Biogeosciences Discussions

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Comment on bg-2021-257

Anonymous Referee #1

Referee comment on "Bacteriohopanetetrol-*x*: constraining its application as a lipid biomarker for marine anammox using the water column oxygen gradient of the Benguela upwelling system" by Zoë R. van Kemenade et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-257-RC1, 2021

Van Kemenade et al report in their manuscript a suite of oceanographic, biomarker and molecular microbiological data on one of the most important marine upwelling regions, the Benguela Upwelling System (BUS), offshore the coast of SW Africa. Suspended particulate matter (SPM) was filtered at distinct water depths at different shelf and offshore stations of the complex marine setting. The focus of the study was on the occurrences of biomarkers specific to certain bacteria, performing the anaerobic oxidation of ammonia (anammox). These bacteria are known to produce specific ladderane lipids as well as a bacteriohopanetetrol isomer ("BHT-x" *sensu* Schwartz-Narbonne *et al*., 2020). The distribution of these lipids in a complex, spatially and temporarily changeable setting is so far only insufficiently understood and the BUS was highly suitable for such an investigation. Van Kemenada and co-authors present respective data in detail and the multidisciplinary data sets suite excellently to the scope and readership of *Biogeosciences*. In the manuscript the authors demonstrate that BHT-x, ladderane lipids (intact polar lipids and fatty acids) and 16S rRNA, in concert with oceanographic data (O_2 depletion and N-deficit), record at many of the situations anammox bacteria. At specific sites, however, particularly the more recalcitrant biomarkers BHT-x and ladderane fatty acids were also observed, where autochthonous anammox bacteria were an unlikely explanation. In accordance with other studies (e.g., Mollenhauer *et al*., 2007), the authors conclude that transported SPM in such dynamic settings can complicate the use of such and other biomarkers. The manuscript is of general high quality and well written, but before final acceptance I ask for minor revision of some points:

Main concerns

- The authors present a threshold for the ratio of BHT-x/(BHT-x + BHT), which is introduced to infer "deoxygenation". They use the ratio, which they observed in their data sets (">0.04") and correct these ratio to ">0.18" to also account for potential complications from allochthonous organic matter (in challenging settings like the BUS). I agree that the latter process is an important issue, but it should be better explained what the ratio is exactly suggested for (most likely sedimentary studies), whether it can be transferred to other, so far unstudied settings and what it exactly tells us?

Temporal or stable deoxygenation, deoxygenation in bottom waters or larger water bodies? Must the deoxygenation be occurring at a water body from which transport into the sediment is possible (sedimentary OM is not necessarily an integrated signal of SPM from all water depths). Further, I also ask the authors to cite and discuss a paper, which addressed the distribution of a BHT isomer ("BHT II"), which is tentatively the same as in Sáenz *et al*. (2011) and the study here, in a marine oxic-suboxic-anoxic water column and underlying sediments (Baltic Sea Gotland Deep; Berndmeyer *et al*., 2013). This papers shows that sedimentary OM only partly records water column SPM signals. A further complicating point if trying to establish a fixed threshold is the differences in extraction techniques (Soxhlet, ultrasonication, Bligh & Dyer), derivatisation (acetylated or not) and analytics (APCI *vs*. ESI). Considering these complications the authors should consider giving a less strict number like the suggested ">0.18", because it infers a high robustness or restrict the use to the here studied BUS setting.

- The authors present a large and complicate multidisciplinary data set. Such a paper requires the best possible way of presentation. In general, the Figures are of high quality, but the map showing the sample locations is too small (and it does therefore not cover all information). Figure 2a should therefore be either enlarged or, better, presented as single Figure. Furthermore, station numbers should be better located in the Fig. at the respective symbols (and each stations should be labeled). It would then also be possible and helpful to add the profiles shown in Figure 7.
- At least at two places in the manuscript station numbers appear to be incorrect. At line 383 station "55" is mentioned, which is not in the Figures (potentially the authors refer to station 59?). At line 453 they refer to stations 8 and 55. It appears that both numbers are wrong here. The first is tentatively 18 and the second, again, 59. Station numbers given in the text must therefore be carefully checked!
- The authors use data from a natural setting and compare them with biomarker data from the laboratory. This is good and state of the art, but over interpretation of the lab data should be avoided. This holds also because only relatives of the organisms in the BUS water columns were available for lab studies and it remains unclear how valid these values are for the BUS (and other natural settings). For instance, using the BHT-x ratio from lab cultures would argue for (partly even more) than 100 % of bacterial hopanoid producers to be represented by anammox bacteria. This is unlikely and also far from the 16S rRNA data presented (less than 5 %). The authors discuss this discrepancy, but they should check whether not some of their statements need to be toned down. This refers also to the use of the temperature sensitive "NL_5" ratios. The calculation is interesting and supports the conclusion of transported ladderane fatty acids, but decimal numbers for the temperature calculations appear to exact.
- I did not check all references, but there appears to be a discrepancy between references in the text and the reference list (e.g. Hopmans *et al* 2021 was not cited and Berndmeyer *et al* 2013 is in the list, but not in the text).

Specific comments

- Line 13: Modify for consistency to "(IPLs)"
- Line 24: Change to "ratios"
- Line 25: Introduce "NL_5" here or rewrite.

- Line 45: Delete part of the sentence from ", hereby…"
- Line 56: Better deceased instead of "dead"?
- Line 62: Is BHT-x really "rare"? In marine sediments with relatively high organic matter I would suppose not (e.g. in the Black Sea, the Cariaco Trench, the Baltic Sea this compound is abundantly reported).
- Line 63: I am not convinced that the current knowledge on the appearance of the BHT isomer allows describing it as "uniquely sourced by anammox". There is a convincing accord between anammox bacteria, their niches and BHT-x occurrences, but it does not exclude other sources. The authors may rethink the use of a less strict term here and elsewhere.
- Line 81: here and elsewhere change to "Brüchert"
- Line 135ff: Here the liters filtered should be added
- Line 159: The paper is not referenced in the list.
- Line 193: "Hopmans *et al* 2021" is not in the reference list. I did not went through all references, but there appear to be inconsistencies. For instance, Berndmeyer *et al* 2013 is in the list, but not cited in the paper. This must be carefully checked and corrected!
- Line 236 formula: For consistency write the denominator in brackets.
- Line 243 and 245: Check symbol at "kit" and "Qiagen"
- Line 289: Introduce "ABF" here.
- Figure 3: Colors for station 8 and 9 are hard to distinguish. It is generally complicate to locate station-specific data in the biomarker plots. Why not using smaller symbol sizes, but also using different symbols? What does "NB" in the legend means?
- Line 316: Modify to "…near St. 117 or…"
- Line 321: Modify to "85 mbss"
- Line 346: Modify to "were found in the BUS"
- Figure 5: Please give always the same x-axis for IPL-ladderanes (always 0 to 6 ru L-1). Also, why are numbers in Figure 3b so much higher (" x 10^5")
- Line 473ff: Comment: BHT-x concentrations were also 10 less in the offshore samples. IPL ladderanes were not detected. However, is the sensitivity of both methods similar?
- Line 485ff: Two publications should be added to this discussion, which reported on BHT-II in Benguela sediment (Watson, 2002) and on the problems of allochthonous organic matter in the same region (Blumenberg *et al*., 2010; geohopanoids including a "BHT (isomer 2", which is tentatively and and in analogy with the "BHT II" BHP in Sáenz *et al*. (2011) the BHT-x in this manuscript).
- Line 512ff: Sentence sounds odd and needs rewriting.
- Figure 7: Not sure, but there appears to be a discrepancy between the concentrations compared with Figure 3 (IPL ladderanes maximize in Fig. 3 at 2,5 x 10^5 and in Fig. 7 at 25 x 10^3). The authors should check that.
- Line 564: An example, where a less exact threshold could be introduced. E.g. "…St. 5 at 30 mbss, and 0.2 may thus act as a safer threshold…"
- Line 581: I don't think that the BHT-x ratio is correctly described as a marker for "anoxia", but rather for anammox bacteria and its respective niches.
- Line 584: Better modify to "…and indicate that anammox…"
- Line 587: Better modify to "….the temperature sensitive NL_5 index…"
- Line 591: According to above, I recommend suggesting "0.2" instead of "0.18" here.
- References: See general comment above and delete numbers for references.
- Line 770: Requires splitting into two references.

References

- Berndmeyer, C., Thiel, V., Schmale, O. and Blumenberg, M. (2013) Biomarkers for aerobic methanotrophy in the water column of the stratified Gotland Deep (Baltic Sea) Org. Geochem. 55, 103-111.
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- Schwartz-Narbonne, R., Schaeffer, P., Hopmans, E.C., Schenesse, M., Charlton, E.A., Jones, D.M., Sinninghe Damsté, J.S., Farhan Ul Haque, M., Jetten, M.S.M., Lengger, S.K., Murrell, J.C., Normand, P., Nuijten, G.H.L., Talbot, H.M. and Rush, D. (2020) A unique bacteriohopanetetrol stereoisomer of marine anammox. Org. Geochem. 143., 103994, doi:10.1016/j.orggeochem.2020.103994
- Watson, D.F. (2002) Environmental distribution and sedimentary fate of hopanoid biological marker compounds. University of Newcastle, Ph.D. thesis, Newcastle upon Tyne. UK.