

The reply of the authors is written in bold after each comment of the referee. Additionally to changes suggested by the referees we have done minor changes related with grammar and preciseness. All changes are tracked by means of track changes of Word. We thank all referees for the time taking to comment the Ms and thus participating in improving it.

Reply to Anonymous Referee #1.

The manuscript deals with an experimental study using short sediment cores which were incubated under different temperatures in order to simulate the effects of increasing temperatures and eutrophication on early diagenetic release of nutrients. Incubation was done at three different temperatures representing present conditions as well as those simulated for global change scenarios in 50 and 100 years. A second incubation was done with added ground fish feed in order to simulate the addition of labile organic matter. Part of the study, namely the CO₂ release, oxygen uptake, sulfate release and sulfide enrichment, was published in an earlier paper. The present paper is focused on the mobilization of phosphate and ammonium and uses a regression analyses to determine the efflux of the nutrients with and without organic matter addition. The results are that phosphate release increases linearly with increasing temperatures while ammonium is released according to an exponential fit with an increase starting only at temperatures elevated by >6°C. Another important finding is that only a very small proportion of added phosphorous is released probably due to binding of phosphates to Fe-oxides while up to two thirds of the added nitrogen is released as ammonium. The paper is well written, well illustrated and findings are sound and supported by the data.

COMMENT FROM REFEREE: A problem is, however, that nitrite and nitrate were not measured. It is very likely that nitrification took place under the oxygenated conditions so that the exponential curve may be an artefact of the lack of nitrite and nitrate data. It is feasible that under slightly elevated temperatures nitrate is released while under higher temperatures as more oxygen has been consumed ammonium is released in larger proportions. The authors mention this and discuss the problem shortly but it needs to be stressed and discussed in more detail.

AUTHOR'S RESPONSE: We agree that it would add more information having NO₂- and NO₃- data, but we do not agree that the exponential curves of NH₄⁺ sediment efflux along temperature is an artefact because of lack of NO₂- and NO₃- data. This graph is presenting the amount of NH₄⁺ that is released to the water column. Another different fact is that part of the mineralized organic N could be effluxed as NO₂- and NO₃-. Although NO₃- in the pelagic system can be at comparable concentration levels as NH₄⁺, NO₃- effluxes are generally much lower (around one magnitude order below) than the NH₄⁺ effluxes (e.g. Hansen & Kristensen, 1998; Alsterberg et al. 2012). As regards, NO₂- concentration levels are always notably low compared to NH₄⁺ effluxes (around one or two magnitude orders below) when the water column is under oxic conditions. Anyway, we think that the referee highlights an important point that should be clarified and we followed their recommendation of discuss this in more detail.

AUTHOR'S CHANGES: We have added at the end of this paragraph: "Despite so, NH₄⁺ is mostly the dominating form of dissolved inorganic N effluxing from organic enriched sediments (Christensen et al. 2000; Holmer et al. 2003), while coupled nitrification-denitrification rarely exceeds 1-2 mmol m⁻² d⁻¹ in marine sediments

(Middelburg et al. 1996). We are therefore confident that the NH₄⁺ release rates can act as a proxy for total inorganic N release to the water column.”.

References used in this comment:

Christensen, P. B., Rysgaard S., Sloth N. P., Dalsgaard T., and Schwaerter S.: Sediment mineralization, nutrient fluxes, denitrification and dissimilatory nitrate reduction to ammonium in an estuarine fjord with sea cage trout farms, *Aquat. Microb. Ecol.*, 21, 73-84, 2000.
Holmer, M., Duarte C. M., Heilskov A., Olesen B., and Terrados J.: Biogeochemical conditions in sediments enriched by organic matter from net-pen fish farms in the Bolinao area, Philippines, *Mar. Pollut. Bull.*, 46, 1470-1479, 2003
Middelburg, J. J., Soetaert K., Herman P. M. J., and Heip C. H. R.: Denitrification in marine sediments: A model study, *Global Biogeochemical Cycles*, 10, 661-673, 1996.

Specific comments:

COMMENT FROM REFEREE: Page 22, line 5/6: "especially: : :may just be additive" This statement is rather vague and would need a lot of explanation. I think it is better to delete this as it is beyond the scope of an abstract.

AUTHOR'S CHANGES: **Done**

COMMENT FROM REFEREE: p. 23, l. 21/22: delete "and needs to be fixed again: : :" until the end of the sentence.

AUTHOR'S CHANGES: **Done**

COMMENT FROM REFEREE: p. 27, l. 7: typo "slices to"

AUTHOR'S CHANGES: **Done**

COMMENT FROM REFEREE: p. 29, l. 5ff: this sentence sounds a bit strange to me, may be it is best to end with :was calculated.

AUTHOR'S CHANGES: We have modified the sentence and the followings of the paragraph, but we have started with "was calculated" instead of ending with it. We did so because the active form seemed to us simpler and easier to understand.

Anonymous Referee #2.

This is a study that investigates the combined effects of a predicted seawater temperature combined with an organic nutrient input on the biogeochemistry on shallow-water sediment systems. I like these type of studies for a number of reasons: 1) the authors investigates multiple stressors (since single stressor is very uncommon in the real world), 2) intact, natural sediment communities are used, 3) the experiment is designed to take time into consideration and 4) more than 2 temperature treatments were used. However, I do have major issues with the fact that the experiment was conducted in darkness, especially considering: "The aim of this work is to examine the effects of temperature rise and organic enrichment on sediment nutrient release". See specific

comments in this matter below.
Specific comments:

COMMENT FROM REFEREE:#1 The last paragraph in the introduction. You are only looking at heterotrophic processes, i.e. not the entire sediment community and how it is affected by organic input and warming. Please specify the aim better and also try to incorporate this in the rest of your introduction.

AUTHOR'S RESPONSE: Generally, this type of experiments are run in darkness, when the focus of the study is the heterotrophic processes. Although we may not be able to predict nutrient release rates in shallow photic sediments based on this experiment, the obtained effluxes can be extrapolated to sediments receiving little or no light. Such sediments are very common in coastal waters in the Baltic Sea area due to eutrophication.

AUTHOR'S CHANGES: We have added to the end of the last paragraph of the introduction: "In these areas, heterotrophic processes in sediments prevail due to the generally low availability of light in the seabed due to eutrophication and high input of labile organic matter (Conley et al. 2009)."

We have also added to the sentence of the aim in the last paragraph of the introduction: "... derived from heterotrophic processes."

Reference used in this comment:

Conley, D. J., Bjorck S., Bonsdorff E., Carstensen J., Destouni G., Gustafsson B. G., Hietanen S., Kortekaas M., Kuosa H., Meier H. E. M., Muller-Karulis B., Nordberg K., Norkko A., Nurnberg G., Pitkanen H., Rabalais N. N., Rosenberg R., Savchuk O. P., Slomp C. P., Voss M., Wulff F., and Zillen L.: Hypoxia-Related Processes in the Baltic Sea, Environ. Sci. Technol., 43, 3412-3420, 2009.

COMMENT FROM REFEREE: #2 Although you refer to the paper by Valdemarsen et al., 2009 for specific details regarding the methods, I still would like to know at what depth the sediment was collected without looking at this paper. This is important considering your data and the scope of this paper. Since this sediment was collected in July at 1 m depth (at least it was in Valdemarsen et al. 2009), I wonder why the autotrophic community, i.e. benthic microalgae is not even mentioned? How much benthic microalgae was present at the sediment surface?

AUTHOR'S RESPONSE: Since we performed the experiment under absence of light (which is stated in the M&M in subsection 2.2) we found that it was no relevant to measure data related the autotrophic community. We used homogenized sediments and hence microalgae present on the sediment surface during sampling were mixed into the whole sediment cores.

AUTHOR'S CHANGES: We have added in the sentence where we explain the sediment collection: "...at 1 m depth..."

COMMENT FROM REFEREE: #3 With the approach of only incubating the sediment in darkness you only target heterotrophic processes. However, during light, these shallow sediments often functions as sinks for inorganic nutrient via uptake by benthic microalgae. This is especially true during summer, with far less dark hours than light hours, meaning

that the net flow of nutrients (at least nitrogen) during 24 hours might be the complete opposite to your results. Any thoughts on this?

AUTHOR'S RESPONSE & AUTHOR'S CHANGES: We have added to the discussion before the conclusions the following paragraph: "Additionally it should be considered that the experiment was performed in darkness, as the experiment was designed to simulate the generally low availability of light at the sediment surface in relatively enclosed and shallow coastal areas exposed to eutrophication, such as in the Danish coastal areas. Nevertheless, in other areas with more light availability autotrophic processes are also important. This could lead to different results with regards to nutrient release rates from the sediment."

COMMENT FROM REFEREE: #4 The surface oxygen (in the top layer of sediment???) was maintained due to bioturbation, even though the sediment was in complete darkness during the entire experimental period. Was any oxygen profile in the sediment performed? If the oxygenized layer was reduced (which I suspect it would since no photosynthesis could be performed by the benthic microalgae) this would have contributed to your results with time even though the top surface was oxygenized?

AUTHOR'S RESPONSE: The top layer of the sediment was oxidized as we could see it in the supplementary material in Sanz-Lázaro et al. (2011b) since the sediment when oxidized it showed a yellowish color indicating that Fe was oxidized.

<http://esapubs.org/archive/appl/A021/118/appendix-A.htm>

This was expected to be because the polychaete *Nereis diversicolor* bioirrigated the sediment. The water that *Nereis* introduced in the sediment was oxygenated since there was permanent aeration of the water column to prevent water hypoxia.

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.

COMMENT FROM REFEREE: #5 I would suggest starting the discussion with a brief reminder of the aims and also highlighting the most important results for your study. As it is right now, the discussion come across as a bit boring and to technical and immediately starts discussing the phosphorus fluxes.

AUTHOR'S RESPONSE & AUTHOR'S CHANGES: We have added a paragraph at the beginning of the discussion: "Our results show that temperature rise resulted in different trends of sediment nutrient release of PO₄³⁻ and NH₄⁺ under both organic and non-organic enrichment conditions. While sediment PO₄³⁻ release followed a linear trend with increasing temperature, the NH₄⁺ release from sediment show exponential trends, notably increasing when temperature increments were above 6°C."

COMMENT FROM REFEREE: #6 I would like to see a discussion if you believe your results would look the same if you allowed the temperature to vary, i.e. taking daily and weekly temperature variations into consideration. Because during summer it can be several degrees difference between day and night, between days and weeks which might affect your results.

AUTHOR'S RESPONSE: Because water has a high specific heat index daily variations in relatively large basins are expected to be low. We think that this effect would be minimum and so we rather not add anything related with this comment to the discussion.

COMMENT FROM REFEREE: #7 Page 36, line 24, sure, could be true, on the other hand with your experimental design you cant really say since NH₄ (especially during summer with many light hours in contrast to dark hours) is taken up by the sediment during the day.

AUTHOR'S RESPONSE: We have answered this with the first comments, since our experiment is based in conditions of light absence.

COMMENT FROM REFEREE: #8 Please include in your discussion how your fluxes probably would behave if your they were measured both during light and dark and how this probably changes your conclusions.

AUTHOR'S RESPONSE: Undoubtedly, this could have modified the fluxes from the sediment. We think this idea is considered with the paragraph added derived from the specific comment #3. Otherwise we found it would be too much hypothesising in something we haven't tested.

COMMENT FROM REFEREE: #9 Multiple stressor model. What model is used for investigating the multiple stressor effect? Where you find significant interactions are these results synergistic?

AUTHOR'S RESPONSE: As stated in the M&M: "... we did regression models considering temperature the continuous covariate and OM enrichment as a fixed factor." This is to say ANCOVA (analysis of covariance). The interaction between the covariate and the fixed factor tell us if there are synergisms or antagonisms. We found this to happen for PO₄³⁻ efflux rate but not for NH₄⁺ efflux rate (see table 3). This is explained in the results: "There were significant differences between -OM and +OM treatments at 26°C (p<0.05) and the significant interaction term of the regression (p<0.05) indicated a steeper temperature response in +OM compared to -OM treatments"; "The interaction term of the regression did not show significant differences. Thus, NH₄⁺ efflux in -OM and +OM cores followed a similar trend with temperature although at different ranges (Table 3)."

Anonymous Referee #3.

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 1°C 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.

COMMENT FROM REFEREE: 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.

AUTHOR'S RESPONSE: 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.

COMMENT FROM REFEREE: 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.

AUTHOR'S RESPONSE: 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.

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

AUTHOR'S RESPONSE: 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 differently by temperature, and that these effects are dramatically stimulated in organic enriched sediments. We do not find these findings trivial.

COMMENT FROM REFEREE: 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 NH_4 and PO_4 with the data presented, which makes it 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.

AUTHOR'S RESPONSE: It may not come as a surprise that NH_4^+ and PO_4^+ fluxes are stimulated by temperature (not even to us – we actually expected it). It is, however, extremely valuable information to see exactly how NH_4^+ and PO_4^+ 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.

COMMENT FROM REFEREE: 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.

AUTHOR'S RESPONSE & AUTHOR'S CHANGES: 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)."

COMMENT FROM REFEREE: 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.

AUTHOR'S RESPONSE: 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., and Kristensen, E. (1999). Effects of two polychaete worms, *Nereis diversicolor* and *Arenicola marina*, on aerobic and anaerobic decomposition in sandy marine sediment. *Aquat. Microb. Ecol.* 19, 189–204. Hansen, K., and Kristensen, E. (1998). The impact of the polychaete *Nereis diversicolor* and enrichment with macroalgal (*Chaetomorpha*) detritus on benthic metabolism and nutrient dynamics in organic-poor and organic-rich sediment. *J. Exp. Mar. Biol. Ecol.* 231, 201–223.

COMMENT FROM REFEREE: 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.

AUTHOR'S RESPONSE: The cores sectioned initially were used to quantify natural background pools of the elements in the sediment (such as PON and TP). So no acclimation nor worms were needed.

AUTHOR'S CHANGES: 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.

COMMENT FROM REFEREE: How were the sediment cores and the nutrient fluxes measured?

AUTHOR'S RESPONSE: We think this is clearly stated in the M&M: "NH₄⁺ and PO₄³⁻ 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 (-20°C)."; "NH₄⁺ and PO₄³⁻ 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."

COMMENT FROM REFEREE: 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.

AUTHOR'S RESPONSE: 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.

AUTHOR'S RESPONSE (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 nevertheless 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 from 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.