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Interactive comment on "Sediment release of dissolved organic matter to the oxygen minimum zone off Peru" *by* Alexandra N. Loginova et al.

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In the following, comments by Dr. Tomoko Komada are marked as "TK" and authors' responses are marked as "A".

TK: Benthic DOC and DON flux data are scarce, because they are difficult to obtain. The reactivity of the DOM that diffuse out of sediments is also not well constrained. This study is important in the sense that it contributes new data to both areas. However, as presented, I am not quite convinced that the conclusions drawn by the authors are fully supported by their findings. SPECIFIC COMMENTS: TK: Macrofauna are reported to be abundant in the study area. (In addition to what is discussed in the manuscript, Dale et al. (2015) mention occurrence of polychaetes at these stations, and Bohlen et al.

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Discussion paper

(2011) report a bioturbation depth of 2 cm in the 11deg.S stations.) Centrifuging sediments containing macrofauna has been shown to elevate DOC (Martin and McCorkle 1993, L&O, 38:1464-1479; Alperin et al. 1999, 63:427-448, GCA), and most probably DON. The authors should provide some evidence that assures the reader that their pore water DOM data are free of such artifacts. The authors report very low DOC/DON ratios in the sediment, and some spikes are apparent in DOC and DON in both depth profiles and in the chamber data. While microbial processes may be behind these features, it is also entirely plausible that they were due to occurrence of macrofauna (e.g., stirring up sediment during benthic chamber deployment; getting squashed in the centrifuge). This is a very important point to consider when comparing diffusive vs net (benthic chamber) DOM fluxes. A: This is a good point, and we thank Dr. Komada for mentioning that. We had no control over this question; however, we may refer to logistical reasons and previous studies in order to explain our choice of the centrifugation as a method for DOM extraction. First of all, we have chosen centrifugation over direct squeezing, as the latter method would imply numerous soft plastic parts, that were not possible to be pre-cleaned in advance. The centrifugation tubes (PP), in turn, were pre-cleaned with HCl, for each sample individual PP tube could be used. We will add the following to the methods section: “Studies conducted in areas with abundant macrofauna suggested that pore waters isolated by centrifugation exhibit higher DOC concentrations compared to for non-invasive methods, such as sip-isolation (Alperin et al. 1999). Macrofauna cell rupture during centrifugation was suggested to influence the extracted DOC, and the removal of macrofauna from sediments before centrifugation and whole-core squeezing was shown to reduce elevated DOC concentrations (Martin and McCorkle, 1993). In turn, our study site did not exhibit signatures of significant bioturbation (Dale et al., 2015). Herewith, at sites similar to our study area (low oxygen - low bioturbation), DOC concentrations extracted by centrifugation were in agreement either with those obtained by sip-isolation method (Komada et al., 2004) or with those obtained from in situ and ex situ incubations (Holcombe et al., 2001). Furthermore, Holcombe et al. (2001) suggested that sip-isolated pore-water DOC gradients may lead

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to underestimation of diffusive DOC fluxes in low bioturbation regions. Thus, varying strength of organic matter–mineral associations may create different solute reservoirs around the surface of a mineral. Sip-isolation method was suggested to extract only loosely bound DOM out of the marine sediments, while centrifugation would sufficiently perturb sediments and sample the majority of the pore-water DOM that may efflux out of the sediment. In connection with the above, the centrifugation method was preferred as pore water extraction method for DOM analyses."

TK: Syringe filters can give large DOC background (and possibly DON also), but there is no mention about how the filters were cleaned. Please provide additional information showing that the data do not contain high (and variable) levels of blank. A: We thank the Dr. Komada for noticing that. Indeed, we did the mistake not to add the details behind choosing a filter type or their cleaning. Prior to the research cruise, we did several checks for different filters of that pore size, which are commonly used during pore water work, including PES, nylon, CA and RC. All the filters gave one or another background level, therefore, we tested which volume of ultrapure water was the optimal and reasonable for cleaning. CA and RC filters gave the minimal values for DOC and for DON after rinsing with 60 ml of ultrapure water among all filters. CA was chosen over RC due to lower binding affinity to macromolecules and proteins, as we did not want to influence recovery of organic components during filtration. Following will be added to the page 4 lines 25-30 to the revised version of the manuscript: "All samples were passed through pre-washed (60 mL of ultrapure water) cellulose acetate (CA) membrane syringe filters (0.2 μm). The preference for the CA filters was given as a result of a home-based test that occurred before the research cruise. Then, several types of filters (PES, nylon, CA and regenerated cellulose (RC)) were examined for background DOC and total dissolved nitrogen (TDN) signal. CA and RC filters gave the minimal background signal for both parameters after rinsing with 60 ml of ultrapure water (Fig. S4). CA was chosen over RC due to lower binding affinity to macromolecules and proteins"

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Figure S4 will be also added to the Supplement.

TK: The authors state that microbial N turnover and DOM fluxes are likely related (page 9, line 11). I wholeheartedly agree with this statement, and find that this is an area that is ripe for further study. The authors go on to discuss N dynamics quite a bit, but the problem with this is that, other than DON, none of the inorganic N data are included in this manuscript. This renders most of the nitrogen-related discussion speculative at best. The authors should either scale back on the N discussion, include the DIN data, or perhaps plan on publishing a companion paper that includes relevant DIN data. At the very least, chamber data should include nitrate, assuming that was the major electron acceptor. The DIN data are also relevant to the extremely low DOC/DON ratios in sediments. The authors originally declare that nitrate/nitrite concentrations in sediments were negligible (bottom of page 5), then resurrect this issue as a possible explanation for the low DOC/DON ratio (bottom of page 9), only to dismiss it again (top of page 10). The authors provide a few other possible explanations for the low DOC/DON ratios, but this discussion would be a lot more convincing in the presence of a more complete DIN data showing that the DON values were not overestimated. A: Unfortunately, we were restricted by the data legacy and could not report on DIN from benthic chambers and pore waters, but could only use the data for our calculations of DON. The data on DIN from the benthic chambers will be published soon in a different manuscript by MSc David Clements and co-authors. However, MSc Clements has agreed to provide us the data for DIN for publishing from one of the stations. Therefore, data for DIN components from one benthic chamber at station 3 will be added to a Supplement as a Figure S6. Due to reviewers' suggestions, measurements of ammonia may now be published for all six stations and will be added to a Supplement as a depth profile plot (Figure S5).

TK: There seems to be an underlying assumption that sediment DOM is all refractory (e.g., page 1 line 5; page 2, line 21; page 11, line 15). As far as I am aware, this is not supported by the current literature. If anything, the opposite is more likely; a con-

siderable fraction of DOM, especially near the sediment-water interface, is labile, and only a small fraction appears to be refractory (e.g., Bauer et al. 1995, Nature 373:686-689; Burdige et al. 2016, GCA 195:100-119; Komada et al. 2013, GCA 110:253-273). Therefore, assuming that the DOC and DON data presented here are indeed free of artifacts, it makes sense that the flux data point to microbial consumption of DOM that diffused out of the sediments. The authors should adjust the wording to better reflect the literature data. A:We thank Dr. Komada for her suggestion. Following will be added to the revised version of the manuscript on Page 2, line 33: "It was suggested previously that DOM in sediments consist of recalcitrant low molecular weight (LMW) compounds (Burdige and Gardner, 1998; Burdige and Komada, 2015), therefore, the sediment outflux of DOM was hypothesized to serve an important source of recalcitrant DOM to the water column (e.g. Burdige and Komada, 2015; Burdige et al., 2016). Herewith, elevated concentrations of dissolved organic nitrogen (DON) within sediments suggest the presence of labile proteinaceous organic matter in pore waters, that have escaped degradation within the water column (e.g. Faganeli and Herndl, 1991). Furthermore, measurements and modelling of isotopic carbon composition in the anoxic and suboxic sediments off California, suggest that about 50 % of DOM within upper sediments represents isotopically young and labile DOM components, that are readily released to the water 5 column, where they are actively utilized by heterotrophs (Bauer et al., 1995; Komada et al., 2013; Burdige et al., 2016)."

Page 12, Line 8: "On the other hand, isotopic carbon composition suggests that a substantial fraction of pore-water DOM is isotopically young and is readily utilized by heterotrophic communities, when released to the water column (Bauer et al., 1995; Komada et al., 2013; Burdige et al., 2016)." Page 13 Line 3: "Thus, the production of humic-like LMWDOM along with the utilization of proteinaceous DOM suggest active microbial DOM utilization occurring in the near bottom waters (e.g. Alkhatib et al., 2013). Therefore, our results from the benthic chambers support the idea that DOM release to the water column may stimulate its utilization by water–column microbial communities (Komada et al., 2016; Burdige et al., 2016)." TECHNICAL COMMENTS:

TK: I had difficulty reading Fig. 3, because the panels are so small. I am also unable to tell the difference between dark grey and blue (DOC vs DON). A: DOC and DON will be separated in different panels in the revised version of the manuscript, we also will increase the size of the plot.

TK: Black and grey arrows in Fig. 9 also look identical in color. A: We will change the description under the plot to: "Conceptual view of DOM cycling near the sediment off Peru. Arrows directed out of the sediment represent diffusive fluxes of DOC (JDOC(Diff)) in mmolm-2d-1. Circular arrows indicate microbial DOM reworking, calculated as a difference of DOC (JDOC(Diff)) and net in situ flux DOC (JDOC(Net)) at each station."

TK: Written English is OK, but not in publishable shape (parts that would benefit from editing are too numerous to list here). A: We will address those issues with care.

TK: The narrative meanders in some places (e.g., discussion about DIN as I pointed out above). A: To avoid meandering we will omit following from the chapter 4.1: "For instance, NO3 that is present at high concentrations in intracellular vacuoles of Marthioploca (Dale et al., 2016) could be leaked to the pore water during sediment handling and centrifugation. An ammonium oxidizing bacteria were shown previously to be able profiting from nitrous oxide, produced by denitrification (e.g. Kartal et al., 2013). Thus, the production of NH+4, as a result of DNRA occurring at the inner shelf stations in combination to nitrous oxide production via denitrification occurring at outer shelf, may produce a convenient niche for anammox bacteria at the rim of the inner shelf at 12oS. The intermediate product of anammox, hydrazine (e.g. Kartal et al., 2013), may, in turn, accumulate in the inner space of anammox bacteria, and be released in the pore water samples as a consequence of the cell rapture induced by centrifugation. However, the concentrations of those intermediate products are likely very small and may not explain elevated TDN values."

TK: I also recommend streamlining the Introduction; I found the transition to DOC (line

16) a bit jarring. A: We will address this suggestion, e.g. following will be added to Page 2 line 16: “While POM degradation in sediments is mostly associated with its full remineralization to dissolved inorganic carbon (DIC) and inorganic nutrients, the mechanism of POM remineralisation implies important intermediate stages of dissolved organic matter (DOM) production, reworking and mineralization processes (Smith et al., 1992; Komada et al., 2013). Thus, around 10% of remineralized particulate organic carbon (POC) may accumulate as dissolved organic carbon (DOC) in the pore waters (Alperin et al., 1999). In turn, DOM efflux may represent an important escape mechanism for carbon from sediments (e.g. Ludwig et al., 1996; Burdige et al., 1999) and a source of organic matter to the water column (e.g. Burdige et al., 2016).Despite the acknowledged importance of sediment DOM for organic matter cycling, the measurements of benthic DOM fluxes remain scarce and the reactivity of the pore-water DOM is not well constrained.”

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