

## 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  $K_m$  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:*

Historically, these blooms have been associated with toxin producing, non-N<sub>2</sub> 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).

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 (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 Nicklisch 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  $\mu\text{M}$  (Yang et al., 2017). We will add the following text to the Discussion section:*

With a high saturation threshold and reported  $K_m$  values from 26.5  $\mu\text{M}$  to 37  $\mu\text{M}$  (Baldia et al., 2007; Nicklisch and Kohl 1983) in culture and up to 112.9  $\mu\text{M}$  in Taihu populations (Yang et al., 2017), *Microcystis* should be able to outcompete nitrifiers at the high ambient NH<sub>4</sub><sup>+</sup> 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  $\text{NH}_4$  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: Chlorophyll a data showed seasonal variation. Overall, lowest values were recorded in March 2015 (mean =  $11.1 \mu\text{g L}^{-1}$ ), but bloom conditions ( $> 20 \mu\text{g L}^{-1}$ ; Xu et al., 2015) were observed at some locations ( $20.3 \mu\text{g L}^{-1}$  at station 3, and visual observations at Station 1 and several other areas of the lake). Bloom conditions were also observed in June 2014 (mean =  $36.6 \mu\text{g L}^{-1}$ ), July 2016 (mean =  $58.1 \mu\text{g L}^{-1}$ ), and August 2013 ( $43.7 \mu\text{g L}^{-1}$ ).*

*We will also provide a supplemental table of field notes and observations describing bloom status during each sampling event.*

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  $\text{NH}_4^+$  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  $\text{NH}_4^+$  dynamics. We pointed this out in lines 518–523. 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:*

Taihu Lake has a relatively long residence 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 NH<sub>4</sub> concentrations, or at least 8 μM (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 NH<sub>4</sub> 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.*

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:*

*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 NH<sub>4</sub><sup>+</sup> 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<sub>4</sub><sup>+</sup> = 0.04 μM; NO<sub>x</sub> = 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 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  $\text{NH}_4^+$  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  $\text{NH}_4^+$ . Palatinszky et al. (2015) shows lower protein concentration in AOA grown on cyanate than on  $\text{NH}_4^+$ . Tolar et al. (2016) shows lower oxidation rates of urea than  $\text{NH}_4^+$  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:*

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 a different 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:*

This study highlights the importance of water column  $\text{NH}_4^+$  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  $\text{NH}_4^+$  demand during cyanobacterial blooms in Taihu.

Line 528: Assuming  $\text{NH}_4$  is the preferred substrate

*Many studies have shown that  $\text{NH}_4^+$  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>