

# ***Interactive comment on “Microbial communities associated with sediments and polymetallic nodules of the Peru Basin” by Massimiliano Molari et al.***

**Massimiliano Molari et al.**

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Received and published: 4 April 2020

Authors' Response ('AR') to the interactive comment on “Microbial communities associated with sediments and polymetallic nodules of the Peru Basin” by Massimiliano Molari et al.

Review by Anonymous Referee ('RC2')

RC2> General comments to authors: The manuscript by Molari et al. describes the microbial community structure associated with sediments and manganese nodules from 3 and 2 sites, respectively, within the Peru Basin.

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The authors find that Gammaproteobacteria and Alphaproteobacteria are the dominant bacterial classes in sediments and manganese nodules while all archaeal communities investigated were dominated by Thaumarchaeota. However, sediment and nodule communities were found to differ significantly at the OTU level, as assessed by calculating Jaccard dissimilarity. The authors note differences in the nodule community composition (specifically, a lower relative abundance of Archaea, and a different nitrifier community) in their study in the Peru Basin as compared with communities in the Clarion-Clipperton Fracture Zone (CCZ), where previous work on microbial community composition of nodules has been done.

The strengths of the manuscript include the following: i. There is a lack of studies of the prokaryotic diversity in the surface sediments and nodules of the Peru Basin, which has different environmental conditions than the relatively well-studied CCZ. ii. The molecular and bioinformatic methods are well-documented and the microbial community analysis is thorough.

Weaknesses of the manuscript include the following: i. The lack of metadata associated with the various sites makes interpretation of the differing community structures among sites difficult.

AR> We appreciate the suggestion of the reviewer. However, the primary aim of this study was not to investigate and explain the variability of microbial community between sites, but between habitats (nodules and sediments). Only sedimentary metadata (e.g. pigments and organic carbon content, porewater profiles, and porosity) are available for sites investigated, and not for nodules, which precludes the quantitative characterization of nodule habitat setting. Thus sedimentary setting alone does not help to understand differences in microbial community structure and diversity that are observed between sediments and nodules. However, this metadata, as well as the discussion of variability of sedimentary environmental settings and microbial communities, will be soon (scheduled publication at the 29th of April) available in “Vonnahme T.R, Molari M., Janssen F., Wenzhöfer F., Haeckel M., Titschack J., Boetius A. Effects of a deep-

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sea mining experiment on seafloor microbial communities and functions after 26 years. Science Advances, in press”.

We point the reader to this publication in the revised version of the MS.

RC2> Specific comments to the authors:

RC2> Major concerns:

RC2> 1. Page 3 – 4. Somewhere in this discussion of the CCZ versus the Peru Basin I think it would be helpful to briefly let the reader know the state of hypothetical mining in each of these regions. In the CCZ, the ISA has entered into contracts with various contractors for exploration for polymetallic nodules. Is this the case in the Peru Basin as well?

AR> We will clarify this point in the revised MS modifying the introduction as follows (added/replaced text in italics): “Nodule accumulations of economic interest have been found in four geographical locations: the Clarion-Clipperton Fracture Zone (CCZ) and the Penrhyn Basin in the central north and south Pacific Ocean, respectively; the Peru Basin in the south-east Pacific; and in the center of the north Indian Ocean (Miller et al., 2018). However to our knowledge there are no exploration activities and no plans for mining in the Peru basin so far.”

RC2> 2. Page 5, line 113. “Samples were collected at three sites. . .” For clarity I think the authors should explicitly state in the text that nodules were only collected at 2 of these 3 sites.

AR> The Reviewer is right and we will clarify this issue in the revised MS as follows (added/replaced text in italics): “Manganese nodules where sampled, using a TV-MUC, or a Remotely Operated Vehicle (ROV Kiel6000): one nodule at Reference West and four nodules at Reference South.”

RC2> 3. Page 5, line 115. “. . . called “Reference Sites.” I suggest directly listing the Reference Sites here in the text instead of making the reader consult Table 1, especially

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since the authors refer to Reference South later in the text. Could change to “. . . called “Reference Sites”: Reference East, Reference West, and Reference South.”

AR> We will modify the revised MS according to the Reviewer’s suggestion as follows (added/replaced text in italics): “Samples were collected at three sites outside the seafloor area selected in 1989 for a long-term disturbance and recolonization experiment (DISCOL; Thiel et al., 2001), for this reason they were called “References Sites”: Reference East, Reference West, and Reference South.”

RC2> 4. Page 5, line 116. Here a map of the Peru Basin (in addition to the Table already provided), with the study sites and DISCOL experiment sites marked, would be very helpful to the reader.

AR> An appropriate map is available in the Vonnahme et. al study mentioned above. We would suggest pointing the reader to that publication to avoid duplication. On request of the editor we are happy to provide a similar map for inclusion in the revised version of the MS.

RC2> 5. Page 8, lines 226 – 232. “. . . significant differences were detected in sediment microbial community structure among the different sites. . . “Site” defined by geographic location and “Substrate” . . . explained a similar proportion of variation in bacterial community structure. . .” This was a bit surprising to me and this is where I think some physical/chemical/biological metadata about each site would be really helpful. If any is available, perhaps from other groups on the cruise, it would help add context to some of the observations here.

AR> A detailed environmental characterization of sites investigated and focused discussion of baseline condition (i.e. variability of environmental settings, and community structure and diversity between “Reference sites”) will be soon available in the Vonnahme et al.” study mentioned above. Primary aims of this study were: i) to compare the microbes of nodules fields with the microbiome of other deep-sea sediments, in order to identify specific features of microbial communities of nodule fields; ii) to elucidate

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differences in diversity and in microbial community structure between sediments and nodules, and their potential implications for microbially-mediated functions. Thus, we believe that in order to achieve these aims it is neither needed nor beneficial to provide and discuss sedimentary metadata. However to meet demands of readers to better understand the effect of environmental settings on differences in microbial community structure between the sites investigated we will point out that these information can be found in “Vonnahme et al.” in the revised MS.

RC2> 6. Page 8, lines 226 – 229. “. . .significant differences were detected in sediment microbial community structure . . . between communities associated with nodules and sediments at Reference South.” I think it is important to state directly in the text that this site, Reference South, was the only site that had enough nodule sampling to allow the authors to do this analysis (at least I assume this is what occurred). Otherwise this sentence could be taken to mean that differences in community structure between nodules and sediments were also investigated at the other 2 sites, and no differences were found.

AR> We thank the Reviewer for highlighting this point. We will clarify this issue in the revised MS as follows (added/replaced text in italics): ‘Also, significant differences were detected in sediment microbial community structure among the different sites (PERMANOVA; Bacteria:  $R^2 = 0.384$ ;  $p = 0.003$ ;  $F_{2,8} = 1.87$ ; Archaea:  $R^2 = 0.480$ ;  $p = 0.013$ ;  $F_{2,8} = 2.31$ ; Table S1), and between communities associated with nodules and sediment at Reference South (PERMANOVA; Bacteria:  $R^2 = 0.341$ ;  $p = 0.023$ ;  $F_{1,6} = 2.59$ ; Archaea:  $R^2 = 0.601$ ;  $p = 0.029$ ;  $F_{1,6} = 7.53$ ; Table S1), which was the only site where the number of samples allowed for the test.’

RC2> Minor issues to be addressed:

RC2> 7. Page 8, line 238. “Aphaproteobacteria” should be “Alphaproteobacteria”.

AR> It will be corrected.

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RC2> 8. Page 9, line 282. “Aphaproteobacteria” should be “Alphaproteobacteria”.

AR> It will be corrected.

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2020-11>, 2020.

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