

## ***Interactive comment on “Intact polar lipids of Thaumarchaeota and anammox bacteria as indicators of N-cycling in the Eastern Tropical North Pacific oxygen deficient zone” by M. Sollai et al.***

**M. Sollai et al.**

martina.sollai@nioz.nl

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We are grateful for Anonymous Referee #1's comments. Below we respond to the comments and indicate how we have modified the manuscript. As you will see, we have followed most of the reviewer's suggestions. The revised manuscript will be uploaded when required from the editorial system. In particular, see Q for questions and A for answers.

1. General comment Q1. Microbial ecology associated with the expanding oxygen

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minimum zone has a crucial impact on the global nitrogen and carbon cycles. AOA and anammox are two key components of the metabolic processes of nitrification and denitrification. Using lipid biomarkers this manuscript discussed the distribution and potential interaction of AOA and anammox in two different sites of ETNP. Both chemistry and lipids data are very well presented. The distribution of Thaumarchaeota and anammox bacteria in the water columns indicated by the lipid profiles is convincing, and consists with other previously reported data. Indeed, it is shown that these two kinds of microbes are more sensitive to oxygen content rather than nutrients.

R1. We thank referee # 1 for this positive assessment and the time spent on reviewing our paper.

Q2. What I am concerned is whether the somehow overlapped crenarchaeol and ladderane profiles in the open ocean site really represents an actual interaction between the AOA and anammox communities. At the coastal site the ammonia spike close to oxycline must have stimulated the growth of Thaumarchaeota, while anammox bacteria is more restricted to nitrite. Therefore, under a suitable oxygen condition these two communities are divided by their favorite nutrients. The nitrite peak in the middle of OMZ is likely not related to AOA. Their overlap at the open ocean site with constant low ammonia concentration is more constrained by oxygen content. The direct metabolic link or network between Thaumarchaeota and anammox bacteria seems really weak. Other species involved in the nitrogen metabolism are probably more closely interacted with either one of them.

R2. We agree with the referee that the slight overlap in the occurrence of crenarchaeol and ladderane at certain depths at the open ocean station does not prove that the AOA and anammox communities interact. However, to our opinion we carefully phrased this; e.g. “but a partial overlap of the two niches of these microbial species in the open water setting. The latter distribution suggests the potential for an interaction between the two microbial groups at the open ocean site, either as competition or cooperation” (lines 17-20; page 4834).

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Specific comments Q3. 1. Introduction: A N-cycle diagram showing the metabolic feature of AOA and anammox will be straightforward. The font of 'Thaumarchaeota' does not need to be in italic.

R3. We do agree with the suggestion and we will add a N-cycle diagram showing how AOA and anammox metabolisms are integrated in the global marine nitrogen cycle. We will correct the font of Thaumarchaeota in the text.

Q4. 2.2 Sampling: Station names in Table. 1 and Fig. 1 are not consistent. "147-149" is labeled as "147" in Fig. 1. And, "106" is missing in Table 1.

R4. We will correct the labels in Fig. 1 to 147-149 as in Table 1. Station 106 will also be included in Table 1.

Q5. 2.3 Intact polar lipid analysis: It is ok to show the relative abundance of HPH-crenarchaeol with peak areas, but as I know, the response factor of a given compound may also vary due to instrument condition in different times. Did you analyze all your samples in the same batch?

R5. The reviewer correctly points out that the response factor of a given compound may vary as a consequence of instrument condition, in different times. In order to minimize this inconvenience we indeed did analyse our samples in the same batch; we will mention this in the experimental section 2.3, page 8, line 27, of the revised manuscript.

Q6. 4. Results: 3.1 please give a brief statement why water density is so different at the two studied sites. . . .Salinity? Please explain the unite (r.u. L-1) of HPH-crenarchaeol, and the same as in Fig. 3.

R6. Indeed the difference in the density anomaly depends on the different salinity of the water masses found at some depth of the two sampling sites. In the revised version we will add at page 9 line 20 this statement: "As the salinity along the water column at the two sampling sites diverges (data not shown), also  $\sigma_\theta$  diverges and has an effect

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on the distributions we observe." The unit for HPH-crenarchaeol relative abundance, as response unit L-1 (i.e. r.u. L-1) is specified, in the Material and method section 2.3, page 9, line 5 but in the revised version we will repeat this in the legend of Figure 3.

Q7. Discussion: P4845, lines 9-11, At the coastal site there is low ladderane concentration detected at the oxycline, first three data points in Figure 2f. There is also typo in this sentence, please rewrite.

R7. We thank the referee for spotting this typo. We will rewrite the paragraph in this way: "Moreover, similarly to De Brabandere et al. (2014) who also reported low anammox rates at the oxycline in one of their sampling stations in the ETSP, we observed low ladderane concentration in the ETNP coastal setting."

Q8. P4848, lines 5-13, Terrestrial input could be the main cause. A more detailed discussion will be better.

R8. This comment is not entirely clear to us; we agree that coastal settings will receive a higher terrestrial input but do not really see that this will affect the nutrient profiles to a large extent. Therefore, we feel we should refrain from bringing this into the discussion.

Q9. Figure caption, What do the yellow shades in Fig. 2 represent?

R9. With those yellow shades we wanted to highlight the differences we observed in terms of HPH-crenarchaeol, PC-monoether, nutrients and oxygen distribution between the two sampling sites. We will add an explanation to the caption of Fig. 2.

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