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Discussions

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

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

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Dear Reviewer 1,

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

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

âĂć Reviewer 1, Point 1. Lines 112 – 115: the amounts of aquaculture waste added daily to the incubation chambers are given as '26.8 mg day-1 wet weight'. It is not clear if this amount refers to aquaculture waste or to carbon. If it refers to carbon, then it cannot be 'wet weight'. Please clarify.Âă o In line 145, it is stated that 400 mmol C/m-2/day is added to the incubation chambers. Considering the chambers have an inner diameter of 8.4 cm (Line 119), then about 26.6 mg C/incubation chamber/day is added. This carbon represents dry weight. Please make statements in lines 112-115 and line 145 to concur.Âă o Line 114: 'Of soluble starch 7.5 mg DM is added daily'. Here too, it is not clear if this refers to starch or to carbon in starch. Please clarify.Âă o Even if above refers to carbon in starch, then the amount is too small to raise the C:N ratio from 5 to 20, assuming the fish waste contains 400 mmol C/m2/day and 80 mmol N/m2/d (= C:N ratio 5). Adding 7.5 mg C per chamber, concurs with 113 mmol C/m2/d. The C:N ratio of the combined fish waste & starch then becomes 6.4. Please clarify.Âă

Reviewer 1 has picked up a number of unfortunate errors in the calculations of the elemental ratios (section 2.2) of the aquaculture waste and carbon additions (section 2.4) that have occurred during the editing of the thesis and manuscript. These errors have now been fully corrected and the rationale taken in the study has been more fully explained to facilitate interpretation by readers.

The approach taken in the study was to target the upper loading for benthic organisms of 400 mmol C m-2 day-1 for the treatments that received additional carbon to increase the C:N from 5.21 to 20:1. Thus, 400 mmol C m-2 day-1 does not refer to the C:N

of the fish waste alone, rather it refers to the target C:N of 20:1 to be achieved in the aquaculture waste + carbon (+C) treatments. The overall C:N ratio of 20:1 at 400 mmol C m-2 day-1 represents the carbon present in the aquaculture waste (104.06 mmol C m-2 day-1) plus the carbon present in the starch (295.58 mmol C m-2 day-1). The sentence referring to the equivalent rate of carbon loading has been clarified and moved to the experimental design section. Lines 119-120 now read: The carbon addition treatments (+C) were standardised at a concentration of 400ÂămmolÂăC m-2 d-1.

As the carbon loading was different between treatments, the quantity of aquaculture waste was standardised between treatments at 215.06 mg of wet waste per chamber per day. To calculate the quantity of aquaculture waste and carbon to add, the molarity of carbon was converted into mass of carbon and the C:N of 20:1 is thus expressed on a mass basis and not as a molar ratio. This has been clarified, such that lines 118–119 now read:. . .... 'to increase the C:N to 20:1 (mass ratio) from day zero (Table 1)'.

There was an error in the quantities reported in the previous version of the manuscript. In particular, the 26.8 mg day -1 wet weight reported in reference to the aquaculture waste was in fact referring to the target quantity of carbon to be added to achieve a C:N of 20:1 at 400 mmol C m-2 day-1 for the aquaculture waste plus carbon treatments. During the experiment, the carbon addition treatments received 215.06 mg of wet waste per chamber per day plus 44.50 mg of dry starch per chamber per day. The errors have now been corrected and the manuscript text has been re-written to clarify the volumes of aquaculture waste, starch and their equivalents in mmol of C added. Lines 115-119 now read: The 'no added carbon' treatment (-C) with a C:N of 5:1 received aquaculture waste only (215.06 mg day-1 wet weight). The 'added carbon' treatment (+C) received aquaculture waste (215.06 mg day-1 wet weight) and carbon in the form of soluble starch (44.50 mg day-1 dry weight) to increase the C:N to 20:1 (mass ratio) from day zero (Table 1).

Reviewer 1, Point 2. Lines 370-373: The information that the sea cucumbers lost

weight is useful, but comparing to the final weight obtained in similar conditions in another experiment, without giving details on nutrient loading, is not useful. If additional information is given it should give insight why or how the animals lost weight.Âă

Reviewer 1 helpfully pointed out that the rate of nutrient loading in the previous study of Robinson et al. (in review) was not reported. This information has been included following the suggestion and Lines 380-383 now read: The biomass density decreased from 1,034.00 $\pm$ 12.73 g m-2 to 874.97 $\pm$ 18.31 g m-2, although the initial stocking density was comparable to the final densities (1,011.46 $\pm$ 75.58 g m-2) achieved in previous carbon amended cultures standardised at 200 mmol C m-2 day-1 (Robinson et al., in review).

In addition, a new paragraph has been added to the discussion to highlight possible reasons for the difference in growth performance. Lines 626-640 now read: Our findings indicate that carbon addition may partly bioremediate nitrogen-rich effluent by retaining nitrogen within the system, however longer-term trials are necessary to determine whether this translates into improved sea cucumber biomass yields. In the current study, the sea cucumbers decreased in mass with growth rates of 0.02 g.day-1, however there was no significant difference in mean wet weight of the sea cucumbers at the start or end of the experiment. Two key factors are likely to have accounted for the differences in growth performance of Holothuria scabra in the present study and the previous study of Robinson et al. (in review). Firstly, chambers were shaded from direct sunlight in this experiment to mitigate against water temperature spikes that would likely have caused hypoxia in the small sealed chambers. However, because high light levels may be important for Holothuria scabra growth (Battaglene et al. 1999), this may have resulted in the lower growth performance. Secondly, the duration over which the sediment microbial community was allowed to develop differed between the studies. In Robinson et al. (in review) the trials lasted 112 days compared with the current 28 day study (14 day preconditioning and 14 day experimental).

ÂăMinor comments

line 84: Start sentence with: The molar C:N ratio...Âă As the C:N ratios used are presented as mass ratios and not molar ratios, this suggestion has not been adopted to maintain consistency in the revised manuscript.

whole manuscript: when listing cited references in the text, in some cases, the author names should be written outside the brackets.Âă Thank you for highlighting this inconsistency in the citations. All references have now been checked and corrected.

Line 154: a standard deviation is given extrapolating the stocking density from 3 animals per chamber to 541 animals per m2. This cannot be correct.Âă The standard deviation reported was based on the average weight of all animals for all treatments, however since this is misleading and not accurate, it has been removed.

Lines 162 and 163: delete 'approximately two hours'. The duration ranges of incubations are given in paragraph lines 183-190.Âă The suggested text has been deleted

Lines 215-216: remove hard return at end of line 215.Âă The hard return has been deleted

Line 464: delete 'or'.Âă Here, the sentence has been re-written to improve clarity. There were 3 phlya identified and a number of taxa that were not assigned at phylum level. Lines 477-478 now read: Taxa from three archaeal phyla were present, including Euryarchaeota, Thaumarchaeota and Woesearchaeota.

Line 466: not clear why a reference is given on an observation of own data?Âă The reference refers to the classification of Natronorubrum, however its placement at the end of the sentence is misleading. Lines 478-480 now read: Natronorubrum (Euryarchaeota), a halophilic aerobic chemoorganotroph (Xu et al., 1999), was the most abundant genus representing 14 of the 27 archaeal reads.

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