We greatly appreciate the time and effort referee 2 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 respond (**in bold**) to the reviewers' comments (*in italic*), point by point.

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

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. **We thank the reviewer for pointing this out, the listing order of the various stations is indeed confusing. In Table 1, the reason for not listing the station numbers in a consecutive order was to highlight the division of 'shelf' and 'offshore' stations. In addition, within these two subdivisions, the stations are listed according to their sampling dates. We will highlight this in the caption of Table 1. Additionally, the odd numbering of the stations (e.g. jumping from station number 117 to 140) is due to the fact that during the second cruise (64PE450) stations were numbered according to activity (*e.g.* CTD sampling, multicoring etc.) rather than location. We will clarify this in the revised manuscript.**

Line 171. "twice" instead of "thrice". **Our intent with this phrasing was to clarify that after the first round of extraction, the supernatant was extracted three more times, where during the last two extractions the phosphate buffer was replaced with trichloroacetic acid. The phrasing might have been confusing, so we have rephrased this sentence to: "… re-extracted thrice (i.e. total of four extraction rounds), where during the last two extractions…"**

Line 125. Please specify here how these standards were obtained (after having been isolated from sediments I imagine). **The ladderane FAME standards were isolated from biomass of an anammox enrichment culture, grown in sequencing batch reactors, containing both *Ca.* Scalindua wagneri and *Ca.* Kuenenia stuttgartiensis (described in Kartal et al., 2006). We will add this information to the revised manuscript.**

Line 322. Even 750 mbs for station 2. **We have now included the specific bottom depths for St. 2 and 1 at which BHT-x was found.**

Line 359. The point at 125 mbs is difficult to visualize. **We will amend the figure (a.o. decrease symbol size) to clarify the visualization.**

Line 383. Station 59 instead of 55. **Amended.**

Line 453. Station 18 and 59 instead? **The reviewer is correct and is thanked for catching this error. Station number 8 is corrected to 18 and 55 to 59.**

Lines 462-464. The seasonal effect should be better discussed here. **We agree with the reviewer that a more detailed discussion about the seasonal shift of the Angola Benguela frontal zone and corresponding physicochemical changes in the water column would be appropriate. We propose to include the following text:**

"The northern BUS is strongly influenced by the meeting of the warm, poleward flowing Angolan Current (AC) and the cold, equatorward flowing Benguela current (BC), which converge at the seasonally dynamic Angolan Benguela frontal zone. The balance between the intensities of the AC and BC determine the position of the front. At the end of austral summer (i.e. the timing of expeditions 64PE449 and 64PE450), the ABF reaches its most southern point and is generally

found around 20°S. At this time, strongest oxygen depletion is known to occur around ~24-26°S, while less severe oxygen depletion is observed near the ABF. In contrast, during austral winter, the ABF is located furthest north (~14-16°S) and the most severe oxygen depletion occurs between 16-20°S (Chapman and Shannon, 1987; Boyer et al., 2000). Thus, the absence of anammox biomarkers north of the ABF during expeditions 64PE449 and 64PE450, is concurrent with the latitudes of the ABF (~19.8°S) and the most severe oxygen depleted waters (~26°S). Considering the seasonal northward shift of the ABF and the oxygen-depleted waterbodies, the occurrence of anammox bacteria and associated biomarkers will likely shift northwards too."

Line 469. Affect abundance. **Amended.**

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.
Amended to: "At St. 2, BHT-*x* was observed at 250, 310 and 710 mbss, with the highest abundance found at the lowest depth. Ladderane FAs were observed at 125, 250 and 710 mbss, with peak concentrations observed at 250 mbss."

Line 481. The persistence degree of ladderanes in the water column should be discussed here. **The reviewer rightly points out that the degree of persistence of ladderane FAs in the water column is not properly discussed in this section. We propose to include the following text:**

"The degradation rate of ladderane FAs is slower than that of ladderane IPLs (i.e. ladderane FAs have been observed in sediments of 140 kyr BP; Jaeschke et al. 2009b, whereas ladderane IPLs are thought to reflect living or recently dead anammox cells; *e.g.* Jaeschke et al., 2009a). Accordingly, ladderane FAs are likely not degraded immediately upon cell death and could be transported to other water bodies. Indeed, the offshore NL_5 derived temperatures suggest a higher *in situ* ambient temperature during ladderane FA synthesis than the CTD recorded temperatures. In contrast, NL_5 derived temperatures from shelf stations were similar to the CTD recorded temperatures, suggesting *in situ* synthesis of ladderane FAs on the shelf. This indicates that ladderane FAs observed offshore likely originated in the warmer shelf waters and were transported offshore."

Line 482. "likely indicating". **Amended.**

Line 493. I would define the Lüderitz upwelling cell here. **We agree with the reviewer that the Lüderitz upwelling cell is not properly introduced here. We propose to amend line 493 to:**

"The Lüderitz upwelling cell has been identified as one of the most intense upwelling regions in the BUS. In austral winter, the water column near the Lüderitz upwelling cell is relatively oxygenated, due to the upwelling of oxygen-rich South Atlantic Central Water (Bailey et al., 1991). However, low-oxygen conditions and even anoxia prevail during austral summer due to the respiration of sinking organic matter supplied by phytoplankton blooms (Bailey et al., 1991; Brüchert et al., 2006). Consequently, continental shelf waters between 24–26°S display large temporal variations in DO concentrations under the influence of the Lüderitz upwelling cell."

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? **Line 502-504 is amended to:**

"Ladderane IPLs could have been present at St. 6 in abundances that were below the detection limit of our method. Alternatively, BHT-x and ladderane FAs at this station could have been laterally transported from more southern shelf sites (Mollenhauer et al., 2007), whereas ladderane IPLs which degrade quickly upon cell death (e.g. Jaeschke et al., 2009b) and would not withstand this transport."

Please check the salinity scale in Fig. 5c. **We thank the reviewer for spotting this error in the number of decimal places, which should have been increased to two (giving 35.20, 35.25 and 35.30 as scale intervals). We will amend this in the revised manuscript.**

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. **We will further highlight the potential explanations for the offset between ladderane IPL and ladderane FA concentrations.**

Line 537. What do you mean by "well-known PCR biases"? This is unclear for the non-specialists. **Amended to: "Unequal amplification efficiency of PCR products could result in the preferential amplification of certain 16S rRNA genes, whilst others might be inhibited for amplification (*e.g.* Pinto & Raskin, 2012). This could theoretically also have led to a low coverage of *Ca.* Scalindua spp. reads."**

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

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

We agree with the reviewer that comparing the BHT-x ratios observed in the BUS with BHT-x ratios observed at other sites would be a valuable contribution to the manuscript. We propose to include a new figure in the revised manuscript (see figure 1), which shows the relationship between oxygen concentrations and the BHT-x ratio in water columns from the Sáenz et al., (2011) and Matys et al., (2017) studies, as well as our own study. In order to compare our datasets, oxygen concentrations of our own study are converted to µmol kg^{-1}. Based on the data presented in this figure and the discussion in the rebuttal to referee 1, we propose rounding the threshold value up to 0.2. The combination of our own dataset with those of by Sáenz et al. (2011) and Matys et al., (2017), shows that when $[O_2]$ is <50 µmol kg^{-1}, the BHT-x ratio is ≥ 0.2 (except 1 sample in the Cariaco Basin; see figure 1). Considering the large variety in marine settings (including four different upwelling regions and one restricted anoxic basin) and in methodologies (Soxhlet versus modified Bligh & Dyer; UHPLC-APCI-MS versus UHPLC-ESI-MS), we believe that a BHT-x ratio of 0.2 provides a robust threshold to estimate lox oxygen conditions (<50 µmol kg^{-1}) in sedimentary records of various marine settings, including upwelling regions.

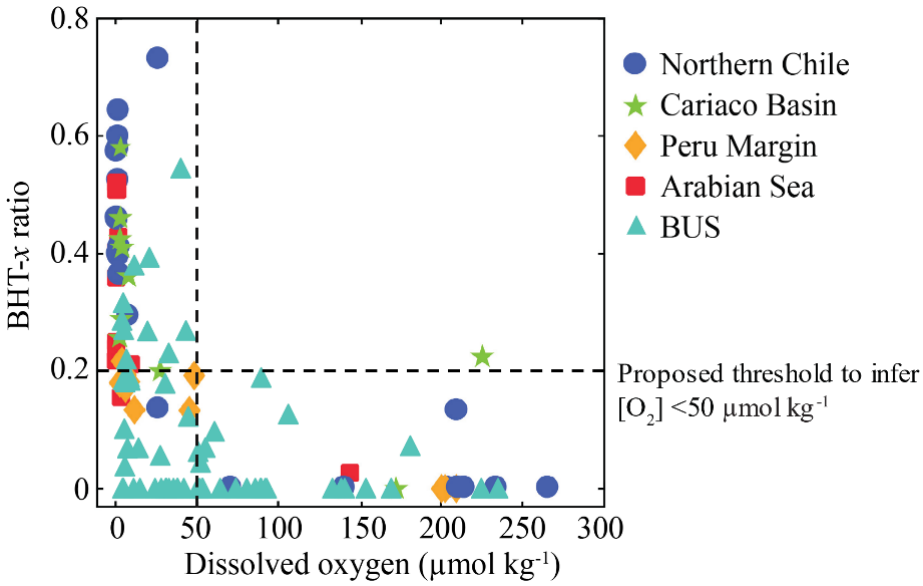

Figure 1 (proposed new figure): Relationship between dissolved oxygen concentration and the BHT-x ratio in suspended particulate matter (SPM) collected from the water columns of Northern Chile (Matys et al., 2017), the Cariaco Basin, Peru Margin and Arabian Sea (Sáenz et al., 2011) and the Benguela upwelling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

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