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Comment on bg-2021-257

Anonymous Referee #2

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-RC2>, 2021

Review of "Bacteriohopanetetrol-x: constraining its application as a lipid biomarker for marine anammox using the water column oxygen gradient of the Benguela upwelling system" by Z.R. van Kemenade et al.

The authors of this paper investigated the applicability of the bacteriohopanetetrol-x (BHT-x) as a marker for marine anammox in the Benguela upwelling system. To this aim, they concomitantly analyzed BHT, ladderanes (both in their intact polar forms and as fatty acids) and *Ca. Scalindua* 16S rRNA genes. The combination of these different tools revealed a strong spatiotemporal variability in the presence/abundance of anammox bacteria and associated lipids in the region. They showed the suitability of BHT-x as a marker for marine anammox and demonstrated the effect of lateral organic matter transport on the distribution of ladderane fatty acids and BHT-x vs. total BHTs. Such an effect should be taken into account when interpreting lipid data (based on core lipids) in sedimentary archives.

This is my opinion that this manuscript is of interest for the readers of Biogeosciences. The combination of microbiological and organic geochemical tools to better understand the marine anammox process is welcome. The paper is well-written, well-presented and easy to read.

I have only a few minor comments which should be easy to address before publication.

Line 49. I would invert Figs. 1a and b, as ladderanes are presented first in the text.

Fig. 2 and Table 1, as well as materials and methods section. I do not understand why the sampling stations are not numbered consecutively. This should be explained somewhere.

Line 171. "twice" instead of "thrice".

Line 125. Please specify here how these standards were obtained (after having been isolated from sediments I imagine).

Line 322. Even 750 mbs for station 2.

Line 359. The point at 125 mbs is difficult to visualize.

Line 383. Station 59 instead of 55.

Line 453. Station 18 and 59 instead?

Lines 462-464. The seasonal effect should be better discussed here.

Line 469. Affect abundance.

Lines 476-477. High concentrations in BHT-x were observed at 720 mbs at to a much lesser extent at 270m mbs, whereas the opposite was noted for ladderanes. This should be clearly specified.

Line 481. The persistence degree of ladderanes in the water column should be discussed here.

Line 482. "likely indicating".

Line 493. I would define the Lüderitz upwelling cell here.

Lines 502-504. Here you should provide some hypotheses to explain why ladderane IPLs were not detected throughout the water column, whereas ladderane FA concentration

increased with depth. Where are ladderane FAs derived from? What about potential influence of lateral transport?

Please check the salinity scale in Fig. 5c.

Line 515. Similarly here, the relationship between ladderane IPLs and FAs should be better explained. Despite high abundance of ladderane IPLs, high abundance of ladderane FAs is not observed. This temporal offset should be discussed in more detail than just the sentence in lines 515-517.

Line 537. What do you mean by "well-known PCR biases"? This is unclear for the non-specialists.

Fig. 7. The numbering in the caption and in the figure is not consistent.

Lines 563-566. This threshold should be tested in other sites, this could be mentioned.