

Reply to Referee #1

We are grateful to Referee #1 for his/her generally positive comments on the previous version of the manuscript. Moreover, we would like to thank him/her for the key critical points raised about the way some of the data were analyzed and presented. We agree with many of the points raised by the Referee #1. These, indeed, were all kept into consideration in the preparation of an amended version of the manuscript. Below we report point-by-point responses to his/her comments.

My first point of concern is with the way the authors formulated and tested their hypothesis. In particular, as the authors stated in their Introduction, they set out to test that the cascading event affected the biodiversity and functioning of the deep-sea benthic ecosystem. However, while they provide enough data and discuss in detail how the cascading affected the biodiversity, they don't provide any data nor do they discuss the second part of their hypothesis, namely how the cascading affected the functioning. For this, it would be necessary to provide an independent measure of functioning in the canyon and the deep basin. I strongly suggest to either include, if of course available, such data and analysis in their Results and Discussion or, in case this is not possible, reformulate their hypothesis and adjust the Discussion by excluding the part on the effects on ecosystem function.

As also detailed below in the reply to the comments raised by Referee#2, having no independent measurement of ecosystem functioning, any reference to “ecosystem functioning” has been eliminated in the amended version of the manuscript.

Another part where the manuscript could be largely improved is the part on the effects on nematode diversity. There is still great potential in the dataset, which, in my opinion, has not been fully explored. More specifically, while the authors provide convincing evidence for a significant decline in the number of major taxa and nematode species and a consecutive recovery, they do not discuss how the cascading affected the nematode community structure. I think with a little more effort, this can be easily achieved since the data on the nematode community structure are available. It would be very interesting to know how the cascading event shaped the nematode community structure, which particular species were the most tolerant or vulnerable to the disturbance, which species re-occurred after the recovery, if these species that re-occurred were the same as those that were extinct and if any of these patterns was consistent along the depth gradient. All these are important ecological questions which, if discussed, would greatly improve the manuscript.

We can agree with the Referee#1 about this point. Actually, we made the multivariate PERMANOVA tests for assessing variations in the nematode assemblages composition between event and post-event conditions. We even mentioned this in the methods (P.17863; L20-22), cited it in the text, but missed to include the relative Table 5. In the amended version of the manuscript we include Table 5. Nevertheless, the large differences among nematode assemblages during and after the cascading were illustrated in the bi-plots in Figure 5. In this regard, and also detailed below in the reply to Referee#2, we also included a Supplementary Table S3 showing the results of the CAP analyses. In more detail, in the amended version of the manuscript: “The multivariate PERMANOVA tests reveal significant temporal variations in the composition of the nematode assemblages at all depths in both the Cap de Creus canyon and deep margin (Table 5). The bi-plots produced after the canonical analysis of principal coordinates identify a clear and significant difference in the compositions of the nematode assemblages of the Cap de Creus and deep margin during the cascading when compared to those observed in all other sampling periods (Fig. 5)” **now reads** “The bi-plots produced after the canonical analysis of principal coordinates, that identifies a clear and significant segregation of the different sampling periods (Supplementary Table S3), show that the compositions of the nematode assemblages observed in canyon and deep margin sediments during the cascading are clearly different from those observed in all other sampling periods after cascading (Figure 5)”.

As far as the inclusion of a formal analysis of which species went extinct (locally), were more resistant to or recovered from the cascading, we think that this couldn't be reliably carried out for two main reasons. **First:** as detailed elsewhere (eg Canals et al 2006) the cascading event in 2005 was an exceptional episode characterized by bottom currents of up to 1 m s^{-1} . The intensity of the cascading was so huge that any inference on species “resistance” is difficult to be reliably supported: most probably, the very few (see data

on total meiofaunal abundance) individuals remaining after cascading were not “resistant” per se rather were “random survivors”. This issue is now explicated in the Discussion (see below). **Second:** as also argued by the Referee#2, having no data about nematodes “before” the cascading avoids any reliable contention about (local) extinctions. To partly accomplish the referee’s request we reported the results of the SIMPER tests in another Supplementary Table S4, providing also indication of the species explaining cumulatively up to 50% of the dissimilarity between communities during and after to the 2005 cascading event. To highlight for possible consistencies at different depth ranges, the results of the SIMPER are provided separately for the two depths in the canyon (ca. 1000 and ca. 1800 m) and the deep margin (>2100 m) and an explanatory sentence added in the discussion. This sentence now reads *“Our results suggest that the low values of meiofaunal abundance and diversity during the 2005 cascading event are mostly likely an effect of the massive disturbance caused by cascading flows rather than controlled by an increased food availability. Moreover, the results of the SIMPER analyses show that different groups of nematode species explained the dissimilarity between assemblages during and after the 2005 cascading event at the different depth ranges. This result suggests that species encountered in DSWC-impacted sediments were random survivors and the 2005 cascading event hit massively the benthos leaving no room to any possible selection of specifically resistant species and possibly leading to random (local) extinctions.”*

A final point of confusion is the fact that the authors present and discuss their results in light of the cascading event that occurred in 2005 (i.e. all graphs, analyses and comparisons are performed focusing on the values found in April 2005 and comparing these values with the values found just before and after that time point). However, as the authors state on Page 17858 and Line 5, according to Palanques et al. (2012), there was another cascading event in 2006 in a nearby canyon. Taking into account the proximity of the two canyons, this event in 2006 should have equally impacted the CCC canyon. Thus I wonder why the authors don’t consider their 2006 sampling point as another cascading event as Palanques et al. (2012) suggests? If indeed there was a cascading event in 2006, then one would expect that a similar to the 2005 event collapse and recovery would appear just before and after this sampling point as well.

In principle, we could agree with the Referee#1 about possibly including the 2006 cascading in the formal analysis. The two events in 2005 and 2006 were indeed both “intense” as described in more details in the amended version of the manuscript. Having unfortunately no data from April 2006 (i.e. during or just after the 2006 cascading event), we feel that conclusions or inferences about the possible effects of the 2006 event cannot be reliably made. We explicated this problem in the amended version of the manuscript. Moreover, to let the reader appreciating also the potential effects of open-sea convection - as shown in Stabholz et al *Biogeosciences Discuss.*, 9, 12845–12894, 2012- we implemented the discussion with a short related paragraph. This sentence now reads: *“Besides the evidences provided in this study, we must consider also that, for the deep margin (>2100 m depth), also open-sea convection can be a concurrent causative agent of the observed variations after cascading (Stabholz et al., 2012). Strong near-bottom particle flux, due partly to local sediment resuspension by strong currents occurred during bottom-reaching convection (like in 2005 and, possibly, 2006), are associated to the DSWC plume spreading. For milder winter (like in 2007 and 2008) the open-sea convection did not reach the bottom and thus had possibly only little impacts on bathyal ecosystems.”*

1. (Page 17863, Lines 5-6): Here the authors say that the calculated b-diversity between sites, sampling times and depths but later on Page 17864 and Lines 2-3, they say that turnover diversity (which, if I understand this correctly is practically the same thing) was estimated between sampling times and depths only.

Correct. As also detailed below in the reply to the comments raised by Referee#2, in the amended version of the manuscript we clarified that the SIMPER tests were carried out among sampling periods only, separately for the different depth ranges.

2. (Page 17865, Line 3): I believe that (Fig. 2c) should read (Fig. 2b,c) since it refers to the bioavailable C and bioavailable fraction respectively.

Correct. The text has been amended accordingly.

3. (Page 17865, Lines 12-14): This statement is not correct for biomass since, as can be seen in Fig. 3b, the biomass in Apr. 2008 is significantly lower compared to all other sampling events.

Correct. The text has been amended accordingly. In particular, the new sentence now reads: *"A few months after the cascading (e.g. in October 2005), at all depths, meiofaunal abundance and biomass display values similar to those observed in all other sampling periods, with exception of April 2008 for meiofaunal biomass (Figure 3a-b)"*.

4. Statistical analyses: The PERMANOVA analysis is either incorrect or the authors did not explain well what they did. In a two-way PERMANOVA, similar to its two-way ANOVA analogy (see the PERMANOVA manual) , when the interaction term is significant, the usual advice is that you should not test the effects of the individual factors. For example, it would be misleading to examine the individual factors and conclude in page 17865, and lines 25-26 that "nematode biodiversity, expressed either as species richness, expected species number [ES(100)] or as Shannon's (H) index, is significantly lower ". As all statistical textbooks advise, what you can do, if the interaction term is significant, is look at each factor separately, using a one-way anova (or PERMANOVA in our case).

We think that, most probably, we were not clear enough in describing how we treated our data. As a matter of fact, the presence of a significant interaction in a 2-way (PERM)ANOVA design including orthogonal factors both with fixed levels (as in our study) does not imply that comparisons between levels of one factor can be reliably carried out solely with one-way ANOVAs carried out separately for each level of the other factor (and *viceversa*). The presence of a significant interaction, indeed, indicates that differences between levels in one factor vary in each level of the other one. In our case, for example, the recurrent presence of a significant Time x Depth interaction suggests that differences between sampling periods vary at different depths (and possibly *viceversa*, which however was not the main effect we were testing). This was clearly stated in the previous version of the manuscript. In the previous version of the manuscript it was, most probably, partly unclear that, pending the presence of the abovementioned significant interactions and according to what proposed in all environmental statistics textbooks (eg Underwood 1991 or Qion & Keough 2009) and as also suggested in the PERMANOVA+ for PRIMER handbook (the last paragraph at page 35 of the handbook reads *"... Appropriate logic dictates that the next step after obtaining a significant interaction is to do pair-wise comparisons of the factor of interest – during/after cascading in our case – separately within each level of the other factor – depth in our case*). Indeed, we used pairwise tests for ascertaining, separately at each sampling depth, whether values before/ during the 2005 cascading were significantly different from those after the event. These results (contrasting pre/during vs. after cascading conditions) were actually reported in the Supplementary Tables available in the previous version of the manuscript. Nevertheless, to accomplish the Referee#1 comment, we better explained in the description of the statistical analyses this possible misleading issue. More in details, the amended description of the sampling design and the use of PERMANOVA and pairwise comparisons is now as follows: *"We determined the effects of the cascading on each variable separately: the design included two orthogonal factors: sampling time (5-6 and 4-5 fixed levels for meiofauna and OM data in the canyon and the deep basin, respectively) and water depth (2 fixed levels: ca. 1000 m and ca. 1800 m depth), with n=3 for the combination of factors. Since the information for the deep margin did not include data from April 2008, to avoid unbalanced designs a separate one-way test (with sampling time as the unique source of variation with 4 fixed levels, April 2005, October 2005, August 2006 and April 2009) was carried out to ascertain the effects of cascading at >2100 m depth in the deep margin. Since most Time x Depth interactions were found to be significant, pairwise comparison tests were also carried out to discriminate the effects of the cascading, separately for the different depth ranges (Table S1 and S2 in Supplement)"*.

5. (Page 17865, line 26). I think Table 3 should read Table 4. Same on the next page, Table 5 should read Table 4?

Correct for Table 3 that should read Table 4. Table 5, as detailed above, was not included in the early version of the manuscript, but is now included in the amended version.

6. (Page 17867, line 6). "...to the deep-sea margin ..." should read "... to the deep-sea basin ..."

Amended as follows: “... to the deep margin”

Reply to Anonymous Referee #2

We would like to thank very much Referee #2 for his/her words of appreciation of our study. We also would like to thank Referee #2 for his/her honest criticism about the strong statements we included in the previous version of the manuscript. We definitely agree with his/her position and, accordingly, we have smoothened our conclusions about the impacts on meiofauna. To accomplish this, we also modified the title as follows “**Major consequences of an intense Dense Shelf Water Cascading event on deep-sea benthic trophic conditions and meiofaunal biodiversity**” and eliminated any further reference to the deep-sea ecosystem functioning (see also comments to Referee#1). Also, the abstract has been slightly modified and the following changes were carried out (page numbers refer to the previous version of BGD manuscript).

Abstract P.17856 L.6-7: “*Their effects on the deep-sea biodiversity and ecosystem functioning are almost unknown*” **replaced with** “*Their effects on deep-sea ecosystems are almost unknown*”.

Abstract P.17856 L.16-23: “*During the cascading event the meiofaunal abundance and biodiversity in the studied deep-sea sediments dropped down by a factor of 5 to 10. Benthic assemblages in the impacted seafloor recovered to pre-cascading conditions after only six months from the cessation of the cascading. Since the present climate change is expected to increase the intensity and frequency of these episodic events, we anticipate that they will increasingly impact biodiversity and functioning of the benthic bathyal ecosystems, which may eventually challenge their resilience*” **replaced with:** “*During the cascading event the meiofaunal abundance and biodiversity in the studied deep-sea sediments were 5 to 10 lower than after the event. Benthic assemblages during the cascading were significantly different from those in all other sampling periods in both the canyon and deep margin. After only six months from the cessation of the cascading benthic assemblages in impacted sediments were again similar to those observed in other sampling periods. Since the present climate change is expected to increase the intensity and frequency of these episodic events, we anticipate that they will increasingly affect benthic bathyal ecosystems, which may eventually challenge their resilience*”

Below we report detailed point-by-point responses to all technical issues raised by the Referee #2.

Page 5 line 28. Stating that samples are perfectly undisturbed is a pleonasm. If it was not perfectly the samples would not be undisturbed.

We agree, and therefore: “At all sites, replicate sediment samples were collected, using a NIOZ-type box corer, allowing the collection of perfectly undisturbed sediment samples” is **replaced with** “At all sites, replicate sediment samples were collected, using a NIOZ-type box corer (May 2005, Apr 2005) or an OCTOPUS multi-corer (Oct 2005, Aug 2006, Apr 2008, Apr 2009), allowing the collection of undisturbed sediment samples”.

Page 6 line 1/ specify number of cores taken. In the amended version of the manuscript we clarified the number of cores analyzed. In particular: “At each station, the top 1 cm of sediment cores (internal diameter 3.6 cm), obtained from independent deployments of the box corer” **replaced with** “At each station, the top 1 cm of three sediment cores (internal diameter 3.6 cm), each obtained from independent deployments of the box or multi-corer”

Page 8 line 2 specify number of cores. In the amended version of the manuscript we clarified that the diversity data were calculated from n= 3 cores. In particular: “These indices were calculated from the sum of the individuals of the three replicates of each of the sampling sites” **replaced with** “These indices were calculated from the sum of the individuals of the three cores from each of the sampling sites”

Page 8 line 20 What is depth of each station? We did not find a link to this request at P.8 L.20. This information however was already given in the previous version of the manuscript at P.5 L.25. Specifically, the exact depth of sampling site at each sampling date is given in Table 1 (P. 17859 L. 26).

Page 9 upper paragraph/ what is difference between sites, areas and depths? Clarify. In the amended version of the manuscript we clarified that the SIMPER test was carried out among sampling periods only, separately for the canyon and the deep margin. In particular: *“SIMPER analyses were also carried out to estimate the turnover of nematode diversity between sampling times and depths”* **replaced with** *“SIMPER analyses were also carried out to estimate the turnover of nematode diversity between sampling times”*

Page 9 line 13 : 46 fixed levels? I guess you mean 6 fixed levels?. Yes, obviously. Most likely, an error occurred in the conversion of the text from the original PDF to the journal format (that was missed in the revision of the BGD proofs). Furthermore, in the amended version of the manuscript we explicitly declare the different number of levels for the different arrays of variables or sampling depths considered (5-6 and 4-5 fixed levels for meiofauna and OM data in the canyon and the deep basin, respectively). Also, as requested below, we clarified that the data from the deep basin were not available in April 2008. Therefore: *“We determined the effects of the cascading on each variable separately: the design included two orthogonal factors: sampling time (46 fixed levels, depending on the variable)”* is **replaced with** *“We determined the effects of the cascading on each variable separately and the design included two orthogonal factors: sampling time (5-6 and 4-5 fixed levels for meiofauna and OM data in the canyon and the deep basin, respectively)”*

Page 9 line 17 Justify better why two separate analysis are done (2200 m separated). In the amended version of the manuscript we explained that, to avoid unbalanced designs, this dual approach was chosen because the information from the deep margin did not include data for April 2008. Therefore: *“An additional one-way test (with sampling time as the unique source of variation with 4 fixed levels, April 2005, October 2005, August 2006 and April 2009) was also carried out to ascertain the effects of cascading at 2200m depth in 20 the deep margin”* is **replaced with** *“Since the information for the deep margin did not include data from April 2008, to avoid unbalanced designs a separate one-way test (with sampling time as the unique source of variation with 4 fixed levels, April 2005, October 2005, August 2006 and April 2009) was carried out”*

Page 10 line 26 You mean with the exception of aug 2008? In the amended version of the manuscript *“April 2009”* is replaced with *“August 2006”*.

Page 11 line 6 replace ‘than in all other sampling events’ by “than after the DSWC event” In the amended version of the manuscript *‘than in all other sampling events’* is replaced with *“than after the DSWC event”*

Page 11 line ??. *There is no depth comparison done (not shown at least) , so how do you get significant differences here?* Correct. This sentence has been removed.

Page 11 line 16. *I would not say that cascading had a major impact since we do not know the pre situation. Maybe diversity increased only after DSWC, but never decreased as a consequence of DSWC. There is no evidence for that.* In the amended version of the manuscript the sentence *“The cascading had apparently a major impact on benthic assemblage biodiversity”* has been removed.

Page 11 line 26 replace ‘than in all other sampling events’ by than after the DSWC event. In the amended version of the manuscript *“than in all other sampling events”* is **replaced by** *“than after the DSWC event”*

Page 12 line 14 CA results are not shown? Explain why? To accomplish this request we included an additional Supplementary Table S3 showing the CA outputs of the analysis.

Page 13 line 7 weaken the statement that “ this event had a major impact on benthic diversity and functioning”. Since it is an overstatement with no evidence available here for functioning, and even not for biodiversity since we do not know the pre event situation. Same for line 11. We do not know if effect was devastating since maybe densities were low and just increased due to DSWC; Page 13 Line 14; There were no data from pre cascading periods for nematodes, as stated here; Page 13 Line 15 and 17 and 25. You cannot talk about loss when there are no pre event data to compare with; Page 13 Line 29 How do we know if there are no data from pre? As far as the preceding points are concerned, we agree with the Referee #2. In the amended version of the manuscript we thus smoothed all the statements related with the above mentioned points and thoroughly improved the discussion to make clear that, although the data

for meiofauna were not available from pre-cascading conditions, our results are suggestive of an impact of the 2005 DSWC event on deep-sea biodiversity. In particular:

P. 17867 L. 6-8: “We report here that this event had a major impact on the trophic conditions, benthic biodiversity and functioning of the deep-sea ecosystem” **replaced with** “We report here that this event had a major impact on the trophic conditions of the deep-sea ecosystem and was associated with major changes in the biodiversity of meiofauna”.

P. 17867 L. 11-14. “we observed a devastating effect on all of the meiofaunal variables (abundance, biomass, richness of higher taxa and nematode biodiversity). These, indeed, were reduced by up to one order of magnitude at all sampling sites when compared to the pre- and post-cascading periods. The loss of meiofaunal abundance, biomass and biodiversity along the canyons can be due to the hydrodynamic stress that resuspended and dispersed the surface sediment layer, whereas the loss of benthic fauna could be the result of suffocation due to the massive deposition of sediments transported by the cascading to the distal part of the canyon” **replaced with:** “we observed that the abundance, biomass, richness of meiofaunal higher taxa and nematode biodiversity during the cascading were generally much lower than after the DSWC event. At all depths, these variables, indeed, were up to one order of magnitude lower than those observed in post-cascading periods. The low values of meiofaunal abundance, biomass and biodiversity along the canyon can be due to the hydrodynamic stress that resuspended and dispersed the surface sediment layer during the cascading (Canals et al., 2006), whereas the low values in the deep margin meiofauna could be the result of suffocation due to the massive deposition of sediments transported by the cascading to the distal part of the canyon”.

P. 17867 L. 11-14. “The strong impact of DSWC-mediated flushing of the canyon’s sediment determined also a negative impact on the composition of the nematode assemblages.” **replaced with** “Accordingly, the compositions of the nematode assemblages during the cascading were very different from those in post-cascading conditions, at all depths”

P.17867 L. 24: “However, our results suggest that the loss in meiofaunal abundance and diversity is inevitably linked to the massive disturbance caused by cascading flows rather than controlled by food availability” **replaced with:** “However, our results suggest that the low values of meiofaunal abundance and diversity during cascading is mostly likely an effect of the massive disturbance caused by cascading flows rather than controlled by an increased food availability.”

P.17868, L. 1-3: “Six months after the event, meiofaunal abundance, biodiversity and community composition recovered to values observed before. Such values remained stable also in the subsequent years, during which intense cascading events were not observed. The quick recovery of the deep-sea assemblages impacted by cascading can be explained by the high turnover (up to > 10 generations yr^{-1}) and opportunistic life strategies of meiofauna. However, the increased food availability observed in the deep margin and the ecological space released by the meiofauna killed or brought away by cascading could have favoured the fast recovery of meiofaunal assemblages. As such, the impact of DSWC on the deep-sea benthos had a very limited temporal effect” **replaced with:** “Six months after the event, meiofaunal abundance, biodiversity and community composition recovered to values typically observed in all other sampling periods. The apparent quick recovery of the deep-sea assemblages after cascading can be explained by the high turnover (up to >10 generations y^{-1}) and opportunistic life strategies of meiofauna. However, also the increased food availability observed in the deep margin and the ecological space released by the meiofauna killed or brought away by cascading could have favoured the fast recovery of meiofaunal assemblages. As such, we could infer that the impact of DSWC on the deep-sea meiofauna had a limited temporal effect”

P. 17868 L. 18-21: “we anticipate that biodiversity and ecosystem functioning of the benthic bathyal ecosystems will be increasingly impacted by them in the future, which may eventually challenge their resilience” **replaced with:** “we anticipate that benthic bathyal ecosystems will be increasingly impacted by them in the future, which may eventually challenge their resilience”.