

**Anonymous Referee #1**

**Received and published: 29 January 2013**

**RC: General comments**

**This paper is on the turnover of nematode species composition along a west-east gradient in the Mediterranean. It is a well written paper which includes some interesting (although not novel) aspects on regional diversity of nematodes in relation to habitat heterogeneity.**

**Specific comments**

**The main problem I have with the paper is the statement that turn-over diversity drives large scale patterns in biodiversity (with other words the regional diversity). This sounds like 'kicking in on an open door'. What else than turnover diversity drives regional diversity? However the results on page 12 line 7 suggest that both alpha and beta diversity explain similar amounts of the regional diversity (28 and 32% respectively) which again seems like obvious since regional diversity is calculated based on alpha and beta. What is maybe most remarkable is that they do not explain more than only 60% together since regional diversity is calculated based on them. Also is the paper sometimes difficult to follow since the terms habitats, sites and areas are not clearly defined.**

**AC:** We thank Referee#1 for the useful comments. We think that regional and macro-regional diversity are driven by different diversity components, both local (i.e., alpha diversity) and the turnover diversity (i.e., beta diversity), but our hypothesis was to test the relative importance of the 2. For example, at regional level, high values of biodiversity could result either from high levels of local (alpha) diversity but low levels of turnover diversity among sites/habitats within the region, or, conversely, from low levels of local (alpha) diversity but high levels of turnover diversity among different sites/habitats within the region (Loreau 2000, Ecology Letters). In this context, while we can agree that both alpha and beta diversity contribute to the regional diversity, it is not so obvious that only (or even mostly) turnover diversity drives large scale patterns of biodiversity. Several studies have been dedicated to the analysis of the relationships between  $\alpha$ -,  $\beta$ - and  $\gamma$ -diversity since the '70s (eg, Whittaker 1972 Taxon 21; Gray 2000 J. Experimental Marine Biology and Ecology 250; Loreau 2000 Ecology Letters 3) but this issue is still strongly debated (Thrush et al 2010 Plos One 5; Leduc et al 2012 Mar Ecol Progr Ser 454). Our results, with very few previous records for the deep sea, provide almost unprecedented insights on how alpha and beta diversity can explain with different proportions large scale patterns of nematode biodiversity in the Mediterranean Sea.

At page 12 line 7, we reported the results of the DISTLM forward test (a multivariate multiple regression analysis). In this analysis we used both trophic resources and local diversity (both alpha and beta) as possible factors influencing the observed variability among different values of regional diversity (i.e., North-Western, Central and Eastern Mediterranean), and not the values of regional diversity themselves. Table 6 allows showing that food resources cumulatively explain a minor proportion of regional diversity variability. This result, as argued by the Referee#1, leaves unsolved what other factors – not included in this study – explain the remaining fraction of variation. The discussion of this issue has been implemented in the amended version of the manuscript.

We fully agree with the referee about the potential confusion in using sites/habitats terms in the previous version of the manuscript. Consequently, in the amended version of the manuscript the use of these terms was accurately made consistent. More specifically, we used the terms "region" for NW, Central and E Mediterranean, "habitat" referring to canyon, open slope, coral rubble, and abyssal plain, and "site" for the sampling stations. This is now clarified in the Methods section as follows: "Sediment samples were collected from three regions of the Mediterranean Sea: the North-Western, Central and Eastern basins. In each region, samples were collected from different habitats (i.e., canyon, open slope, coral rubble or bathyal plain) at the same bathymetric range, comprised between ca. 600 and 1300 m in depth, from a total of 18 sampling areas sites (Fig. 1; Table 1)."

## **RC: Technical comments**

**Therefore in the first place the title needs to be revised. It seems like obvious that turnover drives large scale biodiversity patterns. In addition I would specify the taxonomic group in the title too.**

**AC:** Following our previous reply (as for the role of turnover diversity) and accepting the Referee's suggestion about the need of specifying the taxonomic group, we modified the title as follows: "Turnover diversity drives large-scale nematode biodiversity patterns in bathyal sediments of the Mediterranean Sea".

**RC: Page 2 (17820) line 9 The depth varies between 600 and 1200 m so it actually doubles. Stating it is fixed is therefore not correct.**

**AC:** According to the Referee#1's comment, we have changed in "bathymetric range comprised between 600 and 1200 m".

**RC: Page 3 (17821) line 6 Remove "or they can arise from a single component". The nomenclature can be more consistent (sampling areas, sites, habitats used for the same or not?? This is often very confusing)**

**AC:** We changed this phrase, better explaining what we meant, according to Loreau (2000, Ecology Letters 31). "*High values of regional diversity can arise from a combination of local and turnover diversities, or they can be mostly driven by one single component (Loreau, 2000; Koleff and Gaston, 2002; Witman et al., 2004)*". As commented above, the nomenclature has been revised throughout the entire manuscript. The relative references have been also included in the amended references' list.

**RC: Pag 5 (17823) habitat heterogeneity is not equally represented over all three regions. Is that taken into account when comparing regional diversity? Explain.**

**AC:** We thank Referee#1 for this comment. In the amended version of the Discussion, a new paragraph explaining that estimates of the regional diversity could be affected by the different number of habitats analyzed has been included. However, to provide the needed relevance to this possible bias, and according also to the suggestion by the Referee#2, we calculated the regional diversity also by means of other species richness estimators (Chao1 and Chao2), considering different numbers of samples (habitats in our case). This now reads: "The total number of species retrieved from the Central Mediterranean region (210 species) was higher than Western and Eastern Mediterranean. Despite such difference could be imputable to the higher number of investigated habitats in the Central Mediterranean, it is worthy of notice that such pattern was consistent, whatever the biodiversity index (total number of species, ES(100)) or species richness estimator (Chao1, Chao2) used".

**RC: Page 5 (17823) line 26: there is some contradiction between the two deployments per site indicated in this line, while two lines further three cores from independent deployments are indicated.**

**AC:** We thank Referee#1 and Referee#2 for this note and apologize for the mistake in the previous version of the manuscript. According to the comments on this section raised by the two Referees the paragraph now reads: "*At all of the sampling sites, replicate sediment samples were collected using a NIOZ-type box corer, except for the North-Western Mediterranean, where the sediment samples were collected by means of a multiple corer. Both sampling devices allowed the recovery of undisturbed sediment samples. A total of 3 cores (internal diameter 3.6 cm) from two or three independent deployments were analysed for nematode species diversity (from 0-1 cm sediment layer).*"

**RC: Page 6 (17824) line 9 What is meant by previously here? Explain.**

**AC:** This means that first we have extracted the entire meiofaunal community from the sediment and then only nematodes were picked up and mounted on slides. For consistency we have removed “previously” from the original sentence.

**RC: Line 14 replace passed by sieved.**

**AC:** Done.

**RC: Line 25 since replicates are pooled the small scale turnover between replicates is not considered while these small scale patchiness also may be important.**

**AC:** We are aware about this, but, unfortunately, there is no way to go back. Indeed, the analysis of the micro-scale was not a purpose of the present study.

**RC: Page 7 (17825) Line 26 remove the line on Moens et al since not relevant here.**

**AC:** Done.

**RC: Page 8 (17826) line 10. Bongers modified the CP score of the monhysterids in a latter paper. Is this correction taking into account?**

**AC:** The Referee#1 is referring to the paper “Bongers et al. Proposed changes of c-p classification for nematodes, Russian Journal of Nematology 61-62.” We are aware that Bongers et al revised the CP scores in 1999, since this paper has been cited several times (see also Pape et al, Biogeosciences Discussion, this issue). However, we do not have the access to this paper, since it is not available online. Indeed, it is not possible to have access to the paper from the journal and the paper does not appear in the main search engines (e.g., Scopus, Isi Web of Science).

**RC: Page 9 (17827) line 3 There is no indication if assumptions for ANOVA were fulfilled.**

**AC:** According to the Referee#1’s (and also Referee#2) suggestion, we added the sentence (Methods section, 2.4 Statistical analyses): “Before the analyses, the homogeneity of variances was checked using the Cochran’s test on appropriately transformed data, whenever necessary. For those data sets for which the transformation did not obtain homogeneous variances, a more conservative level of significance was considered (Underwood, 1991).”

**RC: Page 9 (17827) line 7 and how were differences between sites (habitats) investigated if replicates were pooled?? (also on page 10 line 7 sites are compared but there are no replicates?)**

**AC:** We thank the Referee#1 for this comment. Actually, Table 2 reports the indexes calculated on the pooled data from the replicates, whereas the statistical analyses were carried out on data from all replicates. To avoid this confusion, we amended the caption of the Table as follows: “Nematode diversity indices calculated cumulatively from the individuals retrieved from the three replicates of the investigated sampling sites. SR, species richness; ES(100), expected species number for 100 individuals; H’2, Shannon’s index; J, species evenness; 1-ITD, index of trophic diversity; MI, maturity index.”

**RC: Page 10 (17828) from line 5; the degrees of freedom are not provided so it is difficult to see what is exactly compared, also because in material and methods it was mentioned that replicates were pooled?**

**AC:** Correct. In the amended version of the manuscript we added the degrees of freedom in Table 3.

**RC: Page 11 (17829) line 5 The saturation pattern is a methodological artifact and explained by the fact that only 200 nematodes were identified in these samples. You cannot calculate an ES for a number of individuals higher than the one you have actually identified.**

**AC:** We thank Referee#1 for this comment. Nevertheless, we would like to draw the reviewer's attention on the fact that we calculated the rarefaction curves based on the species cumulatively retrieved from different habitats in each investigated region, so that they have been always calculated on at least 100 individuals per habitat.

Since this was possibly unclear in the previous version of the manuscript, we modified the Methods section including the following sentence: "At almost all sampling sites, 100 randomly-selected individuals were identified from each replicate, however at some sites the abundances were less than 100 individuals per replicate, in particular in Central and Eastern Mediterranean, where the meiofaunal abundances were typically lower than the Western Mediterranean (Bianchelli et al., 2010). For this reason we pooled together the data from different replicates, to have a minimum of 100 identified individuals, in order to calculate the expected species number on >100 individuals and to construct the rarefaction curves cumulatively for the sites of all the investigated habitats."

**RC: Page 12 (17830) from line 5. Regional diversity is calculated based on alpha and beta diversity so it is obvious that you will find a strong correlation.**

Again, this is not so obvious. According to the Referee#2, in the amended version of the manuscript we included marginal tests and correlations among explanatory variables. As now it clearly appears in the marginal tests, gamma diversity is not significantly correlated with beta-diversity in the univariate context.

**RC: Page 12 (17830) line 21-24. This sentence is not clear. what is meant here?**

**AC:** We thank Referee#1 for this comment. Accordingly, we changed the last phrase into: "Conversely to our results, previous studies conducted in other oceanic regions (e.g., the Portuguese margin, NE Atlantic ocean) find out relevant differences in the local ( $\alpha$ ) diversity between sampling sites (García et al., 2007; Ingels et al., 2009). This finding suggests that in the Mediterranean sea, whatever the habitat and region considered, the level of local ( $\alpha$ ) diversity results very low at all the sampling sites."

**RC: Page 13 (17831) line 28. The eight fold increase is not seen from fig 2.**

**AC:** As explained in the Methods section, we took samples from different sites belonging to the same kind of habitat. For example, in the Western Mediterranean, we took samples from two sites within the canyons, one in the Cap de Creus and one in the Lacaze-Duthiers canyon. The figure 2 shows the overall habitat diversity, for example, for the Western Mediterranean, the cumulative species richness found in the canyon habitat (i.e., the total nematode species found in both canyons). The same approach was utilized for each habitat in all the investigated regions. In Figure 2, we showed the overall habitat diversity and not the local ( $\alpha$ ) diversity. The correct reference for this phrase is Figure 5. To be clearer, we added the reference to the Figure 5 in the text, as follows: "*Conversely, the comparison of the  $\alpha$ -diversity at larger spatial scales (i.e., amongst basins, instead of among habitats or sites), shows the presence of clear differences between the sites of the Western, Central*

and Eastern Mediterranean basins, with the  $\alpha$ -diversity in the Western Mediterranean up to eight-fold higher than in the Eastern Mediterranean (Figure 5).”

**RC: Page 14 (17832) from line 5 (see my earlier comment on rarefaction curves).**

**AC:** See above.

**RC: Page 14 line 23 when you have a low number of nematodes with many rare taxa it is obvious you get a high turnover.**

**AC:** We agree with the Referee#1 but we simply used a standard definition. The term “rare” refers to the relative abundance of a species within the whole assemblage. There might be many rare species that are shared between samples or not. Nematode assemblages in different samples/sites/habitats/regions could be composed by only “rare” species (e.g., ideally each represented by one single individual), but these “rare” species could be the same (i.e. have the same identity) or totally different in the contrasted sites. In these ideal cases the turnover between the assemblages would be low (in the former) and high (in the latter). At Page 14 line 23 we did not mention “rare species”, but we are just assessing that we observed high levels of  $\beta$ -diversity among assemblages.

**RC: Page 16 line1 to 10. The east has less predators but higher maturity index while predators in general have high CP scores. This seems like contradictory?**

**AC:** It does not seem contradictory, since also the bacterial feeders (1A) have high scores of CP (typically 4), and in the east, this group is dominant. As commented above, we would like to stress out that we used the CP values reported in Bongers et al 1991, since we have not access to the revision of CP values reported in Bongers et al 1995. Moreover, the MI index is calculated on the frequency of each genus in the whole assemblage, so that it depends on the relative frequency of predators and bacterivorous genera, and not on the absolute abundance of a single trophic group.

**RC: Page 16 from line 15; It is not clear if the exclusive species per habitat are also the dominant ones or rather rare taxa, while this is relevant information. Can you provide this information.**

**AC:** We agree with the Referee#1. The requested information (indeed relevant) is now included in the amended version of the manuscript, in the Results section. “In all the investigated regions, the exclusive species in different habitats can be dominant (up to 6.76% in the coral rubble, in the Central Mediterranean) or rare. In particular we found that i) in the Western Mediterranean, the exclusive species accounted, each, for 0.16-1.13 and 0.17-1.03% of the entire assemblages, in canyon and slope, respectively; ii) in the Central Mediterranean, the exclusive species accounted, each, for 0.21-2.48, 0.30-1.81, 0.40-4.86 and 0.24-6.76%, in canyon, open slope, bathyal plain and coral rubble, respectively and iii) in the Eastern Mediterranean, the exclusive species accounted, each, for 0.81-14.52, 0.76-9.92, 0.57-8.06%, in canyon, open slope, bathyal plain, respectively.”

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**Anonymous Referee #2**

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**RC: 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.

**AC:** We thank Referee#2 for his/her criticism. All general and specific comments have been taken into consideration in preparing the amended version of the manuscript.

**Specific comments**

**RC: 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 *Universitatis*, 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).

**AC:** The Referee is absolutely right. All missing information about spatio-temporal distribution of sampling activities are now included in Table 1. Moreover, the description of sampling activities now reads "At all of the sampling sites, replicate sediment samples were collected using a NIOZ-type box corer, except for the Western Mediterranean, where the sediment samples were collected by means of a multiple corer. Both sampling devices allowed the recovery of undisturbed sediment samples. A total of 3 cores (internal diameter 3.6 cm) from two or three independent deployments were analysed for nematode species diversity (from 0-1 cm sediment layer)."

**RC:** 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).

**AC:** We always analyzed a total of 3 cores at all sampling sites, but retrieved from 2 or 3 independent deployments. We added all the requested info in the Table 1 and 2: sampling dates, number of cores (Table 1) and number of individuals (Table 2).

**RC: 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).**

**AC:** When the sample contained >100 nematodes, we picked up all nematodes from the sample and put them into a plastic vial. After a gentle shaking, the nematodes were put into a Delfuss cuvette. A random number  $i$  comprised between 1 and 3 is extracted and the first 100 nematodes collected from the  $i^{\text{th}}$  cell of the Cuvette were used for identification. We identified 100 randomly-selected individuals at almost all sampling sites, however at some sites the abundances were less than 100 individuals per replicate (corresponding to 10 cm<sup>2</sup> of sediment), in particular in the Central and Eastern Mediterranean regions, where the meiofaunal abundances were typically lower than the Western Mediterranean. For this reason, when the nematode number per replicate was <100, to have 100 individuals per sampling site to be included in the ES(100) diversity estimate, we pooled together the data from different replicates. This, obviously does not fully solve the possible bias of considering diversity estimates from variable size samples (and thus variable chance of random extraction). In the amended version of the manuscript we acknowledge this possible bias. The new relevant methods section (2.2) now reads: “At almost all sampling sites, 100 randomly-selected individuals were identified from each replicate. However, at some sites the abundances were less than 100 individuals per replicate, in particular in Central and Eastern Mediterranean, where the meiofaunal abundances were typically lower than the Western Mediterranean (Bianchelli et al., 2010). For this reason, to have minimum 100 individuals per sampling site to be included in the ES(100) diversity estimate, we pooled together the data from different replicates”.

**RC:** 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 (ES<sub>n</sub>). 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.

**AC:** The Species Richness has been defined by Gray (2000) in several ways. Gray (2000) recognized “four different scales of species richness: point species richness, sample species richness, large area species richness and biogeographical province species richness”. Moreover, Gray (2000) identified two “types of species richness, habitat species richness and assemblage species richness, which are not scales and thus are separate measures referring to habitat or assemblage” (see Table 1 in Gray 2000 J Exp Mar Biol Ecol 250). Regarding the scale, we used the Species Richness *sensu* Point Species Richness (species richness of a single sampling unit), as we explicated in the Methods section: “We measured local ( $\alpha$ -diversity), regional ( $\gamma$ -diversity) and macro-regional ( $\epsilon$ -diversity) species richness as the numbers of different nematode species within each site (local, *sensu* Point Species Richness), region and macro-region (i.e., the whole Mediterranean basin; Gray, 2000; Danovaro et al., 2009a).” As we are aware that SR can be affected by the number of identified individuals, at the smaller scale (i.e. habitat) we also used the expected number of species relative to number of specimen (ES<sub>n</sub>), and in particular ES(100).

**RC:** 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).

**AC:** According to the Referee#2's comment, we added the references Higgins and Thiel (1988) and Pfannkuche and Thiel (1988) dealing with meiofaunal extraction and identification analyses.

**RC:** 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 statistical 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.

**AC:** As commented above, we analyzed a total of 3 cores from 2 or 3 independent deployments at all sampling sites and we corrected the text accordingly, adding all the missing information.

Moreover, as commented above, we are aware that having pooled together the data from different replicates led to lose an estimate of the variability at microscale (as commented by the Referee#1), but in that way we managed a larger sample size, which allows us to compare different habitats and regions, even when characterized by a low number of individuals per replicate. Moreover, as previously commented, we pooled together the three replicates of samples to have the estimate of the Species Richness (and the others diversity indices) of each sampling site, taking into account cumulatively all the species in common to all replicates and those exclusive of each replicate. For consistency, and as commented above, we corrected the Methods section, 2.2 Nematode biodiversity.

**RC:** 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<sup>2</sup>)? Please specify.

**AC:** In Table 2 we reported the values of diversity indexes determined cumulatively from the species retrieved from the three replicates, as explained in the Methods section "These indices were calculated from the sum of the individuals of the three replicates of each of the sampling sites, using PRIMER v6.0+ (Plymouth Marine Laboratory, UK; Clarke and Gorley, 2006)."

To be even more clear, we added an additional explanation in the Result section. "The different indices of nematode biodiversity, calculated cumulatively from the individuals retrieved from the three replicates, are reported in Table 2: SR, ES(100), and H<sup>2</sup>." Moreover, we changed accordingly the caption of the Table 2 into: "Nematode diversity indices, calculated cumulatively from the individuals retrieved from the three replicates in the investigated sampling sites."



**RC:** 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).

**AC:** Following the Referee#2's comment, we calculated the regional ( $\gamma$ ) diversity for Western, Central and Eastern Mediterranean regions also with both abundance- (i.e. Chao1 and ACE) and incidence based estimators (Chao2 and ICE), by means of EstimateS software v8.2.0 (Colwell, 1997) available at <http://viceroy.eeb.uconn.edu/estimates/>.

We added the following paragraph in the Methods section: "*The regional ( $\gamma$ ) diversity for Western, Central and Eastern Mediterranean regions, was also assessed separately by computing estimates of total species richness using non-parametric estimators (Leduc et al., 2012, and citations therein). We used both abundance- (i.e. Chao1 and ACE) and incidence based estimators (Chao2 and ICE). Estimates of species richness using these estimators were computed using the EstimateS software v8.2.0 (Colwell, 1997). Result from species and genus data were compared by plotting randomised, cumulative species richness estimates against number of samples.*"

We added the Table 5 and Figure 8 showing the estimators values. We added the paragraph in the Results section: "*Even when the regional ( $\gamma$ ) and macro-regional ( $\epsilon$ ) diversity were calculated by means of estimators (Chao1 and Chao2), the same patterns were observed, with the highest level recorded in the Central Mediterranean. Plots of randomised, cumulative Chao2 estimates against number of samples show that species curves for all the investigated regions (except for the Eastern Mediterranean), and for regions combined, approached an asymptote (Fig. 8).*"

We added the paragraph in the Discussion section: "*The total number of species retrieved from the Central Mediterranean region (210 species) was higher than Western and Eastern Mediterranean. Despite such difference could be imputable to the higher number of investigated habitats in the Central Mediterranean, it is worthy of notice that such pattern was consistent, whatever the considered index (total number of species, ES(100)) or estimator (Chao1, Chao2).*"

**RC:** 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.

**AC:** We checked and changed the terms to be consistent throughout the entire manuscript. We used the terms "region" (i.e. West, Central and Eastern Mediterranean), "habitats" (i.e., canyon, open slope, coral rubble, abyssal plain) and "site" (i.e., the sampling stations). We explained the terms used in the Materials and Methods section as follow: "Sediment samples were collected from three regions of the Mediterranean Sea: the North-Western, Central and Eastern basins. In each region, samples were collected from the different habitats (i.e., canyon, open slope, coral rubble or bathyal plain) at the same bathymetric range, comprised between ca. 600 and 1300 m in depth, from a total of 18 sampling areas sites (Fig. 1; Table 1)."

We added all the missing info in Tables 1, 2 and 5.

**RC:** 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...).

**AC:** Done.

**RC:** 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).

**AC:** Done.

**RC:** 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?

**AC:** We only analyzed resemblance matrices based on Bray-Curtis dissimilarity using a presence/absence matrix, since our aim was to emphasize the role of exclusive species in structuring the diversity at different spatial scales.

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

**AC:** Done.

**RC:** 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).

**AC:** Done.

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

**AC:** Done.

**RC:** 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.

**AC:** As commented above with the Referee#1; if we understood well, the Referee is referring to the paper Bongers et al. "Proposed changes of c-p classification for nematodes", Russian Journal of Nematology 61-62.

As already commented, we are aware that Bongers et al revised the CP scores in 1999, since this paper has been cited several times (see also Pape et al, Biogeosciences Discussion, this issue). However, we do not have the access to this paper, since it is not available online. Indeed, it is not possible to download the paper from the journal and the paper does not appear in the main search engines (e.g., Scopus, Isi Web of Science).

**RC: 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).**

**AC:** We added the reference, both in the main text and in the reference list.

**RC: 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?**

**AC:** According to the suggestions of Referee#1 and 2, we added the sentence (in Methods section, 2.4 Statistical analyses): *“Before the analyses, the homogeneity of variances was checked using the Cochran’s test on appropriately transformed data, whenever necessary. For those data sets for which the transformation did not obtain homogeneous variances, a more conservative level of significance was considered (Underwood, 1991).”*

**RC: 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<sup>2</sup>? Adjusted R<sup>2</sup>? Or others? Did you check out the relationships of the particular predictor variables with one another (correlation of variables) to avoid co-linearity?**

**AC:** We used the Fortran-written “DISTLM forward”, by Anderson (2003), which performs forward selection on the basis of R<sup>2</sup> criterion.

**RC: 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).**

**AC:** No, we used the Fortran-written DISTLM forward, by Anderson (2003) and we added the relative citation.

**RC: 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 labeling. The choice of the symbols is rather unfavorable. It is sometimes difficult to recognize the results for single sites/habitats.**

**AC:** We thanks the Referee for the suggestion and modified the figures accordingly.

**RC: 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?**

**AC:** According to the Referee, we changed the Y-axes label in *“Habitat diversity (N. species per habitat)”* and the caption of figure 2 in *“Species richness of the nematodes in the different habitats in each of the regions investigated, calculated as number of cumulative species retrieved in each habitat. Mean  $\beta$ -diversity among the habitats in each investigated region is also shown.”*

**RC: Figure 3: What is meant by relative importance? Percentages? If so, are the percentages based on the number of species or the number of individuals found?**

**AC:** The relative importance is the percentage of exclusive/common species based on the total of species retrieved in each region. To be even more clear, we changed the caption of Figure 3 in “*Pie charts showing the relative importance of the exclusive species (as percentage of exclusive species on the total number of species retrieved in the region) in each of the habitats investigated, along with the species in common to all of these habitats in the North-Western, Central and Eastern Mediterranean regions*”.

**RC: 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.**

**AC:** We agree with the Referee’s comment and we corrected the figures accordingly.

In the figure 6a, the species richness is referring to local ( $\alpha$ ) diversity, i.e. the species richness of the nematodes in the different sampling sites (cumulatively for the three replicates). We changed the label of the X-axis into “ $\alpha$ -diversity (N. species per sampling site)”.

**RC: 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.**

**AC:** In figure 7, species richness is referring to the number of species cumulatively retrieved from the different investigated regions. To be even more clear, we modified the Y-axis label into “Regional  $\gamma$ -diversity (N. of species per region) and the caption into “Nematode species richness at regional and macro-regional spatial scale:  $\gamma$ -diversity (as total number of species retrieved from each region),  $\delta$ -diversity between regions and the  $\varepsilon$ -diversity (as total species richness in the Mediterranean Sea).”

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

**AC:** Done, we modified the Table accordingly.

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

**AC:** Done, we modified the Table accordingly.

#### **RC: 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. 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.

**AC:** In Danovaro et al (2009), the authors investigated biodiversity patterns along bathymetric gradients, from 196 down to 4987m. Here, we investigated sampling sites at depths ranging from ca. 600 to 1300 m. According to Referee#1, we modified the definition “fixed water depth” into “bathymetric range comprised between 600 and 1200 m”, throughout the ms.

**RC: 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.

**AC:** As commented above, in response to Referee#1, regional or macro-regional diversity could be driven by different factors, and, in particular, by the level of local diversity, by the turnover diversity or a combination of both. In this sense, at regional level, high values of biodiversity could result from high levels of local diversity but low levels of turnover diversity between habitats, or, conversely, to low levels of local diversity but high levels of turnover diversity between different habitats (Loreau 2000, Ecology Letters).

We are aware that DistLM can tell little about the mechanisms driving gamma diversity. This is why we did not and cannot primarily based the discussion on these outputs.

**RC: 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 20% (see Table 5), indicating that some untested/unmeasured processes/parameters affect gamma diversity.**

**AC:** We did not use  $\alpha$ - and  $\beta$ -diversity as proxies for habitat heterogeneity, as habitat heterogeneity is related to the complexity of physical, chemical, topographic, biological characteristics of the habitat and it has to be valuated through appropriate measurements or proxies (Cordes et al 2009, Marine Ecology 31). Conversely, we used  $\alpha$ - and  $\beta$ -diversity as properties of the biological assemblages inhabiting one or more habitats.

In our study, we used  $\alpha$ - and  $\beta$ -diversity as different components of biodiversity, possibly influencing the biodiversity at larger spatial scale (i.e., regional diversity).

**RC: 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).**

**AC:** According to the Referee's suggestions, we implemented this paragraph of the Discussion as follows.

*"The Mediterranean basin is considered as a hot spot of biodiversity with a uniquely high percentage of endemic species (Danovaro et al., 2010). However, this information is almost completely confined to coastal ecosystems, while data on deep-sea assemblages are still limited, indeed the deep Mediterranean sea has been considered for decades as diversity-depleted (Danovaro et al., 2010 and citations therein).*

*The results of the present study confirmed that, in the Mediterranean deep sea, the high levels of  $\beta$ -diversity are responsible for unexpected high levels of regional  $\gamma$ -diversity, even if local  $\alpha$ -diversity results low. Conversely to our results, previous studies reported that whilst deep-sea nematode diversity may be very high at the local scale, diversity at the regional scale may result relatively limited (Lamshead and Boucher, 2003;*

Leduc et al., 2012, and citations therein). Moreover, these studies suggested that low levels of regional diversity in deep-sea environments (despite the greater local diversity) may be related to the lack of dispersal barriers and/or relatively low macro-habitat heterogeneity (Lambshhead and Boucher, 2003; Leduc et al., 2012).

The number of species recorded during the present study from the different Mediterranean regions (North-Western, Central and Eastern Mediterranean) appears to be relatively high. Though comparisons with other deep-sea regions result difficult (due to different bathymetric range and sampling efforts; Leduc et al., 2012), the biodiversity levels found in the Mediterranean deep-sea sediments result similar to those retrieved in other deep-sea regions, as in NE Atlantic (Portuguese margin, Danovaro et al., 2009) or in Polar regions (Gallucci et al., 2008; Fonseca and Soltwedel, 2009). Conversely, the number of species recorded in the Mediterranean regions were lower than the regions in the SW Pacific ocean (Leduc et al., 2012), but this difference probably reflects differences in water depth range, sediment depth or the utilized methods (e.g., the mesh size, Leduc et al., 2010).

The total number of species retrieved from the Central Mediterranean region (210 species) was higher than Western and Eastern Mediterranean. Despite such difference could be imputable to the higher number of investigated habitats in the Central Mediterranean, it is worthy of notice that such pattern was consistent whatever the considered index (total number of species, ES(100)) or estimator (Chao1, Chao2).

We also reported significant differences in nematode species compositions between the different deep-sea Mediterranean regions, which such differences are highlighted by the  $\delta$ -diversity (measured as the turnover of nematode species among the different regions), which was always >80%. This suggests that each deep-sea region is characterised by a specific nematode assemblage and species composition, thus letting us hypothesizing a high habitat heterogeneity, possibly related to low dispersal potential (Leduc et al., 2012)."

We would like to underline that the paper by Leduc et al 2010 (Deep-Sea Research I 57 (10): 1354-1362), dealing with nematode diversity, was conducted in only one deep-sea sampling station and demonstrated that there is an effect of different mesh size in assessing the nematode biodiversity. So maybe, it is difficult to make any comparison with this paper, since we used a different mesh size (20 $\mu$ m in the present study vs 63, 45 and 42 $\mu$ m in Leduc et al 2010).

**RC:** Turnover is not only driven by 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.

**AC:** At Page 17833, Lines 5-8, we reported: "Altogether, the data obtained in the present study are supportive of the hypothesis that different habitats, such as deep canyons, open slopes, basins and deep-water corals, host particular assemblages, and that the higher the number of habitat in a region, the higher the number of exclusive (and potentially endemic) species." This suggests that apparently the dispersal is for evident reasons limited, otherwise the species composition would be more homogeneous. We hope that the reviewer can agree on this conclusion.

Moreover, Fonseca & Soltwedel (2009) analyzed a North-Atlantic region, with peculiar characteristics: "There are two apparent aspects of the area studied that may promote such low turnover of species between margins. First, the eastern and western margins of the northern North Atlantic are relatively close to each other, the water corridor is ca. 500 km wide in the northern part (80°N) and less than 1,500 km at latitude 70°N between Norway and Greenland. Second, the WSC and EGC form an oceanic gyre which is constantly interrupted by numerous eddies and recirculation currents mixing the waters between both currents (Swift 1986). Both

aspects may promote the exchange of nematodes by passive transport from one margin to the other.” In this paper, the authors only suggest or hypothesized a high potential dispersal, without any demonstration.

Conversely, the Mediterranean Sea presents opposite features: deep-sea floor is very heterogeneous and characterized by a continuum of open slopes, submarine canyons, landslides, seamounts, dead and alive coral mounds, mud volcanoes interacting with water masses and biotic components (as examples see Weaver et al 2004, 2009 Oceanography; Canals et al 2006 Nature; Company et al 2008 Plosone; Bongiorno et al 2010 Biological Conservation; Danovaro et al 2010 Plosone, Coll et al 2010 Plosone; Zeppilli et al 2011 Progress in Oceanography). In this context, it appears hazardous making any speculation about the potential of dispersal.

To cope with the Referee#2's suggestion we added in the Discussion section: “The results of the present study confirmed that the high levels of  $\beta$ -diversity are responsible for unexpected high levels of regional  $\gamma$ -diversity, even if local  $\alpha$ -diversity results low. Conversely to our results, previous studies reported that whilst deep-sea nematode diversity may be very high at the local scale, diversity at the regional scale may result relatively limited (Lamshead and Boucher, 2003; Leduc et al., 2012, and citations therein). Moreover, these studies suggested that low levels of regional diversity in deep-sea environments (despite the greater local diversity) may be related to the lack of dispersal barriers and/or relatively low macro-habitat heterogeneity (Lamshead and Boucher, 2003; Leduc et al., 2012).”

And later in the Discussion: “We also reported significant differences in nematode species compositions between the different deep-sea Mediterranean regions, such differences are highlighted by the  $\delta$ -diversity (measured as the turnover of nematode species among the different regions), which was always >80%. This suggests that each deep-sea region is characterised by a specific nematode assemblage and species composition, thus letting us hypothesizing a high habitat heterogeneity, possibly related to low dispersal potential (Leduc et al., 2012).”

**RC: 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.**

**AC:** The Referee is right. However, we would like to stress out that only samples from the bathyal plain in Central and Eastern Mediterranean were collected in 1998 and 1989, respectively. Moreover, even if we calculate the  $\beta$ -diversity among habitats excluding the sites located in the bathyal plain, the levels of  $\beta$ -diversity remain quite the same, higher than 80% in both cases (i.e., Central and Eastern Mediterranean).

However, to cope with the Referee#2's suggestion, we added the sampling date in the Table 1, and a paragraph in the Discussion section critically explaining the bias related to the temporal component: “Overall, our findings related to  $\beta$ -diversity and the percentage of exclusive species may be affected by the temporal shift of the collection of samples (from 1989 to 2006, Table 1; Steiner and Leibold; 2004). However it is worthy of notice that only samples from the bathyal plain in Central and Eastern Mediterranean were collected in 1998 and 1989, respectively. Moreover, even if we calculate the  $\beta$ -diversity among habitats excluding the sites

*located in the bathyal plain, the levels of  $\beta$ -diversity remain quite the same, higher than 80% both in Central and Eastern Mediterranean, data not shown."*

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

**AC:** Done. We checked and corrected the English form throughout the ms and corrected the spelling of Sabatieria.