We greatly appreciate the time and effort referee 1 has taken to review our manuscript and are grateful for the positive assessment. The provided constructive comments will significantly contribute to a further improved manuscript. Below, we will we will respond (**in bold**) to the reviewers' comments (*in italic*), point by point.

*"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"….(***see point 2 below***)…."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."*

We agree with the reviewer that establishing a BHT-x ratio threshold to infer deoxygenation poses multiple challenges. We will aim to better highlight these challenges in the revised manuscript, and to elaborate further on the proposed threshold. The following points will be discussed in further detail:

1) ***The value of the BHT-x threshold value*. One of the aims of our study was to establish a threshold that can be used to determine past water column deoxygenation in sedimentary records of upwelling regions. As the reviewer notes, we established a relatively high BHT-x ratio threshold of 0.18 to infer water column oxygen levels of <50 µmol L^{-1} to account for allochthonous anammox products. Nonetheless, as laid out by the reviewer, different extraction and/or analytical techniques may result in a different ratio. For instance, the BHT-*x* ratio derived from an acetylated culture analysed by Peiseler and Rohmer (1992) using HPLC (0.1), was different than that measured by Schwartz Narbonne et al. (2019) in an aliquot of the same non-acetylated culture using UHPLC (0.2). Thus, in accordance with the reviewer's comment, we will further emphasize that caution must be applied when comparing the BHT-x ratio between different studies with different methodologies. We therefore agree that a BHT-x ratio threshold of 0.18 determined in this study may be too constrained. Thus, in the revised manuscript we will remove a decimal point (rounding 0.18 up to 0.2). We show that our findings align well with those from different marine systems as investigated by Sáenz et al. (2011; Arabian Sea, Peru Margin and Cariaco Basin) *and* Matys et al., (2017; Humboldt current system). Combining these datasets with ours (oxygen concentrations converted to µmol kg^{-1}; see figure 1) indicates that when $[O_2]$ is <50 µmol kg^{-1}, the BHT-x ratio (i.e. BHT-II ratio) is ≥ 0.2 (except in 1 sample from the Cariaco Basin). Considering the large variety in marine settings (four different upwelling regions and one restricted anoxic basin) and in methodologies (Soxhlet versus modified Bligh & Dyer; UHPLC-APCI-MS versus UHPLC-HESI-MS), a BHT-x ratio of 0.2 is likely to provide a robust threshold to estimate low-oxygen conditions (<50 µmol kg^{-1}) from sedimentary records. We will include this new figure in the revised manuscript, which shows the relationship between oxygen concentrations and the BHT-x ratio from all previous BHT-x water column studies.**

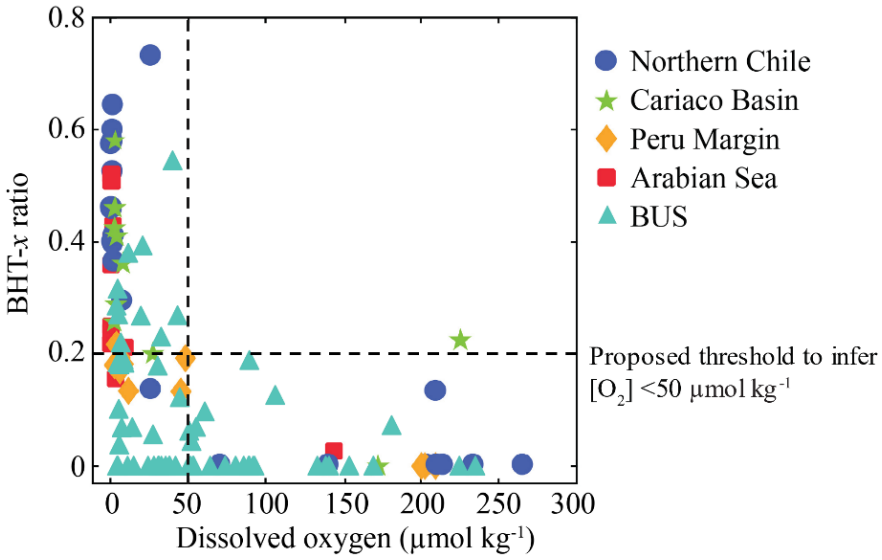

Figure 1 (proposed new figure): Relationship between dissolved oxygen concentration and the BHT-x ratio in suspended particulate matter (SPM) collected from the water columns of Northern Chile (Matys et al., 2017), the Cariaco Basin, Peru Margin and Arabian Sea (Sáenz et al., 2011) and the Benguela upwelling system (BUS; this study).

….."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."……

2) ***Application of the BHT-x ratio*. As the reviewer points out, an integrated water column signal is not always recorded in the sedimentary record. We agree that the Berndmeyer et al. (2013) study valuably contributes to this discussion. Berndmeyer et al., (2013) show that bacteriohopanepolyols (BHPs) recorded in the sediment of the Gotland Deep are not an exact integrated signal of the entire water column, but instead more mirror the distinctive BHP distribution of the suboxic zone. In accordance, Matys et al. (2017) found that the BHT II isomer ratio (i.e. BHT-x ratio) values observed in surface sediments of the Humboldt current system are comparable to those observed in the OMZ core of the overlying water. This suggests that though BHPs found in sedimentary records might not be an integrated signal of the entire water column, it appears that the BHT-x and BHT-x ratio signal observed in the suboxic zones of the water column is preserved in the sediment. Though we have not analysed the BHT-x ratio in the underlying sediments of the BUS, based on the findings of Berndmeyer et al., (2013) and Matys et al., (2017), it is likely that the BHT-x ratio observed in the oxygen-deficient interval of the BUS water column is retained in the sediments. We aim to further discuss these facets in the discussion of the revised manuscript.**

"*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."*

In accordance with reviewer #2's suggestion, we will aim to clarify the station map of figure 2a further by: i) separating figure 2a from the other figures (b, c and d), ii) enlarging the figure and iii) locating the station numbers nearer to their respective station location, as indicated with the dots. All station numbers for the CTD and nutrient measurements are provided in the supplementary material (with coordinates). The profiles of figure 7 will be presented earlier in the manuscript (after figure 2), and figure numbers will be adjusted accordingly.

"*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 reviewer is correct and we thank the referee for spotting these errors. We will correct this, and carefully check all station numbers provided in the text.

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 "NL5" ratios. The calculation is interesting and supports the conclusion of transported ladderane fatty acids, but decimal numbers for the temperature calculations appear to exact.

We agree that the discrepancy between BHT-x ratios found in the anammox biomass enrichment culture (cultivated in the laboratory) and the values observed in the BUS warrant further discussion. We will highlight potential discrepancies in the paragraph starting at line 567, by discussing that, to date, it is unknown if -and how BHT and BHT-x synthesis by *Ca.* Scalindua spp. is influenced by 1) environmental conditions and 2) species diversity. Concerning the NL_5 derived temperatures, we agree that the reported temperature values do not reflect the accuracy of the proxy. Thus, decimal numbers will be removed, as suggested by the reviewer.

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).

The Berndmeyer et al., 2014 study (2013 was not in the reference list) was incorrectly included in the reference list. We thank the reviewer for pointing this out and we will carefully check that only in text references are listed. The Hopmans et al., 2021 study is cited in the text in section '2.4.2 BHP and IPL analyses'.

Specific comments

Line 13: Modify for consistency to "(IPLs)" **intact polar (IPL) ladderane lipids will be amended to ladderane intact polar lipids (IPLs).**

Line 24: Change to "ratios" **Amended.**

Line 25: Introduce "NL5" here or rewrite. **Amended.**

Line 45: Delete part of the sentence from ", hereby…" **Amended.**

Line 56: Better deceased instead of "dead"? **We feel that 'dead' is the more appropriate term for bacteria, and therefore propose to keep this term in the sentence.**

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). **Agreed, the word 'rare' is removed from the sentence.**

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. **Amended to "So far reported to be uniquely synthesized by marine anammox bacteria"**

Line 81: here and elsewhere change to "Brüchert" **Amended.**

Line 135ff: Here the liters filtered should be added. **Amended. The range in liters filtered will be added.**

Line 159: The paper is not referenced in the list. **The paper the citation refers to is number 40 in the reference list (Redfield et al., 1963), indeed, the year cited in the text was incorrect (1960 instead of 1963). This is now amended.**

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! **The Berndmeyer et al., 2014 study was indeed included in the reference list (but not the 2013 study) without any in-text reference. We thank the reviewer for pointing this out and will carefully check all references. The Hopmans et al., 2021 study is cited in the text in section '2.4.2 BHP and IPL analyses'.**

Line 236 formula: For consistency write the denominator in brackets. **Amended.**

Line 243 and 245: Check symbol at "kit" and "Qiagen" **Amended.**

Line 289: Introduce "ABF" here. **Amended.**

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? **Symbol size will be decreased and different colors will be used for station 8 and 9, to ensure they are distinguishable. NB is an abbreviation for 'Nota bene', i.e. Latin for 'note well'.**

Line 316: Modify to "…near St. 117 or…" **In this sentence, our intent was to indicate stations near the ABF (station 117) and north of the ABF (stations 18 and 59). We have amended this sentence to enhance clarity.**

Line 321: Modify to "85 mbss" **Amended.**

Line 346: Modify to "were found in the BUS". **Not all ladderane IPLs that were present in the anammox enrichment cultures were found in the BUS SPM samples. For clarity, we will amend this paragraph to read: "All the ladderane IPLs reported for the Ca. Scalindua brodae enrichment culture (Table S4) and those previously reported for Ca. Scalindua spp. (Rattray et al., 2008) were evaluated in the BUS SPM samples. However, at the time of sampling, only the PC and PG ladderanes (Fig. 1c) were detected in the BUS water column. Furthermore, these ladderane IPLs were found in SPM from a limited number of shelf stations located …"**

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"). **We thank the reviewer for pointing this out, as indeed the figure is missing the factor by which the axis value should be multiplied. We will also provide the same scales for the ladderane IPL axis.**

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?

A study by Wörmer et al. (2015) provides a detailed overview of lipid biomarker analysis using HPLC/ESI-MS. Their results show a drastically expanded analytical window and sensitivity for IPLs when using reversed phase HPLC/ESI-MS, which we also applied here. In accordance, both Sturt et al. (2003) van Mooy & Fredricks (2010) report a high sensitivity for intact polar lipids (IPLs) using HPLC/ESI-MS. Though these latter two studies did not include analysis of BHPs, it is likely that the PC ladderane observed in our study has in fact a higher sensitivity than BHT (when analyzed using HPLC/ESI-MS), as the PC ladderane has a charged quaternary amine moiety, and therefore does not need to be ionized. The relative response factor of IPLs with a PC headgroup, in comparison to betaine lipids and glycolipids, was therefore observed to be relatively high (van Mooy & Fredricks, 2010). In addition, Wörmer et al., (2015) observed that IPLs with a PC headgroup had the highest response factor (and lowest ion suppression) in comparison to other IPLs. Nonetheless, it could be that ladderane IPLs at offshore stations were simply below the detection limit of our method. We aim to discuss all of the beforementioned points in further detail in the revised manuscript.

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). **Agreed. These publications will be included in the discussion.**

Line 512ff: Sentence sounds odd and needs rewriting. **Entire section from line 506 to 513 amended to:**

"**In March (Fig. 5c), the same sampling location showed distinct differences in physiochemical properties. This is consistent with previously reported seasonality: lower temperatures and increased upwelling commence in austral autumn, resulting in decreased SSTs (Monteiro et al.,**

2008; Louw et al., 2016). Indeed, the strong redoxcline observed in February was absent in March. SST in March was also ~1.5°C lower than observed in February, indicating water column mixing and weakened stratification. Likewise, the nutrient-rich sub-thermocline waters mixed with the surface waters, resulting in similar NO_2^-, NO_3^-, and NH_4^+ concentrations throughout the water column (Fig. x). Additionally, salinity was relatively high throughout the water column (35.2–36.2 psu), indicating the late summer (Feb–April) salinity maximum (S > 35.1 psu) had set in, which is known to co-occur with the oxygen minimum (Monteiro et al., 2008). Indeed, in March, surface waters (<10 mbss) were more oxygen-depleted than observed in February."

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. **The scale multiplication factor in figure 7 for the ladderane IPLs is corrected to 2.5x10^5 (i.e. 25x10^4), as the factor was indeed incorrect.**

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…" **Amended.**

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. **Agreed, this will be rephrased**

Line 584: Better modify to "…and indicate that anammox…" **Amended.**

Line 587: Better modify to "….the temperature sensitive NL5 index…" **Amended.**

Line 591: According to above, I recommend suggesting "0.2" instead of "0.18" here. **Amended. See our comments above.**

References: See general comment above and delete numbers for references.

Line 770: Requires splitting into two references. **Amended.**

References:

1. **Berndmeyer, C., Thiel, V., Schmale, O. and Blumenberg, M.: Biomarkers for aerobic methanotrophy in the water column of the stratified Gotland Deep (Baltic Sea), Org. Geochem., 55, 103-111, doi: https://doi.org/10.1016/j.orggeochem.2012.11.010, 2013.**
2. **Louw, D. C., van der Plas, A. K., Mohrholz, V., Wasmund, N., Junker, T. and Eggert, A.: Seasonal and interannual phytoplankton dynamics and forcing mechanisms in the Northern Benguela upwelling system, J. Mar. Sy., 157, 124–134, doi:10.1016/j.jmarsys.2016.01.009, 2016.**
3. **Matys, E.D., Sepúlveda, J., Pantoja, S., Lange, C.B., Caniupán, M., Lamy, F. and Summons, R.E.: Bacteriohopanepolyols along redox gradients in the Humboldt Current System off northern Chile, Geobiology., (6), 844-857, doi: 10.1111/gbi.12250, 2017.**
4. **Monteiro, F. M., Pancost, R. D., Ridgwell, A. and Donnadieu, Y.: Nutrients as the dominant control on the spread of anoxia and euxinia across the Cenomanian-Turonian oceanic anoxic event (OAE2): Model-data comparison, Paleoceanography, 27(4), 1–17, doi:10.1029/2012PA002351, 2012.**
5. **Peiseler, B. and Rohmer, M.: Prokaryotic triterpenoids of the hopane series. Bacteriohopanetetrols of new side-chain configuration from *Acetobacter* species, J. Chem. Res., 298–299, 1992.**

6. **Rattray, J. E., Van De Vossenberg, J., Hopmans, E. C., Kartal, B., Van Niftrik, L., Rijpstra, W. I. C., Strous, M., Jetten, M. S. M., Schouten, S. and Damsté, J. S. S.: Ladderane lipid distribution in four genera of anammox bacteria, Arch. Microbiol., 190(1), 51–66, doi:10.1007/s00203-008-0364-8, 2008.**
7. **Sáenz, J. P., Wakeham, S. G., Eglinton, T. I. and Summons, R. E.: New constraints on the provenance of hopanoids in the marine geologic record: Bacteriohopanepolyols in marine suboxic and anoxic environments, Org. Geochem., 42(11), 1351–1362, doi:10.1016/j.orggeochem.2011.08.016, 2011.**
8. **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.: A unique bacteriohopanetetrol stereoisomer of marine 770 anammox, Org. Geochem., 143, doi:10.1016/j.orggeochem.2020.103994, 2020.**
9. **Sturt., H. F, Summons, R.E., Smith, K., Elvert, M., Hinrichs, K.: Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry—new biomarkers for biogeochemistry and microbial ecology, Rapid Commun. Mass Spectrom., 18(6), 617–628, doi: https://doi.org/10.1002/rcm.1378, 2004.**
10. **Van Mooy, B.A.S and Fredricks, H.F.: Bacterial and eukaryotic intact polar lipids in the eastern subtropical South Pacific: Water-column distribution, planktonic sources, and fatty acid composition, Geochim., 74(22), 6499-6516, doi: https://doi.org/10.1016/j.gca.2010.08.026, 2010,**
11. **Wörmer, L., Lipp, J. S. and Hinrichs, K. U: Comprehensive analysis of microbial lipids in environmental samples through HPLC-MS protocols, In Hydrocarbon and lipid microbiology protocols, Springer, Berlin, Heidelber, 289-317, 2015.**