

*Reviewer2:*

This manuscript by Koho et al presents descriptive as well as experimental data from field work in the Arabian Sea investigating the drivers and constraints of OM cycling under oxygen limitation. Results confirm previous findings on the quality and quantity of organic matter preserved in OMZs, but describe an interesting contrast between measurements of OM degradability and other frequently used methods to describe OM quality. But while the data presented are interesting and deserve publication, I feel the manuscript would benefit from significant revision before publication.

*Authors' response:*

The authors thank the reviewer for the thorough review of the manuscript and constructive criticism.

General comments:

*Reviewer2:*

1. At the end of the discussion the authors state that the purpose of the paper is to describe OM quantity and quality along a bottom water oxygen gradient, and experimentally test bioavailability and potential microbial remineralisation. While work was clearly carried out in a context of investigating the drivers and constraints of OM degradation in marine sediments, I missed a clear statement of the wider relevance of this work, or any specific hypotheses to be tested. Which specific research question was addressed?

*Authors' response:*

We assume that reviewer means "at the end of the introduction" and not "at the end of the discussion", as the statement outlined by the reviewer appears in the introduction. The final paragraph of introduction will be modified to:

"This study was motivated by applying two independent techniques (microbial mineralisation and biochemical quality) to investigate the drivers and constraints of OM degradation in marine sediments. Despite existing studies on biochemical quality of OM in Arabian Sea sediments (e.g. Cowie and Levin 2009, Vandewiele et al. 2009, Woulds and Cowie, 2009) or experimental approach investigating microbial degradation (Moodley et al. 2011), a combined study is lacking. Here we report biochemical quality of OM, including amino acid and pigment analyses and the potential (oxic) microbial remineralisation rates of OM along a bottom water oxygen gradient. In addition, biological mixing, i.e. metazoan activity was assessed, using downcore phaeopigment and <sup>210</sup>Pb profiles. OM burial was estimated using <sup>14</sup>C-dating."

*Reviewer2:*

2. The methods section needs some clarification and missing information needs to be added. For example, why was bioavailability tested under identical oxic conditions? Would it not have been more interesting to run comparative incubations under both standardised and in situ O<sub>2</sub> conditions? Why were sediments stored under (I believe) anoxic conditions at 4°C prior to oxic incubation? To kill off fauna? What sediment volume was incubated, and at what oxygen concentration? As slurries in plastic bags, or bottles? At some point it appears the incubations were run exactly as in Moodley et al 2011, but even if so, the most important parameters need to be briefly repeated here as not every reader will know the Moodley paper by heart.

*Authors' response:*

The experiments were conducted in order to examine the *potential net* remineralisation rates of organic matter. This is clearly stated throughout the manuscript. Nowhere do

we suggest that the rates are prevailing in situ. We decided that this approach is the most suitable for indicating the potential net rates of organic matter as oxic conditions have been shown to produce higher, or similar, remineralisation rates than anoxic ones (e.g. Hulthe et al. 1998, Moodley et al. 2011), depending on the origin of organic matter. The sediment was stored in airtight, sealed plastic bags at 4 °C. This was chosen over freezing of the sediment, which could have been detrimental to bacterial community. The storage in sealed plastic bags most likely also resulted in anoxic conditions, although this was not monitored.

The incubations were carried out in 80ml bottles, and not in bags. 10 ml of sediment was inserted into the bottles. Bottles that were then filled with well-aerated 0.2 µm filtered seawater (low nutrient deep Atlantic water). Total water volume, as well as accurate conversion of wet and dry weight sediment, was obtained by direct weighing. Throughout the experiment the bottles were periodically shaken to mix the slurry. At the end of the incubations the oxygen content was measured with an oxygen optode (Presens, Germany). The oxygen content in each bottle at the end of the incubation was always >20 µM.

More details on the experimental set up will be added to the final manuscript.

*Reviewer2:*

Why were the upper 3 sediment cm combined, although pigment and 210Pb profiles clearly confirm the steep gradients to be expected within this sediment layer? And why were only two replicates incubated, which were then combined for analyses? The lack of replicates compromises the results so this should be carefully justified.

*Authors' response:*

The incubations were designed to examine the microbial bioavailability of the marine surface sediments. Surface sediments typically contain higher quantities of organic matter and also of higher biochemical quality. When the sediments were sliced on board we did not know how the pigment profiles would look like. Therefore, an arbitrary top 3 cm sample was taken for the slurry incubations. The top 3 cm slicing is also consistent with previous studies of Moodley et al. (2011) and thus allows a direct data comparison between the studies.

Deep-sea research is challenging and it is not always possible to gain enough study material containing multiple replicates. Here we were able to get material for two replicates. The replicates were analysed separately where possible (i.e. net remineralisation rates and associated parameters) but the sediments from the incubation were pooled at the end of the experiment for bacterial biomass measurements due to expected low numbers of bacterial polar lipid-derived fatty acids (PLFAs). This is also stated in the methods section 2.1.

*Reviewer2:*

3. The discussion seems to lack a clear focus and conclusion, and the paragraph on the potential role of macrofauna for OM degradation is somewhat misleading. For example, diversity is not necessarily directly correlated to sediment mixing, burial or feeding rates, and the fact that, as the authors state, 'the role of fauna in OM processing is thus limited to a few taxa' is by no means synonymous with it being negligible. This is actually highlighted by the 210Pb and pigment profiles which indicate deep bioturbation at several stations. In addition, the discussion should take into account recent literature on macrofauna – bacteria interactions in OMZ sediments (Hunter et al 2012, ISME Journal).

*Authors' response:*

In the resubmission we will restructure this part of the manuscript, giving it more detail and focus. We will also discuss the recent paper by Hunter et al 2012, which is indeed highly relevant.

The reviewer is right that the diversity may not be directly correlated to enhanced sediment mixing, burial or feeding rates, however, it is very likely that if more diverse groups of fauna are present the bioturbation patterns become more complex. For example, the dominant polychaete (*Linopherus* sp) in the low O<sub>2</sub> OMZ sites is known to burrow relatively shallow and only vertically, thus mixing is more limited as burrows are relatively simple (Gooday et al. 2009 DSR II, vol 56p. 488-502; Levin et al. 2009 DSR II, vol 56, p. 449–471). In addition, if organic matter is processed by more diverse groups of organisms the actual break down of the organic matter in the animals' guts may vary from species to species. Thus with more diverse community more organic matter substrates will become available to the microbial community. We will include these issues in the revised manuscript.

*Reviewer2:*

While the present manuscript clearly confirms previous data regarding the quantity and quality of OM preserved in OMZ sediments, the interesting discrepancy found between quality indices and microbial degradability remains almost unexplored and a paragraph or two should be dedicated to discuss this and develop ideas of potential underlying causes.

*Authors' response:*

In the current version we have tried to do this in the end of the section "3.3 OM bioavailability versus biochemical OM quality". As this was not clear for the reviewer we will rephrase and put more emphasis on our conclusion. Also see a related response above, concerning the same matter.

Specific comments:

*Reviewer2:*

Method descriptions/ references need to be checked and completed, some are missing (e.g. bioturbation, statistics etc). Reference list needs to be checked, some publications cited in the text do not appear in reference list.

*Authors' response:*

This will be done for the resubmission.

*Reviewer2:*

Fig 1 inset should be enlarged or area of inset reduced to make it easier to identify the location of the Murray Ridge within the northern Arabian Sea.

*Authors' response:*

This will be done for the resubmission.

*Reviewer2:*

Fig. 4 will likely be very difficult to read, unless set on a whole page in the final layout

*Authors' response:*

A high-resolution image was submitted to the editorial office. Image submitted had a size of A4.