Object:
Submission of a **revised** manuscript to Biogeosciences special issue *Assessing environmental impacts of deep-sea mining – revisiting decade-old benthic disturbances in Pacific nodule areas*

From Paulo,
To Editor and Associate Editor of the Biogeosciences

Brest, November 21st 2019.

Dear Dr. Treude,

We are pleased to submit our **revised** manuscript entitled "*Alpha and beta diversity patterns of polychaete assemblages across the nodule province of the eastern Clarion-Clipperton Fracture Zone (Equatorial Pacific)*" by P. Bonifácio, P. Martinez-Arbizu & L. Menot for consideration to be published in Biogeosciences special issue "Assessing environmental impacts of deep-sea mining – revisiting decade-old benthic disturbances in Pacific nodule areas".

We are thankful for the useful and positive comments and corrections of all four referees which were mostly accepted. Two of them suggested minor revisions while two of them suggested major revisions. Following all suggested modifications our revised manuscript has been improved in terms of methodology and results; and in topics concerning management of nodule mining; but, also, rephrasing and rewording some parts of the text in order to facilitate and clarify the manuscript.

As solicited by you, below you can find the answers to each comment for the two referees who suggested major revisions. Ours answers are in green and make references to the pages/lines of the revised manuscript (submitted separately). In the final part of this document you will find a marked-up manuscript version as well.

We thank you and we are looking forward to hearing from you.

Best regards,
Paulo Bonifácio, Pedro Martinez-Arbizu & Lénaick Menot

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Author's response to Referee's comments (Dr Dando)

The paper presents the data on the diversity patterns in polychaetes collected on a cruise that was "aimed at improving species inventories, determining species ranges - -" in the polymetallic nodule area of the Clarion-Clipperton Fracture Zone (CCFZ).

Given the importance of the results, for areas that may be subject to disturbance by nodule mining, I do not understand why there is no reference to the distribution of the rest of the major macro-infaunal groups, given that Wilson (2017) showed that different groups respond in different ways. It is important to know whether similar distribution patterns to those found for polychaetes occurred in the other groups in the studied area.

We agree that, for the time being, the diversity and distribution patterns of different taxonomic and functional groups must be assessed. To achieve this goal, considering the diversity of the abyssal fauna, as well as the large proportion of new species, each taxonomic group has been processed by its own set of specialists. This is a chance as the lack of taxonomic expertise is a major impediment to our knowledge of the abyssal fauna. As a consequence, also, the results for each group taxonomic group will be published separately – we all have to valorize our work. Results on tanaids for example, the second most abundant group of the macrofauna after polychaetes have just been published (Blazewicz et al. 2019). Tanaid assemblages show similar patterns as polychaetes, this is mention page 12 line 16 (the reference has been updated from Pabis et al. submitted to Blazewicz et al. 2019). Eventually, the results for each taxonomic group, from this project as well as other ongoing projects will be synthesized to provide a global picture of distribution and diversity patterns of the benthos in the CCFZ.

It is clear that a lot of effort has gone into obtaining and working up this data. It is therefore unfortunate that a poor description of what was done and the reasons for this make it difficult to understand what fraction of the infauna was sampled and the consequences for the overall polychaete biodiversity and the comparisons between different box cores.

This general comment is addressed more specifically below where are discussed the epifauna versus infauna and nodule infauna versus sediment infauna.

Given that the topography of the polymetallic nodules affects local water flow patterns and creates different microhabitats (Mullineaux, 1989). The cruise report gives information on the nodule differences between individual cores but this information does not appear to have been used in analyzing polychaete numbers and distributions, although this should have a major influence on species composition and numbers. I would like to see an analysis of polychaete species distribution and numbers with respect to the differences in nodule topography, numbers and sizes between cores.

The influence of nodules on the structure and composition of polychaete assemblages has been tested. The variable named "nodules" is the wet weight of nodules per box-core, extrapolated to a square meter (see page 6 lines 26, the data are given in Table 1). This measure of nodules density was related to the abundance and richness of polychaetes from each box core sample using Spearman correlations (Figure 3); and related to polychaete composition by the Redundancy Analysis (RDA, Figure 7). Unfortunately, the number and size of individual nodules were not recorded during the cruise.

The variable "Nodules" has been renamed to "Nodule density" throughout the manuscript and figures for clarity.

Treating all box core samples within an area as replicates does not appear to be valid.

Box-core samples within areas have been used as replicates in order to assess regional-scale variations in the abundance, richness and composition of polychaete assemblages. We think that the approach is valid because the distance between areas is much larger than the distance between individual samples within areas. The samples within an area can be considered as representative of the populations within that area compared to the other areas. There are two instances where the assumption is questionable:

- In the BGR area, two sub-areas were sampled: a prospective Area (PA); and a reference Area (RA), with a low nodule density. There was no statistical difference in the abundance and richness of polychaetes between the two sub-areas. Samples from the two sub-areas were thus considered as replicate samples for the BGR area.

- In the IOM area, three sub-areas were also sampled: one that had been disturbed by a BIE-experiment, one that had been impacted by the plume and one control, undisturbed area. The three sub-areas had otherwise similar environmental settings and there were no statistical differences in the abundance or richness of polychaetes.

This has been clarified in the sampling strategy with the addition of the following paragraph (page 4 line 26 – page 5 line 5) and the reference in reference list:

"The sampling strategy resulted from a combination of objectives that were unique to each area, together with the overarching aim of describing alpha and beta diversity patterns across a productivity gradient that included both contract areas for nodule exploration and an APEI (Martínez Arbizu and Haeckel, 2015). In the BGR area, two sub-areas were sampled: a Prospective Area (PA) that could be mined in the future and a Reference Area (RA) that could serve as a preservation area. In the IOM area, three sub-areas were sampled: one that had been directly disturbed by a BIE-experiment (Radziejewska, 2002), one that had been impacted by the plume and one control, undisturbed area. These levels of sampling stratification are however out of the scope of the present study, which focuses on variations between contract areas. After checking that there was no statistically significant difference on the abundance and richness of polychaetes between sub-areas, all samples within an area were deemed representative of that area and considered as replicate samples. The level of replication within areas accordingly varied as a function of sampling stratification. The aim was to collect a minimum of five replicate samples per strata but due to sampling failures and time constraints, it couldn't be systematically achieved (Table 1)."

Similarly no use has been made of the data on species distribution with respect to sediment depth.

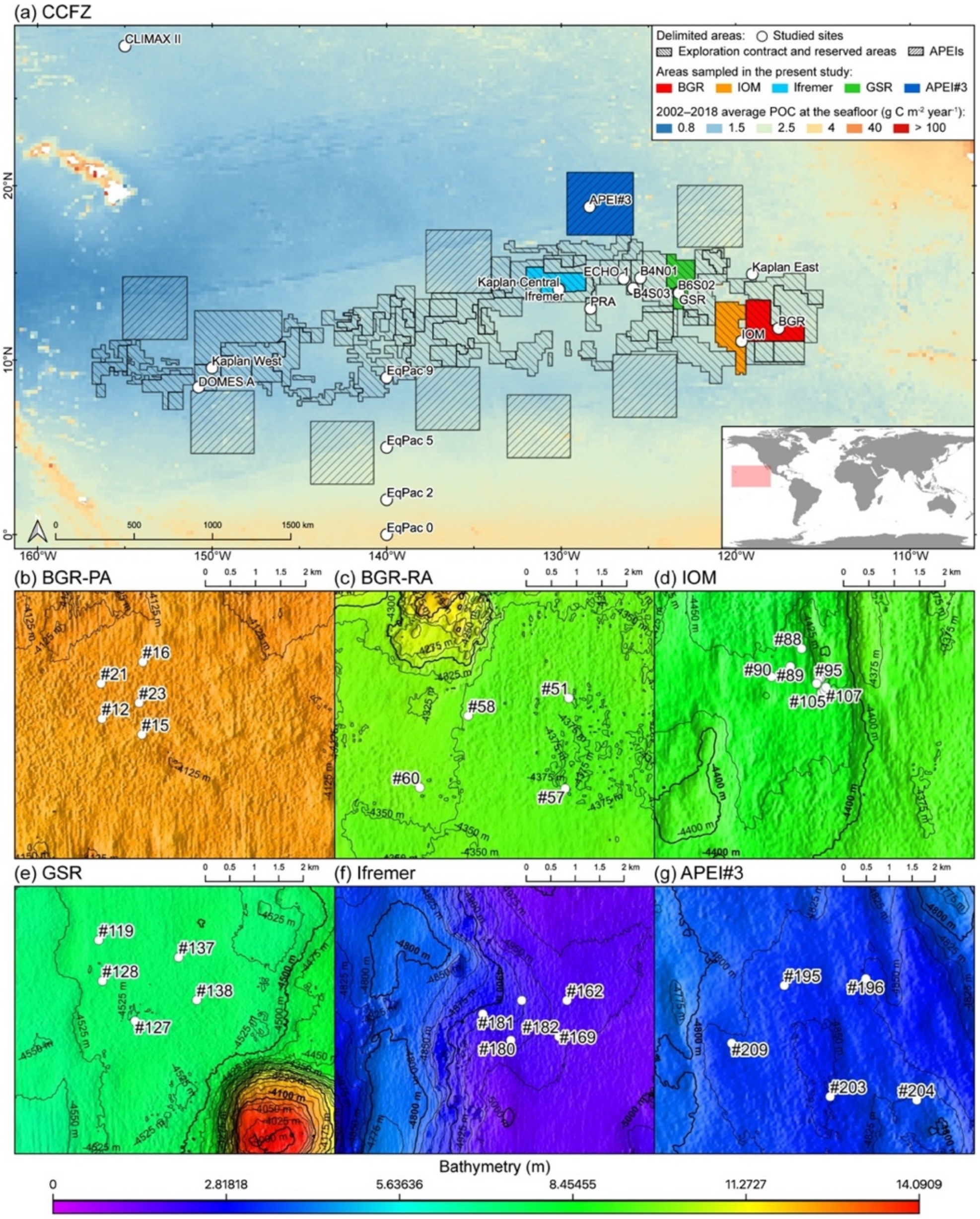
We considered that the vertical distribution of the species was not relevant to the questions we asked. Moreover, patterns in vertical distribution are difficult to test because sub-samples from each sediment layer can hardly be considered as independent samples, which violates the most basic assumption of all statistical tests.

There should be an enlargement of the sampled area in Fig. 1 to allow for the individual core stations to be plotted. A section on the differences between samples taken within an individual area would be beneficial, since a lot of information on the ecology has been lost by only comparing means for each area.

Figure 1 (below) has been modified to show the distribution of individual core stations within area or sub-area. The caption has been modified accordingly:

From "Figure 1. Map of the nodule exploration contract, reserved areas and areas of particular interest (APEI) in the Clarion-Clipperton Fracture Zone (CCFZ) showing the sampling areas from this study and previous macrobenthic surveys. The areas sampled during the SO239 cruises are shown in color. The background map shows average particulate organic carbon (POC) flux at the seafloor during the 2002–2018 period."

To "Figure 1. (a) Map of the nodule exploration contracts, reserved areas and Areas of Particular Environmental Interest (APEI) in the Clarion-Clipperton Fracture Zone (CCFZ) showing the sampling areas from this study (in color) and previous macrobenthic surveys ; the background map shows the average particulate organic carbon (POC) flux at seafloor during the 2002-2018 period. The areas sampled during the SO239 cruises are enlarged in following figures: BGR (b and c), IOM (d), GSR (e), Ifremer (f) and APEI#3 (g); with detailed local hydroacoustic maps based on multibeam system EM122 (Martínez Arbizu and Haeckel, 2015; Greinert, 2016) in background."



Sampling strategy

The sampling strategies need to be clearly explained with reference to the following questions, either by amplification of the text or by giving a references.

1. Since there are 9 APEIs, why was the only one sampled from an oligotrophic area when the exploration blocks sampled were all in mesotrophic areas?

The question was about the representativeness of APEIs given the fact that these APEIs were pushed at the periphery and even beyond the Fracture Zones that delimitate the nodule province and thus into a different productivity regime. Two APEIs could potentially be sampled APEI#3 and APEI#6. APEI#6 was sampled by other teams in the framework of other projects the same year as the SO239 cruise– in order to avoid duplication of efforts and increase knowledge on APEIs, the APEI-3 was sampled during SO239.

2. Within each block how was it decided where to sample, given the problem of determining the geographic range of species? [see Wilson (2017) for one approach to this.]

The sampling strategy resulted from a combination of objectives that have been explicated in the text (Page 4 line 27 – page 5 line 6):

The sampling strategy resulted from a combination of objectives that were unique to each area, together with the overarching aim of describing alpha and beta diversity patterns across a productivity gradient that included both contract areas for nodule exploration and an APEI (Martínez Arbizu and Haeckel, 2015). In the BGR area, two sub-areas were sampled: a Prospective Area (PA) that could be mined in the future and a Reference Area (RA) that could serve as a preservation area. In the IOM area, three sub-areas were sampled: one that had been directly disturbed by a BIE-experiment (Radziejewska, 2002), one that had been impacted by the plume and one control, undisturbed area. These levels of sampling stratification are however out of the scope of the present study, which focuses on variations between contract areas. After checking that there was no statistically significant difference on the abundance and richness of polychaetes between sub-areas, all samples within an area were deemed representative of that area and considered as replicate samples. The level of replication within areas accordingly varied as a function of sampling stratification. The aim was to collect a minimum of five replicate samples per strata but due to sampling failures and time constraints, it couldn't be systematically achieved (Table 1).

3. Given the known high percentage of species represented by only a single individual in box core samples from red clay polymetallic nodule areas (Hessler & Jumars, 1974 and several later papers), why were such a small number of box cores taken in each area, as opposed to taking the same overall number of box core samples from fewer areas.

The SO239 cruise was a multidisciplinary cruise in the framework of a collaborative European project. The sampling strategy is thus a tradeoff between multiple objectives, optimization of transit time and adaptive management. As explained above, the sampling design within area was guided by specific objectives for each of the area. The sampling effort was constrained by ship time even though the cruise lasted for 50 days, which is close to the endurance of the RV Sonne.

4. Why was it decided (apparently post-sampling) to treat every box core sample from a single area as a replicate, given the geochemical differences within some of the areas and also differences in the number, size and depth distribution of the polymetallic nodules in box cores from the same area?

The reason for considering box core sample from a single area as replicates has been explained above. It should be noted here that variations in organic content and sediment grain size were low within each area (see Table below from Hauquier et al. 2019). Variations in nodule density were high in some instances but the influence of nodule density on the abundance, richness and composition of polychaete assemblages was assessed (Figure 3 and Figure 7).

	CPE (µg ml^{-1})	TN (weight %)	TOC (weight %)	clay (%)	silt (%)
APEI-3	0.06 ± 0.00	0.10 ± 0.00	0.29 ± 0.02	35.48 ± 5.40	61.63 ± 4.91
IFREMER	0.08 ± 0.02	0.12 ± 0.03	0.40 ± 0.07	15.41 ± 1.77	71.89 ± 3.80
GSR	0.11 ± 0.05	0.16 ± 0.07	0.47 ± 0.11	15.64 ± 1.66	70.89 ± 2.42
IOM	0.17 ± 0.03	0.12 ± 0.01	0.53 ± 0.12	10.74 ± 0.47	73.39 ± 0.89
BGR_RA	0.20 ± 0.09	0.10 ± 0.02	0.43 ± 0.12	11.21 ± 0.89	72.90 ± 1.27
BGR_PA	0.28 ± 0.11	0.12 ± 0.01	0.58 ± 0.08	12.21 ± 0.65	70.31 ± 3.01

5. Each box core was sliced into 0-3, 3-5 and 5-10 cm depth sections that were sieved separately. Why was this done when the data from each layer were then added for the data analysis? The slicing procedure is not described but when slicing box cores polychaetes are frequently fragmented. What precautions were taken that an individual was not counted more than once, for example by only counting head-ends.

The layering was mostly used in order to facilitate sieving and sorting. At the ECHO I site in the CCFZ, Spiess et al. (1987) reported that about 70% of the macrofauna was concentrated in the top water and 0-1 cm depth, while less than 10% was found in the 5-10 cm depth. To prevent double counting, we have only counted head-ends.

This has been clarified (page 5 lines 10-11):

From "The upper 10 cm of each core was sliced into three layers (0–3, 3–5 and 5–10 cm), each layer transferred into cold seawater and sieved using the same mesh size."

To "The upper 10 cm of each core was sliced into three layers (0–3, 3–5 and 5–10 cm) ***to facilitate sieving and sorting;*** *each layer was transferred into cold seawater (4 °C) and sieved using the same mesh size..."*

A precision about the counting head-ends was added page 6 lines 9-10:

From "Preserved specimens were examined under a Leica M125 stereomicroscope and a Nikon Eclipse E400 microscope and morphologically identified..."

To "Preserved specimens were examined under a Leica M125 stereomicroscope and a Nikon Eclipse E400 microscope, ***counted (anterior-ends only)*** *and morphologically identified ..."*

6. The banked data shows that, although most individuals were found in the 0-3 cm layer, in some cores over 20% of the individuals were present in the 5-10 cm layer. It is therefore reasonable to assume that an unknown fraction of the biodiversity was lost in the samples because the cores were not sampled for macrofauna deeper than 10 cm. In some deep-sea sediments (at > 2000 m) polychaetes are known to penetrate over 100 cm below the sediment surface and the major infaunal biomass can often be found below 10 cm sediment depth. Given that some box cores were sampled below 10 cm depth, since nodules were recorded at 25 cm depth, why is no mention made of animals being present below 10 cm? Can the authors cite any reference to deeper sampling for infauna in the working areas? It might have been assumed that only the upper 10 cm of sediment would be disturbed by

nodule harvesting, however most nodule-mining prototypes have been based on bottom crawlers that would cause sediment compression and affect deeper-burrowing organisms.

Hessler and Jumars (1974) sampled the CCFZ macrofauna down to 20 cm but did not report on the vertical distribution of the fauna. Since then, we are not aware of any study that sampled the macrofauna below 10 cm in the CCFZ and in its recommendations for contractors, the International Seabed Authority also suggest to sample down to 10 cm. In fact, the 5-10 cm layer of sediment is already very sticky with little evidence of bioturbation and difficult to sieve. After sample processing, the box-corers were emptied by hand. Nodules were occasionally found buried in sediments, but this was rare. These buried nodules won't be a target for the mining industry. Yet, on one occasion a large maldanid polychaete was found at about 50 cm depth. We thus agree that large burrowers can live below 10 cm but their densities are so low that a box-core sample is too small to provide a precise and accurate estimate of these populations. For all these reasons, we followed the widely use standard of sampling down to 10 cm only.

7. Surface polymetallic nodules do penetrate the sediment to a degree. We are not told now deep this was in the different cores. Even if it was only 1 cm, given the large number and size of the nodules in some cores (see photographs in the cruise report) this would greatly decrease the volume of sediment available for sieving in the 0-3 cm layer and bias the results, considering that all cores were equated only on an area. The fauna results should also be considered with respect to sediment volume.

We agree that quantifying the volume occupied by nodules would have been an important factor to consider. The depth penetration, the size and area covered by the nodules were not assessed during the SO239 cruise, but we acknowledge that this should be done (and in fact has been done during a subsequent cruise).

The following phrase was added in the Discussion to point this out page 11 lines 14-15:

"In our study, the volume and surface occupied by nodules were not quantified but the positive relationship between nodule density and polychaete abundance shows that space is not a limiting factor for polychaetes."

8. It is unclear what happened to animals collected when the nodules were washed free of sediment. Were these animals added to the 0-3 cm layer and if so was this done before picking animals off the nodules?

The larger epifauna attached to the nodules was immediately picked up before nodules were removed from box core sample. The nodules were then gently washed with cold sea water to remove most of the sediment sticking to the nodules and possible associated fauna. The smaller sessile fauna remained attached to the nodules and was processed on its own. The sediments washed from the nodules were added to the 0-3 cm layer.

The text has been changed to precise sample processing (page 5 lines 7-16):

From "The overlying water column was siphoned and filtered using a sieve of 300 µm of mesh size. The box core sample surface was photographed, and all nodules picked up from the sediment surface, washed and individually measured and weighed. The upper 10 cm of each core was sliced into three layers (0–3, 3–5 and 5–10 cm), each layer transferred into cold seawater and sieved using the same mesh size. The overlying water residue and the 0–3 cm layer were immediately sieved in the cold room with cold seawater (4 °C) and then live-sorted. All polychaete specimens were photographed, individualized and preserved in cold (-20 °C) 80 % ethanol and then kept at -20 °C (DNA-friendly). The 0–3 cm residue, 3–5 and 5–

10 cm layers were fixed in formalin for 48 to 96 h and preserved in 96 % ethanol and later sorted in the laboratory (not DNA-friendly). The sieve residues from the overlying water and the washed nodules were combined with all layers for the community analysis."

To "The overlying water was siphoned and sieved using a sieve of 300 µm of mesh size. The box core sample surface was photographed, and all nodules picked up from the sediment surface, washed with cold seawater over a 300 µm-mesh sieve and individually weighed. ***Sessile polychaetes, if present, remained attached to the nodules and were not considered in this study.*** *The upper 10 cm of each core was sliced into three layers (0–3, 3–5 and 5–10 cm) to facilitate sieving and sorting; each layer was transferred into cold seawater (4 °C) and sieved using the same mesh size. The 0–3 cm layer was immediately sieved in the cold room with cold seawater (4 °C).* ***The sieve residues from the overlying water and nodule washing were added to the 0-3 cm layer and live-sorted.*** *All polychaete specimens were photographed, individualized and preserved in cold (-20 °C) 80 % ethanol and then kept at -20 °C (DNA-friendly). The 0–3 cm residue, 3–5 and 5–10 cm layers were fixed in formalin for 48 to 96 h and preserved in 96 % ethanol and later sorted in the laboratory (not DNA-friendly). All layers were combined for the community analysis."*

9. What happened to the animals picked off the nodules, were these treated as epifauna and not considered here? Some serpulids are included in the species listed in the dataset – were these all epifaunal on the nodules?

Yes, the sessile epifauna, including sessile polychaetes such as serpulids, has been treated separately and are not considered in this study. The serpulids present in the samples can be from nodule washing residue.

The following sentence was added page 5 lines 9-10:
"Sessile polychaetes, if present, remained attached to the nodules and were not considered in this study."

10. Polychaetes are known to occur as infauna within the polymetallic nodules (Thiel et. al. 1993). Some polychaete species were only found in crevices within the nodules and knowledge of differences in species composition in nodules from the different exploration blocks would be important information. Was this part of the infauna sampled? Will there be a separate publication dealing with the nodule-associated fauna, since the present manuscript does not cover the habitat of the species within the core samples?

No, the nodule crevice fauna was not sampled during this cruise. We recognize that this is a neglected component of the benthos. Sampling the crevice infauna requires to break up the nodules, this was not done during the cruise.

11. Both the biomass and the biovolume of the infauna can affect the geochemistry, were these measured?

Each polychaete specimen was sized and those measures could be used to calculate a biovolume. Conversion to biomass however is a rough approximation due to the large number of damaged specimens and not included in this paper.

Specific Comments

Table 2: the data should be checked and the header clarified. Does the data refer to only polychaetes or all macroinfauna? For example, Table 7 in Wilson (2017) gives a mean polychaete density of 21 individuals 0.25 m-2 for Domes A compared with the 16 given in the present Table 2. It is also necessary to know if like is being compared with like with

respect to combined fractions of samples from the box cores. Since most of the previous studies did not look for cryptic species I think you should give the number of morphologically identified species in parenthesis for the sites recorded in the present study.

Data in Table 2 refer to polychaetes only. Glover et al. (2002) and Wilson (2017) published results from the same dataset, except that for DOMES A, Glover et al. have considered 47 box cores while Wilson has considered 41 box cores, which may explain the differences in mean polychaete density between the two studies. In Table 2 of our manuscript, data for DOMES A and ECHO 1 were taken from Glover et al. (2002), except for Bootstrap values, which were taken from Wilson (2017).

Rows were added in Table 2, for each of the three sites DOMES A, PRA and ECHO 1 in order to make the difference between data taken from Glover et al., 2002 and data taken from Wilson (2017). The correspondent rows have been changed, from:

Area	**Year**	**References**	**Depth (m)**	**Latitude**	**Longitude**	**Number of box cores**	**Mean abundance (ind. 0.25 m^{-2})**	**Total number of species**	**ES163**	**Bootstrap**	**2002–2018 average POC at the seafloor (g C m^{-2} year^{-1})**
DOMES A	1977/78	Glover et al. (2002); Wilson (2017)	5100	8.45	-150.78333	47	16	104	56	203 (based on 41 box cores)	1.46
PRA	1989	Glover et al. (2002); Wilson (2017)	4800	12.95	-128.31667	16	65	100	47	310	2.04
ECHO 1	1982	Glover et al. (2002); Wilson (2017)	4500	14.6666667	-126.41667	15	42	113	60	274 (based on 14 box cores)	2.05

To:

Area	**Year**	**References**	**Depth (m)**	**Latitude**	**Longitude**	**Number of box cores**	**Mean abundance (ind. 0.25 m^{-2})**	**Total number of species**	**ES163**	**Bootstrap**	**2002–2018 average POC at the seafloor (g C m^{-2} year^{-1})**
DOMES A	1977/78	Glover et al. (2002)	5100	8.45	-150.78333	47	16	104	56		1.46
		Wilson (2017)				41				203	
PRA	1989	Glover et al. (2002)	4800	12.95	-128.31667	16	65	100	47		2.04
		Wilson (2017)								310	
ECHO 1	1982	Glover et al. (2002)	4500	14.6666667	-126.41667	15	42	113	60		2.05
		Wilson (2017)				14				274	

Figure 3 needs a lot more explanation and labelling. Below the diagonal some of the plots appear to use mean data from areas and others data from individual cores without a clear explanation.

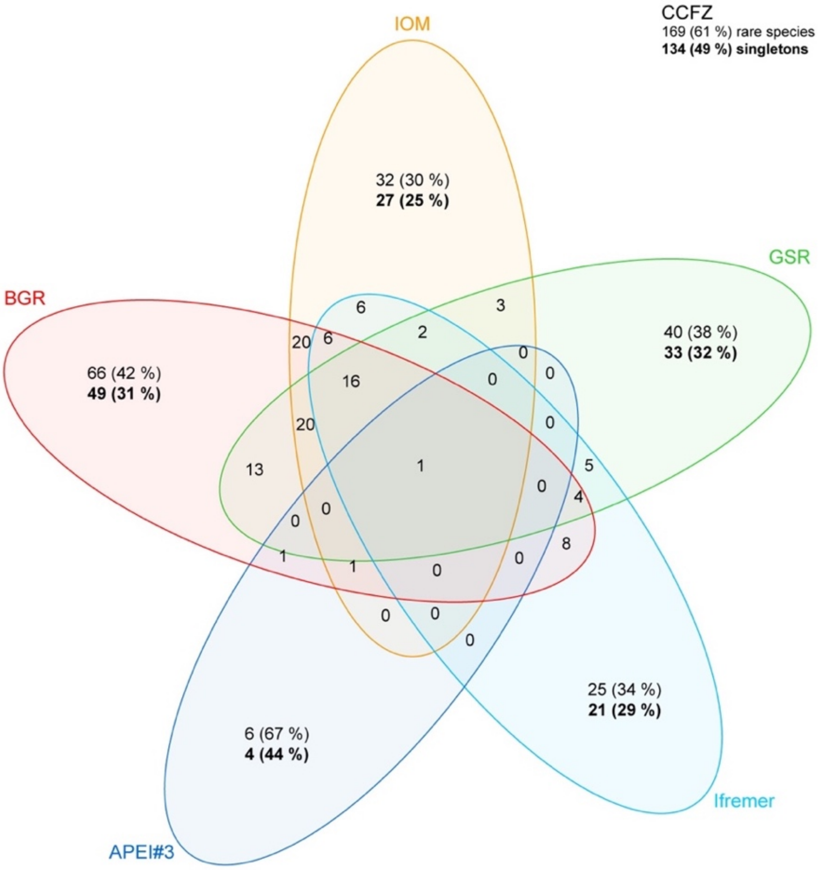
Indeed, to make it clearer the Figure 3 has been separated in two: (a) by box-core samples and (b) by area. Thus, the caption of Figure 3 has been changed:

From "Figure 3. Correlation matrix between biotic and abiotic variables from sampled areas within the eastern CCFZ. Diagonal panels show the distribution frequency of values for each variable. Below-the-diagonal panels show the correlation plot between pairs of variables. Above-the-diagonal panels show the Spearman coefficient correlations between pairs of variables. "" indicates p < 0.05, "**" p < 0.01 and "***" p < 0.001."*

To "Figure 3. Correlation matrix between biotic and abiotic variables from sampled areas within the eastern CCFZ. Diagonal panels show the distribution frequency of values for each variable. Below-the-diagonal panels show the correlation plot between pairs of variables. Above-the-diagonal panels show the Spearman coefficient correlations between pairs of variables. ***Abundance, richness and nodule density per box-core (a) and average biotic and abiotic variables per area (b).*** *POC Eastern values provided by Volz et al. (2018); POC NE Pacific values were estimated in the present study. "*" indicates p < 0.05, "**" p < 0.01 and "***" p < 0.001."*

In Figures 7 and 9 yellow text does not show well on a white background – use a coloured background for the text.

The yellow has been replaced by a dark orange in all figures to better highlight the graph and texts. See for example Figure 9 below as an example:



Conclusions: The first paragraph does not belong here – it should be in the introduction.

This has deleted from Conclusions but partially integrated into Introduction (page 4 lines 3-10) as suggested.

Supplementary data: It would be useful to have a species list available as a supplement to the paper, although I realise that the taxonomic studies are ongoing.

We agree that a species list would be quite helpful, but about 90% of the polychaete species in the CCFZ are new to Science (Glover et al., 2002). The task of identifying and then naming most of the 275 morphotypes found in this study is thus huge and out of the scope of this paper. The best we can do is to provide the list of morphospecies together with their DNA barcodes when available. This information are available as supplementary material on PANGEA in the abundance file.

The following sentence was added in the beginning of Results to clarify the availability of the dataset page 8 lines 23-24: "The dataset has been archived in the information system PANGAEA and is available in open access (Bonifácio et al., 2019)."

References

Hessler, R. R. & Jumars, P. A. (1974) Abyssal community analysis from replicate box cores in the central North Pacific, Deep SeaRes., 21: 185-209

Mullineaux, L. (1989) Vertical distributions of the epifauna on manganese nodules: implications for settlement and feeding. Limnol. Oceanog., 32: 1247-1262

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Radziejewska, T.: Responses of deep-sea meiobenthic communities to sediment disturbance simulating effects of polymetallic nodule mining, Int. Rev. Hydrobiol., 87, 457-477, 2002.

Spiess, F.N., Hessler, R., Wilson, G., Weydert, M.:Environ- mental effects of deep-sea dredging. SIO Reference Report 87-5, Scripps Inst Oceanogr, San Diego, 1987.

Wilson, G. D. F.: Macrofauna abundance, species diversity and turnover at three sites in the Clipperton-Clarion Fracture Zone, Mar. Biodivers., 47, 323-347, 10.1007/s12526-016-0609-8, 2017.

Author’s response to comments of Referee #2

The scientific methods are valid and up-to-date and the experiments and calculations are adequately described. The authors acknowledge related work and clearly discriminate their data from data obtained by others. In some cases, where they discuss their data (e.g., regarding winners and loosers of the ‘competition’ of meio- and macro-fauna) in relation to that of others they should be more specific about the exact content of the cited data so the reader can better comprehend the authors’ discussions and conclusions. The data presented are sufficient to reach conclusions part of which – and that is not criticizing the ambitions work behind this study – are pointing out gaps in knowledge. The paper is well structured and reads mostly fine – in some parts of the results the language may seem a bit repetitive (abundances and species numbers are presented with almost the same wording). The abstract provides a good summary. In the way it is currently presented, the first part of the discussion (about the relative success of meio an macrofauna in different deep-sea environments) should be either significantly reduced or more specific with a better and quantitative presentation of data on Meiofauna from the study area from other studies. I would vote for a reduction of that part as it seems to be a bit off the main focus anyway.

To me, the main shortcoming of the paper is, that the authors are very carefully when stating their conclusions and overcautious if it’s about the consequences of their findings. As the work touches societal concerns and areas of strong debates the authors should address in more depth the implication of their work regarding procedures and management of nodule mining.

In our opinion, the main message of this study is that we don’t know enough about species diversity and species ranges to be conclusive about the potential impact of nodule mining. We fully understand the need and eagerness to get answers to acute societal concerns, but we must be cautious of not over-interpreting the results of this study. Yet we agree that there are some questions that we can address and recommendations that can be made. In the following lines we will try to address the concerns where we can or explain why we can’t.

e.g., can we decide on regional management based on the available knowledge and are the APEIs appropriate as they are?

The answer to these questions is ‘No’ but not for scientific reasons. The location of the APEIs has been initially defined based on available knowledge (see http://www.soest.hawaii.edu/oceanography/faculty/csmith/MPA_webpage/MPAindex.html) and then severely constrained by contractor areas and reserved areas. A comparison of Figure 3 in Wedding et al. 2013 with Figure 1 in Lodge et al. 2014 clearly shows the difference between recommendations and implementation.

This has been clarified in the Introduction (page 3 lines 17-28):

From ‘Due to the paucity of biological data in the CCFZ, the spatial management plan was designed mainly based on nitrogen flux at 100 m depth (a proxy for trophic inputs to the seafloor), modeled nodule densities, the distribution of large seamounts and the dispersal distances of shallow water taxa (Wedding et al., 2013). The nine proposed 400 x 400 km managed (non-mining) areas were included in the regional management plan for the CCFZ and designated as APEIs (Lodge et al., 2014). Most of the CCFZ however has already been preempted to current exploration contracts and areas reserved for future exploration. The distribution of APEIs at the periphery of the CCFZ thus deviates from an optimal design.’

To "Due to the paucity of biological data in the CCFZ, the recommendations issued by Wedding et al. (2013) for the design of a network of protected areas were mainly based on nitrogen flux at 100 m depth (a proxy for trophic inputs to the seafloor), modeled nodule densities, the distribution of large seamounts and the dispersal distances of shallow water taxa. ***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. As a result, Wedding et al. (2013) divided the spatial domain of the CCFZ into 3 x 3 subregions and suggested to create one large no-mining area in each subregion. The size of the no-mining areas was defined with the aim of maintaining viable population sizes for species potentially restricted to a subregion, taking into account the inferred dispersal distances of species and of the plumes created by nodule mining (Wedding et al., 2013). Those principles were implemented in the regional management plan for the CCFZ, which resulted in the designation of 9 APEIs (Lodge et al., 2014).*** *Most of the CCFZ however had already been preempted to current exploration contracts and areas reserved for future exploration. Consequently, the APEIs were located at the periphery of the CCFZ thus deviating from an optimal design.*

Are there any specific recommendations that can be provided, e.g., regarding the size or arrangement of mining patches?

It would be premature to provide specific recommendations, first because the level of confidence on our estimates of species ranges is too low, second because polychaetes may not have the smallest species ranges (see also below).

What should be the focus of future studies and what would be the expected effort needed to come to scientifically sound conclusions?

In an ideal world, 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.

The conclusions part is so far rather summarizing what has been stated already before and may be a good place to discuss these things. The fact, that those discussions are rather limited in the current version of the manuscript is the reason for my general proposition that the manuscript should undergo a major revision before publication.

Below some more detailed comments

MAJOR ISSUES – IMPLICATIONS FOR MINING / MINING REGULATION

Regarding the main shortcoming of the paper mentioned above I urge the authors to significantly extend the discussion of their results towards the implications of their work with regard to nodule mining and its regulation. This could be distributed in several parts of the discussion as well as in a separate section in the discussion or in the conclusions. This, of course, has to be done with some caution to not extend beyond the scope of the study and has to take into account that this is a scientific publication and not a policy paper. However, it is clear that the motivation of the study – and certainly of societies providing the funding for mining-related investigations these days – is to provide the basis for scientifically sound procedures and decisions regarding deep-sea mineral exploration and exploitation. This should be better reflected in the text. This includes recommendations regarding the management and regulations - where the data of the study allow this - but also specific requests for future investigations where the results reveal significant gaps. Below I am providing some examples where I think the discussion needs to move beyond where it currently terminates.

Page 3, line 15/16 'The distribution of APEIs at the periphery of the CCFZ thus deviates from an optimal design.' Page 11, line 20-23 'The biogeochemical settings as well as the biological patterns of the three size groups of the benthic fauna thus converge to conclude that the structure and functioning of the benthic ecosystem in APEI#3 is not representative of any of the four exploration contract areas included in this study.' Page 12, line 26-29 'The influence of the fracture zones on the dispersal of the abyssal fauna remains to be better understood as the Clarion and Clipperton fractures may act as a barrier for species with low dispersal abilities such as infaunal brooders. If so, the representativeness of seven out of the nine APEIs, which are partly lying beyond the fractures, may be questionable.' If these statements hold true, the concept of APEIs and the regional management plan as a whole don't seem to be appropriate.

What is the advice of the authors to overcome this problem?

As for now, this is a hypothesis that needs to be further tested. Our main advice is to foster research in the APEIs and to support this research we propose the future Environmental Compensation Fund to be created by the regulations on exploitation of mineral resources in the Area. This recommendation has been added to the Conclusions:

Pages 16 lines 21-24:

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).

What do we know about the other APEIs and how their environmental conditions and faunal communities compare to license areas?

Studies on other APEIs are ongoing but few results have been published yet. Simon-Lledó et al. (2019a, 2019b) recently described megafaunal community patterns as a function of seafloor heterogeneity and nodule density from imagery surveys. Comparisons with similar megafaunal surveys undertaken in contract areas is however difficult due to a current lack of standardization of methods, both for the surveys and the image-based taxonomy.

What would be an optimal APEI layout and how would you – from the results of your study – address the question whether an area is suited as APEI or not.

Our results suggest that the boundaries of the management area and sub-regions used by Wedding et al. (2013) could be improved but do not contradict the conceptual bases of the current management plan. Again, the implementation rather than the design of APEIs is problematic. APEIs had several conservation objectives, including the maintenance of sustainable and healthy populations of minimum viable sizes and a full range of habitat types. The topic of the representativeness of the APEIs is too broad to be addressed here.

Can we use some easily measured sedimentological (grainsize?) or biogeochemical measurement to assess the probability that an APEI will host similar faunal communities than a specific license area? Should the assessment of correlations of habitat characteristics and fauna in APEIs be a focus of future studies?

In our constrained multivariate analysis (RDA), the environmental factors that were available explained 13% of the local and regional variations in polychaete community composition. The explanatory power of the model is low and could certainly be improved to some extent by a better understanding of the physico-chemical niche of species. However, the main unknown is most likely about the biology and biotic interactions of species: how long do they live, how do they reproduce and disperse, do they interact and how are they interacting between others. These would be key questions to answer, although much more challenging than looking at correlations of abiotic factors and biological variables.

If you consider the lack of knowledge potentially only a few years before exploitation commences: Should the ISA setup a scheme by which contractors carry out or fund baseline studies in the APEIs?

There is not such funding mechanism in the mining code for exploration nor recommendations towards contractors to carry out baseline surveys beyond their contracted area and it's beyond our expertise to assess whether such a mechanism could be implemented in the framework of current exploration contracts. The draft regulations on exploitation of mineral resources in the Area provides for the establishment of an Environmental Compensation Fund (ISBA/25/C/WP.1). The purpose of the Fund does not include the promotion of baseline studies in the APEIs but this is a recommendation we can make.

The following lines were added in conclusion (page 16 lines 20-24):

'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).'

One consideration that lead to the APEIs' current position outside the area covered by license areas was to allow for very large areas. In light of the fact that, according to the current planning, only part of the license areas will be used for nodule extraction and the seemingly low species' ranges: do we need APEIs to conserve biodiversity or would the areas inside the patch of license areas, that are not mined do the job? Or do we need APEIs somewhere else, e.g., smaller ones between license areas?

In the current draft regulations on exploitation of mineral resources in the Area, a plan of work in the case nodule mining shall not exceed 75000 km² (ISBA/25/C/WP.1), which is the size of the exploration contracts areas. Thus, in the current planning, the only area that won't be mined are Preservation Reference Zones (PRZ) and non-mineable areas (slopes, no-nodule, etc…). Since the PRZ haven't been clearly defined yet and since non-minable area do not represent the full range of habitats in the CCFZ, and especially not the most threatened habitats, we believe that APEIs are required.

In addition, APEIs are very large for two reasons: 1) To allow for the self-sustainability of populations within the APEI. An alternative would be to create a higher number of smaller inter-connected APEI but we lack data on dispersal range of species, which is different from the geographic range of species, to discuss this alternate design. 2) Avoid the impact of the sediment plume, again we don't have the data to discuss the relevance of this point.

Page 13, line 1-5 'However, based on the best knowledge we have, our study suggests that [. . .] nodule mining would affect each year an area that is equivalent to the average geographic range of a polychaete species.' Spatial ranges – especially if they are indeed that small – are highly relevant.

Can we use polychaetes as key species here or would we need to have similar data also for other size classes and other groups of macrofauna? What data are available already?

Polychaetes are the most abundant and most diverse among the macrofauna. Polychaete might however be less threatened than peracarids, which are brooders and show narrower species ranges on average. Polychaetes might also be less functionally important than nematodes, which dominate the metazoan biomass or foraminifera, off which we know very little. If the aim is to monitor and preserve all levels of biological diversity, from gene, to species, to functions then polychaetes are likely not enough. The good news is, numerous studies have recently been undertaken in the CCFZ and are still going on. Some have been published recently but there is still a lot come. Our knowledge of benthic biodiversity in the CCFZ is going to significantly increase in the years to come.

What are the implications of theses results for mining operations and their regulation? Do we need more research to understand whether the estimated spatial range is really true or just mirrors the inappropriate sampling effort available scientific knowledge is based upon? Or do we 'know enough' and could provide specific suggestions as for how to spatially arrange mined patches? Taking this further: if we take the precautionary approach seriously: if we have an average species range of 20km (and, for some of the species obviously a smaller one) wouldn't we need to restrict the mining operations by contractors including secondary impacts by the plume to that size until it is proven, that the high turnover of beta diversity is an artifact of undersampling?

Indeed, if the species ranges are narrow AND if the environmental objectives are to avoid species extinction then the spatial footprint of mining impact would need to be severely restricted. This is the meaning of the sentence "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". "If true" in this sentence refers to the fact that we cannot yet exclude that the average species range that we have estimated is biased by singletons. Thus, with the data we have we can't provide specific suggestions regarding the spatial arrangement of mining. That's what we meant by "The assessment of potential risks and scales of biodiversity loss thus requires an appropriate inventory of species richness in the CCFZ."

We further underlined the uncertainty regarding species range in the conclusion, page 16 lines 31-32:

"non-parametric estimators of species richness suggest that total species richness across the five study areas does not exceed 498 species which likely implies a species range much larger than 25 km."

Page 15, line 27/28 'The assessment of potential risks and scales of biodiversity loss thus requires an appropriate inventory of species richness in the CCFZ.' While the conclusions are basically a summary up to this point, this is where the discussion in implications starts: How should this goal should be achieved?

As outlined above we can suggest 1) a stratified random sampling at nested scales, from region down to seascapes and 2) an intensive sampling of selected habitats up to the point where the number of singletons decreases with sample size. We agree that these general *recommendations would need to be more specific. There is a need to carefully think the sampling design and sampling effort together with statisticians. This would be a topic for another paper.*

We can already say is that it won't be cheap. And we can also paraphrase Coddington et al. (2009) here and share their hopes "we suggest that inventory analyses continue to assess undersampling bias in order to justify the budgets required to obtain adequate data. Funding sources and consumers of these essential data can scarcely argue that inadequate results are acceptable. If results continue to demonstrate that much greater sampling intensities are required, such will eventually become the norm, rather than the exception."

The following lines were 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.

How much of this work is, according to the knowledge of the authors, already done by baseline work of the different contractors and just needs metaanalysis of the pooled contractor's data (e.g., by an independent scientific consortium)? Or are samples and data lacking and more dedicated sampling campaigns needed? What effort would this take? Or - if that is too hard to estimate, how would you control if enough data are available (based on rarefaction curves? Based on biodiversity descriptors merging?)? If you compare this to what is found in the ISA regulations and guidelines: how does that compare?

This is a good point. Significant progress has been made during the last 5 years. Stratified random sampling has been carried out in the framework of the European project MIDAS (e.g. Simon-Lledo et al., 2019a, 2019b for megafaunal communities) and contractors are producing a large amount of data on macrofaunal communities in the Eastern CCFZ (e.g BGR: Janssen et al., 2015; GSR: De Smet et al., 2017; UKSR: Glover et al, 2016). A meta-analysis is going to be conducted that should provide insight onto species richness and species ranges (https://www.isa.org.jm/news/deep-ccz-biodiversity-synthesis-workshop). By the end of this Deep CCZ Biodiversity Synthesis we should be able to tell where we collectively stand in terms of what we know and what we don't know. In order to provide an accurate estimate of species richness, we would look for a decreasing trend in the accumulation curve of singletons.

What about the key-species concept? Could that become appropriated once the necessary knowledge was obtained or do you think we always have to cope with the full complexity when we want to address environmental impacts of deep-sea operations (assessment of the risks prior to operations, assessment of impacts happening during operations).

We must stress here that there is a difference between studies aiming at assessing the potential risks of mining and studies aiming at monitoring the impact of mining (i.e. Environmental Impact Assessment, EIA). The key species concept would apply to the EIA, which is beyond the scope of our study.

OTHER MAJOR ISSUES

Page line 3-27 The discussion of meiofauna in nodule areas comes as a bit of a surprise in the context of this paper, that does only provide data on macrofauna. If you want to leave this so prominent and detailed, you should first state what the data show. Does the Pape et al. study provides data from the same station so a quantitative comparison to other deep sea areas is possible? > In this case I would suggest to provide that quantitative information here. Otherwise consider reducing the discussion or move it to a less prominent part of the discussion. Maybe you could also connect it more to the paper, e.g. as an argument for focusing environmental impact studies on macro-fauna because they seem more relevant in terms of biomass and ecosystem function as in typical abyssal areas.

Page 11 lines 8-22. All meiofauna discussion has been reduced as suggested:

From 'Food supply, sediment grain size and the density of nodules are the three main environmental factors that seem to drive the structure and composition of polychaete assemblages in the CCFZ.

Nodules have antagonistic influences on different size groups of benthic communities. Meiofaunal assemblages are less abundant in nodule-rich than in nodule-free sediments (Miljutina et al., 2010; Pape et al., 2018). Nodules however increase habitat heterogeneity, providing hard substrate for sessile organisms and generally enhancing the standing stocks of both sessile and vagile megafauna (Amon et al., 2016; Vanreusel et al., 2016; Simon-Lledó et al., 2019). Similarly, nodules seem to enhance macrofaunal density (De Smet et al., 2017) and diversity (Yu et al., 2018). Our results support the reported positive and significant relationship between polychaete abundance and nodule density (De Smet et al. (2017). The macrofauna in nodule fields may benefit from increased food supply and the release from competition with meiofauna. Nodules increase seafloor roughness, thereby increasing friction (Sternberg, 1970; Boudreau and Scott, 1978) and potentially sediment deposition rates. The large sessile suspension feeders may similarly enhance biodeposition (Graf and Rosenberg, 1997). Both processes may stabilize sediments and increase organic carbon supply as tube lawns do, for example (Michael et al., 2000). An increase in food supply may explain the higher densities of polychaetes in nodule-rich areas. The divergent response of meiofauna to the presence of nodules further suggests some sort of competition between meiofauna and macrofauna. The contribution of meiofauna to benthic biomass generally increases along a bathymetric gradient to outweigh that of macrofauna at abyssal depths (Thiel, 1975; Rex et al., 2006; Wei et al., 2010). This pattern is assumed to reflect a selective advantage for small size at very low levels of food input (Thiel, 1975, 1979; Sebens, 1982, 1987; Rex and Etter, 1998). Sibuet et al. (1989) reported however a linear relationship between meiofaunal and macrofaunal biomass at abyssal sites. Both size classes indeed co-varied with organic carbon burial flux, which suggests the occurrence of a dynamic equilibrium between meiofauna and macrofauna at abyssal depths. Due to its small size, meiofauna is likely more efficient at exploiting the low level of food input, but this interstitial fauna may also be more sensitive to high nodule coverage because its ambit is largely limited to superficial sediments. The opposite effects of nodule coverage on meiofaunal and macrofaunal densities may thus lie in a release from the advantage of being smaller in the abyss, inducing a shift in size-group equilibrium toward increased macrofaunal densities. These results suggest that nodule coverage have an influence on the functioning of the ecosystem, because it modifies biotic interactions and resource allocation among functional groups.'

To 'Food supply, sediment grain size and the density of nodules are the three main environmental factors that seem to drive the structure and composition of polychaete assemblages in the CCFZ.

The abundance and richness of polychaetes were positively correlated with nodule density, which is consistent with previous studies showing that nodules enhance macrofaunal densities and polychaete diversity (De Smet et al., 2017; Yu et al., 2018). Nodules may have antagonistic influences on different size groups of benthic communities. Meiofaunal assemblages are less abundant in nodule-rich than in nodule-free sediments, which may be due to the lower volume of sediment available in nodule areas (Miljutina et al., 2010; Hauquier et al. 2019). In our study, the volume and surface occupied by nodules were not quantified but the positive relationship between nodule density and polychaete abundance shows that space is not a limiting factor for polychaetes. Nodules also increase habitat heterogeneity, providing hard substrate for sessile organisms and generally enhancing the standing stocks of both sessile and vagile megafauna (Amon et al., 2016; Vanreusel et al., 2016; Simon-Lledó et al., 2019). Nodules increase seafloor roughness, thereby increasing friction (Sternberg, 1970; Boudreau and Scott, 1978) and potentially sediment deposition rates. The large sessile suspension feeders may similarly enhance biodeposition (Graf and Rosenberg, 1997). Both processes may decelerate water current, stabilizing sediments and, thus, increase organic carbon supply as polychaete tube lawns do, for example (Friedrichs et al., 2000). An increase in food supply may explain the higher densities of polychaetes in nodule-rich areas.'

Connected to this: Page 10, line 16 'The contribution of meiofauna to benthic biomass generally increases along a bathymetric gradient [. . .] which suggests the occurrence of a dynamic equilibrium between meiofauna and macrofauna at abyssal depths.' Again - this is very detailed for a study that does not focus on size class comparisons. I assume the Sibuet paper focuses on non-nodule areas? I understand you want to put forward that macrofauna is particularly important in the CCZ / in nodule areas as they – different to what was previously reported - show but a relative increase as compared to Meiofauna (i.e., neither do they show a relative decrease at depth as compared to meiofauna nor do they scale with meiofauna). This really would need a quantitative basis, i.e., a comparison of macrofauna abundances (better biomass) to meiofauna abundances at your study sites relative to other areas. > consider adding more quantitative information

This has been deleted following previous comment.

Connected to this: Page 10, line 23 'Due to its small size, meiofauna is likely more efficient at exploiting the low level of food input, but this interstitial fauna may also be more sensitive to high nodule coverage because its ambit is largely limited to superficial sediments.' Do you mean, that the meiofauna is restricted to the top layer where the available sediment volume is limited by the presence of nodules? I think you dont show the data but I assume that also for polychaetes the top sediment layer is the one where most individuals are found. In any way you could strengthen this idea by comparing your abundance vs. depth relationship with that of meiofauna in nodule areas. > please comment, explain and consider including this information in the manuscript

This has been deleted as previous comment.

Page 14, line 7/8 'Overall, the combination of high local diversity, unsaturated rarefaction curves, high levels of cryptic diversity and high rates of species turnover suggest that polychaete diversity in the CCFZ is large and vastly under-sampled.' It needs a discussion of the most appropriate technologies (sampling gear, analysis) and the expected effort it needs to raise our knowledge to a level appropriate to decide on mining (yes or no, spatial organization of operations and protected areas), and allow for scientifically sound impact assessment and management

Differences in sampling gear, type of preservation and how the diversity estimators consider the large presence of singletons were discussed along the Discussion. The expected efforts needed to reach an appropriate biodiversity assessment was suggested in the Conclusions with the following sentences:

Page 16 lines 20-24:

'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).'

And page 17 line 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. Both strategies are needed to assess the potential risks and scales of biodiversity loss due to nodule mining in the CCFZ'.

Maybe in this context it should also be discussed, if (and why!) the authors believe, that polychaetes may serve as a model group for baseline and impact assessments. Or is this just the 'pet group' of the authors and any other group should be similarly addressed before taking decisions? > please extend discussions to include these points.

Polychaetes are the most abundant and most diverse among the macrofauna. Polychaete might however be less threatened than peracarids, which are brooders and show narrower species ranges on average. Polychaetes might also be less functionally important than nematodes, which dominate the metazoan biomass or foraminifera, off which we know very little. If the aim is to monitor and preserve all levels of biological diversity, from gene, to species, to functions then polychaetes are likely not enough.

The following text was added in aim explaining the relevance of studying polychaetes:

Page 4 line 6-10

'To tackle these issues [study aims], we focused on polychaete assemblages. Polychaetes are the dominant and most diverse group of the macrofauna; they can be quantitatively sampled, and identify down to species level using a combination of morphological and molecular methods (Hessler and Jumars, 1974; Janssen et al., 2015; Wilson, 2017). Polychaetes also show a wide range of biological traits, from trophic behaviors to life history strategies, and play a major role in the functioning of benthic communities (Hutchings, 1998; Jumars et al., 2015).'

MINOR ISSUES

Page 1, line 1-3 Including the metaanalysis performed the study indeed addresses the entire CCZ. However, the stations of this study are all rather in the eastern part. > rephrase the title to not raise false expectations, e.g., by replacing ‘across the nodule province of the Clarion-Clipperton Fracture Zone’ with add ‘across the nodule province of the eastern Clarion-Clipperton Fracture Zone’

Page 1 lines 1-3. Done as suggested.

Page 1, line 17 ‘. . .the SO239 cruise aimed at improving species inventories. . .’. Was this really the subject of the cruise as a whole or of this expedition? > consider rephrasing

*Page 1 line 17. This has been changed to ‘. . . the SO239 cruise **provided data to improve** species inventories.’*

Page 2, line 6 ‘Only about 1 % of abyssal plains have been explored to date’. In this context of this paper I would restrict the use of the term ‘explore’ / ‘exploration’ to deep- sea mining-related activities > consider rewording

*Page 2 line 8. This has been changed to ‘Only about 1 % of abyssal plains have been **investigated** to date…’*

Page 2, line 7 ’In particular’ seems to connect to the previous sentence but in fact does not. > consider remove

Page 2 line 9. Done as suggested (removed).

Page 2, line 9 ‘. . .mainly manganese and iron,. . .’ > I would also mention copper, nickel and cobalt right away here - than you don’t have to repeat that in line 13/14.

*Page 2 lines 10-11. Done as suggested. It has been changed to ‘…, mainly manganese and iron **but also copper, nickel and cobalt …**’*

The repeated words were removed.

Page 2, line 15/16 ‘. . .the International Seabed Authority [. . .] is in charge of protecting fauna against any pollution or other hazards...’ Pollution is not the main concern in the context of nodule mining and expected impacts related to this study. > I would rephrase. Maybe just refer to harm (i.e., ’protecting fauna agains harm’)?

Page 2 line 19. Done as suggested.
*It has been changed to ‘. . .the International Seabed Authority [. . .] and is in charge of protecting fauna against any **harm**...’*

Page 2, line 25 I dont understand what is meant with ’scaling issue’. Is this referring to the uncertainties connected to effects of the full scale, long-term operations with large plumes as compared to single experimental tracks? > please rephrase / be more specific

*Page 2, line 31. This has been changed from ‘Beyond the scaling issue…’ to ‘Beyond the **unpredictable effects of the full-scale mining**…’*

Page 2, line 27 > replace ’the high diversity’ by ’a high diversity’

Page 3 line 1. Done.

Page 3, line 23 ‘test the hypotheses that support spatial conservation planning in the CCFZ’. I don’t think that these hypotheses (that the authors think would sever as guidance or that form the basis of the current regional management plan are explicitly stated somewhere in the publication. > consider being more specific here or state them elsewhere in the paper

Page 3 lines 20-22. The following sentence was added in Introduction: “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."*

Furthermore, we have reworded the aims (page 4 lines 3-6):

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 1/2 > replace '. . .were located between 4000 and 5000 m depth. . .' by 'had water depths between 4000 and 5000m'

Page 4 lines 19-20. Done as suggested.

Page 4, line 12/13 '. . .all nodules picked up from the sediment surface, washed and individually measured and weighed. . .' It should be mentioned already here that the water that was used for washing the nodules was sieved after washing. Have the nodules themselves been inspected for small polychaetes, e.g, living in tubes attached to the nodules? > rephrase and make sure to mention somewhere in the paper, if the data also include nodule-associated polychaetes

Page 5 lines 8-10. This has been changed and the nodule-associated polychaetes clarified:

From 'The box core sample surface was photographed, and all nodules picked up from the sediment surface, washed and individually measured and weighed.'

To 'The box core sample surface was photographed, and all nodules picked up from the sediment surface, ***washed with cold seawater over a 300 µm-mesh sieve*** *and individually weighed.* ***Sessile polychaetes, if present, remained attached to the nodules and were not considered in this study.'***

Page 4, line 18 '. . .The sieve residues from the overlying water and the washed nodules were combined with all layers for the community analysis...' Was the material combined (i.e., before analysis) or the data? > specify in the text

These layers (overlying water, the nodule washing water and the 0–3 cm layer) were combined after sieving, before sorting. It has been clarified in previous sentences (page 5 lines 13-14):

From 'The overlying water residue and the 0–3 cm layer were immediately sieved in the cold room with cold seawater (4 °C) and then live-sorted.'

To 'The sieve residues from ***the overlying water and nodule washing were added to the 0-3 cm layer and live-sorted****."*

The correspondent lines (page 5 line 16) cited by Referee #2 were changed:

From 'The sieve residues from the overlying water and the washed nodules were combined with all layers for the community analysis'

To 'All layers were combined for the community analysis.' making reference to all layers (overlaying water and washed nodules added to 0-3 cm; 3-5 and 5-10 cm).

Page 4, line 20/21 '. . .(see Section 2.3 DNA extraction, amplification, sequencing, and alignment)' No need to refer to a section that follows directly > remove

Done (removed).

Page 4, line 24/25 '. . .and 1600 bp of 18S genes. . .' ? Are 18S data really used in this study (I could not find it later on)? If not: restrict M&M to 16S and COI or discuss why that approach was not successful or not included in the analyses.

This is correct, the 18S data is not considered in the present study. However, we will available them concomitantly with the other genes. Because this, the methods used to amplify them must figure in the manuscript.

We have added (page 9 lines 17-19) the following sentence in the Results section to clarify: 'The 18S gene was sequenced for phylogenetic purposes on a restricted number of specimens. The 21 sequences of the 18S gene that have been obtained are mentioned here because they were archived concomitantly with COI and 16S sequences in GenBank and BOLD public datasets but they are not further considered in this study.'

Page 5, line 13 'To separate closely related species...' [...] observed between intraspecific and interspecific variations' What does 'closely related species' mean? Specimen that could not be discriminated based on morphology? > specify Page 5, line 13-17 To separate closely related species [...] observed between intraspecific and interspecific variations' This section is describing the principle not what acutally was done. This does not fully qualify for a Materials and Methods part > Move to another part of the study (introduction?) or rephrase.

Page 6 lines 11-12. This has been clarified with the addition of the suggested information and rephrasing:

From 'To separate closely related species, the principle of phylogenetic species was used, [...] observed between intraspecific and interspecific variations'

To 'We separated closely related ***species (specimens that could not be discriminated morphologically****) using the principle of phylogenetic species, whereby the genetic divergence among specimens belonging to the same species (intraspecific) is smaller than the divergence among specimens from different species (interspecific) (Hebert et al., 2003b)'*

Page 5, line 25 '. . .to calculate nodule density. . .' Is nodule mean size or size distribution also considered in this study? If not, why was this not included as a parameter that may shape communities? > explain, consider adding explanation to the paper

The nodules were weighted but not sized.

Page 5 lines 8-9. It was corrected:

From 'The box core sample surface was photographed, and all nodules picked up from the sediment surface, washed and individually measured and weighed.'

To 'The box core sample surface was photographed, and all nodules picked up from the sediment surface, washed with cold seawater over a 300 µm-mesh sieve and individually weighed.'

Page 5, line 25-29 ‘Particulate organic carbon flux (POC, mg C m−2 d−1) at the seafloor for our study areas [. . .] applying the Suess algorithm (POC at the seafloor as a function of the net primary production scaled by depth; Suess, 1980; Table 2).’ How do POC fluxes estimated with different methods compare where they overlap (i.e. in the study area?) > consider adding that information to the paper.

Spearman correlation shows a significant correlation (rho 0.90, $p < 0.05$). This was included in the Figure 3.

Page 6, line 1 ‘2.6 Regional-scale data’ Also the Ocean Productivity-based POC fluxes in the previous section refer to the regional scale > choose another headline, e.g., ’Regional scale polychaete community data’

Page 7 line 1. Done as suggested but also modified in order to clarify the range of the meta-analysis: ‘2.6 NE Pacific-scale polychaete community data’

Page 6, line 6 > add references for ES163 and bootstrap

References of the papers providing ES163 and bootstrap are given in the text (page 7 line 5) as well as in Table 2. Reference of the paper describing bootstrap are given in the text (page 7 line 19).

Furthermore, ES163 has been explained in the text with the addition of the following sentence:

Page 7 lines 14-15 ‘Based on these data the expected number of species was calculated for 12 individuals (ES12) and 163 individuals (ES163); as well as for three samples (S3).’

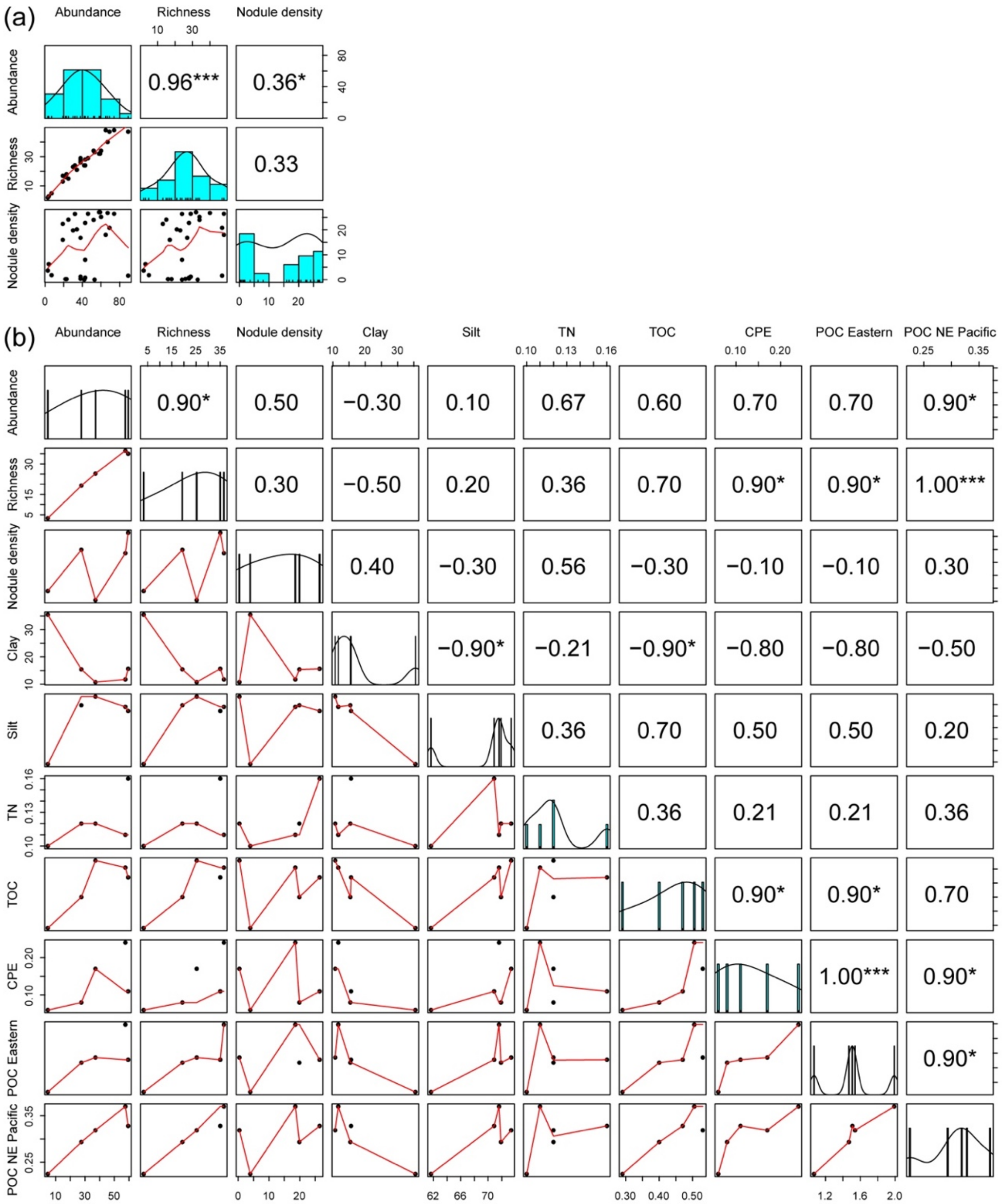
Page 6, line 20/21 ‘Spearman correlations were sought between biotic and abiotic variables, using data from the SO239 cruise in the CCFZ and data compiled from the literature.’ The data used for these correlations should match the data sources listed in section 2.5 > to avoid confusion I suggest to just refer to section 2.5. here. If the ’biotic and abiotic variables’ include data not mentioned in section 2.5 add them there.

Figure 3 (below). The variables POC flux at seafloor from Volz et al. (2018) and the POC flux at seafloor estimated in the present study were included in the revised Figure 3 as POC Eastern and POC NE Pacific, respectively. The following sentence was added in the caption ‘POC Eastern values provided by Volz et al. (2018); POC NE Pacific values were estimated in the present study.’

Moreover, in order to be clear, the figure was separated in (a) when having data from each box-core sample such abundance, richness and nodule density (n=30); and (b) when having data averaged by area (n=5) such clay, silt, TN, TOC, CPE, POC Eastern and POC NE Pacific, and averaged abundance, richness and nodule density.

The corresponding caption was changed from ‘Figure 3. Correlation matrix between biotic and abiotic variables from sampled areas within the eastern CCFZ. Diagonal panels show the distribution frequency of values for each variable. Below-the-diagonal panels show the correlation plot between pairs of variables. Above-the-diagonal panels show the Spearman coefficient correlations between pairs of variables. “” indicates $p < 0.05$, “**” $p < 0.01$ and “***” $p < 0.001$.’*

To ‘Figure 3. Correlation matrix between biotic and abiotic variables from sampled areas within the eastern CCFZ. Diagonal panels show the distribution frequency of values for each variable. Below-the-diagonal panels show the correlation plot between pairs of variables. Above-the-diagonal panels show the Spearman coefficient correlations between pairs of *variables.* ***Abundance, richness and nodule density per box-core (a) and average biotic and abiotic variables per area (b). POC Eastern values provided by Volz et al. (2018); POC NE Pacific values were estimated in the present study****. "*" indicates p < 0.05, "**" p < 0.01 and "***" p < 0.001.'*



Page 6, line 23 to Page 7, line 14 Also in this 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 (e.g., ' Low values of m give a high weight to dominant species, high values of m give a high weight to rare species.'). > Rephrase, possibly move parts to other sections

The NNESS and CNESS distances are not commonly used in community ecology although they have interesting properties, in particular to rationalize the weight given to abundant and rare species rather than the arbitrary choice of a data transformation (e.g square root, double square root…). For this reason we think that it is worth providing some information on the use of these metrics in the M&M section.

Page 7, line 21 '...tended to decrease from east to west with high spatial variation' 1. the main axes does not seem to go strictly longitudinal > replace 'east to west' by 'southeast to northwest' 2. 'high spatial variation' would make more sense in a study design, that follows a clear geographical transect. > consider rephrasing, e.g., 'high variability between neighboring areas'.

Page 8 line 25-26. Done as suggested.

Page 8, line 2 'The relative contributions of trophic guilds also varied among the areas...' Is there an explanation found somewhere, how trophic guilds were determined? > If not, add description and references to M&M.

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

The following phrase 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 name (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, line 6 'Off the 1223 polychaetes, 1118 specimens belonging to 78 possible genera within 40 families were identified down to Morphospecies. . .' What are 'possible genera'? > consider rewording, e.g., '. . .possibly belonging to 78 genera. . .'?

This makes reference to possible new genera. It was corrected to valid genera only. Also, we have corrected the number of sampled polychaetes which is 1233 instead of 1223.

*This was changed (page 9 line 11) to 'Off the **1233** polychaetes, 1118 specimens belonging to **62 genera** within 40 families were identified down to morphospecies.'*

Page 8, line 6/7 '1118 specimens [...] were identified down to morphospecies (see Section Data availability)' Not sure why you refer to that section here. > please provide explanation and consider including it in the text.

This has been removed.

Page 8, line 14 'The mean number of species tended to decrease from east to west with high spatial variation. . .' see comment above (regarding Page 7, line 21, second comment)

Page 9 lines 20-21. Done as suggested.

Page 10, line 13/14 'Both processes [i.e., increased friction and sediment deposition / biodeposition rates] may stabilize sediments and increase organic carbon supply as tube lawns do' I dont see the connection to sediment stability. > please explain better what your idea is here

It has been showed (Graf and Rosenberg, 1997; Friedrichs et al., 2000) that biological structures in the sediment-water interface favored the biodeposition and avoid erosion (e.g. polychaete tube lawns) by deceleration of water flow leading to a possible increase in food supply.

*Page 11 lines 20-21. It has been rephrased to 'Both processes **may decelerate water current stabilizing sediments and, thus,** increase organic carbon supply as **polychaete** tube lawns do'.*

Also, the correspondent reference was incorrectly written (names in the place of surnames), we have changed to 'Friedrichs et al., 2000'

Page 10, line 15/16 'The divergent response of meiofauna to the presence of nodules further suggests some sort of competition between meiofauna and macrofauna.' I can see that - if nodules increase food supply but meiofauna abundances are relatively small, meifauna may be unable to make full use of the additional food. What I don't understand is why the reason does need to involve competition with macrofauna (see also my major comment on the meiofauna discussion above). > please provide explanation and consider including it in the text.

This has been deleted as suggested.

Page 11, line 34/35 'No significant correlation was however found between alpha diversity and productivity, neither at the NE Pacific scale nor at the scale of the whole CCFZ.' Do the authors have a hypothesis why this can be the case? Could it be related to the fact that most of the tested areas lie within more or less similar mesotrophic conditions and that this 'biased' data set is not fully appropriate to address this question? > please consider discussing the reason for the missing significant correlation of diversity and productivity on larger scales.

Sorry but the sentence was not completely right. We have changed (page 12 lines 30-31) to:

"Species richness and productivity were significantly correlated at Eastern CCFZ scale, but no significant correlation was found between alpha diversity and productivity in the meta-analysis at the scale of the NE Pacific".

Furthermore, we believe that the missing significant correlation between diversity estimators and productivity at whole CCFZ scale is mostly due the differences in methods, in particular integrative vs. *morphological taxonomy.*

The following phrase was added (page 12 lines 31-32):

"The reason diversity and productivity were not correlated in the meta-analysis that included data from the literature could be mainly methodological. In particular, the use of integrative taxonomy in this study versus morphological taxonomy in previous works might hinder comparisons of diversity metrics."

Page 12, line 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.' This statement reads quite vague as the idea of geographical barriers is not mentioned and elaborated before the next section > please consider adding (see next section) after the statement.

This has been deleted because was vague and the next section will better discuss it.

Page 12, line 12/13 '...characterized by a peak and through ...' Typo > change 'through' to 'trough'

Page 13 line 14. Done as suggested.

Page 12, line 24-26 'However, species identification was based on morphology only, although cryptic species are common among scavenging amphipods, even in abyssal lineages (Melo, 2004; Havermans et al., 2013)' Another reason is, of course, that scavenging amphipods are typically highly motile. > consider adding mobility as an argument why scavenging amphipode distribution is not limited by fracture zones.

Page 12 lines 27-28. It has been changed:

From 'In the abyssal Pacific, the CCFZ and the Peru Basin share nine species of scavenging amphipods (Patel et al. (2018), which thus potentially cross the Clipperton and Galapagos Fracture Zones''

To 'In the abyssal Pacific, the CCFZ and the Peru Basin share nine species of scavenging amphipods (Patel et al. (2018), which are ***highly motile*** *and thus potentially cross the Clipperton and Galapagos Fracture Zones'*

Page 13, line 5 'In other words, nodule mining would affect each year an area that is equivalent to the average geographic range of a polychaete species.' This sounds like one mining operation would lead to the extinction of one polychaete ('only' - as some may argue). > consider removing 'a', i.e., write 'equivalent to the average geographic range of polychaete species.. . .'

Page 14 line 8-10. Done as suggested.

Page 13, line 27/28 '...suggesting that such extreme environmental conditions...' I don't share the view that the deep sea is per se an extreme environment. > replace 'such extreme' with 'the specific' or explain what specifically is considered extreme

Page 14 lines 31-32. Done as suggested.

Page 14, line1/2 'This highlights a shortcoming of COI-based barcoding because success rates for COI sequencing are generally low...' ? Are current molecular approaches appropriate if only are relatively small proportion could be identified based on 16S and COI and even less with both? Where is the problem and can it be overcome? If there new promising methods that base on other regions of the genome: how can we safeguard comparability of the full data set including new and older data?

The current molecular approach using COI and 16S genes has proved to be appropriate in delineating species (e.g., Carr et al., 2011), but the sequencing success, especially for COI, is low. The reasons for failure can be numerous from bad DNA preservation to inappropriate DNA primers, annealing temperatures, etc…To some point, the only way to overcome the problem is to invest more time and efforts to get DNA sequences out of reluctant samples. For now, the most parsimonious method in our opinion is to associate morphology and DNA.

Page 15 lines 5-7. It has been changed:

From 'This highlights a shortcoming of COI-based barcoding because success rates for COI sequencing are generally low and a combination of several genetic markers plus morphology is essential to accurately assess species diversity.'

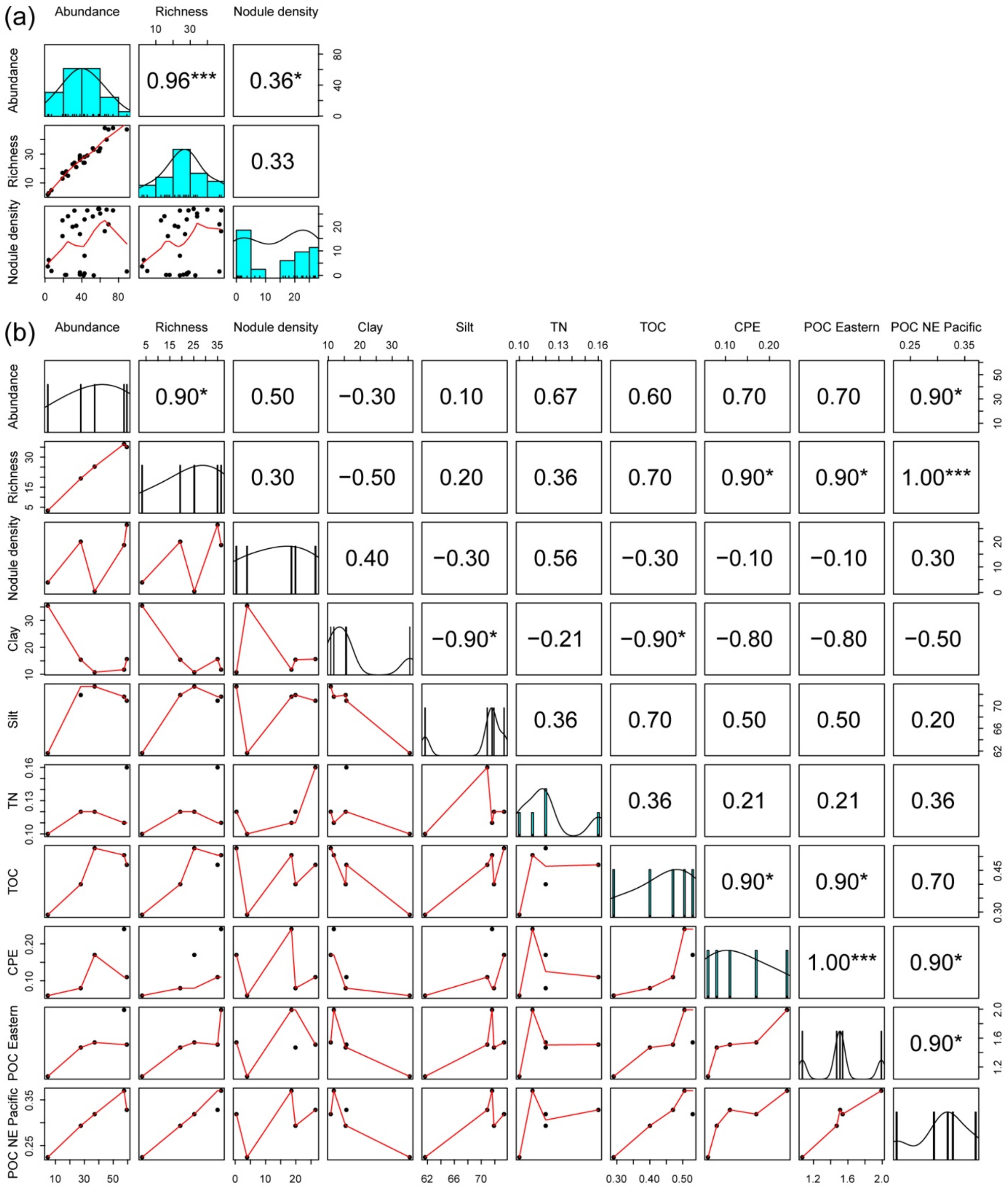
To 'This highlights a shortcoming of COI-based barcoding because success rates for COI sequencing are generally low. A combination of ***several genetic markers associated to formal morphological descriptions are thus essential*** *to accurately assess species diversity.'*

Page 15, line 2-4 'The latter estimate assumes that we have sampled 0.1 % of the polychaete species in the CCFZ and that these species have narrow geographical ranges about the size of a yearly mined area.' If I understand right, this refers to the expected annual area exploited as part of one mining operation – not the total annually mined area > replace 'a yearly mined area' with 'the area that will presumably mined in one year by a single mining operation'.

Page 16 line 9. This has been changed.

Page 31, Fig. 3 Irrespective of the fact that the variables are provided in the diagonal panels I would prefer if to the side of the plot the variables would be indicated like in https://images.app.goo.gl/oFQRE6xD7fvFwxJR6

Done as suggested, please see the revised figure 3 below:



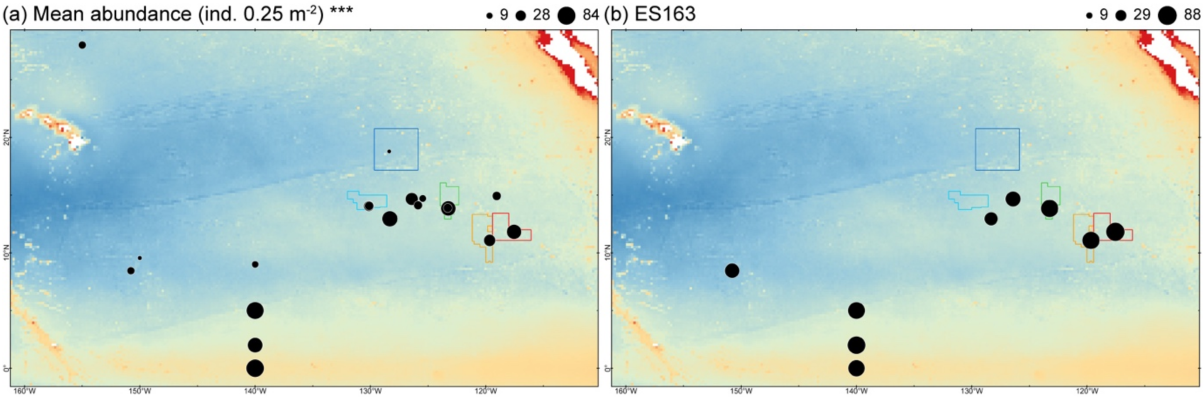
Page 32, Fig. 4 '...in relation to the 2002–2018 average particulate organic carbon (POC) concentration at the seafloor along the CCFZ. The background map shows average POC flux at the seafloor during the 2002–2018 period.' How can the maps show relations to POC concentration and flux at the same time? > consider rephrasing the caption. The caption should also state that this shows / includes data from published studies and refer to section 2.6

Page 38 Figure 4. This has been rephrased:

From 'Figure 4. Map of mean abundance (a) and diversity estimators, ES163 (b) and bootstrap (c), from the Northeast Pacific in relation to the 2002–2018 average particulate organic carbon (POC) concentration at the seafloor along the CCFZ. The background map shows average POC flux at the seafloor during the 2002–2018 period.'

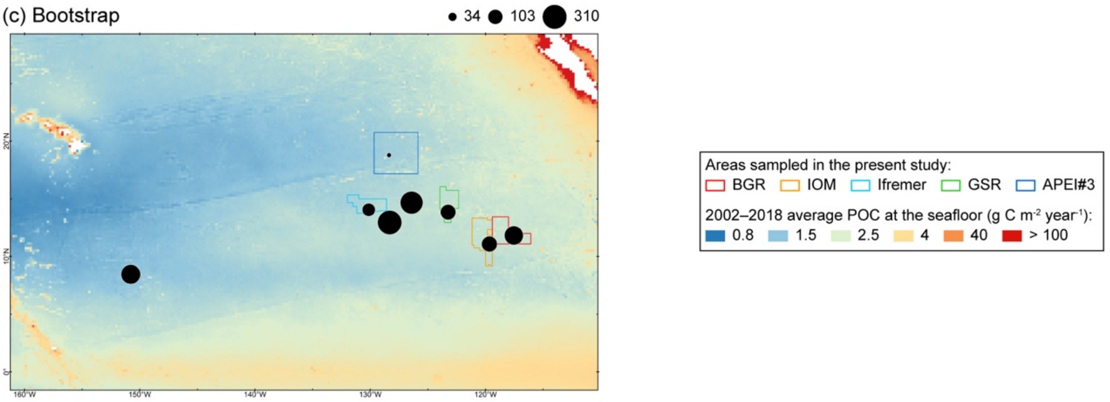
*To 'Figure 4. Plot of mean abundance (a) and diversity estimators, ES163 (b) and bootstrap (c), from previous and the present study (Table 2) in relation to the 2002–2018 average particulate organic carbon (POC) flux at the seafloor along the CCFZ (background). "***" indicates significant (p < 0.001) Spearman correlation.'*

Furthermore, an asterisk was included in Figure 4a in order to highlight that the relation between abundance and POC at seafloor was significant (figure below):


(a) Mean abundance (ind. 0.25 m^{-2}) ***

(b) ES163

(c) Bootstrap



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Alpha and beta diversity patterns of polychaete assemblages across the nodule province of the eastern Clarion-Clipperton Fracture Zone (Equatorial Pacific)

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Abstract. In the abyssal Equatorial Pacific Ocean, most of the seafloor of the Clarion-Clipperton Fracture Zone (CCFZ), a 6 million km^2 polymetallic nodule province, has been preempted for future mining. In light of the large environmental footprint that mining would leave, and given the diversity and the vulnerability of the abyssal fauna, the International Seabed Authority has implemented a regional management plan that includes the creation of nine areas of particular environmental interest (APEIs) located at the periphery of the CCFZ. The scientific principles for the design of the APEIs were ~~defined~~ based on the best – albeit very limited – ~~scientific~~ knowledge for the area. The fauna and habitats in the APEIs are unknown, as are species' ranges and the extent of biodiversity across the CCFZ.

As part of the Joint Programming Initiative Healthy and Productive Seas and Oceans (JPI Oceans) pilot action "Ecological aspects of deep-sea mining", the SO239 cruise ~~aimed at improving~~provided data to improve species inventories, ~~determining~~determine species ranges, ~~identifying~~identify the drivers of beta diversity patterns and ~~assessing~~assess the representativeness of an APEI. Four exploration contract areas and an APEI (APEI#3) were sampled along a gradient of sea-surface primary productivity that spanned a distance of 1440 km in the eastern CCFZ. Between 3 and 8 quantitative box cores (0.25 m^2; 0–10 cm) were sampled in each study area, resulting in a large collection of polychaetes that were morphologically and molecularly (COI and 16S genes) analyzed.

A total of 275 polychaete ~~morphotypes~~morphospecies were identified. Only one ~~morphotype~~morphospecies was shared among all five study areas and 49% were singletons. The patterns in community structure and composition were mainly attributed to variations in ~~food~~organic carbon fluxes to the seafloor at the regional scale and nodule density at the local scale~~.~~, thus supporting the main assumptions underlying the design of the APEIs. The ~~four exploration contract areas belong to a mesotrophic province. The distance-decay of similarity among the four areas provides an estimated species turnover of 0.04 species km^{-1} and an average species range of 25 km. The polychaete assemblage in~~ APEI#3 ~~showed the lowest densities, lowest diversity as well as very low, distant-independent similarity with the other four study areas. Given that APEI#3~~however, which is located in an oligotrophic province and separated from the CCFZ by the Clarion Fracture Zone, ~~our results call into question~~showed the lowest densities, lowest diversity, as well as a very low and distant-independent similarity in community

composition compared to the contract areas, thus questioning the representativeness and the appropriateness of APEI#3 to meet its purpose of ~~preserving the biodiversity of the CCFZ fauna. Two methods for estimating the total number~~diversity preservation. Among the four exploration contracts, which belong to a mesotrophic province, the distance-decay of similarity provided a species turnover of 0.04 species km^{-1}, an average species range of 25 km and an extrapolated richness of up to 240,000 polychaete species ~~gave estimates that ranged from~~in the CCFZ. By contrast, non-parametric estimators of diversity predict a regional richness of up to 498 ~~to 240 000~~ species. Both ~~methods~~estimates are biased by the high frequency of singletons in the dataset, which likely result from under-sampling~~; our estimates thereby~~, and merely reflect our level of uncertainty. The assessment of potential risks and scales of biodiversity loss due to nodule mining thus requires an appropriate inventory of species richness in the CCFZ.

1 Introduction

The abyssal plain is vast, covering 54 % of the Earth's surface and 75 % of the ocean floor, typically located between 3000–6000 m depth; it generally features low temperature, low current, and well-oxygenated, oligotrophic waters (Gage and Tyler, 1991; Smith and Demopoulos, 2003; Ramirez-Llodra et al., 2010). Only about 1 % of abyssal plains have been ~~explored~~investigated to date: much remains to be discovered. ~~In particular, polymetallic~~Polymetallic nodule fields are one of the unique habitats in the abyss (Ramirez-Llodra et al., 2010; Vanreusel et al., 2016). Nodules are potato-shaped, variably sized aggregations of minerals, mainly manganese and iron but also copper, nickel and cobalt, that are patchily distributed (~~Michael et al., 2000~~Hein and Petersen, 2013; Morgan, 2000). Polymetallic nodules were discovered during the Challenger expedition in the 1870s at depths below 4000 m in the Pacific, Atlantic and Indian oceans (Boudreau and Scott, 1978). 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 ~~(Michael et al., 2000~~nodules (Hein and Petersen, 2013; Morgan, 2000~~).~~), which may represent a minimum sale value of 25,000 billion US Dollars (Volkmann et al., 2018). The presence of abundant metal resources~~ (e.g. manganese, iron, copper, nickel and cobalt)~~ has attracted the interest of industries. Established by the United Nations Convention on the Law of the Sea (UNCLOS), the International Seabed Authority (ISA) manages the deep-sea mineral resources in international waters and is in charge of protecting fauna against any ~~pollution or other hazards~~harm (Articles 145, 156, UNCLOS~~) (~~; Lodge et al., 2014). Currently, the ISA has granted 16 nodule exploration contracts and approved nine "areas of particular environmental interest" (APEIs) for preservation (Lodge et al., 2014) in the CCFZ.

Among the current seabed mining technologies, the hydraulic collector seems the most effective for commercial utilization (Jones et al., 2017). The mining pre-prototype vehicle (4.7 x 12 m) presented by GSR is a pick-up system based on four nodule collector heads (1 m wide each) using jet water pump and suction to collect nodules until 15 cm depth (Global Sea Mineral Resources NV, 2018). A mining operation is anticipated to directly affect over 100 km^2 yr^{-1} of the seabed (Volkmann and

Lehnen, 2018) and create sediment plumes that can indirectly increase the footprint of mining by a factor 2 to 5 (Oebius et al., 2001; Glover and Smith, 2003). Nodule mining will clearly have detrimental effects on the benthic ecosystem, but the severity of the impacts is difficult to predict. Long-term surveys of small-scale disturbances or mining tests have shown that the direct impacts of seafloor disturbances may last for over 30 years in the CCFZ (Vanreusel et al., 2016; Jones et al., 2017). Such small experiments however hardly mimic the cumulative impacts of any single nodule mining operation that could last for 20 years~~. A mining operation is anticipated to directly affect over 100 km^{2} yr^{-1} of the seabed and create sediment plumes that can indirectly increase the footprint of mining by a factor 2 to 5 (Oebius et al., 2001; Glover and Smith, 2003; Volkmann and Lehnen, 2018). Beyond this scaling issue~~ (Glover and Smith, 2003; Jones et al., 2017). Beyond the unpredictable effects of the full-scale mining, the extent of biodiversity and species' ranges in the CCFZ are two major unknowns that prevent the assessment of potential biodiversity loss due to nodule mining. The few biodiversity studies undertaken so far in the CCFZ have revealed ~~the~~a high diversity of communities of megafauna [over 130 ~~morphotypes~~morphospecies (Amon et al., 2016; Simon-Lledó et al., 2019)], polychaetes [over 180 ~~morphotypes~~morphospecies (Paterson et al., 1998; Glover et al., 2002; Wilson, 2017)], isopods [over 160 ~~morphotypes~~morphospecies (Wilson, 2017)], tanaids [over 100 ~~morphotypes~~morphospecies (Wilson, 2017; ~~Pabis~~Błażewicz et al., ~~submitted~~2019)] and nematodes [over 300 ~~morphotypes~~morphospecies (Miljutina et al., 2010)]. Overall, over 870 ~~morphotypes~~morphospecies are already known in the CCFZ, but almost none have been named and 90 % of them are likely new to science (Glover et al., 2002; Miljutina et al., 2010). Therefore, new inventories of CCFZ biodiversity cannot be compared with these previous ones. To overcome this bias, DNA taxonomy is increasingly used (Glover et al., 2016). In the CCFZ, in two exploration contract areas separated by 1300 km, the first assessment of macrofaunal diversity based on DNA taxonomy already increased the number of known polychaetes to 233 "molecular operational taxonomic units" (MOTUs~~) (~~; Janssen et al., 2015). This study further highlighted three characteristics of abyssal biodiversity: (a) high rates of species turnover (i.e. species replacement) with only 12 % of polychaete MOTUs and 1 % of isopod MOTUs shared between the two areas; (b) high frequencies of singletons (MOTUs known from a single unique DNA sequence) ranging from 60 % to 70 % for polychaetes and isopods, respectively; and (c) cryptic ~~richness~~diversity within polychaete and isopod ~~morphotypes~~morphospecies, suggesting that previous surveys have underestimated alpha and beta diversity of these two taxa. Considering the large environmental footprint of nodule mining disturbances on the seafloor, as well as the diversity and vulnerability of the abyssal fauna, the need for marine spatial planning to preserve species, habitats and functions in the CCFZ has emerged, concomitant to a renewed interest for deep-sea mineral resources (Wedding et al., 2013). Due to the paucity of biological data in the CCFZ, the ~~spatial management plan was designed~~recommendations issued by Wedding et al. (2013) for the design of a network of protected areas were mainly based on nitrogen flux at 100 m depth (a proxy for trophic inputs to the seafloor), modeled nodule densities, the distribution of large seamounts and the dispersal distances of shallow water taxa ~~(~~. 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. As a result, Wedding et al~~.,~~. (2013~~). The nine proposed 400~~) divided the spatial domain of the CCFZ into 3 x ~~400 km managed (non~~3 subregions and suggested to create one large no-mining~~)~~ area in each subregion. The size of the no-mining areas was defined with the aim of maintaining viable population sizes for species potentially restricted to a subregion, taking into account the inferred dispersal distances of species and of the plumes created by nodule mining (Wedding et al., 2013). Those principles were ~~included~~implemented in the regional management plan for the CCFZ~~ and designated as~~, which resulted in the designation of 9 APEIs (Lodge et al., 2014). Most of the CCFZ however ~~has~~had already been preempted to current exploration contracts and areas reserved for future exploration. ~~The distribution of~~ Consequently, the APEIs were located at the periphery of the CCFZ thus ~~deviates~~deviating from an optimal design.

The European project "Managing Impacts of Deep-seA reSource exploitation" (MIDAS) and the "Joint Programming Initiative Healthy and Productive Seas and Oceans" (JPI Oceans) pilot action "Ecological aspects of deep-sea mining" aimed at improving the scientific ~~grounds~~basis on which to assess and manage the potential impacts arising from nodule mining. In this context, four exploration contract areas and one APEI (separated by 240 to 1440 km) were sampled along a sea-surface primary productivity gradient from ~~east~~southeast to ~~west~~northwest across the eastern portion of the CCFZ nodule province~~. Polychaetes~~ (Martínez Arbizu and Haeckel, 2015). The four exploration contract areas were ~~identified using a combination of morphological and molecular criteria. The structure and composition~~located within the eastern central subregion defined by Wedding et al. (2013), where an APEI did not fit. One of the nearest APEIs, was thus sampled instead.

The aims of ~~polychaete assemblages~~our study were ~~analyzed to describe and identify alpha and beta diversity patterns,~~(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 ~~potentially~~(c) to improve the assessment of potential risks ~~to~~of biodiversity loss due to nodule mining. To tackle these issues, we focused on polychaete assemblages. Polychaetes are the dominant and most diverse group of the macrofauna; they can be quantitatively sampled, and identify down to species level using a combination of morphological and molecular methods (Hessler and Jumars, 1974; Janssen et al., 2015; Wilson, 2017). Polychaetes also show a wide range of biological traits, from trophic behaviors to life history strategies, and play a major role in the functioning of benthic communities (Hutchings, 1998; Jumars et al., 2015).

2 Materials and methods

2.1 Clarion-Clipperton Fracture Zone

The CCFZ is located in the Equatorial Pacific Ocean between the Clarion Fracture to the north and the Clipperton Fracture to the south, and between Kiribati to the west and Mexico to the east (Fig. 1). This area covers about 6 million km^2 being composed of a high variety of habitats such as abyssal hills or seamounts, as well as polymetallic nodule fields (Glover et al., 2016). As part of the JPI Oceans project "Ecological aspects of deep-sea mining", the EcoResponse cruise SO239 took place from 9 March to 30 April 2015 aboard the RV *Sonne* (~~Martinez~~Martínez Arbizu and Haeckel, 2015). Sampling during the cruise focused on four exploration contract areas as well as APEI number 3 (APEI#3; Fig. 1). All five study areas ~~were~~

~~located~~had water depths between 4000 and 5000 m ~~depth~~ (Fig. 1). The four exploration areas were licensed by the ISA to the Federal Institute for Geosciences and Natural Resources of Germany (BGR); the InterOceanMetal Joint Organization (IOM); the G-TEC Sea Mineral Resources NV (GSR); and the *Institut Français de Recherche pour l'Exploitation de la Mer* (Ifremer). Furthermore, the ISA administrates APEI#3 as part of the regional environmental plan for the CCFZ. The distances separating the areas ranged from 243 km (BGR to IOM) to 1440 km (BGR to Ifremer or APEI#3).

2.2 Sampling strategy

The sampling strategy resulted from a combination of objectives that were unique to each area, together with the overarching aim of describing alpha and beta diversity patterns across a productivity gradient that included both contract areas for nodule exploration and an APEI (Martínez Arbizu and Haeckel, 2015). In the BGR area, two sub-areas were sampled: a Prospective Area (PA) that could be mined in the future and a Reference Area (RA) that could serve as a preservation area. In the IOM area, three sub-areas were sampled: one that had been directly disturbed by a BIE-experiment (Radziejewska, 2002), one that had been impacted by the plume and one control, undisturbed area. These levels of sampling stratification are however out of the scope of the present study, which focuses on variations between contract areas. After checking that there was no statistically significant difference on the abundance and richness of polychaetes between sub-areas, all samples within an area were deemed representative of that area and considered as replicate samples. The level of replication within areas accordingly varied as a function of sampling stratification. The aim was to collect a minimum of five replicate samples per strata but due to sampling failures and time constraints, it couldn’t be systematically achieved (Table 1).

Within each area, macrofaunal samples were collected using an USNEL spade box corer of 0.25 m^2 (Hessler and Jumars, 1974~~) with five to nine replicates in each area, totaling~~). A total of 34 box ~~core~~cores were sampled of which 30 samples were deemed quantitative (Table 1). ~~Although in the BGR and IOM areas nine box cores had been sampled from different localities within the study area, they were considered as replicates (as were environmental variables).~~ The overlying water ~~column~~ was siphoned and ~~filtered~~sieved using a sieve of 300 µm of mesh size. The box core sample surface was photographed, and all nodules picked up from the sediment surface, washed with cold seawater over a 300 µm-mesh sieve and individually ~~measured and~~ weighed. Sessile polychaetes, if present, remained attached to the nodules and were not considered in this study. The upper 10 cm of each core was sliced into three layers (0–3, 3–5 and 5–10 cm~~),~~) to facilitate sieving and sorting; each layer was transferred into cold seawater (4 °C) and sieved using the same mesh size. The ~~overlying water residue and the~~ 0–3 cm layer ~~were~~was immediately sieved in the cold room with cold seawater (4 °C~~) and then~~). The sieve residues from the overlying water and nodule washing were added to the 0-3 cm layer and live-sorted. All polychaete specimens were photographed, individualized and preserved in cold (-20 °C) 80 % ethanol and then kept at -20 °C (DNA-friendly). The 0–3 cm residue, 3–5 and 5–10 cm layers were fixed in formalin for 48 to 96 h and preserved in 96 % ethanol and later sorted in the laboratory (not DNA-friendly). ~~The sieve residues from the overlying water and the washed nodules~~ All layers were combined ~~with all layers~~ for the community analysis. In the laboratory, from each DNA-friendly polychaete specimen and from very few fragments, a small piece of tissue was dissected, fixed in cold 96 % ethanol and frozen at -20 °C for molecular studies ~~(see Section 2.3~~

~~DNA extraction, amplification, sequencing, and alignment).~~ . 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.

2.3 DNA extraction, amplification, sequencing, and alignment

The DNA of the subsampled tissues was extracted using a NucleoSpin Tissue kit (Macherey-Nagel), following the manufacturer's protocol. Approximately 450 base pairs (bp) of 16S, 700 bp of COI (cytochrome c oxidase subunit I) and 1600 bp of 18S genes were amplified using the following primers: Ann16SF and 16SbrH for 16S (Palumbi, 1996; Sjölin et al., 2005); polyLCO, polyHCO, LCO1490, and HCO2198 for COI (Folmer et al., 1994; Carr et al., 2011); and 18SA, 18SB, 620F, and 1324R for 18S (Medlin et al., 1988; Cohen et al., 1998; Nygren and Sundberg, 2003) for 18S. The polymerase chain reaction (PCR) mixtures of 25 µL contained 5 µL of Green GoTaq® Flexi Buffer (final concentration of 1X), 2.5 µL of $MgCl_2$ solution (final concentration of 2.5 mM), 0.5 µL of PCR nucleotide mix (final concentration of 0.2 mM each dNTP), 9.875 µL of nuclease-free water, 2.5 µL of each primer (final concentration of 1 µM), 2 µL template DNA and 0.125 U of GoTaq® G2 Flexi DNA Polymerase (Promega). The temperature profile was as follows: 95 °C/240 s – (94 °C/30 s – 52 °C/60 s – 72 °C/75 s (for 16S and COI) or 180 s (for 18S) *35 cycles (for 16S or 18S) or *40 cycles (for COI)) – 72 °C/480 s. PCR products, visualized after electrophoresis on 1 % agarose gel, were sent to the MacroGen Europe Laboratory in Amsterdam (the Netherlands) to obtain sequences, using the same set of primers as used for the PCR.

Overlapping sequence (forward and reverse) fragments were aligned into consensus sequences using Geneious Pro 8.1.7 2005–2015 (Biomatters Ltd). For COI, the sequences were translated into amino-acid alignments and checked for stop codons to avoid pseudogenes. The minimum length coverage was 207 bp for 16S, 327 bp for COI and 1615 bp for 18S.

The sequences were blasted in GenBank to check for the presence of contamination. Each set of genes was aligned separately using the following plugins: MAAFT (Katoh et al., 2002) for 16S and 18S; and MUSCLE (Edgar, 2004) for COI. 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/).

~~2.4 Operational taxonomic units (OTUs)~~

2.4 Taxonomic identification and feeding guilds classification

Preserved specimens were examined under a Leica M125 stereomicroscope and a Nikon Eclipse E400 microscope, counted (anterior-ends only) and morphologically identified using the deep-sea polychaete fauna bibliography (Fauchald, 1972, 1977; Böggemann, 2009) at the lowest taxonomic level possible (~~species-morphotype). To separate~~morphospecies). We separated closely related species~~,~~ (specimens that could not be discriminated morphologically) using the principle of phylogenetic species ~~was used~~, whereby the genetic divergence among specimens belonging to the same species (intraspecific) is smaller than the divergence among specimens from different species (interspecific) (Hebert et al., 2003b). In the distribution of pairwise divergences among all sequences of a typical barcode data set, a gap can be observed between intraspecific and interspecific variations. Molecular operational taxonomic units (MOTUs) were generally recognized using a threshold of 97 % or 99 % similarity between COI and 16S sequences, respectively (Hebert et al., 2003a; Hebert et al., 2003b; Brasier et al., 2016). The similarity of sequences within species was considered when identifying morphologically similar species. As genetic data was only used to separate closely related species, the delimited taxa entities in the present study are referenced as morphospecies. Trophic guilds were determined at family level following Jumars et al. (2015).

2.5 Environmental data

Environmental data were compiled from Hauquier et al. (~~2018~~2019) and Volz et al. (2018). Sediment samples were collected with a multi-corer or a gravity corer during the same cruise and in the same areas (see ~~Martinez~~Martínez Arbizu and Haeckel, 2015 for details). The sediment characteristics studied by Hauquier et al. (~~2018~~2019) included a clay fraction (< 4 µm), a silt fraction (4–63 µm), total nitrogen (TN in weight%), total organic carbon (TOC in weight%) and chloroplastic pigment equivalents (CPE in µg ml^{-1}). Nodules were weighed onboard for each box-core sample to calculate nodule density (kg m^{-2}; Table 1). Particulate organic carbon flux (POC, mg C m^{-2} d^{-1}) at the seafloor for our ~~study~~studied areas (Eastern CCFZ) were provided by Volz et al. (2018). At the ~~regional~~NE Pacific Basin scale, POC flux (mg C m^{-2} d^{-1}) at the seafloor was approximated using net surface primary production provided by the Ocean Productivity site (Westberry et al., 2008) averaged over years 2002 to 2018 and applying the Suess algorithm (POC at the seafloor as a function of the net primary production scaled by depth; Suess, 1980; Table 2). POC flux at seafloor was considered as a proxy for food supply to benthic communities.

2.6 ~~Regional~~NE Pacific-scale polychaete community data

To put the results of our study in ~~a regional~~the larger context of the NE Pacific Basin, we compiled data from previous surveys of polychaete assemblages in the Northeast Pacific, including CLIMAX II sampled in 1969, DOMES A, B and C in 1977 and 1978, ECHO I in 1983, PRA in 1989, EqPac in 1992, Kaplan East in 2003, Kaplan West and Central in 2004, KR5 in 2012, 2013 and 2014 and GSRNOD15A (B4N01, B4S03 and B6S02) in 2015 (Paterson et al., 1998; Glover et al., 2002; Wilson, 2017). From these studies, we compiled (when available) the mean abundance (ind. 0.25 m^{-2}), total number of species, ES163 and bootstrap (Table 2).

2.7 Data analysis

2.7.1 Univariate analyses

Mean abundance and mean number of species per box-core were used as descriptors of alpha diversity. A few cryptic or damaged ~~species~~specimens that could not be classified to a lower taxonomical level were included in total abundance calculations but excluded from subsequent diversity analyses. To compare diversity among the studied areas~~,~~ and for all samples (Eastern CCFZ), rarefaction curves were computed based on the total number of individuals and the total number of box core samples (Hurlbert, 1971; Gotelli and Colwell, 2001). Based on these data the expected number of species was calculated for 12 individuals (ES12) and 163 individuals (ES163); as well as for three samples (S3). Non-parametric estimators of species richness were used to estimate the total number of species at local and regional scales. Abundance-based estimators included Chao1 and abundance-based coverage estimator (ACE~~) (~~; O'Hara, 2005; Chiu et al., 2014). Incidence-based estimators included Chao2 (Chao, 1984), first- and second-order jackknife (Jackknife 1st and Jackknife 2nd, respectively~~) (~~; Burnham and Overton, 1979) and bootstrap (Smith and van Belle, 1984). A Venn diagram was used to show the distribution of rare, wide and common species across the CCFZ.

Univariate analyses relied on non-parametric tests. The Kruskal-Wallis rank sum test was used to test differences among areas (Hollander and Wolfe, 1973); and the Conover multiple pairs-rank comparisons (adjusted p-value by Holm) was used to identify the pairs showing differences (Conover and Iman, 1979; Holm, 1979). Spearman correlations were sought between biotic and abiotic variables, using data from the SO239 cruise in the CCFZ and data compiled from the literature. The latter analysis aimed at testing correlations between biotic variables and POC fluxes at the regional scale.

2.7.2 Multivariate analyses

Three indices of faunal similarity were used in multivariate analyses, the chord-normalized expected species shared (CNESS), the new normalized expected species shared (NNESS~~) (~~; Trueblood et al., 1994; Gallagher, 1996) and the Jaccard-family indices (Baselga, 2010; Legendre, 2014). The CNESS and NNESS ~~are~~were computed from probabilities of species occurrence in random draws of m individuals~~. Low~~; with low values of m ~~give~~giving a high weight to dominant species, and high values of m ~~give~~giving a high weight to rare species. The best trade-off value of m is the one providing the highest Kendall correlation between the similarity matrix for $m = 1$ and the similarity matrix for $m = m$max. The value of $m$max ~~is~~was given by the total abundance of the least abundant sample considered. CNESS ~~is a~~was the distance metric ~~that can be~~ used to perform a redundancy analysis (RDA~~) (~~; Legendre and Legendre, 2012). The RDA is a constrained multivariate analysis that ~~tests~~tested the influence of multiple environmental covariates on multispecific assemblages. Species contributing significantly to the ordination were plotted out of the equilibrium circle in RDA (scaling 1). The best set of environmental variables was selected using a forward~~, backward and stepwise~~ selection ~~procedures~~procedure (Borcard et al., 2011) among the environmental variables available (see Section 2.5 Environmental data): clay fraction, silt fraction, TN, TOC, CPE, nodule density and POC flux at the seafloor. Furthermore, when selected variables had more than 80 % co-correlation, they were excluded, and the selection procedure started over again. Also, the variance inflation factor (VIF) was used to verify the possible linear dependency among variables in the RDA model.

The NNESS index was used to perform a distance-decay analysis as in Wilson (2017). Distance-decay screens for a negative correlation between faunal similarities and geographic distances among pairs of areas. Wilson (2017) used the slope of linear regression between NNESS and distance to compute the rate of change (species km^{-1}) and the species range (km species^{-1}). The rate of change is the slope of linear regression between NNESS and distance multiplied by the mean total estimated species from all areas. The species range is the inverse of the rate of change.

The Jaccard-family indices were used to partition beta-diversity into its three components: similarity; turnover – which is dissimilarity due to species ~~turnover~~replacement – and nestedness – which is dissimilarity due to differences in the number of species (Baselga, 2010).

All analyses were conducted using the R language (R Core Team, 2018) with RStudio (RStudio Team, 2015) and the following specific packages or functions: adespatial (Dray et al., 2019), BiodiversityR (Kindt and Coe, 2005), fossil (Vavrek, 2011), vegan (Oksanen et al., 2015), VennDiagram (Chen, 2018), beta.div.comp (Legendre, 2014), ness (Menot, 2019).

3 Results

3.1 Abundance and alpha diversity

During the SO239 cruise, 1233 polychaete specimens were sampled in the five study areas. The dataset has been archived in the information system PANGAEA and is available in open access (Bonifácio et al., 2019).

The mean abundance in each study area tended to decrease from ~~east~~southeast to ~~west~~northwest with high ~~spatial variation~~variability between neighboring areas (Fig. 2a). Mean densities ranged from 58 ± 18 ind. 0.25 m^{-2} in the BGR area to 5 ± 2 ind. 0.25 m^{-2} in APEI#3. The mean abundance differed significantly between areas (Kruskal-Wallis test, $p < 0.001$). The pairwise comparison test (Conover-Holm) showed that (a) APEI#3 had significantly lower abundance than the other areas ($p \leq 0.01$) except Ifremer; (b) the Ifremer exploration area (28 ± 8 ind. 0.25 m^{-2}) had significantly lower abundance than the BGR and GSR areas (59 ± 10 ind. 0.25 m^{-2}) ($p < 0.001$); and (c) the IOM area (37 ± 10 ind. 0.25 m^{-2}) had significantly lower ($p < 0.01$) abundance than the BGR and GSR areas. Furthermore, within the Eastern CCFZ, the mean abundance per box~~-~~-core was significantly correlated (Spearman correlations; Fig. ~~3~~3a) with the mean number of species ($rho = 0.96$, $p < 0.001$~~), POC at the seafloor (rho = 0.61, p < 0.001), CPE (rho = 0.52, p < 0.01), clay fraction (rho = -0.43, p < 0.05), silt fraction (rho = 0.40, p < 0.05),~~, $n = 30$) and nodule density ($rho = 0.36$, $p < 0.05$~~).~~, $n = 30$); the mean abundance per area was significantly correlated only with POC flux at seafloor ($rho = 0.90$, $p < 0.05$, $n = 5$; Fig. 3b). At the scale of the NE Pacific, polychaete ~~densities~~abundances were also significantly correlated with POC flux at the seafloor ($rho = 0.75$, $p < 0.001$, $n = 19$; Fig. 4a).

The polychaetes belonged to 41 families (Fig. 5a) with the most abundant being spionids (20 %), cirratulids (13 %), ~~paranoids~~paraonids (11 %), and lumbrinerids (6 %). Spionids showed the highest relative abundance at the Ifremer (34 %), GSR (27 %) and IOM (19 %) areas, whereas cirratulids were dominant at APEI#3 (36 %) and the BGR (17 %) areas. The relative contributions of trophic guilds also varied among the areas (Fig. 5b). In particular, carnivores were more common at BGR, IOM and GSR (23–28 %) than at Ifremer and APEI#3 (12–14 %), whereas deposit feeders were overwhelmingly dominant at Ifremer and APEI#3 (78–86 %) and less so at BGR, IOM and GSR (63–65 %). Suspension feeders, omnivores and scavengers contributed to less than 13 % of abundance in each area and were not found in APEI#3.

Off the ~~1223~~1233 polychaetes, 1118 specimens belonging to ~~78 possible~~62 genera within 40 families were identified down to morphospecies~~ (see Section Data availability).~~. The 115 remaining specimens were too damaged or cryptic/doubtful to be assigned to a morphospecies and were thus not included in diversity and composition analyses. The DNA-friendly samples totaled 430 specimens; 265 of which were successfully barcoded with either or both the COI and 16S genes. The success rates were 17 % for COI and 60 % for 16S. The COI gene was successfully sequenced for 71 specimens totaling 45 MOTUs; for the 16S gene, 259 specimens were successfully sequenced covering 104 MOTUs; only 65 specimens were successfully sequenced using both genes and yielded 40 MOTUs. The 18S gene was sequenced for phylogenetic purposes on a restricted number of specimens. The 21 sequences of the 18S gene that have been obtained are mentioned here because they were archived concomitantly with COI and 16S sequences in GenBank and BOLD public datasets, but they are not further considered in this study.

Based on both morphological and molecular identification, a total of 275 morphospecies ~~(i.e. OTUs)~~ were recognized. The mean number of species tended to decrease from ~~east~~southeast to ~~west~~northwest with high ~~spatial variation~~variability between neighboring areas (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. The number of species differed significantly among areas (Kruskal-Wallis test, $p < 0.001$). The pairwise comparison test (Conover-Holm) showed that the number of species per box core was (a) significantly lower at APEI#3 than all other areas ($p \leq 0.01$) except Ifremer; (b) significantly lower at Ifremer (19 ± 5 taxa 0.25 m^{-2}) than at BGR and GSR (35 ± 7 taxa 0.25 m^{-2}) ($p < 0.001$); and (c) significantly lower at IOM (25 ± 6 taxa 0.25 m^{-2}) ($p < 0.05$) than at BGR and GSR. A total of 156 species (observed species richness, Sobs) were sampled at BGR from eight box core samples, 107 species at IOM from eight box cores, 104 species at GSR from five box cores, 73 species at Ifremer from six box cores and 9 species at APEI#3 from three box cores (Table 3). Species rarefaction curves, based on individuals or samples, did not reach an asymptote at the local scale (Fig. 6a, b). Individual-based rarefaction curves did not show any clear diversity patterns among study areas (Fig. 6a). Sample-based rarefaction curves followed a pattern similar to abundance (Fig. 6b). From a random draw of three box cores, BGR and GSR, with 82 and 77 species, respectively, had higher expected numbers of species than did IOM and Ifremer with 58 and 45 species, respectively; APEI#3, with only 9 species, had the lowest expected number of species (Fig. 6b, Table 3). The non-parametric estimators of local diversity followed the same patterns with the highest values for BGR and the lowest for APEI#3 (Table 3).

~~The~~Within the Eastern CCFZ, the mean number of species in each study area was significantly correlated (Spearman correlations; Fig. ~~3~~3b) with POC flux at seafloor (rho = ~~0.62~~1.00, p < 0.001~~)~~, n = 5) and CPE (rho = 0.~~55, p < 0.01), clay fraction (rho = -0.45~~90, p < 0.05~~), silt fraction (rho = 0.38, p < 0.05), and TOC (rho = 0.39, p < 0.05).~~
, n = 5). At the scale of the NE Pacific, neither ES163 (rho = 0.59, p = 0.09, n = 9) nor bootstrap (rho = 0.10, p = 0.8, n = 8) were correlated with POC flux at the seafloor (Fig. 4b, c).

3.2 Beta and gamma diversity

In the RDA, the forward selection procedure kept CPE, clay fraction and nodule density as the best explanatory variables. The model explained 13 % (R^2_{adj}) of the total variance in the composition of polychaete assemblages (Fig. 7a). The first axis of the RDA discriminated the eastern areas (BGR, IOM, GSR) from the western areas (Ifremer, APEI#3). The second axis of the RDA discriminated Ifremer from APEI#3 but also captured local-scale variation, because replicate samples within areas were distributed along this second axis. The CPE concentrations mostly explained variance along the first axis. CPE was also positively and highly correlated with POC flux at seafloor and TOC (Fig. 3). The first axis of the RDA thus illustrates the influence of food inputs on species composition. The clay fraction contributed to the first and the second axis of the RDA. Grain size distribution ~~indeed~~ differentiated APEI#3 from all other areas in the CCFZ (see Hauquier et al., ~~2018~~2019 for details). In the RDA, the clay fraction accounted for the large dissimilarity in species composition of the APEI#3. Nodule density was the main contributor to the second axis of the RDA. Variation in nodule density likely accounted for some of the local variation in species composition. The ordination of species (Fig. 7b) showed that *Lumbrinerides* sp. 2107 was the species most characteristic of the eastern areas; a cirratulid (*Aphelochaeta* sp. 2062) and a maldanid (Maldanidae sp. 121) were characteristic of APEI#3; and two spionids (*Aurospio* sp. 249 and *Laonice* sp. 349), a paraonid (*Levinsenia* sp. 498) and an opheliid (*Ammotrypanella* sp. 2045) were characteristic of the Ifremer area.

The distance-decay of similarity showed two different patterns (Fig. 8a, b). APEI#3 had very low values of NNESS compared with all other areas, irrespective of distance (Fig. 8a). There was no statistically significant correlation between NNESS and distance (R^2_{adj} = 18 %, p = 0.12). However, without APEI#3, the NNESS values among pairs of exploration contract areas (Fig. 8b) within the CCFZ per se were negatively correlated with distance (R^2_{adj} = 0.85, p = 0.006). The slope of the linear regression (-0.0003) multiplied by the mean of species richness estimators for each area (Table 3) provided a rate of species change that ranged from 0.04 species km^{-1} with the bootstrap estimator (mean species richness of 135 species) to 0.07 species km^{-1} for the ACE estimator (mean species richness of 234 species). The inverse of these rates of species change predicted geographic ranges of 14 to 25 km.

Beta diversity was thus high across the CCFZ and particularly so between the exploration contract areas, south of the Clarion Fracture Zone, and APEI#3, north of the Clarion Fracture Zone. In addition, the decomposition of beta diversity showed that dissimilarity was mainly due to species turnover (91 %) and not nestedness (9 %). However, species turnover was driven by singletons. The Venn diagram (Fig. 9) showed that, in each area, at least 30 % and up to 67 % of species were unique to one area, so that overall 169 out of 275 species were unique to a given area. Of these, 134 species were singletons~~.~~ (i.e. morphospecies known from a single specimen). Only one species, *Aurospio* sp. 249, was sampled in all five areas, 16 species (6 %) were sampled in four areas, 33 species (12 %) were shared among three areas and 56 species (20 %) were shared between two areas.

When all individuals and samples were pooled together, rarefaction curves did not level off (Fig. 10a, b) and the number of singletons steadily increased with increasing sample size (Fig. 10b). At this regional scale, non-parametric estimators of species richness ranged from 334 to 498 species (Table 3).

4 Discussion

4.1 Major forces driving local- and regional-scale patterns in community structure and composition

Food supply, sediment grain size and the density of nodules are the three main environmental factors that seem to drive the structure and composition of polychaete assemblages in the CCFZ.

~~Nodules~~The abundance and richness of polychaetes were positively correlated with nodule density, which is consistent with previous studies showing that nodules enhance macrofaunal densities and polychaete diversity (De Smet et al., 2017; Yu et al., 2018). Nodules may have antagonistic influences on different size groups of benthic communities. Meiofaunal assemblages are less abundant in nodule-rich than in nodule-free sediments~~ (Miljutina et al., 2010; Pape et al., 2018). Nodules however~~, which may be due to the lower volume of sediment available in nodule areas (Miljutina et al., 2010; Hauquier et al. 2019). In our study, the volume and surface occupied by nodules were not quantified but the positive relationship between nodule density and polychaete abundance shows that space is not a limiting factor for polychaetes. Nodules also increase habitat heterogeneity, providing hard substrate for sessile organisms and generally enhancing the standing stocks of both sessile and vagile megafauna (Amon et al., 2016; Vanreusel et al., 2016; Simon-Lledó et al., ~~2019). Similarly, nodules seem to enhance macrofaunal density (De Smet et al., 2017) and diversity (Yu et al., 2018). Our results support the reported positive and significant relationship between polychaete abundance and nodule density (De Smet et al. (2017). The macrofauna in nodule fields may benefit from increased food supply and the release from competition with meiofauna.~~2019). Nodules increase seafloor roughness, thereby increasing friction (Sternberg, 1970; Boudreau and Scott, 1978) and potentially sediment deposition rates. The large sessile suspension feeders may similarly enhance biodeposition (Graf and Rosenberg, 1997). Both processes may ~~stabilize~~decelerate water current, stabilizing sediments and, thus, increase organic carbon supply as polychaete tube lawns do, for example (~~Michael~~Friedrichs et al., 2000). An increase in food supply may explain the higher densities of polychaetes in nodule-rich areas. ~~The divergent response of meiofauna to the presence of nodules further suggests some sort of competition between meiofauna and macrofauna. The contribution of meiofauna to benthic biomass generally increases along a bathymetric gradient to outweigh that of macrofauna at abyssal depths (Thiel, 1975; Rex et al., 2006; Wei et al., 2010). This pattern is assumed to reflect a selective advantage for small size at very low levels of food input (Thiel, 1975, 1979; Sebens, 1982, 1987; Rex and Etter, 1998). Sibuet et al. (1989) reported however a linear relationship between meiofaunal and macrofaunal biomass at abyssal sites. Both size classes indeed co-varied with organic carbon burial flux, which suggests the occurrence of a dynamic equilibrium between meiofauna and macrofauna at abyssal depths. Due to its small size, meiofauna is likely more efficient at exploiting the low level of food input, but this interstitial fauna may also be more sensitive to high nodule coverage because its ambit is largely limited to superficial sediments. The opposite effects of nodule coverage on meiofaunal and macrofaunal densities may thus lie in a release from the advantage of being smaller in the abyss, inducing a shift in size-group equilibrium toward increased macrofaunal densities. These results suggest that nodule coverage have an influence on the functioning of the ecosystem, because it modifies biotic interactions and resource allocation among functional groups.~~

At regional to global scales, food input is among the main forcing factors of the structure and functions of the abyssal ecosystem, which mainly rely upon 0.5–2 % of the organic carbon derived from sea-surface primary production (Rowe et al., 1991; Smith et al., 1997; Smith et al., 2008a). Variations in sea-surface primary productivity divide the NE Pacific abyss into three main areas (Sokolova, 1997; Hannides and Smith, 2003; Smith and Demopoulos, 2003): the eutrophic abyss in the equatorial upwelling zone (-5˚S–5˚N) with POC flux about 1–2 g C m^{-2} $year^{-1}$; the mesotrophic abyss in the Equatorial North Pacific (5–15˚N) with a POC flux of about 0.5–1.5 g C m^{-2} $year^{-1}$ and the oligotrophic abyss underlying the North Pacific Subtropical Gyre (15–35˚N) with a POC flux lower than 0.5 g C m^{-2} $year^{-1}$. Our metadata analysis confirmed that polychaete abundance was significantly and positively correlated with POC flux at seafloor, distinguishing areas in the oligotrophic abyss (APEI#3, CLIMAX II, DOMES A, EqPac 9 and Kaplan West) with low abundance (4–21 ind. 0.25m^{-2}) from areas in the mesotrophic abyss (Kaplan Central, Ifremer, PRA, ECHO 1, GSRNOD15A, GSR, IOM, Kaplan East and BGR) with average to high abundance (14–85 ind. 0.25m^{-2}) and areas in the eutrophic abyss (EqPac 0, 2 and 5) with abundance in the highest range (60–84 ind. 0.25m^{-2}~~) (~~; see Table 2).

The exploration areas sampled in our study all lie within the mesotrophic zone, but APEI#3 lies within the oligotrophic zone. An analysis of biogeochemical processes confirmed the very low POC fluxes to the seafloor at APEI#3 ~~(<~~(1 mg C m^{-2} d^{-1}) and found respiration rates that were 2-fold lower than in the exploration areas of the mesotrophic zone (Volz et al., 2018). APEI#3 was also characterized by higher clay content, which may be caused by lower sedimentation rate and a different sedimentation regime (Hauquier et al., ~~2018~~2019; Volz et al., 2018). Polychaete assemblages in APEI#3 consistently showed lower abundance, lower species richness and lower alpha diversity. Species turnover was also very high, with APEI#3 showing the highest rate of species unique to an area and the lowest NNESS for all pairs of comparisons. The redundancy analysis also suggested that, in addition to food supply, the higher relative proportion of clay contributed to variation in species composition at APEI#3. The polychaete assemblage was dominated by cirratulids, with one species significantly contributing to ordination (*Aphelochaeta* sp. 2062). Some cirratulids are recognized as surface deposit-feeders (Jumars et al., 2015), and may prefer the smaller particles predominantly present at APEI#3 ($D_{4\text{-}3}$ = 15.71 µm). At least two cirratulid species can effectively select particle sizes in the clay-size range using their tentacles (Magalhães and Bailey-Brock~~ (~~, 2017). The strong shift in community structure and composition of polychaete assemblages between the APEI#3 and the exploration areas echoes that of megafaunal (Vanreusel et al., 2016), nematode (Hauquier et al., ~~2018~~2019), and tanaid assemblages (~~Pabis~~Błażewicz et al., ~~submitted~~2019). The biogeochemical settings as well as the biological patterns of the three size groups of the benthic fauna thus converge to conclude that the structure and functioning of the benthic ecosystem in APEI#3 is not representative of any of the four exploration contract areas included in this study.

Within the mesotrophic zone, the species composition of polychaete assemblages in the Ifremer exploration area differed from the other exploration areas. Differences were driven by species belonging to common deep-sea deposit feeders such as spionids, paraonids, and opheliids (Jumars et al., 2015), whereas a lumbrinerid species characterised the eastern exploration areas (BGR, IOM and GSR). Furthermore, other carnivorous families were relatively more abundant in the eastern areas as well, such as paralacydoniids and sigalionids. These results agree with Smith et al. (2008b) who observed higher abundances of lumbrinerids and amphinomids, two families of carnivorous polychaetes (Jumars et al., 2015), in the eastern CCFZ (Kaplan East). The upper trophic levels indeed tended to be less represented in the Ifremer and APEI#3 areas than in the eastern areas. This pattern matches model predictions that food-chain length is positively correlated with resource availability in very low productivity systems (< 1–10 g C m^{-2} year^{-1}~~) (~~; Moore and de Ruiter, 2000; Post, 2002). McClain and Schlacher (2015) formulated this food-chain length-productivity relationship as the "one-more-trophic-level" hypothesis to account for a positive productivity–diversity relationship. ~~No~~Species richness and productivity were significantly correlated at Eastern CCFZ scale, but no significant correlation was ~~however~~ found between alpha diversity and productivity~~, neither at~~ in the ~~NE Pacific scale nor~~meta-analysis at the scale of the ~~whole CCFZ.~~NE Pacific. The reason diversity and productivity were not correlated in the meta-analysis that included data from the literature could be mainly methodological. In particular, the use of integrative taxonomy in this study versus morphological taxonomy in previous works might hinder comparisons of diversity metrics.

To conclude, our study supports the assumptions behind the creation of nine large APEIs, namely that gradients of sea-surface primary productivity determine large-scale patterns, and that nodule densities determine local-scale patterns in community structure, species composition and functioning (Wedding et al., 2013). However, ~~environmental conditions at the APEI#3~~among exploration contract areas, there is a shift in community composition and trophic structure between BGR, IOM and GSR on one hand, and Ifremer on the other hand, suggesting that these two groups do not belong to the same subregion as hypothesized by Wedding et al. (2013). Environmental conditions at the APEI#3 also seem to be beyond the range of those found in exploration contract areas, which may explain why the community structure and species composition of benthic assemblages are so different. ~~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.~~

4.2 Species turnover and geographic ranges

Species turnover was best illustrated by the distance-decay of NNESS similarity, which showed two different patterns. Firstly, APEI#3 showed very low similarity with all other areas, irrespective of distance. Secondly, similarity decayed linearly with distance among the exploration contract areas located within the CCFZ. Beyond variation in food inputs, as discussed above, the large dissimilarity of polychaete assemblages in APEI#3 may suggest a major physiographic barrier between the north and south of the Clarion Fracture. The Clarion Fracture Zone is a long and narrow submarine mountain range characterized by a peak and ~~through~~trough exceeding 1800 m difference in elevation (Hall and Gurnis, 2005), which may be a barrier to dispersal for abyssal fauna. In the Atlantic, the Vema-TRANSIT expedition tested the influence of the Mid-Atlantic Ridge (MAR) and the Vema Fracture Zone (VFZ) on distribution and connectivity patterns of abyssal fauna with contrasting results (Riehl et al., 2018a). The MAR is not a barrier to dispersal for nematode species of the genus *Acantholaimus* (Lins et al., 2018), a pattern already found for 61 copepod species of the genus *Mesocletodes* (Menzel et al., 2011). However, the MAR is differently permeable to dispersal for three families of isopods, depending on their habits and swimming abilities (Bober et al., 2018). In particular, connectivity was very low for Macrostylidae species, a family of burrowing isopods with limited dispersal abilities (Riehl et al., 2018b). The species composition of the two polychaete families Spionidae and Polynoidae also differed on both sides of the VFZ, which may be due to limited dispersal and different habitat characteristics (Guggolz et al., 2018). This was however not the case for species of *Laonice*, which tended to show large ranges of up to 4000 km across the Eastern and Western Atlantic (Guggolz et al., 2019); or species of *Aurospio* and *Prionospio* which could show pan-oceanic distribution (i.e., Pacific and Atlantic oceans; Guggolz et al., submitted). In the CCFZ, Bonifácio and Menot (2019) described 17 new species of polynoids based on morphology and DNA, of which four species are shared between APEI#3 and the exploration areas. In the abyssal Pacific, the CCFZ and the Peru Basin share nine species of scavenging amphipods~~ (Patel et al. (2018)),~~, which are highly motile and thus potentially cross the Clipperton and Galapagos Fracture Zones~~.~~ (Patel et al., 2018). However, species identification was based on morphology only, although cryptic species are common among scavenging amphipods, even in abyssal lineages (Melo, 2004; Havermans et al., 2013). The influence of the fracture zones on the dispersal of the abyssal fauna remains to be better understood as the Clarion and Clipperton fractures may act as a barrier for species with low dispersal abilities such as infaunal brooders. If so, the representativeness of seven out of the nine APEIs, which are partly lying beyond the fractures, may be questionable.

Moreover, the slope of the linear decay of NNESS similarity within the CCFZ suggests an average range of 14 to 25 km per species. This average range masks large variance between a small pool of widespread species, known from two or more areas, and a large pool of rare species, yet only known from one study area and in most cases only known from a single individual. This high frequency of singletons may also significantly bias the estimation of species ranges (see below for a discussion on singletons). However, based on the best knowledge we have, our study suggests that, on average, the spatial range of polychaete species in the CCFZ is on the order of 20 km. This figure can be compared with the scale of a mining operation (Volkmann et al., 2018; Volkmann and Lehnen, 2018): rounding production rate to 1.5 Mt year^{-1} and with a nodule ~~abundance~~density of 15 kg m^{-2}, an area of a 100 km^2 would be mined each year. In other words, nodule mining would affect each year an area that is equivalent to the average geographic range of ~~a ~~polychaete species.

4.3 How many polychaete species live in the CCFZ? The under-sampling bias

Considering that the economic feasibility of nodule mining requires, for any single operation, mining a minimum of ca. 100 ~~km^2~~km^2 of abyssal seafloor per year for a couple of decades (Volkmann et al., 2018; Volkmann and Lehnen, 2018), there is no doubt that the benthic ecosystem will be subjected to adverse environmental impacts and that recovery, if any, will take centuries (Miljutin et al., 2011; Vanreusel et al., 2016; Gollner et al., 2017; Jones et al., 2017). The main issue that has to be addressed is how significant these adverse impacts will be; will they cause “serious harm” (Levin et al., 2016) and in particular what will be the magnitude of biodiversity loss (Van Dover et al., 2017)? To assess the significance of adverse impacts due to nodule mining, one of the key unknowns is whether the deep sea, including abyssal fauna, is hyper-diverse (Hessler and Jumars, 1974; Grassle and Maciolek, 1992; Paterson et al., 1998) or not (May, 1992; Rex et al., 2005).

Locally, alpha diversity of polychaete assemblages is high in the CCFZ (Paterson et al., 1998; Glover et al., 2002; Wilson, 2017), and it is particularly so for the equitability component of diversity, as exemplified by the slopes of individual-based rarefaction curves and a ratio of individuals to species of two to three at a local scale. At none of the sampling areas does rarefaction curves level off, highlighting that species richness has been systematically under-sampled, even at DOMES A, where 41 box-cores have been sampled (Wilson, 2017). At a regional scale, Glover et al. (2002) reported a total of 177 polychaete species in 2.94 ~~m^2~~m^2 along a 3260 km latitudinal gradient of productivity in the NE Pacific and a total of 183 species in 21 ~~m^2~~m^2 along a 2800 km longitudinal transect crossing the CCFZ. Janssen et al. (2015) found 233 MOTUs of polychaetes from epibenthic sledge samples of the BGR and Ifremer areas separated by 1400 km. Along this same transect, and using an integrative taxonomy approach, we report here a total of 275 species from 30 quantitative box cores, covering an area of 7.5 ~~m^2.~~m^2. The two latter studies, relying partly or totally on DNA barcoding, yield higher numbers of species than the two former regional assessments based on morphology only. Our personal observations during the identification process effectively allowed the identification of cryptic species sometimes sympatrically distributed. This presence of cryptic species has been already observed by Janssen et al. (2015) and Bonifácio and Menot (2019) with the former suggesting that ~~such extreme~~the specific environmental conditions have already selected for the best morphological characters, resulting in convergent speciation in other aspects as well, such as behavior or physiology. Integrative taxonomy thus not only provides more accurate estimates of species diversity, but also facilitates comparisons across datasets. 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), although DNA sequences can easily be matched. Therefore, 26 MOTUs are shared between Janssen et al. (2015) and our study. The overlap is low but it should be noted that we had only 71 COI sequences belonging to 45 MOTUs to compare with the 556 COI sequences belonging to 233 MOTUs from Janssen et al. (2015). This highlights a shortcoming of COI-based barcoding because success rates for COI sequencing are generally low~~ and a~~. A combination of several genetic markers ~~plus morphology is~~associated to formal morphological descriptions are thus essential to accurately assess species diversity. In addition, Janssen et al. (2015) used an epibenthic sledge and we used a box corer. These two devices sample different components of benthic communities. During the SO239 cruise, epibenthic sledge samples provided a collection of 278 specimens and 80 MOTUs of polynoids, a family of larger epifaunal polychaetes (Bonifácio et al., 2016; Bonifácio and Menot, 2019), but in our box core samples, we only found one polynoid.

Overall, the combination of high local diversity, unsaturated rarefaction curves, high levels of cryptic diversity and high rates of species turnover suggest that polychaete diversity in the CCFZ is large and vastly under-sampled. Within the eastern CCFZ, the linear decay of NNESS similarity suggests a species turnover of 0.04 to 0.07 species km^{-1}, and decomposition of the beta diversity shows that 90 % of dissimilarity is due to spatial turnover. This rate of species change is one order of magnitude higher than the rate found by Wilson (2017) for polychaetes (0.0056 species km^{-1}) and even higher than the rate for isopods (0.012 species km^{-1}). These discrepancies may again reflect a high level of cryptic ~~richness~~diversity. Wilson (2017) acknowledged that the rates of change he found may be underestimated, particularly for polychaetes, due to the fact that identifications were based on morphology only. The rate of species turnover that we report here for a 1440 km transect across the eastern CCFZ is however 20 times lower than the rates of 1 species km^{-1} reported by Grassle and Maciolek (1992) from a 180 km transect at 2100 m in the Northwest Atlantic. This difference is roughly consistent with Grassle and Maciolek (1992) hypothesis that in the deepest and most oligotrophic parts of the ocean, species richness may be lower by one order of magnitude. Still, an extrapolation of our rate of species turnover to the 6 million km² of the CCFZ, as Grassle and Maciolek (1992) did for the whole deep sea, yields predictions of at least 240,000 polychaete species, i.e. a number of species equivalent to the number of accepted marine species globally (WoRMS, 2019).

This prediction is in sharp contrast with the outcome of non-parametric estimators of species richness such as Chao or Jackknife, which provides a maximum estimate of 498 species. Such estimators however implicitly assume that the number of singletons decreases with increasing sample size (Melo, 2004), but the number of singletons steadily increased with sample size in this study. In such circumstances, the non-parametric species estimators underestimates species richness (Melo, 2004; Coddington et al., 2009). In an intensive survey of spiders in 1 ha of tropical forest, Coddington et al. (2009) found 29 % of singletons and tested the null hypothesis of under-sampling against ecologically driven hypotheses to explain this “anomalously” high frequency of singletons. They concluded that under-sampling was the most parsimonious explanation for the high frequency of singletons and that it causes a systematic negative bias of species richness estimators. In the deep-sea, an anomalously high rate of singletons ~~of (~~(about one-third of the sampled species) is ~~in fact~~usually the rule of macrofaunal surveys (Gage, 2004) and the most parsimonious hypothesis that still needs to be tested thus is that the deep-sea macrofauna has been systematically under-sampled.

Although under-sampling causes an underestimation of species richness, it may also lead to an overestimation of the distance-decay of similarity, because singletons, considered as endemic to an area in the analysis, may have much wider distributions. 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. The former estimate assumes that we have already sampled about half of the regional diversity and further suggests that most species have a large geographical range. The latter estimate assumes that we have sampled 0.1 % of the polychaete species in the CCFZ and that these species have narrow geographical ranges about the size of ~~a yearly mined area~~the area that will presumably be mined in one year by a single mining operation.

5 Conclusions

~~As part of the JPI Oceans project “Ecological aspects of mining impact”, four~~ Food inputs and nodule ~~exploration contract areas and one APEI were sampled across the eastern half of the CCFZ to characterize the benthic communities found there. The main objectives of the present study were to describe and identify alpha and beta diversity patterns of polychaete assemblages with the aim of increasing basic knowledge on the ecology of these abyssal communities, testing the hypotheses that supported spatial conservation planning~~ density influence the structure and composition of polychaete assemblages in the CCFZ~~, assessing the representativeness of an APEI and improving the assessment of potential risk to biodiversity due to nodule mining.~~

~~In the abyssal NE Pacific, patterns of polychaete abundance follow the northward and westward gradients of decreasing sea-surface primary productivity. The increasingly~~. This is a confirmation of hypotheses underpinning the design of the APEIs. Increasingly oligotrophic conditions cause ~~a shift~~two shifts in the trophic structure and species composition of polychaete assemblages~~ that is consistent with the assumption that led to the creation of nine APEIs ~~. The first shift suggests that within the eastern central subregion defined by Wedding et al. (2013) and sampled in the ~~CCFZ~~present study, the eastern contract areas (BGR, IOM, GSR) and the western contract (Ifremer) belong in fact to different subregions and if so should be represented by two different APEIs. The most significant shift in community structure and composition was however found between the APEI#3 and the nodule exploration areas. APEI#3 is found in oligotrophic conditions, north of the Clarion Fracture Zone, whereas exploration areas experience mesotrophic conditions south of the Clarion Fracture Zone. The scantiness of food supply and a barrier to dispersal may thus compromise the representativeness of APEI#3 and ~~thus ~~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 ~~fauna.~~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).

The efficiency of the regional environmental management plan for the CCFZ is crucial in face of the yet uncertain but potentially adverse impacts of nodule mining. Within the CCFZ per se, the diversity of polychaete assemblages is even higher than previously thought due to a high level of cryptic ~~richness~~diversity. 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. On the contrary, non-parametric estimators of species richness suggest that total species richness across the five study areas does not exceed 498 species~~.~~ which likely implies a species range much larger than 25 km. Both methods of estimating species richness can however be severely biased by singletons. Singletons represent 49 % of the 275 species of polychaetes that were sampled. The most parsimonious hypothesis to explain such a high rate of singletons is under-sampling. ~~The assessment of potential risks and scales of biodiversity loss thus requires an appropriate inventory of species richness in the CCFZ.~~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. Both strategies are needed to assess the potential risks and scales of biodiversity loss due to nodule mining in the CCFZ.

Data availability

~~Abundance~~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 (Bonifacio~~PANGAEA database (Bonifácio et al., 2019~~) whereas DNA sequences are available in BOLD or GenBank databases.~~).

Author contribution

LM and PMA conceived the project and designed the sampling. LM and PB performed the sampling and processed the samples. PB identified (morphology and DNA) the polychaetes. LM and PB analyzed and interpreted the data. All authors prepared and contributed to the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgement

The research leading to these results has received funding from the Ifremer program "*Ressources Minérales Marines*" (REMIMA), the JPI Oceans pilot action "Ecological Aspects of Deep-Sea Mining" and the European Union Seventh Framework Program (FP7/2007–2013) under the MIDAS project, grant agreement no. 603418. We are grateful to the crew of the RV *Sonne*, and all people involved in the field sampling and sample processing during the SO239 cruise. We would like to thank for their expertise in washing and sieving samples to Dr. Stefanie Kaiser, Sarah Schnurr and Dr. Ana Hilário; and for live-sorting the worms to Lenka Neal. Also, thanks to Baptiste François for sample sorting in the laboratory. We are grateful, as well, to all people involved in molecular analysis: Dr. Aliou Dia, Guillaume Lannuzel, Emmanuelle Omnes, Alana Jute, Mohamed Dosoky and Gavin Campbell. Special thanks to Dr. Thomas Dahlgren for taking care and sharing data of some families of polychaetes; to Prof. Dr. Ann Vanreusel, Dr. Freija Hauquier and Dr. Felix Janssen for providing the abiotic data of CCFZ.

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Tables

Table 1. Area, locality, station, date, depth, geographical position and nodule density of all 34 box corer deployments across the CCFZ during the SO239 cruise. "*" indicates box cores considered as non-quantitative, not included in the analyses.

Area	Locality	Station	Date	Depth (m)	Latitude	Longitude	Nodule density (kg m^{-2})
BGR	BGR-PA	12	20/03/15	4118	11.8471667	-117.05933	26.40
BGR	BGR-PA	15	21/03/15	4133	11.8443333	-117.05217	26.80
BGR	BGR-PA	16	21/03/15	4122	11.8573333	-117.052	24.00
BGR	BGR-PA	21	22/03/15	4120	11.8535	-117.0595	22.80
BGR	BGR-PA	23	22/03/15	4122	11.85	-117.05267	20.80
BGR*	BGR-RA	51	27/03/15	4348	11.8236667	-117.52367	0.00
BGR	BGR-RA	57	28/03/15	4370	11.8075	-117.52433	8.00
BGR	BGR-RA	58	28/03/15	4350	11.8205	-117.54167	1.60
BGR	BGR-RA	60	29/03/15	4325	11.8076667	-117.55033	18.00
IOM	IOM-control	88	02/04/15	4433	11.079	-119.65883	0.00
IOM	IOM-control	89	02/04/15	4437	11.0758333	-119.66083	1.20
IOM	IOM-control	90	03/04/15	4434	11.074	-119.66417	0.00
IOM	IOM-disturb	94	03/04/15	4414	11.0736667	-119.6555	0.40
IOM	IOM-disturb	95	03/04/15	4418	11.0735	-119.65583	0.80
IOM	IOM-disturb	97	04/04/15	4421	11.0728333	-119.65617	0.20
IOM*	IOM-resed	105	05/04/15	4423	11.0711667	-119.65533	0.00
IOM	IOM-resed	106	05/04/15	4425	11.0716667	-119.65483	0.20
IOM	IOM-resed	107	05/04/15	4425	11.0721667	-119.6545	0.30
GSR	GSR	119	08/04/15	4516	13.8591667	-123.25267	26.47
GSR	GSR	127	09/04/15	4514	13.8443333	-123.246	27.10
GSR	GSR	128	09/04/15	4511	13.8516667	-123.252	27.10
GSR	GSR	137	11/04/15	4510	13.856	-123.238	25.20
GSR	GSR	138	11/04/15	4503	13.8481667	-123.23467	26.47
Ifremer	Ifremer	159	15/04/15	4921	14.049	-130.13433	19.80
Ifremer	Ifremer	162	16/04/15	4951	14.049	-130.126	20.20
Ifremer	Ifremer	169	17/04/15	4964	14.0421667	-130.12733	24.10
Ifremer	Ifremer	180	18/04/15	4936	14.0416667	-130.13633	16.00
Ifremer	Ifremer	181	18/04/15	4896	14.0465	-130.1415	16.80
Ifremer	Ifremer	182	18/04/15	4957	14.0423333	-130.1275	22.40
APEI#3	APEI#3	195	21/04/15	4833	18.7958333	-128.36217	6.28
APEI#3	APEI#3	196	21/04/15	4847	18.7971667	-128.34617	1.80
APEI#3*	APEI#3	203	23/04/15	4843	18.774	-128.35317	2.88

APEI#3	APEI#3	204	23/04/15	4816	18.7733333	-128.33617	3.65
APEI#3*	APEI#3	209	24/04/15	4819	18.7845	-128.3725	3.65

Table 2. Data available from previous studies and from the present study included in ~~regional~~NE Pacific-scale analyses.

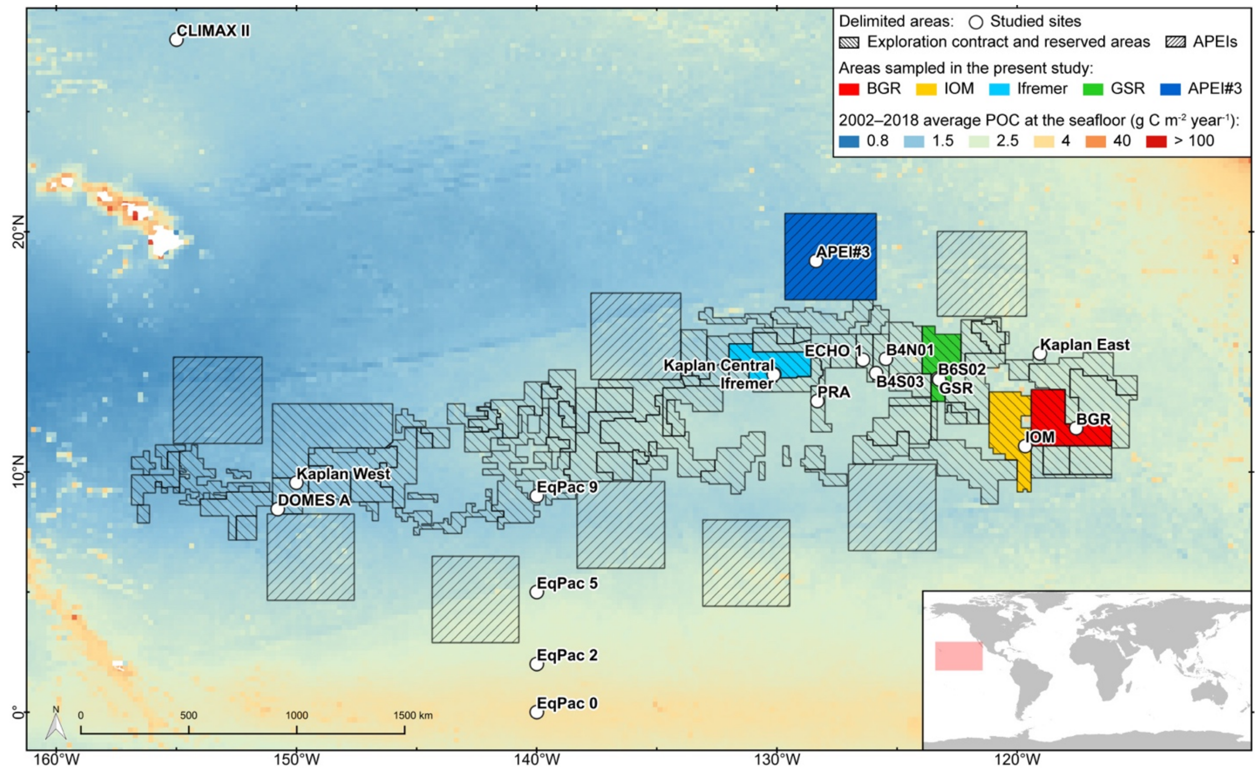
Area	Year	References	Depth (m)	Latitude	Longitude	Number of box cores	Mean abundance (ind. 0.25 m^{-2})	Total number of species	ES163	Bootstrap	2002–2018 average POC at the seafloor (g C m^{-2} year^{-1})
DOMES A	1977/78	Glover et al. (2002~~);~~ ~~Wilson (2017))~~ Wilson (2017)	5100	8.45	-150.78333	47 41	16	104	56	~~203 (based on 41 box cores)~~ 203	1.46
Kaplan West	2004	Smith et al. (2008b)	5000	9.55195	-150.00845		5				1.50
Kaplan Central	2004	Smith et al. (2008b)	5000	14.0710333	-130.109		21				1.88
Ifremer	2015	Present study	4937	14.049	-130.13433	6	28	73		91	1.90
PRA	1989	Glover et al. (2002~~);~~ ~~Wilson (2017~~) Wilson (2017)	4800	12.95	-128.31667	16	65	100	47	~~310~~ 310	2.04
ECHO 1	1982	Glover et al. (2002~~);~~ ~~Wilson (2017~~) Wilson (2017)	4500	14.6666667	-126.41667	15 14	42	113	60	~~274 (based on 14 box cores)~~ 274	2.05
B4S03	2015	De Smet et al. (2017)	4500	14.1124806	-125.87147	4	20	12			2.11
B4N01	2015	De Smet et al. (2017)	4500	14.7064111	-125.46118	5	14	10			2.04
B6S02	2015	De Smet et al. (2017)	4500	13.8940389	-123.29704	3	21	14			2.11
GSR	2015	Present study	4510	13.8443333	-123.246	5	59	104	79	126	2.11
IOM	2015	Present study	4425	11.0758333	-119.66083	8	37	107	79	131	2.07
Kaplan East	2003	Smith et al. (2008b)	4000	14.9308333	-119.0495		21				2.27
BGR	2015	Present study	4206	11.8205	-117.54167	8	58	156	88	192	2.23
APEI#3	2015	Present study	4832	18.7845	-128.3725	3	5	9		11	1.66
CLIMAX II	1969	Paterson et al. (1998)	5010	28	-155	10	16	46			1.91
EqPac 0	1992	Glover et al. (2002)	4300	0	-140	3	84	73	71		3.55
EqPac 2	1992	Glover et al. (2002)	4400	2	-140	4	60	82	82		2.68

EqPac 5	1992	Glover et al. (2002)	4400	5	-140	3	80	76	75		2.33
EqPac 9	1992	Glover et al. (2002)	4900	9	-140	3	13	23			1.78

Table 3. Observed species richness (Sobs) and estimators of species richness for each sampled area and for eastern CCFZ.

Area	Sobs	Individual-based				Sample-based					
		n	Chao 1	ACE	ES12	n	Chao 2	Jackknife 1st order	Jackknife 2nd order	Bootstrap	S3
BGR	156	415	355 ± 61	334 ± 11	11 ± 1	8	311 ± 46	240 ± 34	295	192 ± 15	82 ± 9
IOM	107	274	191 ± 30	225 ± 10	11 ± 1	8	182 ± 26	162 ± 22	195	131 ± 10	58 ± 6
GSR	104	263	157 ± 19	196 ± 9	11 ± 1	5	161 ± 20	153 ± 26	178	126 ± 12	77 ± 6
Ifremer	73	154	163 ± 38	181 ± 8	11 ± 1	6	160 ± 36	115 ± 19	142	91 ± 9	45 ± 5
APEI#3	9	12	20 ± 10	27 ± 2	9 ± 0	3	30 ± 27	14 ± 4	17	11 ± 2	9 ± 0
CCFZ	275	1118	450 ± 41	484 ± 13	11 ± 1	30	467 ± 44	411 ± 29	498	334 ± 14	66 ± 13

Figures


Delimited areas: ○ Studied sites
Exploration contract and reserved areas
APEIs
Areas sampled in the present study:
BGR
IOM
Ifremer
GSR
APEI#3
2002–2018 average POC at the seafloor (g C m-2 year-1):
0.8
1.5
2.5
4
40
> 100
CLIMAX II
APEI#3
Kaplan East
ECHO 1
B4N01
Kaplan Central
B6S02
Ifremer
B4S03
GSR
PRA
BGR
IOM
Kaplan West
DOMES A
EqPac 9
EqPac 5
EqPac 2
EqPac 0
N
0
500
1000
1500 km
20°N
10°N
0°
160°W
150°W
140°W
130°W
120°W

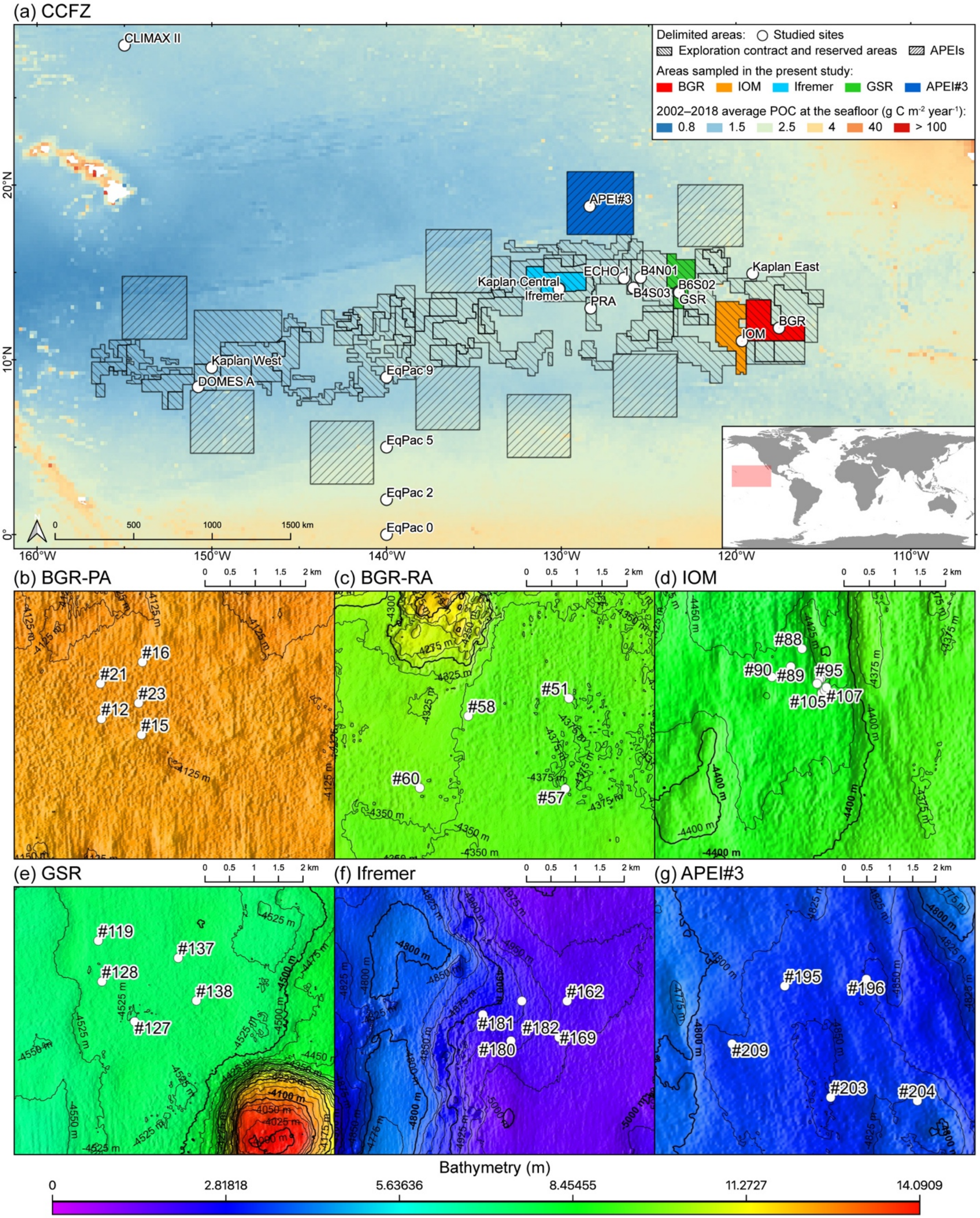
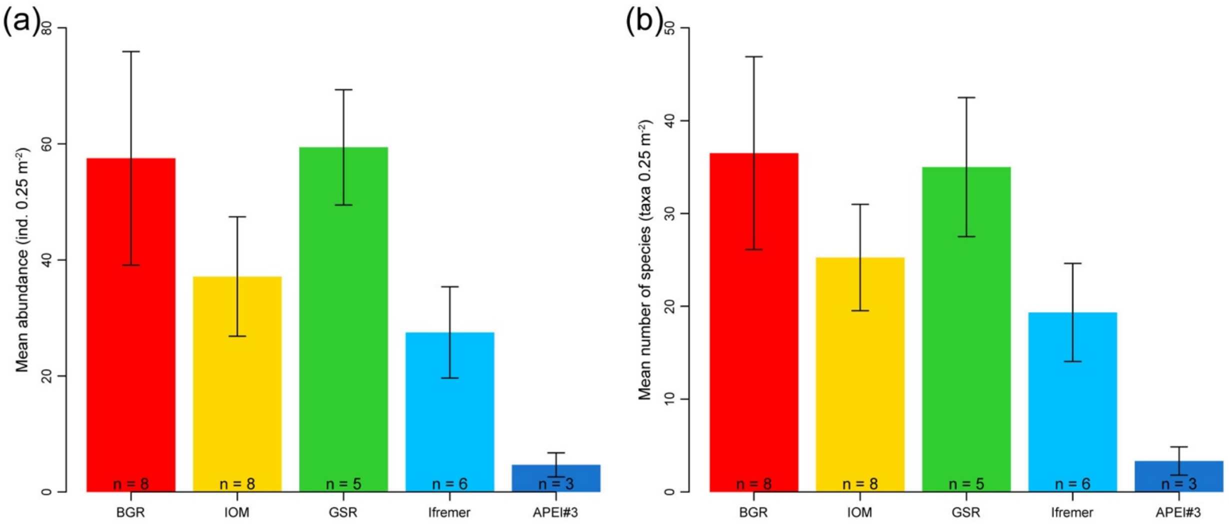

(a) CCFZ
Delimited areas: ○ Studied sites
▨ Exploration contract and reserved areas ▨ APEIs
Areas sampled in the present study:
🟥 BGR 🟧 IOM 🟦 Ifremer 🟩 GSR 🟦 APEI#3
2002–2018 average POC at the seafloor (g C m⁻² year⁻¹):
0.8 1.5 2.5 4 40 > 100
CLIMAX II
APEI#3
Kaplan Central
Ifremer
ECHO 1
B4N01
B4S03
B6S02
GSR
PRA
Kaplan East
BGR
IOM
Kaplan West
DOMES A
EqPac 9
EqPac 5
EqPac 2
EqPac 0
0 500 1000 1500 km
20°N
10°N
0°
160°W
150°W
140°W
130°W
120°W
110°W
(b) BGR-PA
0 0.5 1 1.5 2 km
#16
#21
#23
#12
#15
4125 m
(c) BGR-RA
#51
#58
#60
#57
4275 m
4325 m
4350 m
4375 m
(d) IOM
#88
#90
#89
#95
#105
#107
4375 m
4400 m
4450 m
(e) GSR
#119
#137
#128
#138
#127
4525 m
4550 m
4500 m
4100 m
4050 m
4025 m
(f) Ifremer
#162
#181
#182
#169
#180
4800 m
4850 m
4900 m
5000 m
(g) APEI#3
#195
#196
#209
#203
#204
4775 m
4800 m
4825 m
4850 m
Bathymetry (m)
0
2.81818
5.63636
8.45455
11.2727
14.0909

Figure 1. (a) Map of the nodule exploration ~~contract~~contracts, reserved areas and ~~areas~~Areas of ~~particular interest~~Particular Environmental Interest (APEI) in the Clarion-Clipperton Fracture Zone (CCFZ) showing the sampling areas from this study (in color) and previous macrobenthic surveys~~. The areas sampled during the SO239 cruises are shown in color. The~~ ; the background map shows the average particulate organic carbon (POC) flux at ~~the~~ seafloor during the 2002~~–~~-2018 period. The areas sampled during the SO239 cruises are enlarged in following figures: BGR (b and c), IOM (d), GSR (e), Ifremer (f) and APEI#3 (g); with detailed local hydroacoustic maps based on multibeam system EM122 (Martínez Arbizu and Haeckel, 2015; Greinert, 2016) in background.



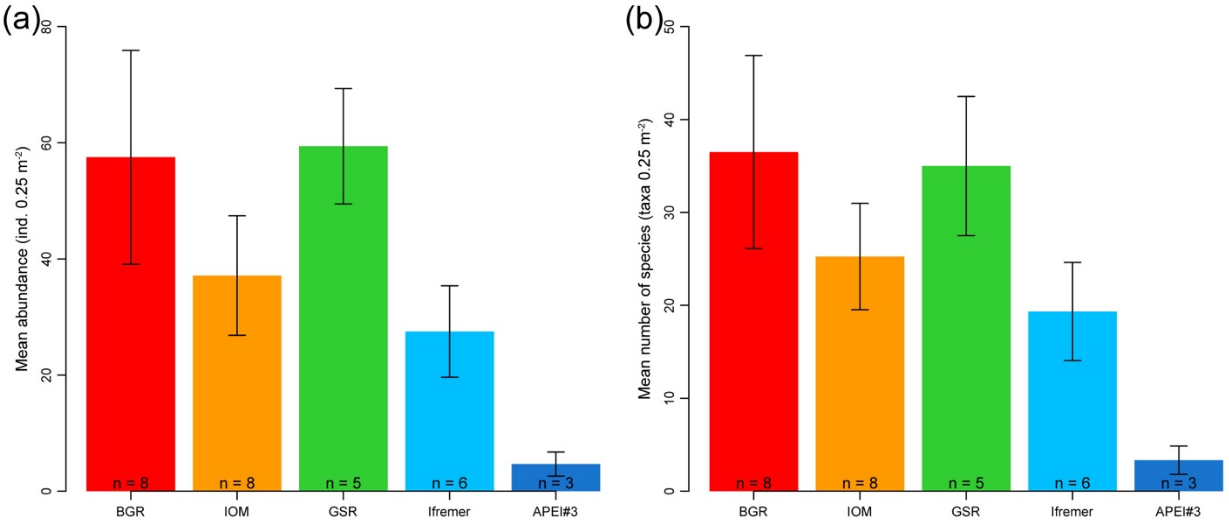
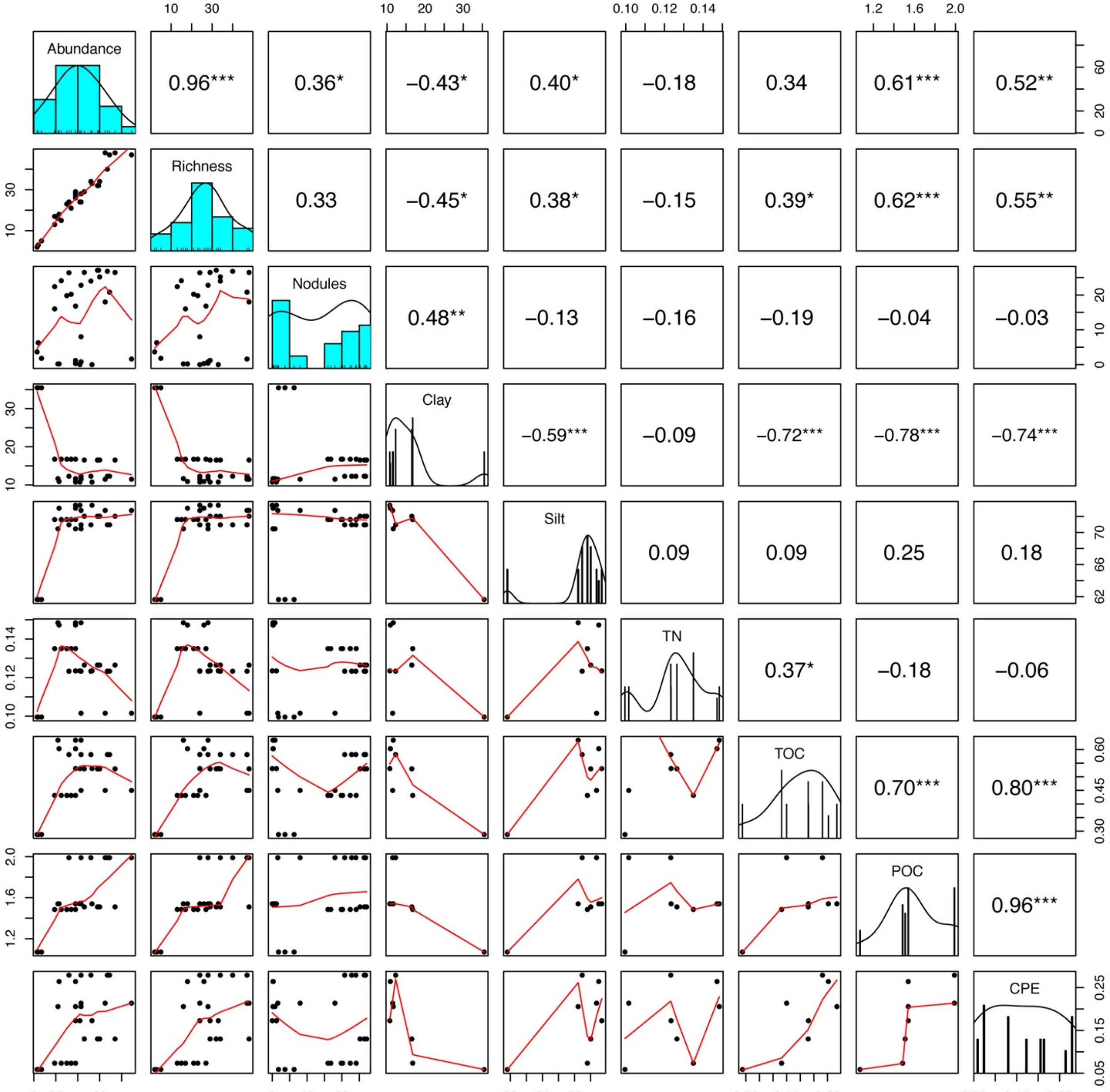


Figure 2. Bar plots of mean abundance per box core (a) and mean species ~~density~~richness per box core (b) of polychaete assemblages for each sampled area within the eastern CCFZ. “n” indicates the number of box cores samples; error bars show the standard deviation.


Abundance
Richness
Nodules
Clay
Silt
TN
TOC
POC
CPE
0.96***
0.36*
−0.43*
0.40*
−0.18
0.34
0.61***
0.52**
0.33
−0.45*
0.38*
−0.15
0.39*
0.62***
0.55**
0.48**
−0.13
−0.16
−0.19
−0.04
−0.03
−0.59***
−0.09
−0.72***
−0.78***
−0.74***
0.09
0.09
0.25
0.18
0.37*
−0.18
−0.06
0.70***
0.80***
0.96***
10
30
10
20
30
0.10
0.12
0.14
1.2
1.6
2.0
0
20
60
0
10
20
62
66
70
0.30
0.45
0.60
0.05
0.15
0.25

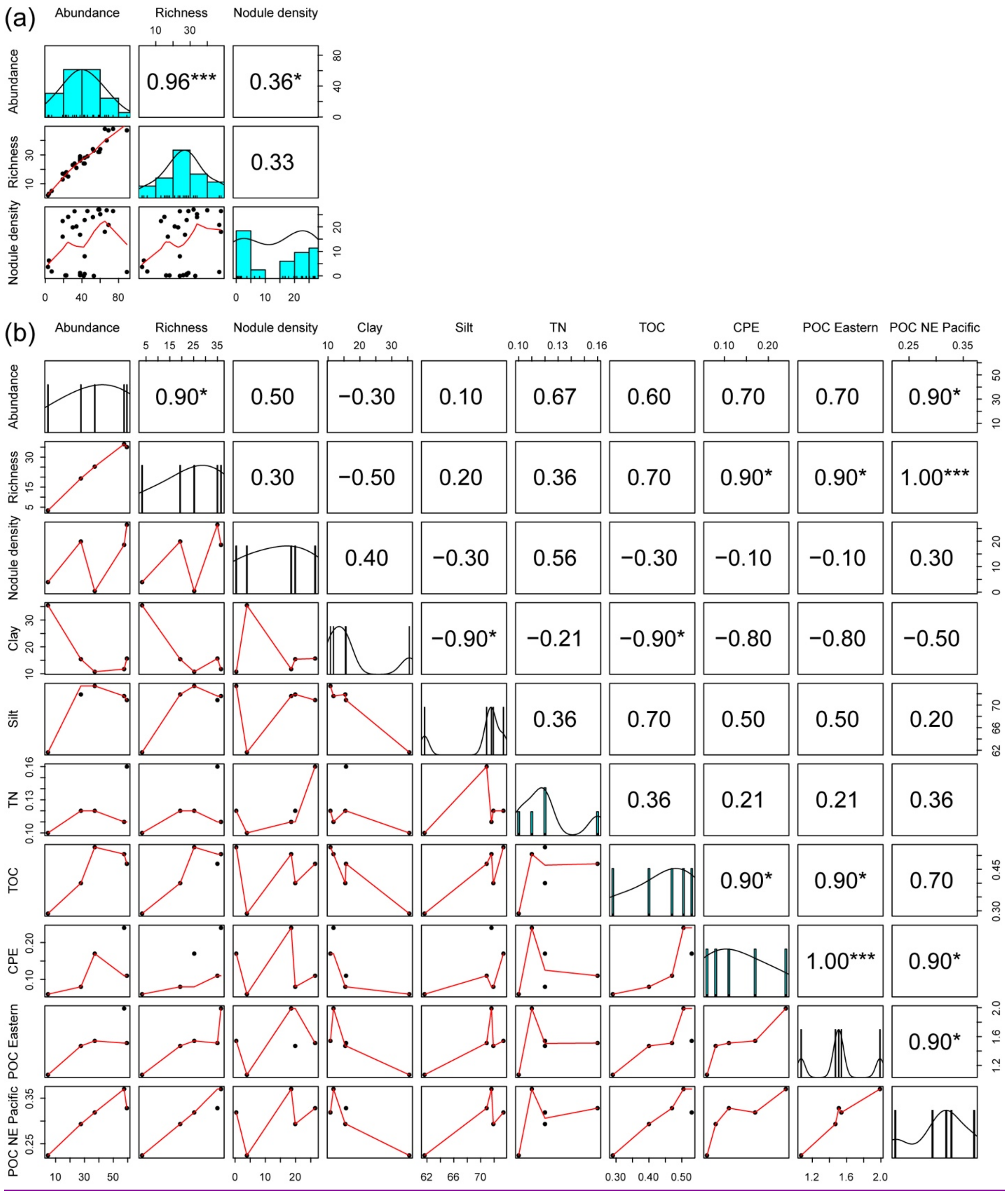
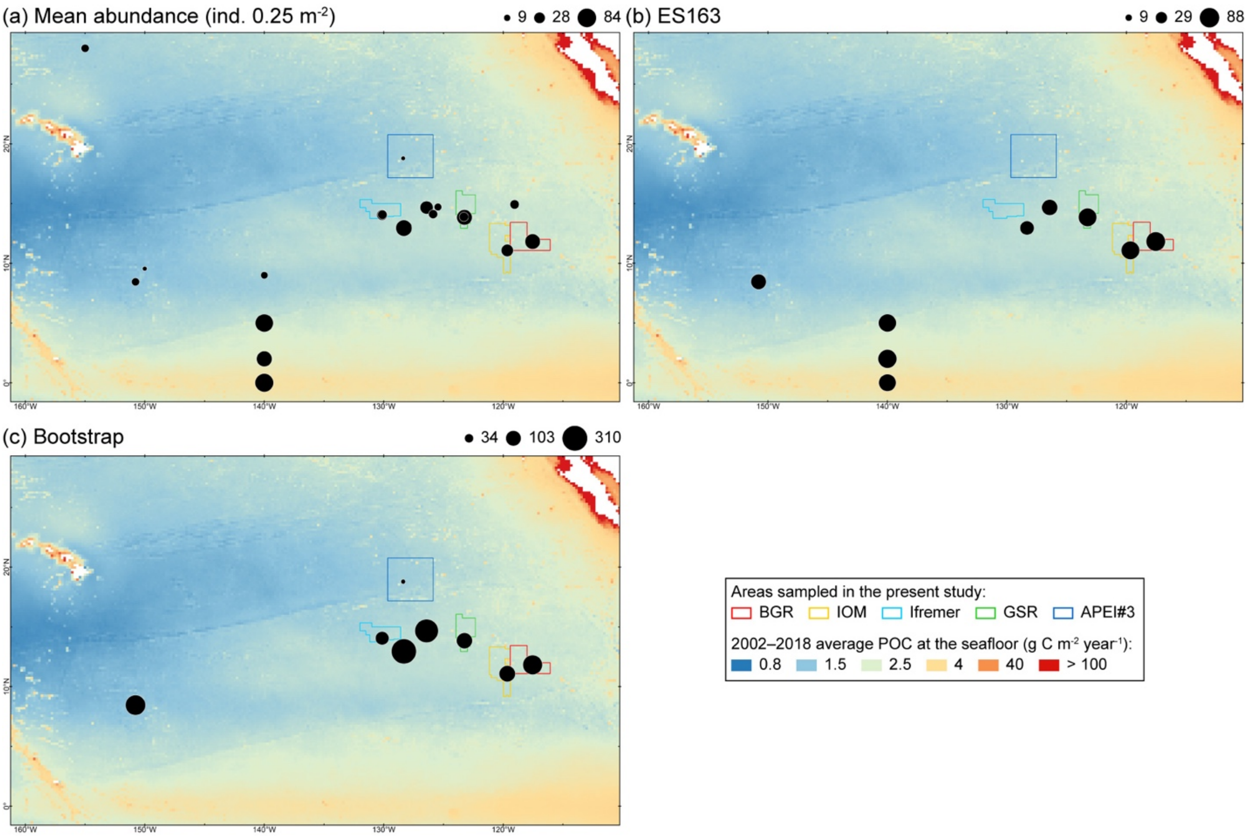


Figure 3. Correlation matrix between biotic and abiotic variables from sampled areas within the eastern CCFZ. Diagonal panels show the distribution frequency of values for each variable. Below-the-diagonal panels show the correlation plot between pairs of

variables. Above-the-diagonal panels show the Spearman coefficient correlations between pairs of variables. Abundance, richness and nodule density per box-core (a) and average biotic and abiotic variables per area (b). POC Eastern values provided by Volz et al. (2018); POC NE Pacific values were estimated in the present study. "*" indicates $p < 0.05$, "" $p < 0.01$ and "***" $p < 0.001$.**


(a) Mean abundance (ind. 0.25 m^{-2})
9
28
84
(b) ES163
9
29
88
(c) Bootstrap
34
103
310
20°N
10°N
0°
160°W
150°W
140°W
130°W
120°W
Areas sampled in the present study:
BGR
IOM
Ifremer
GSR
APEI#3
2002–2018 average POC at the seafloor (g C m^{-2} year^{-1}):
0.8
1.5
2.5
4
40
> 100

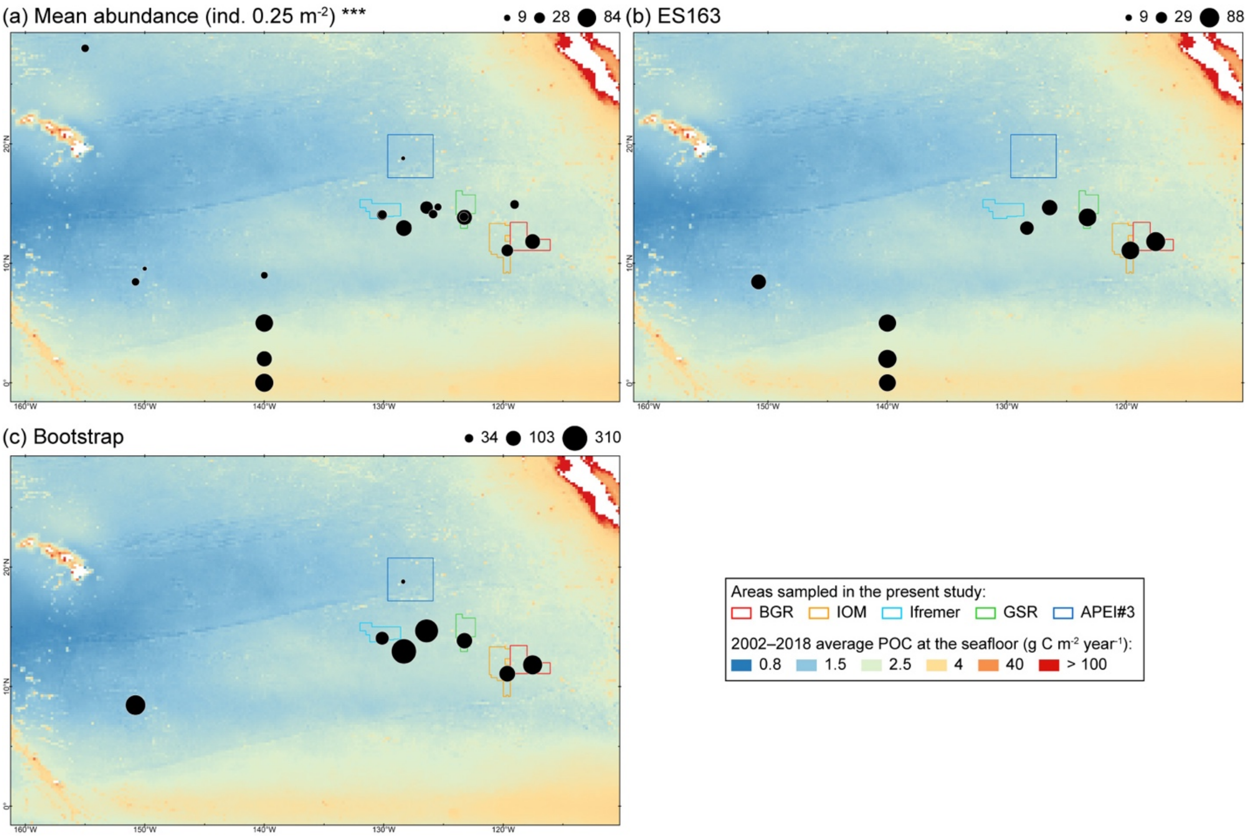
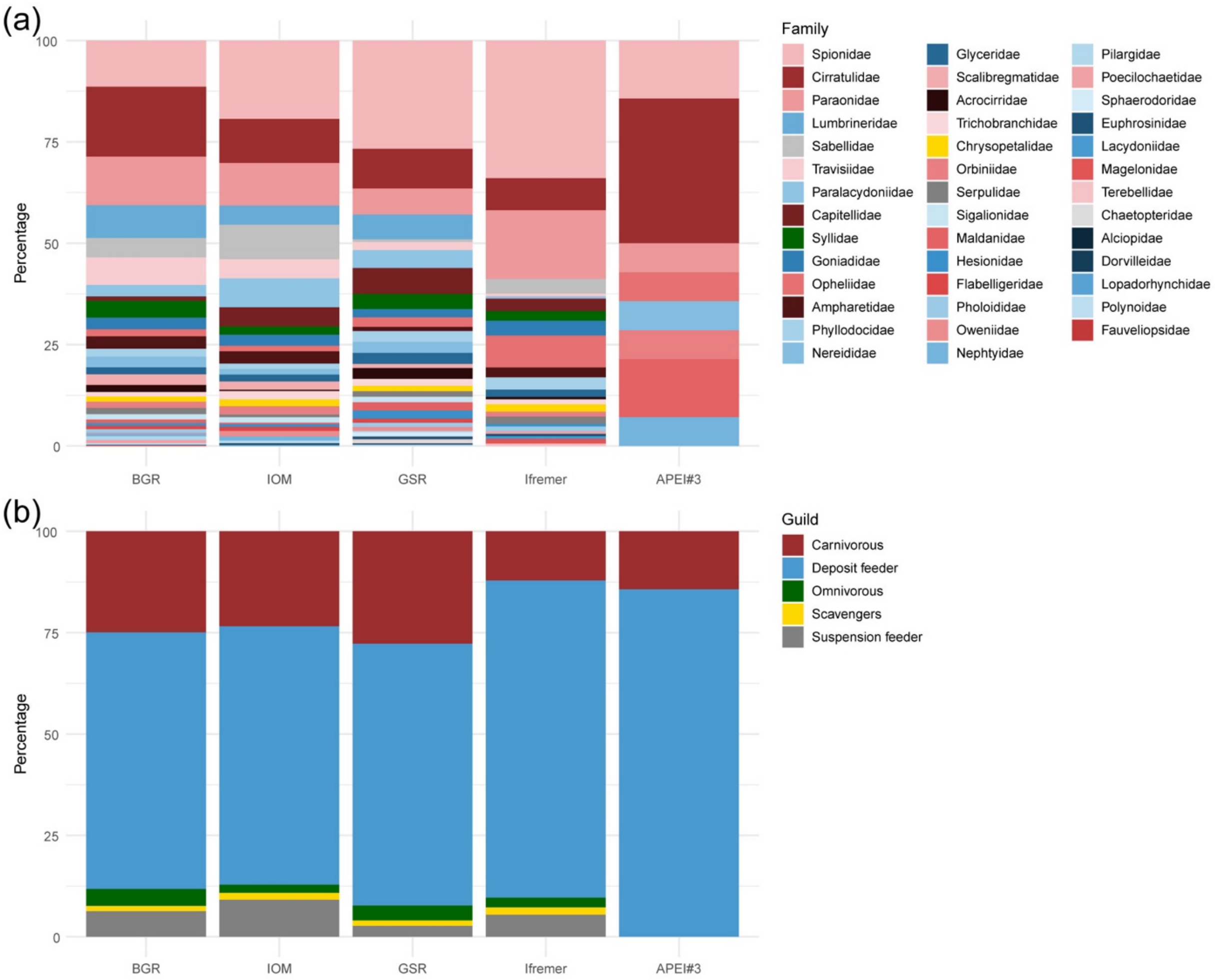


Figure 4. ~~Map~~Plot of mean abundance (a) and diversity estimators, ES163 (b) and bootstrap (c), from ~~the Northeast Pacific~~previous and the present study (Table 2) in relation to the 2002–2018 average particulate organic carbon (POC) ~~concentration~~flux at the seafloor along the CCFZ~~. The~~ (background~~ map shows average POC flux at the seafloor during the 2002–2018 period~~). "***" indicates significant ($p < 0.001$) Spearman correlation.


(a)
100
75
50
25
0
Percentage
BGR
IOM
GSR
Ifremer
APEI#3
Family
Spionidae
Cirratulidae
Paraonidae
Lumbrineridae
Sabellidae
Travisiidae
Paralacydoniidae
Capitellidae
Syllidae
Goniadidae
Opheliidae
Ampharetidae
Phyllodocidae
Nereididae
Glyceridae
Scalibregmatidae
Acrocirridae
Trichobranchidae
Chrysopetalidae
Orbiniidae
Serpulidae
Sigalionidae
Maldanidae
Hesionidae
Flabelligeridae
Pholoididae
Oweniidae
Nephtyidae
Pilargidae
Poecilochaetidae
Sphaerodoridae
Euphrosinidae
Lacydoniidae
Magelonidae
Terebellidae
Chaetopteridae
Alciopidae
Dorvilleidae
Lopadorhynchidae
Polynoidae
Fauveliopsidae
(b)
100
75
50
25
0
Percentage
BGR
IOM
GSR
Ifremer
APEI#3
Guild
Carnivorous
Deposit feeder
Omnivorous
Scavengers
Suspension feeder

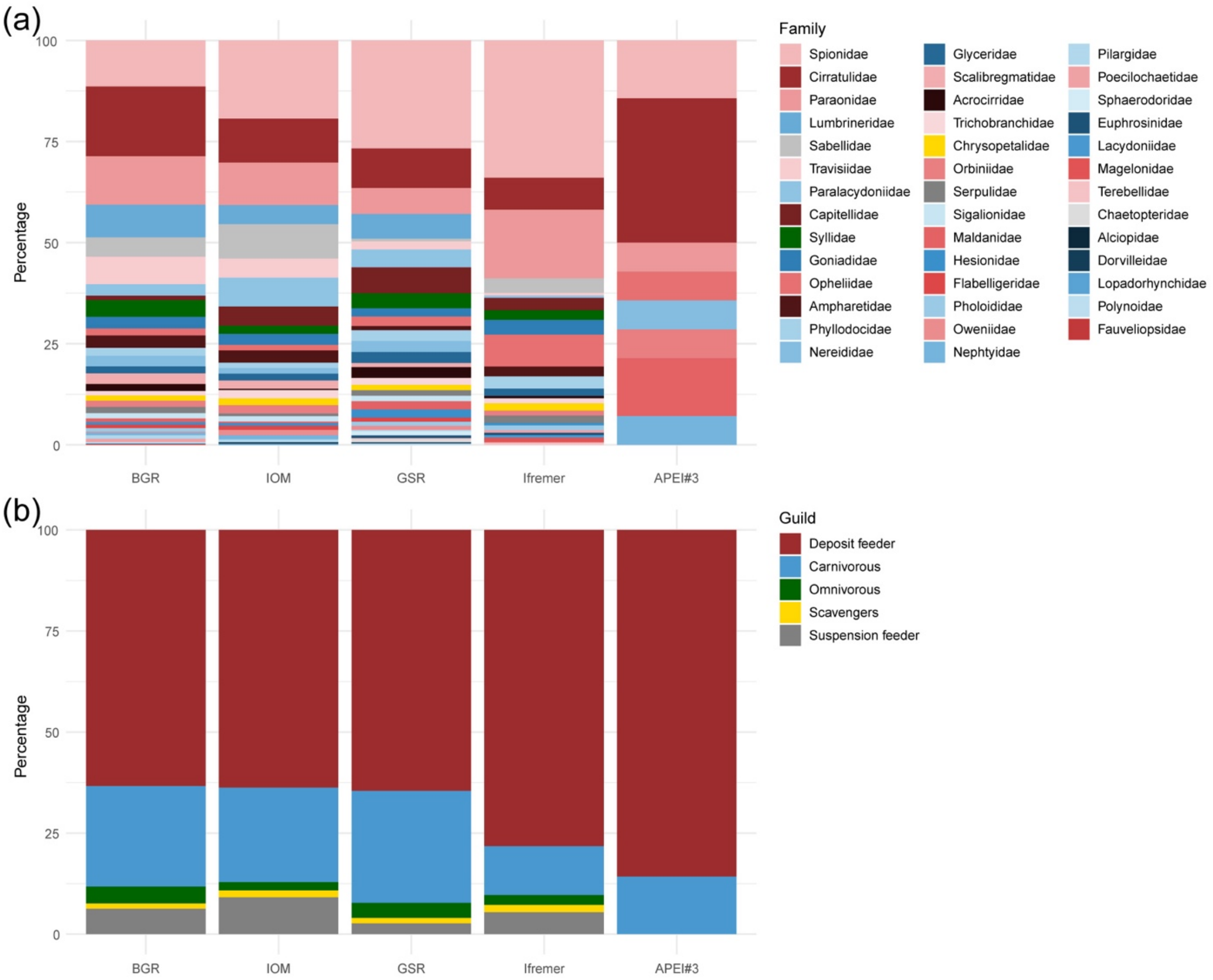
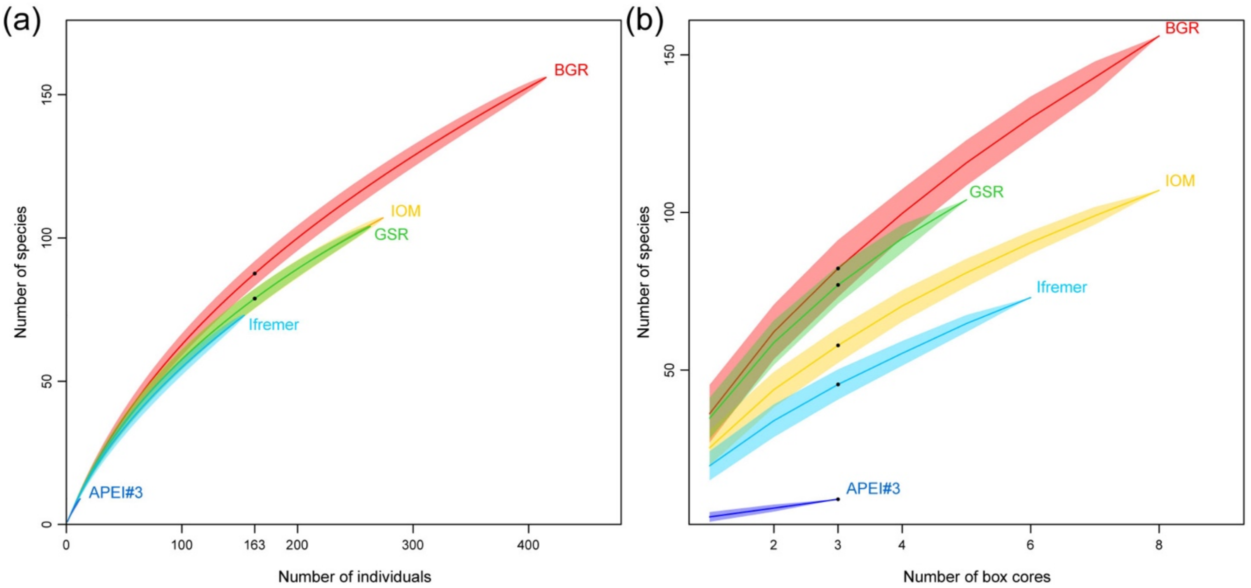


Figure 5. Bar plots of the relative abundance of families (a) and trophic guilds (b) for each sampled area within the eastern CCFZ. Gradient color in (a) corresponds to the different guilds in (b).



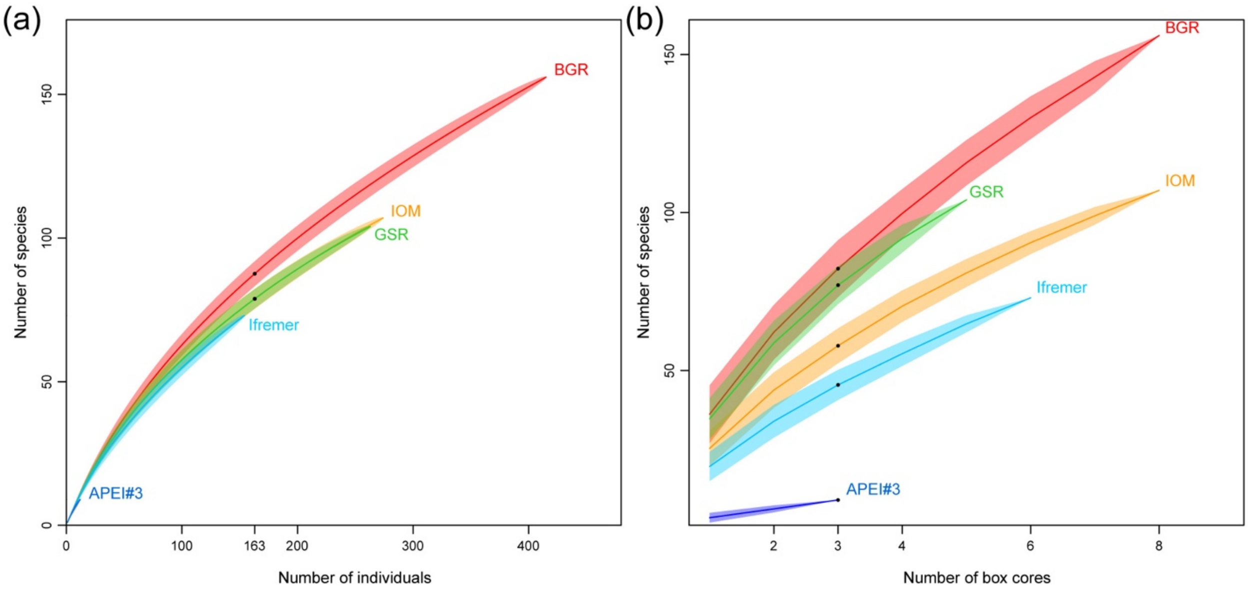
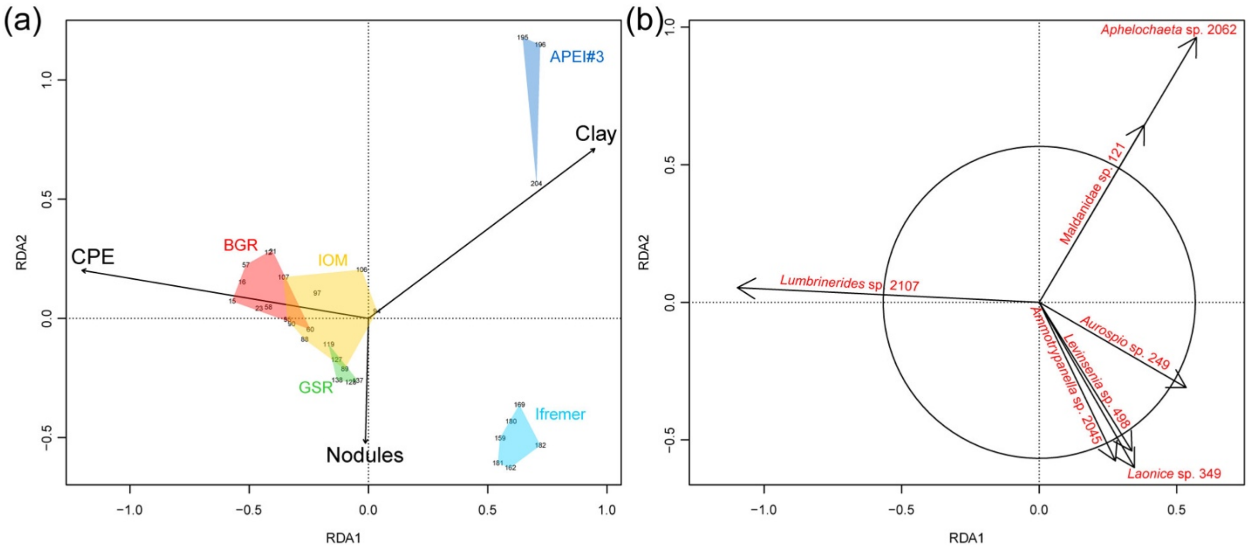


Figure 6. ~~Individual-based rarefaction~~Rarefaction curves based on individuals (a) and ~~sample-based accumulation curves~~samples (b) for each sampled area within the eastern CCFZ.



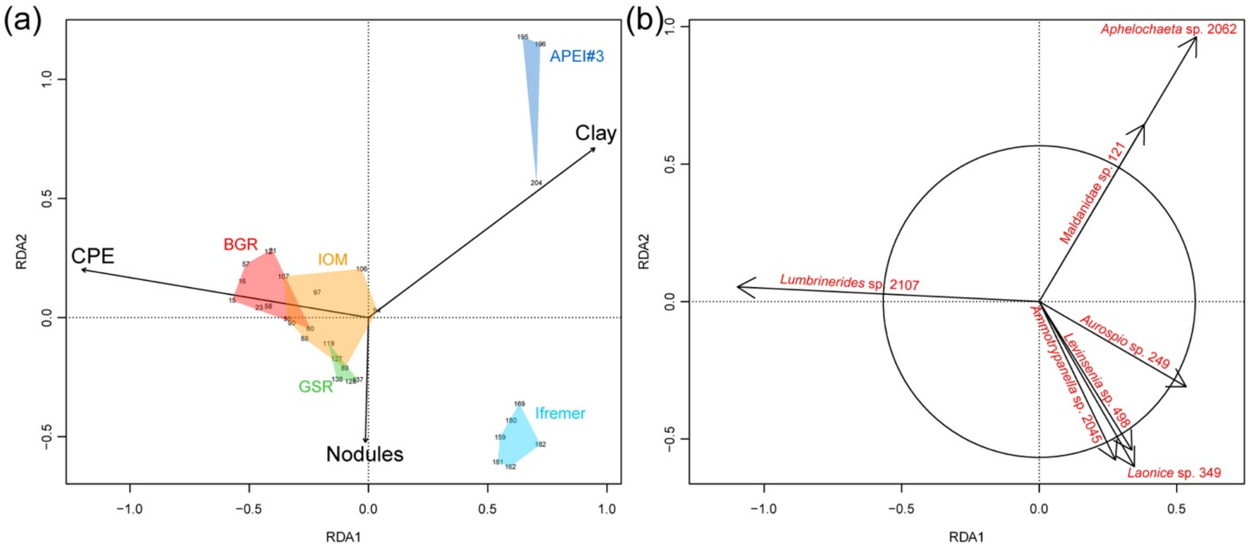


Figure 7. Redundancy analysis (RDA) biplot based on the chord-normalized expected species shared (CNESS) distance constrained by the selected variables (a, scaling 2) and showing species significantly contributing to the ordination diagram (b, scaling 1).

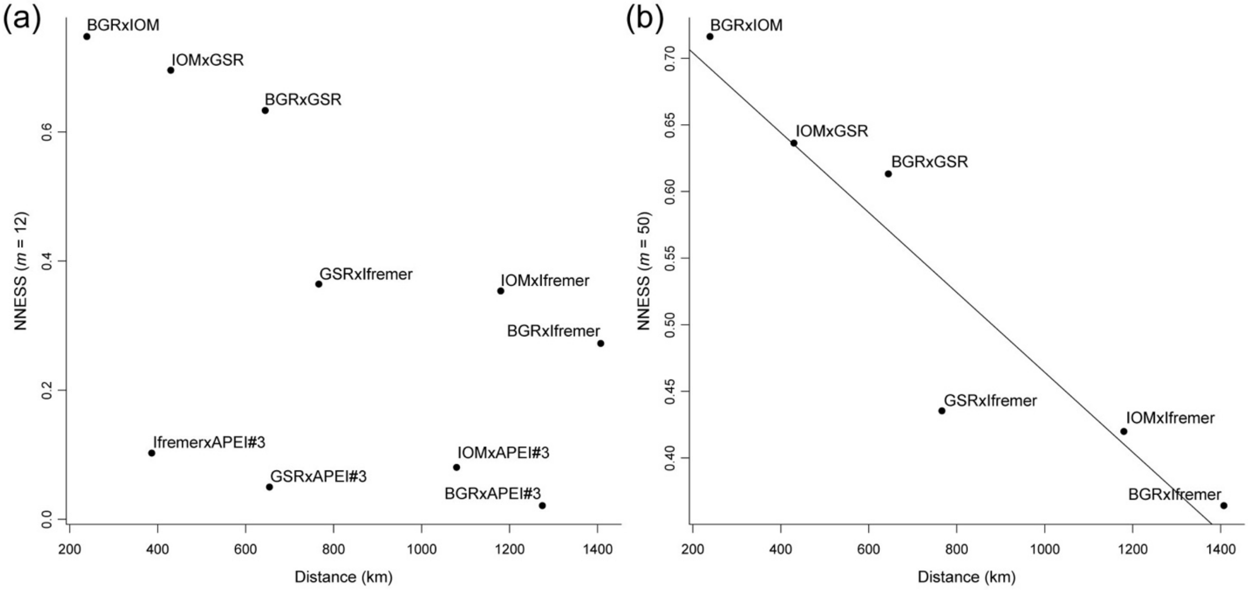
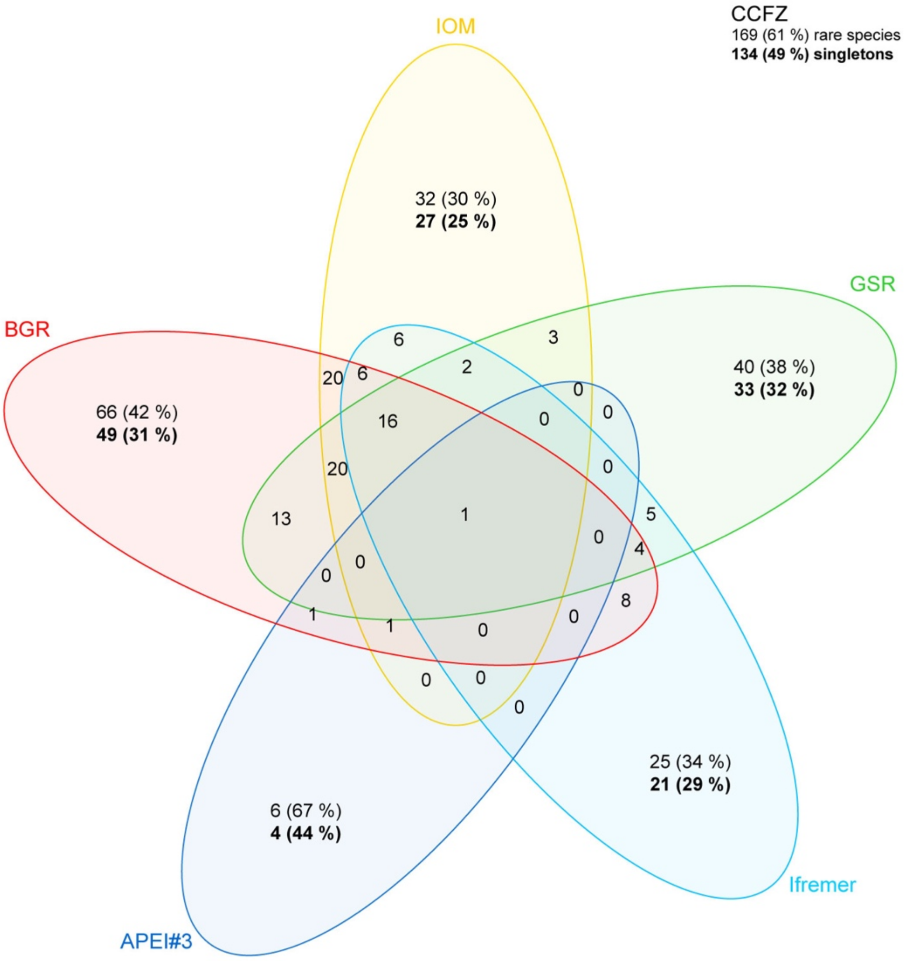


Figure 8. Distance-decay of new normalized expected species shared (NNESS) between BGR, IOM, GSR, Ifremer and APEI#3 using *m* = 12 (a); and between BGR, IOM, GSR and Ifremer using *m* = 50, regression with *y* intercept: 0.7642, slope: -0.0002999 (b).


CCFZ
169 (61 %) rare species
134 (49 %) singletons
IOM
32 (30 %)
27 (25 %)
GSR
40 (38 %)
33 (32 %)
BGR
66 (42 %)
49 (31 %)
Ifremer
25 (34 %)
21 (29 %)
APEI#3
6 (67 %)
4 (44 %)
6
3
2
20
6
0
0
16
0
0
20
13
1
5
0
4
0
0
8
1
1
0
0
0
0
0
0

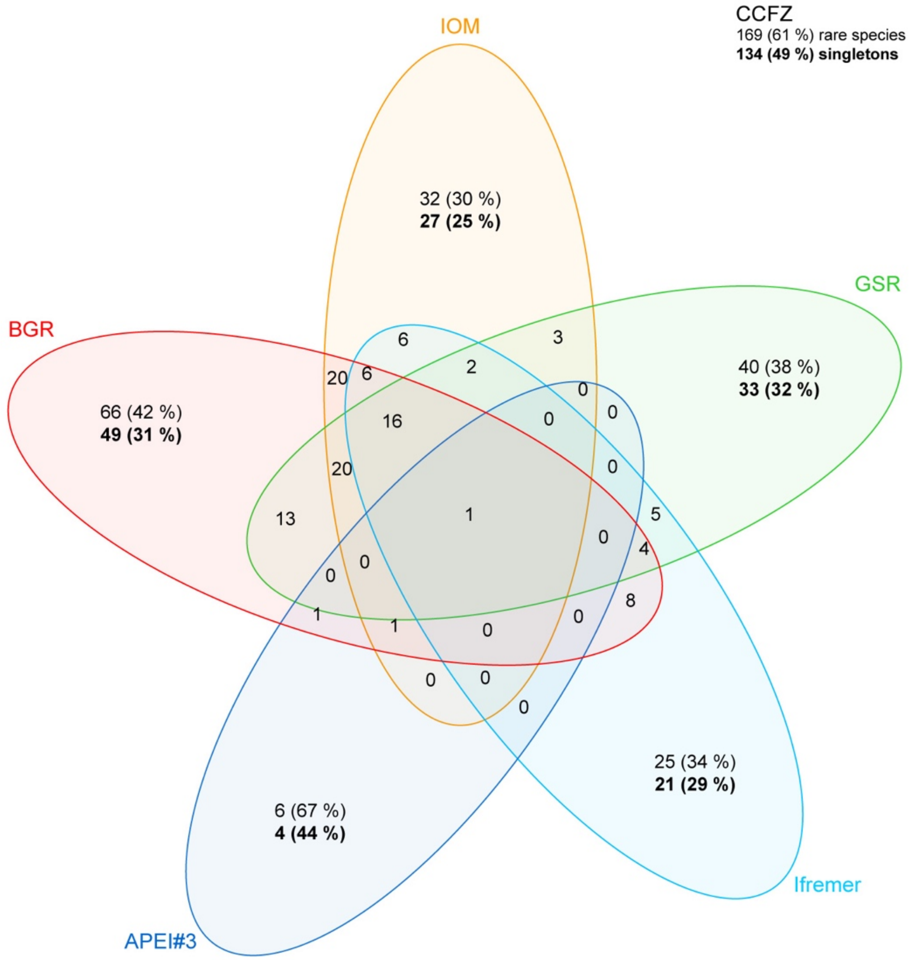


Figure 9. Venn diagram with the records of rare (being recorded in only one area, with corresponding percentage) and common species among sampled areas and for the eastern CCFZ. Bold values indicate the number of species with a single specimen (singletons, with corresponding percentage).

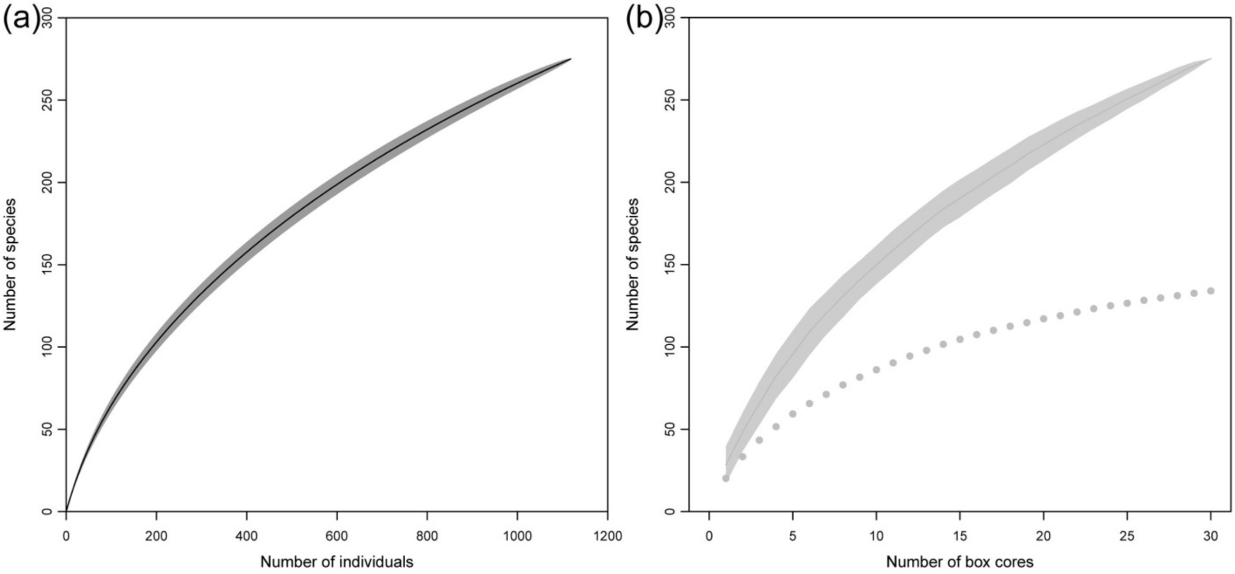


Figure 10. ~~Individual~~ Rarefaction curves based ~~rarefaction curve~~ on individuals (a) and ~~sample-based accumulation curves~~ samples (b) within the eastern CCFZ. The dotted curve shows the sample-based ~~accumulation~~ rarefaction curve of singletons.