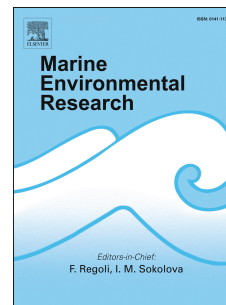


# Journal Pre-proof

Bioturbation may not always enhance the metabolic capacity of organic polluted sediments

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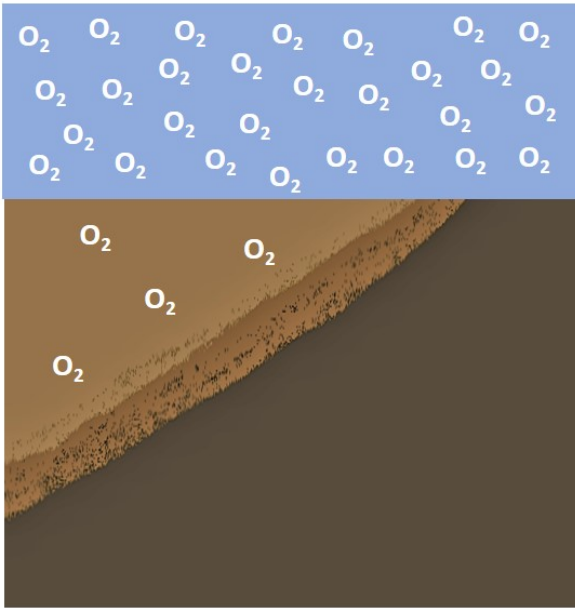
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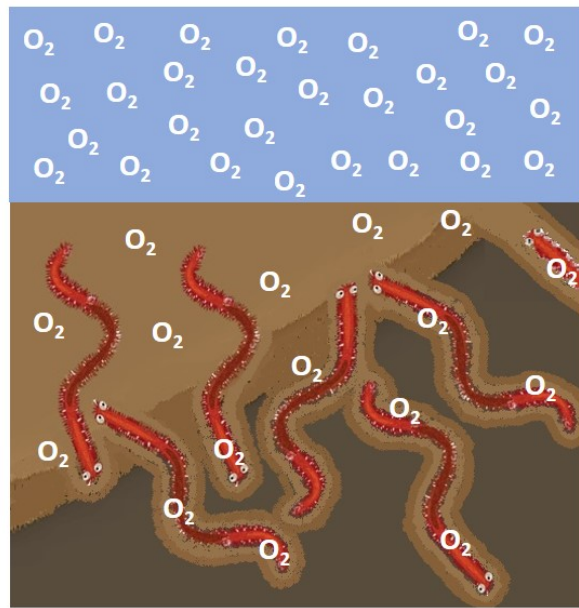
## Author Statement

**Nuria Casado-Coy:** Methodology, Writing- Original draft preparation, Formal analysis, Visualization, Investigation. **Pablo Sánchez-Jerez:** Supervision, Validation, Funding acquisition. **Marianne Holmer:** Writing-Reviewing and Editing. **Carlos Sanz-Lázaro:** Data Curation, Conceptualization, Validation, Supervision, Funding acquisition, Writing-Reviewing and Editing.

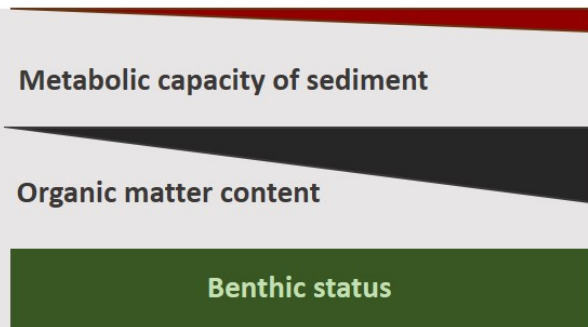
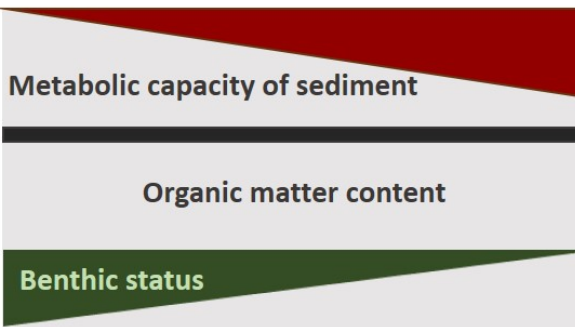
Journal Pre-proof



Organic pollution gradient



Organic pollution gradient



**1 Bioturbation may not always enhance the metabolic capacity of organic polluted sediments.**

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**10 Abstract**

11 Marine sediments are a major sink of organic matter, playing a crucial role in the global cycling  
12 of major elements. Macrofauna, through the reworking of particles and movement of solutes  
13 (bioturbation), enhances oxic conditions and the sediment metabolic capacity. Increases in the  
14 inputs of organic matter can lead to profound changes in the seabed and impact benthic  
15 ecological functions. Through a microcosm experiment, the effect of bioturbation of the  
16 polychaete *Lumbrineris latreilli* on biogeochemical fluxes under scenarios of increasing loads of  
17 organic matter was quantified. We found that bioturbation can buffer the negative  
18 consequences of anoxic conditions produced by organic enrichment, preventing the build-up  
19 of toxic by-products derived from anaerobic metabolic pathways by maintaining oxic  
20 conditions. However, the maintenance of oxic conditions by bioturbation is at the expense of  
21 limiting the sediment metabolic capacity. The maintenance of oxic conditions may limit  
22 anaerobic metabolic pathways, and consequently, the metabolic capacity of sediment. Thus,  
23 under organic matter pollution conditions, bioturbation may lessen the metabolic capacity of  
24 the sediment.  
25

26 **Capsule:** Bioturbation can promote oxic conditions while diminishing the metabolic capacity of  
27 sediment under a certain level of organic pollution

28 **Keywords:** aquaculture; bioirrigation; bioturbation; metabolic capacity; organic matter

29

## 30 1. Introduction

31 The seabed is an important sink of organic matter (OM) and plays a main role in the global  
32 cycling of major elements including carbon, nitrogen and phosphorus (Middelburg et al.,  
33 1997). These processes are principally performed by microorganisms through a wide variety of  
34 metabolic functions (Battin et al., 2008; Emerson and Hedges, 2003). Oxygen respiration is the  
35 most thermodynamic favourable metabolic pathway, followed by denitrification, manganese  
36 and iron reduction (Holmer and Barry, 2005). However, sulphate reduction can account for up  
37 to half of the total benthic metabolism, being the most important metabolic pathway in  
38 marine sediments (Jorgensen, 1982). The metabolic capacity of the sediments, i.e. the amount  
39 of OM that the sediments can mineralize, greatly depends on the prevailing metabolism  
40 (Holmer and Barry, 2005; Kristensen, 2000). This prevalence of specific metabolic pathways is  
41 subjected to several abiotic and biotic factors related to the supply of electron acceptors to the  
42 sediment (Arndt et al., 2013; Welsh, 2003).

43 Among these factors, macrofauna plays a crucial role in the sediment biogeochemistry  
44 through the active displacement of sediments particles and solutes (bioturbation) (Kristensen  
45 et al., 2012), which enhances habitat complexity (Meysman et al., 2006) and electron acceptor  
46 supply (Aller and Aller, 1998). These processes stimulate element cycling (Kristensen and  
47 Kostka, 2013; Mermillod-Blondin and Rosenberg, 2006). The biogenic structures created by  
48 macrofauna maximize the area of the sediment that is in contact with the water column  
49 promoting oxic conditions (Bergström et al., 2017; Kristensen and Mikkelsen, 2003). Under  
50 these circumstances, aerobic respiration prevails, (Holmer and Barry, 2005; Kristensen, 2000;  
51 Storey et al., 1999), and consequently, the sediment metabolic capacity is expected to be  
52 enhanced (Banta et al., 1999; Heilskov et al., 2006; Kristensen and Kostka, 2013). Therefore,  
53 bioturbation produced by macrofauna promotes the good ecological status of the benthic  
54 systems by favouring beneficial oxic conditions for the inhabiting communities, not only  
55 directly, but also indirectly enhancing the sediment metabolic capacity, which prevents OM  
56 accumulation in the seabed.

57 Marine sediments are impacted by a range of anthropogenic pressures, especially in  
58 coastal areas where human population concentrates (Griffen et al., 2016). OM enrichment is a  
59 common type of pollution, derived from several sources, such as domestic sewage, mining,  
60 dredging, industrial, agricultural and aquaculture waste (Aguado-Giménez et al., 2015; de-la-  
61 Ossa-Carretero et al., 2009; Simboura et al., 2007). Aquaculture waste is a source of OM  
62 enrichment in coastal areas. This activity is rapidly expanding due to the steep population  
63 growth and the stagnation of fisheries and other food production systems (Moffitt and Cajas-  
64 Cano, 2014).

65 The environmental drawbacks of OM enrichment in the sediment are derived from  
66 oxygen depletion, which enhance anaerobic metabolic pathways (Zhang et al., 2010) and,  
67 consequently, the release of their toxic by-products such as sulphides and methane. These  
68 conditions negatively affect macrofauna, leading to the predominance of opportunistic species  
69 which in general have a low bioturbation capacity (Heilskov and Holmer, 2001; Pearson and  
70 Rosenberg, 1978). Eventually, if the level of OM enrichment is large enough, macrofauna can  
71 be depleted (Diaz and Rosenberg, 1995; Lu and Wu, 2007) decreasing, even more, the  
72 metabolic capacity of the sediment (Sanz-Lazaro and Marin, 2011). Additionally, OM  
73 enrichment can affect the water column by increasing the nutrient supply from the sediment,  
74 causing eutrophication (Dimitriou et al., 2015).

75 Understanding how the sediment conditions and its metabolic capacity change with  
76 OM enrichment are mandatory to estimate the carrying capacity of marine sediments, a key  
77 parameter for the environmental agencies to establish the maximum OM enrichment that  
78 each benthic habitat can receive. Applying these carrying capacities to human activities that  
79 produce OM enrichment, is compulsory to promote their sustainability by maintaining a good  
80 status of marine ecosystems and of the ecological services that provide to society.

81 The aim of this study is to test the effect of the bioturbation produced by macrofauna  
82 in the sediment biogeochemistry along an OM enrichment gradient. To do so, a microcosm  
83 experiment was designed using a gradient of OM enrichment from aquaculture as a model of  
84 this type of pollution. We quantified the effect of bioturbation by macrofauna on the  
85 biogeochemical fluxes under different scenarios of OM enrichment levels.

## 86 2. Material & Methods

### 87 2.1 Experimental Set-Up

88 This study was performed through a microcosm experiment where coastal sediment conditions  
89 were simulated. For the microcosm, the sediment from the coast of Alicante, Spain, was  
90 collected (ca. 60L) close to the shoreline from the seabed surface (0-10 cm) and sieved through  
91 a 0.5 mm mesh to remove macrofauna. The sediment was graded as a very fine and fine sand  
92 grain (0.063-0.25 mm) according to the Wentworth (1922) classification. The sediment was  
93 enriched with sodium sulphate ( $50 \text{ mmol L}^{-1}$ ) to prevent sulphate depletion. A total of 24  
94 methacrylate cores (with an internal diameter of 6 cm and a length of 32 cm) were filled with  
95 sediment to a depth of 20 cm. The bottom part of the cores was sealed at the bottom by  
96 rubber stoppers, and above the sediment, the core was filled (ca. 12 cm) with seawater. The  
97 cores were maintained for four days to allow the sediment to stratify (Papaspyrou et al., 2007)  
98 and then, were separated into four groups: a control group without sediment OM addition and  
99 the other three groups with a gradient of OM enrichment. To simulate OM enrichment, OM  
100 was added to the surface of the sediment in the form of finely ground fish feed. We used three  
101 levels of OM enrichment by adding, 14.5, 29 and 58 g of labile OM per kg of the sediment. The  
102 treatment with 29 g of OM per kg of the sediment corresponded to  $255.6 \text{ mmol C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ ,  
103 which has been applied in previous enrichment studies (Casado-Coy et al., 2017; Valdemarsen  
104 et al., 2010) as a realistic organic pollution in natural sediment, such as underlying fish or  
105 mussel farms (Callier et al., 2009; Morrisey et al., 2000). The other two enrichment were half  
106 and double the amount of POC used in the first-mentioned addition. During the experiment,  
107 the corresponding OM enrichment was done weekly.

108 To test the effect of the bioturbation of macrofauna (+W), three worms (*Lumbrineris*  
109 *latreilli* Audouin & Milne Edwards, 1834) of length 14–20 cm were added to half of the cores  
110 (12) (c. 1300 individuals  $\text{m}^{-2}$ ), density observed in the coast of Alicante (personal  
111 communication). Bioturbating worms are frequently used in mesocosm experiments as a  
112 model of the bioturbation effect of macrofauna (Banta et al., 1999; Kristensen, 2000). The  
113 other 12 cores were left without worms (-W). Worms were bought from a fishing bait supplier  
114 and the three healthy worms were added 7 days after the enrichment of OM load in each core.  
115 When the worms were added it was considered that the experiment had started ( $t=0$ ). The  
116 seawater (salinity 37.6) was collected from the same area than the sediment and previously  
117 filtered ( $\phi 0.001 \text{ mm}$ ) to remove large particles. The cores were maintained in darkness in an  
118 environmental chamber, submerged in aired seawater at  $16^\circ\text{C}$ . Temperature was controlled by  
119 means of two coolers and a pump that recirculated the water through the tank and the

120 coolers. Seawater column inside the cores was stirred by magnetic bars (4 cm length), which  
121 were placed a few centimetres above the sediment surface, driven by a rotating magnet  
122 placed close the cores to favour water circulation from the cores and mix with the rest of the  
123 water of the environmental chamber. Additionally, every 2 days, *ca.* half of the seawater of the  
124 chamber was renewed to prevent unnatural accumulation or depletion of molecules in the  
125 water. The chamber was set up as done by Piedecausa et al. (2012).

## 126 2.2 Biogeochemical Fluxes

127 The experiment lasted 27 days and during this period six incubations were carried out to  
128 determine the fluxes of total CO<sub>2</sub> (TCO<sub>2</sub> flux) and the sediment oxygen uptake (SOU), by  
129 estimating the production or consumption rates during the incubation by measuring the  
130 concentration of the molecules before and after the incubation. The incubations were done by  
131 sealing the upper part of the cores with a rubber stopper for a relatively short time periods (2–  
132 4 h for OM enriched cores and 4–5 h for cores without OM enrichment) to prevent  
133 experimental artefacts derived from excessive oxygen depletion (Glud, 2008). Initially, the  
134 incubations were performed 2 days per week during the first two weeks, and then once per  
135 week. TCO<sub>2</sub> was estimated using total carbon titration with Titrisol HCl (0.1 mol L<sup>-1</sup>, Applichem  
136 Panreac, Germany Methyl Red.) at two pH ranges (Gran et al., 1950). O<sub>2</sub> was measured with an  
137 oximeter (CRISON OXI 45 P).

## 138 2.3 Sediment Analyses

139 After the experiment, the cores were sectioned in eight sections with the first and second sliced  
140 in 1 cm intervals, and the rest in 2 cm. On each section, sediment density was calculated by  
141 weighing a known volume of the sediment and the OM content was measured by weight loss  
142 by ignition for four h at 450 °C. Redox was determined in each section by an electrochemical  
143 sensor (Hamilton Liq-Glass ORP). Acid-volatile sulphide (AVS) content in the sediment was  
144 determined by distillation and then quantified following Allen's et al. method (1993). The same  
145 analyses were done to analyse 4 initial cores to know the sediment initial conditions. The initial  
146 cores were set up following the same steps that the rest of the cores, being maintained for  
147 four days to stratify before the analyses were done to ensure that the conditions were similar  
148 to those of the cores of the experiment. Additionally, in the cores after the experiment,  
149 bioturbation rates were quantified by modelling the bromide (Br<sup>-</sup>) excess in the porewater  
150 (bromide profiles) along with the sediment depth and corrected for diffusion (Heilskov et al.,  
151 2006). The Br<sup>-</sup> concentration in the porewater was analysed by ion-exchange chromatography  
152 with a Dionex auto-suppressed anion system (IonPac As9-HC column and AG9-HC suppressor,  
153 Thermo Fisher Scientific, Sunnyvale, CA, USA) and bicarbonate/carbonate eluent. Due to  
154 economic constraints bromide profiles were only measured in the cores with the highest level  
155 of OM enrichment.

## 156 2.4 Data Analysis

157 Significant differences in the TCO<sub>2</sub>, SOU, OM content, AVS accumulation and the mean depth-  
158 integrated redox values in the sediment were tested by pairwise t-tests between cores *with*  
159 and *without worms* for each level of OM enrichment. The trends of the measured variables  
160 along the OM enrichment gradient were modelled through regression considering OM  
161 enrichment as the continuous variable and *worm* as a fixed factor. Several regression models  
162 were applied and the Akaike information criterion (AIC) was used to choose the best model  
163 (Akaike, 2011). Homoscedasticity was checked using Levene's test and normality with p–p  
164 plots. Analyses were run in R (v. 3.4.2), and linear regressions were implemented using the lm



165 function (R Development Core Team, 2012). The data are reported as mean  $\pm$  standard error  
166 (SE), and the significance level of the statistical analyses was  $\alpha=0.05$ .

### 167 3. Results

#### 168 3.1 Visual results

169 *Lumbrinereis latreilli* produced burrows that were *ca.* 0.5 cm width and 7-14 cm deep (Fig. S1).  
170 There were no visual differences among cores with different enrichment of OM as regards the  
171 depth and width of the tubes. In all cases, the sediment close to the burrow showed a  
172 yellowish colour, that was less dark than the surrounding sediment. The sediment was light  
173 grey and became darker with increasing enrichments of OM.

#### 174 3.2 Carbon & Oxygen

175 TCO<sub>2</sub> fluxes in cores without OM enrichment were  $44.70 \pm 10.48$  mmol·m<sup>-2</sup>·d<sup>-1</sup> and  $83.37 \pm 7.43$   
176 mmol·m<sup>-2</sup>·d<sup>-1</sup> in cores *without* and *with worms*, respectively. TCO<sub>2</sub> fluxes showed a positive  
177 increase along the gradient of OM enrichment (Fig. 1; Table S1), with higher rises in cores  
178 *without worms* than in the ones *with worms* (Table 1). At the highest level of OM enrichment,  
179 *worms* had a marked effect diminishing TCO<sub>2</sub> fluxes to around 100 mmol·m<sup>-2</sup>·d<sup>-1</sup>. For SOU, in  
180 sediment without OM enrichment, the effect of *worms* was a stimulation of the rates with  
181 around 50 mmol·m<sup>-2</sup>·d<sup>-1</sup> along the gradient of OM enrichment (Fig. 1; Table 1 and S1). The  
182 TCO<sub>2</sub>:SOU ratio differed along the gradient of OM enrichment, wherein cores *without worms*  
183 showed marked increases up to 7 at intermediate levels of OM enrichment, whereas in cores  
184 *with worms* remained relatively stable and close to 1 (Fig. 1; Table 1).

#### 185 3.3 OM content

186 The sediment OM content showed a stable trend along the gradient of OM enrichment in the  
187 absence of *worms*. However, *worms* showed a variable effect on the OM content of the  
188 sediment along the gradient of OM enrichment. In cores without OM enrichment, *worms*  
189 lessened the sediment OM content to 0.39 kg·m<sup>-2</sup> corresponding to 83 % of the initial content.  
190 In cores with OM enrichment, the sediment OM content increased with increasing OM  
191 enrichment, up to 0.53 kg·m<sup>-2</sup> (Fig 2; Table 1 and S1).

#### 192 3.4 AVS accumulation

193 *Worms* had a marked effect on lessening the pools of AVS by *ca.* 20-40 mmol·m<sup>-2</sup> at all levels of  
194 OM enrichment (Fig. 2). No significant trends along the gradient of OM enrichment were found  
195 in cores either *with* and *without worms* (Table 1).

#### 196 3.5 Redox

197 In cores without OM enrichment, the mean depth-integrated redox values were similarly  
198 disregarding the presence of *worms* (*ca.* -230 mV). However, along the gradient of OM  
199 enrichment the mean depth-integrated values redox showed, generally, higher values in cores  
200 *with worms* than *without worms* (Fig. S2). The mean depth-integrated redox values in cores  
201 without OM enrichment were similar disregarding the presence of worms (around -230 mV)  
202 (Fig. 2). However, the mean depth-integrated redox values along the gradient of OM  
203 enrichment in cores *without worms* were lower (*ca.* -250 mV) than in cores *with worms*. In  
204 these cores, the mean depth-integrated redox values increased with increasing levels of OM  
205 enrichment, reaching a maximum value of  $-84.9 \pm 0.9$  mV (Tables 1 and S1).



## 206 3.6 Bioturbation activity

207 Depth-profiles of  $\text{Br}^-$  concentration were significantly higher in cores *with worms* ( $2.4 \cdot 10^{-3} \pm 1 \cdot 10^{-4}$  mmol  $\text{Br}^- \cdot \text{cm}^{-3}$ ) than in cores *without worms* ( $3 \cdot 10^{-4} \pm 1 \cdot 10^{-4}$  mmol  $\text{Br}^- \cdot \text{cm}^{-3}$ ) suggesting  
208 that the worm produced bioturbation (Fig. S2).  
209

## 210 4. Discussion

211 Our experiment confirms that the bioturbation produced by *L. latreilli* can modify the  
212 biogeochemical conditions of the sediment, leading to important consequences in the cycling  
213 of elements, which agrees with previous studies (Callier et al., 2009; Casado-Coy et al., 2017;  
214 Mermillod-Blondin et al., 2004). Disregarding the level of OM enrichment, bioturbation  
215 promotes oxic conditions of the sediment, limiting anaerobic metabolic pathways and  
216 preventing their by-products. More interestingly, our experiment shows that bioturbation can  
217 lower the metabolic capacity of the sediment.

## 218 4.1 Carbon, oxygen &amp; sulphur dynamics

219 Our study suggests, that under non-OM enrichment conditions, bioturbation enhanced  $\text{TCO}_2$   
220 fluxes indicating an enhancement of the sediment metabolic pathways, which agrees with  
221 other studies (Banta et al., 1999; Kristensen and Kostka, 2013; Sanz-Lázaro et al., 2011c).  
222 Contrastively, when the sediment suffered OM enrichment, above a certain level of OM  
223 enrichment, bioturbation diminished  $\text{TCO}_2$  fluxes, indicating a decrease of the sediment  
224 metabolic rates. Thus, bioturbation seems to have opposing effects depending on OM  
225 enrichment. This finding partially contradicts the current paradigm that bioturbation of  
226 macrofauna enhances benthic metabolic pathways (Arndt et al., 2013; Callier et al., 2009).  
227 Despite it has been presumed that this assumption is true, some studies, even not directly  
228 testing this hypothesis, also suggest this opposite effect under OM enrichment conditions  
229 (Andersen and Kristensen, 1992; Casado-Coy et al., 2017; Welsh, 2003).

230 Our data on the sediment OM content are coherent with  $\text{TCO}_2$  fluxes. In non-  
231 bioturbated sediments, incremental addition of OM enrichment led to steep increases in  $\text{TCO}_2$   
232 production, which could be due to a priming effect (Guenet et al., 2010). The priming effect is  
233 a complex process that modifies mineralization rates of the sediment organic matter due to  
234 inputs of labile organic matter (Gontikaki et al., 2015). In agreement with this hypothesis, the  
235 level of OM content in non-bioturbated sediments remained similar irrespectively of the  
236 amount of OM added. Contrastingly, in bioturbated sediments, the enrichment of OM led to a  
237 less marked increment in  $\text{TCO}_2$  production. Accordingly, OM content increased with increasing  
238 levels of OM enrichment. These results suggest that the decrease of the sediment metabolic  
239 capacity caused by bioturbation resulted in the accumulation of OM content in the sediment.

240 As regards oxygen, SOU rates were always 2-3 fold higher in bioturbated than in non-  
241 bioturbated sediments and the difference between both sediments remained constant along  
242 the OM enrichment gradient. This fact suggests that bioturbation can maintain aerobic  
243 respiration, as well as promote the reoxidation of reduced by-products derived from anaerobic  
244 metabolic pathways disregarding the level of OM enrichment (at least up to  $500 \text{ mmol C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$   
245 sedimentation rate). Part of the increment of the aerobic respiration comes, not only from  
246 the enhancement of the metabolic pathways of the microorganisms but also from the worms  
247 itself. Macrofauna increases the sediment  $\text{TCO}_2$  fluxes and SOU due to its own aerobic  
248 respiration, but generally constitutes a low percentage of the total sediment metabolism  
249 (Banta et al., 1999; Braeckman et al., 2010; Heilskov et al., 2006).

250 In our study, bioturbated sediments had a  $\text{TCO}_2:\text{SOU}$  ratio close to 1, indicating the  
251 prevalence of oxic conditions and aerobic respiration, which agreed with . Accordingly, the  
252 mean depth-integrated redox values, which were generally higher in bioturbated than in non-  
253 bioturbated sediments. This fact indicates that bioturbation can keep aerobic respiration as  
254 the main metabolic pathway disregarding the level of OM enrichment (at least up to  $500 \text{ mmol}$   
255  $\text{C}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  sedimentation rate). This regulatory capacity seems to be lost in the absence of  
256 bioturbation, where ratios were 2 to 7 times higher and the mean depth-integrated redox  
257 values lower than when worms were present. Thus, these results suggest that bioturbation  
258 promote aerobic respiration and the reoxidation of reduced by-products, which agrees with  
259 previous studies (Casado-Coy et al., 2017; Heilskov et al., 2006; Valdemarsen et al., 2009).

260 Sulphate reduction is a prevalent anaerobic mineralization pathway of OM in marine  
261 sediments (Jørgensen, 1982) and produces sulphides as a by-product, which can be  
262 sequestered in the sediment as AVS. In our experiment, bioturbated sediment showed lower  
263 values of AVS accumulation than non-bioturbated sediment along the gradient of OM  
264 enrichment. This fact indicates that bioturbation can promote oxic conditions, lessening the  
265 prevalence of anaerobic metabolic pathways and their derived by-production formation and  
266 the reoxidation of buried AVS (Holmer and Barry, 2005) lowering AVS accumulation, as shown  
267 in previous experiments under organic pollution conditions (Casado-Coy et al., 2017; Martinez-  
268 Garcia et al., 2015).

269 Bioturbation is expected to be due to organisms such as the worm used in this  
270 experiment produce. The bromide profiles demonstrated that, at the highest level of OM  
271 enrichment, the species used was able to actively produce the exchange of solutes between  
272 the water column and the sediment down to 16 cm, indicating the bioturbation capacity of the  
273 species used. Accordingly, redox values along depth profiles were generally higher in the  
274 sediment with worms than in the sediment without worms. Due to economic constraints, the  
275 bromide profiles could not be done at other levels of OM enrichment. Nevertheless, although  
276 bioturbation rates may be affected by OM enrichment, if at the highest level of OM  
277 enrichment worms can produce a notable bioturbation effect, it is expected this effect was  
278 also occurring at other levels of OM enrichment, as it has already been demonstrated with the  
279 same species (Casado-Coy et al., 2017).

#### 280 *4.2 Ecological Implications*

281 Bioturbation has a main role in the mineralization of OM, especially coastal sediments (Glud,  
282 2008), enhancing its metabolic capacity (Hedges and Keil, 1995). Among marine sediments,  
283 coastal ones are prone to OM enrichment since many anthropogenic activities that are sources  
284 of this type of pollution are based in coastal areas (Griffen et al., 2016). The present  
285 experiment suggests that bioturbated sediments can buffer the negative consequences of  
286 anoxic conditions produced by OM enrichment (Aller and Aller, 1998; Valdemarsen and  
287 Kristensen, 2005). This buffering capacity seems to be achieved by maintaining oxic conditions  
288 disregarding the level of OM enrichment (at least up to  $500 \text{ mmol C}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  sedimentation  
289 rate). The prevalence of oxic conditions enhances the metabolic capacity of sediments under  
290 conditions of no OM enrichment . Contrastingly, above a certain level of OM enrichment,  
291 bioturbation may reduce the metabolic capacity of sediments. Oxic conditions may limit  
292 sulphate reduction and other anaerobic metabolic pathways, and consequently, the metabolic  
293 capacity of sediments (Jørgensen, 1982). Thus, the metabolic capacity of sediments may not be  
294 always maximized by bioturbation produced by macrofauna. Under OM enrichment

295 conditions, anaerobic pathways may increase the metabolic capacity of sediments at the  
296 expense of producing high amounts of toxic by-products derived from these pathways.

297 Macrofauna, through bioturbation, plays a key role in coastal sediments processes and  
298 functions, such as oxygenation, recycling of nutrients and metabolic capacity (Sanz-Lazaro and  
299 Marin, 2011), that sustain key ecosystem services, such as food provision. Thus, the  
300 preservation of macrofauna with bioturbation capacity is a priority for environmental  
301 managers to maintain a good environmental status of the sediment. However, our study  
302 suggests that above a certain level of OM enrichment, bioturbation can diminish the sediment  
303 metabolism capacity, lowering the ability to mitigate this type of pollution. Thus, this study  
304 suggests that, when defining OM enrichment thresholds to set up the carrying capacity of  
305 sediments, not only the preservation of macrofauna must be considered. Other variables, such  
306 as the sediment metabolic capacity, are important parameters to be taken account. Thresholds  
307 must be established seeking for preservation of the natural metabolic capacity of sediments,  
308 which depend on the sediment parameters such as the grain size (Martinez-Garcia et al.,  
309 2015), the type of benthic habitat (Sanz-Lázaro and Marín, 2008) and the bioturbation capacity  
310 of macrofauna. Therefore, the thresholds must be adaptive and measurable (e.g. Sanz-Lázaro  
311 et al., 2011a) to make sure that the carrying capacity of an ecosystem is not surpassed. Using  
312 these ideas as a framework will foster the good status of coastal sediments and, consequently,  
313 a correct functioning of the ecological processes and the derived services to society.

#### 314 4.3 Study limitations

315 Microcosm experiments are always an approximation of natural ecosystems;  
316 therefore, results must be interpreted carefully. Nevertheless, the microcosm experiments let  
317 us control environmental variables and hence to study cause-effect relationships. The range of  
318 the values of the measured parameters in this study is comparable to other microcosm studies  
319 (Bergström et al., 2017; Sanz-Lázaro et al., 2015; Valdemarsen et al., 2010, 2009). The  
320 bioturbation rates, estimated as bromide profiles, were comparable to other microcosm  
321 experiments using natural macrofauna assemblages (Valdemarsen et al., 2010) or estimated *in*  
322 *situ* (Kristensen and Holmer, 2001) Additionally, TCO<sub>2</sub> and O<sub>2</sub> fluxes, as well as the AVS  
323 accumulation, the OM content and the mean depth-integrated redox values were comparable  
324 with *in situ* measurements (Callier et al., 2009; Christensen et al., 2000; Giles et al., 2006;  
325 Sundby et al., 1992). Thus, the results obtained in this experiment can, at least to some extent,  
326 be comparable to natural bioturbation in the sediment.

327 Nevertheless, more experiments should be performed with other species of  
328 macrofauna and with a combination of them, using sediments with different grain sizes and in  
329 different habitats, to contrast the results and give a wider perspective. Also, it must bear on  
330 mind the relatively short duration of the experiment. Additionally, epibenthic invertebrates  
331 and benthic/demersal fish can also produce relevant bioturbation (Sanz-Lázaro et al., 2011b;  
332 Vita et al., 2004). Accordingly, complementary *in situ* experiments with more types of  
333 bioturbating species should be desirable to test these effects. In general, more research effort  
334 is needed to better predict the biogeochemical consequences of OM enrichment in coastal  
335 sediments.

#### 336 5. Conclusions

337 This study demonstrates that bioturbated sediments, by maintaining oxic conditions,  
338 can buffer the negative consequences of anoxic conditions derived from OM enrichment. As  
339 the levels of OM enrichment increase, this maintenance of oxic conditions can hamper the

340 sediment metabolic capacity, increasing the accumulation of OM content in the sediment. The  
341 results of the present study suggest that, under high levels of OM enrichment, the prevalence  
342 of oxic conditions through the bioturbation of macrofauna hamper anaerobic metabolic  
343 pathways, resulting in the diminution of the sediment metabolic capacity. These findings  
344 question the current paradigm that bioturbation produced by macrofauna enhances the  
345 sediment metabolic pathways. Thus, this study suggests that, when defining OM enrichment  
346 thresholds to set up the carrying capacity of sediments, not only the preservation of  
347 macrofauna must be considered. The sediment metabolic capacity is an important parameter  
348 to be taken account and may not follow a linear relationship with the level of OM enrichment.  
349 Therefore, keeping sediments with healthy macrofauna and under the least possible levels of  
350 OM enrichment must be a priority to guarantee a good ecological status of marine sediments  
351 and the preservation of the ecological services they provide.

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553 **Figures & Table**

554

555 **Figure 1.** A) total CO<sub>2</sub> (TCO<sub>2</sub>) release in sediment–water-column flux (i.e., efflux), B) sediment oxygen  
556 uptake (SOU) sediment–water-column flux rates and C) TCO<sub>2</sub>:SOU ratio (n=3, mean ±SE) versus organic  
557 matter inputs [additional C sedimentation rates (mmol C·m<sup>-2</sup>·d<sup>-1</sup>)] without worms (-W) and with worms  
558 (+W). Lines indicate significant (p < 0.05) regressions for cores -W and +W treatments. Type of  
559 regression was chosen according to the AIC (Table S1). R<sup>2</sup> refers to the regression model for each  
560 variable, which includes the factor *worm*.

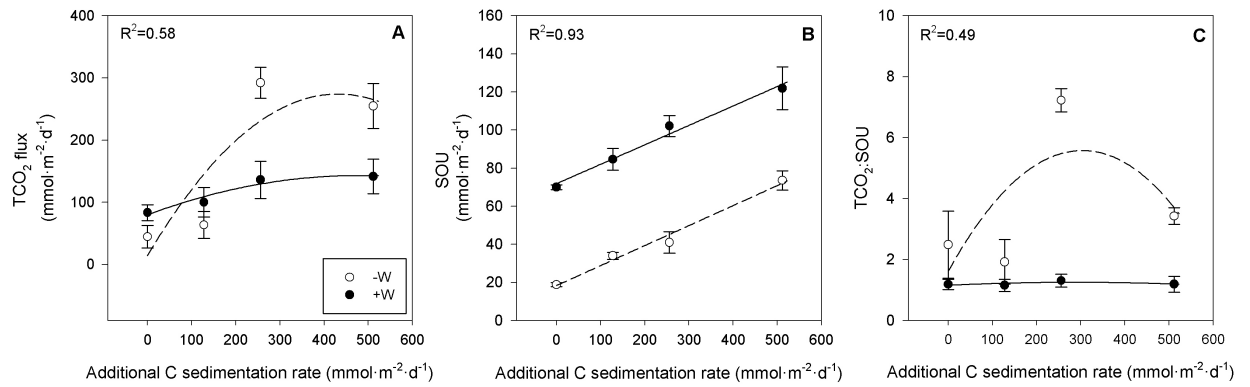
561 **Figure 2.** A) organic matter (OM) content in sediment B) depth-integrated accumulation acid volatile  
562 sulphide (AVS) accumulated in sediment and C) mean depth-integrated redox values (16 cm) (n=3, mean  
563 ±SE) versus organic matter inputs [additional C sedimentation rates (mmol C·m<sup>-2</sup>·d<sup>-1</sup>)] without worms (-  
564 W) and with worms (+W). Lines indicate significant (p < 0.05) regressions for cores -W and +W  
565 treatments. Type of regression was chosen according to the AIC (Table S1). R<sup>2</sup> refers to the regression  
566 model for each variable, which includes the factor *worm*.

567 **Table 1.** Coefficients (mean ± SE) of the regression model for total CO<sub>2</sub> (TCO<sub>2</sub>) release in sediment–  
568 water-column flux (i.e., efflux), sediment oxygen uptake (SOU), TCO<sub>2</sub>:SOU ratio, organic matter (OM)  
569 content of sediment, depth-integrated accumulation acid volatile sulphide (AVS) accumulation in  
570 sediment (16 cm) and the mean depth-integrated redox values (16 cm) along the gradient of additional  
571 organic matter in the absence of worm (intercept; -W) and presence (+W) of worm. The first coefficient  
572 indicates the value at the baseline (without organic matter input) and the second coefficient indicates  
573 the linear term of the regression. When the regression was a second order polynomial one, there is a  
574 third (quadratic) coefficient. The type of regression model is selected according the AIC (see Table S1).  
575 Significant effects (p < 0.05) are indicated in bold.

576

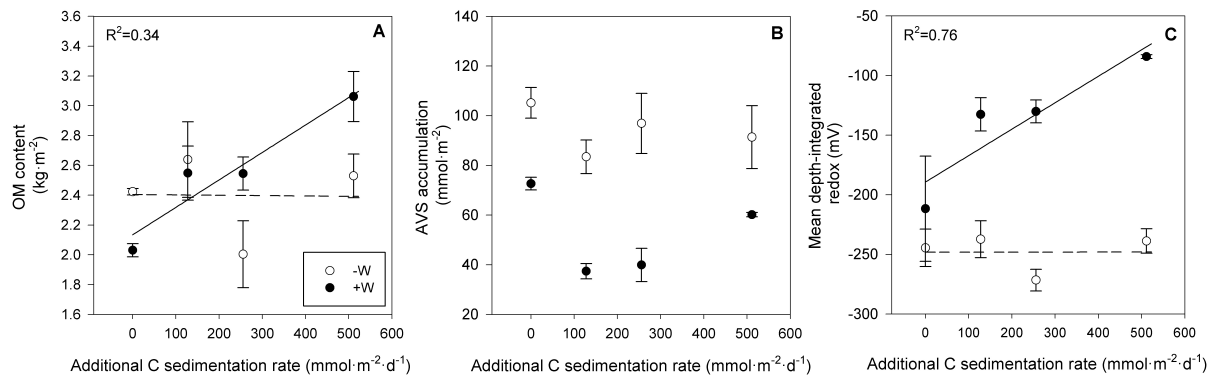
577 **Figure 1.**

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580

581 **Figure 2.**

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583

584 **Table 1.**

		Without additional organic matter	Additional organic matter lineal term	Additional organic matter quadratic term
TCO <sub>2</sub> flux	-W	14.09 (33.43)	<b>1.21</b> (0.33)	-0.94 (0.47)
	+W	65.95 (47.28)	<b>-0.001</b> ( $6 \cdot 10^{-4}$ )	0.001 ( $8 \cdot 10^{-4}$ )
SOU	-W	<b>18.38</b> (4.09)	<b>0.10</b> (0.01)	-
	+W	<b>53.48</b> (5.78)	-0.003 (0.02)	-
TCO <sub>2</sub> :SOU	-W	1.62 (0.83)	<b>-0.26</b> (0.008)	<b>-0.025</b> (0.01)
	+W	-0.46 (1.18)	<b>-4.30</b> ·10 <sup>-2</sup> ( $1.50 \cdot 10^{-5}$ )	$4.19 \cdot 10^{-5}$ ( $2.13 \cdot 10^{-5}$ )
OM content	-W	<b>2.40</b> (0.14)	$-2.35 \cdot 10^{-5}$ ( $4.96 \cdot 10^{-4}$ )	-
	+W	-0.27 (0.20)	<b>1.87</b> ·10 <sup>-3</sup> ( $7.01 \cdot 10^{-4}$ )	-
Depth-integrated AVS accumulation	-W	<b>90.58</b> (4.67)	0.14 (0.09)	-
	+W	<b>-44.77</b> (6.60)	0.12 (0.13)	-
Mean depth- integrated redox	-W	<b>-248.2</b> (15.5)	$5 \cdot 10^{-4}$ (0.05)	-
	+W	<b>-189.27</b> (21.86)	<b>0.22</b> (0.07)	-

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## Supplementary information

**Table S1.** AIC (Akaike information criterion) of the different regression models for total CO<sub>2</sub> (TCO<sub>2</sub>) release in sediment–water-column flux (i.e., efflux), sediment oxygen uptake (SOU) sediment–water-column flux rates, TCO<sub>2</sub>:SOU ratio, organic matter (OM) sediment content, depth-integrated accumulation acid volatile sulphide (AVS) accumulated in sediment (16 cm) and the mean depth-integrated redox values (16 cm) values versus organic matter inputs [additional C sedimentation rates (mmol C·m<sup>-2</sup>·d<sup>-1</sup>)] without worms and with worms. The significant regression models (p < 0.05) are indicated in bold.

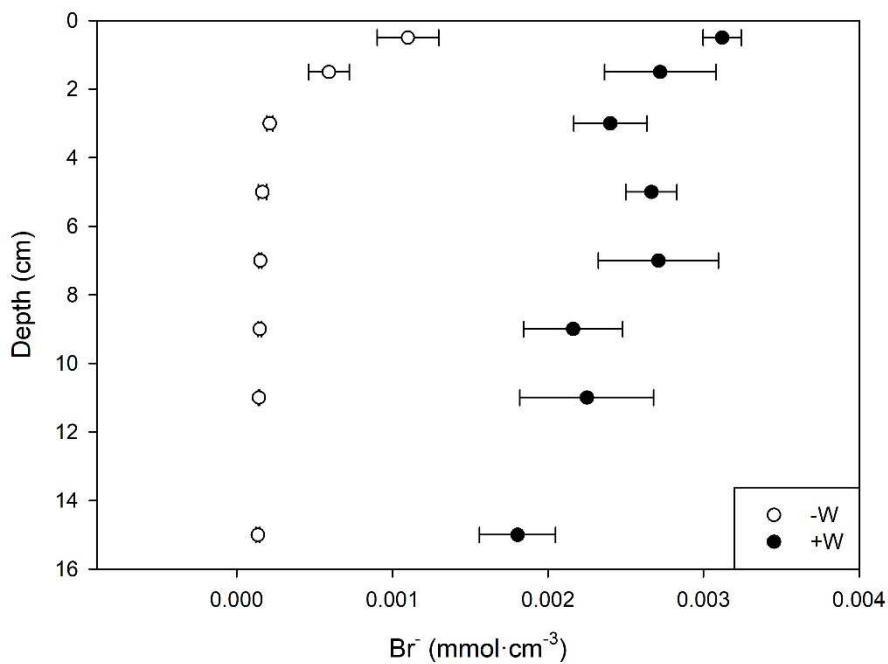
	AIC					
	TCO <sub>2</sub> flux	SOU	TCO <sub>2</sub> :SOU	OM content	AVS accumulation	Mean depth-integrated redox
$Y_i = \beta_0 + \beta_1 X_i$	274.57	<b>179.97</b>	99.94	<b>19.71</b>	210.17	<b>243.79</b>
$Y_i = \beta_0 + \beta_1 X_i + \beta_2 X_i^2$	<b>272.07</b>	182.70	<b>94.93</b>	21.33	201.63	244.51
$Y_i = \beta_0 + \beta_1 (1/X_i)$	283.32	211.77	99.21	23.78	<b>200.41</b>	244.06

589 **Figure S1.** A) Burrows produced by the bioturbation of the polychaete *Lumbrineris latreilli* in the  
 590 sediment of the cores used in the experiment. B) Polychaete *L. latreilli* used in the experiment  
 591 (author of photo B Elena Martinez-García).



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593 **Figure S2.** Bromide ( $\text{Br}^-$ ) concentrations (mean  $\pm$ SE,  $n=3$ ) in porewater along the sediment depth profile  
 594 ( $\text{Br}^- \text{ mmol}\cdot\text{cm}^{-3}$ ) at the end of the experiment in cores without worms (-W) and cores with worms (+W)  
 595 at the highest level of additional organic matter enrichment ( $511.2 \text{ mmol C m}^{-2}\cdot\text{d}^{-1}$ ).



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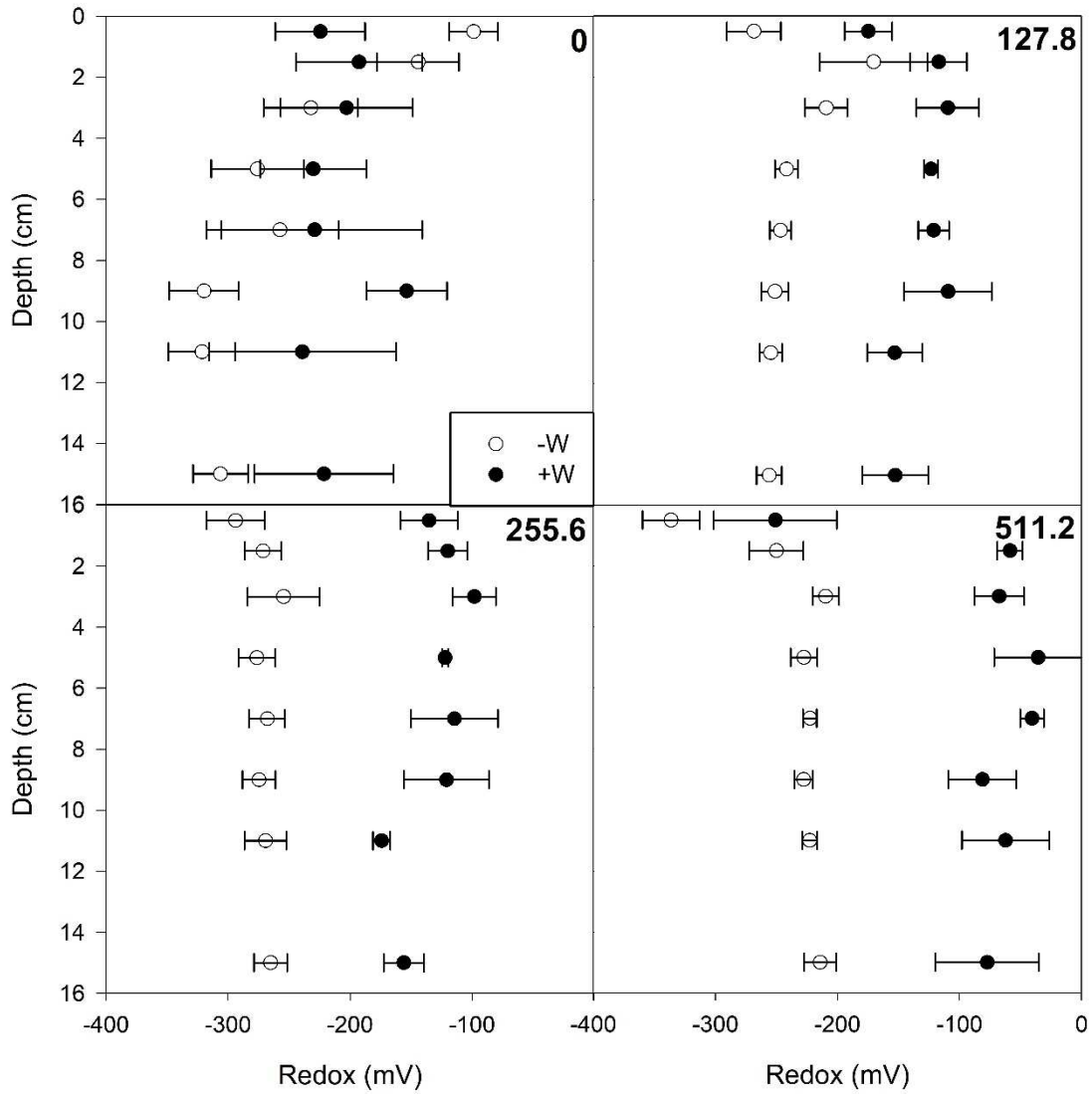
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599 **Figure S2.** Depth profile of redox (mean  $\pm$ SE,  $n=3$ ) of sediment in 16 cm depth in each level of organic matter enrichment (additional C sedimentation rates) in cores without worms (-W) and in cores with worms (+W). The number in the upper right corner of each plot corresponds to the additional C sedimentation rates ( $\text{mmol-C m}^{-2}\cdot\text{d}^{-1}$ ).

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**Highlights**

- Bioturbation can buffer the negative consequences of anoxic conditions produced by organic matter pollution.
- The maintenance of oxic conditions through bioturbation is at the expense of limiting the sediment metabolic capacity.
- Under organic matter pollution conditions, bioturbation may lessen the metabolic capacity of the sediment.

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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