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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.

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We are grateful for Anonymous Referee #2'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 form the editorial system. In particular, see Q for questions and A for answers.

Q1. The paper by Sollai et al. reports on the distribution of archaeal and bacterial

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Interactive Discussion

Discussion Paper

denitrifying organisms in oxygen deficient zones (ODZ) of the Eastern Tropical North Pacific (ENTP). These groups of organisms are of relevance as they account for most of the nitrogen loss in ODZs worldwide. The authors have tracked the occurrence of the aerobic ammonium oxidizing archaea and anaerobic ammonium oxidizing bacteria in the water column by using two diagnostic biomarkers. Their main observations are that these two groups overlap in offshore regions, while there is a clear zonation separating them in coastal waters. They consequently suggest that this distribution represents a potential for either competition or cooperation of these two microbial groups at the open ocean sites. Understanding the distribution and functioning of these two microbial groups is of great relevance for understanding the marine nitrogen cycle. While this study gives insights on their distribution patterns, the authors have invested little effort into further investigating the interactions of these two groups with each other. One could envision for instance 15N-labeling studies to track the cycling of for instance nitrite, a potentially important intermediate between aerobic and anaerobic oxidation of ammonium. This study therefore only provides little in-depth insights into the processes related to nitrogen cycling in ODZs and only adds minimally to the advancement of the field. However, I see no technical problems with the paper and therefore only have minor request for revisions to the paper. Some citations were incorrect that need to be corrected before publication.

R1. We thank referee #2 for the time dedicated to the review of our paper. We respectfully disagree with the assessment of this referee that this manuscript “adds minimally to the advancement of the field”. Our study compares for the first time the simultaneous distribution of AOA and anammox bacteria between the coastal and the open ocean of the ETNP ODZ, which represents one of the most prominent ODZ in the present ocean. Moreover, the resolution of our sampling campaign is very high and therefore provides valuable insights in the distribution of AOA and anammox bacteria in the water column of the two areas investigated in our study, which were beforehand missing. We agree with the referee that this does not provide quantitative rate estimates for nitrification and anaerobic ammonium oxidation. However, we do refer to a previous study, which,

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by employing 15NH4+-labelling, investigated the oxidation rate of this nutrient to nitrite in the vicinity of our coastal sampling station (Beman et al., 2012 Limnol. Oceanog; see also Discussion 4.1, pages 12, line 26 and page 13, lines 1 to 5). The study suggests that AOA are relevant players in the nitrification taking place in this area. Other papers discussing correlated topics include Beman et al., 2008, The ISME J. and Beman et al., 2013, The ISME J. Altogether, this work represents the only systematic attempt to investigate the contribution by AOA and AOB in the nitrification process occurring in the proximity of our coastal sampling station, at least to the best of our knowledge. On the other hand, an assessment of this kind with respect to the offshore regions of the ETNP ODZ is still missing. Regarding anammox bacteria and their metabolism, such kind of evaluation relative to the ETNP ODZ is still lacking.

Q2. 1) Page 3, lines 24-27: It is true that Karner et al., 2001 observed that Archaea (particularly Crenarchaea) account for 20% of picoplankton and ca. 40% of the estimated total number of cells in the ocean, however, these cells are not very likely to be ammonia oxidizing archaea (AOA), since these archaeal cells were found predominantly between 200 to 5000m water depth and AOA thrive in the upper water column, between 100 to 100m water depth (e.g., Lam et al., 2007, Pitcher et al., 2011). The statement as the authors have written is consequently misleading and needs to be corrected.

R2. We disagree with the referee on this matter. All isolated Thaumarchaeota from the ocean have been shown to be oxidizing ammonia so far and the Thaumarchaeota residing in deep ocean waters contain the amoA gene (the gene coding for the enzyme involved in the first step of ammonium oxidation) in their genome (e.g. Francis et al., 2005, PNAS). Although this does not 100% prove that they perform ammonium oxidation, it is judged very likely and another physiology for deep water Thaumarchaeota has not convincingly been demonstrated to the best of our knowledge. Indeed previous studies have suggested that deep water Thaumarchaeota could use urea to fuel nitrification in environments with low ammonia concentration. However, the potential use of

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urea to fuel nitrification has only been reported so far for a soil Thaumarchaeota isolate (Tourna et al., 2011, PNAS). Moreover, these assumptions are only based on the abundance of the urease gene and do not provide any evidence that this metabolic process is actually performed by deep marine Thaumarchaeota. We agree that it is possible that deep water Thaumarchaeota could be performing other metabolisms other than ammonia oxidation but there is no experimental evidences supporting it. Furthermore, the paper that claimed the existence of non-nitrifying Thaumarchaeota (Agogue et al., Nature 456, 788, 2008) was actually based on a primer mismatch. Therefore, we feel that this section is not "misleading" and simply provides an accurate explanation.

Q3. 2) Page 6, line 1: The authors are citing a number of studies in the context that HPH-crenarchaeol and not MH and DH-cren is a more suitable tracer to track living biomass. However, many of the cited papers do not provide support for this statement. To my knowledge there exists no study that systematically investigated the relative liabilities of glycosidic over phosphate-based head groups. Instead, what the authors could say is that HPH crenarchaeol has proven to be an adequate tracer for ammonium oxidizing Thaumarchaeota in past studies (e.g., Pitcher et al., 2011), while other IPLGDGTs could have other archaeal sources in the water column (add the other citations, the authors could also consider adding Lincoln et al., 2012 PNAS) or represent fossil contributions (e.g. Xie et al., 2011).

R3. As suggested by the referee, we will refer in the revised manuscript for our statement only to the Picher et al. (2011) reference, since this is the only study that showed by examining HPH-, MH-, and DH-crenarchaeol and 16S rRNA and amoA gene copy numbers that HPH-crenarchaeol is the best marker for tracking "life" Thaumarchaeota. Harvey et al., (1986, GCA) have experimentally demonstrated that phosphate-ester bond lipids are reported to be more labile compared to the glycosidic ether bond lipids and thus more suitable as biomarker for living cells.

Interactive comment on Biogeosciences Discuss., 12, 4833, 2015.