

Response to Referee # 2

How do you define nitrification? If it is the rate at which NH_4 transforms to NO_3 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 $^{15}\text{NO}_3^-$ must have originated from $^{15}\text{NH}_4^+$ 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 $^{15}\text{NH}_4^+$ additions (i.e., comparing $^{15}\text{NO}_2^-$ to the $^{15}\text{NO}_3^-$ pool). We will revise the text in the methods, results, and discussion accordingly. For example, we will review this issue in the Discussion as follows:

Nitrification rates were positively correlated with ambient NH_4^+ , NO_2^- , and NO_3^- concentrations ($p < 0.05$; Table 2), as expected. Substrate concentrations drive NH_4^+ oxidation rates and, therefore, end-product pools, since it is the rate limiting step of nitrification (i.e., completion of nitrification is dependent on the first step). Accumulation of $^{15}\text{NO}_3^-$ exceeded accumulation of $^{15}\text{NO}_2^-$ by a factor of 9 at stations 1, 3, and 7, across all sampling events (Fig. 3a), indicating that NO_2^- oxidation is keeping pace with or exceeding NH_4^+ oxidation. Higher accumulation of $^{15}\text{NO}_3^-$ was expected, since NO_3^- is the final product of total nitrification.

In contrast, at station 10, while accumulation of $^{15}\text{NO}_3^-$ exceeded $^{15}\text{NO}_2^-$ in March 2015 and June 2014, in July 2016 accumulation of $^{15}\text{NO}_2^-$ was three times higher in surface water and comparable at depth (Fig. 3b). Additionally, there was a significant pool of nitrite: ambient NO_2^- concentration at station 10 in July 2016 ranged from 9.6 μM in surface water to 8.4 μM at depth (Table 1). This accumulation of NO_2^- could indicate that NO_2^- oxidizers were saturated, as reported 418 Km values for NO_2^- oxidation in an oligotrophic, oxygen deficient region in the ocean were $0.25 \pm 0.16 \mu\text{M}$ (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 “ N_2 fixation”

We will revise to N_2 fixation.

L44: idem as L43 L57: maybe useful to say that NH_4 can accumulate in systems when there is O_2 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 O_2 in this uncoupling: There are many “kinetic” studies that show that nitrite oxidation is more sensitive to low O_2 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= cyanobacteria harmful 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:

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% ^{15}N : of ammonium?

Yes, we will clarify in the revised manuscript that we mean atom % of $^{15}\text{N-NH}_4^+$

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:

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 ($43.7 \mu\text{g L}^{-1}$).

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. $\text{nmol L}^{-1} \text{d}^{-1}$ is usually used for these rates (Bristow et al., 2015; Heiss and Fulweiler 2016; Newell et al. 2011; Ward and Kilpatrick 1991). Uptake and regeneration 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 $\mu\text{M/hr}$ units.*

L306 How do you define the total nitrification rate? By definition nitrification is the 2 steps reaction $\text{NH}_4 \rightarrow \text{NO}_2 \rightarrow \text{NO}_3$, as you said in the introduction, so it should be the rate at which NH_4 is transformed into NO_3 . 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 $\text{NO}_2 + \text{NO}_3$? but then as most of the NO_2 is not produced by NH_4 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 $^{15}\text{NO}_2^-$ and $^{15}\text{NO}_3^-$ production), as well as our discussion of the partitioning of the product of $^{15}\text{NH}_4^+$ additions (i.e., comparing $^{15}\text{NO}_2^-$ to the $^{15}\text{NO}_3^-$ pool).

Reference:

Clark et al., 2008 doi:10.2307/40006149

Ward and Kilpatrick 1991 [https://doi.org/10.1016/0278-4343\(90\)90016-F](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 NH_4 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:*

While potential NH_4^+ uptake rates increase with chlorophyll a ($p < 0.05$), the relative proportion of community uptake that can be supported by regeneration remains 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:

“Photoautotrophs may continue to assimilate nutrients in the dark under nutrient limitation (Cochlan et al., 1991). However, Taihu is nutrient replete, so dark uptake rates can likely be attributed 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.

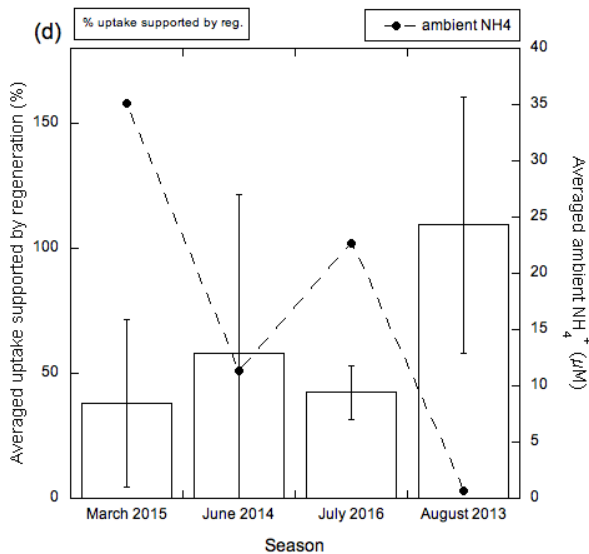
Yes, more runoff = more nutrient inputs.

There is strong bloom evidence looking at July 2016 chlorophyll (above the chl a threshold of $20 \mu\text{g L}^{-1}$; Xu et al., 2015) and high nutrient concentrations (Table 1). We will add a Supplementary table with our field notes and visual observations to help clarify this point.

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 NH₄⁺ 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 NH₄⁺, NO₂⁻ and NO₃⁻ and cycling rates are high; therefore, a nitrification extrapolation would not be informative.

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:

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 $^{15}\text{NO}_3^-$ pool dilution method. Rates in Lakes Superior and Mono (Line 397), however, were measured using the same $^{15}\text{NH}_4^+$ 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:

Published nitrification rates in lakes include the water columns of saline Lake Mono, CA (60–480 $\text{nmol L}^{-1} \text{d}^{-1}$; Carini and Joye, 2008) and Lake Superior, USA (0–51 $\text{nmol L}^{-1} \text{d}^{-1}$; Small et al., 2013), both measured via $^{15}\text{NH}_4^+$ tracer additions, and Lake Okeechobee, FL (67–97 $\text{nmol L}^{-1} \text{hr}^{-1}$; James et al., 2011) measured via the $^{15}\text{NO}_3^-$ pool dilution method (Carini et al. 2010).

Rates on this scale were previously reported only in eutrophic Lake Mendota (WI; 1700 – 26000 $\text{nmol L}^{-1} \text{hr}^{-1}$; Hall, 1986) and the Paerl River Estuary (China; 2100 – 65100 $\mu\text{mol L}^{-1} \text{d}^{-1}$; Dai et al., 2008). However, these rates were measured from accumulation of NO_2^- and NO_3^- , not stable isotope additions.

L402: nitrification or ammonium oxidation?

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

L414-415 “Higher NO_2 oxidation rates were expected, since NO_3 is the product of NO_2 oxidation, and NO_2 oxidation relies on the product of NH_4 oxidation” I don’t understand this statement. NO_2 oxidation also relies on external sources of NO_2 to the lake. It is not clear how you can have 10 times higher NO_2 oxidation compared to NH_4 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 NH_4 oxidation?

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

L451 idem L424 L454 but as NO_2 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 $^{15}\text{NH}_4^+$.

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”