

Response to Referee #1

I would like to thank Referee 1 for considering this manuscript being adequate for publication in Biogeosciences. The comments and thoughts provided by Referee 1 were particularly helpful to improve this manuscript. Questions and comments were addressed as described in the following; modifications are highlighted in yellow in the main text:

General comments:

Referee comment (RC)1: I am fairly surprised to not see organisms of the SUP05 clade being present/abundant here as they are normally found in OMZs. Was the amplicon dataset screened for them?

Author comment (AC)1: SUP05 was indeed present in the dataset; we presented the diversity on the phylum level, SUP05 would only show up on the family level and clusters within the phylum of Proteobacteria, as shown in figure 4. In order to provide a deeper insight into the distribution of SUP05 and other organism groups, we now included a table and a bar plot including all taxa on the family level to the supplement and added the information of SUP05 distribution to the text.

RC2: If the figures are already the size they later should appear in the manuscript, then I would suggest increasing it for the sake of readability. They are really nice, so no need to make them so small.

AC2: Thanks for this nice remark- we will increase the figures in the final manuscript.

RC3: While the manuscript is generally well written, sometimes complicated sentence structure with multiple sub-sentences makes reading bothersome. Streamlining this a little could enhance the reading flow significantly.

AC3: The manuscript has now been checked by a native speaker in order to increase the reading flow.

Specific comments:

RC4: p. 14178, l. 10 ff: "...at depth around 40 to 100 m..." sounds weird. Is "...at depths of around 40..." meant?

AC4: Changed as suggested.

RC5: p. 14180, l. 6-13: Why state the aim and then the ultimate aim? Wouldn't it make more sense to clearly define what the aim was? To identify differences in microbial community structure between eddy and surrounding waters was just a step towards the aim, or?

AC5: This is true, the paragraph has been revised:

'In this study, we investigated differences in microbial community structure in an O₂-depleted eddy, surrounding ETNA open waters, and upwelled waters on the Mauritanian shelf. This was achieved using a combined high-throughput 16S rDNA amplicon sequencing/qPCR approach along with carbon uptake rate measurements and hydrochemical observations. This study aimed to understand the microbial community response to O₂ depleted conditions with regard to primary production and remineralization in these poorly described anomalies to further understand the sensitivity of the ETNA biogeochemistry to future ocean deoxygenation.'

RC6: p. 14190, l. 15: It would be nice to cite the original papers here.

AC6: We replaced the cited overview paper by the original publication of Giovannoni et al., 1990 in Nature.

RC7: p. 14190, l. 21 ff: It is not clear if the methanogens detected are free living in the water column or are particle associated. Judging from the collection of biomass samples on 0.2 µm filters, both options could be possible. Were free living methanogens detected in OMZs previously?

AC7: Sure, as a result of our sampling strategy both options are possible; however, it would not matter with regard to the difference in community structure between samples inside and outside the eddy. Ganesh et al. (2015 in ISME j) show in the supplement of their study that a certain proportion of euryarchaeota are present in the filter fraction of 0.2-1.6 µm. The authors consider this fraction as free-living organisms. They, however, do not show more details on the phylogeny of this group so that it remains unclear whether these are methanogens or not.

RC8: p. 14192, l. 12-15: To confirm if the upper chl a maximum is caused by eukaryotic phototrophs, it might be an idea to look into the sequences classified as cyanobacterial 16S rRNA and see if 16S genes from plastids were detected and are hidden in there.

AC8: The applied sequence analysis method removes sequences of chloroplasts and mitochondria in the initial step. So, they are not hidden in the cyanobacterial part of the dataset. However, it is possible to recover them, and we added the information to the text. Actually, the idea of recovering information on eukaryotic phototrophs from the plastids is interesting (although a characterization of eukaryotes remains impossible from plastid sequences) - still the extraction protocol for nucleic acids may not be as efficient for eukaryotes as it is for microbes. This has to be considered. In addition, we tested the applied primer set *in silico* on the SILVA database and determined a relatively low coverage of only 38% for chloroplast sequences. Therefore, the information obtained from chloroplast sequence analysis can only be taken indicative for the presence and abundance of eukaryotic phototrophs; however, using it as a quantitative tool is only possible to a limited extent. Particularly, it can't be quantitatively compared to the abundance of cyanobacteria because of the different coverage as described above.

RC9: p. 14193, l. 23-25: Judging from the phylogenetic information on the metabolisms of the organisms is not always possible, so the statement is fairly strong here. While I think the authors are right I still would word it a little bit more careful.

AC9: I agree, the sentence has been rephrased:

'The close proximity of increased abundances of the HNLC-PCC maximum to the O₂ minimum in the eddy may thus point towards a beneficial relation between the HNLC-PCC and the eddy core water microbial community that largely consists of heterotrophic organisms.'

RC10: p. 14194, l. 7: original studies are missing in the references (Morris 2002, Rappe 2002)

AC10: We added the suggested references.

RC11: p. 14195, l. 4: How do the 3000 copies/L and 100 copies/L compare to the bacterial 16S rRNA gene copy numbers? What part of the population has the capability for denitrification? Because of differences in cell numbers between eddy and the outside the actual difference might not be as high as suggested.

AC11: The part of the community containing *nirS* is very minor in all samples, which does not mean that the corresponding process is not active or important. (For comparison, N₂-fixers are considered to account for only 2% in ocean surface metagenomes, however, they contribute up to 80% of new nitrogen to these waters). The absolute number of *nirS* sequences measured against a standard dilution series cannot be compared to 16S rRNA copy numbers from the amplicon sequencing, as this would mean comparing two different methods.

However, we see a highly pronounced effect of denitrification on nitrous oxide (N₂O) production within the eddy core waters based on molecular and isotopic data (Grundle, Löscher et al.' Extreme N₂O activity in an oxygenated ocean', under review). This effect is not present outside the eddy, where N₂O is supposed to be exclusively produced by nitrification (see, e.g., Löscher et al., 2012, Ryabenko et al., 2012). Therefore, we are convinced that our *nirS* dataset mirrors the presence/activity of the denitrification process, which is the key finding, here. We added the reference (Grundle et al.) to the text.

RC12: p.14195, l. 12: With similar reasoning as in the comment above, it would make sense to compare the transcripts of the *nirS* gene to a housekeeping gene.

AC12: I do not agree on that. The potential for nitrite reduction is not depending on the overall abundance of bacteria or microbes or the biomass. It is more a question of the presence of certain

organisms in an environment that enables the respective process. Absolute gene quantification is also from a methodological perspective preferable as it mitigates the risk of including a second PCR bias.

RC13: p. 14197, l. 22: space missing

AC13: Changed.