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Biogeosciences Discussions

Interactive comment on "Distinctly different bacterial communities in surface and oxygen minimum layers in the Arabian Sea" *by* Mandar Bandekar et al.

Mandar Bandekar et al.

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Dear Dr. Robinson,

Subject: Submission of revised manuscript bg-2016-147

Thank you very much for obtaining excellent set of expert reviews also by Reviewer 2 for our submission. We gratefully thank Reviewer#2 for all the improvement suggestions. As you will kindly see, we have revised the manuscript in accordance with the advices offered. We have addressed all the points suggested and list our replies in 'Responses to reviewers' comments'.

I append below the replies to comments of Reviewer 2 we received for the ms. To

comply with, the suggestions by Reviewer 2, we have now added some text in response to the revision/inclusions. This text is in blue fonts. Please let me know when to upload our revised ms with all changes.

I hope that these changes in response to the reviewers are acceptable to you. We believe that with these changes, the re-revised version meets the requirements and you will kindly accord acceptance to our submission.

I look forward to your kind and early response.

With my Best Regards.

Yours sincerely,

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Responses to reviewer's comments The authors have used DNA sequence analysis of environmental 16S rRNA genes and phylogenetic reconstruction to investigate the community diversity of bacteria present at a time-series station in the Arabian Sea. Samples were obtained from the surface mixed layer and from within the oxygen minimum zone (OMZ) on three separate occasions over the course of several months and under contrasting hydrographic conditions. The authors conclude that while there was no distinct seasonal difference in community structure (lines 21-22), greater diversity and community richness were evident within the OMZ when compared to the surface and deep chlorophyll maximum. While the latter observation is of interest, it is based on a very modest number of sequences from each depth and time point (Table 5) and not that well supported by classical indices of diversity (Shannon and Simpson indexes). As the authors acknowledge at several points in the manuscript (e.g., Section 3.6, lines 334-335, lines 436-438, etc.) their findings are preliminary because due to the undersampling of the community they have not captured or analyzed in sufficient depth the far greater diversity evidently present at the station in order to draw robust conclusions.

We are grateful for these highlights you kindly offered on this submission. In view of your observations below, we have revised the ms to include the changes in accordance with your expert observations. Comment: Where they may be on firmer ground is in reporting that the composition of the surface community was distinct from that of the OMZ (Section 3.5). Much of this difference appears to be explained by the absence of cyanobacteria from the deeper samples (lines 195-198, lines 269-270), however, which is an expected result given their photoautotrophic nature. Indeed, if one ignores the contribution of cyanobacteria to the surface community, the relative percentage contributions of the dominant heterotrophs (alpha and gamma proteobacteria) at all depths would be much less distinct than that shown in Figure 2. Reanalyzing the data (minus cyanobacteria) might proof useful. Reply: We gratefully acknowledge this insightful observation on taking out the contribution of cyanobacteria from the surface community and looking at the contribution of Alphaproteobacteria and Gammaproteobacteria at all depths. Upon reanalyzing the data (minus cyanobacteria) the two following points were clear. 1- The percent contribution of Gammaproteobacteria increased by 9% in the surface and that Alphaproteobacteria by 4%. 2- Further the contribution of both Alphaproteobacteria and Gammaproteobacteria were in general lower in the surface than they were below DCM and in the core OMZ. Therefore, we may be permitted to retain our earlier statement as well as the figure 2 as they are.

The following are the replies for minor points. Comment: Line 13 What does 'Contributions" mean? Numbers, biomass, activity? Reply: This was meant as a general statement; in view of your advises, the sentence is suitably modified. Pl see lines 13-14 in the re-revised version. It reads: 'Role of microbial communities in terms of its biomass, number and activity in oxygen minimum oceanic zones are being realized through the applications of molecular techniques'

Comment: Line 84 What is 131 Tris? Reply: Sorry, It was just Tris and '131' is deleted on line 103 of the re-revised version of the manuscript.

Comment: Section 2.2 Much more detail of the PCR conditions is required. The refer-

ence to the manual of Sambrook (et al.) is insufficient - what were the temperatures, times, cycle numbers, etc.? Reply: Thank you so much. Complete details of PCR condition is now included in section 2.3, lines 118-120 in the re-revised manuscript. "DNA was extracted from Sterivex cartridges following the modified method of Ferrari and Hollibaugh (1999). The precipitated DNA was hydrated in 50 μl sterile deionised water, purified and quantified in a Nano-drop (Thermo Scientific, USA). All extracts were confirmed to be of PCR quality. Using the universal 16S rRNA primers, 27F and 1492R, the 16S rRNA gene was amplified as per the conditions given in Sambrook (1989). These conditions are: initial denaturation at 94°C for 4 min, 35 cycles consisting of denaturation at 94oC for 1 min, annealing at 55oC for 1 min and elongation at 72oC for 2 min and, final extension at 72oC for10 min. PCR amplification was performed in a final volume of 50 μl in a thermocycler (Applied Biosystems, USA) and correct amplification was ensured by checking for the amplicons electrophoretically."

Comment: Section 2.3 TA cloning is very efficient. What explains the poor numbers of transformants recovered in this study? Reply: We are unable to provide anymore explanation on the low transformation efficiency. The fact remains that we did multiple trails to achieve the clone numbers that we could garner.

Comment: Line 103 Manufacturers name and reaction conditions required Reply: As per the reviewer's suggestion, manufacturer's name and reaction condition is now stated in lines 133-135 in the re-revised version. "All positive clones/transformants from each sample were picked out, grown overnight at 37°C on LB plates and subjected to the colony-PCR with primers sets pucM13F/pucM13R using temperature conditions as per TOPO-TA cloning guide(Invitrogen): initial denaturation step of 10 min at 94°C, followed by 30 cycles of 94°C for 1 min, , annealing at 55°C for 1 min with elongation step at 72°C for 1 min and final extension at 72oC for10 mins."

Comment: Line 104-05 which primers and reaction conditions were used for sequencing? Reply: As per the suggestions, primers and reaction conditions used for sequencing is now mentioned in lines 138-140 in the re-revised version. "The PCR products

were purified with the Axyprep-96 PCR Clean up kit (Axygen, Biosciences) and then sequenced using 16S rRNA primers, 27F and 1492R in an ABI 3130XL genetic analyzer (Applied Biosystems, USA) with the temperature profile as follows: initial denaturation at 96°C for 1 minute, 30 cycles consisting of denaturation at 96oC for 10 sec, annealing at 55oC for 10 sec and elongation at 60oC for 4mins and final extension at 60oC for 1mins."

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