Referee comments in black.

Author's responses in green.

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.

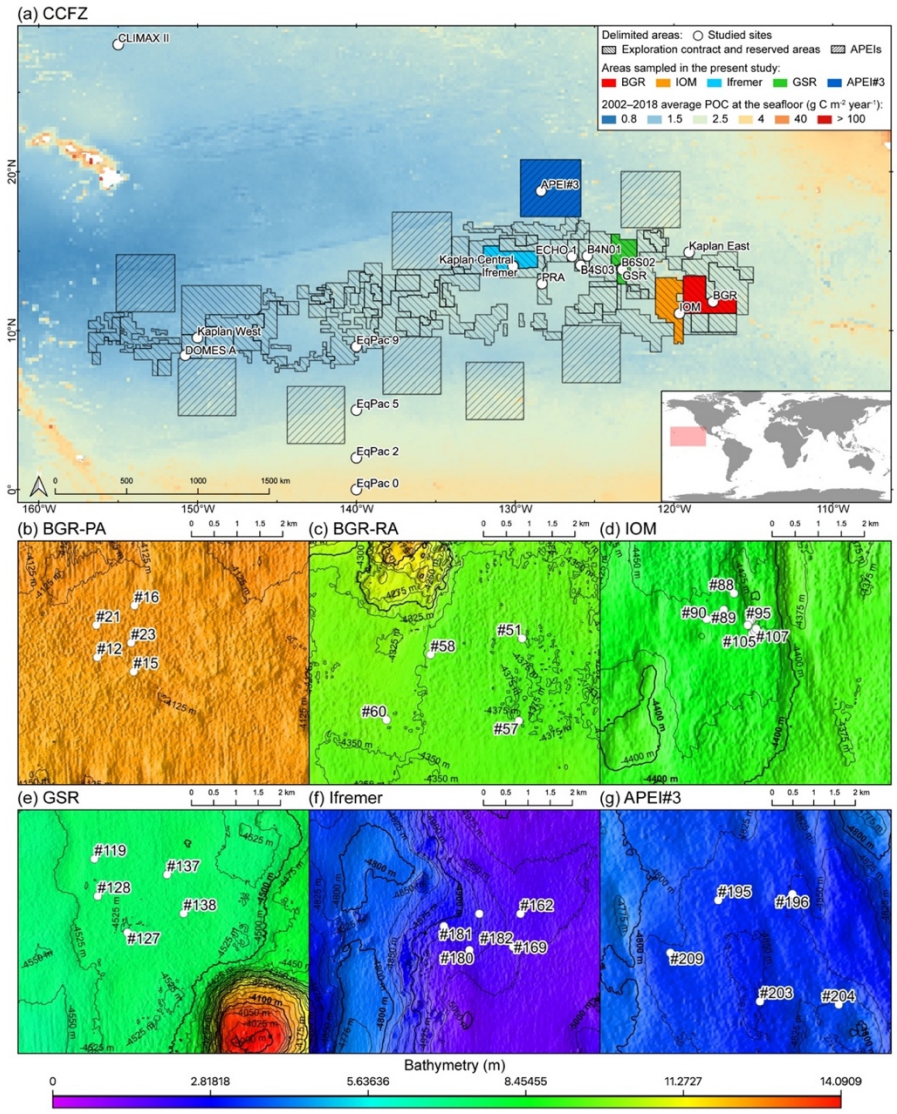
There should be an enlargement of the sampled area in Fig. 1 to allow for the individual core stations to be plotted. A section on the differences between samples taken within an

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.

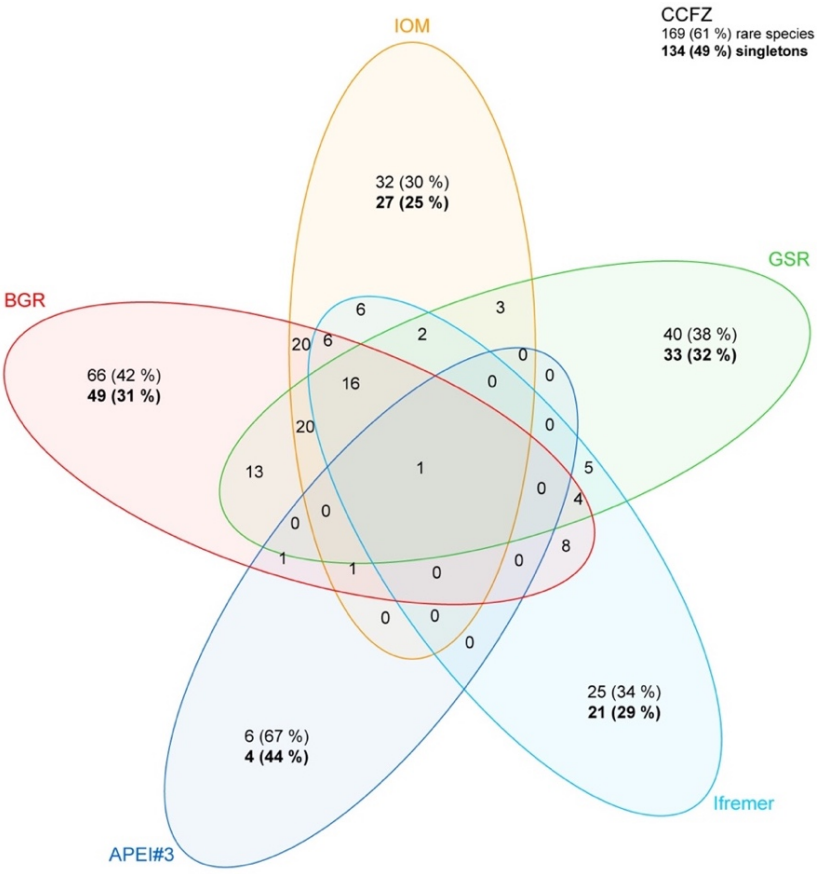
Indeed, to make it clearer the Figure 3 has been separated in two: (a) by box-core samples and (b) by area. Thus, the caption of Figure 3 has been changed:

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)."

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