

Response To Reviews – egusphere 2025-3676

This response to reviews has been updated following revisions to the manuscript.

Reviewer Comment 1

We would like to thank the reviewer (William Hunter) for his thorough and rigorous review of our manuscript, and for the opportunity to respond to the points that he raises. We are confident that the additional clarification and discussion requested will be of benefit to the manuscript.

The reviewer questions the level of replication applied to each of our treatments (each treatment was run in triplicate).

Response: We acknowledge that greater replication will always strengthen an experiment, however those benefits need to be balanced against practical constraints. The chambers used were sizeable (19.4 and 14 cm i.d.), and running a higher number of replicates was not possible for logistical reasons (laboratory space, volume and weight of sediment required, and operator time for carrying out respiration measurements). The triplicate replication used is in line with similar experiments in the literature (e.g. Moodley et al., 2000; Sweetman and Witte, 2008; van Nugteren et al., 2009; Rossi et al., 2009), and shows differences between treatments, even with the level of natural variability present. Further, we have made efforts to ensure that the collection of taxa used in our experiments is representative of the sediment sites on which the experiments are based (see method section). We do not believe we have interpreted data beyond the limitation of the replication that we applied. We agree that the addition of a brief discussion on this topic of replication to the method section is worthwhile as the reviewer suggests.

The reviewer questions the value of using inferential statistical analysis on a data set with the level of replication that we have. Specifically there is the concern that using non-parametric tests may not help with the fact that data distribution can't be assessed in small groups of data. It is also suggested that non-parametric tests may inflate the possibility of a type 2 error (not detecting a pattern that is present).

Response: We acknowledge this point, but would like to stress that for the majority of statistical tests reported, the groups of data compared comprised data across all days for a particular treatment. For comparisons of respiration rates n ranged from 10 to 24. For comparisons of faunal labelling levels by taxon n ranged from 5 to 17. Only a sub-set of tests were comparisons of treatments on a single day, for which $n = 3$, which is the situation about which the reviewer has a concern. We thank the reviewer for the suggestion that either the statistical testing should be removed, or one-way ANOVA used instead of non-parametric tests. For the cases where $n=3$ and a non-parametric test had been used we tried applying a one-way ANOVA instead and found that there was still no statistically significant difference. We note that the questions of which statistical tests are most appropriate, and whether statistical testing should be included at all, are ones on which different statisticians and readers are unlikely to agree, and changing our approach could raise other objections instead. We feel that the approach we have taken is justifiable. We will add an acknowledgement of this point in our method section.

We would like to thank the reviewer for the suggestion of plotting our bar graphs with data points also present. This is a helpful suggestion, and we will work on altering the plots in this way.

The reviewer suggests that de-faunation by sieving would have been a better approach in achieving natural sediment free from macrofauna.

Response: De-faunation by sieving was considered, but we opted for faunal removal by inducing anoxia. We decided against sieving on the basis that it would have caused very extensive disruption to sediment structure and composition, which would have been more problematic than the concern raised by the reviewer. We decided that less of an artifact would be created by the de-faunation through anoxia, and reporting macrofaunal biomass extracted at the end of the experiment as we have done. We will add a section on the rationale behind our defaunation approach to clarify this. We are transparent about the fact that some metazoan meiofauna will still have been present in the de-faunated treatment, and that there could also have been a small amount of live macrofaunal biomass remaining in the de-faunated treatment.

The other minor comments were addressed with appropriate revisions.

Reviewer Comment 2

We thank the reviewer for their thorough review and constructive comments. We feel confident that revisions guided by the comments will definably improve the manuscript.

The reviewer suggests that we could improve the clarity of the introduction in places, in particular to provide a more accessible explanation of the principles on which the study is founded.

Response: We thank the reviewer for this suggestion, and will include such changes in our revision. We will add an explanation of terms such as 'bioirrigation', and provide an introduction to 'the microbial loop' before the 'inverted microbial loop' is introduced (this will also satisfy some of the specific minor comments). Such changes will broaden the context for the study and highlight its wider relevance.

The reviewer suggests to pay attention to the structure of the discussion, and to make sure that summaries are provided in the right places.

Response: We agree with this suggestion and will improve the text in the discussion section accordingly. We will also, as suggested, increase the extent to which some findings (e.g. sections 4.4 and 4.5) are discussed in relation to the literature.

Finally, we thank the reviewer for their insightful comment on the general lack of inclusion of benthic processes in earth system models, and the potential future impact of this study, especially as the field starts to consider how marine carbon storage and burial can be protected and enhanced. This suggestion will be incorporated in our revisions of discussion/conclusion text.

The minor comments will also be addressed with appropriate revisions.

References

Moodley, L., Boschker, H., Middelburg, J., Pel, R., Herman, P., de Deckere, E., and Heip, C.: Ecological significance of benthic foraminifera:: ¹³C labelling experiments, MARINE ECOLOGY PROGRESS SERIES, 202, 289-295, 10.3354/meps202289, 2000.

Rossi, F., Vos, M., and Middelburg, J.: Species identity, diversity and microbial carbon flow in reassembling macrobenthic communities, *OIKOS*, 118, 503-512, 10.1111/j.1600-0706.2009.17112.x, 2009.

Sweetman, A. and Witte, U.: Macrofaunal response to phytodetritus in a bathyal Norwegian fjord, *DEEP-SEA RESEARCH PART I-OCEANOGRAPHIC RESEARCH PAPERS*, 55, 1503-1514, 10.1016/j.dsr.2008.06.004, 2008.

van Nugteren, P., Herman, P., Moodley, L., Middelburg, J., Vos, M., and Heip, C.: Spatial distribution of detrital resources determines the outcome of competition between bacteria and a facultative detritivorous worm, *LIMNOLOGY AND OCEANOGRAPHY*, 54, 1413-1419, 10.4319/lo.2009.54.5.1413, 2009.

Further Response Notes Following Revision

We have re-structured the discussion to reduce switching between themes and thus improve flow. We have also enhanced links with the literature where this was lacking, included summary statements where required, and added a consideration of wider implications and links to current policy relevant questions. It should be noted that track changes was switched off while changes to the order of sections were made, so that alterations within each section would still be clearly marked up.

The data will be available through the University of Leeds data repository. The process of depositing the data has been initiated, and a DOI will be made available in time for a final version of the manuscript.

Responses to Minor Comments

Our response to each comment is provided in italics.

Reviewer 1

All suggested editorial changes have been made. Further notes on changes are as follows:

- Page 23, Line 348-350: 'In summary, for the majority of our timepoints, the observed O₂ consumption rates, which reflect degradation of total sedimentary organic matter, supported the occurrence of the inverted microbial loop.' I think at this point a recap of what is mean by the 'inverted microbial loop' would help. I realize this is outlined in the introduction, but a single sentence would suffice. *Response: The re-structuring of the discussion in response to reviewer 2 has included a re-wording of this section which also provides the clarity / reminder that this comment requests.*
- Page 25. Line 368-369: "The 13C that could not be accounted for presumably remained in the sediment, although data are not available to confirm this. " This statement is partially true. Another major pool of ¹³C that is not measured is the Dissolved Organic Carbon pool. Whilst the authors are not seeking a mass balance, it is worth referencing the potential shunt of organic matter into the DOC pool, which may be important in terms of the biogeochemistry of the system. *Response: This section of text has been*

moved to the results section, and an acknowledgement of the DOC pool is now included.

- Page 30 Lines 456 - 464: The conclusions need to clearly state that the results of this experiment are focused on an estuarine system. I don't believe this diminishes the findings, but highlights the wider knowledge gap around microbial-faunal interactions in marine sediments. *Response: The revised conclusions now include this point.*

Reviewer 2

- Line 20: An odd sentence to end an abstract. Either include a concluding sentence, a summary of your results, or the implications of your study. *Response: Edited to indicate wider implications.*
- Line 45: "In contrast to our knowledge of the external factors that govern total community respiration, we lack an understanding of the internal mechanisms that determine how respiration is partitioned amongst the different groups of organisms that make up the benthic community, and especially, how interactions between those groups can influence the total community respiration. The microbial component is often assumed to be of paramount importance in driving total community respiration, and evidence for this comes from both observational (e.g., Schwinghamer et al., 1986; Hubas et al., 2006) and modelling (Van Oevelen et al. 2006) studies." This section provides a strong introduction to the study. I recommend introducing a brief version of it earlier and then expanding on it here. *Response: A mention of this content has been made earlier. Preceding paragraphs introduce the importance of marine carbon storage, and provide a basic introduction to overall benthic respiration and the factors which control it. This material should help to answer the major comments by the reviewer. We trust that revisions to the early part of the introduction now provide a better level of introduction to the importance and functioning of benthic community respiration.*
- Line 65: Terms like bioirrigation are not common vocabulary; I would introduce these. Also, consider contrasting the standard microbial loop with the inverted microbial loop. I would also define labile and refractory organic carbon. *Response: Terms are now explained where they are first used.*
- Line 90: "more refractory and 'diffusively' distributed sedimentary organic C, and less to the fresh organic C that is concentrated on the sediment surface." I would expand on this; it is unclear what is meant by "diffused" here. *Response: We have added the explanation that 'diffusely distributed' means that the organic matter is mixed through the sediment rather than being concentrated in a layer on the surface.*
- Figure 1: Remind the reader what the ^{13}C -AA experiment is, and what the ^{13}C -MPB experiment is, in the figure caption. *Response: Caption edited.*
- Line 360: I would include a variation of this text in the conclusions, to better place the findings of your study in context. *Response: The conclusions have been edited, and there is now an explicit mention of priming as suggested.*
- Sections 4.4 and 4.5 read like results rather than discussion. *Response: These sections have been extensively re-structured, including moving some text to the results section.*
- Conclusions section: Now that we know your results, so what? What have we learned, and what future relevant questions arise in the context of your findings? *Response: Conclusions section significantly re-written to include these wider points.*