

Interactive comment on “Biomarker evidence for the occurrence of anaerobic ammonium oxidation in the eastern Mediterranean Sea during Quaternary and Pliocene sapropel formation” by Darci Rush et al.

Anonymous Referee #1

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This manuscript is a nice contribution to Biogeosciences and provides valuable new data exploring the applicability of an up and coming biomarker for anammox. The data from the Pliocene S73 sapropel are particularly interesting since they extend the timescale on which BHT isomer has been used as a proxy for anammox. Overall, I have relatively little criticism. Please find some open questions and points that need clarification below.

General comments 1) While I agree that Fig. 3 provides a good argument for an anammox origin of the BHT isomer in the investigated Aegean sapropels, this compound

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has been identified in various non-anammox settings/under different redox conditions (the authors also point this out). So far, the most convincing argument for an anammox origin of the BHT isomer in environmental samples seems to come from BHT isomer/(BHT+BHT isomer) ratios, which are really high in anammox cultures (Rush et al. 2014) and also in OMZ settings (Matys et al. 2017). In this respect, I would strongly encourage the authors to include the BHT data as well, to support the argument that the trends seen in BHT isomer abundances across sapropel horizons reflect the occurrence/increase of anammox during sapropel deposition. The sapropels have very high TOC in comparison to the under- and overlying sediments. Thus, these sediments can be expected to be hot spots for deep biosphere bacterial communities, which could also be (non-anammox) producers of BHT isomer and increasing absolute BHT isomer abundances for example could simply reflect a relative increase of bacterial over eukaryotic biomass (or some other process). Can this be excluded? I think it would be helpful to include the BHT data and to show BHT isomer/(BHT+BHT isomer) ratios for these records (Figs. 4 and 5) to strengthen the argument for an anammox biomarker. If BHT isomer/(BHT+BHT isomer) ratios show different or less obvious trends, please add a respective section/paragraph to the discussion.

2) The occurrence of SC ladderanes and simultaneous absence of ladderanes (or occurrence only at the detection limit) in the S5 sapropel in core 64PE406 warrants some more discussion. While SC ladderanes could only be detected in 3 samples, the authors provide two possible β -oxidation scenarios to explain their occurrence. However, what is missing is the explanation why there are no ladderanes in the maximum sapropel unit for which fully anoxic (euxinic) conditions and peak anammox are invoked. I would expect that the preservation potential for ladderanes was much higher during maximum sapropel deposition. If this was only due to an “unknown” degradation mechanism (as stated earlier), shouldn't i) that mechanism also degrade the SC ladderanes generated during the onset and termination of the sapropel? or ii) if that “unknown” mechanism abruptly starts/ends during the onset/termination of sapropel deposition, what kind of mechanism would work opposite to the “normal” redox-driven preserva-

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tion/degradation mechanisms, i.e., higher degradation under anoxic conditions? Based on the BHT isomer abundances, the ladderane pattern does not seem to be driven by productivity since the onset and termination BHT isomer peaks are not significantly higher than the maximum sapropel concentrations and a productivity argument would also disagree with the peak anammox assumption during maximum sapropel deposition made earlier. Please elaborate. Also, please consider including the SC ladderane abundances on a second axis in the b panel of Fig. 4, it will help guiding the reader through the arguments.

Specific comments l. 140: change to “immediately” l. 163: change to “ratio” l. 257: change to “detect” l. 324-327: since the BHT isomer has also been detected in non-anammox samples, the trace amounts detected in the background non-sapropel samples may also reflect different bacterial sources rather than minimal anammox activity. Again, BHT isomer/(BHT+BHT isomer) ratios would help here. l. 339-340: please elaborate a little more which kind of (unknown) mechanisms you consider may cause ladderane decomposition (see also above comment 2). l. 341: change to “appear” l. 349: change to “S5 formation” l. 403: change to “all samples” l. 439-443: the argument is contradictory, in the first sentence it says that the “BHT isomer displayed a distribution different to that of the S5 record” while the second sentence states “much like the trend seen in the S5 Levantine sapropel.” Please clarify. l. 446-448: “It is possible that euxinia shoaled further into the photic zone during this Pliocene sapropel, forcing anammox at the chemocline to compete for N with phytoplankton.” If the euxinia was even more pronounced, wouldn’t one expect to find isorenieratene (or other biomarkers such as okenone/okenane) in sapropel 73 if it was found in a different sapropel at this site? Please elaborate a little. l. 450-452: “There was a spike in BHT isomer concentration mid-sapropel that coincided with a decrease in TOC (65 – 67 cm core depth; Fig. 5a).” To me it appears that the BHT isomer spike pre-dates the TOC decrease, which is similar to the pattern observed for the S5 in core 64PE406 but opposite to the pattern evident for the termination of the S73 when TOC decreases earlier. How is this explained?

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Fig. 3 Please only show data points, not box plots. The minimum sample size for box plots is $n=5$, which would allow only the S1 data to be visualized this way. See further Krzywinski M. and Altman N. (2014) Visualizing samples with box plots. *Nature Reviews Microbiology* 11, 119–120. However, why not plot the Aegean core like the other two cores (Y-axis could be broken between sapropels)? This would allow better comparison with the other records as well.

Fig. 4 Maybe the quality of this figure could be improved, Fig. 5 has much better resolution. For both figures, a thin line connecting the circles in panel b would aid at seeing the trends.

Supplement The supplementary figure (showing the chromatograms and the mass spectrum of non-acetylated BHT and BHT isomer) is not referred to in the text and it does not have a caption.

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