

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

### **Anonymous Referee #2**

Received and published: 20 March 2012

This paper examines some methods used to remove animals from the marine sediment. According to the authors, such practice is required when assessing the effects of disturbances on, for instance, (i) rates or methods of recovery (ii) strength of biological interactions, (iii) nutrient fluxes and erosion processes. The authors test how different methods can affect the sediment properties, which, in turn may affect patterns of recolonisation and therefore introduce confounding factors in the experiment as the effect of defaunation can be confounded with the effects of the method of defaunation.

The paper is well written and results are clearly presented. However two major points around the main question of the paper are not sufficiently explored. The first is being

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able to distinguish between the effects of disturbance (on both sediment properties and on fauna) and the effects of artificial methods of defaunation that can be used to selectively eliminate certain components of the benthos in order to study their effects on other components. The second is studying how disturbance affect fauna or sediment properties without confounding the effect of disturbance on fauna with the effect of sediment properties on fauna or the effect of disturbance on sediment properties without confounding it with the effect of fauna. For what concerns the first point, in soft sediments using artificial methods to eliminate target 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. These methods however are largely artificial and very unlikely reproduce situations close to the effects of a “real” disturbance. Therefore, a revision of these methods should be considered without any confusion with effects of disturbance.

For what concern the second point, as also the authors say in the introduction, infauna has tight relationships with the sediment and there are reciprocal effects of sediment on infauna and infauna on sediment. 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. When one wants to test the effect of a disturbance on for instance macrofauna recovery, then, inevitably recolonisation patterns could be both a consequence of the type of disturbance and of changes in sediment properties operated by the disturbance and the changes in macrofauna. For soft sediments almost any kind of disturbance that might have an effect on the fauna to some extent has an effect on the sediment properties and viceversa. In addition, very unlikely a disturbance will kill all animals and leave intact sediment properties. During the recovery both sediment properties and fauna will reciprocally affect each others in a dynamic way and their reciprocal effects may continuously change.

Based on the data presented, this paper should be restricted to the first approach and,

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as the title suggest, 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. For instance, strength of fauna grazing on macroalgae or the role of infauna on nutrient fluxes, without any further consideration on testing the effect of disturbance. As such, 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, rather than for Biogeosciences.

Another point is about a widely used method of defaunation that it is also an environmental disturbance often occurring in soft sediments. I refer to the use of plastic layers to provoke sediment anoxia. This type of defaunation is cited by the authors but not considered in their study. Indeed, its consideration might help enlarging the paper objectives, by including an example when defaunation as a method to remove infauna (first approach) is also a disturbance, which is considered as a main driver of ecological change in sediments. An analysis of this defaunation effect on sediment properties could be of major interest. I suggest the authors to refer to several papers recently published such as Montserrat et al , van Colen et al and Rossi et al and to follow the suggestions already pointed out by referee #1

Some other comments: 1 - The reason of the choice of the defaunation methods to be tested are not detailed. 2 - 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 not using degradation products? 3 - 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 4 - 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 5- 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

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for 5 days. This might have consequences when evaluating the differences in the response 6 – 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. 7 - In fig 3, I think there is a missing combination concerning the case when a partial defaunation alters sediment properties and fauna

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Interactive comment on Biogeosciences Discuss., 9, 1377, 2012.

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