

Interactive comment

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.

ramaiah@nio.org

Received and published: 12 July 2016

Dear Dr. Robinson.

Subject: Submission of revised manuscript bg-2016-147

Thank you very much for obtaining excellent set of expert reviews for our submission. 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 all comments we received for the ms.

Printer-friendly version



I hope that these changes are acceptable to you. We believe that with these changes, the 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,

N. Ramaiah Biological Oceanography Division CSIR-National Institute of Oceanography Dona-Paula, Goa, India

Responses to reviewers' comments

Comment: The manuscript by Bandekar et al, entitled 'Distinctly different bacterial communities in surface and oxygen minimum layers in the Arabian Sea' uses 16S rRNA gene sequences to examine seasonal and depth variations in bacterial community structure in the Arabian Sea. The oxygen minimum zone of the Arabian Sea has been relatively poorly studied to date and with the expansion of these regions predicted, an analysis of the bacterial community structure and the drivers of this, makes this manuscript timely. However, I have a number of concerns that I have outlined in detail below, that need to be addressed prior to publication. Reply: We are most grateful for the excellent set of review suggestions you have offered to improve our manuscript. We have strived and accordingly revised the manuscript and complied with all the advices provided.

Comment: The focus of the paper seems a little confusing as it is currently structured. Based on the introduction, beginning of the discussion and last sentence of the abstract, the authors seem to want to make this a nitrogen story, but have minimal data to support this being the focus of the paper. In the discussion of the data they only discuss denitrification for four lines and anammox is never mentioned. I think the authors should restructure the paper, to focus it on community structure and diversity and the

BGD

Interactive comment

Printer-friendly version



drivers of this. Reply: This advice is carefully addressed by revising the introduction almost entirely and suitably altering the discussion. Discussion involving very sparse communities of denitrification and anammox are pointed out. Indeed, this surprising result was highlighted earlier itself. More details provided in revision in lines 447-453 of the revised version as follows:. "It is interesting to note that only a few of the common OMZ OTUs affiliated to known denitrifiers, suggesting that denitrification is spatially heterogenous (niche segregation). We found just two 16S rRNA clones of Planctomycetes (NCBI Accession numbers KJ589907, KJ589971) during just one seasons. We therefore suppose that anammox does occur. Lack of larger numbers of clones Planctomycetes which is a known anammox bacterium may be a result of saturation not being reached at OTU or sequence level. Thus some rarer organisms were not being picked up even after repeated cloning efforts."

Comment: Currently the auxiliary data available is not at all utilized, where it could be used for multivariate statistical analysis or something similar, to potentially reveal what variables are shaping the community, instead of relying purely on the literature. The authors also need to emphasize how this study builds on the work by Jain et al, 2014 'Temporally invariable bacterial community structure in the Arabian Sea oxygen minimum zone', which was conducted at the same time as this study, by a number of the authors of this manuscript. Reply: Thanks a lot for this valuable suggestion. As you will kindly see, in the revision, we have added multivariate analysis in methods (Please see lines 201-205 in the revised version) Results (Please see lines 320-328 Figure 6 and Table 5 in the revised version) and discussion (Please see lines 379-383 in the revised version) sections. All these sections are reproduced later on in this reply itself.

Comment: The referencing throughout needs urgent attention, a large number of references are missing and / or incorrect ones used. Reply: All suggested references replaced.

Comment: I think it would be beneficial for the authors to ask a native speaker to read the manuscript for both language and grammar. Reply: We are extremely sorry for

BGD

Interactive comment

Printer-friendly version



the long sentences with conjunctions (many "ands") in the previous version. Such long statements surely made the reading often awkward and laborious. I am sorry for this. This concern is now taken care of, hopefully very largely.

Comment: Line 16: 'zone' instead of 'region' Reply: The word 'region' is replaced by 'zone' (It will in line 16 in the revised version)

Comment: Line 28 to 29: This sentence seems unnecessary, your data shows no evidence for anammox (discussed more below) and denitrification is only very briefly touched on with respect to your own dataset. Reply: Lines 27, 28 rephrased as follows: Hitherto undescribed diversity of denitrifiers, anammox and sulfur metabolizing bacteria was evidenced.

Comment: Line 34 to 36: Surely OMZs are a result of both poor ventilation and productivity fuelling the rain down of organic matter, this needs to be more explicitly addressed Reply: In fact, most of the introduction section is rewritten keeping your advice in the fore. The Introduction now reads as follows: "Intermediate depths in the oceans with very low oxygen saturations of $< 20 \mu M$ L-1 (Karstensen et al. 2008,) are known as oxygen minimum zones (OMZs). These suboxic layers are usually associated with low oxygen supply but high oxygen demand. This is due to poor ventilation of intermediate depths, higher microbial respiration and organic matter oxidation (Wyrtki 1962, Karstensen et al. 2008,). Major OMZs are found in the intermediate depths of eastern tropical North Pacific (ETNP, Wyrtki 1966), eastern tropical South Pacific (ETSP, Wyrtki 1966), eastern Arabian Sea (Wyrtki 1973, Madhupartap et al. 1996, Nagvi and Jayakumar 2000) and eastern South Atlantic (Karstensen et al. 2008). The OMZs, believed earlier to occur mostly in nutrient rich upwelling regions, are currently expanding and/or intensifying due to anthropogenic impacts (Diaz and Rosenberg 2008). Their expansion is affecting pelagic fisheries and benthic ecosystems due to habitat alterations and/or changes in nutrient cycling (Stramma et al. 2008). Further, the OMZ expansion is ascribed to increased production of climate active trace gases including carbon dioxide (CO2), methane (CH4) and nitrous oxide (N2O, Loscher et al. 2015).

BGD

Interactive comment

Printer-friendly version



Interactive comment

Printer-friendly version

Discussion paper



across the AS- OMZ although microbial communities within OMZs are involved in the biogeochemical processes therein. However, information on spatio-temporal variation

and phylogenetic diversity of bacteria from the AS-OMZ is quite sparse. Coinciding with an active denitrification zone (Naqvi 1994, Naqvi et al. 1998) the AS-OMZ experiences dissolved oxygen (DO) levels sometimes as low as 0.1 μ M (Naqvi 2006, Paulmier and Ruiz-Pino 2009). Previous studies mostly focused on delineating the role of denitrifying and anammox bacteria in the loss of fixed nitrogen in the AS-OMZ (Jayakumar et al. 2009, Bulow et al. 2010, Pitcher et al. 2011, Devol et al. 2006, Ward et al. 2009, Lam et al. 2011). However, detailed analyses to understand bacterial community structure and/or bacterial diversity of the AS-OMZ and its overlying surface waters are a few and far in between (Fuchs et al. 2005, Jain et al. 2014). In the present study we therefore aimed to assess the phylogenetic diversity of the extant bacterial communities in the

AS-OMZ. For this, we analyzed small subunit ribosomal RNA gene (16S rRNA or SSU rRNA) clone libraries prepared from water samples collected from five depths from the Arabian Sea Time Series station (ASTS; 17°0.126'N, 67°59.772'E), during three different seasons. ASTS is akin to well-known HOTS (Hawaii Ocean Time-Series) in the Pacific Ocean and BATS (Bermuda Atlantic Time-Series) in the Atlantic Ocean." Comment: Line 36: Lisa, 2003 is not an appropriate reference here, I suggest Karstensen

et al, 2008; Gruber et al, 2007 and / or Codispoti et al, 2001 Reply: As per the suggestion, the reference Lisa 2003 is replaced by Karstensen et al. 2008 (It will be in line 35 in the revised version)

Comment: Line 40 to 44: I think it is important that you emphasize here the importance of OMZs for global nitrogen loss. Reply: The importance of OMZs for global nitrogen loss is reviewed comprehensively (lines 59-66 in the revised version) as follows: "Accounting for 20% of the global oceanic denitrification (Codispoti et al. 2001), the AS-OMZ alone is responsible for removal of up to 60 Tg of nitrogen annually (Codispoti 2007). Naqvi et al. (2008) estimate that the AS denitrification contributes to \sim 40% of the global pelagic N loss. Within the OMZ, microorganisms capable of denitrification and anaerobic ammonium oxidation (anammox) both convert oxidized forms of nitrogen to gaseous species and thereby cause net loss of fixed N. This loss is indicated by the presence of a broad secondary nitrite maximum (SNM) and large fixed N deficits

BGD

Interactive comment

Printer-friendly version



(Naqvi 1991)." Comment: Line 40: What do you mean by 'intense'? Reply: Sentence suitably revised (lines 63-65 in the revised version) as follows. "Within the OMZ, microorganisms capable of denitrification and anaerobic ammonium oxidation (anammox) both convert oxidized forms of nitrogen to gaseous species and thereby cause net loss of fixed N." Comment: Line 40: Stramma, 2008 is not the appropriate reference for the cycling of N within OMZs. Reply: Reference of Stramma, 2008 deleted

Comment: Line 43: Why are we suddenly discussing hypoxic conditions, this has not been defined and in the previous sentence you are discussing anaerobic pathways. Reply: Line 43 is deleted.

Comment: Line 51: Reference needed. Reply: Reference of Loscher et al. 2015 added. It will be in line 47 in the revised version

Comment: Line 51 to 53: There are a number of manuscripts that discuss both the community structure and metabolism within OMZs, for example Stewart et al, 2012; Wright et al, 2012 etc. I agree that less is known about the Arabian Sea, but surely the Jain et al, 2014 paper is relevant here. Reply: Reference added. Please see lines 66-70 of revised version which read: "Apparently, the role of microbial communities in the AS-OMZ N-loss is vital for elucidating the microbes-mediated denitrification in the context of global biogeochemical and climatic processes (Stewart et al. 2012, Ulloa, 2012 but little is known about their community structure and metabolism from the AS-OMZ (Jain et al. 2014)." Comment: Line 57 to 59: The way this is currently written is confusing - im assuming you are trying to say something along the lines of 'OMZs account for 20 to 40% of global N loss from the oceans, with 10 to 20% thought to occur in the Arabian Sea' Codispoti et al, 2001; Gruber, 2004 etc. Reply: The sentence is revised. Please see lines 59-62 in the revision which read: "Accounting for 20% of the global oceanic denitrification (Codispoti et al. 2001), the AS-OMZ alone is responsible for removal of up to 60 Tg of nitrogen annually (Codispoti 2007). Nagvi et al. (2008) estimate that the AS denitrification contributes to ~40% of the global pelagic N loss." Comment: Line 61: The references Newell et al, 2011 and Bouskill et al, 2012 refer

BGD

Interactive comment

Printer-friendly version



to nitrification not N loss processes, so should be deleted. A large number of studies that have looked at N loss in the Arabian Sea are never mentioned Devol et al, 2006; Nicholls et al, 2007; Ward et al, 2009; Jensen et al, 2011; Lam et al, 2011 Reply: References Newell et al, 2011 and Bouskill et al, 2012 are deleted and replaced with suggested ones of Devol et al. 2006, Ward et al. 2009, Lam et al. 2011 (in line 81 in the revised version). Comment: Methods: How the oxygen and nutrient data were collected and analyzed is not mentioned, please include. Reply: Measurement of Nutrients and dissolved oxygen has been explained in subsection 2.2 of methods section (Lines 107-111 in the revised version) as follows: "2.2 Measurement of nutrients and dissolved oxygen Collection and measurements of physico-chemical parameters from each depth/sample were described in detail earlier in Jain et al. (2014). In brief, the Winkler titration method was followed to measure DO (Carpenter 1965) and standard

Comment: Line 79 to 80: I find the terms 'intense dentirification zone' and 'deep denitrification zone' misleading and confusing as denitrification is only discussed very briefly with respect to your data in this manuscript. Im assuming this is because you want to use the same terms as Jain et al, 2014, but upper and lower OMZ would be more appropriate. Reply: The terms 'intense dentirification zone' and 'deep denitrification zone' substituted with 'upper OMZ' and 'lower OMZ' (It will be in lines 99 and 100 in the revised version).

methods of Grasshoff et al. (1983) to measure the concentrations of ammonia. nitrate.

nitrite, phosphate, silicate."

Comment: Line 105 to 106: What does this sentence mean 'you kept the DO profile in mind'? Reply: The sentence is rephrased (Please see line 134 in the revised version) as: "Clone libraries were constructed for each sample." Comment: Section 3.1: Define your OMZ for the reader. Use values in this section, terms like 'slightly more' and 'quite low' are meaningless to the reader, unless they are relative to something. Reply: Measured values now included in the text (lines 215-218 in the revised version) as: "The average DO concentrations at 1000m increased to $7.22 \pm 4.5 \,\mu\text{M}$ L-1. The

BGD

Interactive comment

Printer-friendly version



Interactive comment

Printer-friendly version

Discussion paper



and diversity. It would be advantageous to compare this to your auxiliary data oxygen.

Interactive comment

nutrients, TOC, to see what variables are shaping the community. Reply: Subsections are now made in the discussion. The first paragraph is an implicit prelude to the introduction. In fact, we are very grateful to the question on "Do the authors think the lack of evidence for anammox is a result of saturation not being reached at OTU or sequence level, so rarer organisms such as Planctomycetes are not being picked up?" We found only two 16S rRNA clones of Planctomycetes during just one seasons. We therefore suppose that anammox does occur. This statement now included along with the NCBI accession numbers for our Planctomycetes clones. Lines 447-453 in the revised version read: "It is interesting to note that only a few of the common OMZ OTUs affiliated to known denitrifiers, suggesting that denitrification is spatially heterogenous (niche segregation). We found just two 16S rRNA clones of Planctomycetes (NCBI Accession numbers KJ589907, KJ589971) during just one seasons. We therefore suppose that anammox does occur. Lack of larger numbers of clones Planctomycetes which is a known anammox bacterium may be a result of saturation not being reached at OTU or sequence level. Thus some rarer organisms were not being picked up even after repeated cloning efforts."

This said, unlike earlier studies targeting only denitrifiers and/anammox, this study is useful to recognize the extant diversity of bacterial communities in the surface layers and OMZ depths of the AS.

Comment: Line 296 to 297: References needed Reply: Reference of Stewart, 2011 added; line 336 in the revised version

Comment: Line 297 to 299: This is not true, a number of studies have examined the community in OMZs and you even cite some of them later in the discussion, for example, Ulloa et al, 2006; Bryant et al, 2012; Stewart et al, 2012; Wright et al, 2012 etc Reply: Reply: Thanks a lot again. This section is revised thoroughly (lines 336-338 in the revised version) as. "For almost a decade now, the AS-OMZ gene surveys have focused extensively on microbes performing denitrification and anammox with less emphasis on the overall microbial community."

Printer-friendly version



Comment: Line 302 to 317: This needs to be moved to the introduction. Reply: Introduction is thoroughly revised in view of the suggestions, the suggested paragraph from discussion is moved into introduction but has undergone revision as was necessary (Please see above).

Comment: Line 329 to 330: What is your evidence for this statement? Reply: The statement is an inference proposed by us based on prior studies carried out around the ASTS. Lines 348-351 in the revised version read: "We therefore propose that the high rates of denitrification might be among the many possible causes for this higher diversity in the OMZ as also observed by Ramaiah et al. (2009). They reported both higher heterotrophic bacterial production and abundance from the locations surrounding ASTS." Comment: Line 338: The variation in diversity with depth in Ganesh et al, 2014 is dependent on the size fraction looked at, this is an important point that is currently missing from this sentence. Reply: The reference and related text deleted.

Comment: Line 344: The difference to the Jain et al, 2014 manuscript warrants further discussion here, you sampled at exactly the same stations / depths at the same time. In general, why are not more comparisons made to the Jain et al, 2014 manuscript and how your study builds on that work? Reply: A comparison of our work to that of Jain et al, 2014 is now explained in lines 367-370 as "It is to be noted however that previously, as reported in Jain et al. (2014), we examined the composition of bacterial communities through DGGE and by sequencing only 20 prominent and disparate DGGE bands. This was unlike building numerous clone libraries as done for this study."

Comment: Line 351: 'proficient'? Reply: Word has been replaced by 'major' (line 372 in the revised version).

Comment: Line 359 to 362: You have TOC data, why are not testing this hypothesis? Why are you not using your auxiliary data to look at what is shaping the community – you never even refer to the oxygen and nutrient data in Figure 1 within the discussion. Reply: Multivariate analyses was carried out to check the role of auxiliary parame-

BGD

Interactive comment

Printer-friendly version



Printer-friendly version

Discussion paper



ters and described in methods (in subsection 2.10, lines 20-205 in the revised version) and results (These will be in subsection 3.7; lines 320-328 Figure 6 and Table 5 in the revised version). Brief discussion on the same is added (in lines 379-383 in the revised version) as follows: "2.10 Multivariate analyses Principal component analysis (PCA) was performed using Primer 6 (PRIMER-E, Plymouth, UK) to infer relationships between hydrographic parameters and sampling depths during different seasons. All data were normalized to reduce the importance of minor differences in relative concentration/s of individual parameters. 3.7 Principal component analysis (PCA) Based on the Scree plot, the first three component was retained which explained 94.6% of the variation in the environmental data (Table 5; PI see attached pdf named: Bandekar et al Table5nFig6). The sum of all eigenvalues indicated an overall variance of 6.61%. Close to 82% variance in the dataset was accounted for by the first two principal components. The ordination plots distinctly separated the surface and DCM (<50 m) concentrations of DO and TOC from those in the OMZ (250m, 500m, and 1000m; Figure 6). In the overall, clustering together of silicate, nitrate and phosphate concentrations from OMZ depths is discernible. Ammonia and nitrite concentrations from SIM-500 and NEM-250 did not cluster with any of the other seven parameters." In Discussion Section: The available data from the AS-OMZ depths suggest seasonally invariant concentrations of DO, TOC, NO3, NH4 as well as NO2 (Jain et al. 2014). The multivariate analysis brings forth that various hydrographic parameters cluster distinctly as surface or OMZ bunch. It is thus likely that the bacterial community adapted to this steady-state niche ought to remain similar, perennially." Comment: Line 362 to 366: References or evidence for this statement is needed. Reply: Reference of Martinez et al. 2008 added (line 391 in the revised version).

Comment: Line 375: Include references Lavin et al. 2010 and Ulloa et al. 2013 Reply: These references added in line 401 in the revised version).

Comment: Line 381: Reference needed. Reply: Reference of Moore et al. 1998, Moore and Chisholm 1999, West and Scanlan 1999, Rocap et al. 2002, Scanlan et al.

2009 added (line 407 in the revised version).

Comment: Line 383: Surely a reference for N cycling in the Arabian Sea would be more appropriate here. Reply: Reference of Bange et al. 2005 added in line 410 in the revised version.

Comment: Figure 1: Use the same scale across all panels. Is it really necessary to have the same oxygen profiles in the upper and lower panels? Reply: As suggested Figure 1 is revised and same scales are used across all panels (pl see file named: Bandekar et al Fig1.pdf). Since we are comparing nitrate and nitrite versus DO, we believe that inclusion of DO in both panels helpful for a quick discernment. An explanatory sentence for this is included in the revision as "DO concentrations are shown in both panels for easy comparison between DO versus nitrate and nitrite concentrations" in lines 1038-39.

Comment: Figure 4 and 5: Where are the reference sequences? Reply: The reference sequences are all the non-bold NCBI accession numbers used in the trees. A statement now added in lines 1079-1080 and 1087-1088 as: Representative sequences from this study used for constructing the tree are in bold type. All the rest are reference sequences from the NCBI data base.

Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-147, 2016.

BGD

Interactive comment

Printer-friendly version



comment

Printer-friendly version



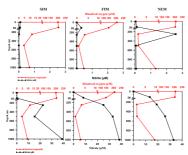


Figure 1 Vertical distribution of dissolved oxygen (DO), nitrate (NO3) and nitrite (NO2) concentrations during spring intermonsoon (SIM), fall intermonsoon (FIM), and northeast monsoon (NEM) at the Arabian Sea Time Series (ASTS) location. DO concentrations are shown in both panels for easy comparison between DO versus nitrate and nitrite concentrations.

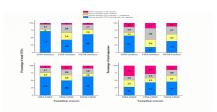


Figure 3 Percentage of OTUs and sequences in the surface and OMZ clone libraries obtained during spring intermonsoon (SIM), fall intermonsoon (FIM), and northeast monsoon (NEM) at the Arabian Sea Time Series (ASTS) location."

Printer-friendly version



Interactive comment

Table 5: Eigen values, variance percentage of correlation coefficients between environmental variables from surface layers and OMZ depths during Spring intermonsoon (SIM), Fall intermonsoon (FIM), and Northeast monsoon (NEM) at the Arabian Sea Time Series (ASTS) location.

Variable	PC1	PC2	PC3
Eigenvalues	4.51	1.21	0.898
% Variation	64.5	17.3	12.8
DO	0.441	-0.053	0.218
Nitrate	-0.45	0.107	0.088
Nitrite	-0.06	-0.674	-0.685
Phosphate	-0.467	0.003	0.013
Silicate	-0.413	-0.043	0.299
Ammonia	0.052	0.726	-0.606
TOC	0.456	-0.049	0.139

Figures in bold indicate significant values at p<0.001

Figure 6:

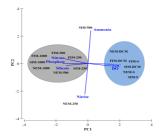


Figure 6. Principal component analysis of environmental parameters from the ASTS. Right hand side circle shows the clustering of hydrographic parameters from the surface samples and the one on left hand side, the clustering from the OMZ depths. Unclustering parameters from SIM-500 and NEM-250 are outside.

SIM - spring intermonsoon, FIM - fall intermonsoon, NEM - northeast monsoon.

S - surface, DCM - deep chlorophyll maxima

PC1 - principal component 1, PC2 - principal component 2

Printer-friendly version

