Referee #3 reply

General comments

This manuscript provides novel information on potential (de)nitrification and anammox rates combined with genomics in 6 abundant cold-water sponges, from which 5 have not been analyzed previously. The data show that denitrification is a common process in deepsea sponges and is relevant for understanding the role of sponges in nutrient cycling. The study seems well planned and conducted.

My main concern is that the potential denitrification rates measured in this study in tissue sections are upscaled to whole sponges and ecosystem level and are even used for future predictions under anthropogenic stress. The rates here should be treated as maximum or potential rates, since they were conducted with 10 times ambient concentrations, on small tissue sections in closed exetainers, under no oxygen and decreasing oxygen concentrations.
We agree that these are maximum potential rates. We make this now more clear in the discussion, beginning of chapter 4.4. Compare also comment from Referee #1, and our reply.

My second major comment is that the MS is focused on and biased towards denitrification, with limited attention for other nitrogen transforming processes, such as nitrification, anammox and perhaps DNRA. I suggest to present all labelling incubations, carefully evaluate the results and present and discuss in a more balanced overview of the different nitrogen transforming processes and also include all data in the published Pangea dataset.
This work focusses on processes which transfer DIN into N2 – denitrification and anammox. The anammox rates were under detection limit. Coupled nitrification-denitrification is of interest in this context, but it was never our intention to provide "a balanced overview of the different nitrogen transforming processes" in sponges. Nitrification and also DNRA in sponges is well explored, while denitrification (and anammox) is not. Our intention was to close this knowledge gap.

Specific comments

- Title: The term "nutrient sink" in the title is confusing and questionable
We disagree and would like to keep it
- P3, L3-11: this is too speculative, see major comment 1.
We agree that this statement sounds provocative, but we still consider this a valid interpretation of our data. The interpretation is sufficiently justified in chapter 4.4.
- P4, L16-19: This sentence doesn't really fit and perhaps the whole part of N fixation can be moved to the discussion, since it disrupts the introduction on DIN release.
Section rephrased
- P7, L20: Add some of the relevant characteristics for this site.
The most relevant characteristic (hard bottom slope of a fjord) is given, details can be looked up in the quoted reference. Community structure of boreal vs arctic sponge grounds is now also described.
- P2.2 and p2.3: A table or flow chart with the experimental incubations would be very useful.
We consider the description in the text to be sufficient
- P10, L:16: On the previous page it is mentioned that all incubations were done with water sampled from the deep, but here surface site water is mentioned for anoxic incubations. This needs to be clarified.
Corrected for clarification

- P11, L4: Can you be sure that 15-NO3 is reduced to 15-NO2, the preferred substrate for anammox? Please elaborate
For the incubation experiment screening for anammox, 15-NH4 was added as substrate. 15-NO3 was added for the denitrification experiment. We realized that this was not clearly written in the method, this is now corrected.
- P 11, L6-7: This is 1000% above ambient concentrations, and ambient concentrations from which site, arctic or boreal grounds or both?
Section rephrased to avoid misunderstanding, see also Ref # 1
- P11, L12: This is not *in situ* temperature for the Arctic species, the temperature increase might increase your potential rates.
Lab experiments can never perfectly mimmick in situ conditions, we chose the best possible solution. We made a comment in the discussion that Arctic rates may be overestimated because incubation temperature was above in situ. See also comment by Ref #1.
- Paragraph 2.3.2: There were no oxic sediment incubations?
No. Since the denitrification was zero under anoxic conditions, there was not need to check for oxic conditions.
- Paragraph 2.3.3: Including the calculations is informative for the reader.
Some equations and calculations are now included.
- P12,L22-P13, L1: The published dataset contains individuals with tissue degradation
We now explained more clearly in chapter 2.3 that these samples were not considered for 15N analyses and rate quantification.
- P13, L:11-13: This needs more explanation. I guess you mean no 29-N2 was detected in the anoxic incubations? What about 29-N2 and 30-N2 production in oxic incubations with labeled ammonium? Some production can be expected from coupled nitrification and denitrification.
The lack of 29N2 production from 15NH4+ (anoxic incubations) suggests an absence of anammox, since N2 production via anammox requires 1 N from NO2- and 1 N from NH4+. It is also important to note that although labelled N2 production can be expected from coupled nitrification-denitrication of 15NH4+ in the oxic incubations, we did not detect labelled N2 in these oxic incubations.

- Paragraph 2.3.4: Also here, the equations would be useful. And following my comment above, can you estimate coupled nitrification-denitrification from your oxic incubations with 15N-NH4?
Equations added
- Paragraph 2.4: In the results and discussion is mentioned that the sponges were also screened for anammox and N2 fixation functional genes. The screening and description of the functional genes should be described here. Was there also screening for other genes relevant for the nitrogen cycle (e.g. nitrification, DNRA)?
Only screening for N2 fixation functional genes. This was relevant for our "nutrient sink" story, since nitrogen fixation would have closed the nitrogen cycle in the sponge. The other processes the reviewer mentions were of less importance for this particular story, and so we did not screen for genes.
- P16, L1-6: Add graphs or tables with the results under oxic and anoxic conditions.
These results are presented in Fig 2.
- P16,L22-23: What about unlabeled N2 production?
Unlabelled N2 production cannot be detected in the IR-MS.
- P18, L5: I would remove "nutrient removal"
This needs to stay, it is the main conclusion of the manuscript, and it is well justified.
- P18, L22: Denitrification has not been directly shown in Fiore et al. 2013, but is given as a potential pathway, together with anammox or DNRA, to explain net consumption of nitrate in some of the sponges.

Changed to "…has been indicated…"
- P20, L6: I won't state that results are representative for normal conditions, but state that these conditions are not atypical (or something similar).
We think this statement is well justified: the quoted literature proves that undersaturation of oxygen in the tissue is a common feature in sponges.
- P20, L9-L15: I would expect year-round higher (dissolved) organic matter concentrations at the Boreal compared to Arctic grounds. Another explanation might be related to the higher incubation temperature (if it was 6°C) compared to the *in situ* temperature.
Good point, text rephrased
- Paragraph 4.2: The relevance of your denitrification rates in view of other nitrogen transforming processes should be discussed in a balanced way (see major comment 2). This paragraph gives the impression that denitrification is more important than nitrification in sponges, even though the majority of sponge studies reveal that sponges are net sources of nitrate, with denitrification being only a fraction of nitrification. Also the possibility of DNRA as competitive process for denitrification should be discussed somewhere.
We removed the calculation of minimum nitrification rates as we see that this was confusing, and keep the statement that nitrification was present. We extensively quote publications which focus on the nitrogen source function of sponges. We do not believe that any reader will get the impression that denitrification is generally more important than nitrification in sponges. DNRA is not relevant for our publication as it conserves bioavailable nitrogen in the system. We focused on processes which remove bioavailable nitrogen from the system. As mentioned above, the aim of our study is not to give a balanced overview of the different nitrogen transforming processes in sponges, but to put the focus on sponges as potential nutrient sinks, and to point out potential scenarios based on calculated potential rates.
- P21, L11-14: What is so different between explants and tissue sections? The tissue sections will also dependent on diffusion? There are more differences between Hoffmann et al. 2009, i.e. in your study you added NH4, which will stimulate nitrification, while in Hoffmann et al. 2009, no NH4 was added. You could discuss the reliability of nitrification measurements.
Section removed and rephrased
- P21, L21-25: "May be higher" should be "are likely higher" and the reported rates are really at the low end of other reported rates.
Section rephrased
- P22, L1, yes, but you added 10 μM (unlabeled) NH4, which can result in 10 μM (unlabeled) NO3 in oxic conditions.
Labelled ammonium was added to the annomox incubations. No ammonium was added to the denitrification experiment. We now discovered that we did not write this clearly in the method section, which has led to confusion. The text is now corrected
P22,L6,9: These last two sentences are not connected to the rest of the paragraph.
Yes they are. This paragraphs lines up several facts that lead to the main conclusion that the nitrogen cycle is not closed in these sponges, so bioavailable nitrogen leaves the system.
- P22, L16-21: I won't use optimized, I guess you want to say there is an active denitrifying community. Perhaps add some statistics to the relationship, this is a nice result.
Rephrased.
- P23, L4-6: I disagree that they are realistic, see major comment one.
Rephrased, but see our reply to major comment one.
- P23, 15-17: Are there reported denitrification measurements of Arctic sediments? What about the other nitrogen transforming processes? A comparison to literature should be added with to this statement.

There is a recent publication about anammox in these Arctic sediments: https://www.biorxiv.org/content/10.1101/729350v1 *, where profiles of nitrate, ammonium and oxygen are included. The group did also predict denitrification rates based on their model but these data are not yet published. Apart from this, we are not aware of any literature on denitrification rates in deep Arctic sediments, only from the continental shelf areas.*

- Paragraph 4.4: The results are not representative for a natural situation, but rather show a potential, so I would be extremely cautious to upscale these numbers and refer to these sponges as efficient nutrient sinks (see major comment 1).

We rephrased the first paragraph to make more clear that we talk about maximum potential rates and possible scenarios. We still think that a presentation of (potential) areal rates is valid and useful to compare denitrification rates of sponge grounds to other ecosystems.

- P24, L6-10: The calculations and conversion factors going from volume to surface integrated measurements are lacking (but see major comment 1).

No, both calculations and conversion factors are given in the data publication https://doi.pangaea.de/10.1594/PANGAEA.899821 which will be publicly available when the manuscript is accepted.

- P25, L13-15: Combined anthropogenic stressors can also lead to changes in nutrient and organic matter availability which might affect microbial composition and biogeochemical processes. It is too speculative to state that sponges will become nutrient sinks in the future if they reduce pumping.

We say "may", not "will". We think it is necessary to point out this potential and so-far overlooked scenario.

- P26, L10: Please expand the dataset in Pangaea with all incubation data and results.

All results of the incubation experiments are completely presented in terms of 29N and 30N accumulations. What is lacking?

- P35, L2: STP is used for the first time, does it stand for standard temperature and pressure?

Removed, as this explanation belongs to the methods and not the figure legend

- Figure 1 and figure 2 should be swapped, based on their reference order in the text.

No, Figure 1 is mentioned for the first time on page 12, while Figure 2 is mentioned for the first time on page 16.

- The order of references in the citations and full references need to be checked.

Reference format is generated automatically in EndNote. We will do the formatting when the manuscript is accepted and no references have to be removed or added.