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

Georgina Robinson et al.

georgina.robinson@sams.ac.uk

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Title: Carbon amendment stimulates benthic nitrogen cycling during the bioremediation of particulate aquaculture waste Journal: Biogeosciences MS Type: Research article

Authors: Georgina Robinson, Thomas MacTavish, Candida Savage, Gary S. Caldwell, Clifford L.W. Jones, Trevor Probyn, Bradley D. Eyre and Selina M. Stead.

Dear Reviewer 2,

We thank you for your prompt and detailed review of our manuscript, which we feel

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has considerably improved the clarity and accuracy of the methods reported in the manuscript. Please find below our point-by-point response to your accurate and helpful comments. Please note that line numbers used here in the response to the reviewers' comments refer to the line numbers in the track changes version of the revised manuscript.

Major Comments

Reviewer 2, Point 1: 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?

The assumption made by Reviewer 2, that increasing nitrogen removal would fulfill the study goal of improving bioremediation of aquaculture effluent, is perfectly valid, since a general perception in aquaculture bioremediation is that processes that permanently remove nitrogen from the system are beneficial, while processes that result in nitrogen retention are detrimental. It is the opinion of the lead author, however that ecologically-based aquaculture bioremediation systems that aim to re-use and recycle nitrogen, by promoting assimilation by heterotrophic biomass or secondary organisms such as sea cucumbers, may provide a more sustainable approach to the future development of aquaculture bioremediation. This is indeed the subject of an opinion piece "As we see it' recently submitted to Aquaculture Environment Interactions. A new sentence and a reference have been added to reflect and clarify this opinion, lines 69-71 now read: This study aims to advance ecologically-based aquaculture bioremediation systems

that may provide an alternative to closing the nitrogen cycle through the promotion of assimilatory processes (Robinson, in review).

The reference 'Robinson, G.: Shifting paradigms and closing the nitrogen loop, Aquaculture Environment Interactions, in review' has been added to the reference list.

As the reviewer points out, however since the amount of nitrogen retained in sea cucumber biomass was not measured in this study, the statement in the abstract has been revised such that lines 43 - 46 now read: These findings indicate that carbon addition may provide a means to successfully bioremediate nitrogen-rich effluents, however longer-term trials are necessary to determine whether this nitrogen retention is translated into improved sea cucumber biomass yields.

Reviewer 2, Point 2: 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 NH4+ 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?

Reviewer 2 makes a very valid point regarding the consideration of a treatment with no sea cucumbers. The actual experimental design was a fully crossed design with the carbon addition (+C/-C) as one factor and the presence or absence of sea cucumbers (+SC/-SC) included in addition to the initial treatments. However, it was decided to analyse and present this data elsewhere (manuscript in prep.) since the presentation of the full set of results may detract from the study goal of determining the effect of carbon addition on aquaculture waste. Also, the effect of sea cucumbers on the mineralization

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of aquaculture waste has been previously studied and reported by two of the co-authors Mactavish, T., Stenton-Dozey, J., Vopel, K. and Savage, C. (2012) 'Deposit-feeding sea cucumbers enhance mineralization and nutrient cycling in organically-enriched coastal sediments', PLoS One, 7(11), e50031 [Online].

Statistical tests (one-way analysis of variance) comparing Initial, -C, and +C on day -1 were run, as explained in lines 303-305 (original pdf of submitted manuscript). The results of these statistical tests were reported in lines 377-378 (original pdf of submitted manuscript) for the gas fluxes and lines 401-406 (original pdf of submitted manuscript) for the nutrient fluxes. However, the helpful suggestion of the reviewer has been adopted and the flux data from the experimental treatments on day -1 has been included in the supplementary material as Fig. S1. The original Fig. S1 has been changed to Fig S2.

Reviewer 2, Point 3: I also think it would be helpful to know more about the ambient environmental conditions in the chambers (e.g. nutrients, oxygen, and salinity). The NOx- fluxes into the sediments are low but NH4+ effluxes are high. If NH4+ effluxes are due to DNRA, where is the NOx- 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)?

Following the suggestion of Reviewer 2, the ambient environmental conditions (mean $\pm$ standard error) recorded in the incubation chambers on day -1, at the start of light and dark incubations, have been included in the supplementary material as Table S1. The original Table S1 has been modified to Table S2.

The comment that Reviewer 2 made regarding the NH4+ effluxes has been fully taken on board and this section of the results have been revised. Lines 534-537 now read: Ammonification and DNRA are therefore the only pathways with the potential to contribute to increased NH4+ production in the +C treatment. The increased NH4+ concentration may have originated from an increase in ammonification consistent with the

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increase in metabolism observed in the +C treatment.

Minor Comments

Line 34: “process nitrogen-rich particles” Does “process” imply removal or retention? The term “process” was used in a neutral sense and could imply permanent removal or retention of nitrogen in the system, however in order to keep the abstract concise and within the word limit, the term ‘process’ has been changed to ‘receive’. Lines 33-36 now read: We present, for the first time, a combined biogeochemical-molecular analysis of the short-term performance of one such system that is designed to receive nitrogen-rich particulate aquaculture wastes.

Line 40: Consider changing “indicating” to “suggesting”? This suggestion has been adopted in Line 41 of the abstract.

Line 74-75: Was the starch treatment a single input or done continuously? The starch was added on a daily basis to the +C treatments, however this had been clarified in the manuscript. Lines 149-152 now read: Additions of waste with (+C) or without (-C) added carbon commenced on day zero. The aquaculture waste was mixed into a wet slurry while the starch was dissolved in seawater and added daily to the respective treatments at 16:00 from day zero to day 14.

Lines 101-102: Was the system designed to retain nitrogen or remove nitrogen (conventional or biofloc)? The experimental system comprised a conventional RAS designed to remove ammonium through conversion to nitrate in the biological system. To clarify, the word conventional has been inserted so that lines 104-105 now read: The study was conducted in a purpose-built bio-secure heated conventional recirculating aquaculture system (RAS) described in Robinson et al. (2015).

Line 114: A single dose of starch or was it per day? The starch addition was done daily, to clarify lines 117-119 now read: The ‘added carbon’ treatment (+C) received aquaculture waste (215.06 mg day-1 wet weight) and carbon in the form of soluble

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starch (44.50 mg day-1 dry weight) daily to increase the C:N to 20:1 (mass ratio) from day zero (Table 1).

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? The wet weight is used in the growth rate calculation, since they were weighed alive at the start and end of the experiment. No sea cucumbers were sacrificed in the experiment, hence the dry weight or C:N ratio of the sea cucumbers was not determined, however this suggestion is useful for future studies.

Line 164: How long were the stirrers paused? The stirrers were interrupted briefly during the start and end of the incubations when data was collected as explained in lines 170-171 and lines 190-193. This has been clarified in the manuscript such that lines 170 to 171 now read: When data were collected the flow from each chamber was interrupted, the stirrers were paused ($\sim$ three min.) and the chambers were uncapped by removing the rubber bung.

Lines 215-216: Move “Equation 3: : :” to line 215? This has been done

Lines 241-242: Can you give a brief description of the carbohydrates method? The sentence has been re-written to include the name of the method and the reference. Lines 248-249 now read: Total sediment carbohydrates (μg g-1) were measured using the phenol-sulphuric acid method (Underwood et al., 1995). The reference has been added to the reference list.

Lines 311-313: Did you do any comparisons (ANOVA) with initial, +C, and –C? This comment has been addressed in the response to Point 2 made by Reviewer 2 in the major comments.

Line 397-400: Given the variability (SE) in the N2 fluxes would you want to say that fixation and removal pathways were approximately equal? Without doing a mass balance, it is not possible to comment on this accurately, however this would be useful in

future studies.

Lines 409-410: It would be helpful to know the ambient nutrient concentrations. Following the suggestion of Reviewer 2, the ambient environmental conditions (mean ± standard error) recorded in the incubation chambers at the start of the light and dark periods on day -1, have been included in the supplementary material as Table S1 and referenced in Section 3.2 of the manuscript. Lines 411 – 412 now read: Ambient environmental conditions recorded in the incubation chambers at the start of the experiment on day -1, during light and dark periods, are presented in Table S1.

Lines 416: Suggests the data is a time-series. Perhaps rewrite as difference between treatments? Reviewer 2 makes a very valid point that the phrasing implied time-series data collection. The sentence has been re-written so that lines 429-430 now read: The sediment organic carbon (OC) content decreased in the experimental treatments after 14 days compared to the initial treatment (Fig. 3a).

Lines 418, 426, 459: This seems like speculation since the oxic-anoxic interface was not measured. Can it be implied with microbial data? We have notes recording the position of the level of the oxic-anoxic interface in each chamber as they were sectioned. We have changed the wording to say “approximate depth” (Line 431).

Line 540-542: Consider major comments above. We have amended the manuscript to incorporate all the major comments suggested by this reviewer and thank them for improving the manuscript.

Line 580: Seems like a reference would be helpful here or are you specifically referring to Welsh 1997 and Newell et al. 2016. Clarify. The references of Welsh 1997 and Newell et al. 2016 have been included again here.

Line 610: Consider changing “for” to “over” We have left this unchanged.

Line 623: See major comments above. Is assimilating nitrogen better than nitrogen removal? We feel that we have addressed this comment under the response to point 1

made by Reviewer 2 under Major comments.

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