

Interactive comment on “Effects of cyanobacterial-driven pH increases on sediment nutrient fluxes and coupled nitrification-denitrification in a shallow fresh water estuary” by Y. Gao et al.

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Here we really appreciate the comments from the reviewer and we have improved our manuscript significantly, based on these comments. The detailed explanations are provided below. 1. pH: Considering the experimental treatment, is the difference in elevated pH's (9.2 vs. 9.4-9.5) large enough to be considered different? Would have been nicer to have a pH value in mid 8's and low 10's. Please comment on why this might not have been possible. Discuss this also in light of the pK_b for NH₃. Also, given the starting pH's of your sampling sites, you should elaborate more on what are normal

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vs. extreme pH's at these sites. The point of departure for the Powerline site was not pH=7, so calling pH=7 a “control” may in fact be misleading in this case. The highest experimental pH was in fact the “control” in this situation. A slight increase in pH leads to great changes in the equilibrium between NH₃ and NH₄⁺. According to the equation (2), NH₃ account for 47% of total ammonium at pH= 9.2 and 65%-60% of total ammonium at pH ranges of 9.4-9.5. Moreover, the pH elevation from 9.2 to 9.4-9.5 caused a significant difference for SRP fluxes, total ammonium fluxes, nitrification rates and N₂ fluxes (Fig.3-6). At Powerline site, in situ pH was above 9 but not the departure point for pH experiments. We removed cyanobacteria by filtration, and then gently bubbled the waters overnight before experiments (in section 2.1). Enrichment of CO₂ from the atmosphere may alleviate C insufficiency during a bloom, resulting in a new carbonate system balance in air-water interface and leading to a decreased pH.. Both pH incubations from Powerline and Budds Landing were started from the similar neutral pH. We agree that data at pH's in the mid to high 8's would have been very useful, potentially leading to an early release of NH₄⁺ prior to the initiation of large fluxes of SRP. We designed these experiments to contrast N and P recycling, there would be a large benefit for future work to more fully examine a broader pH range for effects on sediment N cycling.

2.Comparison of the two different approaches to measure nitrification. Compare and discuss them in discussion, however, and not the methods section (p. 1168, lines 16-18). (Alternatively, include this comparison as an important point in the introduction).

It is a good idea to move the comparison of different nitrification methods to introduction.

3. Porewater profile. 3.1 The confusing discrepancy between the results text (p. 1172, lines 1-9) and Figure 1. The text refers to Powerline (P) but the figure text says Budds Landing (BL). The mistake has been corrected. 3.2 I assume the latter is correct as only the design used at BL allowed pore-water sampling at elevated pH conditions. But aren't there also some porewater data for P as well? Are they not presented?

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We presented porewater SRP profile in Fig. 1C and 1D, and described the vertical profiles in section 3.3.3.3 Please clarify and correct the methods section if necessary. Note, the letters (A-F) are missing from the figure as well. Thank you for suggestion. A-F are added to the Figures.

4. The SRP and NH_4^+ flux data at the high pH treatment for BL presented in Table 4 and Figure 3 do not match. It seems like sites were switched. On the other hand, they were not switched for the control pH level. Such inconsistencies lead me to doubt whether when the authors present the correct data for the right sites, treatments, etc.

Thank you. Powerline sediments were used to compare the estimated nutrient diffusion rates and flux measurements (Table 4 and Fig. 3), which is same as the correction for question 3.1.

5. Please explain better the "cause" of the pH error bars in the NH_4^+ adsorption-desorption experiments (Fig. 2). What "error" or variability is actually shown? Minmax pH? s.d.? It is not stated in the figure text. Also discuss whether these observed changes in pH are consistent (can be solely explained) by the acid-base chemistry of the NH_4^+ - NH_3 reaction. Or are other factors affecting pH during these incubations? Also, state what model is being fit in Fig. 2A and B. Where do the solid lines come from and how are they estimated? A similar comment can be made about Fig. 5A. Is that line in any way related to the kinetics discussed on p. 1176, lines 22-23 for nitrification? Nothing is stated in either the manuscript or figure text.

Thank you. Although a series of pH solutions were prepared for the NH_4^+ desorption experiments, sediment addition decreased pH levels because of buffering. In Figure 2, the error bars of pH (Fig. 2) means standard deviation of pH before and after the incubations ($n=3$). To clarify the description, I will use the end point of pH concerning that water column pH may change quickly after shaking. Thank you. During the incubation, factors affecting pH changes include the release of weak acid (e.g. from sulfide oxidation) from sediments; sediment organic-carbon decomposition preservation associated with

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formation of H_2CO_3 ; and reaction of $\text{HCO}_3^-/\text{CO}_3^{2-}$ with sediment cations (e.g. Ca^{2+} and Mg^{2+}). Thank you. In Fig. 2B, the line represents 'total' exchangeable NH_4^+ , estimated by KCl extraction of pH-neutral sediment. Thank you. Here is a linkage of total ammonium in Fig. 2 with the nitrification (p. 1176, line 22-23). The desorbed ammonium may be lost as NH_3 into headspace and become unavailable for nitrification. Also, pH increases caused an accumulation of dissolved NH_3 in the surrounding water that constrain nitrifying bacteria metabolism. Both processes may contribute for nitrification suppression.

6. p. 1177, lines 18-20 and Fig. 5B : Is what is shown in Figure 5B the augmented NH_4^+ flux rates (with inhibitor) or the calculated (by difference) nitrification rate? The figure text indicates the latter, but the discussion in the text seems to imply the former (and a nitrification rate of $\sim 30 \mu\text{mol m}^{-2} \text{h}^{-1}$). Which is it? It has importance re NH_4^+ relevance for understanding the N_2 flux data later.

Thank you. The position of the CF3-inhibited nitrification (A) and nitrification potential (B) is adjusted, followed by the reorganization in text.

7. p. 1178, lines 2-3: It is not clear to me why a pH driven release of adsorbed NH_4^+ would inhibit nitrification per se. If that NH_4^+ is not lost, then this should still be available (unless adsorbed NH_4^+ is actually more available for nitrifiers). This inhibition of nitrification at higher pH's must also be physiological, as argued with NH_3 inhibition, or that NH_4^+ is lost via diffusive fluxes before it could be nitrified. I am not sure whether the authors can come with some insights or calculations about the relative rates of these fates of NH_4^+ once desorbed, but it would be appreciated.

Thank you. We agreed with the reviewer's opinion that pH and NH_3 formation may inhibit the nitrifying efficiency by constraining physiological bacteria activities. Thank you. While the stoichiometry from these experiments is very good, we are hesitant to mass balance the system since our flux measurements were not continuous. Time is a key to determine the depth of high pH in sediments. With pH penetration continuously, the NH_4^+ - NH_3 equilibrium, DIN fluxes, the efficiency of ammonium oxidation, and denitrification will

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change over time. Using current desorption rates as a function of pH also may be inappropriate to calculate the simulated nitrification rates. The initial NH_4^+ concentration were significantly different for desorption experiments (no-extra addition) and potential nitrification incubation ($[\text{NH}_4^+] = 1.0 \text{ mmol}$). According to equilibrium of exchangeable ammonium to aquatic system, the desorbed NH_4^+ may be different, followed by a large discrepancy of NH_3 at same pH levels. Furthermore, difference of NH_3 , combined with pH elevation, cause the uncertain inhibition on bacteria activity and fate of the remineralized N.

8. The discussion of the controls of denitrification (3.11) and its relationship to nitrification as well as physiological tolerances for pH are good and insightful. The points about the contribution of DNRA to NH_4^+ fluxes is interesting, but could be made more quantitative if it is possible to calculate how much of the elevated diffusive fluxes (Table 4) were supported by porewater NH_4^+ (Fig. 1) increased due to pH-induced NH_4^+ desorption (Fig. 2). I am not sure if this calculation can be made precisely without a model, but it seems to me like you have the parts in place of a good first order calculation.

Again, without a more continuous trajectory of change in the pore water composition, we don't think this is likely to be a useful calculation. DNRA can be a pathway for NH_4^+ flux but is in low fraction in N remineralization. It is hard to tell which proportion of released NH_4^+ coming from pH-induced ammonium desorption or from DNRA.

9. Why not move the presentation of the effects of pH on SRP flux (3.7) closer to the section on SRP profiles (3.1)? It seems awkward to separate them and it breaks up the flow of discussion on N processes (sections 3.4-3.11). Placed here this section seems just like an afterthought.

Agreed, the pH effect on SRP flux and pore water diffusion are now together (Section 3.3 and 3.4). 10. The t-test used to test for effects of pH on SRP and NH_4^+ fluxes (3.7 and 3.8) is inappropriate. You have 3 treatments and thus these should be consid-

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ered simultaneously using an ANOVA. That ANOVA can then be followed up by multiple pairwise comparisons using Tukey's HSD, for example. Why were statistical tests not conducted for other parameters tested (e.g., O_2 flux, potential nitrification, etc.)? Furthermore, no mention is made of whether the data was tested for distributional considerations (normality and homogeneity) necessary before applying parametric statistics. This must also be stated. We will add the statistic evaluation in method and figures as suggested.

I appreciate the conclusion and ecological implication section. It is the only place where the authors attempt to pull the individual processes, discussed individually, together. But I feel that the first section on harmful algal blooms is too long and is too much like an introduction. What is important here is to tell us that such blooms do affect N cycling and thus help promote themselves by enhancing nutrient availability. I would like the authors to state more clearly whether the pH effect on benthic N or P release is greater; it seems like the latter (on a relative basis) and thus while this manuscript has mostly focused on N cycling that in fact the enhanced P flux is a greater effect and will help maintain N-fixing cyanobacteria blooms. This, together with less denitrification (this ms), will lead to even greater N input to the estuary, continuing eutrophication, primary production, and even faster element cycling overall. This does appear to be a troublesome positive feedback system. These comments are appreciated and are reflected in the rewrite. Detailed Comments: p. 1164, line 13-14. What is water depth of collection sites? (move to here) Added p. 1164, line 20-21. What was the effect of this bubbling on pH in the water? This bubbling tended to decrease pH or close to neutral. High pH, such as natural water from Powerline, was sustained by photosynthetic carbon removal during bloom. Aerating CO_2 balance the air-water DIC equilibrium and prepared for the similar neutral pH for experimental incubation. We find it interesting that manual bubbling in the lab relaxes the pH to levels lower than in the field data, possibly suggesting hindrance of gas exchange.

p. 1166, line 9-11. Need to state somewhere that they cores were sealed (and how).

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The whole flow-through system is not very well described. Were peristaltic pumps used? How was this controlled? The flow-through system was set up for each core, in which alkaline water inflow from reservoir and passed through the overlying water (~ 500 ml) into waster tanks. The filtered water was bubbled with air to maintain oxygen saturation and adjusted to experimental pH with NaOH. In order to maintain constant conditions (e.g pH levels, oxygen, flow speed) inside of sediment cores, we connected the targeted pH water to each sediment core and blank core in same group using plastic tubing, and continuously pumped at 10 ml min⁻¹ using a speed-controlled peristaltic aquarium pump

p. 1166, line 23-25. Mention already here that they were sectioned under anoxic conditions.

I will describe the sediment section briefly in p. 1166, line 23-25, and elaborate processes of porewater collection in sections 2.4.

p. 1167, line 13. Were sectioning intervals 1 cm? This is unclear. The sectioning interval for porewater were 0.5 cm in the top 2cm sediments, 1 cm for the 2-5 cm sediments and 2 cm for 5-11 sediments.

p. 1171, line 1. I could not find the reference citation (Larsen et al 2001). Larsen, R. K., Steinbacher, J. C., and Baker, J. E.: Ammonia exchange between the atmosphere and the surface waters at two locations in the Chesapeake Bay, *Environ. Sci. Technol.*, 35, 4731-4738, 2001.

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