

**Climate warming  
effect on nutrient  
cycling**

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# Effects of global climate change and organic pollution on nutrient cycling in marine sediments

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## Abstract

Increasing ocean temperature due to climate change is an important anthropogenic driver of ecological change in coastal systems, where sediments play a major role in nutrient cycling. Our ability to predict ecological consequences of climate change is enhanced by simulating real scenarios especially when the interactions among drivers may not be just additive. 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.  $\text{PO}_4^{3-}$  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.  $\text{NH}_4^+$  release only increased significantly when the temperature rise was above  $6^\circ\text{C}$ , 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  $\text{PO}_4^{3-}$  retention and  $\text{NH}_4^+$  oxidation in the sediment. Bioturbation and bioirrigation appeared to be key processes responsible of this behaviour. Considering that the primary production of most marine basins is N-limited, the excess release of  $\text{NH}_4^+$  at temperature rise  $> 6^\circ$  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.

## 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  $0.2^\circ\text{C}$  per decade (IPCC, 2007), but in relatively enclosed and shallow coastal areas, such as the Baltic Sea, temperature has risen much more rapidly ( $1^\circ\text{C}$

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in last decade; Belkin, 2009; Mackenzie and Schiedek, 2007) and similar rises are expected for the next decades (Doscher and Meier, 2004).

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 this process organic phosphorous (P) and nitrogen (N) are transformed into inorganic forms ( $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$ , 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.

$\text{PO}_4^{3-}$  can be sequestered in the sediment by adsorption to ferric (oxy)hydroxides forming solid ferrous phosphates (Jorgensen, 1983). Sulfides resulting from sulfate reduction may mobilize  $\text{PO}_4^{3-}$  by reducing ferric oxyhydroxides (Roden and Edmonds, 1997) and by dissolving Fe minerals containing  $\text{PO}_4^{3-}$  (Gachter and Muller, 2003). Despite the complex interactions among the Fe, S and P pools,  $\text{PO}_4^{3-}$  release to the water column is mainly dependent on the redox conditions of the sediment surface, where oxidized Fe keep  $\text{PO}_4^{3-}$  immobilized. Thus seasonal increases in sediment metabolic rates due to temperature rise may lead to anoxic conditions in the sediment surface, resulting in  $\text{PO}_4^{3-}$  release during summer (Cowan and Boynton, 1996; Jensen et al., 1995). Mineralized N is mainly released to the water column as  $\text{NH}_4^+$  (Jorgensen, 1983).  $\text{NH}_4^+$  can be further transformed into  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , through nitrification, and end up as  $\text{N}_2$  through denitrification or anammox (Thamdrup, 2012). In contrast to  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$ ,  $\text{N}_2$  exits the biological N-cycle and need to be fixed again so it can be available again for primary producers. Consequently  $\text{N}_2$  production 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 (Kelly et al., 1985; Nixon, 1981). 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

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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 known 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 affects 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).

The aim of this work is to examine the effects of temperature rise and organic enrichment on sediment nutrient release. We used a mesocosm approach and hypothesized that temperature rise due to climate change increases  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  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  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  release.

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## 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),  $125 \mu\text{mol cm}^{-3}$  reactive Fe and 220  $\mu\text{m}$  average grain size) collected in Fænø Sund, Denmark (for further details see Valdemarsen 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  $\text{m}^{-2}$ , 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 26 °C 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 16 °C treatment was chosen as the reference level of present mean SST for summer months in temperate areas

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of the Baltic Sea (Holmer and Kristensen, 1996; Kristensen, 1993). The 22 and 26 °C treatments were chosen as SST climate change scenarios in 60 and 100 years based on SST temperature rise observed in the last decades (Belkin, 2009; Mackenzie and Schiedek, 2007) and expected raises (Doscher and Meier, 2004) (1 °C per decade in both cases).

The sediment in the cores was left 3 days to compact and acclimatize. Then three *N. diversicolor* were added to each core to simulate the natural density (Delefosse et al., 2012). *N. diversicolor* bioirrigation rates were previously reported in Sanz-Lázaro et al. (2011b). They were calculated based on Br<sup>-</sup>-addition experiments and were on average 13–24 L m<sup>-2</sup> d<sup>-1</sup> 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-Lázaro 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).

Additionally, six cores with 5 cm i.d. were also filled with –OM and +OM sediment to determine initial sediment conditions. 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 regularly. 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<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3-</sup> 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

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again. All samples were GF/F-filtered, transferred to 20 mL plastic vials and frozen ( $-20^{\circ}\text{C}$ ).

## 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  $26^{\circ}\text{C}$  was performed after 39, 32 and 25 days, respectively.

## 2.5 Sediment metabolic rates

C mineralization rates were estimated as time-integrated  $\text{TCO}_2$  sediment-water column flux divided by the experiment duration as described in Sanz-Lázaro et al. (2011b). Average C mineralization was  $85 \pm 7$ ,  $138 \pm 15$  and  $257 \pm 24$   $\text{mmol m}^{-2} \text{d}^{-1}$  in  $-OM$  cores at 16, 22 and  $26^{\circ}\text{C}$ , respectively, and  $355 \pm 21$ ,  $486 \pm 43$  and  $775 \pm 43$   $\text{mmol m}^{-2} \text{d}^{-1}$  in the  $+OM$  cores for 16, 22 and  $26^{\circ}\text{C}$ , respectively.

## 2.6 Analyses

$\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  were analyzed spectrophotometrically on a Lachat QuikChem 8500 autoanalyzer. Reactive Fe was extracted on  $\sim 0.2$  g of sediment subsamples with

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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).  $\text{PO}_4^{3-}$  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  $\text{PO}_4^{3-}$  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

15 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 26 °C, so intercepts showed mean values of effluxes at this temperature. The significance of the regression coefficients was tested by ANOVA. Ho-



mocedasticity 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  $\pm$  standard error (SE) and statistical tests were conducted with a significance level of  $\alpha = 0.05$ .

5 The molar ratio of C : N : P in the added OM was calculated based on its composition. The molar C : N : P ratio in the sediment was calculated based on POC, TN and TP content in unenriched and enriched sediment. C : N : P ratios of organic matter being mineralized were estimated based on  $\text{TCO}_2$ ,  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  effluxes. C and  $\text{TCO}_2$  effluxes were reported in Sanz-Lázaro et al. (2011b).

## 10 3 Results

### 3.1 P in sediment

TP concentration in initial -OM and +OM sediment was  $50.8 \pm 3.2$  and  $51.2 \pm 0.3 \mu\text{mol cm}^{-3}$ , respectively. Depth-integrated (0–16 cm) TP thus amounted to  $8.2 \pm 0.5$  and  $8.2 \pm 0.1 \text{ mol m}^{-2}$  in initial -OM and +OM cores, respectively (Table 1). The initial enrichment with fish feed should have resulted in  $0.4 \text{ mol m}^{-2}$  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).  
15  
20 Depth-integrated (0–16 cm) Fe-bound P corresponded to  $2.5 \pm 0.1$  and  $2.1 \pm 0.2 \text{ mol m}^{-2}$  in initial -OM and +OM sediment, respectively. By the end, Fe-bound P ranged between  $2.1 \pm 0.2$  and  $2.6 \pm 0.3 \text{ mmol m}^{-2}$  in -OM cores, and between  $2.0 \pm 0.2$  and  $2.6 \pm 0.3 \text{ mmol m}^{-2}$  in +OM cores (Table 2).

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### 3.2 N in sediment

PON concentration in initial -OM and +OM sediment was  $47.5 \pm 2.4$  and  $61.4 \pm 1.6 \mu\text{mol cm}^{-3}$ , respectively. Thus depth-integrated (0–16 cm) PON was  $7.7 \pm 0.4$  and  $9.8 \pm 0.3 \text{ mol m}^{-2}$  in initial -OM and +OM cores, respectively (Table 1). The enrichment resulted in significantly higher PON in +OM cores ( $p < 0.05$ ). 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  $3.6 \text{ mol m}^{-2}$  (46.8 % of the PON concentration in the sediment), which was close to the measured enrichment (28.6 % of the PON in the sediment).

### 3.3 Fe in sediment

Depth-integrated (0–16 cm) pools of reactive Fe (III) were  $2.5 \pm 0.1$  and  $2.3 \pm 0.2 \text{ mol m}^{-2}$  in initial -OM and +OM sediment, respectively. After the experiment, the pools of reactive Fe (III) were  $2.0 \pm 0.3$ ,  $1.8 \pm 0.1$  and  $2.3 \pm 0.5 \text{ mmol m}^{-2}$  in -OM cores at 16, 22, and  $26^\circ\text{C}$ , respectively, and  $1.9 \pm 0.2$ ,  $2.5 \pm 0.3$  and  $2.3 \pm 0.5 \text{ mmol m}^{-2}$  in +OM cores at 16, 22, and  $26^\circ\text{C}$  (Table 2). Fe (III) concentration was high in the first cm of the sediment being  $32.9 \pm 8.0$ ,  $35.5 \pm 4.3$  and  $25.1 \pm 5.6 \mu\text{mol cm}^{-3}$  in -OM cores at 16, 22, and  $26^\circ\text{C}$ , respectively, and  $12.7 \pm 3.6$ ,  $24.0 \pm 6.9$  and  $28.5 \pm 6.2 \mu\text{mol cm}^{-3}$  in +OM cores at 16, 22, and  $26^\circ\text{C}$  (Fig. 1).

### 3.4 Nutrient release

$\text{PO}_4^{3-}$  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  $\text{PO}_4^{3-}$  efflux were stimulated by OM enrichment and temperature. In -OM cores  $\text{PO}_4^{3-}$  efflux ranged from -0.3 to 0.3, -0.4 to 0.8 and -0.2 to  $0.7 \text{ mmol m}^{-2} \text{ d}^{-1}$  at 16, 22 and  $26^\circ\text{C}$ , respectively. In +OM cores  $\text{PO}_4^{3-}$  efflux ranged

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from  $-0.2$  to  $1.0$ ,  $-0.2$  to  $1.9$  and  $0.0$  to  $1.4$   $\text{mmol m}^{-2} \text{d}^{-1}$  at  $16$ ,  $22$  and  $26$  °C, respectively.

Average  $\text{PO}_4^{3-}$  efflux over the whole experiment was  $1.7 \pm 1.2$ ,  $3.5 \pm 1.7$  and  $4.3 \pm 1.1$   $\text{mmol m}^{-2}$  in  $-$ OM cores at  $16$ ,  $22$ , and  $26$  °C, respectively. In  $+$ OM cores total  $\text{PO}_4^{3-}$  effluxes were  $6.6 \pm 1.2$ ,  $11.4 \pm 2.0$  and  $13.2 \pm 1.8$   $\text{mmol m}^{-2}$  in  $+$ OM cores at  $16$ ,  $22$ , and  $26$  °C, respectively, corresponding to  $4.2$ ,  $7.6$  and  $9.3$  % of the total P added in fish feed (Table 1). Average  $\text{PO}_4^{3-}$  efflux showed a linear increasing trend along temperature in both  $-$ OM and  $+$ OM cores (Fig. 3a). The AICc showed that  $\text{PO}_4^{3-}$  effluxes were best fitted with a linear regression (Table A1). The regression analysis showed that average  $\text{PO}_4^{3-}$  efflux increased linearly with temperature ( $p < 0.05$ ) in  $-$ OM and  $+$ OM cores. There were significant differences between  $-$ OM and  $+$ OM treatments at  $26$  °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 (Table 3).

The overall trend of  $\text{NH}_4^+$ -efflux was similar to  $\text{PO}_4^{3-}$ -efflux.  $\text{NH}_4^+$  effluxes increased at the beginning, peaked and then decreased towards the end (Fig. 2). The range of the  $\text{NH}_4^+$  effluxes was stimulated by both organic enrichment and temperature. In  $-$ OM cores  $\text{NH}_4^+$  efflux ranged from  $-12.5$  to  $27.8$ ,  $-20.7$  to  $35.8$  and  $-16.2$  to  $54.6$   $\text{mmol m}^{-2} \text{d}^{-1}$  at  $16$ ,  $22$  and  $26$  °C, respectively. In  $+$ OM cores  $\text{NH}_4^+$  efflux ranged from  $0.0$  to  $58.8$ ,  $0.0$  to  $60.0$  and  $3.1$  to  $55.6$   $\text{mmol m}^{-2} \text{d}^{-1}$  at  $16$ ,  $22$  and  $26$  °C, respectively.

Average  $\text{NH}_4^+$  efflux over the whole experiment was  $217 \pm 48$ ,  $112 \pm 58$  and  $339 \pm 106$   $\text{mmol m}^{-2}$  in  $-$ OM cores at  $16$ ,  $22$ , and  $26$  °C, respectively. Total  $\text{NH}_4^+$  effluxes in  $+$ OM cores were  $559 \pm 113$ ,  $525 \pm 102$  and  $577 \pm 132$   $\text{mmol m}^{-2}$  at  $16$ ,  $22$ , and  $26$  °C, corresponding to  $17.9$ ,  $17.4$  and  $20.1$  % of N added in fish feed (Table 1). The trend of average  $\text{NH}_4^+$ -effluxes over the whole experiment was not as clear as for  $\text{PO}_4^{3-}$  effluxes (Fig. 3b).  $\text{NH}_4^+$  effluxes were clearly stimulated at  $26$  °C in both sediment types, however. According to the AICc, the trend of  $\text{NH}_4^+$  efflux over temperature was best fitted with an exponential regression (Table A1). The regression analysis showed significant

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respectively. This indicated that  $\text{PO}_4^{3-}$  release occurred in proportion to total sediment metabolism. However, the release of  $\text{PO}_4^{3-}$  was much lower than that of  $\text{TCO}_2$ , as indicated by C : P ratios above 600 in all treatments. This was far below the C : P ratio in the sediment (11.6 and 13.5 for -OM and +OM treatments, respectively) and the C : P ratio in the added OM (21.3), suggesting that organic P was either less labile compared to organic C or that inorganic  $\text{PO}_4^{3-}$  was retained in the sediment to a larger extent than C. For each temperature, we estimated the mineralized OM out of the OM added by subtracting the  $\text{TCO}_2$  production in the +OM treatments to the  $\text{TCO}_2$  production in the -OM treatments. We found that 55–77 % of the added OM was mineralized, whereas P efflux only corresponded to 4.2–9.3 % of the added P (Table 2). Taking into account that the OM added, fish feed, is highly labile, our data suggest  $\text{PO}_4^{3-}$  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 pools of P, Fe and S in the sediment, interactions between these pools and the sediment redox-conditions (Gachter and Muller, 2003; Roden and Edmonds, 1997). Generally, in sediments with an oxic surface layer, oxidized Fe acts as a lid for  $\text{PO}_4^{3-}$ , which is adsorbed to ferric Fe preventing its mobilization to the water column (Rozan et al., 2002). All the treatments maintained a considerable pool of oxidized Fe(III) ( $1.8$  to  $2.5 \text{ mol m}^{-2}$ ), in the same range as measured at the beginning of the experiment ( $2.3$  to  $2.5 \text{ mol m}^{-2}$ ) indicating that the  $\text{PO}_4^{3-}$  retention capacity of the sediment was maintained despite elevated temperature and organic enrichment. Similarly, levels of  $\text{PO}_4^{3-}$  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

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the absence of macrofauna (Valdemarsen et al., 2009). All these results suggest that the capacity of the sediment to retain  $\text{PO}_4^{3-}$  was maintained despite high metabolic rates due to organic enrichment and elevated temperatures.

The  $\text{PO}_4^{3-}$  retention may be diminished in sediments with a low pool of Fe(III) (Roza et al., 2002) and/or with no bioturbating macrofauna (Bartoli et al., 2009). Hence under such conditions the  $\text{PO}_4^{3-}$  efflux could be much higher than measured in this experiment. A longer experiment could also have resulted in decreased  $\text{PO}_4^{3-}$  retention, because of exhaustion of the binding sites of  $\text{PO}_4^{3-}$  with Fe (III) (Jorgensen, 1996). Nevertheless, the capacity of the sediment to retain  $\text{PO}_4^{3-}$  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 $\text{NH}_4^+$ release

Rates of  $\text{NH}_4^+$ -effluxes at 16 °C were within the range reported from previous laboratory experiments at similar conditions with non-enriched or enriched sediment (Valdemarsen et al., 2009, 2010). The 1–3 times temperature stimulation of  $\text{NH}_4^+$ -efflux were in the same range observed in sediments from coastal areas (i.e. temperature rise ca. 10 °C during summer resulted in one- to four-fold increases in  $\text{NH}_4^+$ -efflux) (Kristensen, 1993). As with  $\text{PO}_4^{3-}$ , the release rates of  $\text{NH}_4^+$  were always higher in +OM cores than in –OM treatments for a given temperature, highlighting that OM enrichment had a stimulatory effect on  $\text{NH}_4^+$  release. This was expected since high  $\text{NH}_4^+$ -release is often observed in sediments enriched with labile organic matter (Christensen et al., 2000; Valdemarsen et al., 2012).  $\text{NH}_4^+$  release was not stimulated by temperature between 16–22 °C, but above 22 °C  $\text{NH}_4^+$  release increased exponentially in both –OM and +OM

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treatments. The notable increase of  $\text{NH}_4^+$  release at a temperature increment of  $10^\circ\text{C}$  in both  $-OM$  and  $+OM$  treatments, could led us to speculate that the efficiency of  $\text{NH}_4^+$  oxidizing pathways is lowered at high temperatures, nevertheless the data of C : N ratios pointed to an opposite hypothesis.

5 The C : N ratio of the added OM was 5.2, whereas the C : N ratios of the sediment OM at the beginning of the experiment was close to 12 in both  $-OM$  and  $+OM$  cores. 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 (C : N ratios of C and N release were 25.3 and 25.0 for a temperature increment of  $6^\circ\text{C}$ , 15.1 and 26.8 for a temperature increment of  $10^\circ\text{C}$ , in  $-OM$  and  $+OM$  cores, respectively).  
10 This suggests that part of the mineralized N is not released to the water column as  $\text{NH}_4^+$ , but transformed into other N compounds.  $\text{NH}_4^+$  could be nitrified in the oxidized surface sediment or in the burrows, and subsequently reduced to  $\text{N}_2$  via anammox or denitrification (Thamdrup, 2012). Previous studies show that both denitrification and anammox are stimulated by increasing temperatures (Alsterberg et al., 2012; Canion et al., 2013; Nowicki, 1994) and by availability of OM (Engstrom et al., 2005; Nowicki, 1994; Thamdrup and Dalsgaard, 2002) which supports the data from this experiment. Nevertheless, evidence based on  $\text{NO}_3^-$  and  $\text{NO}_2^-$  data should be needed to confirm this hypothesis.

20 Despite bioturbation and bioirrigation produced by macrofauna promotes OM mineralization, in the present experiment, both  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  effluxes were below the expected levels of the mineralized organic matter. In the case of  $\text{PO}_4^{3-}$ , this can be explained by the  $\text{PO}_4^{3-}$  retention capacity of the sediment that is promoted by macrofauna activity (Bartoli et al., 2009). In the case of  $\text{NH}_4^+$ , these results could be explained because macrofauna promotes  $\text{NH}_4^+$ -oxidizing processes and the coupling between nitrification and denitrification (Gilbert et al., 1995). Thus, sediment reworking processes of macrofauna promote nutrient recycling while prevent eutrophication.

25 While there is, to some extent, experimental manipulative experiments that focus on the effects of carbon mineralization rates of marine sediments on warming scenarios

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(Finke and Jorgensen, 2008; Robador et al., 2009; Sanz-Lazaro et al., 2011b), less information is on the mineralization rates of N and P. Marine sediments act as an important source of nutrients for water column primary production (Nixon, 1981). Global warming may lead to eutrophication of the water column by increasing the nutrient release from sediments. For both nutrients, the effluxes increased with temperature, but followed significantly different trends.  $\text{PO}_4^{3-}$  effluxes increased linearly, highlighting that P mineralization is temperature dependent. On the other hand,  $\text{NH}_4^+$  effluxes showed an exponentially increasing trend suggesting that N mineralization is expected to be independent from temperature rises of up to  $6^\circ\text{C}$ . Since in most marine basins, primary production in the water column is limited by N (Zehr and Kudela, 2011), the predicted increases in  $\text{NH}_4^+$  effluxes may have important consequences for temperature rises above  $6^\circ\text{C}$ . As an example, in the Baltic Sea, N demand of phytoplankton is  $5.2 \text{ mmol m}^{-2} \text{ d}^{-1}$  (Koop et al., 1990). Thus, in this case, phytoplankton demand of N would be surpassed above temperature increments of  $6^\circ\text{C}$  or with no increments in temperature, under non- and organic enrichment conditions, respectively. Under these scenarios, the excess of  $\text{NH}_4^+$  efflux could stimulate primary production, which may lead to excessive proliferation of algae.

In conclusion, in future scenarios of climate change, sediment  $\text{PO}_4^{3-}$  release is expected to increase following the trends observed for OM mineralization rates in the sediment. The magnitude of the release of  $\text{PO}_4^{3-}$  is influenced not only by local OM deposition rates but also by sediment characteristics, particularly, the amount of reactive Fe in sediment.  $\text{NH}_4^+$  release from sediment is not expected to increase substantially with temperature increments of up to  $6^\circ\text{C}$ , in organic polluted as well as in non-polluted sediments. However, more severe temperature rises may be associated with an exponential increase in the release of  $\text{NH}_4^+$  from sediments. As most marine basins are N-limited, the excess release of  $\text{NH}_4^+$  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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**Table 1.** Depth-integrated (0–16 cm) pools of P and N (mean  $\pm$  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 <sup>-2</sup> )	Mineralized (mmol m <sup>-2</sup> ), by temperature		
		16 °C	22 °C	26 °C
<b>–OM</b>				
P	8178 $\pm$ 548	2.65 $\pm$ 1.12	4.35 $\pm$ 1.71	5.64 $\pm$ 1.42
N	7662 $\pm$ 401	252 $\pm$ 57.5	141 $\pm$ 59.3	441 $\pm$ 138
<b>+OM</b>				
P	8205 $\pm$ 72.1	7.54 $\pm$ 1.33 [4.2]	13.54 $\pm$ 2.4 [7.6]	16.49 $\pm$ 2.24 [9.3]
N	9854 $\pm$ 292	641 $\pm$ 130 [17.9]	623 $\pm$ 121 [17.4]	721 $\pm$ 165 [20.1]

Notes: the 16 °C treatment corresponds to the present mean sea surface temperature (SST) for summer months in the Baltic Sea. The 22 and 26 °C 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 (1 °C decade<sup>-1</sup> 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<sup>-2</sup>, 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 26 °C in separate tanks containing filtered seawater.

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

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





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**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<sub>2</sub> flux over the experimental period. C and total CO<sub>2</sub> 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

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**Table A1.** AICc (corrected Akaike Information Criterion) of the different regression models for  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  efflux rates.

	AICc	
	$\text{PO}_4^{3-}$ efflux rates	$\text{NH}_4^+$ 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

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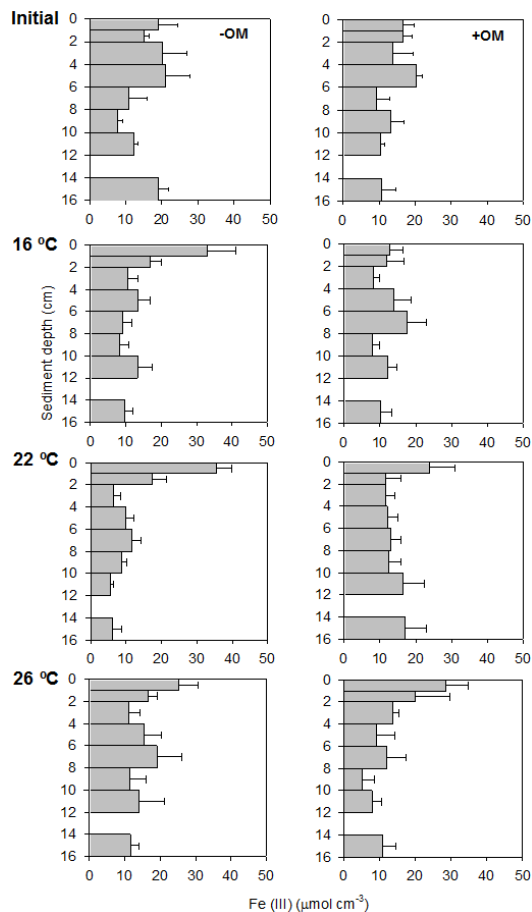
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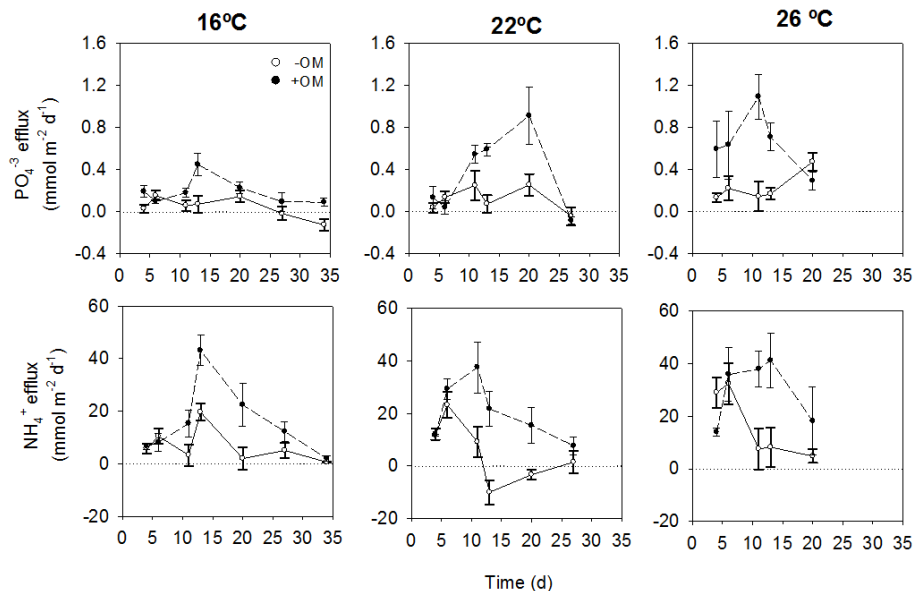




**Figure 1.** Pools (mean  $\pm$  SE) of reactive Fe(III) vs. depth for initial cores ( $n = 3$ ) and final cores ( $n = 6$ ) in the different temperature treatments.

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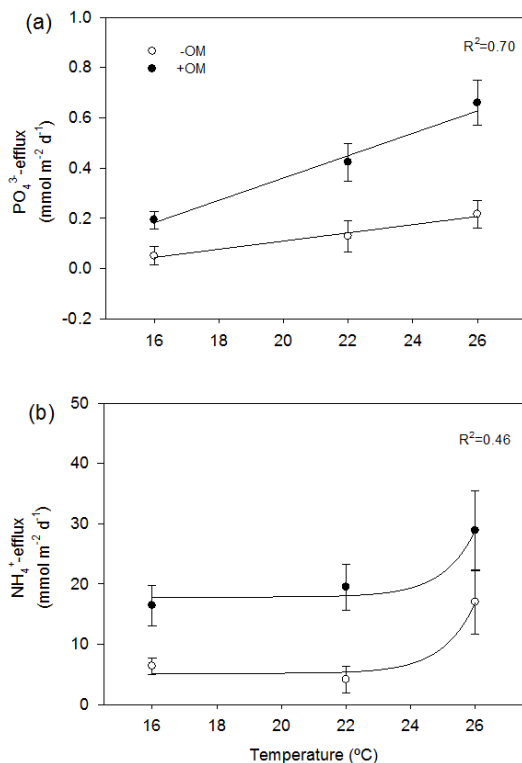
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**Figure 2.**  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  efflux rates ( $n = 6$ , mean  $\pm$  SE) during the experiment at the three temperature scenarios under non- (–OM) and organic enrichment (+OM) conditions. Dotted lines are the line of reference corresponding to 0.

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**Figure 3.** Nutrient sediment-water column flux rates ( $n = 6$ , mean  $\pm$  SE) vs. 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 chosen 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.