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## ***Interactive comment on* “The effects of five different defaunation methods on biogeochemical properties of intertidal sediment” by T. J. Tolhurst et al.**

**T. J. Tolhurst et al.**

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We would like to thank the reviewers for their comments, they raise some interesting points.

Reviewer 1

The reviewer states that there was ‘rapid colonization of diatoms.’ Actually these habitats are usually dominated by green filamentous algae, diatoms are present, but rarely in sufficient numbers for a biofilm to occur. At no point in the study did we see a diatom biofilm.

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The reviewer states that ‘none of the methods used for conducting larger scale experiments, such as those that use different plastics for defaunation are tested.’ We did not test the use of plastic, because one of our aims was to remove fauna without removing MPB. This was, for example, to enable manipulative experiments investigating biotic effects on the erosion of sediments. Plastics will kill MPB as well as fauna, so were inappropriate in this case.

The reviewer states that ‘The authors argue that recovery experiments are confounded in their methods, as they do not explicitly test recovery to undisturbed sediments.’ (emphasis added). This is not true, we are arguing that if the effects of the defaunation process on the sediment are unknown, then responses could be due to effects on sediments, or the fauna, or a combination of both (see fig 3), which means interpretations in terms of fauna could be confounded by changes in the sediment. (see for example the discussion, which states that many previous studies ‘assume that responses of fauna colonizing these sediments are not confounded by responses to any abnormal conditions created in the sediment by the defaunation process.’) As the reviewer states, ‘they do show that these confounding effects are surprisingly minor and transient!’ This is an important finding of our research, as it means we can be more confident in the use of some of the methods we tested.

The reviewer states that ‘Most ecologists working on disturbance and recovery recognize the potential problem.’ This may be true, but it does not mean that the effects on sediments do not need to be investigated and, as stated in the introduction; our aim was not to just test these methods for ecologists, but for other disciplines, such as sedimentologists investigating erosion/deposition processes.

The reviewer states that ‘The relevance of this work to those conducting larger scale experiments is unclear.’ We make no claims about any relevance to larger scales, our results may or may not be relevant to larger scales, and further experiments are required to determine this.

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The reviewer states that ‘Perhaps most importantly though, I do not think there is any kind of disturbance (natural or anthropogenic, experimental or not) that would selectively defaunate the sediments without also affecting sediment structure and microphytobenthic community structure in some way.’ And yet workers often do not quantify these changes and continue to assign responses to fauna that are actually due to some combination of fauna and/or microphytobenthos and/or sediments. Thus, this paper improves our understanding, by quantifying effects on microphytobenthos and sediments. Also, many experiments requiring defaunation are investigating effects on functioning (e.g. nutrient flux) that require methodologies for removing fauna, without directly affecting either sediments or MPB. The same is true for experiments investigating erosion/deposition processes. The methods we investigated were (potentially) appropriate for these types of experiments, which is why they were chosen.

The reviewer goes on to discuss recovery after disturbance. Much of this is true, but misses the point that many studies continue to assign changes solely to fauna and that we were not only interested in recovery after disturbance. These defaunation methods have applications across a wide range of experiments and are not limited to ecological recovery after defaunation. Finding methods that minimise the effects of defaunation on sediments and MPB, for experiments including (but not limited to) those outlined above, is an important goal.

The reviewer states that ‘The authors cite Beukema et al’s. (1999) observation on transient overshoots in abundance early in the colonization processes and highlight that their conclusions that relate to biotic interactions and resource supply may be construed because they did not measure sediment characteristics; in this case it is a mute point as their first sampling was done after half a year and the timescales considered are contrasting.’ We do not agree that it is a moot point. As stated in our discussion, this paper ‘did not address whether the methods of defaunation changed these resources directly, rather than by the removal of animals.’ Historical alterations to the initial ecological structure and/or sediment properties can introduce changes that can

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persist for a long time. The fact remains that without knowing what effect the treatment has had on the sediments and MPB (as well as the fauna), then interpretations could be confounded, by assigning causes directly to fauna that could be due (directly or indirectly) to sediments and/or MPB.

The reviewer states 'see the Dutch papers by Montserrat, Van Colen, Rossi et al.' These recent defaunation papers that have addressed sediment characteristics more directly are to be applauded, but they do not negate the relevance of our study.

The reviewer states that 'Most more recent work has also used different types of plastic sheeting to induce hypoxia to the seafloor – it is a shame that their potential artefacts was not tested in this paper.' As stated above, the use of plastic is inappropriate for experiments that require effects upon MPB to be minimised.

The reviewer states that 'I think the paper either needs to (a) be cut down to either report the mere experiments (which were robust in their design), or (b) be expanded considerably to more generally discuss the importance of the methods used in relation to the questions asked.' We do not think this is necessary, but if required by the editor, we could do either of these.

The reviewer states that 'But why not measure pheophytin to get a quantitative measure of chl degradation products?' This would have been useful, but we were more interested in the effects on the living MPB. We leave it to other workers to investigate degradation products. The reviewer touches on an important issue, there are many properties that could affect the results of an experiment and by not measuring them the interpretation of the results could be confounded. It is essential, therefore, that the effects of defaunation on these properties are quantified, this paper does this for some of these properties, there remain many more that need to be investigated.

Reviewer 2

The reviewer states that 'in soft sediments using artificial methods to eliminate target

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components such as certain infauna is methodologically very difficult because almost all the fauna is distributed over a 3D matrix and it cannot be removed without invasive methods such as those considered in the present paper. ' Some of the methods we investigated (such as the liquid nitrogen) are not at all invasive. This was one aim of the paper, to see if there were less invasive/disruptive methods that had a similar defaunation effect as more invasive methods.

The reviewer states that 'These methods however are largely artificial and very unlikely reproduce situations close to the effects of a "real" disturbance.' As for reviewer 1, investigating disturbance is only one application of these methods. The different methods we used have differing levels of applicability to "real" disturbances. Specifically testing how similar these methods are to "real" disturbances is outside the scope of this paper. It seems likely, however, that the liquid nitrogen and H<sub>2</sub>O<sub>2</sub> treatments (which appeared to have a primarily surface effect) would be applicable to certain pollution disturbances.

The reviewer states that 'Trying to disentangle the effects of sediment on infauna from those of infauna on sediment should be relative to the main questions addressed and take into account what happens in nature, especially when doing field studies.' Yes, this is one of the reasons for doing the study. Having defaunation methods that remove all, or specific groups of fauna from sediments, without altering the properties of sediment and/or MPB, is necessary for doing manipulative experiments to disentangle sediment/fauna/MPB interactions. The first step is to identify those methods which have the desired defaunation effect, whilst minimising effects on sediments and MPB, which is what this study does.

The reviewer states that 'very unlikely a disturbance will kill all animals and leave intact sediment properties.' There are disturbances that would do this, such as anthropogenic release of toxic pollutants. As stated earlier, disturbance was only one of the processes that these methods could be used to investigate.

The reviewer states that 'During the recovery both sediment properties and fauna will

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reciprocally affect each others in a dynamic way and their reciprocal effects may continuously change.’ Yes, this is precisely why it is vital to know how the defaunation method affects the sediments (and MPB). Highlighting the fact that effects on sediment are important, but often ignored, was an aim of the paper.

The reviewer states that ‘Based on the data presented, this paper should be restricted to the first approach and, assess methods of defaunation that can be used to eliminate fauna from the sediment and test the effect of this fauna on certain other components.’ We disagree with this, the data we present is useful for experiments investigating disturbance, if only to steer researchers away from inappropriate methods.

The reviewer states that ‘the paper’s scope appears restricted in its scientific significance and its scope seems to be in line with a journal that specifically consider methodological approaches in marine systems.’ We disagree, the results are relevant to researchers across a range of disciplines and as such is relevant to a multidisciplinary journal such as Biogeosciences.

This reviewer also suggests we should have looked at the use of plastic layers to induce anoxia. We did not investigate the use of plastic layers for the reasons outlined in our reply to reviewer 1.

The reviewer states that ‘The reason of the choice of the defaunation methods to be tested are not detailed.’ As outlined in the introduction, freezing and oven treatment were chosen because these have been used before. The in situ treatments were chosen because they involve fewer disturbances to the sediment. Formalin was expected to be a press disturbance, whilst liquid Nitrogen and H<sub>2</sub>O<sub>2</sub> were expected to be pulse. We omitted to state that formalin has been used in previous studies (although it is listed in table 1) and that liquid Nitrogen was expected to have a similar defaunation effect to freezing in the lab.

The reviewer states that ‘I do not understand why the availability of chlorophyll is a sediment properties while this is a measure of the biomass of living microalgae. Why

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not using degradation products?’ It is one of the properties that makes up the bio component of ‘biogeochemical properties.’ We use the term ‘sediment property’ loosely to refer to any property measured in the sediment. We did not look at degradation products, because one of the aims was to find a defaunation method that minimised the effect on the MPB, therefore it was necessary to know about the living MPB biomass.

The reviewer states that ‘The Organic Matter composition should be investigated in more detail, to especially assess its quality (using C/N, for instance), since organic matter nitrogen availability may be a limiting factor for fauna.’ We did not do this for the same reasons we did not look at pigment breakdown products (see reply to reviewer 1). We selected those properties of most interest to us for later experiments. There are also many other possible properties that should be measured, but it was not possible for us to measure all of these (given the available time and funding). We leave the investigation of these to future researchers.

The reviewer states that ‘the efficacy of defaunation was tested against the (macro) fauna response. Then also macrofauna was a response variable. Methods of collections and analyses are however not in M&M.’ For the purposes of this study the focus is on the properties of the sediment, fauna are only considered in terms of being alive or dead. We are hoping to publish a sister paper detailing the faunal response.

The reviewer states that ‘Concerning the procedural control of the treatments where fauna was removed by freezing or heating: after mixing, the time to allow sediment settling is important for sediment properties on the surface. Here, PC2 was left for 1 day, while the treatments for 5 days. This might have consequences when evaluating the differences in the response.’ None of the samples were fluid, and the oven dried and frozen samples became solid blocks quite quickly, after which no settling could occur, so we do not consider settling to be particularly important. It is possible that there may have been some difference, but this would have been over hours, not days. For most variables, there was no significant difference between the oven and freezer treatment and PC2, so any effect of settling was minor.

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The reviewer states that ‘Concerning the in situ treatments: is 20 min enough for liquid nitrogen or H<sub>2</sub>O<sub>2</sub> to kill animals? The inefficacy of methods could be partly due to the time of treatment or sampling after treatment.’ This is probably true for the liquid nitrogen; the lead author is currently investigating this. H<sub>2</sub>O<sub>2</sub> reacts very quickly, so this is less likely to be a factor in this case.

The reviewer states that ‘In fig 3, I think there is a missing combination concerning the case when a partial defaunation alters sediment properties and fauna.’ This is a possible combination; it was not our intention to present all possible combinations. These 3 examples were presented to illustrate the issues we raise.

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