Effects of temperature change and organic pollution on nutrient cycling in marine sediments

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Running title: Climate warming effect on nutrient cycling

Abstract

Increasing ocean temperature due to climate change is an important anthropogenic driver of ecological change in coastal systems. In these systems sediments play a major role in nutrient cycling. Our ability to predict ecological consequences of climate change is enhanced by simulating real scenarios. Based on predicted climate change scenarios, we tested the effect of temperature and organic pollution on nutrient release from coastal sediments to the water column in a mesocosm experiment. PO_4^{3-} release rates from sediments followed the same trends as organic matter mineralization rates, and increased linearly with temperature and were significantly higher under organic pollution than under non-polluted conditions. NH_4^+ release only increased significantly when the temperature rise was above 6ºC, and was significantly higher in organic polluted compared to non-polluted sediments. Nutrient release to the water column was only a fraction from the mineralized organic matter, suggesting PO_4^{3-} retention and NH_4^+ oxidation in the sediment. Bioturbation and bioirrigation appeared to be key processes responsible for this behaviour. Considering that the primary production of most marine basins is N-limited, the excess release of NH_4^+ at a temperature rise > 6 degrees could enhance water column primary productivity, which may lead to the deterioration of the environmental quality. Climate change effects are expected to be accelerated in areas affected by organic pollution.

Keywords: climate change, eutrophication, global warming, nitrogen, organic matter enrichment, phosphorus.

1. Introduction

Ocean temperature rise due to climate change is considered among the most severe anthropogenic drivers of ecological change in marine systems, especially in coastal areas (Halpern et al. 2008). Sea surface temperature (SST) in marine systems is expected to rise 0.2ºC per decade (IPCC 2007), but in relatively enclosed and shallow coastal areas, such as the Baltic Sea, temperature has risen much more rapidly (1ºC in last decade; (Mackenzie and Schiedek 2007;Belkin 2009) and similar rises are expected for the next decades (Doscher and Meier 2004). 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).

Coastal areas are climate-sensitive systems (IPCC 2007) that play an important role in the mineralization of organic matter (OM) (Middelburg et al. 1997). During mineralization, organic phosphorous (P) and nitrogen (N) are transformed into inorganic forms (PO_4^{3-} and NH_4^+, respectively). OM is mineralized mainly by microbial processes, which are strongly influenced by temperature (Robador et al. 2009). Consequently, climate change may affect nutrient regeneration rates in coastal ecosystems.

PO_4^{3-} can be sequestered in the sediment by adsorption to ferric (oxy)hydroxides and by forming solid ferrous phosphates (Jorgensen 1983). Sulfides resulting from sulfate reduction may mobilize PO_4^{3-} by reducing ferric oxyhydroxides (Roden and Edmonds 1997) and by dissolving Fe minerals containing PO_4^{3-} (Gachter and Muller 2003). Despite the complex interactions among the Fe, S and P pools, PO_4^{3-} release to the water column is mainly dependent on the redox conditions in the sediment surface, where oxidized Fe keep PO_4^{3-} immobilized. Thus seasonal increases in sediment metabolic rates due to temperature rise may lead to reducing conditions in the sediment surface, resulting in PO_4^{3-} release during summer (Jensen et al. 1995;Cowan and Boynton 1996). Mineralized N is mainly released to the water column as NH_4^+ (Jorgensen 1983). NH_4^+ can be further transformed into NO_3^- and NO_2^-, through nitrification, and end up as N_2 through denitrification or anammox (Thamdrup 2012). In contrast to NH_4^+, NO_3^- and NO_2^-, N_2 can not be directly used by primary producers. Consequently denitrification results in N removal from the ecosystem preventing eutrophication (Thamdrup and Dalsgaard 2008). Marine coastal sediments play a major role in supporting primary productivity of the water column by supplying a large part of the nutrient demand for phytoplankton (Nixon 1981;Kelly et al. 1985). Increases in sea water temperature and input of OM to the sediment, are both expected to stimulate the release of inorganic nutrients from the sediment to the water column, which may in turn stimulate primary productivity. Nutrient increase above a certain threshold may lead to algal blooms and subsequent hypoxic/anoxic events, resulting in the deterioration of ecological status (Gray et al. 2002).

Coastal areas gather the greatest human population densities in the world, resulting in high anthropogenic pressure on coastal ecosystems. Thus, many coastal areas are subject to multiple stressors (Halpern et al. 2008) such as different kinds of pollution and global warming. Among the different types of pollution, organic pollution, also know as organic enrichment, is one of the most common in densely populated coastal areas (Islam and Tanaka 2004). As for temperature, organic enrichment enhances sediment metabolism, mainly by stimulating anaerobic pathways and especially sulphate reduction (Valdemarsen et al. 2009). This effect is magnified when organic pollution and temperature rise co-occur (Sanz-Lazaro et al. 2011b).

Our ability to predict the ecological consequences of climate change is enhanced by simulating realistic future scenarios. Hence, it is important to conduct experiments to elucidate not only the effects of individual drivers of ecological change but also the interactions among them, especially when the interactions among these drivers are not just additive (Falkenberg et al. 2013). Despite the current concern of global change, scarce research effort has been taken on understanding how temperature rise affect the cycling of N and P in coastal sediments (Alsterberg et al. 2012). In fact, the accuracy of predictive biogeochemical models related to the effect of climate change on the coastal eutrophication is restrained due to limited knowledge (Meier et al. 2011). Thus, to improve our forecasting capacity related to climate change, estimates of temperature driven changes in sediment nutrient release are needed.

The aim of this work is to examine the effects of temperature rise and organic enrichment on sediment nutrient release derived from heterotrophic processes. We used a mesocosm approach and hypothesized that temperature rise due to climate change increases PO_4^{3-} and NH_4^+ release from the sediments as a consequence of increased metabolic rates. We expected an increase of the release of nutrients with temperature, with a more marked effect in organic enriched than in non-organic enriched sediments, due to stimulation of anaerobic processes resulting in PO_4^{3-} and NH_4^+ release.

2. Materials and methods

This experiment is the second part of a study. The results on carbon and sulphur cycles are presented in Sanz-Lázaro et al. (2011b), where detailed information on the methods it is given.

2.1. Collection of sediment and polychaetes

The sediment used for experiments was well sorted, organic poor, Fe-rich sand [0.4 %DW particulate organic carbon (POC), 125 µmol cm^{-3} reactive Fe and 220 µm average grain size] collected at 1 m depth in Fænø Sund, Denmark (for further details seeValdemarsen et al. 2009). The macrofaunal organism used for experiments was the polychaete *Nereis diversicolor*, which was chosen based on its ability to bioirrigate surface sediment and influence microbial reaction rates (Kristensen 2000;Mermillod-Blondin et al. 2004). *N. diversicolor* was collected from Fællesstrand in the outer part of Odense Fjord, Denmark.

2.2. Experimental setup

Sediment was split into either control (-OM) or organically enriched sediment (+OM). Enrichment was performed by adding 92 g labile OM [finely ground fish feed, Ecolife, Dansk Ørredfoder with 49.4% DW POC, 8.1% DW total nitrogen organic N (TN), 0.9% DW total P (TP)] to 20 L of sediment. The enrichment corresponded to 26 mol POC m^{-2}, which is comparable to the annual OM deposition at fish or mussel farms (Callier et al. 2006;Holmer et al. 2007;Sanz-Lazaro et al. 2011a).

Sediment was packed into 36 core liners (8 cm internal diameter; i.d., 35 cm sediment depth) which were distributed in three tanks maintained at 16, 22 or 26ºC containing 65 L GF/F-filtered seawater from Fænø Sund with a salinity of 17 psu. Each tank contained 6 cores of each –OM and +OM, sediment. The 16ºC treatment was chosen as the reference level of present mean SST for summer months in temperate areas of the Baltic Sea (Kristensen 1993;Holmer and Kristensen 1996). The 22ºC and 26ºC treatments were chosen as SST climate change scenarios in 60 and 100 years based on SST temperature rise observed in the last decades (Mackenzie and

Schiedek 2007;Belkin 2009) and expected raises (Doscher and Meier 2004) (1ºC per decade in both cases).

The sediment in the cores was left 3 days to compact and acclimitize. Then three *N. diversicolor* were added to each core to simulate the natural density (Delefosse et al. 2012). The time of polychaete addition was assumed the beginning of the experiment (t = 0). *N. diversicolor* bioirrigation rates were previously reported in Sanz-Lázaro et al. (2011b). They were calculated based on Br^--addition experiments and were on average 13-24 L m^{-2} d^{-1} in the different treatments. There were no significant ($p < 0.05$) effects of temperature or of OM enrichment on bioirrigation rates and all cores were bioirrigated to 6–8 cm depth (Sanz-Lazaro et al. 2011b). Bioirrigation rates in the present experiment were similar to the bioirrigation measured previously for other polychaetes (Quintana et al. 2013) and within the range of the bioirrigation performed by natural macrofaunal communities (Valdemarsen et al. 2010). Unfortunately irrigation rates and visual observations showed that the added worms died in three +OM cores at 26 degrees, and results from these cores were omitted from further analysis. For the rest of the cores no polychaetes were found dead during the experiment, and active N. diversicolor were observed in all the rest of the cores. The bromide incubations indicated that N. diversicolor ventilated their burrows with the same intensity regardless of OM-enrichment level.

Additionally, six cores with 5 cm i.d. were also filled with –OM and +OM sediment to determine initial element pools in the sediment. Cores were closed at the bottom with rubber stoppers and ~20 cm sediment was added, leaving a 10-12 cm headspace above the sediment. During the experiment the water in each tank was kept aerated and changed every week. The cores were kept submerged and in darkness throughout the experiment (25-39 days). More specific details can be found in Sanz-Lázaro et al. (2011b).

2.3. Nutrient fluxes

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 (-20˚C).

2.4. Sectioning of cores

The three 5 cm i.d. sediment cores of each sediment type (-OM and +OM) were initially sectioned (t = 0; henceforth referred to as initial cores) and the remaining cores from every temperature treatment were sectioned at the end of the experiment (henceforth, final cores). Cores were sectioned into 1 cm slices to 2 cm depth and into 2 cm slices to 16 cm depth. Every sediment slice was homogenized and subsampled for different analysis. One subsample from each sediment slice was used to measure reactive Fe and phosphate bound to reactive Fe. A subsample of sediment was used for TP measurement. The remaining sediment from each slice was used to determine TN, sediment density and water content. All analytical methods are described below. Since temperature has a strong stimulatory effect on microbial reaction rates, the duration of the temperature treatments varied to prevent porewater sulfate depletion and a shift in sediment metabolism towards methanogenesis. Thus, the sectioning of the sediments at 16, 22 and 26ºC was performed after 39, 32 and 25 days, respectively.

2.5. Sediment metabolic rates

C mineralization rates were estimated as time-integrated total CO_2 (TCO_2) sediment-water column flux divided by the experiment duration as described in Sanz-Lázaro *et al.* (2011b) (Fig. 1). TCO_2 fluxes were measured following same procedures as for nutrient fluxes and TCO_2 concentration was analyzed by flow injection analysis (Hall and Aller 1992).

2.6. Analyses

NH_4^+ and PO_4^{3-} were analyzed spectrophotometrically on a Lachat QuikChem 8500 autoanalyzer. Reactive Fe was extracted on ~0.2 g of sediment subsamples with 5 ml of 0.5 M HCl shaken for 30 min. Then samples were centrifuged for 5 min (3000 rpm, ca. 1000 × g) and GF/F filtered. The supernatant was analyzed by the ferrozine method (Stookey 1970). Fe (II) was determined on the untreated extract while total Fe [Fe(II)+Fe(III)] was determined after reduction with hydroxylamine (Lovley and Phillips 1987). Fe (III) was determined as the difference between total Fe and Fe(II). PO_4^{3-} in HCl extracts was also analyzed spectrophotometrically after addition of Molybdate reagent (Koroleff 1983) to get a measure of Fe-bound P. Total P was determined on combusted sediment subsamples (520°C, 2 h), which were boiled in 1 M HCl for 1 h. The supernatant was analyzed for PO_4^{3-} as described above. TN was determined on a Carlo Erba CHN EA1108 elemental analyzer according to Kristensen and Andersen (1987). Sediment density was determined gravimetrically by weighing a known volume of sediment using cut off syringes. Water content was measured as weight loss after drying (105°C, >12 h).

2.7. Data analysis

Significant differences in the content of solid phase nutrients were tested by pair-wise t-tests. Comparisons were done between initial –OM and +OM cores, and between initial and each final treatment within each sediment type (-OM and +OM).

Average nutrient efflux rates were estimated as time-integrated nutrient effluxes divided by the experiment duration. To compare the trends of the time-integrated effluxes between –OM and +OM treatments along temperature we did regression models considering temperature the continuous covariate and OM enrichment as a fixed factor. According to the data on the trends of the time-integrated effluxes with increasing temperature we hypothesized different regression models. We used the second order Akaike Information Criterion (AICc) to choose the best model, since it is a good compromise between fitting and complexity of the model and it is recommended when the sample size is relatively low (Burnham and Anderson 2002). In all regressions temperature was centered at 26ºC, so intercepts showed mean values of effluxes at this temperature. The significance of the regression coefficients was tested by ANOVA. Homocedasticity was checked using Levene's test and normality with p-p plots. Analyses were run in R (v. 2.15.0) and linear regressions were implemented using the *lm* function (R Development Core Team 2012). All data were reported as mean ± standard error (SE) and statistical tests were conducted with a significance level of $\alpha = 0.05$.

We calculated the molar ratio of C:N:P in the added OM based on its composition and the molar C:N:P ratio in the sediment based on POC, TN and TP content in unenriched and enriched sediment. We estimated C:N:P ratios of organic matter being mineralized based on TCO_2, NH_4^+ and PO_4^{3-} effluxes. C and TCO_2 effluxes were reported in Sanz-Lázaro *et al.* (2011b).

3. RESULTS

3.1. P in sediment

TP concentration in initial –OM and +OM sediment showed similar values (Table 1). The initial enrichment with fish feed should have resulted in 0.4 mol m^{-2} higher TP in +OM cores than –OM cores (4.7 % of the TP in the sediment), but this was not evident from our measurements probably due to high variability between subsamples. Hence, there were no detectable differences in the TP content between initial –OM and +OM cores ($p>0.05$). Additionally, there were no significant differences on TP content between the initial and each final treatment within each sediment type (-OM and +OM). Fe-bound P at the beginning of the experiment also showed similar values between–OM and +OM cores (Table 2).

3.2. N in sediment

PON content in initial –OM cores was significantly lower ($p<0.05$) than +OM cores. Additionally, there were no significant differences on PON content between the initial and each final treatment within each sediment type (-OM and +OM). The initial enrichment with fish feed should have resulted in extra 3.6 mol m^{-2} (46.8 % of the PON concentration in the sediment), which was close to the measured enrichment (28.6 % of the PON in the sediment) (Table 1).

3.3. Fe in sediment

Pools of reactive Fe (III) were very similar between initial –OM and +OM cores, as well as between treatments at the end of the experiment (Table 2). Fe (III) concentration was high in the first cm of the sediment being 32.9 ± 8.0, 35.5 ± 4.3 and 25.1 ± 5.6 µmol cm^{-3} in –OM cores at

16, 22, and 26ºC, respectively, and 12.7 ± 3.6, 24.0 ± 6.9 and 28.5 ± 6.2 µmol cm^{-3} in +OM cores at 16, 22, and 26ºC.

3.4. Nutrient release

PO_4^{3-} efflux showed a similar temporal pattern in the different treatments: an increase in the first days, a peak at intermediate stages and then decreasing effluxes towards the end (Fig. 2). The peaks of PO_4^{3-} efflux were stimulated by OM enrichment and temperature. In –OM cores PO_4^{3-} efflux ranged from -0.3 to 0.3, -0.4 to 0.8 and -0.2 to 0.7 mmol m^{-2} d^{-1} at 16, 22 and 26ºC, respectively. In + OM cores PO_4^{3-} efflux ranged from -0.2 to 1.0, -0.2 to1.9 and 0.0 to 1.4 mmol m^{-2} d^{-1} at 16, 22 and 26ºC, respectively.

Average PO_4^{3-} efflux over the whole experiment was 1.7 ± 1.2, 3.5 ± 1.7 and 4.3 ± 1.1 mmol m^{-2} in –OM cores at 16, 22, and 26ºC, respectively. In +OM cores total PO_4^{3-} effluxes were 6.6 ± 1.2, 11.4 ± 2.0 and 13.2 ± 1.8 mmol m^{-2} in +OM cores at 16, 22, and 26ºC, respectively, corresponding to 4.2, 7.6 and 9.3 % of the total P added in fish feed (Table 1). Average PO_4^{3-} efflux showed a linear increasing trend along temperature in both –OM and +OM cores (Fig 3a). The AICc showed that PO_4^{3-} effluxes were best fitted with a linear regression (Table A1). The regression analysis showed that average PO_4^{3-} efflux increased linearly with temperature ($p<0.05$) in –OM and +OM cores. There were significant differences between -OM and +OM treatments at 26ºC ($p<0.05$) and the significant interaction term of the regression ($p<0.05$) indicated a steeper temperature response in +OM compared to –OM treatments (Table 3).

The overall trend of NH_4^+-efflux was similar to PO_4^{3-}-efflux. NH_4^+ effluxes increased at the beginning, peaked and then decreased towards the end (Fig. 2). The range of the NH_4^+

effluxes was stimulated by both organic enrichment and temperature. In –OM cores NH_4^+ efflux ranged from -12.5 to 27.8, -20.7 to 35.8 and -16.2 to 54.6 mmol m^{-2} d^{-1} at 16, 22 and 26ºC, respectively. In +OM cores NH_4^+ efflux ranged from 0.0 to 58.8, 0.0 to 60.0 and 3.1 to 55.6 mmol m^{-2} d^{-1} at 16, 22 and 26ºC, respectively.

Average NH_4^+ efflux over the whole experiment was 217 ± 48, 112 ± 58 and 339 ± 106 mmol m^{-2} in –OM cores at 16, 22, and 26ºC, respectively. Total NH_4^+ effluxes in +OM cores were 559 ± 113, 525 ± 102 and 577 ± 132 mmol m^{-2} at 16, 22, and 26ºC, corresponding to 17.9, 17.4 and 20.1 % of N added in fish feed (Table 1). The trend of average NH_4^+-effluxes over the whole experiment was not as clear as for PO_4^{3-} effluxes (Fig. 3b). NH_4^+ effluxes were clearly stimulated at 26ºC in both sediment types, however. According to the AICc, the trend of NH_4^+ efflux over temperature was best fitted with an exponential regression (Table A1). The regression analysis showed significant increasing NH_4^+ effluxes with temperature following a non-linear trajectory ($p<0.05$) in both –OM and +OM sediment. There were significant differences between -OM and +OM treatments at 26ºC ($p<0.05$). The interaction term of the regression did not show significant differences. Thus, NH_4^+ efflux in –OM and +OM cores followed a similar trend with temperature although at different ranges (Table 3).

3.5. C:N:P ratios

The C:N, C:P and N:P ratios of the added OM were 5.2, 21.3 and 4.1, respectively. C:N, C:P and N:P ratios were similar in the initial -OM and +OM cores. The ratios among C, N and P at the end of the experiment were calculated based on CO_2, NH_4^+ and PO_4^{3-} effluxes. C:N, C:P and N:P ratios at the end of the experiment were generally greater in –OM than in +OM cores, althought there was not a clear trend along temperature treatments (Table 4).

4. Discussion

Our results show that temperature rise resulted in different trends of sediment nutrient release of PO_4^{3-} and NH_4^+ under both organic and non-organic enrichment conditions. While sediment PO_4^{3-} release followed a linear trend with increasing temperature, the NH_4^+ release from sediment show non-linear trends, notably increasing when temperature increments were above 6ºC.

4.1. PO_4^{3-} release

The measured PO_4^{3-} effluxes at 16ºC were within the range of effluxes measured in coastal sediments in the Baltic Sea (Sundby et al. 1992;Jensen et al. 1995). PO_4^{3-} effluxes increased linearly with temperature in both –OM and +OM treatments, but the slope of the regression was significantly steeper in +OM cores compared to –OM cores. This suggests that the temperature dependent increase in PO_4^{3-} effluxes is enhanced by OM enrichment. The temperature effect on PO_4^{3-} release was comparable to the temperature effect on total C-metabolism in both organic enriched and non-organic enriched conditions (Sanz-Lazaro et al. 2011b). However, the release of PO_4^{3-} was much lower than that of TCO_2, as indicated by high C:P ratios. suggesting that organic P was either less labile compared to organic C or that inorganic PO_4^{3-} was retained in the sediment to a larger extent than C. Taking into account that the added OM (fish feed) is highly labile, our data suggest PO_4^{3-} retention in the sediment, which could be associated with adsorption to oxidized forms of Fe (Jensen et al. 1995).

P-retention in marine sediments is controlled by the forms of P, Fe and S in the sediment, interactions between these pools and the sediment redox-conditions (Roden and Edmonds

1997;Gachter and Muller 2003). Generally, in sediments with an oxic surface layer, oxidized Fe acts as a lid for PO_4^{3-}, which is adsorbed to ferric Fe preventing its release to the water column (Rozan et al. 2002). In this experiment, the PO_4^{3-} binding capacity was not easily exhausted due to the large pool of Fe (III) (tens of mmoles) in the first cm of the sediment. This hypothesys was also supported by the fact that the levels of PO_4^{3-} bound to Fe (III) showed low variation between initial and final cores. The oxic conditions of the sediment surface were maintained in all treatments despite metabolism enhancement and subsequent increase in sulphide production, which was especially notable in the +OM treatments. The continuous oxygenation of surface sediment due to *N. diversicolor* bioturbation and bioirrigation (Sanz-Lazaro et al. 2011b) was probably critical for maintaining and oxic sediment surface with high Fe(III) concentrations, since dramatically stimulated sediment metabolism are expected to notably diminish the Fe(III) pool of the sediment in the absence of macrofauna (Valdemarsen et al. 2009). All these results suggest that the capacity of the sediment to retain PO_4^{3-} was maintained despite high metabolic rates due to organic enrichment and elevated temperatures.

The PO_4^{3-} retention may be diminished in sediments with a low pool of Fe(III) (Rozan et al. 2002) and/or with no bioturbating macrofauna (Bartoli et al. 2009). Hence under such conditions the PO_4^{3-} efflux could be much higher than measured in this experiment. A longer experiment could also have resulted in decreased PO_4^{3-} retention, because of exhaustion of the binding sites of PO_4^{3-} with Fe (III) (Jorgensen 1996). Nevertheless, the capacity of the sediment to retain PO_4^{3-} is notable considering that the amount of OM added in this experiment corresponded to the total OM deposited on the seabed below fish farms during a year, and that the OM added has a high reactivity, which implies that most of the OM is mineralized initially. Thus, marine sediment can act as a relevant sink of P, even under severe scenarios of temperature rise and organic enrichment, as long as it has a large enough pool of Fe and the macrofauna keeps the surface of the sediment under oxic conditions.

4.2. NH_4^+ release

Rates of NH_4^+-effluxes at 16ºC were within the range reported from previous laboratory experiments at similar conditions with non-enriched or enriched sediment (Valdemarsen et al. 2009;Valdemarsen et al. 2010). The 1-3 times temperature stimulation of NH_4^+-efflux were in the same range observed in sediments from coastal areas (i.e. temperature rise ca. 10ºC during summer resulted in one- to four-fold increases in NH_4^+-efflux) (Kristensen 1993). As with PO_4^{3-}, the release rates of NH_4^+ were always higher in +OM cores than in –OM treatments for a given temperature, highlighting that OM enrichment had a stimulatory effect on NH_4^+ release. This was expected since high NH_4^+-release is often observed in sediments enriched with labile organic matter (Christensen et al. 2000;Valdemarsen et al. 2012). NH_4^+ release was not stimulated by temperature between 16-22 ºC, but above 22ºC NH_4^+ release increased non-linearly in both –OM and +OM treatments. The notable increase of NH_4^+ release at a temperature increment of 10ºC in both –OM and +OM treatments, could led us to speculate that the efficiency of NH_4^+ oxidizing pathways is lowered at high temperatures, nevertheless the data of C:N ratios pointed to an opposite hypothesis.

The C:N ratios of C and N release during the experiment were always higher than the C:N ratios of the sediment OM, in particular at high temperature rise. This suggests that part of the mineralized N is not released to the water column as NH_4^+, but transformed into other N compounds. NH_4^+ could be nitrified in the oxidized surface sediment or in *N. divericolor* burrows, and subsequently reduced to N_2 via anammox or denitrification (Thamdrup 2012).

Previous studies show that both denitrification and anammox are stimulated by increasing temperatures (Nowicki 1994;Alsterberg et al. 2012;Canion et al. 2013) and by availability of OM (Nowicki 1994;Thamdrup and Dalsgaard 2002;Engstrom et al. 2005) which supports the data from this experiment. Nevertheless, evidence based on NO_3^- and NO_2^- data should be needed to confirm this hypothesis. Despite so, NH_4^+ is 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 NH_4^+ release rates can act as a proxy for total inorganic N release to the water column.

Despite that bioturbation and bioirrigation by macrofauna promotes OM mineralization, in the present experiment, both PO_4^{3-} and NH_4^+ effluxes were below the expected levels of the mineralized organic matter. In the case of PO_4^{3-}, this can be explained by the PO_4^{3-} retention capacity of the sediment that is promoted by macrofauna activity (Bartoli et al. 2009). In the case of NH_4^+, these results could be explained because macrofauna promotes NH_4^+-oxidizing processes and the coupling between nitrification and denitrification (Gilbert et al. 1995). Thus, sediment reworking processes of macrofauna promote nutrient recycling while preventing eutrophication.

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.

In conclusion, in future scenarios of climate change, sediment PO_4^{3-} release is expected to increase following the trends observed for OM mineralization rates in the sediment. The magnitude of the release of PO_4^{3-} is influenced not only by local OM deposition rates but also by sediment characteristics, particularly, the amount of reactive Fe in sediment. NH_4^+ release from sediment is not expected to increase substantially with temperature increments of up to 6ºC, in organic polluted as well as in non-polluted sediments. However, more severe temperature rises may be associated with a non-linear increase in the release of NH_4^+ from sediments. As most marine basins are N-limited, the excess release of NH_4^+ may set in motion a cascade of negative effects leading to deteriorating environmental quality. These effects are expected to be more detrimental in organic polluted areas as well as in coastal basins with no or restricted exchange with ocean waters.

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Tables

Table 1: Depth-integrated (0–16 cm) pools of P and N (mean ± SE) in the sediment used in the experiment (n=3, initial concentration) and the estimates on the amount of nutrient mineralized during the experiment based on nutrient sediment-water column fluxes (n=6). Values inside square brackets indicate the % of the nutrient mineralized out of the total added.

	Initial concentration (mmol m^{-2})	Mineralized (mmol m^{-2}), by temperature		
		16°C	22°C	26°C
-OM				
P	8178 ± 548	2.65 ± 1.12	4.35 ± 1.71	5.64 ± 1.42
N	7662 ± 401	252 ± 57.5	141 ± 59.3	441 ± 138
+OM				
P	8205 ± 72.1	7.54 ± 1.33 [4.2]	13.54 ± 2.4 [7.6]	16.49 ± 2.24 [9.3]
N	9854 ± 292	641 ± 130 [17.9]	623 ± 121 [17.4]	721 ± 165 [20.1]

Notes: The 16ºC treatment corresponds to the present mean sea surface temperature (SST) for summer months in the Baltic Sea. The 22 and 26ºC treatments were chosen as SST climate change scenarios in 60 and 100 years based on SST temperature rise observed in the last decades and expected raises (1ºC per decade in both cases). –OM and +OM correspond to non- and organic polluted treatments, respectively. The organic matter addition to +OM cores corresponded to 26 mol POC m^{-2}, which is comparable to the annual organic matter deposition in areas under the influence of mussel or fish farms. The final cores were maintained at 16, 22, or 26ºC in separate tanks containing filtered seawater.

Table 2: Depth-integrated (0–16 cm) pools of reactive Fe (III) and P bound to reactive Fe (mean ± SE) for initial cores (n = 3) and final cores (n = 6). See notes at Table 1 for explanation about treatments.

	Initial concentration (mol m^{-2})	Final concentration (mmol m^{-2}), by temperature		
		16°C	22°C	26°C
-OM				
Reactive Fe (III)	2.5 ± 0.1	2.0 ± 0.3	1.8 ± 0.1	2.3 ± 0.5
P bound to Fe	2.5 ± 0.1	2.1 ± 0.2	2.5 ± 0.2	2.6 ± 0.3
+OM				
Reactive Fe (III)	2.3 ± 0.2	1.9 ± 0.2	2.5 ± 0.3	2.3 ± 0.5
P bound to Fe	2.1 ± 0.2	2.4 ± 0.2	2.6 ± 0.3	2.0 ± 0.2

Table 3: Coefficients (means at 26ºC) of the regression model for PO_4^{3-} and NH_4^+ sediment-water column flux (i.e. efflux) rates along temperature for non-organic polluted (–OM) and organic polluted (+OM) treatments. Regression model for PO_4^{3-} corresponds to a first order polynomial regression, while for NH_4^+ correspond to an exponential regression (Table A1). Significant effects ($p<0.05$) are indicated in bold.

	PO_4^{3-} efflux rate	NH_4^+ efflux rate
	Coefficient (SE)	Coefficient (SE)
Intercept	**0.207** (0.047)	**13.58** (3.26)
OM	**0.420** (0.076)	**12.45** (5.27)
Temperature	**0.016** (0.008)	**0.946** (0.524)
OM x Temperature	**0.028** (0.012)	0.108 (0.803)

Table 4: Initial C:N:P ratio of the organic matter in the sediment estimated from particulate organic carbon, total nitrogen and total phosphorus and overall C:N:P ratio estimated from nutrient and total CO_2 flux over the experimental period. C and total CO_2 flux data was reported in Sanz-Lázaro *et al.* (2011b). See notes at table 1 for explanation of treatments.

	Initial ratio in the sediment	Overall ratio of the effluxes, by temperature		
		16°C	22°C	26°C
-OM				
C:N	12.7	13.3	25.3	15.1
C:P	11.6	849	656	988
N:P	0.9	63.8	25.9	65.3
+OM				
C:N	11.6	21.6	25.0	26.8
C:P	13.5	1866	1157	1174
N:P	1.2	86.5	46.3	43.7

Figure legends

Figure 1: Mineralization rates, shown as flux rates of total CO_2 (mean ± SE, n=6) versus temperature under non- (-OM) and organic polluted (+OM) conditions modified from Sanz-Lázaro et al. (2011b). Lines are shown to visualize the trajectory but do not represent a regression.

Figure 2: PO_4^{3-} and NH_4^+ efflux rates (n=6, mean ± SE) during the experiment at the three temperature scenarios under non- (-OM) and organic enrichment (+OM) conditions. Dotted lines are the line of reference corresponding to 0.

Figure 3: Nutrient sediment-water column flux rates (n=6, mean ± SE) versus temperature under non- (-OM) and organic polluted (+OM) conditions. Lines indicate significant ($p<0.05$) regressions for –OM and +OM treatments. Type of regression was choosen according to the AICc (corrected Akaike Information Criterion) (Table A1). R^2 refers to the whole regression model for each nutrient which includes the factor OM.

Figure 1

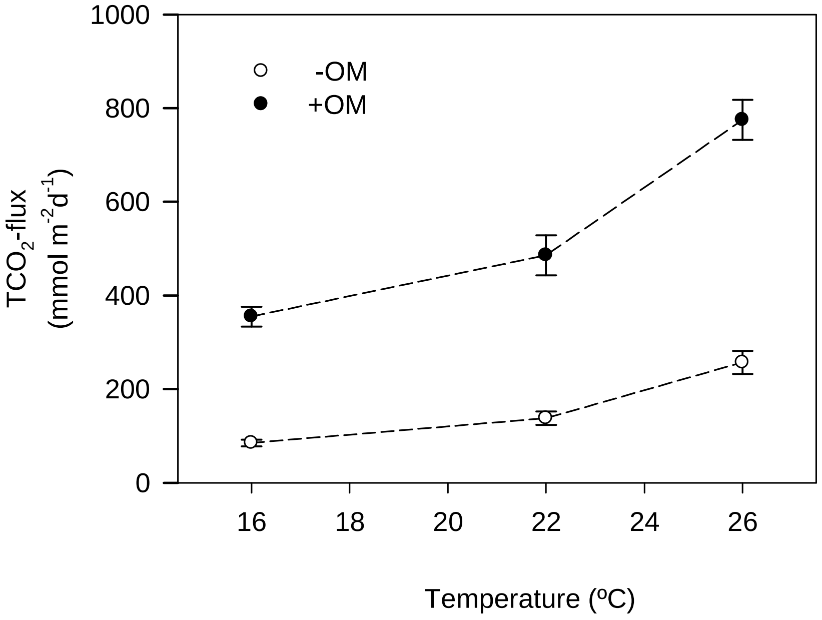

○ -OM
● +OM
TCO2-flux (mmol m-2 d-1)
0
200
400
600
800
1000
16
18
20
22
24
26
Temperature (ºC)

Figure 2

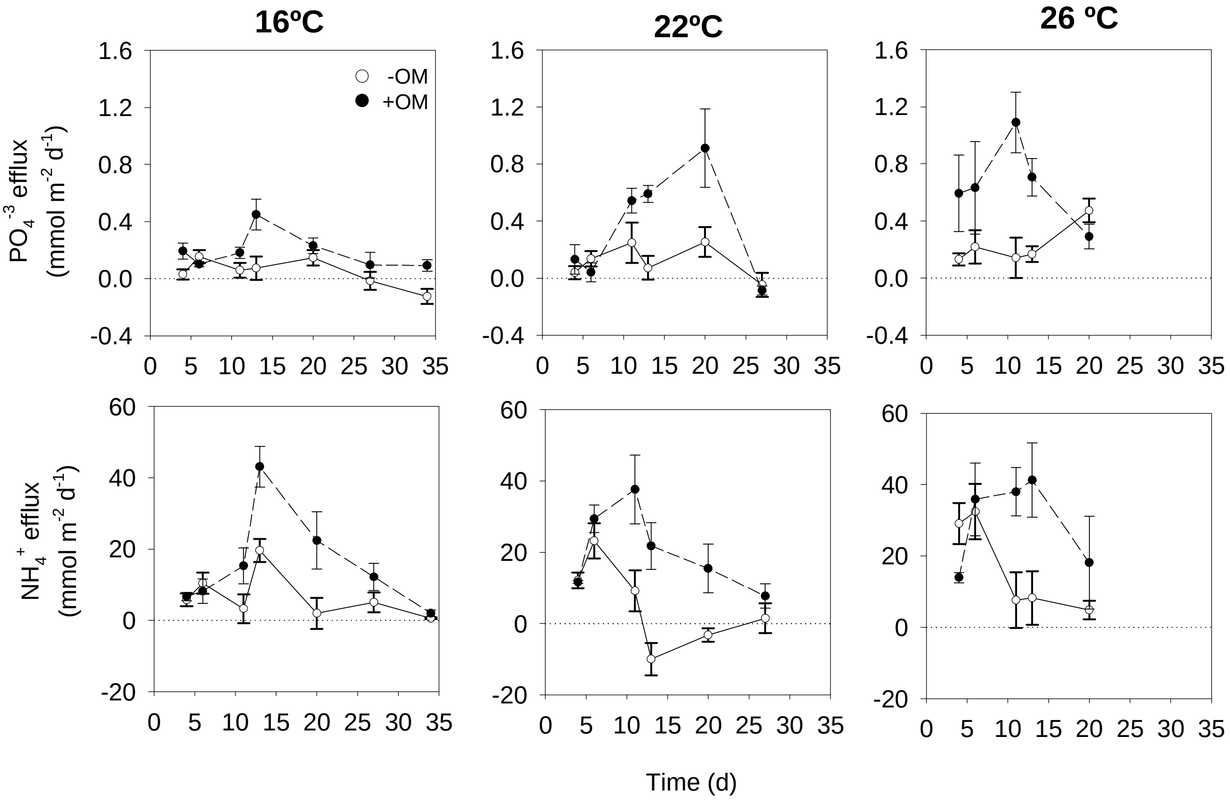
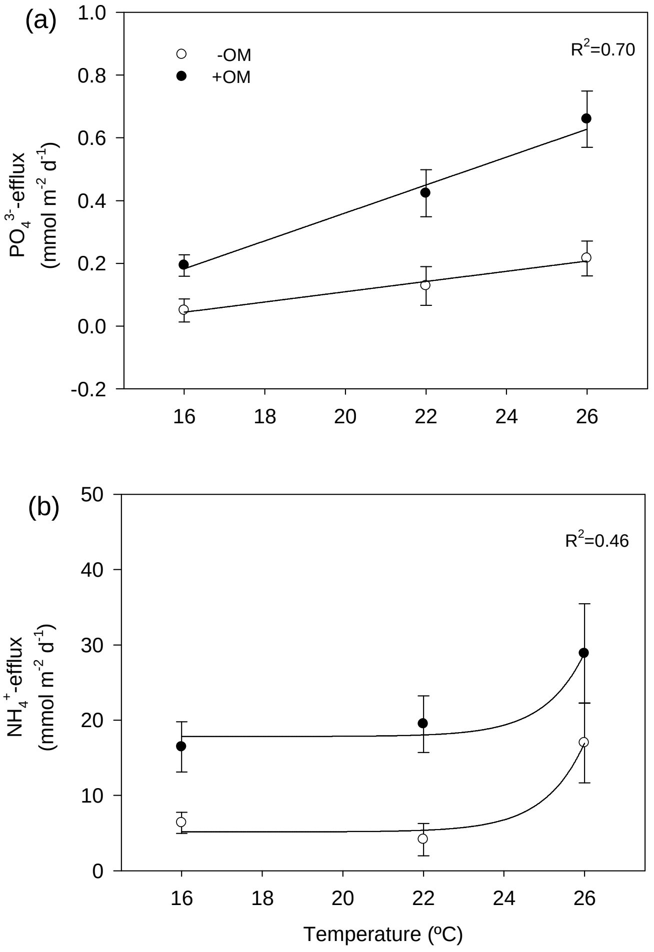

16ºC
22ºC
26 ºC
-OM
+OM
PO4-3 efflux (mmol m-2 d-1)
NH4+ efflux (mmol m-2 d-1)
1.6
1.2
0.8
0.4
0.0
-0.4
60
40
20
0
-20
0 5 10 15 20 25 30 35
Time (d)

Figure 3


(a)
-OM
+OM
R²=0.70
PO4 3- -efflux (mmol m-2 d-1)
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
16
18
20
22
24
26
(b)
R²=0.46
NH4 + -efflux (mmol m-2 d-1)
50
40
30
20
10
0
16
18
20
22
24
26
Temperature (ºC)

Appendix

Table A1: AICc (corrected Akaike Information Criterion) of the different regression models for PO_4^{3-} and NH_4^+ efflux rates.

	AICc	
	PO_4^{3-} efflux rates	NH_4^+ efflux rates
$y_i = \beta_0 + \beta_1 x_i$	-31.940	247.60
$y_i = \beta_0 + \beta_1 e^{x_i}$	-23.032	243.04
$y_i = \beta_0 + \beta_1 x_i + \beta_2 x_i^2$	-26.321	248.63
$y_i = \beta_0 + \beta_1 \frac{1}{x_i}$	-30.336	248.95