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Interactive comment on "Deep-sea sponge grounds as nutrient sinks: High denitrification rates in boreo-arctic sponges" *by* Christine Rooks et al.

Anonymous Referee #1

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The paper presents an experimental study of dissimilatory nitrogen transformations in six cold-water sponge species with particular focus on their potential role as sinks for bioavailable nitrogen. Denitrification and anammox rates were quantified in oxic and anoxic incubations with N-15-labeled substrates, and nitrification rates were inferred from patterns of isotope pairing in N2. The process rates were supplemented with quantification of relevant functional genes.

The main result of the study is that the sponge microbiomes support substantial rates of denitrification under both oxic and anoxic conditions, and that denitrification under oxic conditions is driven to a large extent by nitrate produced endogenously by nitrification.

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Upscaling of the rates indicates high nitrogen removal rates in sponge grounds.

Denitrification was previously demonstrated in a few sponge species, but this survey represents a substantial expansion of the small database, particularly for colder waters. It further contributes to the growing literature on nitrogen transformations in “exotic” environments such as marine snow, animal microbiomes, etc. Thus, it is an original and relevant study and well-suited for Biogeosciences. The study was generally well designed and the results are of good quality. The writing and presentation of results are generally clear. While some conclusions are justified others require further discussion and likely need to be moderated.

Major issues 1) Experiments were conducted with nitrate and ammonium added at at least 10 fold higher concentrations than in situ values (100 μM vs. 10 μM and 10 μM vs. $\leq$ 1 μM, respectively, i.e., 1000% above ambient, and not 90% as stated in the text p. 11 l. 6). This means that the measured rates must be treated as potential rates unless the authors can establish an argument for 0th-order kinetics for both denitrification and nitrification. In turn, this implies that the estimated sponge-ground rates may be vastly (10-fold) overestimated. This issue should be discussed and the conclusions modified accordingly. In the oxic experiments, denitrification rates could, in principle, be calculated using the classic isotope pairing calculations for sediment cores (D14 sensu Nielsen 1992), but then the incubations should have been performed without addition of unlabelled ammonium and with maintenance of steady state.

2) Nitrification-based denitrification rates are calculated from the accumulation of single labelled 29N2. Firstly, it is not entirely clear how these rates and relative contributions were calculated, and I suggest to include the essential equations in Methods. Secondly, the concept of water-based and nitrification-based denitrification was developed by Nielsen for sediment cores with steady state distributions of oxygen and nitrate (and it was challenged by Middelburg in L&O 41:1839). In the present study, oxygen was clearly not at steady state during the oxic incubations, and it also seems likely that new formed nitrate may have leaked from the sponge tissue thus gradually decreasing the

labelling of the ambient nitrate pool, and increasing 29N2 production from the ambient water. Moreover, the data presented in Fig. 1, for one of the six sponges, suggests that there is an issue with the mass balance of unlabelled N in the incubations. Thus, at the end of the anoxic incubations, excess 29N2 dominated over 30N2 in two of three incubations despite the stated ~90% labelling of the nitrate pool, and the accumulated 29N2, reaching up to ~23 μM, exceeds the amount of unlabelled nitrate initially available (10 μM in situ + 1 μM from the 99% 15N tracer). Also during the first 24 h, 29N2 production in the anoxic incubations seems higher than predicted by nitrate labelling in the absence of nitrification. Altogether, these uncertainties and discrepancies undermine the conclusion concerning the role of nitrification. Plots of excess 29N2 vs. excess 30N2 could potentially help the authors to evaluate and constrain some of these issues.

Specific comments 3, 8-12: The final statement is highly speculative and does not belong in an abstract.

4, 16-7: The statement about nif genes seems out of context.

6, 14: Science should never aim to show specific results but rather test hypotheses!

7, 11

9, 4-5: "Upper few centimetres" is vague – considering the negative result, the question is whether only the oxic surface layer was sampled.

9, 20: There was no "atmosphere" in the vials? However, incubation with a helium/oxygen headspace would have kept the incubations oxic throughout.

10, 7-8: This seems a very shaky assumption. Respiration rates must vary with species, temperature, and trophic state.

10, 18-9: Some oxygen is likely introduced during transfer – did you test the water in the Exetainers?

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11, 6: The values are $\geq$1000% above ambient.

11, 12: According to 7, 11 the in situ temperature was below 0 °C! How would the higher incubation temperature affect the rates?

12, 18: The accumulations in Fig. 1 look only approximately linear – which test gave $p < 0.05$? Did the same apply to the linearity of the anoxic rates (13, 4)?

13, 15: Please specify the equations used here (see major issue #2).

16, 3-5: The opening of the Results is very confusing with the first two sentences referring to two different treatments. Delete the first sentence.

16, 22-3: The sediment experiment has little value. The origin of the sediment is unclear, and it does not seem representative of Arctic sediments.

18, 5: See 6, 14.

18, 18-9: Metabolisms in sponges or what? Please clarify/reference.

18, 20-5: The presence of denitrification genes and isolation of denitrifiers cannot prove "the presence of denitrification activity".

20, 11: How would the "pulse of organic matter in the water column" (where in the water column?) affect potential denitrification in the sponges' tissue?

21, 16: "proves" is an overstatement.

22, 1-2: It is not the in situ concentration but the 10 μM ammonium added, that is of relevance here.

22, 13-5: Please provide a reference for the single copies.

22, 16-20: The curve in Fig. 3 does not look like an exponential function. It there statistical support for this relationship?

22, 20: What is meant by "optimized"?

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23, 9-10: With 6 orders of magnitude variation, this is not very telling.

23, 19 on: The calculations of sponge ground rates need explanation, but see Major issue #1. Furthermore, it seems that results of population density surveys are presented here for the first time. If this is the case, the methods and results should be specified i the appropriate sections. Otherwise, a reference should be included.

24, 24: What was the frequency of non-pumping?

25, 11-2: Is this a short-term or permanent effect? Would reduced pumping rates/increased anoxia not result in reduced growth, reduced biomass, and thereby reduced nitrogen removal in a longer perspective? The system effect of the stressors seems speculative.

Table 1: The number of significant digits should be adjusted.

Fig. 1: Different triangles are used for 29N2 and 30N2.

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