Dear Prof. Dr. Treude,

Thank you for your kind consideration of our manuscript for publication in Biogeosciences. We thank the three reviewers for their time and for the constructive comments on our manuscript. Hereby, we submit a revised version that complies with the comments raised by the reviewers. In this rebuttal letter, all of the reviewers’ comments and suggestions are addressed with clear indications of how they were implemented in the revised manuscript.

We feel that the revision has substantially improved the manuscript and would like to thank you very much in advance for re-considering our manuscript for publication in Biogeosciences.

We are looking forward to your opinion on the revised manuscript.

Sincerely,

On behalf of all co-authors,

Lisa Mevenkamp
Rebuttal letter

Reply to reviewer #1

General remarks:

Reviewers comment: 1. Describe in ‘Introduction’ as to what is the likely source of the crushed nodules during a mining operation and what is the expected concentration and size of the nodule particles that are will be introduced on the seafloor based on which this experiment is planned. Also mention what is the size and concentration of the crushed nodules used in the experiment.

Authors reply: The most likely source of the spreading of nodule particles will be the collecting device. Nodule particles may be abraded and brought into suspension by the water jet used during the collection or, depending on the design of the collector, during separation of nodules and sediment inside the collector. This will result in the distribution of nodule particles, which are smaller than the mesh used for the separation. These particles would then spread as part of the sediment plume and would settle depending on their sedimentation rate. Particle sizes used during this experiment are shown in Supplementary figure S2 (former S3) and the material and method section has been expanded (see specific comment #7)

Changes to the manuscript: Page 2, Line 13: We expanded the sentence to “Therefore, breakage and abrasion of nodule particles is likely to occur during a mining operation with heavy gear, for example during separation of nodules and sediment as part of the collection process or by the force of the water jet used for the collection of nodules.”

Reviewers comment: 2. The study makes certain comparisons with the results of previous benthic impact experiments (BIEs) that were not at all similar either in spatial terms or time scales or volume and nature of re-sedimentation. This study is based on effects of concentrated crushed nodules on meiobenthos over eleven days in a restricted area, whereas the BIEs were studies of impacts of distribution of resuspended sediments (and not crushed nodules) over large areas and longer periods of time (1 year or more). So, it is not correct to compare the results of these two.

Authors reply: We agree with the reviewer in the sense that a direct comparison I indeed not very relevant to compare our results with the JET experiment and we therefore would opt to remove that paragraph from the discussion. Nevertheless, migratory responses by meiofauna have been observed in several studies regardless of the nature of the substrate used. Therefore, we would argue that, to a certain extent, it is valid to use studies using different material to interpret our findings while considering also the differences in approach.

Changes to the manuscript: The comparison with the JET experiment on Page 14, Line 17-28 was removed.

Reviewers comment: 3. It is interesting to see that many of the results of this experiment have shown positive response of meiofauna as well as other groups (upward migration into the re-sedimented layer, no additional accumulation of copper, and no extreme changes in community structure) that should be highlighted. When the understanding of likely impacts of deep-sea mining is limited and mostly negative impacts are being projected by the environmental groups based on little or no data, it is important to bring out positive impacts as well so as to have a balanced approach towards sustainable mining. Also researchers need to appreciate that it is not necessary that all responses to any manmade activity will always be negative, but could be positive as well as shown in this study and this is an important contribution from the marine biologists to this subject.
Authors reply: We understand the concern raised by the reviewer, however, we do not believe that the changes in vertical distribution should be described as positive. Indeed some responses, such as copper accumulation were absent/neutral but the upward migration of the meiofauna from upper layers and changes in feeding type composition should be interpreted with care as long-term effects are unknown and may not necessarily be positive. It is not our intention to highlight negative effects of deep-sea mining but to interpret our findings based on the available information. We rephrased some sentences in the manuscript that may be interpreted in a stronger way than intended.

Changes to the manuscript: Page 1 Line 28-29 “The results indicate that short-term substrate burial requires special attention with regard to ecological consequences of mineral extraction in the deep-sea.” Was changed to “Our results indicate that short-term impacts from burial with crushed nodule particles on meiofaunal communities are limited but that long-term studies are needed, especially with regard to vertical structure, community composition and mortality.”

Page 11, Line 30-31 the sentence “We found that this behavioural response was stronger in polychaetes, copepods and their nauplii compared to nematodes which could result in a shift in meiobenthos community composition.” was removed.

Specific comments

Reviewers comment: 1. Page 1, line 23 - Abstract - change ‘...in covered and undisturbed sediments.’ To ‘...in covered and uncovered sediments.’ (because there is no other disturbance on the seafloor but covering of sediments by crushed nodules).

Authors reply: adjusted as suggested

Reviewers comment: 2. Page 2, line 6 – Introduction – change ‘extraction in’ to ‘exploitation from’ – as ‘extraction’ means ‘removal of metals from ore’, whereas ‘exploitation’ means ‘removal of ore from its original source”.

Authors reply: adjusted as suggested

Reviewers comment: 3. Page 2, line 14 – change ‘such as’ to ‘due to’- as these are causes not impacts.

Authors reply: adjusted as suggested

Reviewers comment: 4. Page 4, line 8 – Correct ‘Fig S1” to ‘Fig 1’,

Authors reply: throughout the manuscript, the label of supplementary figures was adjusted to “Supplementary Fig. S…” to avoid confusion with the figures inside the manuscript.

Reviewers comment: 5. Page 4, line 8 – change ‘substrate distribution device’ to ‘crushed nodule distribution device’ – see below for explanation.

Authors reply: see next reply

Reviewers comment: 6. Page 4, line 8 – According to Cambridge dictionary, the word ‘substrate’ means something lying below or base or bed and cannot be used for crushed nodules being deposited artificially from top. So change ‘crushed nodule substrate’ to ‘crushed nodule particles’ and ‘substrate’ to ‘nodule particles’ in the entire manuscript.

Authors reply: We would like to keep the phrasing “substrate” ins the manuscript. The added material is intended to be used as a new substrate by the fauna after deposition, similar to sediment, and relates to the Cambridge dictionary definition “a substance or surface that an organism grows and lives on and is supported by”. This wording is particularly useful when
referring to different material without the need to specify each material separately (e.g. nodule particles, inert tailings, sediment).

**Reviewers comment:** 7. Page 4, line 10-11 – Add mean size of crushed nodules ‘...substrate of ### micron / mm size that was filled inside the tubes of the device.’

**Authors reply:** Mean sizes of the nodule substrate were not measured and would not be very informative due to the large range of the particle sizes. The Material and Method section was expanded with information about acquisition of the nodule particles including size range: Page 4 Line 15 “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 μm and 1 cm (Supplementary Figure S2).”

**Reviewers comment:** 8. Fig. 1 caption – change ‘Impressions’ to ‘Images’ or ‘Photographs’

**Authors reply:** “Impressions” changed to “Images”

**Reviewers comment:** 9. Page 7, line 14 – Please mention the ‘values for sediment characteristics, metal values, and meiofauna composition’ before the experiment and compare the values after the experiment to evaluate the impact of burial of seafloor sediment by crushed nodule particles.

**Authors reply:** We did not conduct a sampling of the sediment before the experiment. Results are based on a Control-Treatment comparison. However, also in the Control, stainless steel rings were used to achieve the same conditions (e.g. limiting lateral movement, water flow) in both treatments.

**Reviewers comment:** 10. Page 7, line 25 - change ‘burial treatment’ to ‘burial treatment sediment sample’.

**Authors reply:** adjusted as suggested

**Reviewers comment:** 11. Page 9, line 3 – add units ‘cm’ after ‘0-1’ and ‘1-2’.

**Authors reply:** units were added

**Reviewers comment:** 12. Page 10, line 7 – change ‘Table S1’ to ‘Table 1’

**Authors reply:** “Table S1” was changed to “Supplementary Table S1”

**Reviewers comment:** 13. Page 10, line 10 – change ‘control’ to ‘control samples’ and ‘burial treatment’ to ‘burial treatment samples’.

**Authors reply:** In our view, this change does not significantly add to the understanding of the sentence and this distinction would need to be applied also to all other sentences.

**Reviewers comment:** 14. Page 10, line 15 – change ‘Figure S4’ to ‘Figure 4’.

**Authors reply:** “Figure S4” was changed to “Supplementary Figure S4”

**Reviewers comment:** 15. Page 13, line 14-15 – After ‘Changes in oxygen could be one of the factor’ it would help to give either layer-wise oxygen values before and after the experiment or at least give general values for oxygen content in sediment layers from other publications (eg. Rzeznik-Orignac et al, 2004) to support the hypothesis.

**Authors reply:** Unfortunately, as mentioned on Page 13 Line 18 we were not able to measure oxygen concentrations in our sample. We hypothesized oxygen availability as explanation for the
observed behavior as it has been proposed to play a role in meiofauna extraction techniques. Furthermore, oxygen penetration depth was reduced in the experiments of Mevenkamp et al 2017 leading the authors to hypothesize this oxygen reduction as an explanation for the upward migration of nematodes and increased mortality.

**Changes to the manuscript:** “by using natural gradients of oxygen availability” was inserted at Page 13, Line 17

Page 13 Line 17 “Moreover, in a short-term laboratory experiment, Mevenkamp et al. (2017) observed significantly reduced oxygen concentration in the underlying soft sediment after the addition of 0.5 and 3 cm sediment and an upward migration and increased mortality of nematodes.” was added

**Reviewers comment:** 16. Page 15, line 9-10 – ‘Interestingly, all dominant nematode genera responded with upward migration...’ is a positive response further supported by section heading 4.3 ‘Increased copper concentrations in added substrate are not reflected in nematode body copper content’- these need to be highlighted as mentioned in #3 of general comments.

**Authors reply:** See reply on general comment #3.

**Reviewers comment:** 17. Page 15, line 18-19 – ‘The results of our experiment did not indicate that these nematodes were more successful to inhabit added substrate’ contradicts the above statements, unless it refers specifically to monhysterids. So please specify or remove this sentence.

**Authors reply:** Indeed, this sentence refers to the monhysterids. “these nematodes” replace with “monhysterids”

**Reviewers comment:** 18. Page 15, line 30-34 – Effects of resedimentation of sediment over large open area and that of crushed nodules over small enclosed area cannot be compared as the material deposited and the process and concentrations are entirely different. The discussion needs to be modified.

**Authors reply:** We changed the last sentence to acknowledge the difference in the disturbance between both studies and to not mislead the reader. Nevertheless, we believe that the persisting change in nematode communities observed by the cited study should be mentioned here to underline the caution needed in the interpretation of disturbances in the deep-sea as even small changes may be long-lasting and a no-impact-conclusion should not be drawn too fast.

**Changes to the manuscript:** Page 15 Line 33 sentence adjusted to “Although the disturbance studied by Miljutin et al. (2011) strongly differs from our experiment, it indicates that changes in nematode community composition in polymetallic nodules areas may be long lasting and are potentially irreversible and, therefore, underlining the importance of long-term experiments.”

**Reviewers comment:** 19. As the experiment of depositing crushed nodules has shown positive response of nematodes by upward migration and maintaining similar community structure, the sentence on page 16 (line 1-2) ‘...Changes in nematode composition... may be long lasting and positively irreversible...’ need to be revised.

**Authors reply:** Community structure on a higher taxonomic level was indeed similar in both treatments, but at lower taxonomic level, changes in nematode feeding types were observed. Therefore, we do believe that this sentence is still valid in order to alert the reader on the potential long-term risks. Furthermore, samples exhibited a very high diversity and high evenness with many rare (<5%) taxa, also evidenced by the low similarity among replicates, which may increase the risk of losing rare taxa after strong sediment disturbances.
Nevertheless, in response to the comment #18, the sentence was rephrased (see above).

**Reviewers comment:** 20. Page 16 – Conclusions – needs to be revised according to the above discussion

**Authors reply:** This comment relates to our reply to general comment #3. We have done small adjustments to the text; however, also taking into account the remarks of reviewer 2, we would like to keep the overall conclusion as it is.
Reply to reviewer #2

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 though 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 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 CO2 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.

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 yr⁻¹ can mean 0.00000000001mm yr⁻¹ to 250mm yr⁻¹.

Authors reply: The sentence was adjusted to read “with very slow formation and growth rates of 5 to 250mm My⁻¹ (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

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⁻¹ (Volz et al., 2018) while sediment resuspension resulting from nodule mining may result in sediment resuspension of 0.6 m³ s⁻¹ (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 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 10cm² 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 μm 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 sediment 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 Chla, or lipid concentrations, you can at least get an idea from the C:N ratio.

**Authors reply:** Indeed, we do not have information on chla 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 ± 0.037) and the Control sediment (1.951 ± 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 − \( \frac{\sum Ni(Ni−1)}{N(N−1)} \). We changed “Shannon-Wiener, Pielou’s evenness and Simpson)” to “Shannon-Wiener 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 meiofaunus 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 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 O2 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.”
Reply to reviewer #3

General comments:

**Reviewers comment:** This study explores a really challenging question, that of how deep sea meiofauna respond to mining operations. It is an increasingly vital question as we learn more about the diversity and importance of deep sea meiofauna and as deep sea mining operations expand. I applaud the authors efforts to tackle this problem and I think this study should be published but with some clarification and moderate revisions. My biggest issues with the article center around their methodology and interpretation of depositing nodule sediment onto existing sediment. First, there is no indication that I can see of where this nodule sediment came from? How far from the “regular” sediment on which it was deposited was it collected? Are these nearby habitats or hundreds of miles apart? Also, why did the authors choose to deposit nodule sediment alone when in their description of nodule mining practices it seems that there is removal of nodule sediment, disturbance of underlying or neighboring sediment, and deposition of nodule particles mixed with suspended sediment and redeposited. It seems the mining operations are after the nodule sediment in particular, so why would they ever redeposit it onto non-nodule sediment? Unless by accident? Please clarify where the nodule sediment came from and why its direct deposition onto non-nodule sediment was chosen as the primary methodology as this doesn’t seem to mimic any aspect of the mining operations under question. Also, the authors mention (with citations) in the discussion that meiofauna does inhabit the nodule sediment, yet there seems to be no taking this into account when interpreting the behavior of the meiofauna upon burial. Was the nodule sediment sterilized? Was it presumed that the meiofauna washed out upon transport? It seems like the primary interpretation of the presence of meiofauna in the nodule sediment at the end of the study is that it was colonized from the buried sediment below due to upward movement, but couldn’t there have been a meiofaunal community in the nodule sediment upon deposition? If you didn’t remove the meiofauna or examine it beforehand, how do you know that meiofauna found in it afterward came from the buried sediment?

**Authors reply:** For the first remark (origin of the nodule particles), we have added a short paragraph in the Material and Method part on Page 4 Line 15: “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 μm and 1 cm (Supplementary Figure S2). “ Thus, the nodule particles originated from the same area and were treated on board prior to the use in the experiments. Because of the treatment on board (removal of sediment, keeping the particles in plastic bags without the addition of seawater) we are very sure to not have added any meiofauna to the sediment of our experiment. And if meiofauna was present inside the nodule crevices, we would have been able to distinguish them as their shape would appear damaged or dried out from the treatment prior to the experiment.

The choice to use crushed nodule particles was partly determined by practical limitations of the experimental design but also to be able to clearly distinguish impacts from the nodule particles with their specific properties (different grain size and porosity, metal content) from the effects of sediment deposition. Especially with regard to metal uptake it was important to limit the study to one substrate instead of a mixture. But indeed, we agree with the reviewer that in a mining scenario, mixtures of sediment and nodule particles will be much more likely. To elucidate the potential source of nodule particles during mining operations a sentence was added in the
Introduction on Page 2, Line 13: “Therefore, breakage and abrasion of nodule particles is likely to occur during a mining operation with heavy gear, for example during separation of nodules and sediment as part of the collection process or by the force of the water jet used for the collection of nodules.”

Specific comments:

Reviewers comment: Page 2, line 1: “70s” should be “1970’s”

Authors reply: adjusted as suggested

Reviewers comment: Page 2, line 17: Here is where you describe mining operations and what happens to nodule sediment and “regular” sediment. You even mention how a large scale mining operation “is expected to directly impact the nodule associated fauna” so then it seems confusing that you then proceed to assume there is no fauna there until you place it on other sediment in your study.

Authors reply: I believe this question relates to our answer to the first general comment. The added nodule substrate did not contain any undamaged meiofauna anymore. Furthermore, from own (unpublished) data we know that the densities of meiofaunal organisms inhabiting crevices of the nodules from the North East Pacific are very low (ranging from 2 to 31 individuals) and, therefore, constitute only a small fraction (5 ± 5 %) of the densities of meiofauna inside the surrounding sediment.

Reviewers comment: Page 4, line 8: Please specify here where the crushed nodule substrate came from and how/if it was treated.

Authors reply: See reply to first general comment.

Reviewers comment: Page 7, line 1: Citations would be helpful for all of these diversity indices and to specify which Simpson index.

Authors reply: In our case the Simpson’s Index of Diversity was used \[ 1 - D = 1 - \frac{\sum_{i} N_i (N_i - 1)}{N(N-1)} \]. We changed “Shannon-Wiener, Pielou’s evenness and Simpson)” to “Shannon-Wiener index using the natural logarithm (H’), Pielou’s evenness (J’) and Simpson’s index of diversity (1-D))”.

Reviewers comment: Page 13, line 10: Please clarify why you think that all the meiofauna in the nodule sediment came from the lower layer (“adjusting their vertical position in the sediment”).

Authors reply: The added substrate did not contain any meiofauna and most meiofaunal organisms, particularly nematodes, do not actively emerge from the sediment. Therefore, it is most likely that colonization of the new substrate was done from the underlying sediment rather than from the water column. This is also in line with the lower densities seen in the 0-2 cm layer of the underlying sediment suggesting that those organisms migrated into the added substrate.

Reviewers comment: Page 14, line 25: Here you mention a study that showed a decrease in nematode densities “attributed to limited upward migration directly after the disturb (as was seen in our experiment)...” but previously you had indicated that there was considerable vertical migration from the lower sediment. Please clarify this.

Authors reply: This part of the discussion was removed as a response to reviewer #1 who suggested that the two studies should not be directly compared due to their very different experimental approach.

Reviewers comment: Page 15, line 27: Here you indicate that your study found that the addition of crushed nodule substrate “changed the relative abundance of feeding types in the new surface
layer...” yet you don’t seem to have examined the nematodes in the surface layer (nodules) before depositing it, so how can you know this?

**Authors reply:** Also this comment relates to our reply to the first, general comment. We do believe that the added substrate was void of meiofauna or that meiofauna would at least be very damaged due to the treatment of the nodules prior to the experiment.
Responses of an abyssal meiobenthic community to short-term burial with crushed nodule particles in the South-East Pacific

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Abstract. Increasing industrial metal demands due to rapid technological developments may drive the prospection and exploration-exploitation of deep-sea mineral resources such as polymetallic nodules. To date, the potential environmental consequences of mining operations in the remote deep sea are poorly known. Experimental studies are scarce, especially with regard to the effect of sediment and nodule debris depositions as a consequence of seabed mining. To elucidate the potential effects of the deposition of crushed polymetallic nodule particles on abyssal meiobenthos communities, a short (11 days) in situ experiment at the Peru Basin in the South East Pacific Ocean was conducted in 2015. We covered abyssal, soft sediment with approx. 2 cm of crushed nodule particles and sampled the sediment after eleven days of incubation at 4200 m water depth. Short-term ecological effects on the meiobenthos community were studied including changes in their composition and vertical distribution in the sediment as well as nematode genus composition. Additionally, copper burden in a few similar-sized, but randomly selected nematodes was measured by means of µ-X-ray fluorescence. At the end of the experiment, 46 ± 1 % of the total meiobenthos occurred in the added crushed nodule layer while abundances decreased in the underlying 2 cm compared to the same depth-interval in original, undisturbed sediments. Densities and community composition in the deeper 2-5 cm layers remained similar in covered and undisturbed-uncovered sediments. The migratory response into the added substrate was particularly seen in polychaetes (73 ± 14 %, relative abundance across all depth layers) copepods (71 ± 6 %), nauplii (61 ± 9 %) and nematodes (43 ± 1 %). While the dominant nematode genera in the added substrate did not differ from those in underlying layers or the undisturbed sediments, feeding type proportions in this layer were altered with a 9 % decrease of non-selective deposit feeders and an 8 % increase in epistrate feeders. Nematode tissue copper burden did not show elevated copper toxicity resulting from burial with crushed nodule particles. The results Our results indicate that short-term impacts from burial with crushed nodule particles on meiobenthic communities are limited short term substrate burial but that long-term studies are required special attention needed, especially with regard to vertical structure, community composition and mortality, ecological consequences of mineral extraction in the deep sea.

1 Introduction

The interest in mineral deposits from the deep seafloor commenced in the early 1970's, after the discovery of a widespread occurrence of economically valuable polymetallic nodules (Glasby, 2000; Mero, 1977). However, economic and technological limitations of using deep-sea resources at that time hampered further industrial activities. The advancements in deep-sea technology and other
socio-economic developments have led to a new surge for deep-sea minerals in the past decade and legal frameworks are being developed to manage their extraction-exploitation in international waters (Lodge et al., 2014). Polymetallic nodules are decimetres-size concretions of ore lying on the surface of abyssal sediments in 4000 – 6500 m water depth and cover large areas of the Pacific and Indian Ocean (Hein and Koschinsky, 2014). Besides the high content in valuable metals such as copper, nickel and cobalt, nodules exhibit a high porosity, low bulk density and fine grain size with very slow formation and growth rates of less than 5 to 250 mm Myr\(^{-1}\) (million years) in the Peru Basin (Jain et al., 1999; Von Stackelberg, 2000). These properties result in very brittle structures that are easily damaged or broken when applying low force (Charewicz et al., 2001; Jain et al., 1999; Thiel et al., 1993; Zenhorst, 2016). Therefore, breakage and abrasion of nodule particles is likely to occur during a mining operation with heavy gear, for example during separation of nodules and sediment as part of the collection process by the force of the water jet used for the collection of nodules.

Polymetallic nodule mining is hence expected to have various direct and indirect environmental impacts due to such as nodule removal, removal of surface sediment, sediment compaction, sediment suspension and deposition, organic matter dilution and redistribution, discharge of waste material and potential release of toxic amounts of heavy metals (Clark and Smith, 2013; Rolinski et al., 2001; Sharma et al., 2001; Thiel, 2001). Additionally, nodule particles abraded during collection may get mixed with the suspended sediment and redeposited in areas close to or further away from the mined site, depending on their sedimentation rate. An economically viable mining operation would cover an area of 300-800 km\(^2\) per year (Smith et al., 2008) and after 20 years an estimated 8500 km\(^2\) would have been mined per concession area (Madureira et al., 2016). Sedimentation rates in nodule areas are slow and range between 0.2-1.15 cm kyr\(^{-1}\) (Volz et al., 2018) while sediment resuspension resulting from nodule mining may result in sediment resuspension of 0.6 m\(^{3}\) s\(^{-1}\) (Oebius et al., 2001), therefore, greatly exceeding natural sedimentation rates. Such a large-scale mining operation is expected to directly impact the nodule associated fauna (Purser et al., 2016; Vanreusel et al., 2016). However, deposition of sediment and nodule particles on the seafloor resulting from mining activities may also impact the typical abyssal soft-sediment fauna, but knowledge about the direct responses of those organisms to substrate deposition is scarce.

In the abyssal deep sea, the meiothons (32 - 1000 \(\mu\)m) constitute the most dominant metazoan component of infaunal communities in terms of biomass (Rex et al., 2006). Due to their high abundance, meiofauna play an important role for the energy flow inside abyssal sediments but also for the functioning of the infaunal ecosystem through e.g. bioturbation, degradation of organic matter or species interactions (Schratzberger and Ingels, 2017). Moreover, meiofauna contribute greatly to the high biodiversity of abyssal ecosystems with nematodes in particular being the most diverse metazoan taxon in some deep-sea habitats (Sinniger et al., 2016). Typically, meiofaunal generation times vary in the range of weeks to months, depending on the species (Coull, 1999; Gerlach, 1971). However, this has only been assessed for shallow water species so far and generation times may be longer in the deep sea where many taxa are characterized by a high longevity (Cailliet et al., 2001; Giere, 2009).

Due to their residence inside the sediment, nodule mining will inevitably disturb meiobenthic communities, directly or indirectly. Directly through the removal of the sediment surface layers, which causes at worst meiofaunal death, but at least removal and redistribution of meiobenthic organisms, and indirectly through sediment deposition, which may have consequences for the survival and vertical structuring of underlying meiobenthic communities. Previous research on the direct effect of nodule mining suggests that abyssal benthic communities have the capacity to recover from small scale sediment disturbances (Gollner et al., 2017), although effects of stress by pollution, oxygen deficiency, forced migration etc. on overall fitness has not been
investigated. In that respect, full recovery of a disturbed community may be a long lasting process which may still be incomplete several decades after the disturbance (Gollner et al., 2017). Previous findings are based on small-scale disturbance scenarios where nodules were removed or ploughed (overview given in Jones et al., 2017). In general, recovery of mobile fauna occurred faster than that of sessile fauna and small organisms tend to recover faster than large organisms (Gollner et al., 2017; Jones et al., 2017). These deep-sea experiments clearly indicated that substrate deposition led to changes in meiofauna community composition and vertical distribution (Kaneko et al., 1997; Miljutin et al., 2011) which has also been observed in experiments on meiobenthic communities from shallower depths (Maurer et al., 1986; Mevenkamp et al., 2017; Schratzberger et al., 2000). However, it remains unclear which thickness of sediment deposition evoked the meiofaunal response in the deep sea and if it is possible to reproduce the results under more controlled conditions on a short term.

Another possible risk of polymetallic nodule mining is the release of potentially toxic amounts of heavy metals during sediment resuspension and nodule abrasion with largely unknown effects on deep-sea biota (Hauton et al., 2017). Bioavailability and toxicity of metals inside marine sediments strongly depend on the structure and chemical properties of the substrate and these complex processes are not yet fully understood (Eggleton and Thomas, 2004). While bulk sediment concentrations of heavy metals such as Cu, Ni and Cd are high in a polymetallic nodule area, the concentrations in the pore water, which constitute the potentially bioavailable fraction are significantly lower, ranging in the sub- or lower ppb level (Koschinsky, 2001; Paul et al., 2018). However, even if pore water concentrations of heavy metals are known, the effective uptake of those metals by infaunal organisms may still vary due to physiological adaptations to high metal burdens (Auguste et al., 2016). Therefore, direct measurements of metals in animal tissues may be used to inform about changes in metal uptake-induced by polymetallic nodule mining be a more robust and may indicate physiological responses to increased metal burdens way to assess toxic effects induced by polymetallic nodule mining.

To evaluate the short-term effects of substrate burial on the structure of the meiobenthos community and metal uptake by nematodes, we deposited a 2 cm layer of crushed nodule substrate on enclosed, undisturbed abyssal sediments in the South-East Pacific at 4200 m water depth, using the remotely operated vehicle (ROV) Kiel 6000. Density and community structure of the meiobenthos as well as the vertical structuring after eleven days of incubation was assessed in treatments with and without crushed nodule substrate deposition. Furthermore, nematode genus composition was investigated and X-ray spectrometric images were taken of nematodes to assess the usefulness of this technique for toxicity assessments in the deep-sea.

2 Material and Methods

2.1 Experiment set-up and sampling

The substrate burial experiment was performed in situ during RV Sonne cruise SO242-2 (28.08.2015-01.10.2015) at the southern reference site of the DISCOL experimental area in the Peru Basin, Southeast Pacific (7°7.51 S, 88°27.02 W, in 4196 m water depth; Thiel and Schriever, 1989). For this purpose the ROV Kiel 6000 (GEOMAR, Germany) was used to insert six stainless steel rings (ø = 25 cm, height = 15 cm) into undisturbed sediment avoiding enclosure of nodules or megafauna. The rings were gently pushed 10 cm into the sediment until the collar around the rings touched the sediment surface (Figure 1 A). Subsequently, a substrate-distributing device (Supplementary Figure S1) filled with 250 mL crushed nodule substrate was deployed on three of the steel rings (Burial treatment,
Figure 1 B). The other three rings enclosed undisturbed sediments which served as an experimental control (Control). Rotation of the T-handle activated the release of the substrate that was filled inside the tubes of the device. This resulted in a roughly homogenous distribution of crushed nodule substrate onto the sediment surface in the steel ring with a thickness of approximately 2 cm (Figure 1 C). After ~20 h, the sediment distributing devices were removed from the steel rings to allow complete settlement of all nodule particles and to ensure open water exposure during the remaining time of the experiment. After a total incubation time of eleven days, the sediment in each steel ring was subsampled with two push cores (7.4 cm inner diameter, Figure 1 D).

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 μm and 1 cm (Supplementary Figure S2).

![Impressions Images](image)

Figure 1 Impressions Images of the deployment and sampling during the *in-situ* experiment. A) Stainless steel ring pressed into the abyssal sediment; B) Filled substrate-distributing device on top of the stainless steel ring before substrate release; C) Sediment surface after 11 days of incubation; D) Push core sampling at the end of the experiment. Copyright: ROV Kiel 6000 Team/GEOMAR, Germany.

On board, the overlying water in the push cores was carefully siphoned off and sieved (32 μm) to retain any meiofaunal communities. Subsequently, the sediment of each push core was sliced in several depth layers (added substrate layer (NOD), 0-1 cm, 1-2 cm and 2-5 cm sediment depth) in a climate-controlled room at *in situ* temperature (2.9 °C). The sediment of each slice from all push cores was homogenized and a 5 ml subsample was taken for bulk sediment metal content analysis and stored at ~20 °C. Of each set of push cores, one was used for meiofaunal community analysis and the sediment from each slice was fixed in 4% Borax buffered formaldehyde. The retained meiofauna from the overlying water of that core was added to the sample of the uppermost sediment layer. The second push core was used to serve for the analyses of sediment characteristics (granulometry, total organic carbon content, total nitrogen content) and sediment from each slice was stored at-
20 °C until further analysis. Unfortunately, one core of the Control treatment was lost during slicing, leaving 2 replicates for environmental analyses.

2.2 Meiobenthos analysis

Meiobenthos sediment samples were washed on two stacked sieves of 32 μm (lower sieve) and 1000 μm (upper sieve). Meiobuna was extracted from the 32 μm fraction by density gradient centrifugation with the colloidal silica solution Ludox HS40 (specific gravity of 1.18)(Somerfield et al., 2005). After each of three centrifugation rounds (3000 rpm, 12 min), the meiobenthos in the supernatant was retained on a 32 μm sieve. Subsequently, the sample was fixed in 4% buffered formaldehyde and stained with a few drops of Rose Bengal solution. Meiobenthos was identified to higher taxonomic level using a stereo microscope (50 x magnification).

From each sample, approximately 50 nematodes were picked, transferred stepwise to anhydrous glycerine following the formalin-ethanol-glycerol protocol of (De Grisse, 1969) and mounted on paraffin-ring glass slides for microscopic identification. Nematodes were identified with a Leica DMLS compound microscope (10 x 100 x magnification) to genus level consulting mainly the original species descriptions and pictorial keys available on the Nemys website (www.nemys.ugent.be; Bezerra et al., 2018). Furthermore, nematode genera were categorized in four feeding guilds based on their buccal cavity morphology as described by Wieser (1953). Feeding guilds included “selective deposit feeders” (Group 1A, small buccal cavity without teeth), “non-selective deposit feeders” (Group 1B, large buccal cavity without teeth), “epistrate feeders” (Group 2A, small buccal cavity with teeth) and “predators / scavengers” (Group 2B, larger buccal cavity with teeth). The mouthless genus Parastomonomawas grouped separately (“mouthless”).

2.3 Sediment characteristics and metal contents

Sediment grain size analysis was done by laser diffraction with a Malvern Mastersizer 2000 particle analyzer (Malvern Instruments, UK) and sediment fractions were classified according to Wentworth (1922). Total organic carbon (TOC) and total nitrogen (TN) content in the sediments were analyzed with an Element Analyzer Flash 2000 (Thermo Fisher Scientific) after lyophilization, homogenization and acidification with 1 % HCl.

Sediment bulk concentrations of Fe₂O₃ (%), MnO (%), Cu, Ni and Co (ppm) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) following protocol nr. 14869-2:2002(E) of the International Organization for Standardization (2002). Pore water metal content was not measured in this study but has been assessed for the experimental area by Paul et al. (2018)

2.4 Individual nematode copper content

To determine copper contents in nematodes, respectively 6 and 11 similar-sized and shaped nematodes were taken from one sample of the added crushed nodule layer and the uppermost layer of a push core sample from the same experimental area taken during the incubation period and processed as described above. Nematodes were transferred to a drop of water and body length (L, μm, excluding filiform tail) and average width (W, μm, measured at three different positions in the middle body region) were determined under a compound microscope connected to a Leica camera system. These measures were used to estimate nematode wet weight (WW) using an adjusted Andrassy (1956) formula to account for the specific gravity of marine nematodes (i.e. 1.13 g cm⁻³): μg WW = L x W²/1,500,000 (as described in Pape et al., 2013).
Nematodes were then mounted on 500 nm thin silicon nitride membranes (Silson Ltd, United Kingdom) by means of a small drop of MilliQ water and left to air-dry. Subsequently, element contents were assessed by means of micro X-ray fluorescence (μXRF) using the Edax Eagle III (Edax Inc., USA). This instrument is equipped with a 50 W Rh X-ray tube fitted with polycapillary optics, which focus the X-ray beam in a 30 μm spot. A liquid nitrogen cooled Si(Li) detector is employed to capture the fluorescent X-rays. To examine the element content of the organisms, small mappings were performed with 30 μm step size; each measurement point of these mapping contains a full XRF spectrum with 10 s live-time. These spectra are analysed using AXIL, an iterative least squares algorithm yielding the net intensities for each detectable element present in the sample. The points belonging to the organism are extracted from the XRF element maps using k-means clustering. Next, the spectra from these data points are summed to obtain the total intensity generated by the nematode during the measurement. The intensities per nematode are normalized using nematode wet weights. Of the heavy metals, copper (Cu) was the only element visible clearly enough in the XRF spectra to yield reliable results. Due to the small diameter of the organisms (~ 30 μm) the absorption effects on Cu are negligible, so the normalized intensities of the different scans can be compared directly with each other, in other words, a nematode with more Cu present in its body will yield a higher intensity (counts) per unit body mass (in μg).

2.5 Data analysis

Meiobenthos densities are expressed as the number of individuals per 10 cm² in the different depth layers and over the whole sampled depth (total densities). Due to the unequal thickness of the sampled depth layers, differences in community composition were examined based on relative abundances of the different meiobenthic taxa in each depth layer.

K-dominance curves of nematode genera over the whole core were calculated based on untransformed density data (ind. 10 cm²) and plotted in Primer 6 (Clarke and Gorley, 2006). Additionally, diversity indices (Shannon-Wiener index using the natural logarithm (H'), Pielou's evenness (J') and Simpson's index of diversity (1-D)) of the whole core community were compared between treatments in univariate analyses. Differences in nematode genus composition between treatments and depth layers were analysed based on relative abundances, only.

Statistical differences between treatments and depth layers in multivariate datasets (sediment TOC and TN contents, meiobenthos community composition, nematode genus composition, nematode feeding types) were investigated with a cluster analysis (cluster mode = group average) combined with a similarity profile test (SIMPROF) using Primer 6. For abiotic data, a resemblance matrix based on Euclidean distances was used while biotic data (meiobenthos and nematode genus composition, nematode feeding types) were analysed based on Bray-Curtis-similarities. Interpretation of the results was further based on a visualization with multidimensional scaling (MDS) plots and on the similarity percentages analysis (SIMPER) of significant cluster groups.

Differences of univariate measures (bulk sediment metal contents and diversity indices) between treatments were tested with a student’s t-test in R (R Core Team, 2013) after ensuring normality (Shapiro-Wilk test) and homoscedasticity (Levene’s test) of the data or, alternatively, with a Wilcoxon test as non-parametric test.

An α = 0.05 significance level was chosen for all statistical analyses.
3 Results

3.1 Sediment characteristics and metal contents

The sediment in all push cores was characterized by a 10-20 cm thick brown layer of fluffy surface sediment with underlying more compact, whitish subsurface sediment and no differences in the coloration were apparent between the Control and the Burial Treatment at the end of the experiment (Supplementary Figure S23).

The analysis of total organic carbon and nitrogen contents between treatments and depth layers revealed two significant clusters branching at a distance of 0.4 (\( r = 0.03, p = 0.001 \)). The first cluster was composed of all added substrate layers (NOD) and the second cluster contained all remaining samples. Differences were caused by lower TN and TOC contents in the crushed nodule layer (TN: 0.20 ± 0.00 %, TOC: 0.39 ± 0.00 %; mean ± standard error (SE)) compared to the Control (TN: 0.41 ± 0.05 %, TOC: 0.77 ± 0.02 %) and the underlying sediment layers (TN: 0.40 ± 0.02 %, TOC: 0.71 ± 0.02). Despite the lower carbon and nitrogen content in the nodule particles, C/N ratio remained similar between the nodule particles (1.926 ± 0.037) and the Control sediment (1.951 ± 0.177).

The Control sediment mainly consisted of silt (75.6 ± 0.2 %; mean ± SE), clay (12.8 ± 0.2 %) and very fine sand (8.9 ± 0.2 %) with a median grain size of 20.8 ± 0.3 μm, which was similar in the 0-5 cm of the Burial treatment sediment (median grain size: 22.0 ± 0.3 μm). In contrast, the crushed nodule substrate contained much coarser grain fragments in the mm to cm range (Supplementary Figure S22).

Concentrations of Cu, Mn and Ni in the solid phase were more than three times higher in the crushed nodule substrate compared to the Control sediments (Figure 2).
Figure 2 Box-Whisker plot of solid-phase metal contents measured in the 0-1 cm layer of the Control and the added crushed nodule layer of the Burial treatment. Black line depicts the median whereas a filled dot indicates the mean of the measurements.

3.2 Meiobenthic community composition and vertical distribution

After 11 days of incubation, total meiobenthos densities ranged from 275 ± 10 ind. 10 cm² (mean ± SE) in the Burial treatment to 303 ± 24 ind. 10 cm² in the Control and did not differ between both treatments (Figure 3 A). Overall, nematodes dominated the meiobenthos community (91.0 ± 1.1 %, mean ± SE) followed by harpacticoid copepods (4.4 ± 0.6 %), nauplii (3.2 ± 0.7 %) and polychaetes (0.6 ± 0.1 %, Figure 3 B). All other taxa (Ostracoda, Tardigrada, Gastrotichia, Isopoda, Mollusca, Tantulocarida and Loricifera) contributed less than 0.5 % to the meiobenthos community.

In the Control, meiobenthos densities were similar across all depth layers with 40 ± 3 % of the meiobenthos occurring in the 0-1 cm layer, 28 ± 5 % in the 1-2 cm layer and 32 ± 4 % in the 2-5 cm layer (Figure 3 A). This vertical distribution was different in the Burial treatment where 46 ± 1 % of meiobenthos occurred in the added crushed nodule layer, 13 ± 1 % in the 0-1 cm layer, 10 ± 1 % in the 1-2 cm layer and 32 ± 2 % in the 2-5 cm layer (Figure 3 A). While at the end of the experiment 43 ± 1 % of nematodes over all depth layers were found in the added crushed nodule layer, this percentage was much higher for polychaetes (73 ± 14 %), copepods (71 ± 6 %) and nauplii (61 ± 9 %).

Whole core meiobenthos community composition was similar in samples of both treatments. However, when taking depth layers into account, two significant clusters were revealed branching at 92 % similarity (r = 0.99, p = 0.001). The first cluster (Cluster A) was composed of all crushed nodule layers (NOD), all 0-1 cm layers of the Control and one sample of the 1-2 cm layer of the Burial treatment (Figure 4) while the second cluster (Cluster B) was composed of all remaining samples. Similarities between both clusters were caused by lower abundances of nematodes and higher abundances of copepods, nauplii and polychaetes in Cluster A compared to Cluster B (SIMPER contributions: 48 %, 25 %, 15 % and 4 % respectively; Table 1).

Table 1 Results of the SIMPER analysis between the significantly different clusters identified in the dataset of relative meiobenthos abundances in different depth layers. Av.Abund = average abundance, Av.Diss = average dissimilarity, Diss/SD = average contribution divided by the standard deviation, Contrib% = Contribution to the dissimilarities, Cum% = Cumulative contribution to the dissimilarities.

<table>
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Figure 3 Vertical profile of the average meiofhos densities (ind. 10 cm$^2$, ± standard error) in the Control and Burial treatment with a ± 2 cm layer of crushed nodule substrate (NOD).

Figure 4 MDS plot of the meiofhos community in each sample of the Control and Burial treatment (BT) per sediment depth layer with overlying contours of significant (SIMPROF test) clusters at a 92 % similarity level indicated by the letters A and B. NOD = crushed nodule layer

3.3 Nematode genus community composition

The nematode genus community was very diverse and composed of 96 genera from 33 families combining all samples (Supplementary Table S1). Of the total number of genera, 26 were only recorded once (singletons) and 18 were recorded twice (doubletons). The most dominant genera included *Acantholaimus* (14 ± 1 %), *Monhystrella* (11 ± 1 %), *Viscosa* (8 ± 3 %) and *Thalassomonhystera* (5 ± 1 %), of the other genera, each contributed less than 5 % to the overall nematode community. Evenness of nematode genera was higher in the Burial treatment (0.86 ± 0.01) compared to the Control (0.81 ± 0.01; $t_{27} = -3.373, p = 0.0499$, borderline significant, Figure 5). Diversity indices were not significantly different between the Burial treatment (Shannon: 3.23 ± 0.06, Simpson: 0.95 ± 0.01) and the Control (Shannon: 3.16 ± 0.08, Simpson: 0.93 ± 0.01, Figure 5).
The Cluster analysis of relative abundances of nematode genus composition revealed two significant clusters branching at 36 % similarity (π = 1.64, p = 0.002, Supplementary Figure S4). However, due to the low similarity among all samples (likely resulting from the large number of rare genera) and the lack of clear groupings (e.g. samples of similar depth layers or treatments) within the clusters, we could not further interpret this result.

**Figure 5** K-dominance plot of whole core nematode genera in the Burial treatment (BT) and the Control.

When grouping genera into feeding types and, thereby, reducing variability between samples, the Cluster analysis of relative abundances of nematode feeding types resulted in 4 significantly different clusters. Cluster A included all crushed nodule layers (NOD) and was different from cluster B which included most other depth layers from both treatments branching at a similarity of 75 % (π = 1.45, p = 0.004, Figure 6). The two other clusters together included four depth layers of both treatments branching at 72 % and 46 % similarity (Cluster C and D, respectively, Figure 6).

SIMPER analysis indicated that the difference between the main clusters A and B was due to a reduction of non-selective deposit feeders by 9 %, an increase in epistrate feeders by 8 %, a reduction of selective deposit feeders by 4 % and an increase in predators by 4 % (SIMPER contributions: 37 %, 31 %, 16 % and 16 %, respectively, Figure 7).
Figure 6 MDS plot of the relative abundances of nematode feeding types in each sample of the Control and Burial treatment (BT) per sediment depth layer with overlying contours of significant (SIMPROF test) clusters at a 75 % similarity level indicated by the letters A –D. NOD = crushed nodule layer.

Figure 7 Vertical profile of the relative abundance of nematode feeding types per sediment depth layer (percentage, ± standard error) in the Control and Burial treatment with a ± 2 cm layer of crushed nodule substrate (NOD).

3.4 Copper burden in individual nematodes

Copper contents in nematode bodies could be successfully assessed using micro X-ray fluorescence (Figure 8 A). However, copper burden in the measured nematodes did not differ between treatments (Figure 8 B).
4 Discussion

4.1 Crushed nodule substrate burial induces changes in meio-benthos community structure

In a relatively short time span of eleven days, the meio-benthic community in our study responded to burial with crushed nodule particles by migration, adjusting their vertical position in the sediment. Almost half of the meio-benthos (46 ± 1%), represented by all major taxa, had migrated into the added substrate layer at the end of the experiment. This migration was predominantly seen by fauna from the upper surface layers (0-2 cm) which showed strongly reduced densities compared to the same depth layers of the Control. We hypothesize that those organisms from the upper sediment layers are trying to re-establish their position in the sediment by moving upwards, while the mechanism that triggers this response remains unclear. Changes in oxygen penetration could be one such factor.

Migratory responses of meio-benthos have been widely observed and made use of in the past to deliberately extract these organisms from sediments, for example by changing temperature or by using natural gradients of oxygen availability to trigger meio-benthos movement (Rzeznik-Orignac et al., 2004; Uhlig et al., 1973). Moreover, in a short-term laboratory experiment, Mevenkamp et al. (2017) observed significantly reduced oxygen concentrations in the underlying soft sediment after the addition of 0.5 and 3 cm sediment and an upward migration and increased mortality of nematodes. Unfortunately, we were not able to monitor oxygen content over the time course of the experiment, but the burial with crushed nodule substrate could have led to reduced oxygen availability in the surface layer, causing the fauna to migrate upwards. In contrast to the surface fauna, meio-benthos densities in the deeper parts (2-5 cm) of the sediment remained similar between
the Control and the Burial treatments suggesting that the subsurface fauna is less sensitive to the changes in abiotic conditions causing the migratory response.

Changes in vertical nematode distributions have also been reported in a shallow-water study investigating the impacts of the disposal of experimental dredging material (Schratzberger et al., 2000) and a short-term laboratory experiment testing the effect of instantaneous burial with inert tailings and dead, native sediment (Mevenkamp et al., 2017). These studies found that the amount and frequency of sediment burial are interactive factors showing that frequent but low amounts cause less severe changes than high amounts and instantaneous burial (Schratzberger et al., 2000); but also that substrate burial may cause nematode mortality of up to 50% in the added substrate layer, which was measured by using a staining technique (Mevenkamp et al., 2017). Moreover, Mevenkamp et al. (2017) indicated that burial with 0.1 cm of tailings may already reduce the functioning of bathyal, benthic fjord ecosystems in terms of fresh organic carbon remineralization. Especially, nematode uptake of added organic carbon was considerably reduced after burial with 0.5 cm of substrate. In contrast, Leduc and Pilditch (2013) report changes in vertical nematode distribution after experimental resuspension of the upper 5 cm of sediment originating from bathyal depths (345 m), but without marked effects on sediment characteristics or community oxygen consumption after 2 and 9 days following the disturbance. This different response may be attributed to the fact that the same suspended sediment resettled in the experimental units, whereas Mevenkamp et al. (2017) investigated the deposition of additional substrate.

Interestingly, some meiofaunal groups including polychaetes, copepods and nauplii showed a stronger upward migration than nematodes in our experiment. Similarly, Kaneko et al. (1997) observed taxon specific responses to sediment burial in the Clarion Clipperton Fracture Zone (CCFZ); a nodule region in the North East Pacific. During the Japan Deep Sea Impact (JET) experiment, the authors created a sediment disturbance by means of a towed benthic disturber and sampled the area that was potentially impacted by re-sedimentation prior to, directly after and 1 year after the disturbance. In the collected upper 3 cm of the sediment, nematode densities were significantly reduced following the disturbance and remained low even after one year. In contrast, copepod densities remained similar directly after the disturbance and increased significantly after 1 year possibly due to their different mobility and life history compared to nematodes. However, Kaneko et al. (1997) did not mention the amount of resettled sediment and it is possible that the decrease in nematode densities was attributed to their limited upward migration directly after the disturbance (as was seen in our experiment) so that part of the nematodes were still residing in sediment layers >3 cm. While our experiment examined short term and immediate responses to substrate burial, the JET experiment clearly indicated that sediment burial can also alter meiofaunal community structure on longer time scales.

The possibility that the migratory response may be accompanied by elevated mortality of abyssal meiofauna requires further investigation, especially because re-sedimentation of fine clay is expected to occur over large areas in a deep-sea mining context (Oebius et al., 2001; Smith et al., 2008). Clay deposition could substantially change oxygen availability in comparison with the coarse nodule debris assessed here. In our experiment, we were not able to assess meiofaunal mortality resulting from the burial because decompression induced mortality during sample retrieval from abyssal depths would bias the results. Nevertheless, several authors have underlined the importance to assess meiofaunal mortality in short-term experimental studies as it may pass unnoticed due to slow decomposition of organic matter in the deep sea (Barry et al., 2004; Fleeger et al., 2006, 2010). Therefore, potential unnoticed mortality in our study may have masked more severe changes in terms of meiofauna densities and diversity. It should be noted that meiofaunal contribution to the benthic ecosystem in terms of relative abundance and biomass increases with water depth (Rex et
Therefore, it is plausible that the induced changes in meiofauna distribution—and, possibly, mortality—may entail even stronger effects on the overall functioning of abyssal soft sediments with regard to food web interactions, organic matter remineralization and bioturbation.

### 4.2 Nematode community may face alterations in response to burial with crushed nodule particles

Generally, the abyssal seafloor is characterized by a low degree of disturbance and low organic matter input from the euphotic zone. Sedimentation rates in the Peru Basin are generally ranging between 0.4 and 2.0 cm ka⁻¹ (Haeckel et al., 2001). Therefore, in this environment we expect benthic assemblages that are adapted to very stable conditions. Interestingly, all dominant nematode genera responded with upward migration and there was no evidence of specific selection mechanisms, e.g. opportunistic genera taking advantage of the new situation and being more successful in either inhabiting the new substrate or in remaining in the surface layers of the original sediment and, therefore, being more stress resistant. Opportunistic species generally occur under extreme, variable conditions and get outcompeted by less opportunistic species when disturbance is low (Grassle and Sanders, 1973). However, small scale disturbances and habitat heterogeneity in the deep sea may induce a more dynamic environment to allow the persistence of colonizing species (Gallucci et al., 2005). This seems to be supported by the large number of Monhysteridae in our study, which are generally classified as good colonizers at least in shallow water environments (Bongers et al., 1991). However, deep-sea monhysterids are characterised by a high local intrageneric diversity not supporting an opportunistic behaviour (Vanreusel et al., 1997). The results of our experiment did not indicate that these nematodes monhysterids were more successful to inhabit the added substrate.

Nematode communities in the abyssal deep-sea and nodule habitats in particular are characterized by a high diversity, potentially owing to the increased habitat complexity created by the nodules (Miljutin et al., 2011; Singh et al., 2014; Vanreusel et al., 2010). Similarly, the nematode community in our experiment displayed a high diversity with a large proportion of rare genera (45% of the genera recorded only once or twice). This high proportion of rare taxa may increase the vulnerability of the nematode community in this area to disturbances since the risks of local extinction may be greater for those small populations (McCann, 2000; Rosli et al., 2018) and recovery will depend on recolonization success and species connectivity. Information about these two factors is still very limited for nematodes from abyssal nodule regions, especially for the rare taxa. Our study indicated that the addition of crushed nodule substrate changed the relative abundance of feeding types in the new surface layer, which could, depending on the long-term effects (e.g. mortality, vertical restructuring, species interactions) affect the role of the nematode community in the functioning of the benthic environment.

A study of Miljutin et al. (2011) indicated that changes in nematode communities following strong sediment disturbance may persist for up to 26 years. They revisited a disturbed nodule site in the equatorial NE Pacific where sediment and nodules were removed by dredging 26 years ago and found that nematode density, diversity and community structure inside the disturbed track still differed from adjacent non-disturbed areas. Although the disturbance studied by Miljutin et al. (2011) strongly differs from our experiment; it this study clearly indicates that changes in nematode community composition in polymetallic nodule areas due to sediment disturbance, as seen in our experiment, may be long lasting and are potentially irreversible and, therefore, underlining the importance of long-term experiments, with the risk of reducing local biodiversity through extinction of rare taxa.
4.3 Increased copper concentrations in the added substrate are not reflected in nematode body copper content

The very high concentrations of solid phase heavy metals in the crushed nodule substrate raise questions about bioavailability and uptake of these metals in benthic organisms. Previous research in polluted, shallow waters has shown that nematodes play an important role in the transfer of heavy metals to and from the benthic food web in harbour communities (Fichet et al., 1999) and that uptake of different pollutants may vary (Howell, 1982). As such, Howell (1982) reported increased zinc uptake in nematodes exposed to pollution, while copper content was very variable and correlations with habitat pollution were less clear.

In the presence of manganese (Mn) oxyhydroxides, other elements such as the transition metals (Cu, Ni, Zn) are adsorbed in the oxic layer which is up to 5 and locally up to 20 cm deep in the Peru Basin (Stummeyer and Marchig, 2001). Therefore, most metals are bound to the solid phase of the sediment and are not bioavailable, which most likely explains our findings of similar copper burdens in the nematodes from the control and the Burial treatments. A recent study investigated the long-term and short-term effects of sediment disturbance on metal and trace element concentrations in the solid phase of the sediment and the pore water (Paul et al., 2018). The authors found that while some solid phase elements still deviated from pre-disturbance levels even after 26 years, levels of trace metals in the pore water levels returned to pre-disturbance values in a short time frame of several weeks. Under conditions present in the sampled sediment, release of metals during a mining operation may not result in an increased metal toxicity because of the fast oxidation of Mn and absorption of metals (Paul et al., 2018). However, in the case that oxygen conditions change inside the sediment due to re-sedimentation or other processes the release of toxic compounds into the pore water cannot be excluded. The use of new techniques to analyse tissue metal content as used in this study may allow precise detection of changes in heavy metal burden even in smaller benthic organisms due to mining related alterations of the abiotic environment.

4.4 Conclusion and recommendations

The brittle character of polymetallic nodules implies that the deposited material following mining will is likely to be a mixture of natural sediment and nodule particles. In this research, we revealed some important insights in the structuring of meio-benthic communities following burial with freshly crushed nodule substrate. Despite the very different abiotic conditions in the crushed nodule substrate and the natural sediment in terms of grain size and carbon and nitrogen content, an upward migration of meio-benthic organisms was observed. Our results from in-situ experiments at >4000 m water depth confirm previous research in different habitats (Kaneko et al., 1997; Maurer et al., 1986; Mevenkamp et al., 2017; Schratzberger et al., 2000) showing that meio-benthic organisms generally show upward migration following burial with native and non-native substrate and varying thickness of the deposited layer. We found that this behavioural response was stronger in polychaetes, copepods and their nauplii compared to nematodes, which could result in a shift in meio-benthos community composition.

Furthermore, the relative distribution of nematode feeding types was altered indicating that changes in the functional role of the nematode community on the short and long term cannot be excluded. Likely due to the high nematode genus diversity and evenness, changes in nematode genus composition were not detected between treatments.
The effect of vertical meiofauna migration on other benthic size classes and over longer time scales requires further research, especially in a deep-sea mining context where sediment re-deposition is expected over large areas and long timescales (Murphy et al., 2016). Furthermore, we hypothesize would like to emphasize that, although it is technically challenging, standardized methods for mortality assessments in deep-sea sediment samples are needed to advance our understanding of short-term environmental impacts on meiofauna.

**Data availability:** The data used in this publication are deposited in the Pangaea database https://doi.pangaea.de/10.1594/PANGAEA.896027

**Author contribution statement:** AV, AB, KG and LM conceived the study. LM conducted the experimental work and collected the samples. Meiofauna samples were analyzed by LM and KG, nematode copper content was measured and analyzed by BL and LV and sediment metal contents were provided by DV and JGD. LM performed the statistical data analysis; LM, BL, KG and AV interpreted the results and LM wrote the manuscript with the assistance of all authors.

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