

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

2

3

4 Carlos Sanz-Lázaro<sup>1,2\*</sup>, Thomas Valdemarsen<sup>1</sup> & Marianne Holmer<sup>1</sup>

5

6 <sup>1</sup>Department of Biology, University of Southern Denmark, Campusvej 55, 5230 Odense,

7 Denmark

8 <sup>2</sup>Present address: Departamento de Ciencias del Mar y Biología Aplicada, Universidad de

9 Alicante, P.O. Box 99, E-03080 Alicante, Spain.

10

11 \*Corresponding author:

12 E-mail: carsanz@ua.es, [carsanzla@gmail.com](mailto:carsanzla@gmail.com)

13

14 Running title: Climate warming effect on nutrient cycling

15

16

17 Abstract

18 Increasing ocean temperature due to climate change is an important anthropogenic driver of  
19 ecological change in coastal systems. In these systems sediments play a major role in nutrient  
20 cycling. Our ability to predict ecological consequences of climate change is enhanced by  
21 simulating real scenarios. Based on predicted climate change scenarios, we tested the effect of  
22 temperature and organic pollution on nutrient release from coastal sediments to the water column  
23 in a mesocosm experiment.  $\text{PO}_4^{3-}$  release rates from sediments followed the same trends as  
24 organic matter mineralization rates, and increased linearly with temperature and were  
25 significantly higher under organic pollution than under non-polluted conditions.  $\text{NH}_4^+$  release  
26 only increased significantly when the temperature rise was above  $6^\circ\text{C}$ , and was significantly  
27 higher in organic polluted compared to non-polluted sediments. Nutrient release to the water  
28 column was only a fraction from the mineralized organic matter, suggesting  $\text{PO}_4^{3-}$  retention and  
29  $\text{NH}_4^+$  oxidation in the sediment. Bioturbation and bioirrigation appeared to be key processes  
30 responsible for this behaviour. Considering that the primary production of most marine basins is  
31 N-limited, the excess release of  $\text{NH}_4^+$  at a temperature rise  $> 6$  degrees could enhance water  
32 column primary productivity, which may lead to the deterioration of the environmental quality.  
33 Climate change effects are expected to be accelerated in areas affected by organic pollution.

34

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

37 1. Introduction

38 Ocean temperature rise due to climate change is considered among the most severe  
39 anthropogenic drivers of ecological change in marine systems, especially in coastal areas  
40 (Halpern et al. 2008). Sea surface temperature (SST) in marine systems is expected to rise 0.2°C  
41 per decade (IPCC 2007), but in relatively enclosed and shallow coastal areas, such as the Baltic  
42 Sea, temperature has risen much more rapidly (1°C in last decade; (Mackenzie and Schiedek  
43 2007;Belkin 2009) and similar rises are expected for the next decades (Doscher and Meier 2004).  
44 In these areas, heterotrophic processes in sediments prevail due to the generally low availability  
45 of light in the seabed due to eutrophication and high input of labile organic matter (Conley et al.  
46 2009).

47 Coastal areas are climate-sensitive systems (IPCC 2007) that play an important role in the  
48 mineralization of organic matter (OM) (Middelburg et al. 1997). During mineralization, organic  
49 phosphorous (P) and nitrogen (N) are transformed into inorganic forms ( $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$ ,  
50 respectively). OM is mineralized mainly by microbial processes, which are strongly influenced  
51 by temperature (Robador et al. 2009). Consequently, climate change may affect nutrient  
52 regeneration rates in coastal ecosystems.

53  $\text{PO}_4^{3-}$  can be sequestered in the sediment by adsorption to ferric (oxy)hydroxides and by  
54 forming solid ferrous phosphates (Jorgensen 1983). Sulfides resulting from sulfate reduction may  
55 mobilize  $\text{PO}_4^{3-}$  by reducing ferric oxyhydroxides (Roden and Edmonds 1997) and by dissolving  
56 Fe minerals containing  $\text{PO}_4^{3-}$  (Gachter and Muller 2003). Despite the complex interactions  
57 among the Fe, S and P pools,  $\text{PO}_4^{3-}$  release to the water column is mainly dependent on the redox  
58 conditions in the sediment surface, where oxidized Fe keep  $\text{PO}_4^{3-}$  immobilized. Thus seasonal  
59 increases in sediment metabolic rates due to temperature rise may lead to reducing conditions in

60 the sediment surface, resulting in  $\text{PO}_4^{3-}$  release during summer (Jensen et al. 1995; Cowan and  
61 Boynton 1996). Mineralized N is mainly released to the water column as  $\text{NH}_4^+$  (Jorgensen 1983).  
62  $\text{NH}_4^+$  can be further transformed into  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , through nitrification, and end up as  $\text{N}_2$   
63 through denitrification or anammox (Thamdrup 2012). In contrast to  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$ ,  $\text{N}_2$   
64 can not be directly used by primary producers. Consequently denitrification results in N removal  
65 from the ecosystem preventing eutrophication (Thamdrup and Dalsgaard 2008). Marine coastal  
66 sediments play a major role in supporting primary productivity of the water column by supplying  
67 a large part of the nutrient demand for phytoplankton (Nixon 1981; Kelly et al. 1985). Increases  
68 in sea water temperature and input of OM to the sediment, are both expected to stimulate the  
69 release of inorganic nutrients from the sediment to the water column, which may in turn  
70 stimulate primary productivity. Nutrient increase above a certain threshold may lead to algal  
71 blooms and subsequent hypoxic/anoxic events, resulting in the deterioration of ecological status  
72 (Gray et al. 2002).

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

81 Our ability to predict the ecological consequences of climate change is enhanced by  
82 simulating realistic future scenarios. Hence, it is important to conduct experiments to elucidate

83 not only the effects of individual drivers of ecological change but also the interactions among  
84 them, especially when the interactions among these drivers are not just additive (Falkenberg et  
85 al. 2013). Despite the current concern of global change, scarce research effort has been taken on  
86 understanding how temperature rise affect the cycling of N and P in coastal sediments  
87 (Alsterberg et al. 2012). In fact, the accuracy of predictive biogeochemical models related to the  
88 effect of climate change on the coastal eutrophication is restrained due to limited knowledge  
89 (Meier et al. 2011). Thus, to improve our forecasting capacity related to climate change,  
90 estimates of temperature driven changes in sediment nutrient release are needed.

91         The aim of this work is to examine the effects of temperature rise and organic enrichment  
92 on sediment nutrient release derived from heterotrophic processes. We used a mesocosm  
93 approach and hypothesized that temperature rise due to climate change increases  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$   
94 release from the sediments as a consequence of increased metabolic rates. We expected an  
95 increase of the release of nutrients with temperature, with a more marked effect in organic  
96 enriched than in non-organic enriched sediments, due to stimulation of anaerobic processes  
97 resulting in  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  release.

98

99

## 100 2. Materials and methods

101

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

104

### 105 2.1. Collection of sediment and polychaetes

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

113

## 114 2.2. Experimental setup

115 Sediment was split into either control (-OM) or organically enriched sediment (+OM).  
116 Enrichment was performed by adding 92 g labile OM [finely ground fish feed, Ecolife, Dansk  
117 Ørredfoder with 49.4% DW POC, 8.1% DW total nitrogen organic N (TN), 0.9% DW total P  
118 (TP)] to 20 L of sediment. The enrichment corresponded to 26 mol POC  $\text{m}^{-2}$ , which is  
119 comparable to the annual OM deposition at fish or mussel farms (Callier et al. 2006;Holmer et al.  
120 2007;Sanz-Lazaro et al. 2011a).

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

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

130 The sediment in the cores was left 3 days to compact and acclimitize. Then three *N.*  
131 *diversicolor* were added to each core to simulate the natural density (Delefosse et al. 2012). The  
132 time of polychaete addition was assumed the beginning of the experiment (t = 0). *N. diversicolor*  
133 bioirrigation rates were previously reported in Sanz-Lázaro et al. (2011b). They were calculated  
134 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  
135 treatments. There were no significant (p < 0.05) effects of temperature or of OM enrichment on  
136 bioirrigation rates and all cores were bioirrigated to 6–8 cm depth (Sanz-Lazaro et al. 2011b).  
137 Bioirrigation rates in the present experiment were similar to the bioirrigation measured  
138 previously for other polychaetes (Quintana et al. 2013) and within the range of the bioirrigation  
139 performed by natural macrofaunal communities (Valdemarsen et al. 2010). Unfortunately  
140 irrigation rates and visual observations showed that the added worms died in three +OM cores at  
141 26 degrees, and results from these cores were omitted from further analysis. For the rest of the  
142 cores no polychaetes were found dead during the experiment, and active *N. diversicolor* were  
143 observed in all the rest of the cores. The bromide incubations indicated that *N. diversicolor*  
144 ventilated their burrows with the same intensity regardless of OM-enrichment level.

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

151

### 152 2.3. Nutrient fluxes

153  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  fluxes between sediment and water were measured every 2-4 days during the  
154 first 2 weeks and every week during the rest of the experiment. During flux measurements, the  
155 water column of each sediment core was sampled and cores were closed with rubber stoppers.  
156 Incubations were ended after 3-5 h (-OM) or 1-2 h (+OM), where the rubber stoppers were  
157 removed and the water column was sampled again. All samples were GF/F-filtered, transferred  
158 to 20 mL plastic vials and frozen (-20°C).

159

### 160 2.4. Sectioning of cores

161 The three 5 cm i.d. sediment cores of each sediment type (-OM and +OM) were initially  
162 sectioned ( $t = 0$ ; henceforth referred to as initial cores) and the remaining cores from every  
163 temperature treatment were sectioned at the end of the experiment (henceforth, final cores).  
164 Cores were sectioned into 1 cm slices to 2 cm depth and into 2 cm slices to 16 cm depth. Every  
165 sediment slice was homogenized and subsampled for different analysis. One subsample from  
166 each sediment slice was used to measure reactive Fe and phosphate bound to reactive Fe. A  
167 subsample of sediment was used for TP measurement. The remaining sediment from each slice  
168 was used to determine TN, sediment density and water content. All analytical methods are  
169 described below. Since temperature has a strong stimulatory effect on microbial reaction rates,  
170 the duration of the temperature treatments varied to prevent porewater sulfate depletion and a  
171 shift in sediment metabolism towards methanogenesis. Thus, the sectioning of the sediments at  
172 16, 22 and 26°C was performed after 39, 32 and 25 days, respectively.

173



174 2.5. Sediment metabolic rates

175 C mineralization rates were estimated as time-integrated total CO<sub>2</sub> (TCO<sub>2</sub>) sediment-water  
176 column flux divided by the experiment duration as described in Sanz-Lázaro *et al.* (2011b) (Fig.  
177 1). TCO<sub>2</sub> fluxes were measured following same procedures as for nutrient fluxes and TCO<sub>2</sub>  
178 concentration was analyzed by flow injection analysis (Hall and Aller 1992).

179

180 2.6. Analyses

181 NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3-</sup> were analyzed spectrophotometrically on a Lachat QuikChem 8500  
182 autoanalyzer. Reactive Fe was extracted on ~0.2 g of sediment subsamples with 5 ml of 0.5 M  
183 HCl shaken for 30 min. Then samples were centrifuged for 5 min (3000 rpm, ca. 1000 × g) and  
184 GF/F filtered. The supernatant was analyzed by the ferrozine method (Stookey 1970). Fe (II) was  
185 determined on the untreated extract while total Fe [Fe(II)+Fe(III)] was determined after  
186 reduction with hydroxylamine (Lovley and Phillips 1987). Fe (III) was determined as the  
187 difference between total Fe and Fe(II). PO<sub>4</sub><sup>3-</sup> in HCl extracts was also analyzed  
188 spectrophotometrically after addition of Molybdate reagent (Koroleff 1983) to get a measure of  
189 Fe-bound P. Total P was determined on combusted sediment subsamples (520°C, 2 h), which  
190 were boiled in 1 M HCl for 1 h. The supernatant was analyzed for PO<sub>4</sub><sup>3-</sup> as described above. TN  
191 was determined on a Carlo Erba CHN EA1108 elemental analyzer according to Kristensen and  
192 Andersen (1987). Sediment density was determined gravimetrically by weighing a known  
193 volume of sediment using cut off syringes. Water content was measured as weight loss after  
194 drying (105°C, >12 h).

195

196 2.7. Data analysis

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

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

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

218

219

## 220 3. RESULTS

221

### 222 3.1. P in sediment

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

231

### 232 3.2. N in sediment

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

238

### 239 3.3. Fe in sediment

240 Pools of reactive Fe (III) were very similar between initial –OM and +OM cores, as well as  
241 between treatments at the end of the experiment (Table 2). Fe (III) concentration was always

242 high in the first cm of the sediment in all treatments but also showed a high variability, ranging  
243 from  $35.5 \pm 4.3$  to  $12.7 \pm 3.6 \mu\text{mol cm}^{-3}$ .

244

#### 245 3.4. Nutrient release

246  $\text{PO}_4^{3-}$  efflux showed a similar temporal pattern in the different treatments: an increase in the first  
247 days, a peak at intermediate stages and then decreasing effluxes towards the end (Fig. 2). The  
248 peaks of  $\text{PO}_4^{3-}$  efflux were stimulated by OM enrichment and temperature. Average  $\text{PO}_4^{3-}$  efflux  
249 showed a linear increasing trend along temperature in both –OM and +OM cores (Fig 3a). The  
250 AICc showed that  $\text{PO}_4^{3-}$  effluxes were best fitted with a linear regression (Table A1). The  
251 regression analysis showed that average  $\text{PO}_4^{3-}$  efflux increased linearly with temperature  
252 ( $p < 0.05$ ) in –OM and +OM cores. There were significant differences between -OM and +OM  
253 treatments at 26°C ( $p < 0.05$ ) and the significant interaction term of the regression ( $p < 0.05$ )  
254 indicated a steeper temperature response in +OM compared to –OM treatments (Table 3).

255 The overall trend of  $\text{NH}_4^+$ -efflux was similar to  $\text{PO}_4^{3-}$ -efflux.  $\text{NH}_4^+$  effluxes increased at  
256 the beginning, peaked and then decreased towards the end (Fig. 2). The range of the  $\text{NH}_4^+$   
257 effluxes was stimulated by both organic enrichment and temperature. The trend of average  $\text{NH}_4^+$ -  
258 effluxes over the whole experiment was not as clear as for  $\text{PO}_4^{3-}$ -effluxes (Fig. 3b).  $\text{NH}_4^+$   
259 effluxes were clearly stimulated at 26°C in both sediment types, however. According to the  
260 AICc, the trend of  $\text{NH}_4^+$  efflux over temperature was best fitted with an exponential regression  
261 (Table A1). The regression analysis showed significant increasing  $\text{NH}_4^+$  effluxes with  
262 temperature following a non-linear trajectory ( $p < 0.05$ ) in both –OM and +OM sediment. There  
263 were significant differences between -OM and +OM treatments at 26°C ( $p < 0.05$ ). The interaction

264 term of the regression did not show significant differences. Thus,  $\text{NH}_4^+$  efflux in -OM and +OM  
265 cores followed a similar trend with temperature although at different ranges (Table 3).

266 Temperature increase enhanced  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$ -effluxes, especially in +OM cores, but the  
267 amount of total mineralized  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  only corresponded to ~10 and ~20 %, respectively,  
268 of initial P and N in the different treatments (Table 1).

### 269 3.5. C:N:P ratios

270 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  
271 N:P ratios were similar in the initial -OM and +OM cores. The ratios among C, N and P at the  
272 end of the experiment were calculated based on  $\text{CO}_2$ ,  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  effluxes. C:N, C:P and N:P  
273 ratios at the end of the experiment were generally greater in -OM than in +OM cores, although  
274 there was not a clear trend along temperature treatments (Table 4).

275

276

## 277 4. Discussion

278 Our results show that temperature rise resulted in different trends of sediment  
279 nutrient release of  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  under both organic and non-organic enrichment conditions.  
280 While sediment  $\text{PO}_4^{3-}$  release followed a linear trend with increasing temperature, the  $\text{NH}_4^+$   
281 release from sediment show non-linear trends, notably increasing when temperature increments  
282 were above 6°C.

### 283 4.1. $\text{PO}_4^{3-}$ release

284 The measured  $\text{PO}_4^{3-}$  effluxes at 16°C were within the range of effluxes measured in coastal  
285 sediments in the Baltic Sea (Sundby et al. 1992; Jensen et al. 1995).  $\text{PO}_4^{3-}$  effluxes increased  
286 linearly with temperature in both -OM and +OM treatments, but the slope of the regression was

287 significantly steeper in +OM cores compared to –OM cores. This suggests that the temperature  
288 dependent increase in  $\text{PO}_4^{3-}$  effluxes is enhanced by OM enrichment. The temperature effect on  
289  $\text{PO}_4^{3-}$  release was comparable to the temperature effect on total C-metabolism in both organic  
290 enriched and non-organic enriched conditions (Sanz-Lazaro et al. 2011b). However, the release  
291 of  $\text{PO}_4^{3-}$  was much lower than that of  $\text{TCO}_2$ , as indicated by high C:P ratios, suggesting that  
292 organic P was either less labile compared to organic C or that inorganic  $\text{PO}_4^{3-}$  was retained in the  
293 sediment to a larger extent than C. Taking into account that the added OM (fish feed) is highly  
294 labile, our data suggest  $\text{PO}_4^{3-}$  retention in the sediment, which could be associated with  
295 adsorption to oxidized forms of Fe (Jensen et al. 1995).

296 P-retention in marine sediments is controlled by the forms of P, Fe and S in the sediment,  
297 interactions between these pools and the sediment redox-conditions (Roden and Edmonds  
298 1997; Gachter and Muller 2003). Generally, in sediments with an oxic surface layer, oxidized Fe  
299 acts as a lid for  $\text{PO}_4^{3-}$ , which is adsorbed to ferric Fe preventing its release to the water column  
300 (Rozan et al. 2002). In this experiment, the  $\text{PO}_4^{3-}$  binding capacity was not easily exhausted due  
301 to the large pool of Fe (III) (tens of mmoles) in the first cm of the sediment. This explanation  
302 was also supported by the fact that the levels of  $\text{PO}_4^{3-}$  bound to Fe (III) showed low variation  
303 between initial and final cores. The oxic conditions of the sediment surface were maintained in  
304 all treatments despite metabolism enhancement and subsequent increase in sulphide production,  
305 which was especially notable in the +OM treatments. The continuous oxygenation of surface  
306 sediment due to *N. diversicolor* bioturbation and bioirrigation (Sanz-Lazaro et al. 2011b) was  
307 probably critical for maintaining an oxic sediment surface with high Fe(III) concentrations,  
308 since dramatically stimulated sediment metabolism are expected to notably diminish the Fe(III)  
309 pool of the sediment in the absence of macrofauna (Valdemarsen et al. 2009). All these results

310 suggest that the capacity of the sediment to retain  $\text{PO}_4^{3-}$  was maintained despite high metabolic  
311 rates due to organic enrichment and elevated temperatures.

312 The  $\text{PO}_4^{3-}$  retention may be diminished in sediments with a low pool of Fe(III) (Rozaan et  
313 al. 2002) and/or with no bioturbating macrofauna (Bartoli et al. 2009). Hence under such  
314 conditions the  $\text{PO}_4^{3-}$  efflux could be much higher than measured in this experiment. A longer  
315 experiment could also have resulted in decreased  $\text{PO}_4^{3-}$  retention, because of exhaustion of the  
316 binding sites of  $\text{PO}_4^{3-}$  with Fe (III) (Jorgensen 1996). Nevertheless, the capacity of the sediment  
317 to retain  $\text{PO}_4^{3-}$  is notable considering that the amount of OM added in this experiment  
318 corresponded to the total OM deposited on the seabed below fish farms during a year, and that  
319 the OM added has a high reactivity, which implies that most of the OM is mineralized initially.  
320 Thus, marine sediment can act as a relevant sink of P, even under severe scenarios of  
321 temperature rise and organic enrichment, as long as it has a large enough pool of Fe and the  
322 macrofauna keeps the surface of the sediment under oxic conditions.

323

#### 324 4.2. $\text{NH}_4^+$ release

325 Rates of  $\text{NH}_4^+$ -effluxes at 16°C were within the range reported from previous laboratory  
326 experiments at similar conditions with non-enriched or enriched sediment (Valdemarsen et al.  
327 2009; Valdemarsen et al. 2010). The 1-3 times temperature stimulation of  $\text{NH}_4^+$ -efflux were in  
328 the same range observed in sediments from coastal areas (i.e. temperature rise ca. 10°C during  
329 summer resulted in one- to four-fold increases in  $\text{NH}_4^+$ -efflux) (Kristensen 1993). As with  $\text{PO}_4^{3-}$ ,  
330 the release rates of  $\text{NH}_4^+$  were always higher in +OM cores than in -OM treatments for a given  
331 temperature, highlighting that OM enrichment had a stimulatory effect on  $\text{NH}_4^+$  release. This was  
332 expected since high  $\text{NH}_4^+$ -release is often observed in sediments enriched with labile organic

333 matter (Christensen et al. 2000;Valdemarsen et al. 2012).  $\text{NH}_4^+$  release was not stimulated by  
334 temperature between 16-22 °C, but above 22°C  $\text{NH}_4^+$  release increased non-linearly in both –OM  
335 and +OM treatments. The notable increase of  $\text{NH}_4^+$  release at a temperature increment of 10°C in  
336 both –OM and +OM treatments, could led us to speculate that the efficiency of  $\text{NH}_4^+$  oxidizing  
337 pathways is lowered at high temperatures, nevertheless the data of C:N ratios pointed to an  
338 opposite hypothesis.

339         The C:N ratios of C and N release during the experiment were always higher than the  
340 C:N ratios of the sediment OM, in particular at high temperature rise. This suggests that part of  
341 the mineralized N is not released to the water column as  $\text{NH}_4^+$ , but transformed into other N  
342 compounds.  $\text{NH}_4^+$  could be nitrified in the oxidized surface sediment or in *N. divericolor*  
343 burrows, and subsequently reduced to  $\text{N}_2$  via anammox or denitrification (Thamdrup 2012).  
344 Previous studies show that both denitrification and anammox are stimulated by increasing  
345 temperatures (Nowicki 1994;Alsterberg et al. 2012;Canion et al. 2013) and by availability of OM  
346 (Nowicki 1994;Thamdrup and Dalsgaard 2002;Engstrom et al. 2005) which supports the data  
347 from this experiment. Nevertheless, evidence based on  $\text{NO}_3^-$  and  $\text{NO}_2^-$  data should be needed to  
348 confirm this hypothesis. Despite so,  $\text{NH}_4^+$  is the dominating form of dissolved inorganic N  
349 effluxing from organic enriched sediments (Christensen et al. 2000;Holmer et al. 2003), while  
350 coupled nitrification-denitrification rarely exceeds 1-2  $\text{mmol m}^{-2} \text{d}^{-1}$  in marine sediments  
351 (Middelburg et al. 1996). We are therefore confident that the  $\text{NH}_4^+$  release rates can act as a  
352 proxy for total inorganic N release to the water column.

353         Despite that bioturbation and bioirrigation by macrofauna promotes OM mineralization,  
354 in the present experiment, both  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  effluxes were below the expected levels of the  
355 mineralized organic matter. In the case of  $\text{PO}_4^{3-}$ , this can be explained by the  $\text{PO}_4^{3-}$  retention



356 capacity of the sediment that is promoted by macrofauna activity (Bartoli et al. 2009). In the case  
357 of  $\text{NH}_4^+$ , these results could be explained because macrofauna promotes  $\text{NH}_4^+$ -oxidizing  
358 processes and the coupling between nitrification and denitrification (Gilbert et al. 1995). Thus,  
359 sediment reworking processes of macrofauna promote nutrient recycling while preventing  
360 eutrophication.

361         Additionally it should be considered that the experiment was performed in darkness, as  
362 the experiment was designed to simulate the generally low availability of light at the sediment  
363 surface in relatively enclosed and shallow coastal areas exposed to eutrophication, such as in the  
364 Danish coastal areas. Nevertheless, in other areas with more light availability autotrophic  
365 processes are also important. This could lead to different results with regards to nutrient release  
366 rates from the sediment.

367         In conclusion, in future scenarios of climate change, sediment  $\text{PO}_4^{3-}$  release is expected to  
368 increase following the trends observed for OM mineralization rates in the sediment. The  
369 magnitude of the release of  $\text{PO}_4^{3-}$  is influenced not only by local OM deposition rates but also by  
370 sediment characteristics, particularly, the amount of reactive Fe in sediment.  $\text{NH}_4^+$  release from  
371 sediment is not expected to increase substantially with temperature increments of up to  $6^\circ\text{C}$ , in  
372 organic polluted as well as in non-polluted sediments. However, more severe temperature rises  
373 may be associated with a non-linear increase in the release of  $\text{NH}_4^+$  from sediments. As most  
374 marine basins are N-limited, the excess release of  $\text{NH}_4^+$  may set in motion a cascade of negative  
375 effects leading to deteriorating environmental quality. These effects are expected to be more  
376 detrimental in organic polluted areas as well as in coastal basins with no or restricted exchange  
377 with ocean waters.

378

379

380 *Acknowledgements*

381 The authors are grateful to the technicians at Økolab, Department of Biology, University of  
382 Southern Denmark, for their help with the chemical analysis. CS was supported by the Ministerio  
383 de Educación y Ciencia of Spain. We thank the referees for helping to improve the manuscript  
384 with their suggestions.

385 References

386

387 *References*

388

389 Alsterberg, C., Sundback K., and Hulth S.: Functioning of a Shallow-Water Sediment System

390 during Experimental Warming and Nutrient Enrichment, Plos One, 7, 2012.

391 Bartoli, M., Longhi D., Nizzoli D., Como S., Magni P., and Viaroli P.: Short term effects of

392 hypoxia and bioturbation on solute fluxes, denitrification and buffering capacity in a shallow

393 dystrophic pond, J. Exp. Mar. Biol. Ecol., 381, 105-113, 2009.

394 Belkin, I. M.: Rapid warming of Large Marine Ecosystems, Prog. Oceanogr., 81, 207-213,

395 2009.

396 Burnham, K. P., and Anderson D. R.: Model Selection and Multimodel Inference: A Practical

397 Information-Theoretic Approach Springer-Verlag, 2002.

398 Callier, M. D., Weise A. M., McKindsey C. W., and Desrosiers G.: Sedimentation rates in a

399 suspended mussel farm (Great-Entry Lagoon, Canada): biodeposit production and dispersion,

400 Mar. Ecol. Prog. Ser., 322, 129-141, 2006.

401 Canion, A., Kostka J. E., Gihring T. M., Huettel M., van Beusekom J. E. E., Gao H., Lavik

402 G., and Kuypers M. M. M.: Temperature response of denitrification and anammox reveals the

403 adaptation of microbial communities to in situ temperatures in permeable marine sediments that

404 span 50° in latitude, Biogeosciences Discuss., 10, 14595-14626, 2013.

405 Christensen, P. B., Rysgaard S., Sloth N. P., Dalsgaard T., and Schwaerter S.: Sediment

406 mineralization, nutrient fluxes, denitrification and dissimilatory nitrate reduction to ammonium

407 in an estuarine fjord with sea cage trout farms, Aquat. Microb. Ecol., 21, 73-84, 2000.

408 Conley, D. J., Bjorck S., Bonsdorff E., Carstensen J., Destouni G., Gustafsson B. G., Hietanen  
409 S., Kortekaas M., Kuosa H., Meier H. E. M., Muller-Karulis B., Nordberg K., Norkko A.,  
410 Nurnberg G., Pitkanen H., Rabalais N. N., Rosenberg R., Savchuk O. P., Slomp C. P., Voss M.,  
411 Wulff F., and Zillen L.: Hypoxia-Related Processes in the Baltic Sea, *Environ. Sci. Technol.*, 43,  
412 3412-3420, 2009.

413 Cowan, J. L. W., and Boynton W. R.: Sediment-water oxygen and nutrient exchanges along  
414 the longitudinal axis of Chesapeake Bay: Seasonal patterns, controlling factors and ecological  
415 significance, *Estuaries*, 19, 562-580, 1996.

416 Delefosse, M., Banta G. T., Canal-Verges P., Penha-Lopes G., Quintana C. O., Valdemarsen  
417 T., and Kristensen E.: Macrobenthic community response to the *Marenzelleria viridis*  
418 (Polychaeta) invasion of a Danish estuary, *Mar. Ecol. Prog. Ser.*, 461, 83-94, 2012.

419 Doscher, R., and Meier H. E. M.: Simulated sea surface temperature and heat fluxes in  
420 different climates of the Baltic Sea, *Ambio*, 33, 242-248, 2004.

421 Engstrom, P., Dalsgaard T., Hulth S., and Aller R. C.: Anaerobic ammonium oxidation by  
422 nitrite (anammox): Implications for N<sub>2</sub> production in coastal marine sediments, *Geochim.*  
423 *Cosmochim. Acta*, 69, 2057-2065, 2005.

424 Falkenberg, L. J., Connell S. D., and Russell B. D.: Disrupting the effects of synergies  
425 between stressors: improved water quality dampens the effects of future CO<sub>2</sub> on a marine  
426 habitat, *J. Appl. Ecol.*, 50, 51-58, 2013.

427 Gachter, R., and Muller B.: Why the phosphorus retention of lakes does not necessarily  
428 depend on the oxygen supply to their sediment surface, *Limnol Oceanogr.*, 48, 929-933, 2003.

429 Gilbert, F., Bonin P., and Stora G.: Effect of Bioturbation on Denitrification in A Marine  
430 Sediment from the West Mediterranean Littoral, *Hydrobiologia*, 304, 49-58, 1995.

431 Gray, J. S., Wu R. S., and Or Y. Y.: Effects of hypoxia and organic enrichment on the coastal  
432 marine environment, *Mar. Ecol. Prog. Ser.*, 238, 249-279, 2002.

433 Hall, P. O., and Aller R. C.: Rapid, Small-Volume, Flow-Injection Analysis for Sigma-Co<sub>2</sub>  
434 and Nh<sub>4</sub><sup>+</sup> in Marine and Fresh-Waters, *Limnol Oceanogr*, 37, 1113-1119, 1992.

435 Halpern, B. S., Walbridge S., Selkoe K. A., Kappel C. V., Micheli F., D'Agrosa C., Bruno J.  
436 F., Casey K. S., Ebert C., Fox H. E., Fujita R., Heinemann D., Lenihan H. S., Madin E. M. P.,  
437 Perry M. T., Selig E. R., Spalding M., Steneck R., and Watson R.: A global map of human  
438 impact on marine ecosystems, *Science*, 319, 948-952, 2008.

439 Holmer, M., Duarte C. M., Heilskov A., Olesen B., and Terrados J.: Biogeochemical  
440 conditions in sediments enriched by organic matter from net-pen fish farms in the Bolinao area,  
441 Philippines, *Mar. Pollut. Bull.*, 46, 1470-1479, 2003.

442 Holmer, M., and Kristensen E.: Seasonality of sulfate reduction and pore water solutes in a  
443 marine fish farm sediment: The importance of temperature and sedimentary organic matter,  
444 *Biogeochemistry*, 32, 15-39, 1996.

445 Holmer, M., Marbà N., Díaz-Almela E., Duarte C. M., Tsapakis M., and Danovaro R.:  
446 Sedimentation of organic matter from fish farms in oligotrophic Mediterranean assessed through  
447 bulk and stable isotope (delta C-13 and delta N-15) analyses, *Aquaculture*, 262, 268-280, 2007.

448 IPCC.: *Climate Change 2007: Synthesis Report* Cambridge University Press, Cambridge,  
449 2007.

450 Islam, M. S., and Tanaka M.: Impacts of pollution on coastal and marine ecosystems  
451 including coastal and marine fisheries and approach for management: a review and synthesis,  
452 *Mar. Pollut. Bull.*, 48, 624-649, 2004.

453 Jensen, H. S., Mortensen P. B., Andersen F. O., Rasmussen E., and Jensen A.: Phosphorus  
454 Cycling in A Coastal Marine Sediment, Aarhus Bay, Denmark, *Limnol Oceanogr*, 40, 908-917,  
455 1995.

456 Jorgensen, B. B.: Processes at the sediment-water interface, in: *The major biogeochemical*  
457 *cycles and their interactions*. Bolin B. and Cook K. B. (Eds.): John Wiley and Sons, Chichester,  
458 U.K, 1983.

459 Jorgensen, B. B.: Material flux in the sediment, in: *Eutrophication in coastal marine*  
460 *ecosystems*. Jorgensen B. B. and Richardson K. (Eds.): American Geophysical Union,  
461 Washington, DC, 1996.

462 Kelly, J. R., Berounsky V. M., Nixon S. W., and Oviatt C. A.: Benthic-Pelagic Coupling and  
463 Nutrient Cycling Across An Experimental Eutrophication Gradient, *Mar. Ecol. Prog. Ser*, 26,  
464 207-219, 1985.

465 Koroleff, F.: Determination of nutrients, in: *Methods of seawater analysis*. Grasshof K.,  
466 Ehrhardt M., and Kremling K. (Eds.): Verlag Chemie, Weinheim, 1983.

467 Kristensen, E.: Seasonal variations in benthic community metabolism and nitrogen dynamics  
468 in a shallow, organic-poor lagoon, *Estuarine Coastal Shelf Sci.*, 36, 565-586, 1993.

469 Kristensen, E.: Organic matter diagenesis at the oxic/anoxic interface in coastal marine  
470 sediments, with emphasis on the role of burrowing animals, *Hydrobiologia*, 426, 1-24, 2000.

471 Kristensen, E., and Andersen F. O.: Determination of Organic-Carbon in Marine-Sediments -  
472 A Comparison of 2 Chn-Analyzer Methods, *J. Exp. Mar. Biol. Ecol.*, 109, 15-23, 1987.

473 Lovley, D. R., and Phillips E. J. P.: Rapid Assay for Microbially Reducible Ferric Iron in  
474 Aquatic Sediments, *Appl. Environ. Microbiol.*, 53, 1536-1540, 1987.

475 Mackenzie, B. R., and Schiedek D.: Daily ocean monitoring since the 1860s shows record  
476 warming of northern European seas, *Global Change Biol.*, 13, 1335-1347, 2007.

477 Meier, H. E. M., Eilola K., and Almroth E.: Climate-related changes in marine ecosystems  
478 simulated with a 3-dimensional coupled physical-biogeochemical model of the Baltic Sea,  
479 *Climate Research*, 48, 31-55, 2011.

480 Mermillod-Blondin, F., Rosenberg R., Francois-Carcaillet F., Norling K., and Mauclaire L.:  
481 Influence of bioturbation by three benthic infaunal species on microbial communities and  
482 biogeochemical processes in marine sediment, *Aquat. Microb. Ecol.*, 36, 271-284, 2004.

483 Middelburg, J. J., Soetaert K., and Herman P. M. J.: Empirical relationships for use in global  
484 diagenetic models, *Deep-Sea Res.*, 44, 327-344, 1997.

485 Middelburg, J. J., Soetaert K., Herman P. M. J., and Heip C. H. R.: Denitrification in marine  
486 sediments: A model study, *Global Biogeochemical Cycles*, 10, 661-673, 1996.

487 Nixon, S. W.: *Remineralization and nutrient cycling in coastal marine ecosystems* Springer,  
488 1981.

489 Nowicki, B. L.: The effect of temperature, oxygen, salinity, and nutrient enrichment on  
490 estuarine denitrification rates measured with a modified nitrogen gas flux technique, *Estuarine,  
491 Coastal Shelf Sci.*, 38, 137-156, 1994.

492 Quintana, C. O., Kristensen E., and Valdemarsen T.: Impact of the invasive polychaete  
493 *Marenzelleria viridis* on the biogeochemistry of sandy marine sediments, *Biogeochemistry*, 115,  
494 95-109, 2013.

495 R Development Core Team.: *R: A language and environment for statistical computing*,  
496 reference index version 2.15.0 R Foundation for Statistical Computing, Vienna, Austria, 2012.

497 Robador, A., Bruchert V., and Jorgensen B. B.: The impact of temperature change on the  
498 activity and community composition of sulfate-reducing bacteria in arctic versus temperate  
499 marine sediments, *Environ. Microbiol.*, 11, 1692-1703, 2009.

500 Roden, E. E., and Edmonds J. W.: Phosphate mobilization in iron-rich anaerobic sediments:  
501 Microbial Fe(III) oxide reduction versus iron-sulfide formation, *Archiv fur Hydrobiologie*, 139,  
502 347-378, 1997.

503 Rozan, T. F., Taillefert M., Trouwborst R. E., Glazer B. T., Ma S., Herszage J., Valdes L. M.,  
504 Price K. S., and Luther III G. W.: Iron-sulfur-phosphorus cycling in the sediments of a shallow  
505 coastal bay: Implications for sediment nutrient release and benthic macroalgal blooms, *Limnol*  
506 *Oceanogr*, 47, 1346-1354, 2002.

507 Sanz-Lazaro, C., Belando M. D., Marín-Guirao L., Navarrete-Mier F., and Marín A.:  
508 Relationship between sedimentation rates and benthic impact on Maërl beds derived from fish  
509 farming in the Mediterranean, *Mar. Environ. Res.*, 71, 22-30, 2011a.

510 Sanz-Lazaro, C., Valdemarsen T., Marín A., and Holmer M.: Effect of temperature on  
511 biogeochemistry of marine organic-enriched systems: implications in a global warming scenario,  
512 *Ecol. Appl.*, 21, 2664-2677, 2011b.

513 Stookey, L. L.: Ferrozine - A New Spectrophotometric Reagent for Iron, *Anal. Chem.*, 42,  
514 779-781, 1970.

515 Sundby, B., Gobeil C., Silverberg N., and Mucci A.: The phosphorus cycle in coastal marine  
516 sediments, *Limnol Oceanogr*, 1992.

517 Thamdrup, B.: New Pathways and Processes in the Global Nitrogen Cycle, *Annual Review of*  
518 *Ecology, Evolution, and Systematics*, Vol 43, 43, 407-428, 2012.



519 Thamdrup, B., and Dalsgaard T.: Production of N<sub>2</sub> through anaerobic ammonium oxidation  
520 coupled to nitrate reduction in marine sediments, *Appl. Environ. Microbiol.*, 68, 1312-1318,  
521 2002.

522 Thamdrup, B., and T. Dalsgaard.: Nitrogen Cycling in Sediments, in: *Microbial Ecology of*  
523 *the Oceans*. Kirchman D. L. (Eds.): John Wiley and Sons, Hoboken, New Jersey, USA, 2008.

524 Valdemarsen, T., Bannister R. J., Hansen P. K., Holmer M., and Ervik A.: Biogeochemical  
525 malfunctioning in sediments beneath a deep-water fish farm, *Environ. Pollut.*, 170, 15-25, 2012.

526 Valdemarsen, T., Kristensen E., and Holmer M.: Metabolic threshold and sulfide-buffering in  
527 diffusion controlled marine sediments impacted by continuous organic enrichment,  
528 *Biogeochemistry*, 95, 335-353, 2009.

529 Valdemarsen, T., Kristensen E., and Holmer M.: Sulfur, carbon, and nitrogen cycling in  
530 faunated marine sediments impacted by repeated organic enrichment, *Mar. Ecol. Prog. Ser.*, 400,  
531 37-53, 2010.

532

533

534

535 Tables

536

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

541

	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]

542 Notes: The 16°C treatment corresponds to the present mean sea surface temperature (SST) for summer  
543 months in the Baltic Sea. The 22 and 26°C treatments were chosen as SST climate change scenarios in  
544 60 and 100 years based on SST temperature rise observed in the last decades and expected raises (1°C  
545 per decade in both cases). –OM and +OM correspond to non- and organic polluted treatments,  
546 respectively. The organic matter addition to +OM cores corresponded to 26 mol POC m<sup>-2</sup>, which is  
547 comparable to the annual organic matter deposition in areas under the influence of mussel or fish farms.  
548 The final cores were maintained at 16, 22, or 26°C in separate tanks containing filtered seawater.

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

552

	Initial concentration (mol m <sup>-2</sup> )	Final concentration (mmol m <sup>-2</sup> ), by temperature		
		16°C	22°C	26°C
<b>-OM</b>				
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
<b>+OM</b>				
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

553

554

555 Table 3: Coefficients (means at 26°C) of the regression model for  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  sediment-  
 556 water column flux (i.e. efflux) rates along temperature for non-organic polluted (-OM) and  
 557 organic polluted (+OM) treatments. Regression model for  $\text{PO}_4^{3-}$  corresponds to a first order  
 558 polynomial regression, while for  $\text{NH}_4^+$  correspond to an exponential regression (Table A1).  
 559 Significant effects ( $p < 0.05$ ) are indicated in bold.

560

	$\text{PO}_4^{3-}$ efflux rate	$\text{NH}_4^+$ efflux rate
	Coefficient (SE)	Coefficient (SE)
Intercept	<b>0.207</b> (0.047)	<b>13.58</b> (3.26)
OM	<b>0.420</b> (0.076)	<b>12.45</b> (5.27)
Temperature	<b>0.016</b> (0.008)	<b>0.946</b> (0.524)
OM x Temperature	<b>0.028</b> (0.012)	0.108 (0.803)

561

562

563 Table 4: Initial C:N:P ratio of the organic matter in the sediment estimated from particulate  
564 organic carbon, total nitrogen and total phosphorus and overall C:N:P ratio estimated from  
565 nutrient and total CO<sub>2</sub> flux over the experimental period. C and total CO<sub>2</sub> flux data was reported  
566 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
<b>-OM</b>				
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
<b>+OM</b>				
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

567

568

569 Figure legends

570

571 Figure 1: Mineralization rates, shown as flux rates of total CO<sub>2</sub> (mean ± SE, n=6) versus  
572 temperature under non- (-OM) and organic polluted (+OM) conditions modified from Sanz-  
573 Lázaro et al. (2011b). Lines are shown to visualize the trajectory but do not represent a  
574 regression.

575

576 Figure 2: PO<sub>4</sub><sup>3-</sup> and NH<sub>4</sub><sup>+</sup> efflux rates (n=6, mean ± SE) during the experiment at the three  
577 temperature scenarios under non- (-OM) and organic enrichment (+OM) conditions. Dotted lines  
578 are the line of reference corresponding to 0.

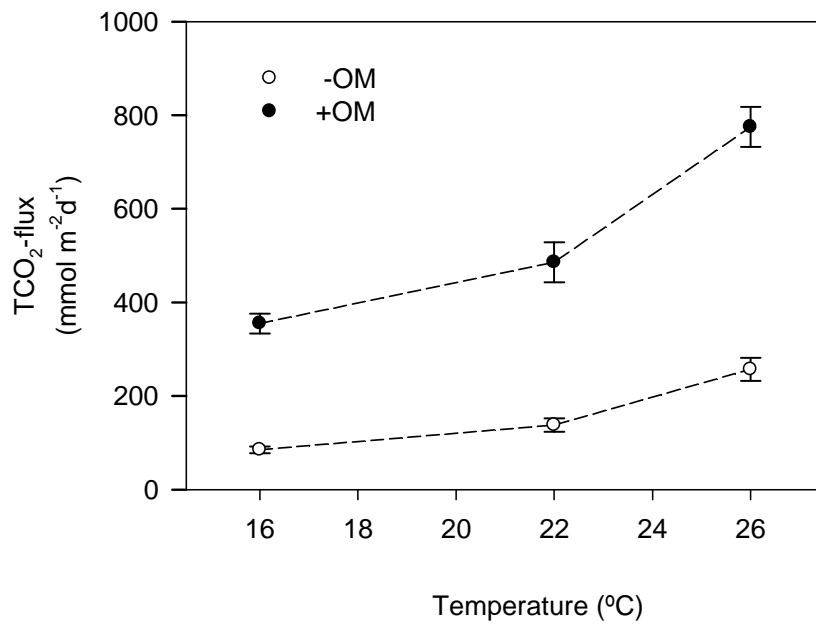
579

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

585

586 Figure 1

587



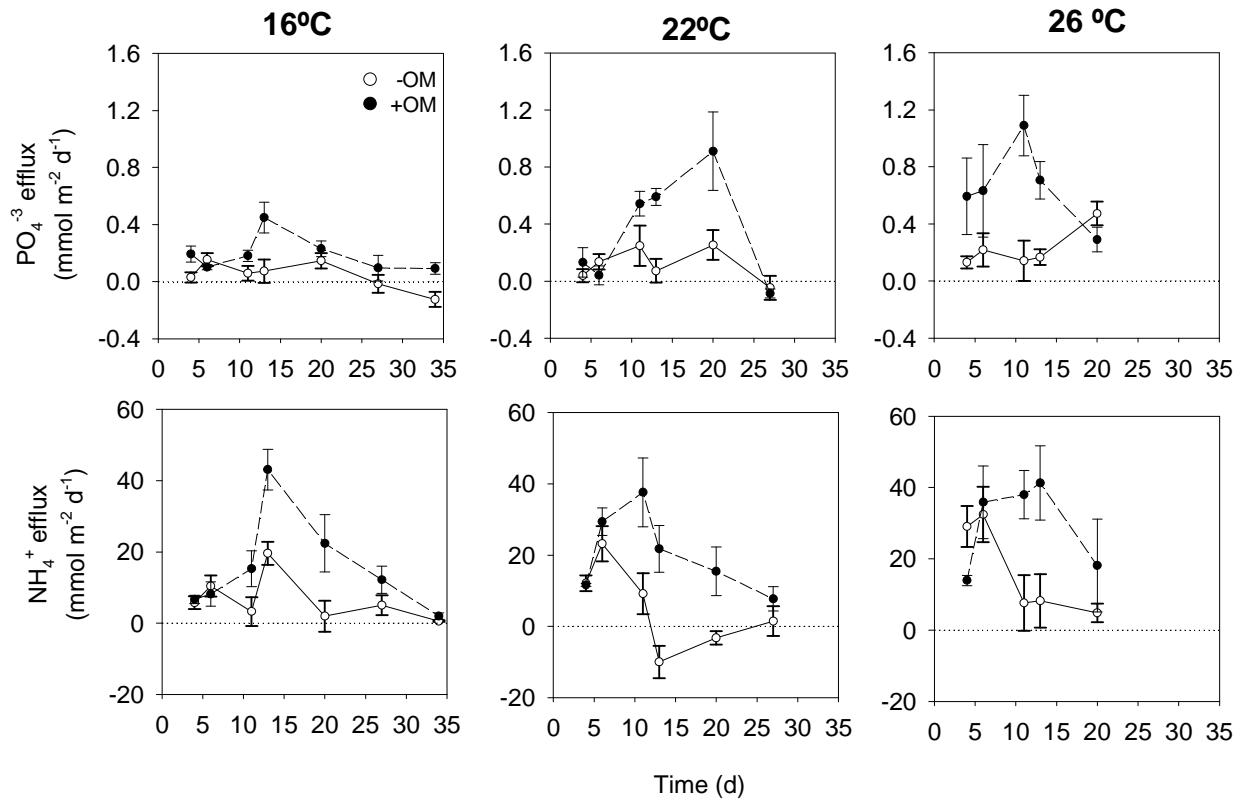
588

589 Figure 2

590

591

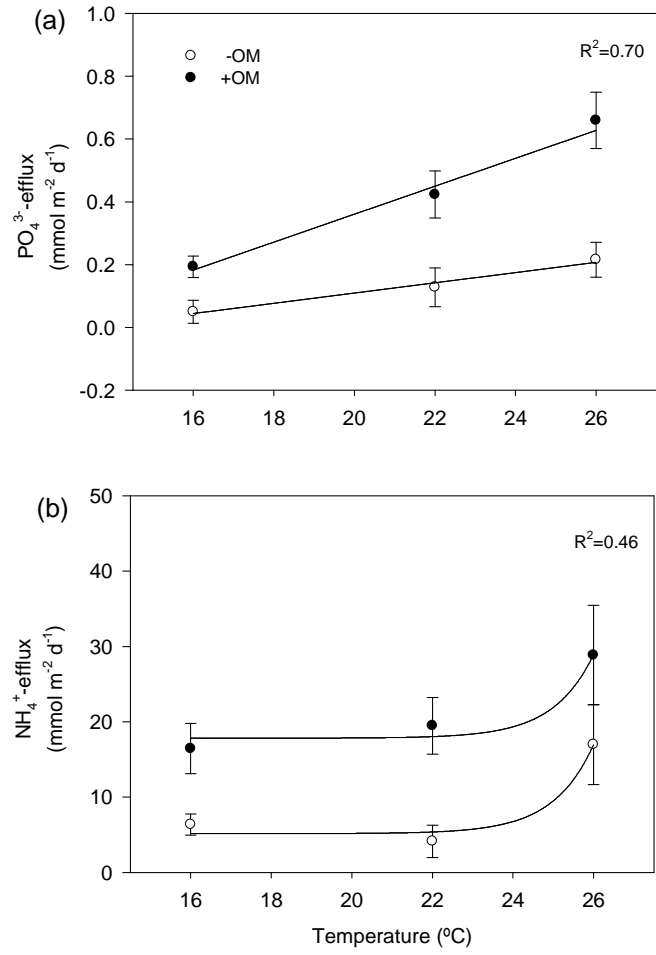
592





593 Figure 3

594



595

596 Supplementary material

597

598 Appendix 1:

599

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

602

	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

603

604

605 Appendix 2

606

607 Table A2: Data of  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  efflux rates obtained in the incubations performed during the  
608 experiment. For each core, average nutrient efflux rate was estimated by integrating the data of  
609 all the incubations along the time span of the experiment as shown in the table. The first written  
610 row indicates the treatment, the number indicates temperature, while –OM and +OM indicates  
611 without or with organic matter addition, respectively.

612