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Interactive comment on "Sink or link? The bacterial role in benthic carbon cycling in the Arabian sea oxygen minimum zone" *by* L. Pozzato et al.

L. Pozzato et al.

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Dear Editor and Reviewers,

we have the pleasure to provide our answers to the comments made to the manuscript "Carbon processing at the deep-sea floor of the Arabian Sea Oxygen minimum Zone: a tracer approach" by Lara Pozzato, Dick van Oevelen, Leon Moodley, Karline Soetaert, and Jack J. Middelburg. We have modified the original manuscript according to the Reviewers' suggestions and many of those have been accepted and integrated directly. We thank the Reviewers for their thorough and useful comments, which helped improving the manuscript. All comments and suggestions have been taken in due

consideration and amendments were made in the text to comply with the Reviewers' requests. Details of our actions are given below: for an easier evaluation we report here the Reviewer's comments between quotation marks, followed by our response. In the attached revised version of the manuscript, added/amended text is in blue for better reading. Line numbers, for obvious reasons, have changed.

Hoping of having been sufficiently thorough in our reviews and to have met the Reviewers' expectations we will wait to hear from you.

Best regards

Lara Pozzato and co-authors

ANONYMOUS REVIEWER #2

We acknowledge this referee for her/his constructive feedback. Below we address the numbered comments point-by-point.

"1.First of all this study is in parts very close to the study by Pozzato et al. (2013) where similar data from the same sites were presented. I guess that the cores incubated in this study originate from the same multiple corer cast as those treated by Pozzato et al. 2013. This provides the opportunity to broaden this study a bit by at least comparing the data between the two different studies – it might even be considered to include some of the data of the previous study when their origin is clearly indicated. This has not been done although treatments were almost identical in both studies. Questions could address the variability of biomass and POM tracer uptake at the two different sites. In case there would be strong variability, how would that affect the conclusions made in this study?"

Indeed locations and time frame of the Pozzato et al. 2013 companion study and this one are identical. More specific, sediment characteristics and background/controls are the same as well. This is now clearly communicated to the reader. We also added citations to other studies done at these stations (organic geochemistry, Koho et al., 2013;

pore-water geochemistry, Kraal et al., 2012). In the revised version we also communicate the difference between this study (microbial loop; DOM and POM treatments) versus the companion study focussing on the effect of in situ and manipulated oxygen levels on POC processing. The depth-layers studied were partly different (upper 6 cm vs. 4 cm), complicating direct comparisons. Because of this difference in approach and research questions we limit comparison to the essential.

"2.During this study the del13C and presumably del15Nbackground values were determined from the different faunal groups but not shown in the results section. These measurements are very valuable and I wonder whether these data could be included in this study? Are there any trophic relationships to discern?" δ13C values were indeed measured and have been added to Table 2 in the manuscript. Trophic relationships are tricky to discern by means of δ13C only and, since the number of replicates is minimal (two per treatment) we will refrain from inferring trophic relationships among groups, to maintain focus on the lack of bacterial predation/grazing. Moreover, this has been presented in more detail in the companion study.

"3. During this study as well as in the study by Pozzato et al. (2013) there was a major difference in the 13C labelled POM and DOM tracer incorporation between the station inside and outside the OMZ. This was hardly discussed and might be considered in more detail. Do the authors have any idea, why is there such a pronounced difference?"

In Pozzato 2013 there wasn't any DOM treatment, only POM treatment and these showed indeed difference due to multiple factors. We have modified the text to discuss the difference between station overall benthic community activity difference (due to carbon availability/supply besides temperature and oxygen), but have kept focus on the main findings that none of the two stations showed a significant flow of carbon from DOM via bacteria to fauna, except for foraminifera at StOurOMZ.

"4.Another issue that I suggest to briefly discuss is the length of the incubations. Witte et al. (2003) covered three different time periods during their in situ incubations, where

it become apparent that the macrobenthos immediately (2.5 days) responded to the input of labelled POM. In contrast, the response of the bacteria, which need to extracellularly hydrolyse particulate organic matter was retarded. This brings me to the point whether the time period of the incubation used in this study (7 days) is sufficiently long to detect the different processes involved in the carbon transfer between the different groups. This is not to question the results presented in this study but I think this issue should at least critically considered."

This is a valid concern and we are aware that 7 days incubation time might be too short to transfer substantial amounts of label over multiple compartments. We have therefore modified the text accordingly (in a new section 4 entitled methodological issues).

"Generally, I think that the study presents interesting results to understand carbon cycling and transfer in marine sediments but nevertheless I have the feeling that the conclusions made are only valid for the experimental conditions chosen in this study hence I suggest to be careful with generalizations."

The text has been thoroughly screened for generalization and modified where needed. We have added a short section on methodological issues (4) which basically discusses all the limitation inherent to this type of studies. The only condition that was manipulated in this study was organic matter type. We offered both POM and DOM and both are to be considered as relatively "fresh" material. Such manipulations were made at in situ temperature and oxygenation, therefore we tried to keep all other variables as close to natural as possible.

"P 10400 L20: "These results, although very informative, left one question unanswered: is organic matter assimilated directly by meio- and macrofauna feeding on phytodetritus, or indirectly via ingestion of bacteria that in turn hydrolyzed particulate organic matter (POM) into dissolved or- ganic matter (DOM) and subsequently incorporatedit? This is not entirely true as Witte et al. 2003 at least indicated that labeled organic material fastly was incorporated into macrobenthic organisms while Bacteria (PLFA)

showed a time lack for incorporation of the labelled substrate."

We thank the referee for identifying this inaccuracy: clarification made as requested.

"Section 2.4 Sediment characteristics: This title is somehow misleading, one really has to read very carefully to understand that these sediments were also used for background determination of the del13C of the fauna. I recommend to already integrate these background measurements in the title. I assume that you also measured PLFA and total lipids in these samples. If so please indicate."

The section the reviewer mentions refers only and exclusively to sediment, as specified in the title. The two cores sampled and used to determine porosity, C:N ratio, carbon content and background sediment background 13C content were not used for any fauna measurement, for which instead other dedicated cores were sampled, as indicated in the material and methods. We believe the reviewer refers to the sentence "Grounded freeze-dried sediment samples were measured for organic carbon content, molar C:N ratio and background δ13C values with a Thermo Electron Flash EA 1112 analyzer (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS)" which, as specified, refers only to sediment, not to faunal background values. None of the samples taken from these two cores were analyzed for PLFAs nor Total lipids. PLFA analysis was done only in samples derived from the background and incubated cores dedicated to biota analysis and are described in section 2.7.1 and 2.7.2. To clarify further core allocation and use, the text in sections 2.3, 2.4, 2.5 has been changed.

"P 10413 Line 28: I am not sure whether it should be generalized beyond the presented study that POM uptake proceeds via ingestion of phytodetritus rather than ingestion of bacterial biomass."

Text modified specifying the investigated setting.

"P 10414 L2: the observation that "in the POM treatment, the 13C values of fauna are much higher than those of bacteria (Table 2), : : : further supporting that tracer

incorporation occurred via direct substrate ingestion" was also made by Witte et al. 2003, see comment above."

Already addressed above.

"P 10400 Line 4: please delete "aquatic" it somehow doubles pelagic." Deleted as requested

"P 10401 Line 17: conditions instead of condition." Changed as requested.

"P10401 Line 17: "She found that biomass and biodiversity of bacterial grazers may explain part of the differences in carbon preservation : : : " for a reader who does not know this particular paper it is difficult to follow of what is meant here. Suggest to better specify and clarify this sentence."

Text changed to improve understanding.

"P10401 Line 25: please specify which particular meiobenthic organisms are addressed in the studies of Nomaki and Guilini." Added as requested.

"P10402 Line 17: "Instead, community functioning and efficiency are more likely to be key factors in determining such phenomenon." It is not really clear what is meant with this sentence and should be a bit more elaborated." Text changed.

"P10403 Line 2: Whats about DOM uptake by nematodes?"

In the manuscript we clarified that "We investigated the specific role of bacteria in the sediments of the Arabian Sea OMZ to clarify if they are a sink or a link in the benthic food web.", therefore this manuscript focus is on bacterial-derived carbon fate in the sediment and different allocation of resources. Our experiments were not designed to specifically target nematode food preferences and, as already found by Moodley, Vanreusel, Pape, Van Oevelen and many others among which Pozzato, it is very difficult to properly label nematodes in pulse-chase experiments, regardless of the tracer or food source offered.

"P10403 Line 19: It would be great if oxygen concentrations could be provided to explain the distribution of the OMZ."

Many other papers have already dealt with this issue, some of which particularly aimed and mapping the exact extent of the Arabian Sea OMZ. It is out of scope of this manuscript to precisely determine the extent on the OMZ and the authors did not collect precise measurements to be able to report such information appropriately .

"P10406 Line 6: please delete “manual” after “: : : standard procedures”. Deleted as requested.

"P10408 Line 6: Linopherus sp is also a polychaete, why did you separate here?" Please see amended text.

"P10410 Line 15: Please indicate that you mean integrated biomass values over the upper 0-4 cm." Clarification made in section 3.2.

"Table 1 provides the same data as have been already provided in Pozzato et al. 2013 but does not refer to this study, it is also not referred to this previous study in the results section."

We decided to present also in this manuscript (as was done in Pozzato et al. 2013) some general background information on the stations, to provide the reader with a complete picture of the sediment characteristics and water temperature of the study sites. This was done to avoid the reader to have to search the paper by Pozzato et al 2013 in order to gather such information. Reference added in table caption.

"Please make clear to what extent data presented here were already presented in your previous study (Pozzato et al. 2013)."

The only data presented here which were already presented in Pozzato et al. 2013 are the ones in Table 1, for which caption was modified. As stated previously, biomasses and uptake data in Pozzato et al 2013 were core integrated on a 0-6 cm horizon, whereas here data are presented separately for each layer. The partitioning of infor-

mation between this and the companion paper is now clearer.

"Did the cores used for the incubations originate from the same multiple corer cast as used for the oxygen experiments described in Pozzato et al. (2013)?" They were from the same station, but different casts.

L. LEVIN

We acknowledge Prof. Levin for her constructive feedback. Below we address the numbered comments point-by-point.

"(1) The work might be more interesting if posed as a series of questions or hypotheses. Did you expect the POC and DOC might have different fates, or that bacteria might play different roles in determining these fates?"

The final part of the introduction has been rewritten to better communicate our research questions and approach.

"(2) The POM and DOM tracers were introduced at different locations in the sediment column (the surface and 4 cm down)."

DOM tracer was introduced throughout the upper 4 cm with multiple injections aimed and distributing the tracer all along the sediment depth, which means that it was present already at few mm depths and all the way down to 4 cm. Text has been modified accordingly.

"How could this have affected the results? Are the animals responsible for feeding on POC (and competing with bacteria) largely surface feeders? What would have happened if you introduced the POC at depth and the DOC on the surface?"

These issues are shortly discussed in the newly created “Methodological issues” section 4.

"(3)Methods – Please give the sampling dates - was the experiment done during monsoon or intermonsoon periods?" Information added to manuscript

"What was the incubation time? I could not find this information in the methods or on the figures although I may have missed it. Water was changed every 3.5 days so it must be longer than this. Is this the same experiment as Pozzato et al. 2013 (7 days?)."

Incubation time was 7 days, as reported in section 2.5 of the manuscript. Delineation with the companion study is now presented clearer.

"(4) Clarify what is different from Pozzato et al. 2013. The information in Fig. 2 and 3 of this ms looks like the same as that in parts of Fig. 2 and 3 off Pozzato et al. 2013 J. of Sea Research. Are these the same or different experiments? I recognize there are multiple treatments in the published paper – is this ms about one of those treatments? Is the information in Table 4 of Pozzato et al. different from that in Figure 4 of this ms?"

Please see also answers given to Reviewer #2. The revised manuscript better incorporates the companion paper's results and conclusions

"(5) Results text in Section 3.2 needs error terms in the text and some statistical text to provide comparisons among taxa and stations."

Error terms have been added to the text. We refrained from running any statistics on the analysed data because the limited amount of replicates per treatment (two) prevents any solid testing. Unfortunately, logistic and time constrains prevented the use of more replicates, despite having been planned.

"(6) Note that megafauna were not included in this experiment. And I believe the experiments occur over a very short time period (unclear about this). Some discussion is merited on the potential that animal ingestion of bacteria might involve deposit feeding megafauna (like holothurians and echiurans). Also allow that macrofaunal ingestion might occur more slowly than the time period of the experiment."

Both the neglect of megafauna and the short incubation period (perhaps too short for bacterial loop detection) are now discussed in the experimental issues section 4 added.

"(7) Conclusions focus on bacteria as a sink but don't say much about the role of the

OMZ: : : it appears in the title so that it seems like there should be mention of whether it plays a role in any aspect of the cycling studied. STOMZ and SToutOMZ are very different in DO, grain size and OM as well as T: : : do any of these factors control aspects of C processing studied here? If there is no influence of these factors that is an important result."

The text has been modified to better communicate that bacterial loop was not important or active (within 7-day period) at both stations, while we anticipated a more active one in the OMZ station with more active fauna.

"Line 28. What about the earliest studies? Blair et al. 1996; Levin et al. 1997 and Others" We have added these pioneering studies

"Line 79 : : : showed that limited faunal activity: : :" Corrected

"Line 84-86. Awkward – please rephrase" Done

""Line 186 The sediment cores were then frozen Corrected

"Line 305 new paragraph" Done

"Table 2 Polychaetes is spell incorrectly. Here and throughout the text Polychaetes and should be listed as Other Polychaetes since Linopherus sp. is a polychaete. Fig. 2 and 3 what is Eukaryia on the charts?"

The meaning of the term "Polychaetes" used in Table 2 and in Figures 2 and 3 has been clarified in each caption. The difference between Polychaetes and Linopherus sp. has been clarified in the text in section 2.7.2.

"Line 312 – Could symbiotic bacteria in the foraminifera be taking up the DOM?"

As stated in the manuscript, we cannot exclude direct foraminiferal DOM uptake of any sort, being it performed by the foraminifera per se or via symbiotic C sharing with endo- or esobacteria. It is an intriguing issue though, surely worth investigating. A short comment about this was added to the manuscript.

"Line 329 DOM tracers were not processed" Corrected

"Line 359-61. Please note there are deep-sea environment where bacteria are a primary food source. Vents and seeps are prime examples."

Thanks for the correction, we added a few lines indicating that chemosynthesis based ecosystems are different and cite Levin (2005) for this.

"Line 386 inducing should be induced." Corrected

"Line 431. It is interesting that in may natural abundance isotope studies foraminifera have d13C values similar to those of sediment Org. C (and slightly lighter than phytoplankton/suspended POC). Could this reflect their reliance on sedimentary bacteria?"

Indeed, this could be the case, but natural abundance isotope studies are non conclusive regarding this. Because of uncertainty in trophic transfer fractionation (0-1 permille), natural variability and experimental error, it will remain spectulative. This said, the similar signature between foraminifera and sediment Corg might indeed suggest a preference of foraminifera for sediment-related food items, rather than suspended POC or phytoplankton. Nomaki et al. (2006), in the experiments specifically designed to evaluate dietary preferences of foraminifera, established that no selective feeding on bacteria was observed, algae were selectively ingested and sediment was randomly ingested. Therefore it seems not to be prudent to infer that, because foraminifera and sediment Corg have similar δ13C, the reason for such similarity must be the protists reliance on sedimentary bacteria. Such signature in the sediment Corg might be due to other OM present between the sediment particles, which might be selectively fed upon by the foraminifera. Lee et al., 1966, Delaca et al., 1981, Delaca, 1982 reported foraminifera feeding directly on DOM so this could also be the source of the similarity in isotopic signature between the protists and the sedimentary OM. We believe that more investigation is needed to fully uncover reality of protistan trophic relationships.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/10/C4987/2013/bgd-10-C4987-2013-supplement.pdf

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