

## ***Interactive comment on “Responses of an abyssal meiobenthic community to short-term burial with crushed nodule particles in the South-East Pacific” by Lisa Mevenkamp et al.***

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Reviewers comment: The manuscript describes results from an experiment to assess the combined effect of burial and manganese nodule particles on abyssal meiofaunal communities. I thought the manuscript was very interesting, and written by a rising star in deep-sea ecology. The paper and data will be very useful to academics as well as policy organisations dealing with the effects of sediment and nodule particle deposition from deep-sea mining for polymetallic nodules. My main concern about the manuscript is that the substrate addition didn't appear to have a huge impact on benthic community structure in the experiments. While these are the results that have been collected and

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need to be reported, my feeling is that a lot of the fauna in the substrate addition treatment were actually dead but hadn't decomposed at the end of the experiment. Then, when the fauna were preserved in formalin after 11 days everything that was alive and dead at the end of the experiment was preserved such that no change in community composition could be detected. I understand that this is difficult to assess using staining methods (as stated in the discussion by the author), but it would have been possible to assess the condition of some of the meiofauna at the end of the experiment (e.g., by looking at the appearance of the striated-muscles of the harpacticoids from the burial treatment, and comparing with the control samples). Similar approaches have been undertaken in the past (see Thistle et al. 2005, Mar. Ecol. Prog. Ser. 289: 1-4) to estimate the proportion of meiofaunal harpacticoids killed in situ by CO<sub>2</sub> perturbations. I would suggest that the lack of information about meiofaunal death is clearly flagged as a possible reason why differences in benthic community composition could not be detected. Although the authors went some way to discuss meiofaunal death in their discussion, this point really needs to be stressed. This is because, at present, mining contractors may use this paper to state that manganese nodule particle/ sediment deposition does not alter benthic community composition, and I am not convinced this will be the case. I recommend that the article be published eventually following some moderate revisions. Minor points to consider:

Authors reply: We thank the reviewer for this comment and for the suggestions made. We are aware of the studies using body conditions (muscles and internal organs) of harpacticoids and nematodes. However, own experiments have shown that body condition of freshly killed nematodes and those that were dead since the start of the experiment were comparable until 16 days into the experiment. This (unpublished) experiment was done on an intertidal sediment community. We therefore fear that this method of assessment may be unreliable for short-term experiments (less than 2 weeks). The issue of possible mortality is discussed on several occasions but due to the lack of data on this from our experiment, we think that a more extensive discussion of this topic is too hypothetical and that the absence of mortality could be equally true.

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## Abstract

Reviewers comment: 1) Line 11: change to “may rive the extraction of deep-sea mineral...”

Authors reply: We replaced “may drive the prospection and exploration” with “may drive the prospection and exploitation”.

Reviewers comment: 2) Line 13: Change to “Experimental studies are scarce and simulated effect studies are small scale relative to the effects that will be seen during deep-sea mining...”

Authors reply: As our conducted experiment is extremely small-scale, this sentence would not particularly highlight/relate to this study. We would like to keep the original sentence.

Reviewers comment: 3) Line 16: Insert “in 2015” after conducted.

Authors reply: adjusted as suggested

Reviewers comment: 4) Line 22: Remove “original”

Authors reply: adjusted as suggested

## Introduction:

Reviewers comment: 1) Page 2, Line 10: It would be good to provide the range of typical manganese nodule growth rates here, because <250mm myr<sup>-1</sup> can mean 0.0000000001mm myr<sup>-1</sup> to 250mm myr<sup>-1</sup>.

Authors reply: The sentence was adjusted to read “with very slow formation and growth rates of 5 to 250 mm My<sup>-1</sup> (million years) in the Peru Basin (Von Stackelberg, 2000).” The citation of Jain et al, 1999 was removed as it refers to nodules from the Central Indian Ocean and does not provide clear estimates of nodule growth.

Reviewers comment: 2) Page 2, Line 15: What about organic matter dilution as well

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Authors reply: We added organic matter dilution and redistribution. The sentence now reads: “... removal of surface sediment, sediment compaction, sediment suspension and deposition, organic matter dilution and redistribution, discharge of waste material. . .”

Reviewers comment: 3) Page 2, Line 18: change “of” to “from”

Authors reply: adjusted as suggested

Reviewers comment: 4) Page 2, Line 22: It would be good to give the reader some idea about the natural sedimentation rates in the abyss, and some indication of the levels of sedimentation that will occur during deep sea mining.

Authors reply: A sentence was added on Page 2, Line 20 that states “Sedimentation rates in nodule areas are slow and range between 0.2-1.15 cm kyr<sup>-1</sup> (Volz et al., 2018) while sediment resuspension resulting from nodule mining may result in sediment resuspension of 0.6 m<sup>3</sup> s<sup>-1</sup> (Oebius et al., 2001), therefore, greatly exceeding natural sedimentation rates.” The citation “Volz, J.B., Mogollón, J.M., Geibert, W., Martinez-Arbizu, P., Koschinsky, A., Kasten, S., 2018. Natural spatial variability of depositional conditions, biogeochemical processes and element fluxes in sediments of the eastern Clarion-Clipperton Zone, Pacific Ocean. Deep Sea Research Part I: Oceanographic Research Papers 140, 159–172. <https://doi.org/10.1016/j.dsr.2018.08.006>” was added to the reference list.

Reviewers comment: 5) Page 3, line 2: Change to “which causes at worse, meiofaunal death, but at least removal...”

Authors reply: adjusted as suggested

Reviewers comment: 6) Page 3, line 24-26: I am confused as to why the amount of metal in the animal tissues is a robust way to assess toxic effects. You could have an animal with a high level of metals in its tissues, but the animal is highly resilient to metal toxicity. Therefore, the amount of metal in its tissue does not really always show the

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degree of toxicity from that particular metal.

Authors reply: We thank the reviewer for this comment and agree. The sentence was rephrased to read: "Therefore, direct measurements of metals in animal tissues may be used to inform about changes in metal uptake induced by polymetallic nodule mining and may indicate physiological responses to increased metal burdens."

Methods:

Reviewers comment: Overall methods. Did you assess the volume of the sediment taken up by solid nodule particles in your 10cm<sup>2</sup> sample from the controls and burial treatments. If some sediments have more solid nodule particles, then there is less sediment to inhabit and this may have an effect on the densities that you found.

Authors reply: The average thickness of the crushed nodule layer in all samples ranged between 1.5 and 2.5 cm. The nodule particle mixture was the same in all 3 cores of the Burial treatment and no additional sediment was added. Therefore, we do not believe this to have influenced meiofauna densities.

Reviewers comment: 1) Page 4, line 8: You need to mention how you sampled the nodule and crushed the nodule to make the substrate. This information is missing.

Authors reply: Page 4 Line 15: We added the missing information. "To obtain the crushed nodule particles, several nodules from the experimental site were collected 2 days prior to the experiment. Upon retrieval, epifauna, if present, was removed from the nodules and nodules were thoroughly washed with fresh water to remove all sediment and fauna. Subsequently, nodules were put inside plastic bags and manually crushed with a hammer. The resulting nodule particles varied in size between 3  $\mu$ m and 1 cm (Supplementary Figure S2). "

Reviewers comment: 2) Page 5, line 8: Change to "The second push core was used to..."

Authors reply: Sentence changed to "The second push core was used to analyse sed-

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iment characteristics ..."

Reviewers comment: 3) Page 5, line 9: Did you try and get an idea of the organic matter quality of the sediment and the added substrate? Given that a lot of meiofauna directly consume labile microbial organic matter (see Bernhard & Bowser. 1992. Mar. Ecol. Prog. Ser. 83: 263-272, Ingels et al. 2010. Mar. Ecol. Prog. Ser. 406: 121–133.), the quality of the substrate, as well as the effects from burial and the content of the manganese substrate may have all had an influence on the meiofaunal response. If you do not have actual Chl-a, or lipid concentrations, you can at least get an idea from the C: N ratio.

Authors reply: Indeed, we do not have information on chl a and lipid concentration. We added a sentence on C/N ratios, that were rather similar between the crushed nodule particles and sediment of the control. A sentence was added in the results section Page 7 Line 23: "Despite the lower carbon and nitrogen content in the nodule particles, C/N ratio remained similar between the nodule particles ( $1.926 \pm 0.037$ ) and the Control sediment ( $1.951 \pm 0.177$ )."

Reviewers comment: 4) Page 6, line 18: Please define "live time". It sounds cool, but I have no clue what this is.

Authors reply: Live time is the real time corrected for the "dead time" when the detector is processing the data and not measuring any signal. To avoid confusion we have removed the term "live time" as it is not essential for the understanding of this sentence.

Reviewers comment: 5) Page 7, line 1: What Simpson metric are you referring to? The term 'Simpson's' can actually refer to any one of 3 closely related indices (Simpson's Index, Simpson's Index of Diversity or Simpson's Reciprocal Index).

Authors reply: In our case the Simpson's Index of Diversity was used  $1-D=1-(\sum_i N_i(N_i-1))/(N(N-1))$ .

We changed "Shannon-Wiener, Pielou's evenness and Simpson)" to "Shannon-Wiener

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index using the natural logarithm (H'), Pielou's evenness (J') and Simpson's index of diversity (1-D)".

Reviewers comment: 6) Page 7, line 1: What univariate analyses were used? Authors reply: Univariate measures were tested as described later on Page 7 Line 10-13. Here, we added "diversity indices" in "Differences of univariate measures (bulk sediment metal contents, total meiobenthos densities and diversity indices) between treatments were tested with a student's t-test"

Results:

Reviewers comment: 1) Page 10, line 13: I think that the biodiversity metrics being the same in both the burial and control treatments may be due to you not being able to differentiate between live and dead fauna. This could have been assessed in the harpacticoids by looking at the condition of the fauna, since dead fauna would appear more degraded even if they've been at abyssal temperatures for a few days. As I stated before, it is important that the manuscript is carefully worded to reflect this as this result could be used as evidence for no impact from re-sedimentation of sediment and nodule particles during mining, and I doubt this will be the case given the low background sedimentation rates in the abyss.

Authors reply: Please see our reply to comment #1

Reviewers comment: 2) Regarding my first point in the methods section above, it would probably have been a good idea to standardise your meiofauna abundances to per unit volume of sediment rather than area. If the nodule substrate layer was full of cm-sized particles then the amount of living space available to the nematodes would be significantly less than in the control samples. Standardising the abundances to unit volume (if you have the data) may show much larger differences, and you may detect differences in community structure, or abundance (at least) between treatments.

Authors reply: Unfortunately, we do not have measurements on the ratios between

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small-sized and large-sized nodule particles. The added substrate was a mixture of very fine to very coarse material and a comparison of "living space" for the meiofauna would be difficult.

Discussion:

Reviewers comment: 1) Page 13, line 19: Given the coarse nature of the nodule particles, wouldn't O<sub>2</sub> penetrate through the manganese substrate layer relatively easily. I understand there is burial, but diffusion will be dependent on the porosity, which should be greater in the substrate layer.

Authors reply: We thank the reviewer for this remark. It is very unfortunate that we were not able to measure oxygen penetration. Since the nodule particle mixture contained coarse and fine material, with different settling velocities, the very fine material likely settled on top of the coarse grains, which could have acted as a "seal". Furthermore, an oxygen consumption of the nodule particles themselves may have reduced oxygen concentrations. However, these are merely hypotheses and could not be verified due to the lack of data.

Reviewers comment: 2) More overall impression of the discussion is that the authors need to acknowledge the weaknesses of the study (e.g., being unable to document meiofauna death) to a much better degree. This is done somewhat, but it really needs to be emphasized that a lot of the responses seen (or lack of them, e.g., in the biodiversity data) may be caused by the inability to distinguish living from dead fauna in the different treatments.

Authors reply: Again, we would like to refer to our reply to comment #1 and add that while we share the fear of unnoticed mortality, it would not be correct to emphasize this too much as the opposite "lack of mortality" could be equally true. Nevertheless, we added a sentence on Page 15 Line3 stating "Therefore, potential unnoticed mortality in our study may have masked more severe changes in terms of meiofauna densities and diversity."

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