RC1: <u>'Comment on egusphere-2023-1498'</u>, Anonymous Referee #1, 11 Mar 2024

This manuscript presents an interesting and detailed study on the benthic nitrogen cycling in the Santa Barbara Basin. The study is original as the authors used a complex approach measuring insitu incubation, quantifying benthic rates of nitrate uptake, denitrification, anammox, nitrous oxide production, and DNRA. They found that the sediments in the Santa Barbara Basin acted a sinks for fixed nitrogen with dominating denitrification and as source for nitrous oxide. The data set is well presented and interpreted and the text well written and organized. I only have a few minor comments.

We thank the reviewer for the positive comments.

L141-142: How big is the effect of additional substrate added to chamber incubations? In-situ bottom water concentrations ranged from 9.9 μ mol L⁻¹ to 27.3 μ mol L⁻¹ (Table 1), after adding ¹⁵N-labeled nitrate concentration varied between 50 and 100 μ mol L⁻¹. Do you suspect to overestimate rates to higher substrate availability?

Table S2 that is already included in the supplementary information shows the effect of additional substrate added to chamber incubations. Lines 220 - 223 acknowledges that:

"the addition of NO_3^- at concentrations that were 1.6 - 6.2 (median = 2.3) times as high as ambient concentrations resulted in NO_3^- uptake rates elevated by a factor of 1.9 - 6.4 (median = 3.8) as compared to those measured in parallel chambers deployed at the same time without any added substrates (Table S2; (Yousavich et al., 2024)."

Indeed, the additional substrate may have stimulated the nitrate reducing activities. On the other hand, because samples from benthic chamber incubations are taken from the overlying water they cannot provide detailed information about all processes that may occur in the underlying sediments. For example, we cannot be certain about the magnitude of nitrate reductions unaccounted for due to reduced products being adsorbed to sediments (e.g. ammonium). As a result, we cannot be sure about whether the reported nitrate reduction rates were overestimates or underestimates (Lines 216 - 219).

L256-257: How would rate changes with seasonal altering oxygen concentrations?

Nitrate reduction processes are inhibited by oxygen, although the oxygen sensitivity of different processes likely differ. Overall, we expect lower rates of nitrate reduction processes at higher oxygen concentrations. This was evident in the long-term monitoring dataset shown in Goericke et al., 2015, where nitrate deficits were correlated with the degree of anoxia in the Santa Barbara Basin.

L258-259: How representative are the results considering seasonal changes in oxygen and nitrate concentrations?

The results are representative of seasonal anoxia in the Santa Barbara Basin (SBB), which develops at least twice a year following upwelling events (winter and spring) (Goericke et al. 2015). Pronounced nitrate deficits accompany the anoxia due to nitrate reduction processes. This is explained in the Introduction in lines 62 – 70. In this study, we sampled during one time of the year, and we do not intend to use the results to represent seasonal changes in the SBB, which is now clarified in the updated version of the summary.

L297 "However, because the porewater NH_4^+ concentration was high [...]": Have pore water or bottom water ambient ammonium concentrations been measured? I cannot find any information about porewater sampling in the method section. If you refer to another paper this statement needs a reference. Both anammox and nitrification, which according to the authors contributes at least in part to N₂O production (L367), are dependent on available ammonium, it would be interesting to know the in-situ concentrations.

Thank you for the suggestion. We will include a reference after this statement at Line 297 (Yousavich et al. 2024), which is where porewater ammonium concentrations were published.

L364-370: Why do you not discuss the potential of DNRA to contribute to N₂O production?

While there are reports of N₂O production from bacteria capable of nitrate ammonification, none of the bacterial lineages are typically found in marine sediments. Bacillus vireti, Bacillus sp., and Citrobacter sp. were isolated from soil (Mania et al. 2014, Streminska et al. 2012), Bacillus licheniformis were isolated from silage, garden soil, and flour (Sun et al. 2016). Moreover, many of these N₂O-producing bacterial strains are capable of both DNRA and canonical denitrification, which confounds the distinction of N₂O produced via denitrification vs. DNRA.