Referee comments in black.

Author's responses in green.

A well written manuscript and a huge contribution of data for an area with limited but increasing data. This will be a useful resource for other researchers and deep-sea management in the region.

A few minor comments to accompany the comments on the attached manuscript. To me the hypotheses were not clear, I think the manuscript would really benefit if they were clearly defined in the introduction and revisited in the conclusions. An overview of why benthic diversity is important to a broader audience would be useful for the bigger picture as well as the ecological role of polychaetes within benthic communities. Why people should care about them? I love polychaetes but a lot of people don't. A brief description of the mining process, not all readers will be aware of this may be with a comment on the current likelihood of these operations happening and if so when. As stated the diversity estimates are very different, is there an additional method that can be used? With two measures that are both biased towards "singletons" is there a method that is not? Some images of the polychaetes would be nice, especially as photography was an important part of the method. These can be really useful for other research groups, will these be made publicly available? Maybe include a plate in the methods or results. I am not 100% familiar with the diversity analysis so can not critically comment on the methods/results for those sections. I leave this to the other reviewer and the editor. Many terms are not clearly defined but really important as often misinterpreted between papers. Table 1 could be supplementary.

In general lines we have clarified the hypothesis in the introduction and aims; added why benthic diversity and polychaetes are important also in Introduction and Conclusions; and added some words in Introduction about mining operation. Unfortunately, the under-sampling and the singletons bias affects all current diversity estimators. A plate with photographs of polychaetes will not help with any aim of the manuscript. Pictures of polychaetes will eventually be available in BOLD database. The table 1 contains important data and we leave it integrated in the manuscript.

Below you can find the answers to each comment.

Page 1, line 9. I felt that the abstract was very long... down to the editor but I think it could be shortened to really highlight the most important findings.

We generally appreciate abstracts that are informative about the context, the methods, and the main results, in addition to the main findings. We would thus prefer to not shorten it.

Page 1, line 14, 'unknown'. This counteracts the previous sentence that they were designated on best knowledge.

It is indeed the paradox that we wished to emphasize by specifying that the design of the APEIs was based on "the best –albeit very limited – scientific knowledge"

We rephrased the sentence to make it clear (page 1, lines 13-14):

From "The APEIs were created based on the best – albeit very limited – scientific knowledge for the area."

To "The scientific principles for the design of the APEIs were based on the best – albeit very limited – knowledge for the area."

Page 1, line 23, 'singletons'. This term is used a lot and I don't think it is defined. It is not always clear if it is a single specimen from an MOTU or morpho species.

Singletons are species known from only one specimen, or MOTUs known from a single sequence.

The text has been modified to define "singleton" where it first appears in the Introduction (page 3, line 12):

From "(b) high frequencies of singletons ranging… "

To "(b) high frequencies of singletons ***(MOTUs known from a single unique DNA sequence)*** *ranging …"*

And later on, in the Results section (page 10, line 32-33):

From "Of these, 134 species were singletons."

To "Of these, 134 species were ***singletons (i.e. morphospecies known from a single specimen)****."*

Page 1, line 23, 'food fluxes'. This was measured indirectly, I'd suggest changing it to represent what was actually measured or explain it is a proxy.

Page 1, lines 23-24. We changed "food fluxes" by "organic carbon fluxes" in order to precise the variable used. We didn't get into the details of the methods here in order to not weigh down the abstract, which is already long.

From "The patterns in community structure and composition were mainly attributed to variations in food fluxes at the regional scale and nodule density at the local scale."

To "The patterns in community structure and composition were mainly attributed to variations in ***organic carbon fluxes*** *to the seafloor at the regional scale and nodule density at the local scale"*

Page 1, line 24, 'regional scale and nodule density at local scale'. Is there a quantitative measure of scale or difference between the two?

Page 1, line 24. The regional scale refers to the scale of the CCFZ and beyond, over 1000-km while, the local scale refers to within-area variations; NE Pacific-scale refers to all meta-analysis data covering the NE Pacific Basin. In order to keep the abstract as short as possible we didn't provided details here but we went through the text and provides explicit definition where needed.

Page 2, line 1, 'reflect our level of uncertainty'. Can you comment using expert opinion about the levels you may expect?

Page2, line 1. In the absence of a clear theoretical background to explain the maintenance of abyssal diversity and in the absence of empirical data to accurately quantify levels of diversity, there is unfortunately no information on which to form an expert opinion.

Page 2, lines 6-7, 'Only about 1 % of abyssal plains have been explored to date: much remains to be discovered'. This is true but there is a lot of research effort trying to change

this. It would be nice to read that there is a positive trend in the number and resources for deep-sea studies.

Page 2, line 8. "A lot of research" is somehow subjective. As far as the CCFZ is concerned, there has been 3 academic research cruises funded over the last 5 years. Whether this is a lot is debatable.

Page 2, lines 9-10, 'possibly containing 34 billion metric tons of manganese (Michael et al., 2000; Morgan, 2000)'. Is there a monetary value for this?

Page 2, lines 13-16. A word was missing in the that phrase. It has been changed 'possibly containing 34 billion metric tons of manganese ***nodules*** *(Michael et al., 2000; Morgan, 2000)'. We also add a value of ore given by Volkmann et al. (2018, Table 1) to provide a monetary value (in bold):*

"In the Equatorial Pacific Ocean, the Clarion-Clipperton Fracture Zone (CCFZ) harbors the largest polymetallic nodule field with nodule densities as high as 75 kg m^{-2} (average 15 kg m^{-2}) and possibly containing 34 billion metric tons of manganese nodules (Hein and Petersen, 2013; Morgan, 2000), ***which may represent a minimum sale value of 16 000 billion US Dollars (Volkmann et al, 2018)."***

Page 2, lines 21-22, 'Such small experiments however hardly mimic the cumulative impacts of any single nodule mining operation that could last for 20 years.'. Reference?

Page 2, line 31. We have added the missing references:

*Such small experiments however hardly mimic the cumulative impacts of any single nodule mining operation that could last for 20 years (****Glover and Smith, 2003; Jones et al, 2017****).*

Page 3, line 4, 'species turnover'. Please define.

"Species turnover" has been defined in the first appearance in the manuscript (page 3, line 10-11):

From "(a) high rates of species turnover with only 12 % of polychaete…"

To "(a) high rates of species turnover ***(i.e. species replacement)*** *with only 12 % of polychaete…"*

And further defined in the Material and methods (page 8, line 15-16):

From "…turnover – which is dissimilarity due to species turnover"

To "turnover – which is dissimilarity due to species ***replacement****"*

Page 3, line 6, 'richness'. Is this the same as cryptic species? sorry to get confused, these terms are widely used but often undefined. Would be good to be clear.

Page 3, line 13. Indeed, "cryptic richness" was not correct, the appropriate term is "cryptic diversity" which indicates the presence of "cryptic species" (species morphologically indistinguishable).

It has been corrected all along the manuscript.

Page 3, line 19, 'grounds'. Basis?

Page 3 line 31. Done as suggested.

Page 3, line 22, 'methods?'

Page 4, line 8. Done as suggested.

Page 3, lines 23-24, 'test the hypotheses that support spatial conservation planning in the CCFZ'. Can these be defined? They are mentioned again in the conclusions but I was still unsure where they are laid out

We have deleted the correspondent sentence from the beginning of Conclusions as suggested by Referee #1.

We have clarified it in Introduction:

Page 3, lines 20-21. Addition of the sentence: "One of the main assumptions underlying the management plan is that longitudinal and latitudinal productivity-driven gradients shape the community structure and species distribution of abyssal communities.".

Page 4, lines 3-6. Changing the following sentence:

From "The structure and composition of polychaete assemblages were analyzed to describe and identify alpha and beta diversity patterns, test the hypotheses that support spatial conservation planning in the CCFZ, assess the representativeness of an APEI and potentially improve the assessment of potential risks to biodiversity due to nodule mining."

To "The aims of our study were (a) ***to test the hypotheses that support spatial conservation planning in the CCFZ, particularly the environmental drivers of alpha and beta diversity such as organic carbon fluxes to the seafloor and nodule density****; (b) to assess the representativeness of an APEI (i.e. APEI#3) and (c) to improve the assessment of potential risks of biodiversity loss due to nodule mining."*

Page 4, line 19, 'from each DNA-friendly polychaete'. Define DNA friendly? Ethanol fixed, whole?

Yes. 'DNA friendly' and 'not DNA friendly' were already defined page 5, lines 14-16. The entire worms were fixed with ethanol and a piece of each one was dissected for molecular studies.

Page 5, lines 7-9, 'All sequences obtained in this study have been deposited in BOLD (http://www.boldsystems.org; (Ratnasingham and Hebert, 2007)) or GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Do you have the accession numbers for these? would be good to be in a table in the supplementary.

Page 5, lines 18-20. The BOLD IDs and GenBank accession number are in the dataset available in Pangaea databases.

The following sentence has been modified in the section Data Availability (page 17, line 8-10):

From "Abundance data analyzed in the present study are available in the Pangaea (Bonifacio et al., 2019) whereas DNA sequences are available in BOLD or GenBank databases."

To "DNA sequences are available in BOLD (http://dx.doi.org/10.5883/DS-GKG001) or GenBank databases. The abundance data analyzed in the present study together with BOLD IDs (Sample ID and Process ID) and GenBank accession numbers are available in the PANGAEA database (Bonifácio et al., 2019)."

Page 6, line 9, 'species'. Species or MOTUs?

As pointed out by Referee #4 there was confusion in the use of terms such as morphospecies, MOTUs and morphotypes. Those were corrected in the revised version. Moreover, in general we consider morphospecies as species.

Page 6, line 10, 'damaged species'. Was DNA not taken from fragmented specimens? Can this be mentioned in the earlier methods sections.

It should be 'damaged specimens'. This has been corrected as suggested (page 7, line 11). DNA was taken from very few fragments but only head-ends were counted.

Page 5, lines 17-18. The following sentence was changed:

From "In the laboratory, from each DNA-friendly polychaete specimen, a small piece of tissue was dissected …"

To "In the laboratory, from each DNA-friendly polychaete specimen ***and from very few fragments****, a small piece of tissue was dissected …"*

Page 5, lines 18-19. The following sentence was added: "DNA sequences from fragments without head were archived in BOLD and GenBank (Bonifácio et al., 2019) but were not further used for the purpose of this paper."

Page 7, line 2, 'forward, backward and stepwise selection procedures'. Can this be explained?

Page 8, line 5. Roughly, the forward procedure adds each predictable variable one by one to the model until be statistically significant; the backward includes all variables in the model and removes one by one until be statistically significant); and stepwise is a combination of both forward and backward. Actually, the selection procedure used in the case was the "forward".

As requested by Referee #2 in Methods section it should be described what has been done while a description of how the methods work does not seem appropriate for the M&M section. Thus, a citation to the reference of Borcard et al., 2011 (and in References) who explain in detail the methods was added.

Borcard, D., Gillet, F., and Legendre, P. (Eds.): Numerical ecology with R, Springer-Verlag, New York, USA, 2011.

Page 7, line 30, 'polychaetes', Of the 1233 polychaetes?

Page 9, line 4. Yes. But it doesn't need to be repeated here.

Page 8, lines 1-2, 'The relative contributions of trophic guilds also varied among the areas'. How were trophic guilds defined? Family level traits from the literatures or another study?

The trophic guilds were defined based on literature (Jumars et al., 2015).

The following sentence was included in the section 2.4 Operational taxonomic units (OTUs) (page 6, lines 19-20): "Trophic guilds were determined following Jumars et al. (2015) at family level."

Consequently, the section (page 6, line 8) has been changed to "2.4 Taxonomic identification ***and feeding guilds classification"***

The changing from "Operational taxonomic units (OTUs)…" to "Taxonomic identification…" followed a comment of Referee #4.

Page 8, lines 13-15, 'total of 275 morphospecies (i.e. OTUs) were recognized. The mean number of species tended to decrease from east to west with high spatial variation (Fig. 2b). Mean richness varied from 37 ± 10 taxa 0.25 m^{-2} in BGR to 3 ± 2 taxa 0.25 m^{-2} in APEI#3.' How do these numbers compare to other published CCZ studies? in terms of MOTUS by area?

Page 9, lines 20-24. The overall species richness is discussed in Discussion section '4.3 How many polychaete species live in the CCFZ? The under-sampling bias' and shown in Table 2. However, this can not be a simple comparison because of differences in sampling effort sampling design and methodologies for species identifications.

Page 9, line 2, 'forward selection procedure kept'. ??

Page 10, line 6. Yes, the "forward selection procedure" selected a best set of variables explaining the variability of polychaete assemblages.

Page 9, line 9, 'indeed'. not needed

Removed as suggested.

Page 9. Line 29, 'Aurospio sp. 249'. Is this a common trait for Aurospio... other examples/oceans where this genus dominates and is well distributed. e.g. https://doi.org/10.1111/j.1439-0485.2008.00265.x

Page 10, line 18. Completely agree. This aspect of Aurospio *is discussed in the submitted paper of Guggolz et al. entitled: High diversity and pan-oceanic distribution of deep-sea polychaetes:* Prionospio *and* Aurospio *(Annelida: Spionidae) in the Atlantic and Pacific Ocean*

We have added the following sentence in order to show differences in distribution of some polychaete species (page 13, lines 23-25) : "… or species of Aurospio *and* Prionospio *which could show pan-oceanic distribution (i.e., Pacific and Atlantic oceans; Guggolz et al., submitted)."*

Page 10, line 1, 'Discussion'. Some switching between abundance and density... may be clarify if different or if the same keep consistent

We have changed "macrofaunal density" into "macrofaunal abundance". Further, we kept "abundance" when referring to numbers of animals and "density" when referring to numbers of nodules.

Page 10, line 3, 'Food supply'. This was measured indirectly, may be mention as a proxy of or indicated by

The following sentence was added in M&M (page 6, line 31): "POC flux at seafloor was considered as a proxy for food supply to benthic communities."

Page 12, lines 5-6, 'The fact that the APEI#3 lies mostly north of the Clarion Fracture Zone may however also contribute to its dissimilarity with the areas located in the CCFZ per se.'. Can you suggest a more suitable APEI area? Would you expect other APEIs given their position to be better?

The main issue with the placement of APEIs is that most of the area to be managed in between the two fracture zones has already been preempted to contract or reserved areas. Moreover, the knowledge on the environment and benthic communities of the APEIs is still

quite limited. Our recommendation is to first gain knowledge on these APEIs. This has been highlighted in the Conclusions (page 16, lines 18-24):

The scantiness of food supply and a barrier to dispersal may thus compromise the representativeness of APEI#3 and question its ability to meet its purpose of preserving the biodiversity from any of the contract areas considered in this study. The sampling effort in both the contract areas and the APEI however remains quite limited. In order to ascertain that the APEIs collectively meet their goal of preserving the biodiversity of the CCFZ an ambitious research agenda is needed, the funding of which could rely on the willingness of contractors and Sponsoring States but could also become a priority of the future Environmental Compensation Fund to be created by the regulations on exploitation of mineral resources in the Area (ISBA/25/C/WP.1).

Page 12, line 28, 'infaunal brooders'. Can you comment on the likely reproductive traits of the polychaetes based on family level data? What proportion of the polychaetes in this study are likely to be brooders?

Page 13, line 32. Reproductive strategies data are limited and sometimes with variation within a family. Also, in the deep-sea we don't know much more about these traits, so we prefer to do no suppositions about.

Page 13, lines 4-5, 'In other words, nodule mining would affect each year an area that is equivalent to the average geographic range of a polychaete species.'. I feel this is really important but not clearly worded. Maybe restructure.

This is potentially important because if true, species extinction due to nodule mining is very likely. We made it clearer in the Conclusions (page 16, lines 27-29):

"Species turnover is high with a minimum estimated rate of species change of 0.04 species km^{-1}, suggesting an average geographical range of 25 km and a number of polychaete species in the CCFZ that may equal the number of all currently known marine species. If true, the risk of species extinction is very high because the environmental footprint of nodule mining would largely exceed the range of many species."

Page 13, lines 31-31, 'Over 90 % of the species in the abyssal Pacific are new to science (Glover et al., 2002) and there are few attempts to try to name them (Paterson et al., 2016; Bonifácio and Menot, 2019)'. Realistically there are not enough experts, time or resource to name species new to scienc :(but there are other approaches such as turbo taxonomy that could be mentioned as an alternative

Summers, M.M., Al-Hakim, I.I. and Rouse, G.W., 2014. Turbo-taxonomy: 21 new species of Myzostomida (Annelida). Zootaxa, 3873(4), pp.301-344.

There are not enough experts in the world, but resources need to be invested in formation and hiring taxonomists. Turbo-taxonomy is not the ideal world neither. It is completely based on molecular approach that is not completely efficient currently; and it did not provide always a detailed morphological descriptions which should be used by ecologist. Morphological identification remains less expensive than barcoding. According to Stein et al. (2014), current barcoding costs using Sanger sequencing are between 1.7 and 3.4 times as expensive as traditional taxonomic approaches, excluding the cost of field sampling (which is common to both approaches). Taxonomy needs to be considered as a science and not a tool.

Page 14, lines 28-29, 'In the deep-sea, an anomalously high rate of singletons of about one-third of the species is in fact the rule of macrofaunal surveys'. Can you clarify or reword?

*Page 15, lines 33-34. This was rephrased to "In the deep-sea, an anomalously high rate of singletons **(about one-third of the sampled species**) is **usually** the rule of macrofaunal surveys …"*

Page 15, lines 9-11, 'testing the hypotheses that supported spatial conservation planning in the CCFZ, assessing the representativeness of an APEI and improving the assessment of potential risk to biodiversity due to nodule mining.'. Can these be clearly laid out in the introduction and then revisited?

As suggested the objectives have been clarified in the Introduction.

Page 4, lines 3-6:

"The aims of our study were (a) to test the hypotheses that support spatial conservation planning in the CCFZ, particularly the environmental drivers of alpha and beta diversity such as organic carbon fluxes to the seafloor and nodule density; (b) to assess the representativeness of an APEI (i.e. APEI#3) and (c) to improve the assessment of potential risks of biodiversity loss due to nodule mining."

And then revisited e.g.

Page 16, lines 11-13

"Food inputs and nodule density influence the structure and composition of polychaete assemblages in the CCFZ. This is a confirmation of hypotheses underpinning the design of the APEIs"

Page 15, line 20, 'even higher'. % range?

The range is between 498 and 240,000 species as stated page 16 lines 5-6:

"In conclusion, our level of certainty on the number of polychaete species inhabiting the CCFZ and potentially threatened by nodule mining ranges from 498 to 240,000 species."

Page 15, line 21, 'cryptic richness'. diversity/species?

This has been changed to "cryptic diversity" across the manuscript.

Page 15, line 23, 'footprint of nodule mining'. Estimate of area?

This was exemplified in the Discussion section '4.2 Species turnover and geographic ranges' Page 14, line 8 "… an area of a 100 km² would be mined each year."

Page 15, lines 27-28, 'The assessment of potential risks and scales of biodiversity loss thus requires an appropriate inventory of species richness in the CCFZ.'. How close are we to achieving this? What would be required - international database or the use of pre-existing databases?

The issue with pre-existing data and databases is that the species lists can't be merged as there is no formal description of species. The use of DNA barcoding and integrative taxonomy in recent years will progressively allow to facilitate data integration. Still, there is a need for a large and coordinated research program in order to fully assess the scales of biodiversity in the CCFZ. The following recommendation was added in the Conclusions (page 17, lines 2-5):

"In the framework of an ambitious and collective effort to inventory species richness in the CCFZ, a stratified random sampling at nested scales, from region down to seascapes, would provide the scales of species turn-over while intensive sampling of selected habitats up to the point where the number of singletons decreases with sample size would provide accurate estimates of species diversity."

References cited in the authors answers:

Bonifácio, P., Neal, L., Omnes, E., Baptiste, F., Dahlgren, T. G., and Menot, L.: The polychaete fauna of the Clarion-Clipperton Fracture Zone from boxcore samples during SONNE cruise SO239, PANGAEA, https://doi.pangaea.de/10.1594/PANGAEA.902860, 2019.

Borcard, D., Gillet, F., and Legendre, P. (Eds.): Numerical ecology with R, Springer-Verlag, New York, USA, 2011.

Guggolz, T., Meißner, K., Schwentner, M., Dahlgren, T.G., Wiklund, H., Bonifácio, P., and Brandt, A.: High diversity and pan-oceanic distribution of deep-sea polychaetes: Prionospio and Aurospio (Annelida: Spionidae) in the Atlantic and Pacific Ocean, Org. Divers. Evol., submitted.

Hein, J. R., and Petersen, S.: The geology of Manganese nodules, in: Deep Sea Minerals: Manganese Nodules, a physical, biological, environmental, and technical review, 1B, edited by: Baker, E., and Beaudoin, Y., Secretariat of the Pacific Community (SPC), Noumea, New Caledonia, 7-18, 2013.

Jumars, P. A., Dorgan, K. M., and Lindsay, S. M.: Diet of Worms Emended: An Update of Polychaete Feeding Guilds, Ann. Rev. Mar. Sci., 7, 497-520, doi:10.1146/annurev-marine-010814-020007, 2015.

Morgan, C. L.: Resource estimates of the Clarion-Clipperton manganese nodule deposits, in: Handbook of marine mineral deposits, edited by: Cronan, D. S., CRC Press, Boca Raton, 145-170, 2000.

Stein, E. D., Martinez, M. C., Stiles, S., Miller, P. E. and Zakharov, E. V.: Is DNA Barcoding Actually Cheaper and Faster than Traditional Morphological Methods: Results from a Survey of Freshwater Bioassessment Efforts in the United States?, edited by M. Casiraghi, PLoS ONE, 9(4), e95525, doi:10.1371/journal.pone.0095525, 2014.

Volkmann, S. E., and Lehnen, F.: Production key figures for planning the mining of manganese nodules, Mar. Georesour. Geotec., 36, 360-375, 10.1080/1064119X.2017.1319448, 2018.

Volkmann, S. E., Kuhn, T., and Lehnen, F.: A comprehensive approach for a techno-economic assessment of nodule mining in the deep sea, Mineral Economics, 31, 319-336, 10.1007/s13563-018-0143-1, 2018.