

**Review of "Marine anoxia initiates giant sulfur-bacteria mat proliferation and associated changes in benthic nitrogen, sulfur, and iron cycling in the Santa Barbara Basin, California Borderland" by Yousavich et al. BG manuscript egosphere-2023-1198.**

General evaluation

This manuscript reports on a study of benthic bacterial mats in the Santa Barbara Basin, including the prerequisites for their formation, how they develop in response to changing bottom water oxygen conditions (which vary in time and space), and the relationship between presence of mats and benthic Fe, P, N and S cycling.

My major concerns with this submission are:

Previous findings and present knowledge are not taken sufficiently into account. It appears that the authors have not properly checked the literature regarding Fe and N cycling in response to changing bottom water oxygen conditions, and regarding benthic bacterial mats in other settings than the Peru, Mauretanian and Arabian OMZs. The findings of this study can then much better be put in the correct context of previous observations and knowledge, which would considerably improve the manuscript.

There are several papers reporting on the influence of declining bottom water oxygen on benthic Fe and P fluxes. Suggestions for papers to look at and cite are given below. There are of course several other papers on this as well.

The manuscript reports on changes in the relative importance of benthic denitrification and DNRA as bottom water oxygen declines and anoxia is approached. This is fine. I suggest that the authors read present literature on corresponding changes in pathways of benthic NO<sub>3</sub>-reduction when the bottom water turns the other way, i.e. from anoxia to oxygenated conditions. That would add a very interesting component to the Discussion of this manuscript. Suggestions for papers to look at and cite in this context are given below.

It has not been clarified enough whether this study presents any new findings, or if process patterns and mechanisms in SBB sediments, presented in this study, have previously been found and explored elsewhere. This should be stated clearer in the manuscript.

The Results section is far too long, and too many details are given. Please shorten it and keep only descriptions of the essential parts of the results. The readers also have the figures and SI to get information from.

In several cases the text is not clear enough, and then it has to be clarified. I have given examples of this below, but I encourage the authors to check throughout the manuscript for unclear text / writing.

Specific comments

L 35-37: "*We found that the presence of mats was associated with a shift from denitrification to dissimilatory nitrate reduction to ammonium.*"

Do you mean the development of mats.... (not presence)? A shift is due to a change (such as a development) of something.

Lines (L) 40-42: “*Our research further suggests that cycles of deoxygenation and reoxygenation of the benthic environment result in extremely high benthic fluxes of dissolved iron from the basin’s sediment.*”

This or similar findings have been published previously – at several occasions. See e.g. Balzer (1982), Sundby et al. (1986), van de Velde et al. (2020), and papers on the Baltic Sea from the group of Caroline Slomp.

References:

W. Balzer. 1982. On the distribution of iron and manganese at the sediment/water interface: thermodynamic vs. kinetic control. *Geochim. Cosmochim. Acta* 46: 1153-1161.

B. Sundby, L. Anderson, P. Hall, Å. Iverfeldt, M. Rutgers van der Loeff and S. Westerlund. 1986. The effect of oxygen on release and uptake of cobalt, manganese, iron and phosphate at the sediment-water interface. *Geochim. Cosmochim. Acta* 50: 1281-1288.

S. van de Velde, A. Hylén, M. Kononets, U. Marzocchi, M. Leermakers, K. Choumiline, P. Hall and F. Meysman. 2020. Elevated sedimentary removal of Fe, Mn, and trace elements following a transient oxygenation event in the Eastern Gotland Basin, central Baltic Sea. *Geochim. Cosmochim. Acta* 271: 16-32. <https://doi.org/10.1016/j.gca.2019.11.034>

L 60-61: “*and promotes organic matter preservation in the sediments*”.

Not necessarily. See e.g. recent paper by van de Velde et al. (2023). Please rephrase this sentence so it better reflects present knowledge.

Reference:

S. van de Velde, A. Hylén, M. Eriksson, R. James, M. Kononets, E. Robertson and P. Hall. 2023. Exceptionally high respiration rates in the reactive surface layer of sediments underlying oxygen-deficient bottom waters. *Proc. Royal Society A*, in press. <https://dx.doi.org/10.1098/rspa.2023.0189>.

L 65-85: Would not this paragraph fit better in the Methods section under (e.g.) “Study site”?

L 89: Remove the second “*mats*”.

L 115-116: “*suggest that sedimentary organisms are responsible for approximately 75% of the total NO<sub>3</sub>- uptake in the SBB*”

This sentence needs clarification. For example, what do you mean by uptake? Accumulation in cells? Assimilation by phytoplankton? Do you also include reduction (denitrification, DNRA and anammox) in uptake? What is the remaining 25% of the uptake due to? Please explain.

L 199: “*DIC detection limit was 0.5 mM.*”

Did not Hall and Aller (1992) report a much lower detection limit? If so, why is yours so high?

L 243-244: There is a factor of 1 000 000 between mmol and nmol (not 1 000).

L 282 and 284: “*Where J is the diffusive flux*”.

Only diffusive? I would call a flux measured in a benthic chamber a total flux. Even if bottom water has a low oxygen concentration, the sediment may contain bioirrigating animals, so molecular diffusion may not be the only transport mechanism.

L 480-482: “*BFC O<sub>2</sub> concentrations were compromised by O<sub>2</sub> release from the chamber’s polycarbonate walls, which prevented an accurate calculation of O<sub>2</sub> fluxes from sensor data.*”

Did not Kononets et al. (2021; which is cited in this manuscript) suggest a way to minimize this problem? Could that procedure not be implemented for your chambers?

L 537-538: “*generally more O<sub>2</sub> and NO<sub>3</sub>- than the southern transect (e.g., 9 μM at NDT3-A and 0 μM at SDT3-A).*”

9 and 0 μM of O<sub>2</sub> or of NO<sub>3</sub>-? Please be comprehensive and clear.

Section 4.1 of Discussion: Benthic microbial mats have also been studied in the central Baltic Sea where they occupy the so called hypoxic transition zone at water depths of about 80-120 m. It would be highly relevant in this manuscript to make comparisons with Baltic benthic mats. The manuscript would benefit from that. See e.g. Noffke et al. (2016), a study which included the influence of these mats on benthic nutrient exchange.

Reference:

A. Noffke, S. Sommer, A. Dale, P. Hall and O. Pfannkuche. 2016. Benthic nutrient fluxes in the Eastern Gotland Basin (Baltic Sea) with particular focus on microbial mat ecosystems. *J. Mar. Syst.* 158: 1–12. <http://dx.doi.org/10.1016/j.jmarsys.2016.01.007>

L 585-586: “*...N<sub>2</sub> production via denitrification and anammox accounted for 86% of NO<sub>3</sub>-removal in the shallow basin (NDT3-D, Fig. 6).*”

Is the implication of this that DNRA accounted for 14%? Please clarify.

L 595: “*Declining nitrate concentrations may be as important as anoxia itself to GSOB mat proliferation.*”

Please explain why it may be so. The sentence needs to be clarified. High nitrate concentrations should stimulate GSOB as long as the condition is oxygen-free. Then, the sentence does not make sense in my mind.

L 611-612: “*While low-nitrate conditions could benefit GSOB mats,...*”

Why? How? This has not been clarified. Please explain.

Section 4.2: There are published studies on the effect of oxygenation of bottom water of anoxic basins on benthic nitrogen cycling including nitrate reduction (especially denitrification and DNRA). That is, going the opposite way (from anoxia to oxygenated conditions) than what this manuscript studies (from oxygenated conditions to anoxia). I think the Discussion of the manuscript would much improve by including comparisons with such studies, e.g. De Brabandere et al. (2015) and Hylén et al. (2022). Topics to discuss may include: Are patterns reversed when one goes from condition A to B compared to from condition B to A? Are systems reversible? See also my comments on this above.

References:

L. De Brabandere, S. Bonaglia, M. Kononets, L. Viktorsson, A. Stigebrandt, B. Thamdrup and P. Hall. 2015. Oxygenation of an anoxic fjord basin strongly stimulates benthic denitrification and DNRA. *Biogeochemistry* 126: 131–152. DOI 10.1007/s10533-015-0148-6

A. Hylén, S. Bonaglia, E. Robertson, U. Marzocchi, M. Kononets and P. Hall. 2022. Enhanced benthic nitrous oxide and ammonium production after natural oxygenation of long-term anoxic sediments. *Limnol. Oceanogr.* 67, 419–433. doi: 10.1002/lno.12001

L 658: “*mechanics*”.

Is mechanisms not a better term?

Section 4.3: Please clearer specify if this study presents any new findings, or if SBB sediments display processes and mechanisms (relevant for this section) which previously have been found and explored elsewhere.

L 661: Section 4.3 appears here a second time. Please rename to 4.4

L 693-694: “*While sulfate reduction rates for B-stations are absent,*”

Do you mean that SRR were not measured, or were they measured and found to be zero? Please clarify.

Second Section 4.3 (which should be called 4.4): There is a lot of text reporting raw results. Can this text be shortened and moved to Results? It fits better there than in Discussion.

L 744: Section 4.4 should be renamed to 4.5.

L 759-760: “*These analogous observations highlight the importance of alternating redox conditions to establish high benthic iron fluxes.*”

This observation has been found and published several times previously. Please better check the literature and put your observations in a correct context of present knowledge. That will improve this manuscript. See also my comments in the beginning of this review.

L 784-785: “*We found that GSOB mats proliferate in the SBB where the bottom water is anoxic and nitrate concentrations are declining, ...*”

It has not been explained or clarified why GSOB mats proliferate when NO<sub>3</sub><sup>-</sup> concentrations go down. Please explain.

L 798-791: “*We conclude that changes in iron mineralogy, specifically the formation of an iron sulfide layer deeper in sediments, encourages the elevation of the sulfate reduction zone.*”

Which is the underlying reason for this, and which is the mechanism? This has not been clarified enough. Please explain and clarify.

L 795-796: “*Further, the same transient deoxygenation that allows for these mats to flourish also allows for a high Fe<sup>2+</sup> and PO<sub>4</sub><sup>3-</sup> flux into the SBB water column.*”

I have commented on the finding of elevated Fe fluxes above.

Already Mortimer (1941) showed that elevated phosphate fluxes from sediment occur upon deoxygenation of the bottom water. Again, please put your findings in the adequate context of present knowledge and previous findings. See also my comments on this above.

L 942-945: This reference is incompletely typed.

I recommend that this submission should undergo a major revision, taking my comments above into account, before it can be considered for acceptance.