

# ***Interactive comment on “Carbon amendment stimulates benthic nitrogen cycling during the bioremediation of particulate aquaculture waste” by Georgina Robinson et al.***

## **Anonymous Referee #2**

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### General Comments:

Robinson et al. aimed to determine if carbon amendments of aquaculture wastewater would promote nitrogen retention in a sea cucumber culture system. The authors hypothesized that the increased carbon would enhance  $\text{NH}_4^+$  assimilation by heterotrophic bacteria and change microbial community composition to favor nitrogen retention. They found that the amendment did not alter microbial community structure, benthic nutrient fluxes, and sediment characteristics. The carbon amendments did, however, enhance nitrogen fixation suggesting greater nitrogen retention. The manuscript is well-written and aims to address an important research need in the

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bioremediation of aquaculture effluent. I also think it provides valuable information on the environmental controls of nitrogen fixation and DNRA. The study results and approaches would be of interest to readers of Biogeosciences, however, I do think the authors need to clarify some of the context of the aquaculture experiment in order for the readers to fully evaluate the study results.

Major Comments:

The authors suggest that improving bioremediation of aquaculture effluent is a study goal. My assumption is that this means increasing nitrogen removal so there is less nitrogen loadings into natural ecosystems. Therefore I find the result of enhanced nitrogen fixation to be conflicting with Lines 43-44 (. . . carbon addition can provide a means to successfully bioremediate nitrogen-rich effluents). I could see fixation and recycling of nitrogen via DNRA being a positive result if the nitrogen was being assimilated by the sea cucumbers. This could then be a removal pathway but that was not measured in this study. Could the authors clarify here? Another way to look at the data set is in terms of a nitrogen budget. Would the carbon amendment result in more nitrogen in the effluent or less?

I appreciate the experimental design and the amount of measurements that were performed in the study. I was surprised by the result of no impact of the carbon addition on sediment carbon content, however, I could see how the sea cucumbers could enhance mineralization. Did the authors consider having treatment(s) with no sea cucumbers? This would have been helpful in interpreting the role of the animals on mineralization/benthic fluxes. For example, how much of the  $\text{NH}_4^+$  efflux is from sediment processes or excretion? Presenting the flux data from the "Initial" trial may help with some of this. Perhaps adding it as a Supplement and including more of this data in the discussion and interpretation of the results. Did the authors run statistical tests comparing Initial, -C, and +C?

I also think it would be helpful to know more about the ambient environmental condi-

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tions in the chambers (e.g. nutrients, oxygen, and salinity). The NO<sub>x</sub>- fluxes into the sediments are low but NH<sub>4</sub><sup>+</sup> effluxes are high. If NH<sub>4</sub><sup>+</sup> effluxes are due to DNRA, where is the NO<sub>x</sub>- coming from? The authors argue that it is not likely due to ammonification (lines 518-421) but they also give data on remineralization ratios that trended higher in the +C treatment (Lines 434-438)?

Minor Comments:

Line 34: “process nitrogen-rich particles” Does “process” imply removal or retention?

Line 40: Consider changing “indicating” to “suggesting”?

Line 74-75: Was the starch treatment a single input or done continuously?

Lines 101-102: Was the system designed to retain nitrogen or remove nitrogen (conventional or biofloc)?

Line 114: A single dose of starch or was it per day?

Lines 151-157: Why did the authors use wet weight instead of dry weight? Why not measure C:N in the sea cucumbers as well?

Line 164: How long were the stirrers paused?

Lines 215-216: Move “Equation 3. . .” to line 215?

Lines 241-242: Can you give a brief description of the carbohydrates method?

Lines 311-313: Did you do any comparisons (ANOVA) with initial, +C, and –C?

Line 397-400: Given the variability (SE) in the N<sub>2</sub> fluxes would you want to say that fixation and removal pathways were approximately equal?

Lines 409-410: It would be helpful to know the ambient nutrient concentrations.

Lines 416: Suggests the data is a time-series. Perhaps rewrite as difference between treatments?

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Lines 418, 426, 459: This seems like speculation since the oxic-anoxic interface was not measured. Can it be implied with microbial data?

Line 540-542: Consider major comments above.

Line 580: Seems like a reference would be helpful here or are you specifically referring to Welsh 1997 and Newell et al. 2016. Clarify.

Line 610: Consider changing “for” to “over”

Line 623: See major comments above. Is assimilating nitrogen better than nitrogen removal?

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