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Discussions

Interactive comment on "Turnover diversity drives large-scale biodiversity patterns in bathyal sediments of the Mediterranean Sea" *by* S. Bianchelli et al.

Anonymous Referee #2

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General comments The authors describe deep-sea nematode diversity patterns at different spatial scales across the Mediterranean basin and presented some spatial aspects of species turnover. Data analysis and interpretation have significant shortcomings. The methods have only been inadequately described and the discussion appears rather superficial. The authors should be more careful with citations. Although the subject would be of interest, significant changes have to be made before the manuscript is ready for publication.

Specific comments

2.1. Study area and sampling

In trying to understand in which year, month or season how many replicates and subsamples were sampled at the different locations, one has to read some of the authors other publications dealing with this data set (e.g. Danovaro et al. 2009, Danovaro et al. 2010, Bianchelli et al. 2010. . ..). It seems that the North Western Mediterranean samples were collected in October 2005 using the RV Universitas, as the North Western Mediterranean results described in the present manuscript are identical to the results given by Danovaro et al. (2009). It seems that these samples were collected using a multiple corer (see Bianchelli et al. 2010). Please state correct in method section (17823, line 1). The authors stated that three sediment cores from independent deployments (whenever possible) were analysed (17823, line 3). Does that mean, it was not always possible to get the same number of samples at each site? Please state how many samples were analysed for diversity measures at the particular sampling sites and explain how you achieve comparability of your results with different sample sizes. Please insert a table with the information about the sampling dates, number of samples (replicates/sub-samples) etc. (see e.g. Supporting Information Table S1 – S4 in Danovaro et al. 2010).

2.2 Nematode biodiversity

Nematode identifications were done for certain subsets of the nematode communities ($\leq$ 100 specimens) per sample (17823, line 17 & 18). How did the authors achieve these sub-samples, how did they decide which nematode to identify? Please describe how you ensure that the nematodes are randomly chosen and each nematode of a sample has an equal probability of selection, respectively. Moreover, if always the same number of nematodes ($\leq$ 100 specimens) was sorted out, regardless of the total number of nematodes per sample, each time different proportions of the entire nematode community will be analysed. This sampling design gives species from larger populations a smaller chance of being selected. The sub-sample size (selection probability for each species) should be set to be proportional to total sample size (in terms of nematode numbers per sample). Please explain how you achieve comparability of your

results with different relative sample sizes (sometimes the entire nematode community was analysed and sometimes only a minor subset).

The authors refer to species richness (SR) as " the total number of different species identified at each site" (17823, line 24 & 25). What the authors describe as species richness is termed species spectrum (the total number of species found in an area), or refers to species density (number of species per unit area), if the values indicated in Table 2 are related to the respective sampling effort (e.g. sampled area, resp. number of grabs). Species richness is defined as "The number of species relative to the number of organisms." (Gotelli & Colwell 2001). The authors' use of the term species richness in this context cause confusion as the authors also give values for the expected number of species relative to number of specimen (ESn). If the authors' definition of SR refers to the definition given by Gray (2000) please give (and use) the correct definition and refer the values for species numbers to a given sample size (see Gray 2000). Please be more exact in using the different diversity describing terms throughout the text.

To my knowledge, the methods described for nematode sampling strategy and processing were initially described by Higgins & Thiel (1988), resp. Pfannkuche & Thiel (1988) (17823, line 8 – 20).

The authors stated that they collected two replicates at each sampling site (independent deployments) and analysed three sediment cores from each replicate (17822 line 26, 17823 line 1-5). Below they described that they analysed nematodes from three replicates (17823 line 18) and that biodiversity was determined as total number of species retrieved from the three independent samplings (line 26-28). What is meant here by replicates resp. three independent samplings?; three sub-samples/sediment cores from each deployment?; or the independent deployments at each site? This cause confusion, as just two replicates/independent deployments were sampled at each side and the three sediment cores from each grab are not replicates, resp. independent samplings, but sub-samples (or pseudo-replicates). Moreover, the major advantages of independent samples or replicates are to measure variation so that sta-

tistical tests can apply, and averaging across replicates enhances precision of measurements when comparing data points. If you really sum up species numbers of three (?) independent samples, sample size will be enlarged, but the advantages of using replicates will be lost. What is it about? Please reformulate the relevant parts of this paragraph (2.2. Nematode biodiversity) and state more clearly how you treated your data for analysing diversity.

The authors stated that they analysed each replicate sample (or sub-sample?) from each site separately. But e.g. in Table 2 they give just one value for diversity measures for each sampling site. How do they treat the results for each replicate (sub-sample) at the different sampling sites? Are the values given in Table 2 means, sums? In general, it remains unclear how the authors treated their data, are the analyses based on raw data or were the data extrapolated (e.g. to 10 cm^2)? Please specify.

According to Table 1 different numbers of samples were collected at three different regions and four different habitats (and perhaps also at the different sites/habitats, depending on the number of possible deployments). The authors analysed/sampled seven habitats/sites at the Central and Eastern Mediterranean Sea, but only four at the North-Western Mediterranean for regional diversity; the authors sampled/analysed five canyon sites, six open slope sites, five bathyal plain sites, and two coral rubble sites for habitat diversity. The number of species found is strongly dependent on sampling effort. What does the uneven number of samples mean for the results of the different diversity measures, and the number of exclusive species in each habitat? Please state how you avoid biases arising from the uneven number of samples per region/habitats (replicates per site).

The definitions of alpha, beta, gamma, delta and epsilon diversity are scale dependent. It becomes not always clear which samples were analysed for the different levels of diversity, in particular as it seems that site, area habitat, region etc. are not consistently used throughout the text. This has to be clearly defined, when measuring species richness at different scale (see Gray 2000). Please be more specific in describing

which samples were analysed for calculating the different levels of diversity.

As the authors solely analysed nematode biodiversity patterns, the differentiation between nematode diversity and biodiversity in analysing the data at each site (17823 line 24 and line 26) is quite confusing. Please reformulate (e.g. 17823 line 26: biodiversity indices/other diversity descriptors for the nematode community were determined. . .).

H' is a diversity measure for the heterogeneity of a community and is called Shannon-Wiener index or Shannon-Wiener diversity (Shannon & Weaver 1963), not Shannon Wiener information function; although it is based on information theory. Please give the reference with the index (17824, line 6 & 7).

The authors stated they calculated beta-diversity as Bray-Curtis similarity between samples based on a similarity matrix using presence/absence data (17824, line 18 – 20). The choice of transformation can have a substantial impact on beta diversity patterns (Anderson et al. 2011). Did you examine differences between beta-diversity patterns derived from abundance and presence/absence data?

Please change ". . ..using a presence/absence matrix" to "using presence/absence data" (17824, line 20)

The authors didn′t use the feeding type classification by Moens et al. (1999). Why is the classification described in the Method section? Please remove the paragraph (17824, line 26-28).

The index of Trophic Diversity (ITD) was initially established by Heip et al. (1985) (17825, line 32). Please give correct reference

The authors calculated the MI based on c-p values given by Bongers et al. (1991) (17825, line 10). Please use the corrected c-p values given by Bongers et al. (1995) and recalculate the MI. If this cause changes in functional diversity please reformulate the corresponding paragraphs in Results and Discussion.

2.3 Quantity and biochemical composition of organic matter (17825, line 12 – 23) To

my knowledge the methods to analyse, at least CPE content (or chlorophyll a and phaeopigments) of the sediment were initially described by Thiel (1978).

ANOVA (17825, line 25 & 26) Did you test for the assumptions of homogeneity of variances and normal distribution? Which tests were used? And where necessary, how were the data transformed?

DistLM (17826, line 15 & 22) The results of DistLM will depend on what selection criterion is used. What did you choose as selection criterion? R^2? Adjusted R^2? Or others?

Did you check out the relationships of the particular predictor variables with one another (correlation of variables) to avoid co-linearity?

The authors stated they used the routine distance-based linear model. How did they calculate the DistLM; by using the DistLM routine in PERMANOVA+? If so, please give the reference (Anderson et al. 2008).

3. Results

Figures in general:

It is not always clear what is shown by the figures. Please be more explicit in the figure captions/axes labelling. The choice of the symbols is rather unfavourable. It is sometimes difficult to recognize the results for single sites/habitats.

Figure 2: As mentioned above the authors terminology of the different diversity measures is rather confusing. Species richness as Y-axis labelling is sufficiently unclear. To be consistent with Y-axes labelling of Figure 6a/b it becomes clearer if the authors change the labelling to alpha-diversity (Figure 2). What is the "unit" of species richness? Number of species per sample/ per number of individuals/ per area? Are the values sums, means?

Figure 3: What is meant by relative importance? Percentages? If so, are the percent-

ages based on the number of species or the number of individuals found?

Figure 5 and 6: Some relevant details are not visible. The symbols for certain stations lie on top of each other. Figure 5a seems to show results only for 17 stations (one open slope stations seems to be missed). Figure 6a: What is the "unit" for species richness? See comments on Figure 2.

Figure 7 What is the "unit" of species richness? See comments on Figure 2. Labelling of the X-axis with gamma diversity is somewhat confusing, as the Y-axis represents gamma diversity.

Table 3: Please give the degrees of freedom with the ANOVA results.

Table 5: Please show results of the marginal test and give the information about the strength of the correlations between parameters and gamma diversity.

4. Discussion

Although the authors stated that in each region the samples were collected at approximately the same water depth (about 1000 m, see Methods 17822, line 9), there are depth differences between the sampling sites of a maximum of 700 m (Central Mediterranean), resp. $\sim$ 300 m (Eastern Mediterranean). Some of the data provided here were also analysed by Danovaro et al. (2009). They demonstrated that bathymetric differences are a key source for beta-diversity (Danovaro et al. 2009). In this regard, the authors should discuss to what extent their results for turnover-diversity (in particular for the Central and Eastern Mediterranean Sea, see Figure 2) were influenced by the depth gradient.

17833, Line 13 & 14 (& Table 5) This approach comprises a circular and therefore invalid logic, as gamma diversity is determined by the mean species diversity of a site or habitat (alpha diversity) and the differentiation among these habitats (beta diversity). Therefore it is obvious that the diversity parameters were significantly correlated with gamma diversity. Moreover, results of the DistLM using alpha- and beta diversity as

explanatory parameters for gamma diversity tell nothing about the underlying mechanisms of variation in gamma diversity.

The authors stated that habitat heterogeneity (and type) is a crucial player of nematode turnover diversity across the Mediterranean basin (1834, Line 20). If I understand the results, resp. the interpretation of the results of the DistLM correctly (Table 5 and Discussion), the authors here used alpha, resp. beta-diversity as proxies for habitat heterogeneity. It would have been more useful, if spatial parameters (e.g. spatial/habitat structure, region, water depth, distance. . .) instead of alpha-/beta-diversity were tested as proxies for habitat heterogeneity. There is still an unexplained variance of $\sim$ 20 % (see Table 5), indicating that some untested/unmeasured processes/parameters affect gamma diversity.

The authors stated that overall species richness of the deep Mediterranean Sea is very high (17834, Line13 & 14). In comparison to what? Other studies of the Mediterranean Sea? Other deep-sea regions? Other water depths? This is an unproven statement without any comparisons to other studies/results.

Although a proper comparison with diversity results of other studies is not possible, since the authors do not refer their result (280 species) to a standard sampling effort (and due to differences in sampling effort and processing methods), other studies reporting comparable or higher total nematode species richness for deep-sea regions/habitats (e.g. Gallucci et al. 2008, Fonseca & Soltwedel 2009, Leduc et al. 2010, Leduc et al. 2012).

Turnover is not only by driven extrinsic factors (environmental characteristics, spatial structure), but also by intrinsic factors (to the organisms' related factors, e.g. trophic position, dispersal rate). The authors analysed intrinsic factors (ITD, MI), but the results were mainly discussed as functional alpha, resp. gamma diversity, than as turnover along biotic gradients. It is intuitively obvious that spatial turnover is connected to the organisms' dispersal ability. Despite their limited ability to swim and lack of pelagic

larvae nematodes can disperse over large distances (e.g. Fonseca & Soltwedel 2009, Miljutin et al. 2010, but see also van Gaever et al. 2010). It would have been interesting, especially as part of a paper dealing with species turnover, if the authors also discussed the nematodes' potential for dispersal as a driving factor of turnover patterns.

Species turnover occurs not only in space but also in time. The main problem with the analyses and interpretation of the present data set is that the authors ignored the temporal component in the dataset. The authors stated in the Methods Section (17822, Line 24 & 25) that the sampling was carried out during cruises from September 1989 to May 2006. This temporal component of 17 years (!) was not considered when analysing/interpreting the data at the different diversity levels. Turnover rates are affected by the spatial AND temporal setting of the observation. The study′s sampling duration (and spatial extent) strongly affects the turnover rates among communities. For example, theory (also not tested) predicts that spatial turnover should be partly driven by temporal turnover due to the decreased probability of sampling a given species repeatedly when temporal turnover is high (Steiner & Leibold 2004). The authors nowhere mentioned/discussed the temporal scale dependence of their data for species turnover (especially of their results for relative importance of rare/exclusive species in the different habitats!).

In this regard it is questionable to what extent analyses/interpretations only based on spatial aspects lead to significant conclusions about species diversity/turnover. The problem is so severe that a proper assessment of the authors' findings concerning turnover diversity is very difficult.

Technical comments The authors changed between American and British English. Appendix S1 A & C: please correct the spelling of Sabatiera (= Sabatieria)

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