Dear editor,

we hereby provide you our detailed answers to all remarks of the referees on our manuscript bg-2012-622 by De Troch et al. Each original remark is retaken and a detailed answer is provided. The revised manuscript is therefore the result of all corrections suggested. We adjusted the line numbers stated in this document according to the lines of the final revised manuscript.

Don't hesitate to contact me in case of any further questions.

On behalf of all co-authors I wish to thank you for the way you handle and appreciate our work.

with my best regards

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Biogeosciences Discuss., 10, C301–C302, 2013

www.biogeosciences-discuss.net/10/C301/2013/

Discussions

Authors' reply to the Interactive comment on "Structural and functional responses of harpacticoid copepods to anoxia in the Northern Adriatic: an experimental approach" by M. De Troch et al.

E. Bonsdorff (Referee)

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Replies of the authors are indicate in bold italics

This is an interesting manuscript, based on well-performed experimental work, and analyzed using appropriate information. Figs and tables are clear and informative.

Meiofauna are seldom included in studies such as this, and yet it is commonly accepted that meiofaunal organisms seem to withstand hypoxia (even anoxia for shorter periods), and also that recovery-rates are generally rapid (in part due to short life cycles, in part due to passive transport.

- ***The authors appreciate these positive words of the referee. It is nice that he likes our work and that he underlines the need for this kind of experiments.***

My minor comment to this valuable contribution is thus that there are numerous studies on meiofauna and hypoxia from the North Sea (Giere's seminal work on meiofauna should be cited), and the Baltic Sea that could and should be included both in the general introduction and in the discussion-part. See e.g. papers by Elmgren & co (roles of meio- vs macro fauna), by Olafsson et al (meio/macrofauna, trophic status under environmental degradation - in press Mar Biol 2013), and Arroyo et al (J Exp Mar Biol Ecol 2012, vol. 420-421, and ydrobiologia 2006, vol. 554). For a general reference to the spreading of coastal hypoxia, see (it is a comprehensive study) Conley et al 2011, Env Sci Technol 45. The interesting issue of nematodes vs harpacticoids should attract more work!

- ***We agree that there is a large number of papers available on anoxia/hypoxia. The ones suggested by the referee are indeed relevant for our study.***
- ***The following information from Elmgren (1978) was added to the introduction:*** **'**Elmgren (1978) stated that oxygen-dependent zonation of the fauna can occur as macrofauna (> 1 cm) disappears at higher oxygen levels than some of the meiofauna. Especially nematodes are known to persist in low numbers in areas which have been anoxic for long periods.' (p. 3 lines 4-7 in the word document).
- ***Unfortunately the paper by Ólafsson et al was not yet available in the online first papers of Marine Biology at the time when we made the revision. Now, after receiving all referee reports, we saw that the paper is available online. However, as it mainly focusses on decomposition of green algae, the authors think it is too far from the core questions in our paper. Furthermore, referee 3 stated that we have far too many references, so we need be selective.***
- ***The following relevant information from Arroyo 2012 JEMBE 420-421 was added to the introduction:*** **'**A comparable phenomenon takes place in the archipelago area of the Baltic

Sea, where algal mats become stagnant in shallow embayments, covering wide areas in whose centre hypoxic and even anoxic conditions develop rapidly (Arroyo et al., 2012). The same study showed that the negative impact of hypoxia induced by drifting algal mats (eutrophication) was propagated to almost all levels of the trophic and functional chain, influencing species interactions even at the lowest levels.' (p. 2, lines 19-25 in the word document).

- Arroyo et al. 2006 Hydrobiologia and Wetzel et al; 2002 ***was cited in the introduction***: 'Moreover, the response to anoxia and the recovery from it can be size- (macrofauna vs. meiofauna) and species-dependent (Wetzel et al., 2002; Arroyo et al., 2006).' (p. 2, lines 25-27 in the word document).
- ***The reference of*** Conley et al 2011 was added to the introduction: 'With worldwide more than 400 systems recognized, covering a total area of ca. 245,300 km^2 (Diaz and Rosenberg, 2008), hypoxia (defined here as DO levels $\leq$ 2ml l^{-1}) and anoxia (no oxygen) are among the top-list of emerging environmental challenges (UNEP, 2004; Rabalais et al., 2010) and were found to expand rapidly (Conley et al., 2011).' (p.2 lines 13-16 in the word document)

The following references were added to the list:

- Arroyo, N. L., Aarnio, K., and Bonsdorff, E.: Drifting Algae as a means of Re-Colonizing Defaunated Sediments in the Baltic Sea. A Short-Term Microcosm Study, Hydrobiologia, 554, 83-95, 2006.
- Arroyo, N. L., Aarnio, K., Mäensivu, M., and Bonsdorff, E.: Drifting filamentous algal mats disturb sediment fauna: Impacts on macro–meiofaunal interactions, J. Exp. Mar. Biol. Ecol., 420–421, 77-90, 2012.
- Conley, D.J., Carstensen, J., Aigars, J., Axe, P., Bonsdorff, E., Eremina, T., Haahti, B.M., Humborg, C., Jonsson, P., Kotta, J., Lannegren, C., Larsson, U., Maximov, A., Medina, M.R., Lysiak-Pastuszak, E., Remeikaite-Nikiene, N., Walve, J., Wilhelms, S. and Zillen, L.: Hypoxia is increasing in the coastal zone of the Baltic Sea, Environ. Sci. Technol., 45, 677-6793, 2011.
- Elmgren, R.: Structure and dynamics of Baltic benthos communities, with particular reference to the relationship between macro and meiofauna, Kieler Meeresforsch. Sonderh., 4, 1-22, 1978.
- Wetzel, M.A., Weber, A. and Giere, O.: Re-colonization of anoxic/sulfidic sediments by marine nematodes after experimental removal of macroalgal cover, Mar. Biol., 141, 679-689, 2002.

I warmly recommend publication; an interesting manuscript well-suited for this special issue on coastal hypoxia

- ***Thank you!***

Biogeosciences Discuss., 10, C259-C263, 2013
www.biogeosciences-discuss.netl1 *0/C259/20131*

Authors' reply to the *Interactive comment* on "Structural and functional responses of harpacticoid copepods to anoxia in the Northern Adriatic: an experimental approach" *by* M. De Troch et al.

Anonymous Referee #1

Replies of the authors are indicate in bold italics

Received and published: 9 March 2013

General comments

This is a very interesting study combining a field and a laboratory experiment in order to determine the effects of anoxia on meiobenthic communities. The manuscript suits well within the focus of Biogeosciences and uses novel approaches (fatty acids, stable isotopes) to answer the questions addressed. The conclusions are well supported by the results and overall the paper is well written. However, there are a few flaws in the design and the presentation of the results which need to be taken into account before the manuscript can be accepted for publication.

Specific comments

The main weakness of this study lies in the uneven design of the field and lab experiments, which makes the manuscript quite difficult to follow and the results at times irrelevant to the aims of the study. Differences between the two experiments include the different vertical sampling scheme, the fact that survival rates, diversity and chlorophyll was only measured in one (not always the same) experiment, different statical tests applied (e.g. two-way vs. one-way Anova's) and so on. To overcome this situation I think the authors should try to unify the analyses and prsentation of results between the two experiments by following the following simple steps:

• Leave out the vertical distribution part of the study. Many such, more carefully designed studies, exists and most of them show what you have also found, namely that the vertical depth plays a significant role in the distribution of meiofauna.

Moreover, your aim, as seen in the Title, Abstract and Introduction was to investigate the short effects of anoxia and in my opinion you have done enough to support your case without the vertical distribution part (Le. clear effects of anoxia on copepods, effects of feeding behaviour etc.). Finally, since you have only detailed vertical distribution on the field experiment this part only complicates matters (see specific comment on ANOVA interactions) and confuses the reader.

- The authors understand the remark of the referee that the removal of the data on the vertical distribution would simply the paper. First, we would like to explain that there was no info on the vertical distribution in the lab experiment because we needed sufficient material to obtain reliable stable isotope data. Therefore, we report only on the top sediment layer for this part of the paper. This issue is now explained in the Material & Methods section. 'To detect a reliable $^{13}C/^{12}C$ ratios in the tissue of the harpacticoids, a minimum of 15 µg C per samples is required. Therefore we used all live copepods from the 0-1 cm layer. There was insufficient biomass of copepods in the deeper sediment layer.' (p. 8 lines 2-4 in the word document)

After all, we decided not to remove the information on the vertical distribution from the field experiment because of the following reasons: (1) it is a standard procedure in meiofauna research to report vertical distribution, so this allows comparison with other studies, (2) our results showed that the effect of the sediment depth is stronger than the effect of anoxia (see Fig. 4 MDS), this is an important outcome of our study that should remain in the paper and (3) we also showed that the anoxia effect differed in different sediment layers (see also remark 6, further in this referee report, where the referee wants us to test this via a post-hoc test). This underlines the importance of reporting the vertical distribution in this study. The specific remarks on ANOVA interactions were clarified as well (remark 11, see further).

• Whenever possible do the same analyses and present the same results for both studies as this would be very helpful to assess whether the effects of anoxia are persistent in both the field and lab experiment or driven by experimental design artifacts. For example, diversity was only estimated and discussed for the field experiment. Survival rate and chlorophyll was only measured for the lab experiment.
Multivariate analysis was only done for the field experiment and so on. I understand that the two experiments may not have been designed together there- fore some analyses, such as survival rate or chlorophyll, are only possible for the lab experiment, however, other analyses such as diversity and multivariate can be performed for both.
- ***First, survival rate and chlorophyll could not be estimated for the field samples as the cores were immediately stored in 4% formaldehyde in order to avoid any impact of oxygen in the anoxic treatment. Further, meiofauna extraction was performed with Ludox, so no survival rates could be estimated because of the formaldehyde and the Ludox.***
The main issue why certain analysis could not be performed for the lab experiment is the fact that all live copepods were needed for the stable isotope analysis. Moreover, it is impossible to put copepods in glycerine slides for identification prior to stable isotope analysis. In order to cope with this, we took 4 control cores at T_0 and those were used to identify the harpacticoid copepods and report the community composition (see results, 3.2 first paragraph). Since these cores were not subject to any treatment it makes no sense to run a multivariate analysis on them as they only show the initial copepod community. Densities of higher meiofauna taxa were not estimated for the lab experiment as the paper focussed on the response of harpacticoid copepods (see title). In response to the referee's remark, we calculated the diversity for the copepods in the T_0 samples of the lab experiment and the Hill indices were added to the revised text. 'Copepod family diversity was within the range of the diversity levels recorded for the field experiment (Table 1): N_0= 6.8 ±1.0, N_{inf} = 3 ±0.8, H' (log_e) = 1.7 ± 1.0. The higher N_{inf} is explained by the lower level of dominance of Cletodidae in comparison to their contribution in the field experiment (collected one year earlier).' (p. 12 line 31-p13. line.2 in the word document)

Technical comments
1. (Page 2484, Line 14). When where the normoxic samples taken at the beginning or at the end of the experiment?
- ***The referee is right, this should be clarified. The sentence was revised as '***One day before the end of the deployment (i.e. at day 4) normoxic samples (3 replicates) were taken at ca. 4-5 m distance from the chamber.' (p. 5 lines 12-13 in the word document)

2. (Page 2486, Line 20 and Fig. 2). Figure 2 and the way the experimental design is presented is a bit confusing. Try to make a better Figure by presenting the time paints linearly and sequentially and including To (start of the experiment).

- Figure 2 was revised according to this suggestion of the referee, T_0 was included. We agree that this clarifies the experimental design.

3. (Page 2487, Line 4). Ccores should read Cores
- This typo was already corrected during proofreading for BG discussions.

4. (Page 2487, Line 16). Why did you use a different extraction method? This is rather strange since the cores were collected from the same area and I wonder if this had some effect on the results (see also point 12 below)
- The main reason to avoid centrifugation with Ludox (was used for the field samples) was that we wanted to collected the animals alive for the stable isotope analysis. In that way, only copepods that survived the experimental lab treatment were used for the stable isotope analysis. Besides the fact that Ludox kills meiofauna organisms, it could also potentially impact the stable isotope signature. There is no carbon or nitrogen in Ludox that could directly affect the stable isotope measurements but there could be impurities in Ludox. Even more important is the osmotic effect of Ludox that can induce the leakage of components of low molecular weight. Also, samples should be prepared quickly in order to avoid leakage of ^{13}C. This reason is now added to the revised text (p. 7 line 29-31 in the word document): 'Centrifugation with ludox was not applied as we targeted live copepods for the stable isotope analysis. Furthermore, ludox could impact the final ^{13}C signal through its osmotic effect on components of low molecular weight.' ***We are expecting only some minor effects on the copepod densities as the decantation was repeated 5 times (see also answer to remark 12).***

5. (Page 2490, Line 13). What were the initial H2S values?
- The initial H2S was zero. This was clarified in the revised text (p.10 line 20 in the word document). 'H_2S started to increase soon after onset of anoxia, from 0 µM to final values reaching ~ 29 µM.'

6. (Page 2491, Lines 4-5). Did you do any post-hoc tests? Did treatment differed in all depths or only at the surface? Its difficult to see from the figure.
- This is indeed a very valid remark. We did some additional post-hoc tests (Tukey HSD). We found that indeed copepod densities (Fig 3b) were only significantly different between normoxia and anoxia in the top sediment layer. This information was added as 'In the latter case, the difference between normoxia and anoxia was only significant for the top sediment layer (0-0.5cm, post-hoc Tukey HSD, p=0.003).' (p. 11 Lines 3-4 in word document)

7. (Page 2491, Line 13). This sentence needs rephrasing as I do not understand it. You probably mean something like this: "For all these taxa there was both a treatment (anova ...) and a depth (anova ...) effect."
- The referee is right that the formulation of this sentence is not clear and it was rephrased as suggested : 'For all these taxa, there was both a treatment (p=0.04) and a sediment depth (p=0.004) effect.' (p.11 line 11-12 in word document).

8. (Page 2491 , Lines 15-16). This is not true and I cant' figure out any such grouping on the MOS. Both the normoxia top layers (i.e. white and light gray triangles) and anoxia (Le. white and light gray circles) are far away and on both sides of the dashed line
- This sentence actually refers only to the top layer (0-0.5 cm) and not to 0-1 cm depth. The 0.5-1 cm samples grouped indeed with the deeper layers. Therefore the text was corrected in this sense. We also added that there was still a considerable spread within the 0-0.5 cm

replicates. The entire paragraph was revised as 'On the MDS plot on the relative meiofauna composition (stress=0.05, Fig. 4A), the top sedimentlayer (0-0.5 cm) grouped separately from the deeper layers (0.5-3 cm), which points to a strong effect of the sediment depth and no clear effect of anoxia on relative meiofauna composition. This separation was further confirmed by ANOSIM (R=0.651, p=0.001). A two-way crossed SIMPER analysis showed an average similarity in taxa contribution of 72.4% in the surface layers (0-0.5 cm) and 79.8% in the deeper layers (0.5-3 cm). Nematodes (60.0%, 95.4%, respectively in 0-0.5 cm and 0.5-3 cm) and copepods (29.5% in 0-0.5 cm) contributed most to the dissimilarity between surface and the deeper layers.' (see p. 11 lines 13-20 in word document)

9. (Page 2492, Lines 10-14). Something is wrong with this sentence. Please rephrase.
- I see now that something went wrong with the Latex format:
The correct text was:
'….Cletodidae (p<0.001), Thalestridae (p<0.01) and Laophontidae (p<0.1**).**
The MDS plot (stress=0.12, Fig. 4B) of the relative copepod families composition revealed a high similarity between the top layers (0-0.5 cm) of normoxic and anoxic samples, while the deeper layers showed a higher variability (i.e. sample points are more spread). The difference in relative family composition between surface (0-1 cm) and deeper sediment layers (1-3 cm) was supported by ANOSIM (R=0.719, p=0.001).' (see p. 12 lines 3-8 in word document)

The bold text disappeared that is why you couldn't read it. I apologize for that. It is still correct in the word version of the manuscript.

10. (Page 2492, Lines 18 and 23). Try to be consistent. Sometimes you refer to the 0-1 cm layer (line 18) and sometimes to the 0-0.5,0.5-1 cm layers (line 23). You do not have a 0-1 cm layer for the field experiment.
- ***We fully agree with the referee, sometimes the two top layers were interpreted as one, but we should note it correctly. This part has been revised as*** 'The difference in relative family composition between surface (0-0.5, 0.5-1 cm) and deeper sediment layers (1-3 cm) was supported by ANOSIM (R=0.719, p=0.001).

SIMPER analysis showed an average similarity in family composition of 53.2% in the surface sediment layers (0-0.5, 0.5-1 cm) and 46.7% in the deeper layers (1-3 cm). Ectinosomatidae (44.9% and 41.5% in 0-0.5, 0.5-1 cm and 1-3 cm, respectively), Cletodidae (42.4%, 42.2%) and Miraciidae (6.7%, 13.6%) were the copepod families that contributed most to the dissimilarity between surface and deeper sediment layers.' (p. 12, lines 6-13 in the word document).

11. (Page 2492, Line 26 but also throughout the manuscript). I wonder if there were any interactions with these two-way ANOVA's. You should mention this explicitly because if you had interactions then you should have taken measures against them.
- ***We agree with the referee. In a case of any significant interactions, these should be reported, if not we didn't reported them. We checked the statistical analyses again and didn't find any significant interaction terms.***

12. (Page 2493, Line 8-9). The To community appears to be quite different from the community of the field experiment (Le. different families are dominant). I would like to see this discussed. Could this be an effect of the different extraction techniques used or is it a matter of temporal change after a year?
-The families Cletodidae and Miraciidae were found in high densities in both field and T_0 cores (collected a year later). The samples collected the year afterwards by Grego et al

(2013b, this volume) also showed a dominance of Cletodidae. For the other families there are indeed some differences that can be linked to interannual variability. Mainly the high relative abundance of Ectinosomatidae in both the normoxia and anoxia cores of the field experiment was not retrieved in the T_0 cores for the lab experiment. The underestimation of this family can be due to the fact that these are mainly rather small species that are closely associated with sediment grains and that were not sufficiently extracted by the decantation method (see before for our arguments why not to use Ludox extraction for the lab experiment and the stable isotope analysis).

These differences are now included in the discussion: 'The initial copepod community (at T_0) differed slightly from the one reported for the field experiment (see before, collected one year earlier). The community was dominated by the families Cletodidae, Laophontidae and Miraciidae but a lower share of Ectinosomatidae was found while they dominated in the normoxic cores of the field experiment. This can be explained by interannual variability in the benthic communities. However, the samples collected the year afterwards by Grego et al (2013b, this volume) were also dominated by the family Cletodidae. Cletodidae were also found to dominate in the anoxic cores of the field experiment in the present study. Next to interannual variability, the underestimation of the family Ectinosomatidae can also be due to the extraction via decantation and not by means of centrifugation with Ludox (see field experiment). Since we wanted to use the live copepods, i.e. the individuals that survived the lab experimental treatment, the use of Ludox was not an option. Species of the family Ectinosomatidae are often rather small and closely associated with sediment grains, it is plausible that they were not sufficiently extracted by the decantation method. In spite of these small differences in copepod family composition, there were no major changes in the overall diversity as the average number of copepod families in the T_0 cores (6.8 ±1.0) falls within the ranges reported for the normoxic (7.7 ± 0.6) and anoxic cores (6.0 ± 0.0) of the field experiment.' (p 16 line 29 and following in the word document)

13. (Page 2493, Line 13). I would be careful with your phrasing here as you can not say that anoxia was successful when you had (even low) evidence of oxygen presence.

- We agree with the referee and rephrased the sentence as follows: 'The induction of anoxia yielded a significant decrease of the oxygen levels in the overlying water (one-way ANOVA, $p<0.00001$) from initial 6.6 ± 0.2 mg/l (T_1N) and 6.4 ± 0.06 mg/l (T_2N) to 0.58 ± 0.29 mg/l (T_2A) after 7 days of closure of the core, independent of the addition of extra diatoms or not.' (p 13 lines 3-6 in the word document).

14. (Page 2493, Lines 17-end of paragraph). This paragraph is rather difficult to follow. Please try to make a Table with the Chi values including maybe also the other measured parameters.

- This paragraph was rephrased as 'Chlorophyll a (Chla) concentrations were measured at time T_1 and T_2 and ranged between 0 and 90 µg/g. The addition of diatoms had a highly significant effect on Chl *a* values ($p<0.0001$) as samples without additional diatoms had <5 µg/g Chl *a* while treatments with extra diatoms had >35 µg/g Chl *a*. Because of the high variance in Chl *a* concentrations in treatments with additional diatoms (83.9 ± 52.2 µg/g (T_1ND), 69.8 ± 33.3 µg/g (T_2ND), 36.6 ± 24.0 µg/g (T_2AD)), there was no significant difference in Chl *a* between the different time intervals, T_1 and T_2, (one-way ANOVA, $p=0.35$). Another pigment, Chlorophyll *c2* (Chl *c2*), showed similar patterns as Chl *a* with max. 3.5 ± 4.2 µg/g (T_1N) in treatments without diatoms and up to 8.7 ± 7.2 µg/g after adding diatoms (T_2ND). In terms of carotenoids, the concentration of fucoxanthin ranged between 1.3 ± 0.1 µg/g and 2.0 ± 0.4 µg/g without additional diatoms and between 18.2 ±19.2 µg/g

(T_2AD) and 39.7 ± 24.8 µg/g (T_2ND) in treatments with extra diatoms.' (p 13 lines 7-18 in the word document).
The authors believe that the differences between the values are very clear but that there are not enough values to list them in an extra table.

15. (Page 2495, Lines 3-5). Something is wrong here. First of all, from the graph it seems that normoxia increased to about 1200 and not 952 as stated in the text.
Moreover, in Figure 7 legent there is a statement that the Figure consists of (A), (8) and (C) but I got only one graph (probably only the (A) part) in my pdf copy.
- ***The referee is absolutely right. We initially wanted to show also the further standardisation towards total uptake per individual and per unit copepod carbon. At the end we decided to remove these figures as they showed the same trend as in Fig. 7a. Apparently we forgot to remove the legends, the authors apologize for that.***
The correct Δδ13C are now reported in the text: **'**Before the onset of the anoxia, the copepods were fed for 3 days with labelled diatoms, which resulted in the increase of their δ^{13}C±stdev from -22.4±1.4‰ (T_1N) to 276.9±192.8‰ (T_1ND) ($\Delta\delta^{13}$C=299.2±192.8‰) . In the normoxic treatments, a significant increase of $\Delta\delta^{13}$C from T_1ND (299.2±192.8‰) to T_2ND (1281.5±667.6‰) was recorded (one-way ANOVA, p=0.03), indicating continuous feeding in normoxia (Fig. 7). In the anoxic treatments, food uptake ceased, with $\Delta\delta^{13}$C values showing no significant difference between T_1ND (299.2±192.8‰) and T_2AD (138.6±43.0‰) (one-way ANOVA, p=0.16). Consequently, the $\Delta\delta^{13}$C value differed significantly between normoxic and anoxic treatment (one-way ANOVA, p=0.014).' (p 14 lines 9-17 in the word document).

16. (2497, Lines 7-9). This sentence is incomplete as it misses a verb. Maybe you intended to have this sentence together with the previous one as one sentence?
- ***This sentence was rephrased as*** 'Typically, a low oxygen demand in combination with a high surface:volume ratio enable some species to survive hypoxia/anoxia for extended times.' (p 15 line33 and following in the word document).

17. (Page 2497, Line 26). Please rephrase. Its no wonder you found effects at lower taxonomic level only for copepods since this is the only group you looked at lower level!
- ***Correct, this sentence was rephrased as:*** **'**At lower taxonomic level, there was a clear effect of anoxia on the harpacticoid copepods' family composition.' (p 16 lines 16-17 in the word document).

18. (Page 2498, Line 1). "by see Grego ... " should probably read "but see Grego ... "
- ***Corrected*** (p 16 line 20 in the word document).

19. (page 2500, Line 27) The "a" in the "a for copepods" is a typo
- ***Corrected as*** '(i.e. see Grego et al., 2013a, for copepods and nematodes)' (p 19 lines 20 in the word document).

20. (Table 1). Explain in the caption that this is only for the field experiment. However, I would also like to see the diversity values from the lab experiment. I do not understand why these were not calculated and discussed. Also, I would suggest to make the table a bit more easy to read by removing the second "Depth" column and by adding another row caption on top indicating the normoxic and anoxic part of the table.
- ***The caption was revised as:*** **'**Table 1. Average Hill's diversity indices (± stdev) for (A) meiofauna taxa and (B) copepod family composition in the field experiment.'

The lay-out of the table was revised and indeed it reads more easily now. Thanks for this suggestion!
In response to the referee's remark, we calculated the diversity for the copepods for the lab experiment as well and the Hill indices were added to the revised text. 'Copepod family diversity was within the range of the diversity levels recorded for the field experiment (Table 1): N_0= 6.8 ±1.0, N_{inf} = 3 ±0.8, H' (log_e) = 1.7 ± 1.0. The higher N_{inf} is explained by the lower level of dominance of Cletodidae in comparison to their contribution in the field experiment (collected one year earlier).' (p. 12 line 12 and following in the word document).

These diversity levels were also further discussed. **'**In spite of these small differences in copepod family composition, there were no major changes in the overall diversity as the average number of copepod families in the T_0 cors (6.8 ±1.0) falls within the ranges reported for the normoxic (7.7 ± 0.6) and anoxic cores (6.0 ± 0.0) of the field experiment.' (p. 17, lines 10-13 in the word document).

21. (Figure 4). What are the dashed lines? Why is not MDS done for the lab experiment? The different gray symbols are difficult to distinguish. Maybe use numbers?

- ***The dashed lines separate the 0-0.5 cm samples from the rest (in the A panel) and 0-0.5 cm and 0.5-1 cm from the rest (in the B panel).***
- ***We didn't aim to do a community analysis for the lab experiment, since only the copepods of T_0 cores were identified (see also the second specific comment by the referee). The copepods from the other treatments were used for the stable isotope analysis.***
- ***Numbers instead of symbols would probably complicate the figure even more. The second referee had no remark on the symbols, but still we made an attempt to clarify the fills of the symbols.***

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Discussions

Authors' reply to the Interactive comment on "Structural and functional responses of harpacticoid copepods to anoxia in the Northern Adriatic: an experimental approach" by M. De Troch et al.

Anonymous Referee #3

Received and published: 8 April 2013

Replies of the authors are indicate in bold italics

General comments

The contribution by de Troch et al. contains interesting information on meiobenthos enduring adverse conditions of anoxia in the Northern Adriatic. The study uses both classic approaches novel techniques and is of good scientific significance. There are results confirming well-known patterns (depth distribution) and results on the surprisingly small impact of short term anoxia, which make this a valuable manuscript. The quality of the presentation could be improved. This is mostly due to technical details outlined below. Also, too many details and small results are presented and discussed as if they were just as important as the main results: short term anoxia survival and reduced feeding during anoxia by copepods. Tables and figures a well designed. The presentation is good, however, the rather high number of citations, statistical information and the details given when referring to other studies, all together render some parts of the text difficult to read.
My criticism mainly refers to specific points outlined below. However, I also consider this discussion as too long and not as streamlined as it could be. I further do think that there are quite a few citations already. This is why adding references in this review process should be avoided. Do not add citations suggested by reviewers without removing others. Please do think about cutting that list.
- ***The authors appreciate it that the referee did a considerable effort to make suggestions to improve our manuscript. We agree that there are many papers cited, we removed 7 references in the revised version. On the other hand, since this study combines a field and a lab experiment we need to explain different techniques and thus more citations. All specific suggestions to improve the text were included, see further.***

Specific comments

Page 2486, Line 7-8: Change to: "The labeling technique resulted in isotope signatures (13C) of 17.29 ‰ for untreated and 8949.51‰ in 13C enriched cultures." (This is more easily read.)
- ***The authors agree with the referee and this was changed in the revised manuscript (p.6 l.25-26 in the word document).***

Page 2487: Line 14: for how long were the cores left before there was no oxygen any more? There are numbers on page 2493, line 15 indicating that concentrations fell to 10% of the initial value within 7 days. This means you witnessed a transient scheme.

The aspect that anoxia was only short (in the experiments in the lab, too) should be made more clear.

- The revised version of figure 2 (as requested by another referee) should clarify that anoxia was induced during 7 days i.e. cores were closed for 7 days. We only measured the oxygen concentration before and after closure of the cores. This implies that we indeed studied a short-term anoxia. We stressed this in the revised text by adding the word 'short-term' to l. 22 p.7. in the word document. The fact that this refers to 7 days is already mentioned in the description of the treatments in the paragraphs before.

- This information only refers to the lab experiment. For the field data it was clearly stated that the EAGU was deployed for 5 days (see 2.2).

Page 2490: Lines 18 ff: It is a bit confusing to use the term "overall meiofauna density" and relate this to depths, i.e. layers in the sediment. I would think that overall density relates to a depth-integrated abundance like Individuals/10 m. Wouldn't the term 'depth-dependant abundance' (or density) be more appropriate? And to be exact: the data demonstrate that depth is the main factor determining a depth-dependant distribution!

Well, is that astonishing? If it was not depth but organic carbon, oxygen availability or sulfide concentrations in the sediment that determined the depth distribution more than depth per se, that would be a result.

That is, I completely agree with ref 1 that your findings on the depth as a major factor are presented in a way that attributes too much weight. It is fine to present the numbers, but the statistical proof that sediment depth is the main factor is not necessary here.

The message is: no difference between anoxic and normoxic. And that is great.

- ***First, we agree that <u>overall</u> meiofauna density is a confusing formulation. Actually we meant the total meiofauna density (so all meiofauna taxa) per depth layer. We revised and clarified this part by removing the word 'overall'. Actually the sentence, as it was initially meant, reads now as '***Overall, a two-way ANOVA on the total meiofauna densities (ind./10cm^2) showed a significant effect of sediment depth (decreasing abundance with depth, p=0.002) while the different oxygen treatments (normoxia/anoxia) interestingly showed no effect (p=0.05, Fig. 3A).**'** p.10 l. 24-27 in the word document.
- ***The authors agree that this paper mainly focusses on the effect of anoxia but still we decided not to remove the information on the vertical distribution from the field experiment because of the following reasons: (1) the factor depth represents a level of variance in our sampling design that we can not neglect by applying for instance a one-way ANOVA, this would be wrong (2) our results showed that the effect of the sediment depth is stronger than the effect of anoxia (see Fig. 4 MDS), it is thus an important outcome of our study and indeed it probably points at other parameters related to depth, (3) we also showed that the anoxia effect differed in different sediment layers (see also remark 6 or referee 1, where he/she wants us to test this via a post-hoc test). This underlines the importance of reporting the vertical distribution in this study and (4) it is a standard procedure in meiofauna research to report vertical distribution, so this allows comparison with other studies,.***

Page 2493: Results show an enormous pigment variation in untreated and treated cores. This patchiness in natural cores, especially given the small diameter of cores, is not uncommon. How do you deal with it? what does it do to your interpretation?

- ***This could be an interesting point, however, we were not really after any change of chl a concentration over time, i.e. from T_1 to T_2. Since the diatoms were ^{13}C prelabelled we mainly aimed to trace the uptake of them by the copepods rather than following their growth in the cores over time (from T_1 to T_2). The large variation in pigments is indeed probably due to patchiness in the cores.***

To answer the research questions of the present study it was essential that the addition of diatoms caused a significant difference with the treatments without extra diatoms. This was the case and this was also included in the discussion: **'**Finally, it is possible that there was already a high amount of initial food present in the sediment and any addition of extra diatoms would not imply any significant difference between both treatments at the onset of anoxia. The contrary, however, was true because the Chl a concentrations increased significantly in the treatments with additional diatoms.' (p. 18 l. 23-26 in the word document).

Page 2494, line 10: "More specifically, the anoxic treatment with extra diatoms (T2AD) had a lower survival rate : : : than the one with diatoms". Why is there with? Should this read: without extra: : :?

- ***This remark is correct, in the last case it should be without extra diatoms. The sentence was revised according to this remark:*** 'More specifically, the anoxic treatment with extra diatoms (T_2AD) had a lower survival rate (66.9±5.8%) than the one without extra diatoms (80.3±21.5%, T_2A).' (p. 13 l. 25-27 in the word document).

The fact that survival is reduced in combination of oxygen lack and diatom addition should be a more prominent thought. These are adverse effects, since additional carbon may intensify oxygen depletion. At least a settling bloom or eutrophication dependant carbon supply often times is responsible/adding to depletion situations.

It is nicely shown that feeding ceased after T1! Good!

In the Discussion Page 2495: the statement in line 15 ("contrasts with previous studies") seems to contradict those in lines 18 and 21. Please clarify.

- ***Actually, lines 15-16 (l.15 p. 14 in the word document)mention that meiofauna <u>decreases</u> because of anoxia while the following lines say that they decrease but never disappear completely ('no complete mortality'). This part was revised as*** **'**In the present field experiment, total meiofauna densities were not significantly affected by anoxic conditions. This is in contrast with previous studies that showed a significant decrease of meiofauna densities due to anoxia (Moodley at al. 1997; Travizi 2000). While studies on macrofauna revealed a peak in mortality at the transition from severe hypoxia to anoxia (Riedel et al., 2012), meiofauna – in general being more tolerant (Moodley et al. 1997) – <u>decreased in density but some</u> may still be alive. Van Colen et al. (2009) created hypoxic conditions in a tidal mudflat for 40 days. While no macrobenthos survived, nematode diversity and abundances, for example, changed but no complete mortality occurred.' ***(p. 14 l.21-28 in the word document)***

Page 2496: line 9 and 13 repeat the same issue. Please shorten.

- ***This part was revised as*** **'**By sealing a 50x50x50 cm volume off from the surrounding environment (Stachowitsch et al., 2007), the field experiment mimics the situation where water column stratification is the main cause of hypoxia, i.e. the isolation of bottom water from oxygen-rich surface water (Diaz, 2001). As this set-up caused a total cut-off of the food supply, eutrophication as an important factor in creating anoxia (Gray et al., 2002) was however neglected.' (p. 15 l.6-11 in the word document).

The repeated sentence on the 'total cut-off of the food supply' in line 13 was deleted.

Page 2497: Lines 6-9 are repetitive of what other found. They do not help in the discussion of your findings and are somewhat superfluous text here. They should be omitted. Lines 21-24 could be omitted completely.

- ***Lines 6-9 try to explain why copepods and nematodes respond differently. This part was revised as '***This difference in response can be due to phylogenetic constraints and lifestyle (Wetzel et al., 2001). Typically, a low oxygen demand in combination with a high surface:volume ratio enable some species to survive hypoxia/anoxia for extended times.'(p. 15 l. 32 and following in the word document).
Lines 21-24 were deleted in the revised version.

Page 2499 line 19: replace "into" by "in". Also: which cores are you referring to? The ones from Gray 2002?
- ***The word 'into' was replaced by 'in'. We refer to what we expected to happen in our experimental cores that received additional diatoms. In order to clarify this, the sentences were revised as*** 'Alternatively, as decomposition of organic matter (here diatoms) typically results in low oxygen concentrations (Gray et al., 2002), the cores with additional diatoms (T_2AD) were expected to have lower DO concentrations and consequently lower survival rates than those without diatoms. This was however not the case in the present study as there was no significant difference in oxygen level in cores with or without extra diatoms.' (l. 14-19 p. 18 in the word document)

Fig. 6. This is a bit confusing. What about: Absolute and relative fatty acid composition of the sediment (0–3 cm) for treatments without extra diatoms (A, C) and for treatments with diatoms (B, D)
- ***We agree that this read easier and the letters in the legend were therefore reshuffled as suggested.***

Fig. 6. There is no figure 6B or 6C in my copy!
- ***The referee is absolutely right. Also referee 1 pointed at this. We initially wanted to show also the further standardisation towards total uptake per individual and per unit copepod carbon. At the end we decided to remove these figures as they showed the same trend as in Fig. 7a. Apparently we forgot to remove/adjust the legends, the authors apologize for that.***