (N and P) delivered, this seems worth considering in interpreting the data.

This is an excellent point. However, an in-depth discussion of these physical factors is beyond the scope of the paper. We are part of a larger collaborative project, and data from this study will be incorporated into a Lake Taihu ecosystem model by the Hellweger lab at Northeastern University. This model will include features like N and P loading, hydraulic residence time, N cycling rates, chlorophyll dynamics, etc.

We agree that there should be a general discussion of the residence time, however. We will add this information to our methods section:

Line 127:Taihu Lake has a relatively long residence time of approximately 280–300 days (Paerl et al., 2014; Xu et al., 2010).

Some more technical comments:

Line 101: maybe within the system, in situ (rather than internally)
We will revise to "within the system"

Line 156: This is, in most cases, an elevated ammonium concentration than measured in situ. Can you justify the concentration added and expand more on the range used - i.e. did lower concentration samples receive less?
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. The substrate additions depended in part on bloom status, and our goal was to add the label concentration at an equivalent value to the most recent monitoring data we could obtain for NH4 concentrations, or at least 8 uM (even when concentrations are low, recycling rates can be quite high). Lower level additions coincided with low ambient concentrations and lighter blooms, while higher level additions were conducted at sites with heavy blooms and/or high ambient NH4 concentrations. We will add text in the methods to clarify our isotope labelling approach. We also point out that the uptake rate is considered potential (line 166), because these substrate additions exceed ambient levels. However, the regeneration rates are actual, not potential.
We added this clarification to the methods:
Lines 171–175: 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).

Line 223: targeting a region, or do these primers amplify the entire gene?
Targeting a region of the amoA gene, as stated in the manuscript (line 223).

Results: The samples capture seasons over several years. Do you have regional precipitation and/or weather patterns to indicate that your samples are representative across this timescale?
Our sampling dates were representative of seasonal conditions in the region, specific to this subtropical climate zone. Our sampling events did not correspond with any extreme weather patterns (e.g., typhoons, droughts). Temperature and precipitation patterns were average for this climate region and are available online.
(https://www.wunderground.com/history/airport/ZSSS/2013/8/1/DailyHistory.html).
We added this sentence to the Methods:
Lines 146–149: 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.

Line 256, Table 1: Can you provide detection limits, especially for NH4+ concentrations that are below limit of detection (should be bdl in your table rather than 0.00).
Thank you for pointing this out. We will change 0.00 values to BDL and provide a footnote with the table stating detection limits for our nutrient analyses:
*Nutrient analysis detection limits: NH_4^+ = 0.04 µM; NO_x = 0.04 µM; OP = 0.008 µM.

Lines 321-322: Please provide the detection limits for qPCR (i.e. from you standard curves).

The detection limit for was 980 copies/ml for AOB and 4807 copies/ml for AOA, calculated from standard deviation of the lowest standard multiplied by student t test value. 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 two orders of magnitude) and AOB.

Lines 265–269: The detection limit was 980 copies/ml for AOB and 4807 copies/ml 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 two orders of magnitude) and AOB.

Lines 361: algal production or cyanobacterial?

Algal production. We can't rule out other organisms.

Line 514: Or utilizing a different substrate? Is it possible that HAB Cyanos successfully compete for NH4+ but AOA can still use the urea - perhaps less efficiently. Its possible that there is no competition.

As stated in the manuscript (lines 512–514), we consider the possibility of AOA utilizing urea and/or cyanate; however, these substrates might be used less efficiently, and at much lower rates than NH_4^+. Palatinszky et al. (2015) shows lower protein concentration in AOA grown on cyanate than on NH_4^+. Tolar et al. (2016) shows lower oxidation rates of urea than NH_4^+ in samples from coastal Georgia and South Atlantic, concluding that urea-derived N does not play a major role in temperate regions.

We will add text as appropriate to clarify this:

Lines 578–581: 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 slower. Therefore, it is possible that AOA might be playing an expanded role in Taihu beyond just ammonia oxidation.

Line 526: See main comment #2 - It doesn't seem like the paper presents data on bloom formation and maintenance in Taihu. As such, I don't think this conclusion can be supported but your data. I suggest removing or presenting the speculative nature of this statements and other similar conclusions about bloom formation / progression.

We will revise this sentence to:

Lines 594–597: 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 cyanobacterial blooms in Taihu.

Line 528: Assuming NH4 is the preferred substrate

Many studies have shown that NH_4^+ is the preferred N source for cyanobacteria, especially non-N-fixers like Microcystis (Blomqvist et al., 1994; Glibert et al., 2015; Gobler et al., 2016; McCarthy et al., 2009; lines 52–56).

Gobler et al., 2016 http://dx.doi.org/10.1016/j.hal.2016.01.010

Response to Referee # 2

How do you define nitrification? If it is the rate at which NH4 transforms to NO3 ultimately, then "total nitrification" should = ammonium oxidation, which is the rate limiting step. Or otherwise, then please justify.

The reviewer makes a good point that these terms need clarification. It is true that produced $^{15}NO_3^-$ must have originated from $^{15}NH_4^+$ and that ammonia oxidation is usually the rate limiting step in nitrification, making total NO_3^- production inclusive of ammonia oxidation. We will rephrase the definition of nitrification (focused on total nitrification; e.g. the sum of NO_2^- and NO_3^- production), as well as our discussion of the partitioning of the product of $^{15}NH_4^+$ additions (i.e., comparing $^{15}NO_2^-$ to the $^{15}NO_3^-$ pool). We will revise the text in the methods (lines 220–227), results (lines 338–355), and discussion accordingly. For example, we will review this issue in the Discussion as follows:

Lines 459–472: 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 $^{15}NO_3^-$ exceeded accumulation of $^{15}NO_2^-$ 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 $^{15}NO_3^-$ was expected, since NO_3^- is the final product of total nitrification.

At Station 10, accumulation of $^{15}NO_3^-$ exceeded $^{15}NO_2^-$ in March 2015 and June 2014. In July 2016, however, accumulation of $^{15}NO_2^-$ was three times higher in surface water and comparable at depth (Fig. 3b). Ambient NO_2^- concentration at Station 10 in July 2016 was 9.6 µM in surface water and 8.4 µM 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 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).

Specific: L43: replace "N fixation" by "N2 fixation"

We will revise to N_2 fixation.

L44: idem as L43 L57: maybe useful to say that NH4 can accumulate in systems when there is O2 limitation – which is relevant in eutrophic systems.

This is true in many eutrophic systems, but Lake Taihu is very shallow (2 m on average) and well-mixed. We do not observe O_2 depletion at depth, and stratification is rare (Qin et al., 2004); therefore, we do not expect O_2 limitation to play a role in N cycling processes in the well-mixed water column.

Reference:
Qin, B., Hu, W., Gao, G., Luo, L. and Zhang, J., 2004. Dynamics of sediment resuspension and the conceptual schema of nutrient release in the large shallow Lake Taihu, China. *Chinese Science Bulletin*, *49*(1), pp.54-64.

L71: It might be useful to cite the role of O2 in this uncoupling: There are many "kinetic" studies that show that nitrite oxidation is more sensitive to low O2 levels than ammonium oxidation and that this causes the decoupling of both processes in many suboxic aquatic systems. See for example Guisasola et al 2005 and references therein.

Indeed, this is true, and thank you for the reference. As stated above, however, Taihu is well-mixed, very shallow, and not susceptible to suboxic conditions in the water column.

L90 and throughout the manuscript: the acronym cyanoHABs= cyonobacteria harmfull algal blooms is often use in the place of "cyanobacteria",as it is the here. Should be checked and corrected when needed.
Thank you for pointing this out. We will correct the inconsistent use of "cyanobacteria" and "cyanoHABs".

L91 Affinity for ammonium: needs a reference
We will add appropriate references here, such as Martens-Habbena et al., (2009) and Baldia et al., (2007).

L96 the term potential uptake rate is a bit confusing
as actually it is not an uptake rate but rather a consumption rate which includes ammonium oxidation
We agree that our wording here is confusing. We will rephrase for clarity:
Lines 99–100: We measured community NH_4^+ uptake and regeneration rates, as well as nitrification rates, under different bloom conditions to help determine how cyanoHABs influence NH_4^+ fluxes.

L149 atom% 15N: of ammonium?
Yes, we will clarify in the revised manuscript that we mean atom % of 15N-NH_4^+

L166 idem L96 L170 Please explain here how you calculate "total nitrification" or "nitrification" and justify. See also comment on L306.
We agree with the reviewer's comments, and we will modify the text per the example above.

L255 You forget Chla in this part of the results
Thank you for pointing this out. We will add chlorophyll results to the results section:
Lines 288–292: Chlorophyll a concentrations were lowest in March 2015 (mean = 11.1 µg L^{-1}), but bloom conditions (> 20 µg L^{-1}; Xu et al., 2015) were observed at some locations (e.g., 20.3 µg L^{-1} at Station 3, and visual confirmation at Stations 1, 3, and several other areas of the lake). Bloom conditions were also present and observed at all sites in June 2014 (mean = 36.6 µg L^{-1}), July 2016 (mean = 58.1 µg L^{-1}), and August 2013 (43.7 µg L^{-1}).
Reference:
Xu et al., 2015 doi:10.1021/es503744q

L256 Do the variables not vary spatially also? I think they do as you discuss the special situation of station 10
Yes, we will add "spatially" to the sentence.

L272: early summer bloom – how do you know this is the early summer bloom? From chla?
L277 early spring bloom: idem as L272 L282 summer bloom: idem as L272.
For clarification, we removed all "early bloom", "mid bloom", and "late bloom" descriptions. Instead, we kept the month names only.

L303 using other units for nitrification is confusing. I would recommend to have similar units especially as later on you consider the fraction of ammonium consumption due to nitrification.
We aimed to report total nitrification rates in units consistent with the majority of the literature. nmol L^{-1} d^{-1} is usually used for these rates (Bristow et al., 2015; Heiss and Fulweiler 2016; Newell et al. 2011; Ward and Kilpatrick 1991). Uptake and regenerations rates are much faster, and are on a micromolar scale (also a standard literature unit for these rates; Gardner et al., 2001; James et al., 2011; McCarthy et al., 2013). For ease of unit conversion/comparison, we will add an additional axis to Fig. 3 to show µM/hr units.

L306 How do you define the total nitrification rate? By definition nitrification is the 2 steps reaction NH4->NO2->NO3, as you said in the introduction, so it should be the rate at which NH4 is transformed into NO3. So as nitrite
oxidation is not the limiting step, it should correspond to ammonium oxidation. You use the sum I believe, which then represents the production of NO2+NO3? but then as most of the NO2 is not produced by NH4 oxidation (much slower rates) but comes from external inputs (or other process) it is not clear what this really represents ecologically? Please, this needs clarification.
In environmental studies, it is not uncommon for nitrite oxidation to exceed ammonia oxidation (Bristow et al., 2015; Clark et al., 2008; Fussel et al., 2012; Heiss and Fulweiler 2016; Ward and Kilpatrick 1991). We agree that the way we reported the rates is confusing; therefore, we will revise the manuscript as stated above. We will rephrase the definition of nitrification (focused on total nitrification; e.g. the sum of $^{15}NO_2^-$ and $^{15}NO_3^-$ production), as well as our discussion of the partitioning of the product of $^{15}NH_4^+$ additions (i.e., comparing $^{15}NO_2^-$ to the $^{15}NO_3^-$ pool).
Reference:
Clark et al., 2008 doi:10.2307/40006149
Ward and Kilpatrick 1991 https://doi.org/10.1016/0278-4343(90)90016-F

L329 in this discussion point it is not clear why you don't you calculate an integrated NH4 uptake rate per station taking into account light/dark rates and surface/depth rates? It would refocus this part of the discussion. Presenting distinct light and dark rates in the discussion is distracting from the major (and most interesting) points.
We did not integrate light and dark rates so that we could highlight and distinguish the differences between total community uptake (light) from non-photoautotrophic uptake (dark). We think this is an important part of the study. We did not integrate the surface and deep rates because the system is shallow and well-mixed, and Microcystis can regulate its buoyancy to form surface scums. Additionally, without high-resolution depth profiles of relevant physicochemical parameters, it is difficult to distinguish differences in surface and bottom water masses.

L330-339 I do not see the use of comparing rates of Lake Taihu in such details with other lakes if there is no discussion on what might explain the differences – and I think it is not the topic of the paper to do so. This could be shorter and table 3 removed.
We agree that our presentation of the system comparisons can be improved. We wanted to include the table to give an overview of uptake and regeneration rates in other freshwater systems, and also to highlight differences in rates relative to trophic status (e.g., eutrophic vs hypereutrophic). We will add chlorophyll a values as an indicator of trophic status in the table and clarify this in the text:

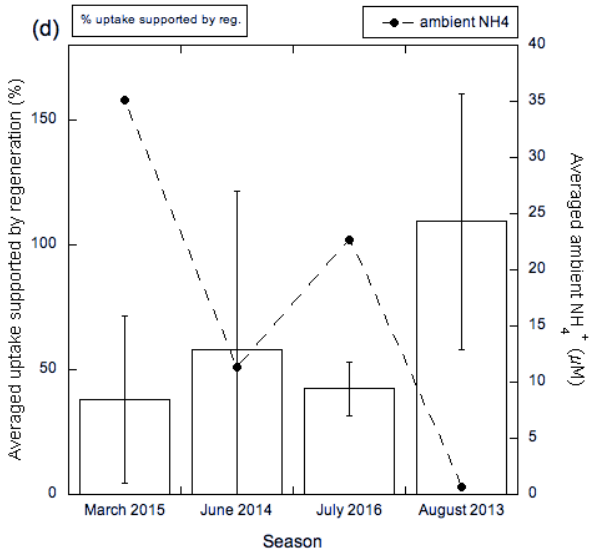
Line 375–381: Potential NH_4^+ uptake rates in these systems, evaluated using the same methods, increase with chlorophyll a ($p < 0.05$), but the proportion of community uptake that can be supported by regeneration remains relatively consistent (Table 3).

L340 Replace "presumably due to photosynthetic phytoplankton activity" by "presumably due to reduced photosynthetic phytoplankton activity"
Thank you for pointing this out. We will revise as suggested.

L340-342 This statement needs a reference: ammonium uptake is by phototrophs is reduced in the dark, not blocked so I don't think you can extrapolate to saying that heterotrophs and chemolithotrophs dominate the uptake. You don't know.
Phototrophs usually take up nutrients in the dark when they are nutrient limited (Cochlan et al., 1991). Taihu is generally nutrient replete, so we speculate that the dark uptake can be mostly attributed to heterotrophs and chemolithoautotrophs. We will revise these two sentences to clarify this information:
Lines 383–386: Photoautotrophs may continue to assimilate nutrients in the dark under nutrient limitation (Cochlan et al., 1991), but Taihu is generally nutrient replete, so we assume that dark uptake rates can be attributed mostly to heterotrophic or chemolithoautotrophic organisms.

L344 "which may have been due to higher precipitation and subsequent runoff" you mean more nutrient inputs? What about the phytoplankton biomass? do you have a bloom that might explain higher rates? I see a max in Chla indeed. And there is also plenty of nutrients.
Yes, more runoff = more nutrient inputs.
There is strong bloom evidence looking at July 2016 chlorophyll (above the chla threshold of 20 $\mu g\ L^{-1}$; Xu et al., 2015) and high nutrient concentrations (Table 1).

L355-358: proportion % cited here do not correspond to the values observed in figure 2d.
Thank you for pointing this out. We accidentally uploaded an outdated graph. Here is the correct version that corresponds to the values in the text and will be included in the revised manuscript.
Figure 2d



L360: describing July as early summer is confusing as June could be early summer: : : Maybe just keep the months names

Thank you for pointing this out. We kept only the month names for clarification.

L369 Why don't you do the same with uptake rates and nitrification? Would be interesting.

The purpose of this extrapolation was to compare external N loading to NH_4^+ provided by regeneration. We think that it is an important highlight of this paper. We will add a sentence comparing the extrapolated uptake rates to total nitrogen load. There are high standing pools of NH_4^+, NO_2^- and NO_3^- and cycling rates are high; therefore, a nitrification extrapolation would not be informative.

Lines 424–429: The same procedure for extrapolation of whole-lake uptake rates yields 3.5 x 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 TN loads are underestimated.

L388-390 "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" I do not understand this.

We agree that this sentence is confusing. We split it into two sentences for clarification:

Lines 437–440: 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 results suggest that microbial denitrification cannot remove enough N to effectively mitigate the high external N loading.

L394 which Nitrification are we dealing with here? Total? Ammonium oxidation? Nitrite oxidation?

We will clarify that we are talking about total nitrification rates here.

L394 "previously reported rates": were these rates measured the same way (as the sum of NH4 and NO2 oxidation)? This can make a big difference on reported rates.

Rates reported in Lake Okeechobee were measured using a $^{15}NO_3^-$ pool dilution method. Rates in Lakes Superior and Mono (Line 397), however, were measured using the same $^{15}NH_4^+$ tracer addition technique. Rates in Lake Mendota and the Paerl River Estuary were not measured using ^{15}N stable isotope methods. We will add this information to the text:

Lines 444–452: 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 (60–480 nmol L^{-1} d^{-1}; Carini and Joye, 2008) and Lake Superior, USA (0–51 nmol L^{-1} d^{-1}; Small et al., 2013), both measured via $^{15}NH_4^+$ tracer additions, and Lake Okeechobee, FL (67–97 nmol L^{-1} h^{-1}; James et al., 2011), measured via the $^{15}NO_3^-$ pool dilution method (Carini et al., 2010). Rates on this scale were previously reported only in eutrophic Lake Mendota (WI; 1700 – 26000 nmol L^{-1} h^{-1}; Hall, 1986) and the Paerl River Estuary (China; 2100 – 65100 µmol L^{-1} d^{-1}; Dai et al., 2008). However, these rates were measured from accumulation of NO_2^- and NO_3^-, not stable isotope additions.

L402: nitrification or ammonium oxidation?

We will clarify in the revision that we are talking about total nitrification rates.

L414-415 "Higher NO2 oxidation rates were expected, since NO3 is the product of NO2 oxidation, and NO2 oxidation relies on the product of NH4 oxidation" I don't understand this statement. NO2 oxidation also relies on external sources of NO2 to the lake. It is not clear how you can have 10 times higher NO2 oxidation compared to NH4 oxidation. Needs more clarification.

We will make necessary changes as stated above.

L424 how do you calculate the contribution of nitrification to the uptake? Do you use NH4 oxidation?

We use total nitrification to determine the contribution of nitrification to total NH_4^+ uptake.

L451 idem L424 L454 but as NO2 oxidation rates are higher everywhere, bot steps are also uncoupled at the other stations of the lake.

We calculated the contribution of nitrification to uptake from total nitrification rates.

L505AOB is an ammonium oxidizer so can only contribute to ammonium oxidation (not total as mentioned)

In this case, total nitrification originated from $^{15}NH_4^+$.

L518 replace "driven by" by "correlated with". Being correlated do not mean they are "driven by"

Good point. We will change "driven by" to "correlated with"

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

Justyna J. Hampel[1*], Mark J. McCarthy[1,2], Wayne S. Gardner[2], Lu Zhang[3], Hai Xu[3], Guangwei Zhu[3], Silvia E. Newell[1]

[1] Department of Earth & Environmental Sciences, Wright State University, Dayton, OH
[2]The University of Texas at Austin, Marine Science Institute, Port Aransas, TX
[3]Taihu Laboratory for Lake Ecosystem Research, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing, China
[*] Corresponding author (hampel.4@wright.edu)

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 0.02–6.80 µmol L^{-1} h^{-1} in the light and 0.05–3.33 µmol L^{-1} h^{-1} in the dark, and NH_4^+ regeneration rates ranged from 0.03–2.37 µmol L^{-1} h^{-1}. Nitrification rates exceeded previously reported rates in most freshwater systems. Total nitrification often exceeded 200 nmol L^{-1} d^{-1} and was ≥1000 nmol L^{-1} 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 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. 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 two and is 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.

Deleted: separate NH_4^+ and nitrite (NO_2^-) oxidation rates and

Deleted: 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.

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 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 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 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 AOO and cyanobacteria were in direct competition. At some point in the bloom progression, cyanobacteria 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 cyanobacteria in hypereutrophic Taihu. 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 AOO. 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 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.

Deleted: internally

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). 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 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. Taihu 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 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, 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-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

Deleted: (late summer bloom),

Deleted: 4 (early summer bloom),

Deleted: (no bloom/early spring bloom),

Deleted: (mid-summer bloom).

161 NH_4^+ concentrations were determined by ammonium retention time shift (AIRTS) high
162 performance liquid chromatography (HPLC) at UTMSI (Gardner et al., 1995). Briefly, the atom
163 % ^{15}N-NH_4^+ and total NH_4^+ concentration are determined by comparing the retention time shift
164 of the sample relative to the natural abundance NH_4^+ standard (Gardner et al., 1996)

165 **2.2 Water column NH_4^+ uptake and regeneration**

166 NH_4^+ uptake and regeneration rates were determined following the protocol of McCarthy
167 et al. (2013). Water collected in 4 l carboys was returned to the Taihu Laboratory for Lake
168 Ecosystem Research (TLLER) for isotope amendments and incubations. 500 ml from each
169 site/depth was amended with 98% $^{15}NH_4Cl$ (Isotec; concentration added 8–96 µM) and
170 distributed into six (triplicates for light and dark) 70 ml, clear tissue culture bottles (Corning;
171 McCarthy et al., 2007). The goal of the substrate additions in these uptake/regeneration
172 experiments was to add more-than-trace levels to ensure that all of the label was not taken up
173 during the incubations; our goal was to add the label concentration at an equivalent value to the
174 most recent monitoring data we could obtain for NH_4^+ concentrations, or at least 8 µM (even
175 when concentrations are low, recycling rates can be quite high). Dark bottles were wrapped with
176 thick aluminum foil. Initial samples (T_0) were withdrawn from each bottle with a rinsed syringe,
177 filtered (0.2 µm filters) immediately into 8 ml glass vials (Wheaton), and frozen until analysis at
178 UTMSI. Light and dark bottles were then submerged (approximate depth 0.2 m) in a mesh bag at
179 in situ light and temperature in the lake. After ~24 h, final samples (T_f) were filtered in the same
180 manner as the T_0 samples. Total NH_4^+ concentrations and atom % ^{15}N for all samples were
181 determined by AIRTS/HPLC (Bruesewitz et al., 2015; Gardner et al., 1995). Potential uptake and
182 actual regeneration rates were calculated using the Blackburn/Caperon isotope dilution model
183 (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 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 $^{15}NH_4^+$ 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 98% $^{15}NH_4Cl$ (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.

Accumulation of $^{15}NO_2^-$ 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.

Deleted: Ammonia and NO_2^- oxidation

Deleted: Ammonia oxidation rates were measured from

Accumulation of $^{15}NO_3^-$ 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 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 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). Nitrification rates were corrected for NaN_3 reduction efficiency, and $^{15}NO_2^-$ production was calculated as:

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

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

And $^{15}NO_3^-$ production:

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

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

Total nitrification rates were calculated from the sum of $^{15}NO_2^-$ and $^{15}NO_3^-$ 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

Deleted: Nitrite oxidation rates were measured from

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. The detection limit was 980 copies/ml for AOB and 4807 copies/ml 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 two 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 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 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 µg L^{-1}), but bloom conditions (> 20 µg L^{-1}; Xu et al., 2015) were observed at some locations (e.g., 20.3 µg L^{-1} at Station 3, and visual confirmation at Stations 1, 3, and several other areas of the lake). Bloom conditions were also present and observed at all sites in June 2014 (mean = 36.6 µg L^{-1}), July 2016 (mean = 58.1 µg L^{-1}), and August 2013 (43.7 µg 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 ± 0.04 µmol L^{-1} 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 ± 0.06 µmol L^{-1} h^{-1}) were significantly higher than deep rates (mean = 0.31 ± 0.08 µmol L^{-1} 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 ± 0.01 µmol L^{-1} h^{-1}) and June 2014 (0.13 ± 0.05 µmol L^{-1} 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 ± 0.04 µmol L^{-1} h^{-1}) were lower than those during August 2013 and June 2014 (mean = 0.43 ± 0.41 µmol L^{-1} h^{-1}) except for Station 10, where the rates were significantly higher (mean = 1.36 ± 0.20 µmol L^{-1} 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, light uptake rates were highest at Stations 1, 7, and 10 (1.31 – 6.82 µmol L^{-1} h^{-1}). Stations 3 and 7 rates were highest in bottom waters (0.80 ± 0.16 µmol L^{-1} h^{-1} and 2.55 ± 0.14 µmol L^{-1} 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 ± 0.67 µmol L^{-1} h^{-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,

Deleted: During the early summer bloom i

Deleted: DuringIn the early spring bloom of

Deleted: summer bloom

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} h^{-1}), but July 2016 regeneration rates (mean = 0.75 ± 0.16 μmol L^{-1} 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 ± 0.16 μmol L^{-1} h^{-1}). In March 2015, mean surface and deep regeneration rates decreased from the river mouth (Station 10; 0.88 ± 0.15 μmol L^{-1} h^{-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} h^{-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. From $^{15}NH_4^+$ additions, 91.8 % of the label was detected as $^{15}NO_3^-$ and only 8.2 % as $^{15}NO_2^-$. 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 ± 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 2016 (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

Deleted: 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).

ranging from 2.00 – 771 nmol L^{-1} d^{-1} (mean = 270 ± 277 nmol L^{-1} d^{-1}). At Station 10 in July 2016, 80% of the $^{15}NH_4^-$ addition was detected as $^{15}NO_2^-$.

Deleted: 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 from 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 (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 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} 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). Potential NH_4^+ 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 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 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 (WorldWeatherOnline.com; accessed on <08/02/2017>) however, it is within the range of typical summer rainfall (185–320 mm; WorldWeatherOnline.com). Dark uptake rates in Taihu 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 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 (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 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 (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 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 (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). The same procedure for extrapolation of whole-lake uptake rates yields 3.5 x 10^8 kg of NH_4^+, which is 4–6 times higher than external N loads.

Deleted: in the later in the summer,

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 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 are 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 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 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 (60–480 nmol L^{-1} d^{-1}; Carini and Joye, 2008) and Lake Superior, USA (0–51 nmol L^{-1} d^{-1}; Small et al., 2013), both measured via $^{15}NH_4^+$ tracer additions, and Lake Okeechobee, FL (67–97 nmol L^{-1} h^{-1}; James et al., 2011), measured via the $^{15}NO_3^-$ pool dilution method (Carini et al., 2010). Rates on this scale were previously reported

only in eutrophic Lake Mendota (WI; 1700 – 26000 nmol L^{-1} h^{-1}; Hall, 1986) and the Paerl River Estuary (China; 2100 – 65100 µmol L^{-1} 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 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^+, 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 $^{15}NO_3^-$ exceeded accumulation of $^{15}NO_2^-$ 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 $^{15}NO_3^-$ was expected, since NO_3^- is the final product of total nitrification.

At Station 10, accumulation of $^{15}NO_3^-$ exceeded $^{15}NO_2^-$ in March 2015 and June 2014. In July 2016, however, accumulation of $^{15}NO_2^-$ was three times higher in surface water and comparable at depth (Fig. 3b). Ambient NO_2^- concentration at Station 10 in July 2016 was 9.6 µM in surface water and 8.4 µM 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 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).

Deleted: Nitrification Ammonia oxidation rates were positively correlated with ambient NH_4^+, NO_2^-, and NO_3^- concentrations ($p < 0.05$; Table 2), as expected.

Deleted: 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.

Deleted: Nitrite oxidation rates were not related to NO_2^- concentrations, perhaps due to the standing pool of ambient NO_2^-. However, at some stations,

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. 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 can assimilated 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 µM to 37 µM (Baldia et al., 2007; Nicklisch and Kohl 1983) in culture, and up to 112.9 µM in Taihu populations (Yang et al., 2017), *Microcystis* should be able to outcompete nitrifiers at the high ambient NH_4^+ concentrations in Taihu as nitrifiers may become saturated as

Deleted: 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).

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.

Deleted: 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.

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 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 in Taihu sediments, where AOA were negatively correlated ($r = 0.53$, $p < 0.05$) with potential nitrification rates (0 – 3.0 μg NO_3^-N g^{-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, 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. 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 slower. Therefore, it is possible that AOA might be playing an expanded role in Taihu, beyond just NH_4^+ oxidation.

Deleted: we speculate

Deleted: different

4.4 Multiple regression model

The best-fitting multiple regression models for N dynamics in Taihu (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 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, model for nitrification rates did not reveal that the seasonal variables, such as temperature, played a major role in the model.

Deleted: driven by

Deleted: , and NH_4^+ and NO_2^- oxidation rates,

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. 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

Deleted: and nitrification in bloom formation and maintenance in Taihu

610 contribute proportionately to nitrification, suggesting that AOA might play another role in the
611 lake.

612 Ammonium inflow into the lake is a large source of reduced N, but external inputs are
613 not the sole source. Extrapolated whole-lake regeneration rates in the water column were twice
614 as high as external N loadings into the lake. To mitigate harmful algal blooms, N loadings into
615 the lake must be reduced so that N can be efficiently removed through denitrification, instead of
616 being recycled in the water column. Our results support the recent calls for dual nutrient (N + P)
617 management strategies (Paerl et al., 2011) and highlight the importance of (chemically) reduced
618 N removal through nitrification and denitrification.

619
620
621 Acknowledgments
622
623 We thank Guang Gao for laboratory space at NIGLAS and Kaijun Lu and other graduate
624 students at NIGLAS and TLLER for help in the field and in the lab. We also thank Richard
625 Doucett at UC Davis Stable Isotope Facility for ^{15}N sample analysis, and Justin Myers, Megan
626 Reed, Ashlynn Boedecker, and Desi Niewinski at WSU for help with nutrient analysis. We also
627 thank Daniel Hoffman at WSU for valuable help with nitrification experiments and Elise Heiss
628 for her input on statistical analysis. This work was jointly supported by the International Science
629 & Technology Cooperation Program of China (2015DFG91980) and the National Natural
630 Science Foundation of China (41573076, 41771519).

631

References:

Akaike, H.: A new look at the statistical model identification, IEEE transactions on automatic control, 19(6), 716–723, 1974.

An, S. and Joye, S. B.: Enhancement of coupled nitrification-denitrification by benthic photosynthesis in shallow estuarine sediments, Limnol. Oceanogr., 46(1), 62–74, doi: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(6), 607–613, doi: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(4), 429–441, doi: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(4), 1285–1289, doi:10.1128/AEM.02018-09, 2010.

Blackburn, T. H.: Method for Measuring Rates of NH(4) Turnover in Anoxic Marine Sediments, Using a N-NH(4) Dilution Technique., Appl. Environ. Microbiol., 37(4), 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(14), 3033–3042, doi: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(2), 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(5), doi: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(5), 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(12), 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(5), 792–812, doi: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 15N isotope dilution technique, Mar. Biol., 54(1), 33–40, doi: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(6), 2546–2557, doi: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(17), 1795–1801, doi:10.1016/j.csr.2010.08.001, 2010.

Chen Y., Qin B., Teubner K., Dokulil M.: Long-term dynamics of phytoplankton assemblages: Microcystis-domination in Lake Taihu, a large shallow lake in China, J. Plankton res., 1988(4), 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= Huanjing kexue, 33(7), 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(8), 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(5), 1227–1244, doi:10.5194/bg-5-1227-2008, 2008.

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 Discussions, 5(2), 1545–1585, 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(7583), 504–509, doi: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, Freshw. Biol., 59(5), 1076–1085, doi: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(10), 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(12), 1135–1142, doi: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, Proc. Natl. Acad. Sci. U. S. A., 102(41), 14683–14688, doi: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(6), 1200–1209, doi: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(2), 185–198, doi:10.1007/s10533-009-9329-5, 2009.

Gardner, W. S., Bootsma, H. A., Evans, C. and John, P. A. S.: Improved chromatographic analysis of 15N:14N ratios in ammonium or nitrate for isotope addition experiments, Mar. Chem., 48(3–4), 271–282, doi:10.1016/0304-4203(94)00060-Q, 1995.

Gardner, W.S., P.A. St. John, C. Evans, and J. Cavaletto.: 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, Limnololgy Oceanogr., 43(8), 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(C3), C03007, doi: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., 2017.

Glibert, P. M., Maranger, R., Sobota, D. J. and Bouwman, L.: The Haber Bosch–harmful algal bloom (HB–HAB) link, Environ. Res. Lett., 9(10), 105001, doi: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(1), 165–

197, doi: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 Spectrom., 23(23), 3753–3762, doi:10.1002/rcm.4307, 2009.

Hall, G.H.: Nitrification in lakes, in: Nitrification, 1st edition, edited by J. I. Prosser, 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 Sci., 178, 110–119, doi: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(7), 2285–2296, doi: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(1), 199–212, doi:10.1007/s10750-011-0683-7, 2011.

Jenkins, M. C. and Kemp, W. M.: The coupling of nitrification and denitrification in two estuarine sediments l v2, Limnol. Oceanogr., 29(3), 609–619, 1984.

Jia, Z. and Conrad, R.: Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil, Environ. Microbiol., 11(7), 1658–1671, doi:10.1111/j.1462-2920.2009.01891.x, 2009.

Jung, M. Y., Park, S. J., Min, D., Kim, J. S., Rijpstra, W. I. C., Damst??, 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. Microbiol., 77(24), 8635–8647, doi: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(7058), 543–6, doi:10.1038/nature03911, 2005.

Kowalchuk, G. A. and Stephen, J. R.: Ammonia-oxidizing bacteria: a model for molecular microbial ecology, Annu. Rev. Microbiol., 55(1), 485–529, doi:10.1146/annurev.micro.55.1.485, 2001.

Krausfeldt, L. E., Tang, X., van de Kamp, J., Gao, G., Bodrossy, L., Boyer, G. L. and Wilhelm, S. W.: Spatial and temporal variability in the nitrogen cyclers of hypereutrophic Lake Taihu, FEMS Microbiol. Ecol., 93(4), 1–11, doi:10.1093/femsec/fix024, 2017.

Li, D. ming, Zheng, H. yan, Pan, J. lin, Zhang, T. qing, Tang, S. kai, Lu, J. ming, Zhong, L. qiang, Liu, Y. shan and Liu, X. wei: Seasonal dynamics of photosynthetic activity, Microcystis genotypes and microcystin production in Lake Taihu, China, J. Great Lakes Res., 43(4), 710–716, doi: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(2), 711–722, doi:10.1002/lno.10246, 2016.

Martens-Habbena, 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(7266), 976–979, doi: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(1), 195–207, doi: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(3), 215–227, doi: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(1), 173–188, doi: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(17), 5589–5595, doi: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(1), 41–46, doi: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(7184), 202–205, doi:10.1038/nature06686, 2008.

Newell, S. E., Babbin, A. R., Jayakumar, D. A. and Ward, B. B.: Ammonia oxidation rates and nitrification in the Arabian Sea, Global Biogeochem. Cycles, 25(4), 1–10,

doi: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(3), 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. Microbiol., 81(2), 745–753, doi: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(4), 907–918, doi: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(5), 1349–1363, doi: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(5), 1973–1983, doi: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(11), doi: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, doi: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(7563), 105–8, doi: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), Bull. Environ. Contam. Toxicol., 99(3), 380–384, doi:10.1007/s00128-017-2149-8, 2017.

Qin, B. Q.: Lake Taihu, China: Dynamics and environmental change. Springer Netherlands, 2008.

Qin, B., Xu, P., Wu, Q. L., Luo, L. and Zhang, Y.: Environmental issues of Lake Taihu, China, Hydrobiologia, 581(1), 3–14, doi: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(1), 105–112, doi:10.1007/s00267-009-9393-6, 2010.

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. Microbiol., 63(12), 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. Bio. Ecol., 404(1–2), 47–56, doi: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(1), 276–286, doi: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(8), 1–9, doi: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., doi: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, Ecsn, 184, 299–308, doi: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. Geoph. Res Lett., 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., 0(October), 1–13, doi: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, Proc. Natl. Acad. Sci. USA, 108(20), 8420–8425, doi:10.1073/pnas.1013488108/-/DCSupplemental.www.pnas.org/cgi/doi/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(5), 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(6), 1067–1071, doi: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. Trans. R. Soc. B Biol. Sci., 368(1621), 20130119, doi:10.1098/rstb.2013.0119, 2013.

Ward, B. B.: Nitrification in marine systems, in: Nitrogen in the Marine Environment, 2nd Edition, edited by: Capone, D., Bronk, D., Mulholland, M., and Carpenter, E., Elsevier, Amsterdam, 2008.

Wu, Y., Ke, X., Hernández, M., Wang, B., Dumont, M. G., Jia, Z. and Conrad, R.: Autotrophic growth of bacterial and archaeal ammonia oxidizers in freshwater sediment microcosms incubated at different temperatures, Appl. Environ. Microbiol., 79(9), 3076–3084, doi:10.1128/AEM.00061-13, 2013.

Xu, H., Paerl, H.W., Qin, B., Zhu, G. and Gaoa, G.: Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. Limnol. Oceanogr., 55(1), 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(2), 1051–1059, doi: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., Xu, J.: Water quantity and pollutant fluxes of inflow and outflow rivers of Lake Taihu, 2009, (Chinese). J. Lake Sci. 6, 855e862, 2011.

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, doi: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(8), 1018–1026, doi: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(3), 327–332, doi:10.1007/s00284-013-0369-7, 2013.

1000
1001
1002

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. Total nitrification rates calculated from accumulation of $^{15}NO_2^-$ (grey) and $^{15}NO_3^-$ (black) ± one standard deviation. (a) Stations 1–7. (b) Station 10. The two axis show different units for total nitrification rates: nmol L^{-1} d^{-1} (left) and μmol L^{-1} h^{-1} (right).

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

1025 Table 1.
1026 Environmental characteristics during sampling events for each station/depth: temperature,
1027 dissolved oxygen (DO), pH, chlorophyll a (chl a; surface only), total dissolved solids (TDS), and
1028 in situ nutrient concentrations. S in station name = surface water (0.2 m), and D = deep, near-
1029 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	53.9	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	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	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	BDL*	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	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: NH4+ = 0.04 µM; NOx = 0.04 µM; OP = 0.008 µM.

1030

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

		Temp	DO	pH	Chl a	TDS	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**
	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
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**

Table 3.

Comparison of ammonium uptake and regeneration rates among different freshwater studies

	Up(L)	Up(D)	Reg Avg	Chl a ($\mu g\ L^{-1}$)	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

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	9.14×10^{-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

Figure 1


Liangxi River
Wuxi
Zhihu Gang
Wujin Gang
Yincun Gang
1
3
CH
TLLER
N
Wangyu River
7
Dapu Gang
10
Suzhou
Lake Taihu
Xu River
Jiapu River
Tiapu River
5km
CHINA
31° 24' N
Lake Taihu
120° 13' N
Changdou Gang
Daojian Gang
Legend
Surrounding River
Station Locations

Figure 2

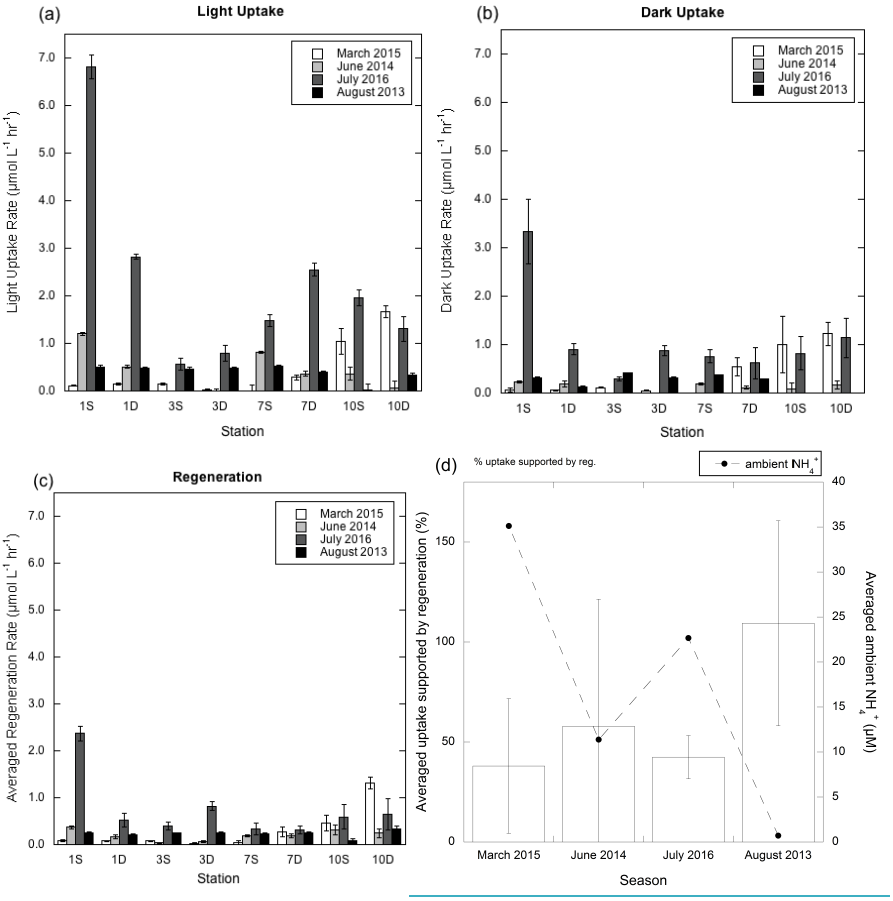

(a)
Light Uptake
March 2015
June 2014
July 2016
August 2013
Light Uptake Rate (μmol L-1 hr-1)
Station
1S
1D
3S
3D
7S
7D
10S
10D
(b)
Dark Uptake
Dark Uptake Rate (μmol L-1 hr-1)
(c)
Regeneration
Averaged Regeneration Rate (μmol L-1 hr-1)
(d)
% uptake supported by reg.
ambient NH4+
Averaged uptake supported by regeneration (%)
Averaged ambient NH4+ (μM)
March 2015
June 2014
July 2016
August 2013
Season

Figure 3

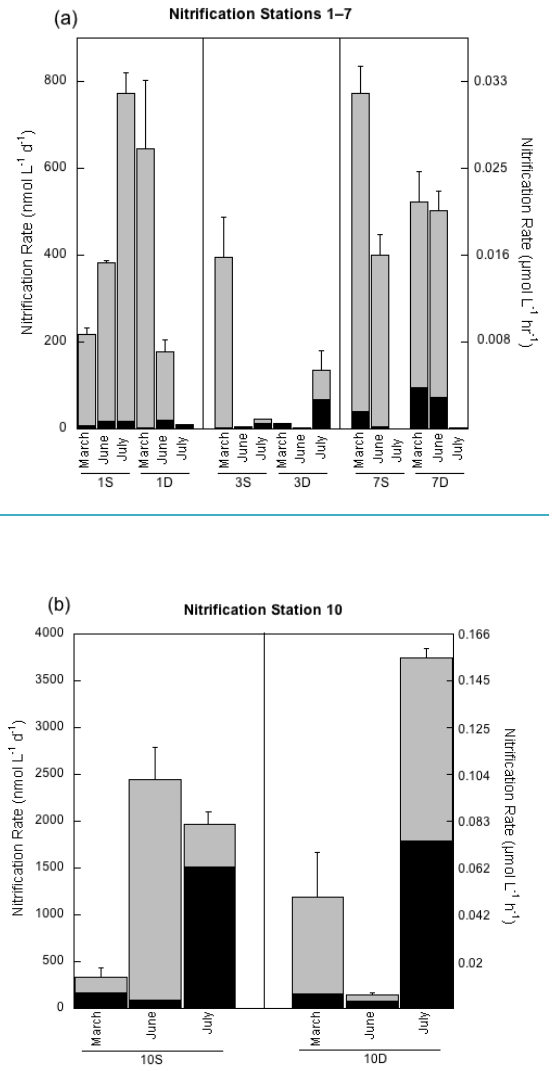
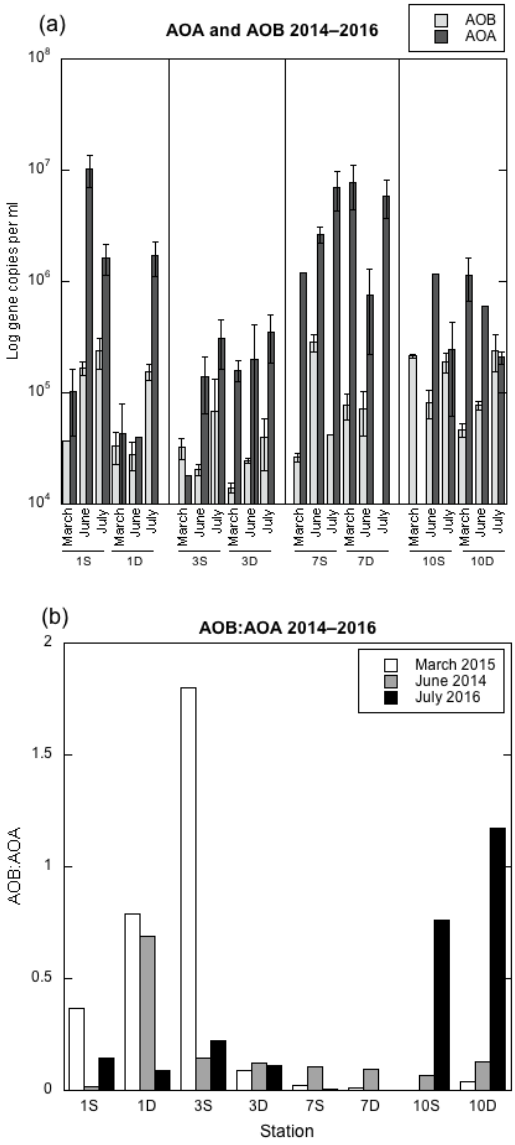

(a)
Nitrification Stations 1–7
Nitrification Rate (nmol L-1 d-1)
Nitrification Rate (µmol L-1 hr-1)
800
600
400
200
0
0.033
0.025
0.016
0.008
March
June
July
1S
1D
3S
3D
7S
7D
(b)
Nitrification Station 10
Nitrification Rate (nmol L-1 d-1)
Nitrification Rate (µmol L-1 h-1)
4000
3500
3000
2500
2000
1500
1000
500
0
0.166
0.145
0.125
0.104
0.083
0.062
0.042
0.02
10S
10D

Figure 4


(a)
AOA and AOB 2014–2016
AOB
AOA
Log gene copies per ml
March
June
July
1S
1D
3S
3D
7S
7D
10S
10D
(b)
AOB:AOA 2014–2016
March 2015
June 2014
July 2016
AOB:AOA
Station