

We thank the reviewer for his/her insightful comments and suggestions. Detailed response to each comment is provided below:

General comments

The present study entitled “Benthic communities in the deep Mediterranean Sea: exploring microbial and meiofaunal patterns in slope and basin ecosystems” by K. Sevestou and colleagues presents analyses on diversity patterns of meiofauna and bacteria in deep-sea sediments of the Mediterranean Sea. The authors have used a large set of samples to span two different habitats, namely slope and basin areas. Their results show decreasing depth trends of meiofaunal abundances, bacterial abundances were not included. Also the number of meiofauna taxa (richness) showed a significant decrease with depth, while bacterial richness rather displayed an increasing trend with water depth. In addition, significant differences in beta diversity were evidenced between depth ranges in the basin habitat, but not for the slope. This seems surprising, as I would expect larger differences along slopes due to stronger environmental gradients. The authors address this question in the discussion, but due to the lack of contextual data, they cannot conclusively suggest an explanation for this result. One of the main results is that depth plays an important role for meiofaunal diversity; this is something that was known before, but was here confirmed with an extensive dataset from the Mediterranean deep sea, including slope and basin samples. Little is known about the distribution patterns and driving environmental factors of deep-sea fauna, and even less about the small size classes, meiofauna and bacteria that were studied here. The present study therefore adds important information on the structuring of deep-sea meiofaunal and bacterial communities.

The Abstract could be phrased a bit more concise, with less listing of the different diversity results, but rather an overview and the most important points/findings highlighted.

Reply: In the new version of the manuscript the abstract has, to certain extend, been rephrased and in such a way so as to follow the suggestions of the reviewer but at the same time to avoid overlaps with the Conclusion

section at the end of the manuscript, where we summarise the main findings of our study.

Due to the large dataset and the different types of analyses and comparisons, special care should be taken to structure and write the results and discussion as clearly as possible. In the Results it is ok. to list all the different results, but I think that the discussion could be improved in order to formulate/present conclusions more clearly. Maybe a summarizing sentence for the different sections would help, with the major message of that sub-section.

Reply: Taking into consideration the specific comment and suggestions, as well as relative comments of Reviewer 3, the results and discussion sections of the manuscript have now been reworked and amended.

I also miss a bit more discussion/speculation on possible reasons for the observed patterns. Maybe it would be possible to include rough extrapolations of certain environmental parameters to test or at least describe possible environmental drivers for the observed differences in the sampled areas.

Reply: Following the reviewer's suggestion, in the revised manuscript we put extra effort on discussing plausible drivers of the observed benthic patterns. As has already been stressed in the manuscript, food availability is very important when dealing with very oligotrophic ecosystems. In all our previous studies in the E. Mediterranean, it was always chlorophyll a and chloroplastic pigments that exhibited the strongest positive correlation with the various benthic indices (Tselepides et al 2000, Tselepides & Lampadariou 2004, Polymenakou et al 2005, Lampadariou & Tselepides 2006, Lampadariou et al 2009). Therefore, as suggested by the reviewer, chloroplastic pigment concentrations and their relationship with the observed patterns is more thoroughly investigated and commented in several parts of the discussion of the revised manuscript.

In such a large sampling effort it would have been desirable to incorporate at least a few environmental parameters. As the authors themselves state, depth has been identified as an important factor in a large number of studies, however the driving factors that may be hidden in the factor depth have not been identified. Unfortunately, no environmental

parameters (other than depth) were considered for the analyses in this study, so most of the discussion on shaping factors/underlying mechanisms (related to depth or habitat type) remain very hypothetical. This study therefore remains very descriptive. However, considering the general sparsity of deep-sea studies, this study contributes important information toward a better assessment of diversity patterns in the deep sea.

Reply: Several environmental variables have been previously investigated at most of the stations included in the present study and several of the papers that we have referred to (e.g. Tselepides et al 2000, Lampadariou & Tselepides 2006, Polymenakou et al 2009) include a wealth of environmental data regarding this study area. In this paper though, our intention was to focus on the smaller benthic components (meiofauna and microbiota), which seem to play a key role in extremely oligotrophic ecosystems, and on the biological aspects of the benthic ecosystem in an attempt to explain the differences in the observed patterns. Nevertheless, following the suggestion of the reviewer (see also reply to previous comment) we have used a subset of available environmental data in an attempt to look further into possible explanations for the observed patterns.

Specific comments

Introduction p. 17542, l. 11-13: rephrase: Hence, increasing the sampling effort and extending research to include more taxa and more areas of the deep oceans will help to further elucidate bathymetric trends of benthic diversity.

Reply: Corrected

p. 17543, l. 9-11: First you refer to bacterial diversity estimates, but then you talk about the occurrence of bacteria in different habitats such as deep-sea sediments, soils and shallow waters (what do you want to compare?). I do not agree with the statement that bacteria found in the deep sea are similar to those living in soil or shallow water. Clear differences in the composition of taxa/the most important taxa have been shown for these different kinds of environments.

Reply: We agree with the reviewer that clear differences in the composition of microbial taxa have been recorded among these different kinds of habitats; however, some cosmopolitan species are commonly found. To avoid any misunderstanding in the revised version of the manuscript this part has been rephrased to “bacterial sequence richness found in the deep sea is similar to estimates for microorganisms living in soil or shallow water”.

p. 17543, l. 12-14: it is unclear to me what you want to say here. “On the other hand” compared to what? Change “and” to “or”, ubiquitous dispersal is the opposite of biogeographic patterns. But, yes, there is little evidence for any of those scenarios because of a lack of studies on bacterial communities in deep-sea sediments.

Reply: “On the other hand” has been replaced by the phrase “in addition” and the word “and” has been replaced by “or” as the reviewer suggested.

p. 17543, l. 26: But you didn’t measure productivity or proxies of input to the deep sea. Also, I don’t really see this discussed in the Discussion section.

Reply: Productivity data were not used in this paper, but as is pointed out in the description of the study area (p. 17544, l. 14-16) the Mediterranean is characterised by a well-documented southward and eastward decreasing productivity gradient. Within this context, we have discussed in several parts of the discussion (e.g. p. 17554, l. 4-20; 17556, l. 2-5) the patterns observed in our study in relation to the Mediterranean productivity gradient. Further to this, following a relevant suggestion from the reviewer, in the revised manuscript chloroplastic pigments are explored as a potential environmental driver of benthic patterns.

p. 17541, l. 1 (see below) p. 17544, l. 1-7: I find the wording of “benthic parameters” to describe diversity estimates very confusing (appears throughout the text). Benthic parameters to me usually describe parameters like oxygen availability or organic carbon content. Better say what you really mean, i.e. diversity estimates/indices. Do not write bacterial species, rather types or groups. In general, the formulation of goals should be a bit more specific, what did you specifically want to test, what were your expectations/hypotheses.

Reply: As opposed to environmental parameters, benthic parameters refer to variables widely used to describe benthos, such as benthic abundance/biomass/diversity etc. In this context, our intention was not to use this term as an alternative to diversity measures, for which "index" or "estimator" would be more appropriate, but rather as a general term to refer to all the measured biological variables of our study, e.g. meiofaunal abundance, nematode genera richness, microbial OTUs. Nevertheless, in the revised version, we have limited the use of this term.

With regard to the second part of the comment ("Do not write bacterial species"), because data of Operational Taxonomic Units, which are known as observed bacterial species, were used in this study, we initially decided to follow the term "bacterial species". In the revised version of the manuscript the term "bacterial species" has been replaced by the term "observed microbial species (OTUs)".

In the last part of the introduction (p. 17544, l. 1-7) we enumerated the aims of our study and tried to state clearly all four aspects of our investigation. Nevertheless, we will further elaborate the text and try to be consistent with the reviewer's opinion on the phrasing of the goals.

Methods You need to give some more detail on the processing of 454 sequences (may also be added to SI). How were 454 sequences processed and cleaned? How did you deal with pyrosequencing noise? What was the number of singletons (sequences occurring only once in the dataset)? Especially richness estimates may be flawed by pyrosequencing noise.

Reply: More details on the 454 pyrosequencing including the amplicon sequencing protocol and the pyrosequencing noise removal are now presented following the reviewer's suggestion. In addition, details on the singletons numbers are also given.

p. 17547, l. 21: check your reference of Schloss and Handelsmann; in the reference list you cite their MiniReview in Genome Biology, but you probably mean Schloss & Handelsmann 2005, AEM 71:1501 (DOTUR)?

Reply: We agree with the reviewer's comment; the original reference of Schloss and Handelsmann has been cited.

p. 17547, l. 7: add reference for DISTLM.

Reply: Reference was added.

Results Is there no information on bacterial numbers?

Reply: Unfortunately, data on bacterial abundances are not available and thus are not presented. All samples were collected onboard and kept frozen for molecular analysis and no samples were collected and preserved for counting. Frozen samples cannot be used for bacteria counting and thus the collected frozen samples were only processed for pyrosequencing.

p. 17548, l. 21: it is not clear in all instances, what you refer to when you speak about “the two habitats”, make sure to remind the reader again that you mean the slope and the basin.

Reply: Accepted. Relevant changes/additions were made in the new version.

p. 17548, l. 22: change: . . . , more pronouncedly at the basin habitat. . . , into: . . . , and were more pronounced for the basin habitat. . .

Reply: Corrected

p. 17550, l. 10: For nematodes you find 30% singletons (only occurring at one station). Can you put this into perspective, in comparison to other studies?

Reply: A relevant short text was added in the discussion.

p. 17551, l. 9-10: move the second part of the sentence to the discussion (and add reference for soil comparison). Be aware that technical differences in 454 analyses may also lead to differences in richness estimates (direct comparisons may be difficult).

Reply: This part has been moved to the Discussion section and a reference for soil comparison has also been added (Nacke et al. 2011). A comment about the technical difficulties in 454 pyrosequencing comparisons has also been added as the reviewer suggested.

p. 17552, l. 21-23: rephrase “meiofaunal major taxa community”. ?

Reply: Rephrased to “of the structure of meiofaunal community”

Tables The results include a large number of Tables. Tables may in general be informative, but I would suggest to design/adjust the information to be a bit more visual, e.g. highlight dissimilarities with color schemes or produce a real heatmap instead.

Reply: Taking into account the comments of all three reviewers we have made relevant changes to most tables.

Table 1: give clear references to the expeditions (ship, cruise). Table 2 and 3: give definition/description of PERMANOVA and DISTLM again. Table 2: footnote 1, “design” instead of “desing” Table 4: Think about more visual representation here, e.g. color scheme according to dissimilarities. Figure 3: plots and legends are too small to read. Try to re-arrange so that they are easier to read. Figure 4: plots and legends are also too small. Re-arrange as 2x2. Did you test for linear trends? Were the results significant (R, p value)? Indicate a trendline in the plots (visual aid). Figure 5: Explain in legend what the “Overall” stars mean (I assume that it is the richness when both habitats are combined). Maybe choose a different sign, e.g. just a bar, to indicate the overall number of taxa. Figure 6: the diamond and the triangle sign at the bottom of the figure are not displayed correctly. Figure 7: better visualize what you want to state/highlight with this figure. Include colors or shapes to separate different depth or habitat groups.

Reply: We have modified tables (including supplementary table) and figures according to all the suggestions of the reviewer, however we need to make the following clarifications to specific parts of his/her comments. (i) Although we have in the new version of the manuscript rearranged the plots in Figures 3 and 4, we believe that this might be just an effect of the specific presentation format in the BGD, while in the pdf version of BG there might not be such problems. The same may stand with regard to the reviewer’s comment for Figure 6. (ii) Bathymetric trends for all variables presented in Figure 4 were tested using DISTLM.

Discussion p. 17554, l. 5: “particularly”, instead of “particular”

Reply: Corrected.

p. 17555, l. 3-10: what are further possible explanations? What were the differences between the studies?

Reply: Having reworked the discussion according to the suggestions of Reviewers 2 and 3 this part has also changed to include comparison of the studies and additional explanations for the observed differences.

p. 17556, l. 11-12: Zinger et al. 2011 also includes deep benthic samples from the Mediterranean.

Reply: After careful consideration of the datasets used by Zinger et al. 2011 as was provided by the Vamps map (<http://vamps.mbl.edu/mapper/index.php>) the statement in lines 11-12 has been corrected accordingly.

p. 17556, l. 12-21: be careful with absolute richness estimates (possible sequencing artifacts may influence them). May technical differences play a role in the large differences obtained? Comparison with further studies?

Reply: Following a previous comment, a more careful consideration of the richness estimates is now presented. A reference for soil comparison (Nacke et al. 2011) and a comment about the technical difficulties in 454 pyrosequencing comparisons have been added as the reviewer suggested.

p. 17558, l. 2: “gradients” instead of “gradient”. “. . . , it is now also widely accepted (or hypothesized) that the high complexity of the seafloor affects. . .”

Reply: Corrected

p. 17558, l. 21: “. . . the only exception being THE eastern Levantine BASIN, . . .”

Reply: Corrected

p. 17558, l. 24: “. . . , the results of THE true richness estimator Jack1. . .” What do you mean with “true”?

Reply: True species richness is used to refer to the actual number of species of a community/habitat/area/etc (see also Gotelli & Colwell 2001, Colwell et al 2004, Magurran 2004).

p. 17559, l. 5: “metazoa” instead of “metazoan”

Reply: Corrected

p. 17559, l. 7-8: . . . and we CAN therefore NOT preclude. . .

Reply: Corrected

p. 17559, l. 25 – p. 17560, l.1: The source-sink hypothesis refers to source and sink of populations, where bathyal populations present the source and abyssal populations the sink. Rex et al. propose an increased significance of these dynamics with increasing depth and attribute it to the exponential decrease in organic carbon flux with depth. There is of course regions in the deep sea where, e.g., depressions may act as a sediment trap accumulating organic matter. But this is not what the source-sink hypothesis relates to! Also, earlier you argue that the food limitation could be a reason for the higher richness observed (here you now say that basins may act as food sinks and therefore lead to higher biodiversity).

Reply: We appreciate the very important remark of the reviewer for the source-sink hypothesis, which was obviously misused. In the revised version of the manuscript we will rephrase this part accordingly.

p. 17560, l. 17: . . . ranges FROM low to moderate values. . . Set numbers into context, what is high and what is low, what does it mean?

Reply: The correction in English was made (“ranges from... to...”). No standard tables categorising percentages into levels (low, moderate, high) exist; however, we believe that we provide throughout the results and discussion section the context in which we use these levels, as in all cases we provide percentages or ranges of percentages.

p. 17562, l. 4-5: please do not say variables, but rather richness and diversity or only diversity. Meiobenthic variables could be anything.

Reply: As has already been stated in a previous comment from the reviewer on benthic parameters, in this part of the discussion we also refer to benthic abundance/biomass/diversity etc. Again, following the reviewers suggestions we have limited the use of this phrase throughout the manuscript.

References

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