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

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Author Statement

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

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Abstract

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

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

Keywords: aquaculture; bioirrigation; bioturbation; metabolic capacity; organic matter
1. Introduction

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

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

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

The environmental drawbacks of OM enrichment in the sediment are derived from oxygen depletion, which enhance anaerobic metabolic pathways (Zhang et al., 2010) and, consequently, the release of their toxic by-products such as sulphides and methane. These conditions negatively affect macrofauna, leading to the predominance of opportunistic species which in general have a low bioturbation capacity (Heilskov and Holmer, 2001; Pearson and Rosenberg, 1978). Eventually, if the level of OM enrichment is large enough, macrofauna can be depleted (Diaz and Rosenberg, 1995; Lu and Wu, 2007) decreasing, even more, the metabolic capacity of the sediment (Sanz-Lazaro and Marin, 2011). Additionally, OM enrichment can affect the water column by increasing the nutrient supply from the sediment, causing eutrophication (Dimitriou et al., 2015).
Understanding how the sediment conditions and its metabolic capacity change with OM enrichment are mandatory to estimate the carrying capacity of marine sediments, a key parameter for the environmental agencies to establish the maximum OM enrichment that each benthic habitat can receive. Applying these carrying capacities to human activities that produce OM enrichment, is compulsory to promote their sustainability by maintaining a good status of marine ecosystems and of the ecological services that provide to society.

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

2. Material & Methods

2.1 Experimental Set-Up

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

To test the effect of the bioturbation of macrofauna (+W), three worms (*Lumbrineris latreilli* Audouin & Milne Edwards, 1834) of length 14–20 cm were added to half of the cores (12) (c. 1300 individuals m⁻²), density observed in the coast of Alicante (personal communication). Bioturbing worms are frequently used in mesocosm experiments as a model of the bioturbation effect of macrofauna (Banta et al., 1999; Kristensen, 2000). The other 12 cores were left without worms (-W). Worms were bought from a fishing bait supplier and the three healthy worms were added 7 days after the enrichment of OM load in each core. When the worms were added it was considered that the experiment had started (t=0). The seawater (salinity 37.6) was collected from the same area than the sediment and previously filtered (ø 0.001 mm) to remove large particles. The cores were maintained in darkness in an environmental chamber, submerged in aired seawater at 16°C. Temperature was controlled by means of two coolers and a pump that recirculated the water through the tank and the
coolers. Seawater column inside the cores was stirred by magnetic bars (4 cm length), which were placed a few centimetres above the sediment surface, driven by a rotating magnet placed close the cores to favour water circulation from the cores and mix with the rest of the water of the environmental chamber. Additionally, every 2 days, ca. half of the seawater of the chamber was renewed to prevent unnatural accumulation or depletion of molecules in the water. The chamber was set up as done by Piedecausa et al. (2012).

2.2 Biogeochemical Fluxes

The experiment lasted 27 days and during this period six incubations were carried out to determine the fluxes of total CO₂ (TCO₂ flux) and the sediment oxygen uptake (SOU), by estimating the production or consumption rates during the incubation by measuring the concentration of the molecules before and after the incubation. The incubations were done by sealing the upper part of the cores with a rubber stopper for a relatively short time periods (2–4 h for OM enriched cores and 4–5 h for cores without OM enrichment) to prevent experimental artefacts derived from excessive oxygen depletion (Glud, 2008). Initially, the incubations were performed 2 days per week during the first two weeks, and then once per week. TCO₂ was estimated using total carbon titration with Titrisol HCl (0.1 mol L⁻¹, Applichem Panreac, Germany Methyl Red.) at two pH ranges (Gran et al., 1950). O₂ was measured with an oximeter (CRISON OXI 45 P).

2.3 Sediment Analyses

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

2.4 Data Analysis

Significant differences in the TCO₂, SOU, OM content, AVS accumulation and the mean depth-integrated redox values in the sediment were tested by pairwise t-tests between cores with and without worms for each level of OM enrichment. The trends of the measured variables along the OM enrichment gradient were modelled through regression considering OM enrichment as the continuous variable and worm as a fixed factor. Several regression models were applied and the Akaike information criterion (AIC) was used to choose the best model (Akaike, 2011). Homoscedasticity was checked using Levene’s test and normality with p–p plots. Analyses were run in R (v. 3.4.2), and linear regressions were implemented using the lm
function (R Development Core Team, 2012). The data are reported as mean ± standard error (SE), and the significance level of the statistical analyses was \( \alpha = 0.05 \).

3. Results

3.1 Visual results

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

3.2 Carbon & Oxygen

TCO\(_2\) fluxes in cores without OM enrichment were 44.70± 10.48 mmol·m\(^{-2}\)·d\(^{-1}\) and 83.37± 7.43 mmol·m\(^{-2}\)·d\(^{-1}\) in cores without and with worms, respectively. TCO\(_2\) fluxes showed a positive increase along the gradient of OM enrichment (Fig. 1; Table S1), with higher rises in cores without worms than in the ones with worms (Table 1). At the highest level of OM enrichment, worms had a marked effect diminishing TCO\(_2\) fluxes to around 100 mmol·m\(^{-2}\)·d\(^{-1}\). For SOU, in sediment without OM enrichment, the effect of worms was a stimulation of the rates with around 50 mmol·m\(^{-2}\)·d\(^{-1}\) along the gradient of OM enrichment (Fig. 1; Table 1 and S1). The TCO\(_2\):SOU ratio differed along the gradient of OM enrichment, wherein cores without worms showed marked increases up to 7 at intermediate levels of OM enrichment, whereas in cores with worms remained relatively stable and close to 1 (Fig. 1; Table 1).

3.3 OM content

The sediment OM content showed a stable trend along the gradient of OM enrichment in the absence of worms. However, worms showed a variable effect on the OM content of the sediment along the gradient of OM enrichment. In cores without OM enrichment, worms lessened the sediment OM content to 0.39 kg·m\(^{-2}\) corresponding to 83 % of the initial content. In cores with OM enrichment, the sediment OM content increased with increasing OM enrichment, up to 0.53 kg·m\(^{-2}\) (Fig 2; Table 1 and S1).

3.4 AVS accumulation

Worms had a marked effect on lessening the pools of AVS by ca. 20-40 mmol·m\(^{-2}\) at all levels of OM enrichment (Fig. 2). No significant trends along the gradient of OM enrichment were found in cores either with and without worms (Table 1).

3.5 Redox

In cores without OM enrichment, the mean depth-integrated redox values were similarly disregarding the presence of worms (ca. -230 mV). However, along the gradient of OM enrichment the mean depth-integrated values redox showed, generally, higher values in cores with worms than without worms (Fig. S2). The mean depth-integrated redox values in cores without OM enrichment were similar disregarding the presence of worms (around -230 mV) (Fig. 2). However, the mean depth-integrated redox values along the gradient of OM enrichment in cores without worms were lower (ca. -250 mV) than in cores with worms. In these cores, the mean depth-integrated redox values increased with increasing levels of OM enrichment, reaching a maximum value of -84.9±0.9 mV (Tables 1 and S1).
3.6 Bioturbation activity

Depth-profiles of Br⁻ concentration were significantly higher in cores with worms (2.4·10⁻³±1·10⁻⁴ mmol Br⁻·cm⁻³) than in cores without worms (3·10⁻⁴±1·10⁻⁴ mmol Br⁻·cm⁻³) suggesting that the worm produced bioturbation (Fig. S2).

4. Discussion

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

4.1 Carbon, oxygen & sulphur dynamics

Our study suggests, that under non-OM enrichment conditions, bioturbation enhanced TCO₂ fluxes indicating an enhancement of the sediment metabolic pathways, which agrees with other studies (Banta et al., 1999; Kristensen and Kostka, 2013; Sanz-Lázaro et al., 2011c). Contrastively, when the sediment suffered OM enrichment, above a certain level of OM enrichment, bioturbation diminished TCO₂ fluxes, indicating a decrease of the sediment metabolic rates. Thus, bioturbation seems to have opposing effects depending on OM enrichment. This finding partially contradicts the current paradigm that bioturbation of macrofauna enhances benthic metabolic pathways (Arndt et al., 2013; Callier et al., 2009). Despite it has been presumed that this assumption is true, some studies, even not directly testing this hypothesis, also suggest this opposite effect under OM enrichment conditions (Andersen and Kristensen, 1992; Casado-Coy et al., 2017; Welsh, 2003).

Our data on the sediment OM content are coherent with TCO₂ fluxes. In non-bioturbated sediments, incremental addition of OM enrichment led to steep increases in TCO₂ production, which could be due to a priming effect (Guenet et al., 2010). The priming effect is a complex process that modifies mineralization rates of the sediment organic matter due to inputs of labile organic matter (Gontikaki et al., 2015). In agreement with this hypothesis, the level of OM content in non-bioturbated sediments remained similar irrespectively of the amount of OM added. Contrastingly, in bioturbated sediments, the enrichment of OM led to a less marked increment in TCO₂ production. Accordingly, OM content increased with increasing levels of OM enrichment. These results suggest that the decrease of the sediment metabolic capacity caused by bioturbation resulted in the accumulation of OM content in the sediment.

As regards oxygen, SOU rates were always 2-3 fold higher in bioturbated than in non-bioturbated sediments and the difference between both sediments remained constant along the OM enrichment gradient. This fact suggests that bioturbation can maintain aerobic respiration, as well as promote the reoxidation of reduced by-products derived from anaerobic metabolic pathways disregarding the level of OM enrichment (at least up to 500 mmol C·m⁻²·d⁻¹ sedimentation rate). Part of the increment of the aerobic respiration comes, not only from the enhancement of the metabolic pathways of the microorganisms but also from the worms itself. Macrofauna increases the sediment TCO₂ fluxes and SOU due to its own aerobic respiration, but generally constitutes a low percentage of the total sediment metabolism (Banta et al., 1999; Braeckman et al., 2010; Heilskov et al., 2006).
In our study, bioturbated sediments had a TCO$_2$:SOU ratio close to 1, indicating the prevalence of oxic conditions and aerobic respiration, which agreed with. Accordingly, the mean depth-integrated redox values, which were generally higher in bioturbated than in non-bioturbated sediments. This fact indicates that bioturbation can keep aerobic respiration as the main metabolic pathway disregarding the level of OM enrichment (at least up to 500 mmol C·m$^{-2}$·d$^{-1}$ sedimentation rate). This regulatory capacity seems to be lost in the absence of bioturbation, where ratios were 2 to 7 times higher and the mean depth-integrated redox values lower than when worms were present. Thus, these results suggest that bioturbation promote aerobic respiration and the reoxidation of reduced by-products, which agrees with previous studies (Casado-Coy et al., 2017; Heilskov et al., 2006; Valdemarsen et al., 2009).

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

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

4.2 Ecological Implications

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

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

4.3 Study limitations

Microcosm experiments are always an approximation of natural ecosystems; therefore, results must be interpreted carefully. Nevertheless, the microcosm experiments let us control environmental variables and hence to study cause-effect relationships. The range of the values of the measured parameters in this study is comparable to other microcosm studies (Bergström et al., 2017; Sanz-Lázaro et al., 2015; Valdemarsen et al., 2010, 2009). The bioturbation rates, estimated as bromide profiles, were comparable to other microcosm experiments using natural macrofauna assemblages (Valdemarsen et al., 2010) or estimated in situ (Kristensen and Holmer, 2001). Additionally, TCO₂ and O₂ fluxes, as well as the AVS accumulation, the OM content and the mean depth-integrated redox values were comparable with in situ measurements (Callier et al., 2009; Christensen et al., 2000; Giles et al., 2006; Sundby et al., 1992). Thus, the results obtained in this experiment can, at least to some extent, be comparable to natural bioturbation in the sediment.

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

5. Conclusions

This study demonstrates that bioturbated sediments, by maintaining oxic conditions, can buffer the negative consequences of anoxic conditions derived from OM enrichment. As the levels of OM enrichment increase, this maintenance of oxic conditions can hamper the
sediment metabolic capacity, increasing the accumulation of OM content in the sediment. The results of the present study suggest that, under high levels of OM enrichment, the prevalence of oxic conditions through the bioturbation of macrofauna hamper anaerobic metabolic pathways, resulting in the diminution of the sediment metabolic capacity. These findings question the current paradigm that bioturbation produced by macrofauna enhances the sediment metabolic pathways. Thus, this study suggests that, when defining OM enrichment thresholds to set up the carrying capacity of sediments, not only the preservation of macrofauna must be considered. The sediment metabolic capacity is an important parameter to be taken account and may not follow a linear relationship with the level of OM enrichment. Therefore, keeping sediments with healthy macrofauna and under the least possible levels of OM enrichment must be a priority to guarantee a good ecological status of marine sediments and the preservation of the ecological services they provide.

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Figures & Table

**Figure 1.** A) total CO₂ (TCO₂) release in sediment–water-column flux (i.e., efflux), B) sediment oxygen uptake (SOU) sediment–water-column flux rates and C) TCO₂:SOU ratio (n=3, mean ±SE) versus organic matter inputs [additional C sedimentation rates (mmol C·m⁻²·d⁻¹)] without worms (-W) and with worms (+W). Lines indicate significant (p < 0.05) regressions for cores -W and +W treatments. Type of regression was chosen according to the AIC (Table S1). R² refers to the regression model for each variable, which includes the factor **worm**.

**Figure 2.** A) organic matter (OM) content in sediment B) depth-integrated accumulation acid volatile sulphide (AVS) accumulated in sediment and C) mean depth-integrated redox values (16 cm) (n=3, mean ±SE) versus organic matter inputs [additional C sedimentation rates (mmol C·m⁻²·d⁻¹)] without worms (-W) and with worms (+W). Lines indicate significant (p < 0.05) regressions for cores -W and +W treatments. Type of regression was chosen according to the AIC (Table S1). R² refers to the regression model for each variable, which includes the factor **worm**.

**Table 1.** Coefficients (mean ± SE) of the regression model for total CO₂ (TCO₂) release in sediment–water-column flux (i.e., efflux), sediment oxygen uptake (SOU), TCO₂:SOU ratio, organic matter (OM) content of sediment, depth-integrated accumulation acid volatile sulphide (AVS) accumulation in sediment (16 cm) and the mean depth-integrated redox values (16 cm) along the gradient of additional organic matter in the absence of worm (intercept; -W) and presence (+W) of worm. The first coefficient indicates the value at the baseline (without organic matter input) and the second coefficient indicates the linear term of the regression. When the regression was a second order polynomial one, there is a third (quadratic) coefficient. The type of regression model is selected according the AIC (see Table S1). Significant effects (p < 0.05) are indicated in bold.
Figure 1.
Figure 2.

A. OM content (kg m$^{-2}$) vs. Additional C sedimentation rate (mmol m$^2$ d$^{-1}$)

B. AVS accumulation (mmol m$^{-2}$) vs. Additional C sedimentation rate (mmol m$^2$ d$^{-1}$)

C. Mean depth-integrated redox (mV) vs. Additional C sedimentation rate (mmol m$^2$ d$^{-1}$)
<table>
<thead>
<tr>
<th></th>
<th>Without additional organic matter</th>
<th>Additional organic matter lineal term</th>
<th>Additional organic matter quadratic term</th>
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<tr>
<td><strong>TCO$_2$ flux</strong></td>
<td></td>
<td></td>
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<tr>
<td>-W</td>
<td>14.09 (33.43)</td>
<td>1.21 (0.33)</td>
<td>-0.94 (0.47)</td>
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<tr>
<td>+W</td>
<td>65.95 (47.28)</td>
<td>-0.001 (6·10$^{-4}$)</td>
<td>0.001 (8·10$^{-4}$)</td>
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<td><strong>SOU</strong></td>
<td></td>
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<tr>
<td>-W</td>
<td>18.38 (4.09)</td>
<td>0.10 (0.01)</td>
<td>-</td>
</tr>
<tr>
<td>+W</td>
<td>53.48 (5.78)</td>
<td>-0.003 (0.02)</td>
<td>-</td>
</tr>
<tr>
<td><strong>TCO$_2$-SOU</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-W</td>
<td>1.62 (0.83)</td>
<td>-0.26 (0.008)</td>
<td>-0.025 (0.01)</td>
</tr>
<tr>
<td>+W</td>
<td>-0.46 (1.18)</td>
<td>-4.30·10$^{-4}$ (1.50·10$^{-5}$)</td>
<td>4.19·10$^{-5}$ (2.13·10$^{-5}$)</td>
</tr>
<tr>
<td><strong>OM content</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-W</td>
<td>2.40 (0.14)</td>
<td>-2.35·10$^{-6}$ (4.96·10$^{-7}$)</td>
<td>-</td>
</tr>
<tr>
<td>+W</td>
<td>-0.27 (0.20)</td>
<td>1.87·10$^{-4}$ (7.01·10$^{-5}$)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Depth-integrated AVS accumulation</strong></td>
<td>90.58 (4.67)</td>
<td>0.14 (0.09)</td>
<td>-</td>
</tr>
<tr>
<td>+W</td>
<td>-44.77 (6.60)</td>
<td>0.12 (0.13)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Mean depth-integrated redox</strong></td>
<td>-248.2 (15.5)</td>
<td>5·10$^{-4}$ (0.05)</td>
<td>-</td>
</tr>
<tr>
<td>+W</td>
<td>-189.27 (21.86)</td>
<td>0.22 (0.07)</td>
<td>-</td>
</tr>
</tbody>
</table>
Supplementary information

Table S1. AIC (Akaike information criterion) of the different regression models for total CO₂ (TCO₂) release in sediment–water-column flux (i.e., efflux), sediment oxygen uptake (SOU) sediment–water-column flux rates, TCO₂: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⁻²·d⁻¹)] without worms and with worms. The significant regression models (p < 0.05) are indicated in bold.

<table>
<thead>
<tr>
<th>Model</th>
<th>TCO₂ flux</th>
<th>SOU</th>
<th>TCO₂:SOU</th>
<th>OM content</th>
<th>AVS accumulation</th>
<th>Mean depth-integrated redox</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yᵢ=β₀+β₁Xᵢ</td>
<td>274.57</td>
<td>179.97</td>
<td>99.94</td>
<td>19.71</td>
<td>210.17</td>
<td>243.79</td>
</tr>
<tr>
<td>Yᵢ=β₀+β₁Xᵢ+β₂Xᵢ²</td>
<td><strong>272.07</strong></td>
<td>182.70</td>
<td><strong>94.93</strong></td>
<td>21.33</td>
<td>201.63</td>
<td>244.51</td>
</tr>
<tr>
<td>Yᵢ=β₀+β₁(1/Xᵢ)</td>
<td>283.32</td>
<td>211.77</td>
<td>99.21</td>
<td>23.78</td>
<td><strong>200.41</strong></td>
<td>244.06</td>
</tr>
</tbody>
</table>
Figure S1. A) Burrows produced by the bioturbation of the polychaete *Lumbrineris latreilli* in the sediment of the cores used in the experiment. B) Polychaete *L. latreilli* used in the experiment (author of photo B Elena Martinez-Garcia).

Figure S2. Bromide (Br$^-$) concentrations (mean ±SE, n=3) in porewater along the sediment depth profile (Br$^-$ mmol·cm$^{-3}$) at the end of the experiment in cores without worms (-W) and cores with worms (+W) at the highest level of additional organic matter enrichment (511.2 mmol C m$^{-2}$·d$^{-1}$).
Figure S2. Depth profile of redox (mean ±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 (mmol·C m⁻²·d⁻¹).
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.
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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