

Effect of sediment grain size and bioturbation on decomposition of organic matter from aquaculture

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Abstract Sediment grain size plays a major role in sediment biogeochemistry and sediments with different grain size are expected to react differently to organic enrichment. Through a mesocosm approach we tested the behavior of sediments with two types of predominant grain size (sandy and muddy sediments) under two levels of organic enrichment, related to mussel and fish farming. The polychaete *Hediste diversicolor* was used to simulate macrofauna bioturbation and bioirrigation. *H. diversicolor* stimulated organic matter (OM) mineralization and nutrient recycling. Muddy sediments had more OM from nature, resulting in higher sulfate reduction rates than sandy sediments. Under low levels of organic enrichment grain size did not have any effect on benthic fluxes (sediment oxygen uptake, total CO₂, ammonium, nitrate, nitrite and phosphate). However, at high

levels of organic enrichment, sandy sediments accumulated less OM, less sulfide and less ammonium than muddy sediments, while sediment oxygen uptake and total CO₂ showed similar levels between sandy and muddy sediments. Thus, grain size should be considered a key parameter for site selection of fish farming facilities when aiming for a sustainable aquaculture industry.

Keywords Aquaculture · Organic enrichment · Benthic fluxes · Sediment biogeochemistry · *Hediste diversicolor*

Introduction

Soft sediments play a major role in biogeochemical cycles on a global scale (Papageorgiou et al. 2010). The functioning of soft sediments is complex due to the multiple variables and their interactions. Marine aquaculture facilities generate waste products that result in increased deposition rate of organic matter (OM), generally one or two orders of magnitude greater than natural background sedimentation (Wildish and Pohle 2005; Sundstein-Carlsson et al. 2009). This organic enrichment may change the sediment biogeochemistry, resulting in oxygen depletion, increased nutrient efflux and diminished benthic macrofauna diversity (Pearson and Rosenberg 1978; Hargrave et al. 2008). Low oxygen concentrations in the benthic environment promote anaerobic metabolic

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pathways, mainly sulfate reduction leading to high concentrations of dissolved sulfide in sediment pore water (Holmer et al. 2005). If sulfate is depleted, methanogenesis becomes the dominant microbial metabolic pathway. Under reduced conditions, sediments are prone to increase the nutrient flux to the water column as processes such as nitrification are inhibited and phosphate is released from iron-bound pools (Christensen et al. 2000; Sundstein-Carlsson et al. 2012). Sulfides and methane have deleterious consequences for the macrofauna inhabiting the sediment (Gray et al. 2002; Hargrave et al. 2008). Burrowing macrofauna are responsible for bioturbation and bioirrigation, which enhance oxic conditions in sediments by maximizing the sediment surface in contact with the water column and through active transport of oxygen into the sediment (Sanz-Lázaro and Marín 2011). Such activities increase the benthic metabolic capacity, promoting phosphate retention by the sediment and nitrogen removal from the system by enhancing the coupling between nitrification and denitrification (Pelegri et al. 1994; Banta et al. 1999; Christensen et al. 2003). Thus, bioturbation and bioirrigation can diminish the possible negative effects of organic enrichment in the sediment, considering that pollution does not reach high levels causing partial or total defaunation (Pearson and Rosenberg 1978).

The environmental effects of OM enrichment from fish farming depend on the cultivation technique, feed composition (Stigebrandta et al. 2004; Holmer et al. 2005) and also site-specific conditions including the prevailing physico-chemical and biological features of the receiving environment (Islam 2005; Sweetman et al. 2014). While studies have focused on the interactions of macrofauna and OM enrichment on sediment biogeochemistry (Kristensen 1988; Kalantzi and Karakassis 2006; Martinez-Garcia et al. 2013) few have focused on these effects in sediments with different grain size (Papageorgiou et al. 2010; Kanaya 2014). Marine aquaculture cages are located over both muddy and sandy bottoms. Muddy sediments are generally found in areas of low physical exposure, relatively deep sediments and in sheltered areas with high sedimentation and naturally high OM pools. Sandy sediments are predominantly found in exposed and shallow areas without naturally high OM pools. Exchange rates of dissolved compounds between sediment and water column depend on lability of

OM. Muddy sediments generally have more reduced conditions than sandy sediments, which influence the mineralization rates of OM in the sediments, and therefore their metabolic capacity (Papageorgiou et al. 2010). Thus, grain size may be an important variable in selecting sites for aquaculture facilities, since it affects the environmental sustainability of this activity and give rise to potential negative interactions with benthic ecosystem (Kalantzi and Karakassis 2006).

The aim of this study is to understand the effects of grain size on biogeochemical processes in sediments affected by aquaculture activities. We focused on C, N, P and S cycles. For that purpose, we reproduced experimentally the effects of two levels of organic enrichment: one produced by mussel farms (low level) and one by fish farms (high level) on sandy and muddy sediments. We considered the effects of bioturbation and bioirrigation by including the polychaete *Hediste diversicolor* in the experiments. We hypothesized that under organic enrichment conditions, sandy sediments can maintain higher metabolic rates due to higher oxygen availability, resulting in low sulfide accumulation, faster nutrient cycling and higher metabolic capacity compared to muddy sediments.

Materials and methods

Study sites and experimental set-up

Sediment cores for the mussel fecal pellets experiment were collected in October 2011 in Denmark. Twelve cores from Limfjorden had muddy sediments (56°47'19.9"N 8°52'56.2"E) and 12 cores from Storebælt had sandy sediments (55°23'24.8"N 10°48'49.6"E). Sediment cores for the fish fecal pellets experiment were collected in June 2013 in Spain. Twelve muddy sediments cores from Almeria bay (36°49'03.7"N 2°30'04.1"W) and 12 sandy sediment cores from Alicante bay (38°15'19.4"N 0°30'24.7"W). The grain size in the sandy sediments was between 63 and 500 µm (>70 %) and in the muddy sediments was <63 µm (>90 %) in both experiments. Sediments were sieved to remove benthic macrofauna (>1 mm). The cores (30 cm length; 5 cm i.d.) were submerged in an incubation chamber (157 dm³) with seawater of 20 psu in Denmark and 37.7 psu in Alicante. The cores were maintained at oxygen saturation in darkness, and the temperature

was kept at 16 ± 1 °C in both experiments. The water overlying the sediment in each core was continuously mixed by rotation (60 rpm) of a 2 cm Teflon-coated magnet attached to the liner wall (Holmer and Kristensen 1992). In the mussel pellet experiment, the surface of half of the cores was enriched with 60 fecal pellets (+FP) from mussels every day for 21 days. This corresponds to $7.7 \text{ g C m}^{-2} \text{ d}^{-1}$ and $1.3 \text{ g N m}^{-2} \text{ d}^{-1}$, similar to sedimentation rates in mussel farms (Carlsson et al. 2010). The other half of the cores were kept without organic enrichment (−FP). Mussels were obtained from the Marine Biological Research Centre of Kerteminde (Odense), and placed in aquariums in the laboratory. The pellets produced were collected every day by 3 mL pipettes and then added to the sediment cores. In the fish pellet experiment, precisely 0.5 g of fecal pellets (+FP) from *Sparus aurata* were added daily for 21 days to half of the cores, corresponding to $72 \text{ g C m}^{-2} \text{ d}^{-1}$ and $12 \text{ g N m}^{-2} \text{ d}^{-1}$, similar to sedimentation rates under fish farms with high organic enrichment (Heilskov et al. 2006; Cromey et al. 2012). Fish fecal pellets were collected from a fish nursery located in Guardamar (Alicante). The other half of the cores were kept without organic enrichment (−FP). In both experiments, in 6 sand cores and 6 mud cores, 3 polychaete worms (*H. diversicolor*) were added (+W_S, +W_M) corresponding to a density of 1061 individuals m^{−2}, within the range of natural abundance (Scaps 2002). Six cores remained without worms, three from sand and three from mud (−W_S, +W_M). Polychaetes were replaced if they died during the experimental period.

Sediment enrichment and sulfides

At the end of the experiments, sediment cores were sliced at 1 cm intervals down to 4 cm depth and at 2 cm intervals down to 8 cm depth. The OM content of dry sediment was estimated by loss on ignition after being ashed at 400 °C for 4 h. In the mussel pellet experiment the sediments were fixed for determination of sulfides by addition of zinc acetate, after which total reduced inorganic sulfides (TRIS) were extracted by distillation in a two-step procedure, whereby acid volatile sulfides (AVS) are separated from the chromium reducible sulfur (CRS) (Fossing and Jørgensen 1989) and quantified spectrophotometrically (Cline 1969). In the fish pellet experiment, total

free sulfides (TFS) content was measured in a sulfide antioxidant buffer solution and ascorbic acid, using a silver/sulfide half-cell electrode following the method described by Wildish et al. (1999). Sediment cores were observed every day for cover by *Beggiatoa* mats.

Benthic fluxes

Benthic fluxes of sediment oxygen uptake (SOU), ammonium (NH₄⁺), nitrate (NO₃[−]), nitrite (NO₂[−]) and phosphate (PO₄^{3−}) were determined every day during the first week, and once a week until the end in both experiments, which lasted 3 weeks. For the mussel pellet experiment, carbon dioxide flux (TCO₂) was also measured. Cores were left for acclimation 24 h, and afterwards water samples were taken and then cores were sealed with rubber stoppers and incubated for 3–6 h. At the end of the incubations, water samples were taken from each core using a 20 ml glass syringe. Incubation times were adjusted to achieve a <20 % decrease in O₂ concentration. Fluxes were calculated from changes in concentration over time in each core, with corrections made for refilled water replacing the volume withdrawn. SOU samples were analyzed within 12 h by precipitating using standard Winkler technique (Parsons et al. 1984). TCO₂ was measured by the flow injection analysis according to Hall and Aller (1992). Samples for nutrients were filtered using GF/C filters and analyzed for NH₄⁺, NO₃[−], NO₂[−] and PO₄^{3−} on a scalar autoanalyzer, (SCAN plusSYSTEM in Denmark and San⁺⁺ Continuous Flow Analyzer in Spain). After incubation, the cores were submerged in the water tank with air saturation until the next incubation.

Statistical analysis

SOU, TCO₂ production and nutrient concentration (NH₄⁺, NO₃[−], NO₂[−] and PO₄^{3−}) were analyzed in time-integrated efflux up to 21 days. OM, TRIS and TFS were analyzed by depth-integration to 8 cm. The results were analyzed by 3-way factorial analysis of variance (ANOVA) to compare between sediments (2 levels: sand and mud), polychaetes (2 levels: with and without polychaetes) and fecal pellets (2 levels: with and without fecal pellets), as well as the interaction between these factors. Heterogeneity of variance was tested with Cochran's test and data were transformed

if necessary (Underwood 1997). All statistical tests were conducted with a significance level of $\alpha = 0.05$. In the case of significant differences, data were subsequently investigated using SNK test (Student–Newman–Keuls) to determine which samples were implicated in the differences. We used the software R (R Development Core Team 2011) with the GAD package (Sandrini-Neto and Camargo 2014).

Results

In the mussel pellets experiment, +FP sediments were characterized by a 0.5 cm thick pale brown oxidized layer and underneath there was a 0.8 cm thick dark gray zone. Beneath this depth, sandy sediments were brown and muddy sediments were dark brown. –FP sediments had a 0.4 cm thick pale brown oxidized layer and beneath this depth the color was brown in sandy sediments and dark brown in muddy sediments, without any dark gray zone. In the fish pellets experiment, +FP were characterized by 1 cm thick black zone and underneath there was a 1 cm thick dark gray zone. Below a depth of 3 cm, sandy sediments were brown and muddy sediments were dark brown. –FP_S sediments were brown and –FP_M sediments had a 0.3 cm pale brown oxidized layer and beneath this depth they were dark brown.

Sediment enrichment

At the end of the mussel pellet experiment, OM percentage in muddy sediments was significantly higher than in sandy sediments (Fig. 1). No significant differences were found between +FP and –FP, or between +W and –W sediments (Table 1). In the fish pellet experiment, there was a significant OM increase down to 3 cm in depth, with higher OM content in muddy than in sandy sediments. Beneath that depth, there was no effect of increased OM levels due to organic enrichment, independently of grain size. OM in +FP_S were 5–8-fold higher than –FP_S samples, and OM in +FP_M was 2–2.5-fold higher than –FP_M samples when integrating over the top 8 cm. The OM content was significantly reduced in +W sediments. +FP+W_S had 43 % less than +FP–W_S, –FP+W_M had 31 % less than –FP–W_M and +FP+W_M had 9 % less than +FP–W_M (supplementary material Fig. A).

Fig. 1 Change in organic matter percentage (OM%) with depth. *Left panels* represent sandy sediments and *right panels* represent muddy sediments. *Mussel* and *fish* symbols indicate the organic enrichment by mussel and fish fecal pellets, respectively. *Error bars* indicate SE ($n = 3$)

Sulfide accumulation

In the mussel pellet experiment, AVS accumulation in the sandy sediments only occurred in +FP in the first cm, where AVS in +FP_S were 2–6-fold higher than in –FP_S (Fig. 2). In the first cm, +FP_M was 1.5-fold higher than –FP_M. Below 1 cm, all samples had a similar range of AVS (9–26 $\mu\text{mol cm}^{-3}$) (Fig. 2). Depth integrated pools of AVS were 24-fold higher in muddy than in sandy sediments (Fig. 3). The presence of polychaetes significantly increased AVS in muddy sediments (Table 1), +W+FP_M being 30 % higher than –W+FP_M, whereas there was no effect in the sandy sediments (Fig. 3). Levels of CRS in sandy sediments were low, except in the first cm, where +FP samples were 3–4-fold higher than –FP. In contrast, in muddy sediments, CRS was the dominant form of reduced sulfur, being significantly higher than CRS in sandy sediments. Maximum levels were found in +W+FP_M (Fig. 2). Depth-integrated CRS showed a significant increase in +W_M (Table 1), where the increases were 43 % in –FP+W_M and 9 % in +FP+W_M (Fig. 3).

In the fish pellet experiment, highest values of TFS were found in the first cm. TFS showed major changes with depth and a wide difference between +FP and –FP in the upper 4 cm. Sandy sediments always had lower TFS concentration than muddy sediments. In sandy sediments, maximum TFS values were found in +W+FP_S. In the first cm, TFS values in +FP_S were 129-fold higher in –W_S and 979-fold higher in +W_S than in –FP_S (Fig. 4). In +FP_M in the first cm, TFS values were sevenfold higher in –W_M and 14-fold higher in +W_M than –FP_M. When TFS were integrated (0–8 cm), TFS values in +FP_M were 2–3-fold higher than +FP_S. In sandy sediments, the increase in +FP_S over –FP_S was 276-fold (Fig. 5). In muddy sediments, levels of TFS in +FP_M were double the levels in –FP_M, but this increase is not significant due to high variability, and the analysis showed an interaction in sediment under organic enrichment conditions. No significant differences were found between +W and –W (Table 1).

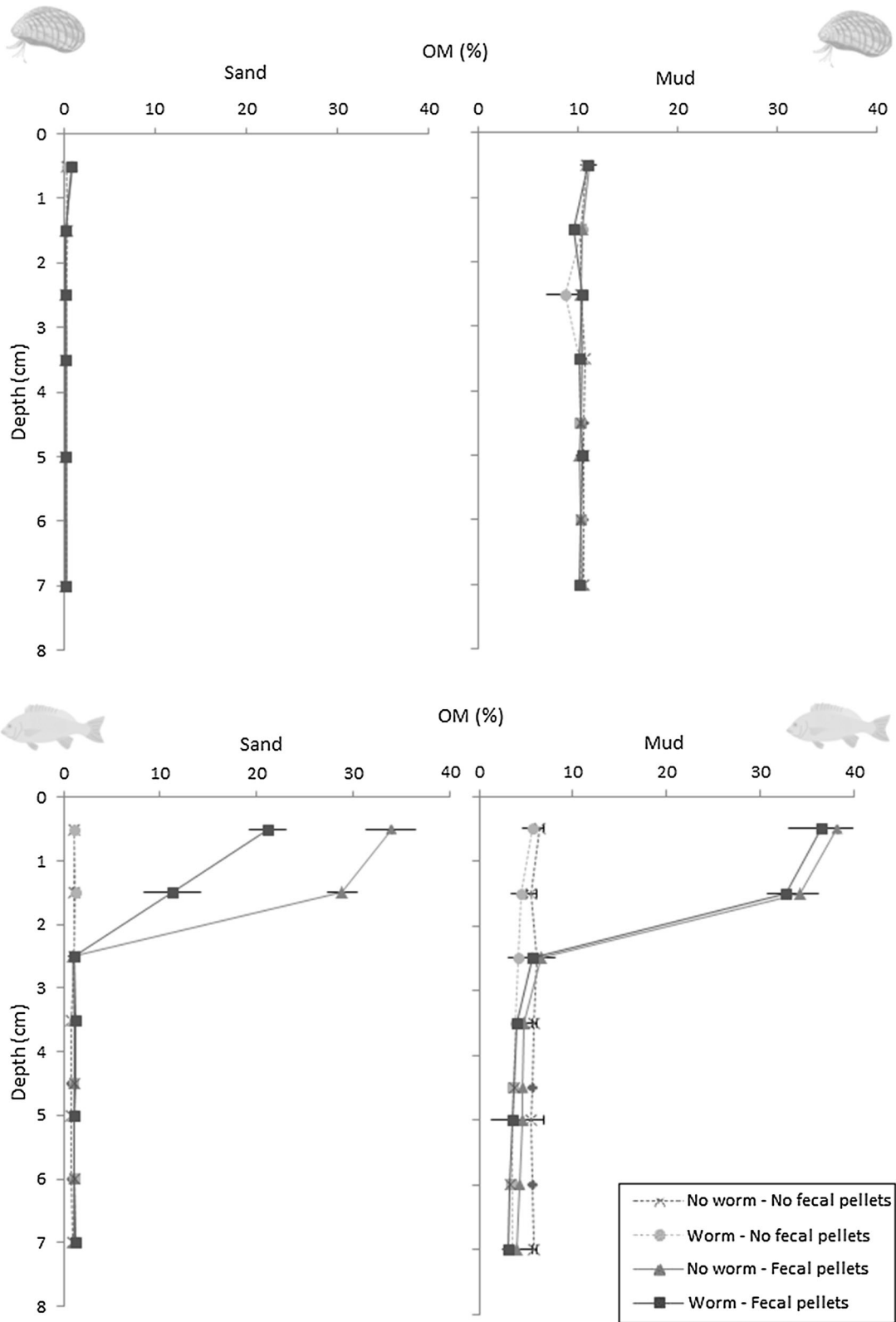


Table 1 continued

Source of variation	dfs	NO ₃ + NO ₂						PO ₄ ³⁻					
		Mussel			Fish			Mussel			Fish		
		MS	F	P	MS	F	P	MS	F	P	MS	F	P
Sediment = s	1	0.62	0.35	0.57	5.52	0.34	0.57	3.65	2.88	0.11	0.06	6.36	0.02
Worm = w	1	2.21	1.24	0.28	0.37	0.02	0.88	0.78	0.62	0.44	0.00	0.03	0.86
Impact = i	1	1.17	0.66	0.43	129.61	8.07	0.01	4.91	3.86	0.07	15.27	1524.38	0.00
s × w	1	1.14	0.64	0.44	93.84	5.84	0.03	0.44	0.35	0.56	0.01	1.31	0.27
s × i	1	0.00	0.00	0.97	0.05	0.00	0.96	0.77	0.61	0.45	0.02	2.42	0.14
w × i	1	0.04	0.02	0.89	22.84	1.42	0.25	0.04	0.03	0.86	0.05	5.32	0.03
s × w × i	1	2.67	1.50	0.24	25.81	1.61	0.22	2.29	1.80	0.20	0.02	1.73	0.21
Residual	16	1.79			16.07			1.13					
Total	23												
Cochran's C test													
Transformation													

Df degrees of freedom, MS mean square, F F-distribution, P significance levels (* P < 0.05, ** P < 0.01 and *** P < 0.001). Significant effects are indicated in bold

In the fish pellet experiment, *Beggiatoa* mats appeared covering the surface in -W+FP_M from day 14, followed by +W+FP_M 2 days after. *Beggiatoa* appeared on day 19 in -W+FP_S and +W+FP_S. After *Beggiatoa* mats appeared, polychaetes started to die; firstly on day 14 in the muddy sediments and 5 days later in sandy sediments. When they were replaced, they died in one or 2 days.

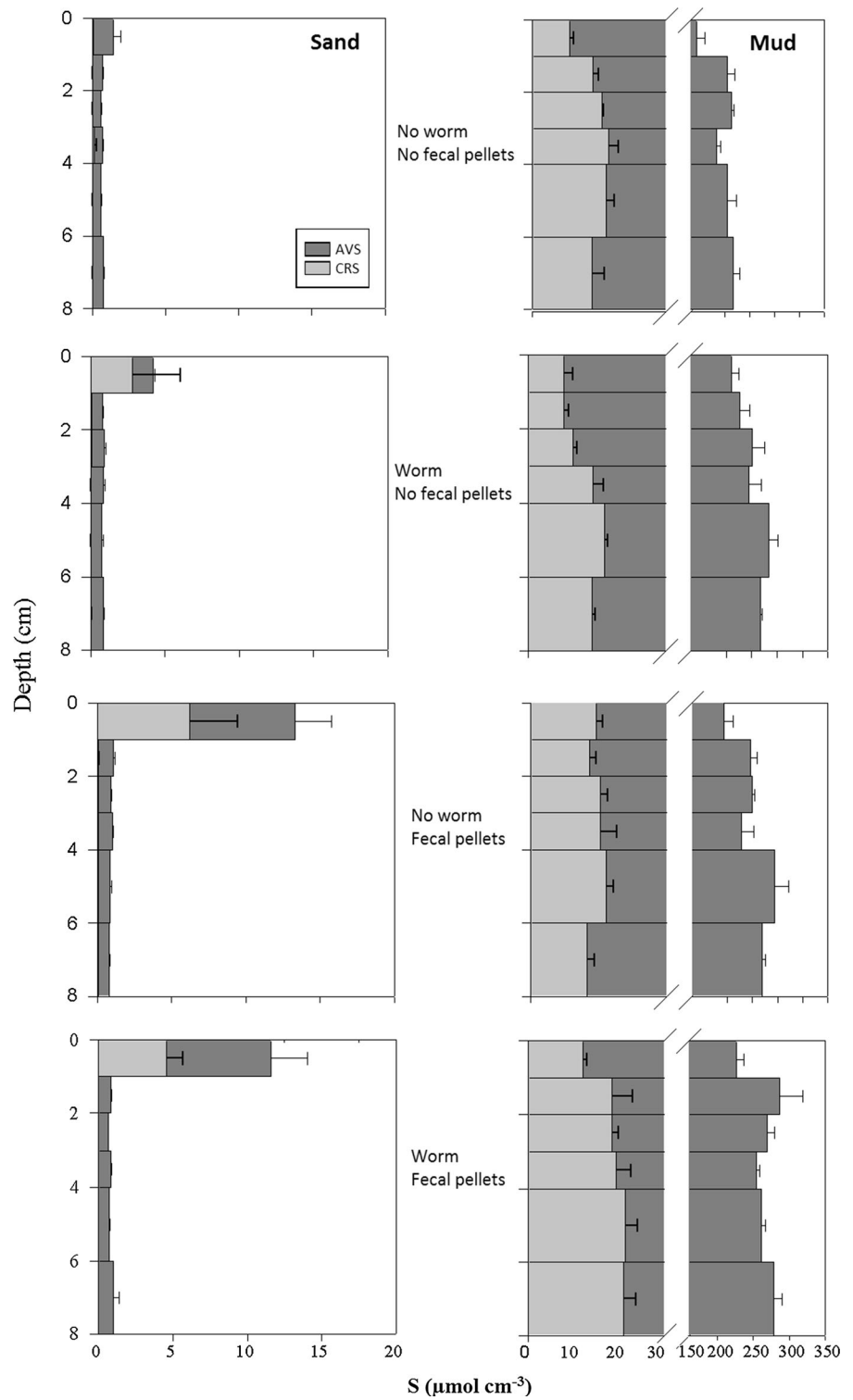
Benthic fluxes

In the mussel pellet experiment, samples with organic enrichment achieved similar SOU levels (Fig. 6), whereas under conditions of no organic enrichment, muddy sediments had higher levels of SOU than sandy sediments (Table 1). +FP_S and +FP_M SOU levels were 6.4 fold higher than -FP_S and 2.5 fold higher than -FP_M (Fig. 6). No differences were observed between +W or -W (Table 1). In the fish pellet experiment, analogous results were observed, in samples with organic enrichment, both sediments reached similar levels of SOU and there were no differences between +W and -W (Fig. 6). Under conditions of no organic enrichment, muddy sediments had higher levels of SOU than sandy sediments (Table 1) and also +W_S had 49 % higher SOU flux than -W_S, and +W_M had 118 % higher SOU flux than -W_M (Fig. 6).

TCO₂ flux in the mussel fecal pellets experiment showed no differences between sandy and muddy sediments (Fig. 6). Only significant differences were found between +FP and -FP (Table 1). The significant increases after organic enrichment were 121 times in +FP-W_S, 5 times in +FP+W_S and 4 times in +FP+W_M compared to -FP (Fig. 6). No differences were observed between -W and +W (Fig. 6).

The NH₄⁺ flux showed no significant differences between sandy and muddy sediments in mussel pellet experiment (Table 1). Rates of NH₄⁺ in +FP increased 7–12-fold respect to -FP (Fig. 7). No differences were found between +W and -W with +FP (Table 1). In the fish pellet experiment, sandy sediment showed a 39 % lower NH₄⁺ efflux than muddy sediments (supplementary material Fig. B). There was a significant 6–10-fold increase in NH₄⁺ in +FP compared with -FP (Fig. 7). Significant differences were found in +FP (Table 1), +W_S had 34 % less NH₄⁺ flux than -W_S and +W_M had 20 % less NH₄⁺

Fig. 2 Vertical profiles of acid volatile sulfides (AVS) and chromium reducible sulfides (CRS) in mussel pellet experiment. *Left panels* represent sandy sediment and *right*, muddy sediments. *Error bars* indicate SE ($n = 3$)



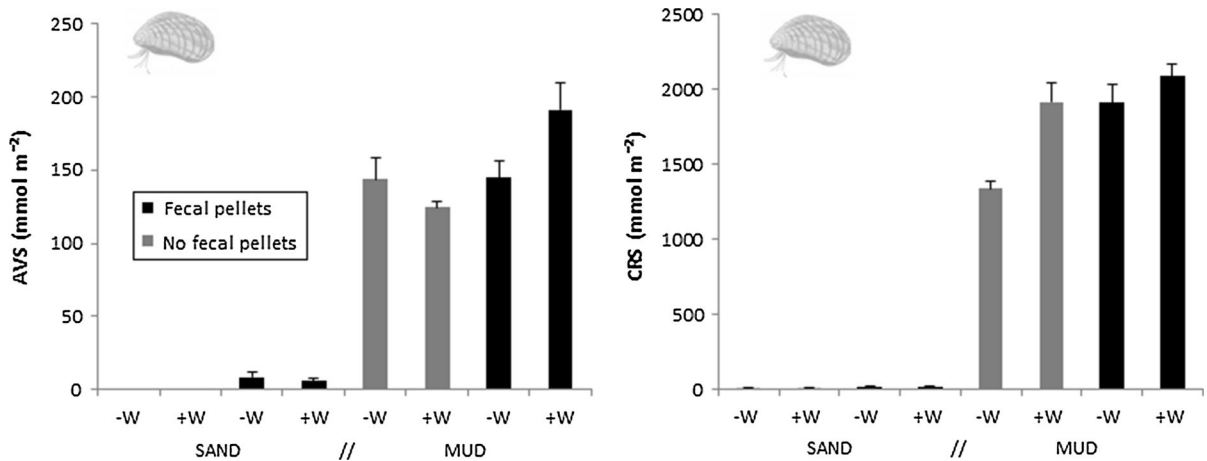


Fig. 3 Depth-integrated (0–8 cm) pools of acid volatile sulfides (AVS) and chromium reducible sulfides (CRS). Mussel symbols indicate the low level of organic enrichment in sediments below a mussel farm. –W symbols represent no

polychaete addition and +W symbols polychaete addition. The left side of the graphs represents sandy sediment and the right, muddy sediments. Error bars indicate SE (n = 3)

flux than –W_M. In contrast, in –FP samples, +W had higher NH₄⁺ flux than –W, increasing eightfold in +W_S and fivefold in +W_M (Fig. 7). Fluxes of NO₃⁻ + NO₂⁻ in mussel pellet experiment were low (supplementary material Fig. C) and there was no difference between any treatments (Table 1). However, in the fish pellet experiment, organic enrichment increased NO₃⁻ + NO₂⁻ fluxes significantly. Significant differences were found between +FP+W_S and +FP+W_M, being +FP+W_S, 130 % higher than +FP+W_M (Fig. 7). +FP–W_S were also higher than +FP–W_M but not significant (Table 1). The increase in NO₃⁻ + NO₂⁻ due to +FP was eightfold in –W_S and twofold in +W_S, threefold in –W_M and fivefold in +W_M (Fig. 7).

In the mussel pellet experiment, PO₄³⁻ fluxes showed no differences between sandy and muddy sediments (Table 1). Whereas PO₄³⁻ fluxes in +FP were significant higher than in –FP (Fig. 7), no differences were found between +W and –W. With fish pellets however, there was a significant difference between sandy and muddy sediments in –FP (Table 1). A large increase in PO₄³⁻ flux was observed between –FP and +FP (supplementary material Fig. D). +FP–W_M had 19 % (significant) higher rates of PO₄³⁻ fluxes than +FP+W_M (Table 1). There was also a notable but non-significant increase of 22 % in +FP–W_S compared to +FP_S+W (Fig. 7).

Discussion

Sediment enrichment

Muddy sediments had a naturally higher content of OM compared to the sandy sediments. Under low organic enrichment conditions, none of the sediments increased their respective OM content at any depth, indicating that the metabolic capacity of the sediments was not exceeded. However, a high level of enrichment from fish pellets resulted in an increase in the OM content in the surface layer, in both sandy and muddy sediments, although this increase was more marked in muddy sediments. This finding indicated that the metabolic capacity of the sediments was exceeded there, particularly in the muddy sediments. Excess OM can change the biogeochemical conditions of the sediment and subsequently diminish its metabolic capacity (McKindsey et al. 2006). Reduced conditions favor anaerobic pathways, which are less efficient than aerobic ones (Kristensen and Holmer 2001).

Macrofauna enhances the metabolic capacity of the sediments (Banta et al. 1999). In our case, sediments with polychaetes had lower OM content compared to defaunated ones even after OM enrichment. This suggests that the metabolic capacity of the sediment was enhanced by the bioturbation and bioirrigation of the macrofauna. These processes enhance mineralization through active transport of electron acceptors to

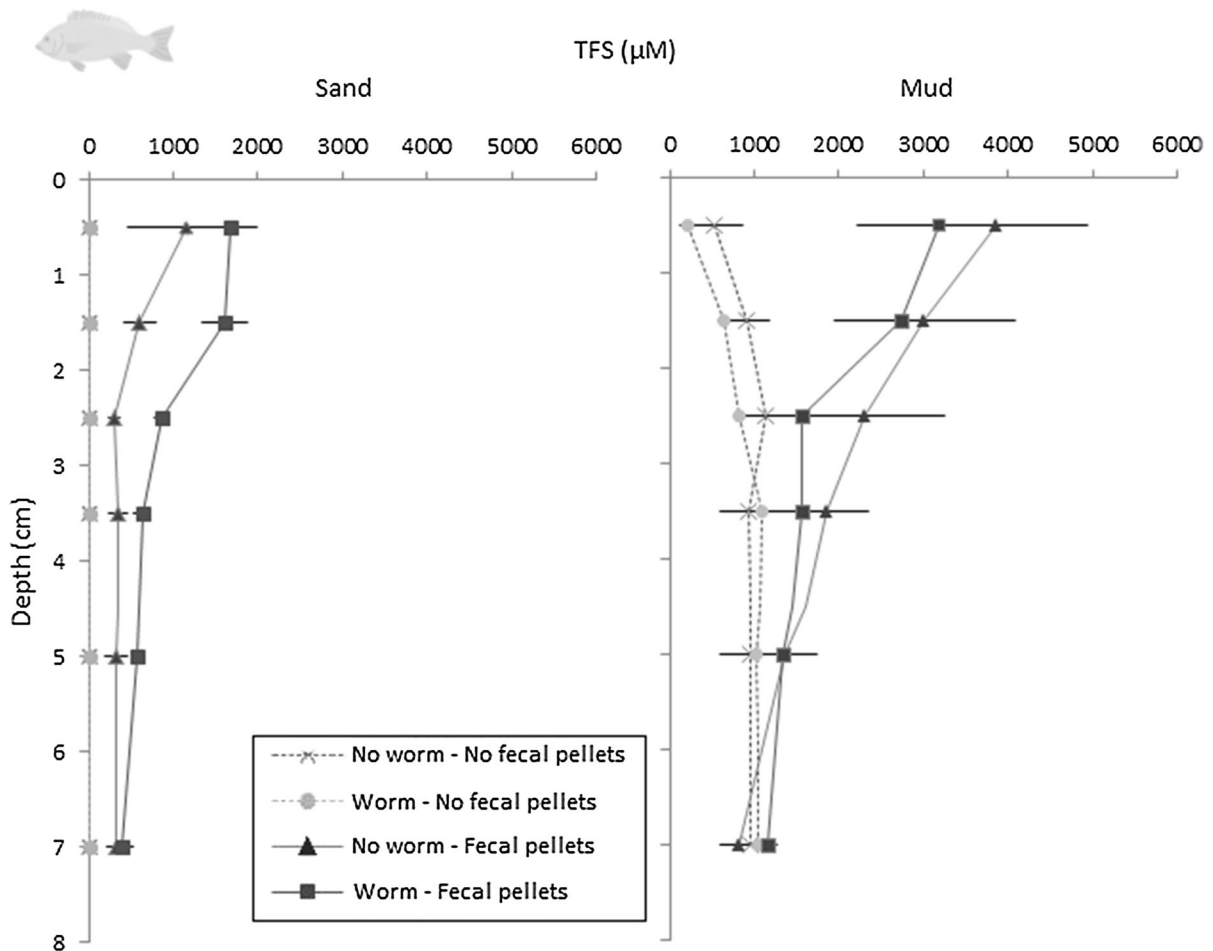


Fig. 4 Vertical profile of total free sulfides (TFS) in fish pellet experiment. *Fish* symbols indicate the high organic enrichment in sediments below a fish farm. *Left panels* represent sandy sediment and *right panels* muddy sediments. *Error bars* indicate SE ($n = 3$)

microbial communities deeper in the sediment and by removal of potential toxic metabolites as well as consuming OM (Kristensen 1988; Heilskov et al. 2006; Kristensen et al. 2012).

Sulfides

Accumulation of sulfides, under low and high levels of organic enrichment, was always higher in muddy than in sandy sediments. Despite the fact that we measured TFS in one experiment and AVS in the other, the increase in sulfides was greater at high than at low levels of organic enrichment. At a low level of organic enrichment, sulfide pools were mainly influenced by sediment type rather than by polychaetes or organic enrichment. In sandy sediments, sulfide content increased only in the first cm

of the sediment under low organic enrichment, while in muddy sediments sulfide content was high throughout the sediment. This difference in sulfide content is possibly due to the naturally high OM content in muddy sediments leading to naturally high sulfide pools (Hargrave et al. 2008). At high levels of organic enrichment, the sulfide accumulation was also higher in muddy than in sandy sediments. Muddy sediments have smaller grain size than sandy sediments, resulting in a low diffusion capacity of solutes between the water column and the sediments (Papageorgiou et al. 2010). This also results in high sulfide accumulation compared to sandy sediments, where sulfides are reoxidized to a greater extent. According to Hargrave et al. (2008), TFS in organic enriched muddy sediments reached Hypoxic level B (between 3000 and 6000 μM S) in the first two cm of

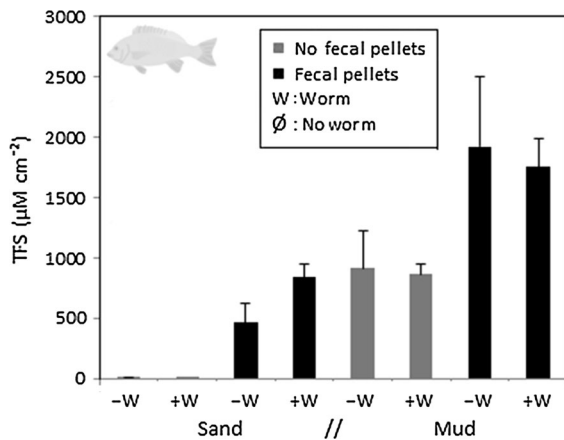


Fig. 5 Depth-integrated (0–8 cm) pools of total free sulfides (TFS). *Fish* symbols indicate the high organic enrichment in sediments below a fish farm. *-W* symbols represent no polychaete addition and *+W* symbols polychaete addition. The *left side* of the graph represents sandy sediment and the *right side*, muddy sediment. *Error bars* indicate SE ($n = 3$)

the sediment. At this level, sulfate reduction is the dominant metabolic pathway (Holmer et al. 2005). Pearson and Rosenberg (1978) described this level as Stage 1 or Polluted in their organic enrichment gradient classification. Also, *Beggiatoa* mat cover on sediments becomes more extensive at TFS higher than 3000 μM , which were found here (Brooks and Mahnken 2003; Hargrave et al. 2008). In sandy sediments, the maximum TFS in the superficial sediment reached Hypoxic A levels (between 1500 and 3000 $\mu\text{M S}$) and *Beggiatoa* mats appeared on the sediment (Hargrave et al. 2008). The bioturbation and bioirrigation produced by the polychaetes in muddy sediments delayed *Beggiatoa* development and reduced sulfide accumulation. *Beggiatoa* grows at the interface of oxic and anoxic conditions and is typically associated with high sulfide levels (Preisler et al. 2007). Sulfides are toxic to the inhabiting fauna at high concentrations and may, along with the depletion of oxidized compounds in the pore water, lead to depletion of the most sensitive species, resulting in sediment defaunation (Brooks and Mahnken 2003; Heilskov et al. 2006). Our results confirm that polychaetes play an important role in inhibiting the proliferation of *Beggiatoa* mats in muddy sediments by increasing the electron acceptor availability in the sediment porewater (Kristensen and Kostka 2005).

Benthic fluxes

Despite the fact that sandy sediments generally have a deeper oxygen penetration than finer grained sediments (Andersen and Helder 1987; Hargrave et al. 2008), the SOU results showed similar maximum levels under organic enrichment, regardless of sediment type and presence of polychaetes. The accumulation of added OM in the upper layer of the sediment could explain these results, since grain size or macrofauna have less effect on SOU. This is because oxygen uptake in the surface layer is controlled primarily by diffusive processes (Glud 2008). However, SOU was three times greater at high than at low organic enrichment. So, high SOU is expected under high organic enrichment conditions because oxygen is used as electron acceptor in aerobic metabolism as well as in the reoxidation of sulfides (Holmer et al. 2005; Piedecausa et al. 2012). The increase in SOU was in the same range as other studies under similar levels of organic enrichment (Glud 2008; Carlsson et al. 2009; Valdemarsen et al. 2009), suggesting similar metabolic rates.

The stimulated TCO_2 at low organic enrichment showed an increase in benthic metabolism, which is consistent with the lack of accumulation of added OM, suggesting that all OM added was mineralized in both sandy and muddy sediments. TCO_2 results at low organic enrichment were in the same range as other mesocosm studies (Banta et al. 1999; Papaspyrou et al. 2010), where yeast and detritus were used as organic enrichment. For high organic enrichment, Valdemarsen et al. (2009) found that TCO_2 flux increased by 5–6 times, which is in the same range or double what we found in our study at low organic enrichment, for sandy and muddy sediments respectively. Differences in TCO_2 between sandy and muddy sediments were only detected in non-enriched sediments, where TCO_2 rates were higher in mud. This could be due to their naturally higher OM content. Surprisingly, we found no difference in TCO_2 between samples with and without polychaetes, maybe due to stimulation of chemoautotrophic processes like nitrification by polychaetes (Kristensen et al. 1991). Despite this, polychaetes also stimulate benthic metabolism by indirectly stimulating microbial metabolism besides contributing to the overall sediment metabolism (Banta et al. 1999; Sanz-Lazaro and Marin 2008).

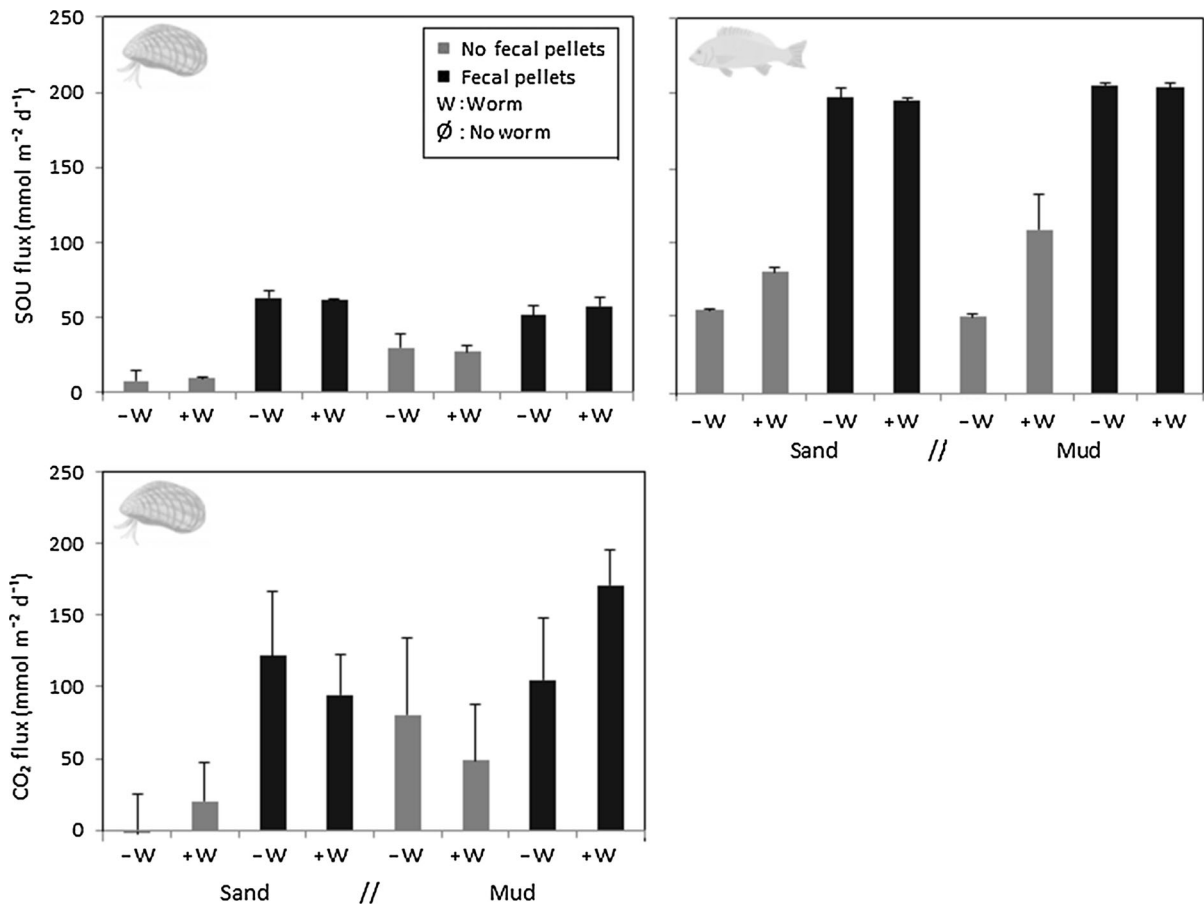


Fig. 6 Sediment oxygen uptake (SOU) and total CO₂ (TCO₂) calculated as time-integrated excess flux. *Fish* symbols indicate the high organic enrichment in sediments below a fish farm. -W symbols represent no polychaete addition and +W symbols

polychaete addition. The *left side* of the graph represents sandy sediment and the *right side* muddy sediments. *Error bars* indicate SE (n = 3)

NH₄⁺ and PO₄³⁻ effluxes were stimulated similarly to SOU at low and high levels of organic enrichment which agrees with previous authors (Carlsson et al. 2009; Stadmark and Conley 2011). However, at high levels of OM, NH₄⁺ and PO₄³⁻ release rates were almost 8 times higher than in low organic enrichment, reflecting high carbon oxidation rates and prevalence of anaerobic metabolism at high OM (Nizzoli et al. 2006). NH₄⁺ values in the fish pellet experiment were higher than expected and higher than found previously in the literature (21–40 mmol m⁻² d⁻¹) (Holmer et al. 2003; Valdemarsen et al. 2009). These high figures possibly could be due to the high level of organic enrichment. This study shows that sediment grain size influenced NH₄⁺ release rates only under high levels of organic enrichment. Under these conditions, muddy

sediments released more NH₄⁺ than sandy sediments. This could be because muddy sediments showed more reduced conditions with higher sulfide levels. When sediments become sulfidic, nitrogen mineralization pathways can shift to production of NH₄⁺ via dissimilatory NO₃⁻ reduction (DNRA) (Christensen et al. 2000; Carlsson et al. 2012). Also under reduced conditions, iron hydroxides become reduced and the PO₄³⁻ adsorbed to hydroxides is released to the water column (Sundby et al. 1993; Papageorgiou et al. 2010).

Release rates of NO₃⁻ + NO₂⁻ were not increased at low organic enrichment as Carlsson et al. (2012) described before, whereas high organic enrichment increased NO₃⁻ + NO₂⁻ efflux, possibly due to the nitrification of ammonium accumulating in the cores. Muddy sediments showed lower efflux of

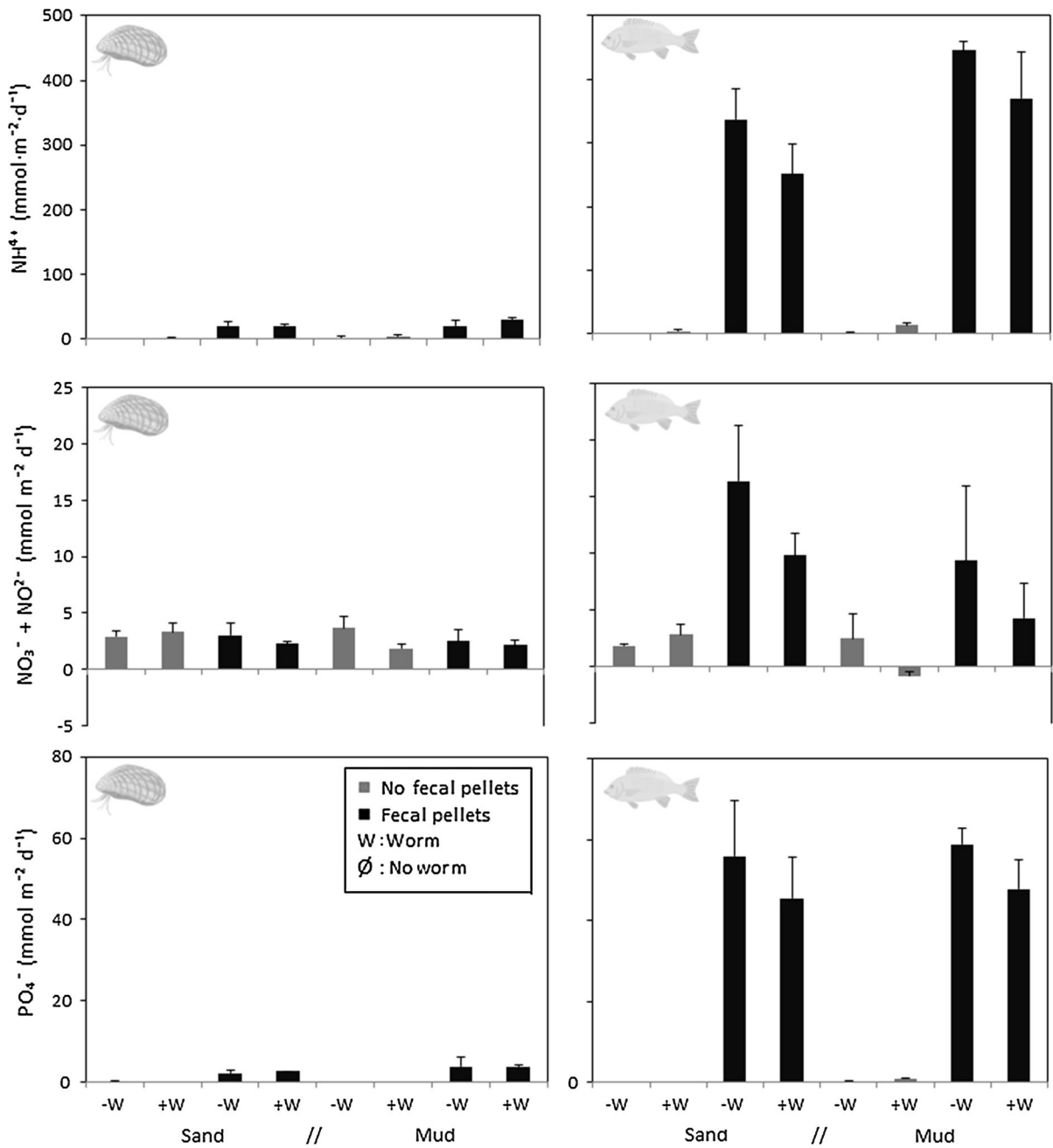


Fig. 7 Ammonium (NH_4^+), nitrate + nitrite ($\text{NO}_3^- + \text{NO}_2^-$) and phosphate (PO_4^-) calculated as time-integrated excess flux. Fish symbols indicate the high organic enrichment in sediments below a fish farm. -W symbols represent no polychaete addition

and +W symbols polychaete addition. The left side of the graph represents sandy sediment and the right side muddy sediments. Error bars indicate SE (n = 3)

$\text{NO}_3^- + \text{NO}_2^-$ than sandy sediments. NO_3^- could be transformed through different metabolic pathways such as denitrification and DNRA, NO_2^- being the first intermediary in this reduction (Thamdrup and

Dalsgaard 2000). *Beggiatoa* likewise uses these components as electron acceptors (Preisler et al. 2007), and the lowest values of $\text{NO}_3^- + \text{NO}_2^-$ in muddy sediments correspond to intense *Beggiatoa* cover.

As mentioned earlier, polychaetes stimulate sediment metabolism (Banta et al. 1999). Indeed, our results showed a contrasting effect on nutrient recycling depending on the organic enrichment content of the sediment. Under conditions without organic enrichment, polychaetes moderately increased sediment release of NH_4^+ and, to some extent of PO_4^{3-} . But, at high levels of organic enrichment, the release of NH_4^+ , $\text{NO}_3^- + \text{NO}_2^-$ and PO_4^{3-} from sediment decreased due to bioturbation. The lower NH_4^+ and $\text{NO}_3^- + \text{NO}_2^-$ release suggests that polychaetes enhance metabolic pathways that transform these nutrients to N_2 , such as coupled nitrification–denitrification (Christensen et al. 2003). Low levels of PO_4^{3-} suggest that bioturbation by polychaetes promotes oxidation of the sediment increasing its capacity to retain PO_4^{3-} by means of iron hydroxides (Sundby et al. 1993).

This study demonstrates that under the low organic enrichment found in sediments below mussel farms, there is no significant difference between sandy and muddy sediments (with contrasting grain size) regarding nutrient effluxes and sulfide accumulation. At high organic enrichment, such as below fish farms, there were however, significant differences between sandy and muddy bottoms. The two types of sediment differed not only in the accumulation of OM, but also in their metabolic capacity. Sandy sediments accumulated less OM, less sulfide and less ammonium, resulting in a less reduced environment. These reduced conditions, higher in muddy sediments, could have important consequences in the recovery process, because sediments that have suffered hypoxia events are prone to undergo again these events (Conley et al. 2009). On the long run, sandy sediments are likely to have a greater sediment buffering capacity than muddy sediments at fish farming sites, since sandy sediments have a greater metabolic capacity resulting in a lower OM accumulation. Polychaetes also contribute to reduce the accumulation of these products by stimulating OM mineralization and nutrient recycling. A balance between OM input and mineralization rates is crucial to allow polychaetes survival, keeping the resistance of the sediment high.

In conclusion, under a low level of organic enrichment, sediment grain size is not a critical parameter to take into account in selecting locations for aquaculture. However, under high organic

enrichment, sandy sediments are more suitable to harbor aquaculture facilities than muddy sediments, since their metabolic capacity is higher. Therefore, sediment grain size should indeed be considered a key parameter for fish farming site selection.

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