

Review

Jayakumar and Ward present an intercomparison of the diazotroph community composition of three prominent OMZs (ETNP, ETSP and Arabian Sea). The approach is used to analyse diversity is clone sequencing, which is arguably outdated nowadays and does not provide enough coverage, and the authors should acknowledge this drawback more clearly and be more cautious when interpreting their results accordingly. Nevertheless, the strength of the manuscript is the intercomparison of the three regions, which has not been addressed in previous studies. Hence, I consider that this work is of interest for the community and deserves publication, but first some minor changes are needed. The ecological role of diazotrophs in OMZs is not properly introduced, nor discussed. The results and discussion section look more like a report than a proper discussion. Finally, interpretations and conclusions need to be reformulated and acknowledge the drawbacks of the methods used. Below I provide specific comments.

Introduction

L26: "sp." should not be italicized.

L29: I suggest using sunlit waters or euphotic zone instead to account for the depth range where cyanobacterial diazotrophs are found in (sub)tropical waters.

L33: The hypothesis tested in Deutsch et al. 2007 has been extensively turned down by several publications in the past years (e.g. Bonnet et al., 2017; Knapp et al., 2016). Please account for this in your text. As it reads now, the reader perceives that this is a confirmed hypothesis still.

L43: But check (Bentzon-Tilia et al., 2015).

Materials and Methods

The methods are described in great detail. However, can the authors confirm that a DNase treatment was used and no-RT controls run?

L49: Please indicate which are the three major OMZ regions you include in your analysis. Perhaps it would fit better in the introduction, a brief description of the three regions, what they have in common, what they differ on...

L51: For no expert readers, an introduction to what ODZ means (which oxygen thresholds are considered), would be helpful.

L93-94: This is unclear. Each sequence that was 3% different from each other was considered an OTU? Can you provide a reference?

Results and Discussion

L109-111: The authors should make it clear that this is a compilation of previously published data.

L174: Groups that are both denitrifiers and N₂ fixers are rather common in low oxygen environments, including coastal sediments. A discussion on the double ecological role of these groups in OMZs would be appreciated.

L205-206: Not sure what the authors mean here, why the separation between alfa and beta was unclear?

L224: Please add references.

L251-253: This is a nice hypothesis but it is not sufficiently explained or referenced. How could this be tested? (idem for L355-357). What references do the authors have of non-cyanobacterial diazotrophs blooming upon important inputs of organic matter?

L357-359: Please add a reference at the end of this sentence.

L366: Replace N for N₂.

L367: Or rather for the nitrogen budget of OMZ zones...

L370: This is the first time that OMZ is included as part of the “dark ocean”. No explanation or comparison with non-cyanobacterial diazotrophy in the dark ocean realm are provided in the manuscript.

Tables

Table 1: A reference of the base of the photic zone at each of these stations would be useful.

References

- Bentzon-Tilia, M., Traving, S. J., Mantikci, M., Knudsen-Leerbeck, H., Hansen, J. L. S., Markager, S., & Riemann, L. (2015). Significant N₂ fixation by heterotrophs, photoheterotrophs and heterocystous cyanobacteria in two temperate estuaries. *ISME Journal*, 9(2), 273–285. <https://doi.org/10.1038/ismej.2014.119>
- Bonnet, S., Caffin, M., Berthelot, H., & Moutin, T. (2017). Hot spot of N₂ fixation in the western tropical South Pacific pleads for a spatial decoupling between N₂ fixation and denitrification. *Proceedings of the National Academy of Sciences*. <https://doi.org/10.1073/pnas.1619514114>
- Knapp, A. N., Casciotti, K. L., Berelson, W. M., Prokopenko, M. G., & Capone, D. G. (2016). Low rates of nitrogen fixation in eastern tropical South Pacific surface waters. *Proceedings of the National Academy of Sciences*, 113(16), 4398–4403. <https://doi.org/10.1073/pnas.1515641113>