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Biogeosciences Discussions

Interactive comment on "Diversity and distribution of Nitrogen Fixation Genes in the Oxygen Minimum Zones of the World Oceans" *by* Amal Jayakumar and Bess B. Ward

Anonymous Referee #2

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In this study, the authors have reported the diversity and phylogeny of putative diazotrophs in the three major OMZs of the Ocean, including ETNP, ETSP and Arabian Sea. By analysing the clone libraries of nifH gene fragments derived from DNA and RNA samples (787 sequences in total), the authors compared the putative diazotroph communities in surface, oxycline and oxygen depleted water of the three OMZs. Basically, my major concerns are the significance of the finding and validity of the approach in this study. It should be noted that the phylogenetic diversity of putative diazotrophs in these three major OMZs have been reported detailedly in previous studies (Jayakumar et al., 2012 & 2017; Loescher et al., 2014; Cheung et al., 2016). Therefore, I afraid that the current study does not provide significant amount of new knowledge to the

field. For the approach, 787 nifH sequences are definitely insufficient to reconstruct the diazotroph communities in different layers of the three OMZs. The authors can calculate the coverage indices to evaluate whether the sequencing depths are enough. With such limited dataset, I doubt if it is meaningful and convincing to compare the diversity and community composition of putative diazotrophs in different waters. The authors stated that most of the OTUs were not shared among the regions (L 297), while it could also be the result of limited sequencing depth. Given that this study is mainly about phylogenetic diversity, the authors should consider using high-throughput next generation sequencing of nifH amplicons to provide more convincing findings.

Specific comments:

1) L30: The authors should briefly talk about the previous studies about the putative diazotrophs in OMZs, including the works done by other teams.

2) L45: Please provide detailed information about the sampling locations and depths.

3) L89-111: Details of qPCR assay were listed in the methodology, while the relevant result was not mentioned at all.

4) L123: Please specify the sequence number of each sample.

5) L137: The diverse nifH phylotypes of 4 different clusters and their affiliated strains have already been discussed in the previous studies. Is there any the new finding worth elaborating? How about the correlation between nifH phylotypes and environmental variables?

6) L281: How about the other stations? How many stations in total? It may be easier to follow if the authors show the diaoztroph community composition in each station clearly.

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