

We thank Dr Blanchet for her thorough and helpful comments which will greatly improve the quality of this manuscript.

We have replied to the reviewer comments below (in bold):

This new paper explores the occurrence of anammox (anaerobic oxidation of ammonium) in the water column of the Eastern Mediterranean during sapropel deposition. Not being a biomarker or anammox specialist but interested in chemical processes in low-oxygen environments, I found this publication very insightful and well presented. I have only minor comments that aim at clarifying the message.

General comments:

1) It might be useful for non-anammox specialists to draw a little sketch to show where you expect anammox to take place in the water column (e.g. from present-day OMZ) and its relationships with euxinia and anoxia (for instance with schematic O₂ and H₂S profiles, chemocline, redoxcline. . .) and competition with phytoplankton. It would also help to visualize the interpretations that you discuss regarding the patterns of anammox in the various sapropels.

We agree that a cartoon showing the different anammox scenarios in the water column during sapropel events will be useful and we will include such a figure in the revised manuscript.

2) In general, I am missing a bit a comparison between the interpretations drawn here in term of water-column chemistry with other type of data. For instance, relationships between the build-up of anoxia or the presence of euxinia are mentioned in the text but do not appear in the figures. For S5 at higher resolution (64PE406), it might be useful to give temporal indications so that it can be compared to other records. Along a similar line, the relationships between deep-water stagnation, eutrophication and eutrophia have been widely explored for S5 and it might be useful to place your record in a wider context (also to highlight its relevance).

We agree with Dr Blanchet that creating a graphical representation of the proposed anammox interpretations that are already made in the text is an excellent idea. We will include these (e.g. references to the build-up of anoxia) in the new cartoon figure (mentioned above).

3) Another point which I am missing is a more structured discussion on the effects of post-depositional diagenesis on your markers. Diagenesis associated with changes in sedimentation rates and level of TOC in and around sapropels has been well documented and generally allows to identify specific horizons in sapropels layers (proto-sapropel, oxidized “burn-down” sapropels). Higher BHT isomer values and the presence of SC ladderanes below and above S5 and the Pliocene sapropel should be discussed in this context.

Though not mentioned in the text or figures, the high-resolution S5 sapropel (Fig. 4) did not show any evidence of post-depositional “burn-down” diagenetic alterations. We will incorporate these findings (as a reference to a recently accepted manuscript that describes XRF measurements of this sapropel (Dirksen et al., 2019)) into the discussion of the revised submission. Unfortunately, the same analyses were not performed on the Pliocene sapropel. However, we would not expect that any potential TOC burndown would selectively preserve organic biomarker lipids. It is more likely that if burndown were to have occurred, BHT isomer and ladderanes would also be subjected to these diagenetic processes. Nevertheless, we will include as part of the discussion burndown as a potential factor affecting the Pliocene core.

4) What can help you decipher whether anammox occurred in the water column or in the sediments? I understand that the presence of free sulfides is preventing anammox to occur but would anammox happen in sediments where the overlying water is not euxinic and where sulfates are present (say until the sulfate-methane transition zone)? This is related to my previous points and questions the role of sediment-bound anammox in your records: would processes occurring during early diagenesis (i.e., when redox and chemical fronts shifted) in the sediments be able to trigger anammox (and overprint the water-column derived biomarker record)? Is it possible for anammox to occur in the sediment core after retrieval and during storage? This might help understand why there are ladderanes in S5 in LC21 but not in 64PE406: i) storage and sediment handling artefact, ii) "unknown degradation mechanism" or iii) spatially nonuniform occurrence of anammox (e.g., in the Aegean but not in the Levantine Basin)?

This is an excellent remark. Anammox is known to occur in certain marine sediments (e.g. Trimmer et al. 2003 Appl. Environ. Microbiol.; Jaeschke et al., 2010 Limnol. Oceanogr.). However, anammox activity has only ever been recorded in the upper surface sediment, which is logical as anammox requires both ammonium (which is often available in anoxic sediment) and nitrite (which is rarely detected in sediments deeper than the upper 5-10 cm). Thus, it is very unlikely that anammox would ever been active in sediments at the sulfate methane transition zone. It was also originally suggested that BHT isomer is a biomarker for pelagic anammox (Rush et al., 2014 Geochim. Cosmochim. Acta). However, follow up studies are needed to confirm this.

Furthermore, sedimentary anammox has previously shown a preference for low carbon mineralisation activity, being outcompeted by heterotrophic sedimentary denitrification in sediments with more available reactive carbon (Thamdrup and Dalsgaard, 2002, Appl. Environ. Microbiol.; Engstrom et al. 2005, Geochim. Cosmochim. Acta; Jaeschke et al., 2010 Limnol. Oceanogr.). Therefore, we would expect that if a sedimentary N removal process was to have occurred during the sapropel (or post sapropel deposition) in sediment with high TOC, denitrification would have been favoured over anammox. However, it is more likely that all of the nitrate and nitrite would have been consumed already in the water column of the sapropel, leaving only the accumulation of ammonium in the sediment.

There were ladderanes in the 64PE406 core, but as Dr Blanchet points out in a comment below, this was not clear. We will amend the manuscript to mention these analyses earlier. As discussed in the manuscript, the ladderanes in the 64PE406 core were at detection limit which did not allow for interpretations of the results. As to whether anammox could have been active in stored cores: this is an unlikely scenario in i) the 64PE406 core as samples were immediately frozen after the core was opened and subsampled, and ii) the LC21 core as anaerobic chemolithoautotrophic anammox would not have been encouraged by the presence of oxygen and lack of N substrates in the cold-stored split core. We believe that the storage of the LC21 core would have rather caused the preferential degradation of ladderanes (as these are more labile than BHPs).

5) Finally, can you rule out that anammox biomarkers were not brought to the core site by runoff (say a "detrital/exogenous" anammox component)? If I am not mistaken, anammox occurs in freshwater and coastal environments as well, but would the BHT isomer biomarker resist fluvial transportation and exposition to oxic conditions?

We thank the reviewer for bringing this point up. Our response below is also of interest to Reviewer 1's comments about additional bacterial sources of BHT isomer. Anammox is indeed a process that also occurs in freshwater and soil environments. However, only the anammox genus *Scalindua* is present in marine environments (Villanueva et al., 2014 Front. Microbiol.). Isomers of

BHT have also been detected in non-anammox bacteria (cf. Rush et al., 2014 *Geochim. Cosmochim. Acta*). However, we are currently working up a manuscript that shows the BHT isomer synthesized by *Scalindua* is different from the isomers synthesized by non-marine anammox genera and non-anammox bacteria. Thus, we conclusively identify the BHT isomer present in these samples as an exclusively marine anammox signature.

Specific comments: I agree with reviewer #1 that information is missing in the figures: Fig. 3: add data for BHT isomers in other cores (S5 for 64PE406 and S73 for ODP 160) Fig. 4: it would indeed be insightful to show ratios and SC ladderanes (see comments by reviewer #1). Drawing a line between points would also be helpful. The depth scale can be removed for the plot 4b (and generally, a and b are not needed).

We agree with Dr Blanchet and the anonymous reviewer who also brought up these points, and we will amend the revised manuscript to include these figure changes.

If possible, indicate the various sub-layers in the sapropel (proto-sapropel, oxidized sapropel) using the Ba and Mn concentrations (or as ratio over Al or Ti). Ba is a good indicator for sapropel extend and Mn shows the upper extend (upper redox front), so the oxidized part of the sapropel (where the TOC is low). If you have some time indication, it might be interesting to indicate/plot some results from other records (isorenioratene, forams, etc. . .) to get a fuller picture of the changes in water-column properties. Such a figure (depth profile) is missing for LC21, although a lot of data has been gathered on this core. This would allow direct comparison between other proxies and the anammox biomarkers, even at low sampling resolution.

To the best of our abilities we will include information about sapropel sections (as discussed above, using the accepted paper of Dirksen et al.) in the revised manuscript and the new figure. However, the low sampling resolution of the LC21 core makes it difficult to draw conclusions about anammox functioning within the sapropels. Rather all we can discuss is presence vs. absence instead of the timing or sequences of events. This was one of the main reasons we chose to study the high resolution 64PE406 core.

Fig. 5: please also connect dots with a line in 5b and if possible, indicate the various horizons in the sapropel (see comments for Fig. 4). While reading section 3.1, I was wondering why ladderanes had not been measured in 64PE406, and it is only when I read section 3.2 that I got my answer. It should be clear from the beginning that ladderanes were measured both in LC21 and 64PE406 (also in the method part, section 2.4.2) but that they could not be detected in the latter one.

Introduction line 48-54: perhaps introduce the meaning of anoxia vs. euxinia for nonspecialists? In general, it would be more accessible if terms would be better introduced (e.g., chemocline vs. redoxcline) or shown on schematic representations.

l. 365-371: I find this part quite obscure: what is meant by "Then, once monsoonal discharge brought in the initial pulse of nutrients from the Nile, [. . .]"? I do not follow the order of events. Perhaps making that appearing on fig. 4 would be helpful (e.g., by comparing to timing of freshwater pulses and development of anoxia)? Or draw small sketches? Similarly, with the proposal that the observed signal might be related to "split-anoxia": not very clear why that happens and might be useful to provide a visualization.

We will amend the MS to include these revision suggestions. A cartoon, as suggested in the earlier comment, will also better explain the order of anammox events in sapropels.

But once again, I enjoyed reading this paper and feel that it will contribute value to our understanding of changes in the marine environment related to deoxygenation processes, which were recently highlighted as a growing concern for present oceanic basins.