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Interactive comment on "Effects of global climate change and organic pollution on nutrient cycling in marine sediments" *by* C. Sanz-Lázaro et al.

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Anonymous Referee #3. The reply of the authors is written after the word "REPLY:", immediately after the comment of the referee.

The objective of the study is to determine the response of benthic ammonium and phosphate fluxes to climate change via an increase in temperature (T) and organic matter (OM) loading. The authors set out to answer this question by incubating homogenized coastal sediment at different temperatures (16, 22 and 26 oC) under control conditions (no additional OM loading) or enhanced loading (OM mixed in with the homogenized sediment) for a period of around five weeks. This follows from other studies that predict a T increase in Baltic Sea coastal waters of around 1oC per decade. They also added worms (Nereis spp.) to the experiment cores to allow for bioirrigation that is observed

at site where the sediments were taken. They broadly conclude that an increase in T and OM loading lead to an increase in NH4 and PO4 fluxes from the sediment, implying that climate change could have important impacts on pelagic productivity. There are a limited number of studies of this nature and the idea is good and certainly timely. The main question addressed by this paper is both interesting and important. The authors should be commended for their effort in tackling this complicated issue because the benthic feedbacks to outside forcings are not well understood. In my opinion, though, the results do not support the conclusions because the experimental period was too short given the study objectives and because the sediment cores were not properly acclimated. If it was the authors' intention to determine how climate change and OM loading affect benthic fluxes, the cores should have been left to reach a quasisteady state. It is obvious from the plots that steady-state was not reached during the incubations, meaning that the final result and thus conclusions could have been very different if the experiment was conducted for, say, another few months or even weeks.

REPLY: Sediment used for the experiment was left for acclimatization at the corresponding temperature after adding organic matter and being set up in the core with its corresponding overlaying water. After that, individuals of Nereis diversicolor were added and the experiment was considered to have started. We don't agree with the reviewer that longer acclimatization or a longer experimental period would have provided more accurate results. Firstly, it is difficult to keep worms alive in an experimental setup for more than a few months without adding new organic matter. Secondly, a "quasisteady state" can never be reached in an experimental setup of this kind, where the natural deposition of organic matter is by-passed in order to study the mineralization of a specific pool of organic matter (i.e. the organic matter present at the beginning). Had the experiment run longer, lets say a few months as the reviewer suggests, sediment cores would not have reached a steady state. Rather, the labile organic matter pools in the different treatments would have been depleted, resulting in successively lower nutrient mineralization rates and more similar mineralization between the –OM and +OM treatments (see e.g. Valdemarsen et al. 2014). Longer incubation periods would therefore have been counterproductive with regards to the specific goal of the experiment, which was to study the mineralization of two different organic matter pools at different temperatures.

A sufficiently long time frame is required to allow the microbial community to respond to the new conditions and for the solute transport fluxes to equilibrate. In that case, the relative change in CNP ratios of the fluxes and the net budget of these elements could have been quantified more accurately and effect of the external variables more readily determined. At present the N,P and Fe contents are hardly different (statistically speaking) among the different treatments.

REPLY: Heterotrophic microbial communities respond extremely fast (within days) to new conditions such as addition of labile organic matter (see e.g. Holmer and Kristensen 1994, Valdemarsen et al. 2009;2010) so the acclimatization period before the addition of worms was more than sufficient to account for this. It is true that in diffusion controlled systems it may take some time before changes in mineralization processes in the sediment can be seen as a change in fluxes, but in faunated sediments, fauna ventilation and bioirrigation results in a rapid coupling between processes occurring in the sediment and nutrient effluxes at the sediment surface. The lack of statistical differences is probably mostly a problem of small scale variability, which is common in ecological studies, rather than lack of adaptation of microbial communities.

The information gained from the present study is of limited value because we see only the initial stages of an evolving system.

REPLY: We do not agree with the reviewer. If the experiment had run for longer we would have created other artefacts, which would have been counterproductive with regards the goals of the experiment as stated above. In this relatively short experiment (which has similar duration as a number of other studies concerning sediment biogeochemistry in bioturbated sediments) we have demonstrated that nutrient effluxes are strongly dependent on temperature, that N and P effluxes probably are influenced dif-

ferently by temperature, and that these effects are dramatically stimulated in organic enriched sediments. We do not find these findings trivial.

There is no careful constraint using mass balances to try and identify the processes in the sediment that are most sensitive response to T and OM loading. This would have been extremely useful information for ecological modelling studies even despite the short incubation length. There is currently no way to isolate the sources and sinks of NH4 and PO4 with the data presented, which makes is almost impossible to evaluate the results in a rigorous manner and greatly devalues their significance. It should come as no surprise to the readership of Biogeosciences that sediment nutrient fluxes increase under the experimental conditions imposed.

REPLY: It may not come as a surprise that NH4+ and PO4+ fluxes are stimulated by temperature (not even to us – we actually expected it). It is, however, extremely valuable information to see exactly how NH4+ and PO4+ fluxes depend on temperature, and this is one of the main findings of this manuscript. It is true that we have some problems creating closed budgets for N and P, because we did not have resources to measure all the potential nutrient pools. However, we have pretty good estimates of the missing nutrients and this is fairly well described in the manuscript. We find it very difficult to answer constructively to this comment.

According to Section 2.2., the sediment cores were accumulated for 3 days at the various T and OM loadings before the worms were added. If the objective is to study the effect of T, the cores should have been acclimated with the worms before increasing temperature. It appears that the opposite is true: the experiment began as soon as the worms were added, although the methods are not very clear on this point.

REPLY: To avoid confusion, in the M&M after the sentence: “Then three N. diversicolor were added to each core to simulate the natural density (Delefosse et al. 2012)”, we have added: “The time of polychaete addition was assumed the beginning of the experiment (t = 0).”.

If this is the case, the results would largely reflect the re-organization of fluxes due to the addition of worms, rather than due to the increase in T.

REPLY: This statement is simply not true. After we add the worms there will be a short period where chemical profiles in the sediment are rearranged. This may result in a peak efflux of nutrients right after the addition of worms, since metabolites that have accumulated in sediment porewater are flushed out by bioirrigation. This peak phase usually lasts 1-2 days in sediment with Nereis and hereafter the “new” chemical profiles are established (Hansen and Kristensen 1998; Banta et al. 1999) and effluxes are a true estimate of total mineralization activity in the sediment. The temperature effects observed in the manuscript are therefore due to e.g. different mineralization rates and not an artefact related to the timing of the addition of worms.

Reference used in this comment: Banta, G.T.,Holmer,M.,Jensen,M.H.,andKristensen,E.(1999 polychaeteworms, Nereisdiversicolor and Arenicolamarina, onaerobicand anaerobicdecompositioninsandymarinesediment. Aquat. Microb.Ecol. 19, 189–204. Hansen,K.,andKristensen,E.(1998).Theimpactofthepolychaete Nereis diversicolor and enrichmentwithmacroalgal(Chaetomorphalinum) detritus on benthicmetabolismandnutrientdynamicsinorganic-poorandorganic- richsediment. J. Exp.Mar.Biol.Ecol. 231, 201–223.

Treatment of the initial conditions is not well justified either because no worms were added to the ‘initial condition’ cores and then allowed to equilibrate. Any comparison of these cores to the experimental cores is highly dubious because the irrigation effect cannot be subtracted from the effect of increasing T and OM.

REPLY: The cores sectioned initially were used to quantify natural background pools of the elements in the sediment (such as PON and TP). So nor acclimation nor worms were needed. We have rewritten on sentence to clarify this: “Additionally, six cores with 5 cm i.d. were also filled with –OM and +OM sediment to determine initial element pools in the sediment.”.We find this comment also not very constructive. If we

understand the reviewer right we cannot compare the solid phase pools in the different treatments because sediments were allowed to acclimatize for a few days before worms were added. This statement simply does not make sense since we clearly demonstrate that the majority of mineralization, and hence changes in sediment pools of various substances, occur in the period after the worms were added.

How were the sediment cores and the nutrient fluxes measured?

REPLY: We think this is clearly stated in the M&M: “NH_4^+ and PO_4^{3-} fluxes between sediment and water were measured every 2-4 days during the first 2 weeks and every week during the rest of the experiment. During flux measurements, the water column of each sediment core was sampled and cores were closed with rubber stoppers. Incubations were ended after 3-5 h (-OM) or 1-2 h (+OM), where the rubber stoppers were removed and the water column was sampled again. All samples were GF/F-filtered, transferred to 20 mL plastic vials and frozen (-20ËŽC).”; “NH_4^+ and PO_4^{3-} were analyzed spectrophotometrically on a Lachat QuikChem 8500 autoanalyzer.”; “Average nutrient efflux rates were estimated as time-integrated nutrient effluxes divided by the experiment duration.”

We are not shown the concentration versus time data for each of the cores to judge the reported values for ourselves. This information should be made available in an appendix or supplement.

REPLY: Concentration data during flux incubations are “raw data” and not really relevant to include in a scientific publication. We provide the temporal trends in calculated fluxes over time (Figure 2) and this is the level of detail we chose to show the data – more detail than that would be excessive in our opinion.

REPLY (as a conclusion): From our point of view, we found some of the considerations of this referee a bit too strong and not very constructive. The experimental set up has already been validated and published (Sanz-Lázaro et al. 2011b). Nevertheless, we do not mean to say that the experimental set up could not be improved - as for almost all lab experiments there were errors and artefacts as we have described in the manuscript. We know from previous experiments that the acclimatization time for the sediment bacteria was sufficient and that the timing of the different steps in the experiment (sediment preparation, acclimatization, addition of worms etc.) was optimal so we have minimized any bias in nutrient budgets. Scientists are aware that mesocosm experiments are simplifications of the real world, but they can nervertheless help us to better understand specific processes. Mesocosm experiments lay its robustness on causality demonstration. We were able to demonstrate that temperature and organic enrichment caused significant effects on nutrient effluxes form the sediment and that NH4+ and PO43- release show different temperature dynamics. This was possible since all the rest of the conditions were controlled and the same. We think that our study is a good starting point to continue investigations and increase our understanding of this timely issue.

Reference used in this comment: Sanz-Lazaro, C., Valdemarsen T., Marin A., and Holmer M.: Effect of temperature on biogeochemistry of marine organic-enriched systems: implications in a global warming scenario, Ecol. Appl., 21, 2664-2677, 2011b.

Nevertheless, we thank the referee for the time taking to comment the Ms and thus participating in improving it.

Interactive comment on Biogeosciences Discuss., 12, 21, 2015.