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Influence of Manila clam aquaculture on rates and partitioning of organic carbon oxidation in sediment of Keunso Bay, Yellow Sea

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ABSTRACT: We investigated the effects of Manila clam aquaculture on the rates and pathways of anaerobic organic carbon (OC) oxidation in highly bioturbated (HB) and poorly bioturbated (PB) sediment in Keunso Bay, Yellow Sea. Due to the labile organic matter supply via sediment reworking by Manila clams, the anaerobic OC oxidation rate in HB sediment (38.8 mmol m^{-2} d^{-1}) was ~1.5 times higher than that in PB sediment (26.8 mmol m^{-2} d^{-1}). Microbial Fe(III) reduction (FeR) dominated OC oxidation pathways in HB sediment, comprising 55 to 76% of anaerobic OC oxidation, whereas sulfate reduction (SR) was the dominant oxidation pathway in PB sediment, accounting for up to 92% of anaerobic OC oxidation. Despite higher anaerobic respiration rates at the HB site, concentrations of NH_4^+, PO_4^{3-}, oxalate-extractable iron ($Fe(II)_{(oxal)}$), and total reduced inorganic sulfur were 2 to 3 times lower in HB than in PB sediment. Conversely, the concentration of reactive $Fe(III)_{(oxal)}$ at the HB site (2243 mmol m^{-2}) exceeded that at the PB site (1127 mmol m^{-2}) by a factor of 2. These results indicate that bioturbation by Manila clams enhances the re-oxidation processes of reduced metabolites in the sediment, thereby prohibiting SR and promoting FeR. Overall, the results suggest that aquaculture activities of Manila clams shift the dominant OC oxidation pathways in sediment from SR to FeR, which generates relatively oxidized and less sulfidic environments.

KEY WORDS: Aquaculture · Manila clam · Bioturbation · Organic carbon oxidation · Sulfate reduction · Iron reduction

1. INTRODUCTION

Aquaculture has expanded rapidly over the last 20 yr due to progressively impoverished natural fish stocks and increased human demand for seafood (Klinger & Naylor 2012, FAO 2018, Ahmed & Thompson 2019). The contribution of aquaculture to global fish production has increased steadily, from 20.9% in 1995 to 46.8% in 2016 (FAO 2018). Shellfish farming represents a major aquaculture activity, accounting for 21.4% of total production, with an annual yield in 2016 of 17.1 million t (FAO 2018). Manila clam *Ruditapes philippinarum* aquaculture increased from 2.6 million t in 2005 to 4.2 million t in 2016 and is ranked third in global shellfish production (FAO 2018).

A major environmental concern associated with intensive aquaculture activities is the accumulation of organic matter in sediments (Holmer & Kristensen 1992, 1994, Holmer et al. 2005, Hyun et al. 2013, Choi et al. 2018). In contrast to finfish farming, where sed-

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iment receives organic-rich uneaten fish food and fecal materials, shellfish aquaculture is considered less environmentally stressful, as filter-feeding shellfish do not require additional food (Nedwell 2004, Dumbauld et al. 2009). Nevertheless, bivalves eject suspended organic matter trapped from the water column during filter feeding either as pseudo-feces or fecal pellets (Zhou et al. 2006, Zhang et al. 2013, Galimany et al. 2017). Ejected organic matter is larger and more prone to deposition on the sediment, since pseudo-feces and fecal pellets are excreted as mucus-bound aggregates (Giles & Pilditch 2004). Unlike oysters and mussels, which are generally cultivated in boxes or attached to longlines in the water column (Christensen et al. 2003, Carlsson et al. 2012, Hyun et al. 2013), Manila clams are farmed directly in intertidal sediment after dispersion (Bendell et al. 2010, Lavoie et al. 2016). Accumulation of organic matter and intense sediment reworking (i.e. physical mixing) can have a substantial influence on sediment biogeochemistry (Welsh 2003, McKindsey et al. 2011, Kristensen et al. 2012, Wendelboe et al. 2013, Koo & Seo 2017, Powilleit & Forster 2018).

In organic-rich coastal sediments, oxygen is depleted within a few millimeters below the surface (Rasmussen & Jørgensen 1992, Cai & Sayles 1996). Thus, most organic carbon (OC) oxidation is performed by a variety of anaerobic microorganisms utilizing different electron acceptors, such as nitrate, manganese oxides, iron oxides, and sulfate (Canfield et al. 2005, Jørgensen 2006). Previous studies have suggested that OC oxidation in coastal sediment is dominated by sulfate reduction (SR) because of the high sulfate concentration in seawater (Howarth 1993, Alongi 1998). However, recent evidence suggests that microbial Fe (III) reduction (FeR) may be comparable to or higher than SR in Fe(III)-rich environments, especially in highly bioturbated sediment (Kostka et al. 2002a, Hyun et al. 2007, 2009, Kristensen 2008, Kristensen et al. 2011, van de Velde & Meysman 2016). The construction of burrows and subsequent ventilation introduce oxygen from the overlying water during submersion or directly from the air during exposed periods, which stimulates OC oxidation coupled with both aerobic (i.e. O_2 reduction) and suboxic (i.e. FeR) processes (Kostka et al. 2002a,b, Gribsholt et al. 2003, Hyun et al. 2007, 2009).

Most previous studies on the significance of FeR in OC oxidation associated with macrofauna activities in coastal sediment have focused on the burrows constructed in natural sediment by polychaetes (*Nereis diversicolor*, *Arenicola marina*, and *Marenzelleria viridis*) (Kristensen 2000, 2001, Quintana et al. 2013), clams (*Mya arenaria* and *Mytilus galloprovincialis*) (Hansen et al. 1996, Nizzoli et al. 2006b), and crabs (*Uca pugnax* and *Cleistostoma dilatatums*) (Kostka et al. 2002a,b, Gribsholt et al. 2003, Nielsen et al. 2003, Ferreira et al. 2007, Hyun et al. 2007). However, despite the extent of Manila clam aquaculture, little is known about its influence on sediment biogeochemistry. In this paper, we investigate the biogeochemical impact of burrows created by Manila clam aquaculture on (1) the distribution of chemical constituents in pore water and sediment and (2) the rates and partitioning of OC oxidation by SR and FeR, the 2 most dominant anaerobic OC oxidation pathways in intertidal sediment.

2. MATERIALS AND METHODS

2.1. Study area and sampling

The west coast of the Korean Peninsula, i.e. the eastern part of the Yellow Sea, is characterized by extensive intertidal wetlands, with an area of approximately 1800 km^2, covering 75% of the total coastal wetlands in South Korea (approximately 2400 km^2) (Koh & de Jonge 2014). Commercial production of Manila clams in this area accounts for 70% of the total production in Korea (Choi et al. 2016). This study was carried out on the intertidal flat in Keunso Bay near the Taean Peninsula (Fig. 1). Keunso Bay covers 87 km^2 without influx of rivers or fresh water and has a semi-diurnal macrotidal regime with an average tidal amplitude of approximately 6 m (Eom et al. 2012, www.khoa.go.kr/eng). The tidal range results in a low hydraulic turnover time (1.32 tidal cycles), indicating that Keunso Bay is a well-flushed environment (Choi et al. 2011). Approximately 70% of the bay is exposed to air at low tide, and water depth is 2 to 4 m during high tide (Eom et al. 2012).

Seed clams (~1.0 cm in shell length) are spread on the sediment by farmers and harvested when they exceed 3.5 cm in size (Lim 2016). Manila clams usually inhabit a depth of 3 to 6 cm in sandy silt sediment with clay content <10% (Cho et al. 2001, Nam et al. 2018). They create I-shaped burrows using their foot and have a basket-like filter inside the body cavity that opens to the outside through 2 siphons. Water enters through the inhalant siphon and exits through the exhalant siphon after passage through the filter where particles are retained. Weight-specific filtra-

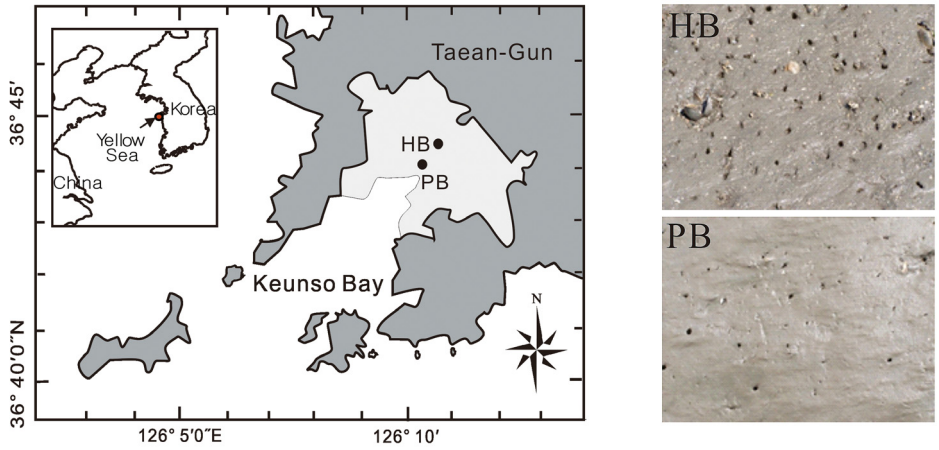

Fig. 1. Study sites of Manila clam aquaculture in the intertidal sediment of Keunso Bay. Light grey: intertidal flats exposed during low tide. Photos: small holes in sediment highly bioturbated (HB) or poorly bioturbated (PB) by Manila clams

tion, or ventilation, of Manila clams ranges from 1.2 to 6.0 l g^{-1} h^{-1} (Nizzoli et al. 2006a), which is lower than rates reported for oysters (8.8 l g^{-1} h^{-1}, Riisgård 1988) and mussels (3.5–8.0 l g^{-1} h^{-1}, Riisgård et al. 2013). During filtration, re-oxidation occurs by oxygen diffusion through the siphon wall or passive advection of water coupled with the siphon reaction (Hansen et al. 1996). Feces and pseudo-feces are ejected through an atrial siphon after digestion, providing labile organic matter to microorganisms in the sediment.

Manila clams cultivated at the study sites show highest growth (e.g. filtration and ingestion rates) at temperatures of 15 to 20°C in spring (Han et al. 2008). Growth decreases rapidly at temperatures above 25°C in summer (Laing et al. 1987), whereas mass mortality occurs during winter because of low temperatures (Kim et al. 2017). To optimize the effects of reworking and ventilation by Manila clams on the rates and pathways of anaerobic OC oxidation, we conducted experiments at a temperature of 18°C in the spring of 2008. Sediment samples were collected in 2 contrasting habitats at the same tidal elevation, one in a highly bioturbated (HB) site in the center of the farm and the other in poorly bioturbated (PB) sediment at the edge of the farm (Fig. 1). Average burrow density was estimated by counting burrow openings within 1 quadrat (25 × 25 cm) that was cast randomly onto the sediment more than 10 times.

2.2. Sediment handling

Triplicate sediment cores were collected using polycarbonate tubes (10 cm i.d.) to analyze for pore water solutes and solid phase Fe. The cores were transferred to an N_2-filled glove bag, and subsamples were collected from depths of 0–1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–8, and 8–10 cm and transferred into polypropylene centrifuge tubes. The tubes were tightly capped and centrifuged for 10 min at 3500 rpm. After reintroduction to the N_2-filled glove bag, pore water was sampled and filtered through 0.2 µm cellulose acetate syringe filters (Advantec). To determine inorganic nutrient concentrations (NH_4^+, NO_x, and PO_4^{3-}), 2 ml aliquots of pore water were fixed with 18 µl of saturated $HgCl_2$ and then frozen (–20°C) until analysis. Fe^{2+} in pore water was acidified with 12 N HCl and stored at 4°C. After collection of pore water, the remaining sediment was frozen at –20°C for later analysis of oxalate-extractable $Fe(II)_{(oxal)}$. Duplicate sediment cores were collected for chl *a*, organic carbon, and nitrogen analyses. The sediment was sectioned as above, loaded into polypropylene conical tubes, and frozen until further laboratory processing.

2.3. Geochemical analysis

Sediment temperature was measured using a digital thermometer (Cole-Parmer). Total organic carbon (TOC) and total nitrogen (TN) content was analyzed using a CHN analyzer (EA 1110, CE Instruments) after removal of $CaCO_3$ with HCl (10%). Chl *a* concentration in the surface sediment (0–2 cm) was determined using spectrophotometry according to Parsons et al. (1984). The concentration of NH_4^+ in the pore water was measured by flow injection analysis with conductivity detection (detection limit = 5 µM,

SD = 2%, Hall & Aller 1992). Concentrations of NO_x and PO_4^{3-} in pore water were measured using an autoanalyzer (detection limit: 0.02 µM, SD = 2% for NO_x; 0.006 µM, SD = 2% for PO_4^{3-}; Proxima, Alliance). Dissolved Fe^{2+} in pore water fixed with 12 N HCl was determined by a colorimetric method using ferrozine (detection limit = 1 µM, SD = 2%, Stookey 1970).

Wet chemical extraction was used to determine poorly crystallized iron minerals. The sediment was well mixed and subsampled (ca. 0.4 g) before extracting total Fe and Fe(II) in an anoxic chamber (vinyl anaerobic chamber, Coy Laboratory Products). Fe(II) was extracted in anoxic oxalate solution prepared by purging N_2 gas to prevent oxidation during Fe(II) extraction (Phillips & Lovley 1987). Total oxalate-extractable $Fe_{(oxal)}$ was extracted from air-dried sediment in a 0.2 M oxic oxalate reagent (pH 3) for more than 4 h (Thamdrup & Canfield 1996). Total $Fe_{(oxal)}$ and $Fe(II)_{(oxal)}$ were determined with ferrozine as described above. Oxalate-extractable solid $Fe(III)_{(oxal)}$ was defined as the difference between total $Fe_{(oxal)}$ and $Fe(II)_{(oxal)}$. Total reduced inorganic sulfur (TRIS), including acid-volatile sulfide (AVS = FeS + H_2S) and chromium-reducible sulfur (CRS = S^0 + FeS_2), was determined with the methylene blue method (detection limit: 3 µM, SD = 1%, Cline 1969) after single-step distillation of approximately 1 g of sediment with cold 12N HCl in boiling 0.5 M Cr^{2+} solution (Fossing & Jørgensen 1989).

2.4. Metabolic rate measurements

To determine the biogeochemical effect of Manila clams at depths of 3 – 6 cm, sediment cores down to 10 cm were collected using acrylic tubes (10 cm i.d.). The sediment was cut into depth intervals of 0–2, 2–4, 4–6, and 6–10 cm in N_2-filled glove bags. Sediment from parallel sections was pooled, mixed, and loaded into gas-tight plastic bags (Hansen et al. 2000). The bags were sealed without head space and incubated in the dark at *in situ* temperatures in larger anaerobic bags to ensure anoxic conditions. Subsamples were collected at Days 0, 1, 2, 3, and 5 to measure the accumulation of dissolved inorganic carbon (DIC) in the pore water. Two 50 ml centrifuge tubes were filled completely with sediment from each bag in N_2-filled glove bags, and pore water was extracted by centrifugation. For DIC analysis, 2.0 ml aliquots were transferred into glass vials without a gas space, fixed with 18 µl of saturated $HgCl_2$, and stored at 4°C for analysis within 2 wk. Total DIC was measured by flow injection analysis with conductivity detection (Hall & Aller 1992). Total anaerobic OC mineralization rates were determined by linear regression of the accumulation of total DIC with time during anoxic bag incubations (Fig. A1 in the Appendix).

After pore water retrieval from the centrifuge tubes, sediment was homogenized in an N_2 atmosphere, and $Fe(II)_{(oxal)}$ was extracted in oxalate as described in Section 2.3. FeR was determined by linear regression of the solid-phase $Fe(II)_{(oxal)}$ concentration with time during anoxic bag incubations (Kostka et al. 2002a, Fig. A1). Dissimilatory microbial FeR rates were calculated by subtracting abiotic FeR coupled with oxidation of sulfide produced by SR as described in Eq. (1) (Gribsholt et al. 2003):

$$\text{Dissimilatory FeR} = \text{total FeR} - \text{abiotic FeR} \quad (1)$$

Abiotic FeR was calculated from FeR coupled with oxidation of H_2S produced by SR using a stoichiometry of 2Fe:3S (Pyzik & Sommer 1981, Melton et al. 2014). Finally, to elucidate the contribution of FeR in anaerobic OC oxidation, a 4:1 stoichiometry of FeR coupled with OC oxidation was used (Canfield et al. 1993).

SR was determined in triplicate on intact cores (3 cm i.d., 35 cm long) by the radiotracer method of Jørgensen (1978). Carrier-free $^{35}SO_4^{2-}$ (2.5 µCi, Institute of Isotopes) was injected into ports at 1 cm intervals, and cores were incubated for 2 h at *in situ* temperature. The sediment was then sliced into sections, fixed in Zn acetate (20%), and frozen at –20°C until distillation in the laboratory. The reduced ^{35}S was recovered by distillation with cold 12N HCl and a boiling acidic Cr^{2+} solution (Fossing & Jørgensen 1989). Radioactivity of the reduced ^{35}S was quantified using a liquid scintillation counter (Tri-Carb 2910 TR, Perkin Elmer). To estimate OC oxidation by SR, the SR values were converted to carbon oxidation using a stoichiometry of 2C:S (Thamdrup & Canfield 1996).

3. RESULTS

3.1. Physico-chemical properties of sediment

Surface sediment (0–2 cm depth) temperature was 18°C at both sites (Table 1), and chl *a* content (which is often used as a proxy for the availability of labile organic matter in surface sediment) was (mean ± SD)

Table 1. Physico-chemical properties of surface sediment (0–2 cm depth) and burrow density at highly bioturbated (HB) and poorly bioturbated (PB) sites. Data are mean ± SE. **Bold**: significant difference between HB and PB sites ($p < 0.05$)

Physico-chemical properties	HB site	PB site
Temperature (°C)	18	18
Porosity	0.59 ± 0.11	0.50 ± 0.10
Density (g cm^{-3})	1.61 ± 0.16	1.71 ± 0.09
Water content (%)	36.7 ± 7.3	29.1 ± 6.0
Chl *a* (mg m^{-2})	185 ± 21	173 ± 62
Burrow density (no. m^{-2})	**326 ± 25**	**37 ± 12**

185 ± 21 and 173 ± 62 mg m^{-2} at the HB and PB sites, respectively. The burrow density at the HB site was 9 times higher than that at the PB site (*t*-tests, $p < 0.05$). Except for burrow density, the overall physico-chemical properties of surface sediment at the HB and PB sites were not statistically different (IBM SPSS Statistics 20).

3.2. Pore water and solid-phase constituents

NH_4^+ concentration increased with depth at both the HB and PB sites (Fig. 2a) and was higher at the PB site (160–351 µM) compared with the HB site (26–248 µM). Concentrations of NO_x at the HB site (4.3–16.5 µM) were higher than those measured at the PB site (1.1–16.8 µM). NO_x concentration decreased with depth to 4–5 cm and remained relatively constant deeper down at both sites (Fig. 2b). Concentrations of PO_4^{3-} only increased slightly with depth at the HB site but increased steeply below 4 cm at the PB site (Fig. 2c). Dissolved Fe^{2+} concentration at the HB site increased from 1 to 4 cm depth and thereafter decreased with depth, whereas the concentration at the PB site increased gradually with depth from 0 to 3 cm and then stabilized (Fig. 2d). Concentrations of SO_4^{2-} ranged from 25.5 to 27.7 mM and from 26.2 to 28.2 mM at the HB and PB sites, respectively (Fig. 2e). Depth-integrated inventories of NH_4^+, PO_4^{3-}, and Fe^{2+} at the HB site were 2-, 3-, and 3-fold lower, respectively, than those measured at the PB site (*t*-tests, $p < 0.05$), whereas concentrations of NO_x and SO_4^{2-} did not differ between sites (Table 2).

The concentration of TOC at the HB site decreased with depth, from 0.80% at the surface to 0.64% at a depth of 1 to 2 cm, and then remained constant to 6–8 cm, whereas that at the PB site decreased gradually from 0.81% at the surface to 0.39% at a depth of 6 to 8 cm (Fig. 3a). The concentration of TN ranged from 0.06 to 0.14% and from 0.04 to 0.14% at the HB and PB sites, respectively, and showed a pattern similar to the vertical profile of TOC (Fig. 3b). TOC concentration with sediment depth was higher at the HB site than at the PB site (2-way ANOVA, IBM SPSS Statistics, $p = 0.037$), and the averaged TOC to a depth of 0 to 10 cm was higher at the HB site than at the PB site (Table 2). TRIS content was low (<3 µmol cm^{-3}) to a depth of 2 to 3 cm and then increased rapidly with depth at the PB site to 15.6 µmol cm^{-3} at 9 to 10 cm (Fig. 3c). In contrast, TRIS content at the HB site was uniformly low with depth, showing a slight increase to 7.4 µmol cm^{-3} at 10 cm (Fig. 3c). At the HB site, $Fe(III)_{(oxal)}$ content decreased from 36.9 µmol cm^{-3} near the surface to 14.6 µmol cm^{-3} at a depth of 5 to 6 cm but decreased steeply from 35.7 µmol cm^{-3} at 0 to 1 cm to 5.3 µmol cm^{-3} at 5 to 6 cm at the PB site and then remained relatively constant below this depth at both sites (Fig. 3d). $Fe(II)_{(oxal)}$ content increased from 0.6 µmol cm^{-3} near the surface to 22.4 µmol cm^{-3} at 5–6 cm depth at the HB site and from 4.7 µmol cm^{-3}

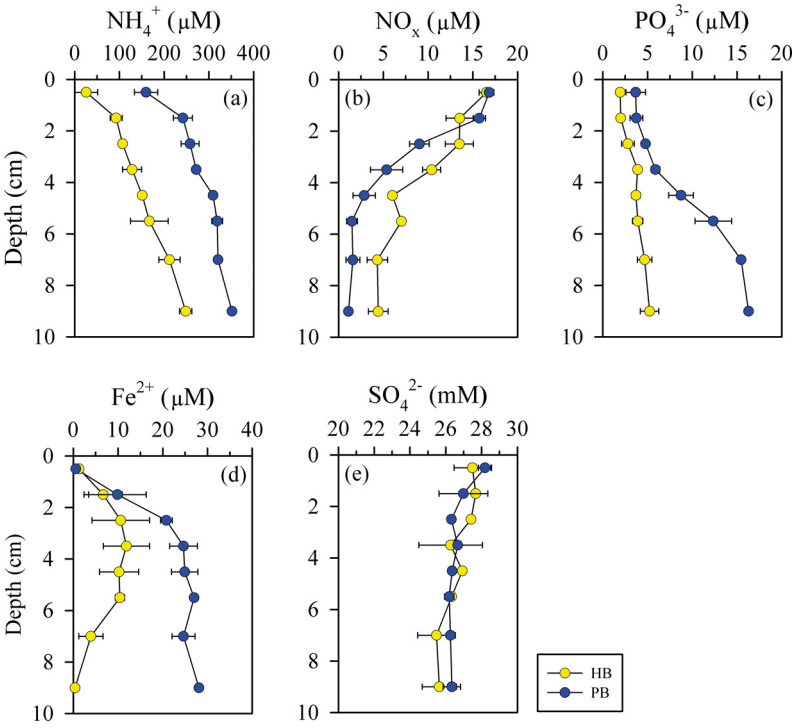

Fig. 2. Vertical profiles of NH_4^+, NO_x, PO_4^{3-}, Fe^{2+}, and SO_4^{2-} in pore water. Horizontal bars indicate SE. HB: highly bioturbated; PB: poorly bioturbated

Table 2. Depth-integrated (0–10 cm) concentrations of pore water and solid phase constituents (mean ± SD) of the duplicate or triplicate samples. **Bold**: significant difference between highly bioturbated (HB) and poorly bioturbated (PB) sites ($p < 0.05$). TOC: total organic carbon; TN: total nitrogen; TRIS: total reduced inorganic sulfur

Site	Pore water (mmol m^{-2})					Solid phase (%)		Solid phase (mmol m^{-2})		
	NH_4^+	NO_x	PO_4^{3-}	Fe^{2+}	SO_4^{2-}	TOC[a]	TN[a]	TRIS	$Fe(II)_{(oxal)}$	$Fe(III)_{(oxal)}$
HB	**7.93 ± 0.40**	0.46 ± 0.01	**0.19 ± 0.01**	**0.31 ± 0.14**	1371 ± 36	0.65 ± 0.06	0.09 ± 0.02	**412 ± 41**	**1546 ± 56**	**2243 ± 155**
PB	**14.17 ± 0.29**	0.29 ± 0.03	**0.49 ± 0.01**	**1.04 ± 0.03**	1313 ± 17	0.53 ± 0.03	0.07 ± 0.01	**946 ± 39**	**2570 ± 104**	**1127 ± 107**

[a]0 to 10 cm averaged

near the surface to 31.7 µmol cm^{-3} at 5–6 cm depth at the PB site, mirroring the decrease in $Fe(III)_{(oxal)}$ (Fig. 3d,e). $Fe(III)_{(oxal)}$ comprised >80% of total $Fe_{(oxal)}$ (i.e. Fe(II) + Fe(III)) at 0–4 and 0–2 cm depth at the HB and PB sites, respectively (Fig. 3d,e). The $Fe(III)_{(oxal)}$ fraction of total $Fe_{(oxal)}$ decreased to <20% below 4 cm at the PB site, whereas it remained >40% in the upper 6 to 8 cm of sediment at the HB site (Fig. 3d,e). The solid-phase $Fe(III)_{(oxal)}$ content was higher at the HB site than at the PB site (2-way ANOVA, $p = 0.020$), whereas solid-phase $Fe(II)_{(oxal)}$ and TRIS contents were higher at the PB site than at the HB site (2-way ANOVA, $p = 0.033$ and $p < 0.001$, respectively).

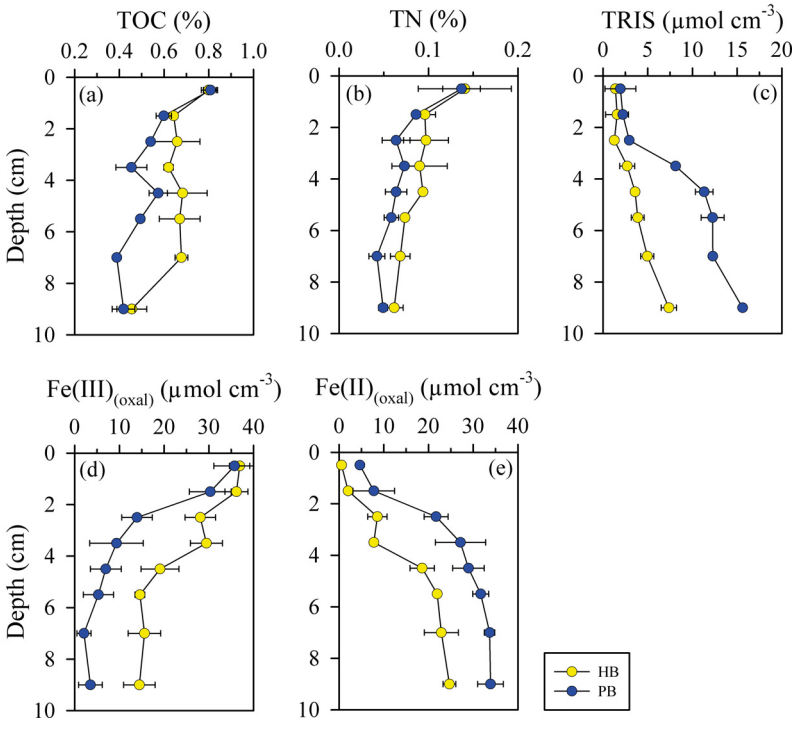

Fig. 3. Vertical profiles of total organic carbon (TOC), total nitrogen (TN), total reduced inorganic sulfur (TRIS), solid-phase Fe(II), and Fe(III) in the sediment. Horizontal bars indicate SE. HB: highly bioturbated; PB: poorly bioturbated

3.3. Rates of SR and FeR

SR at the HB site was generally low (<100 nmol cm^{-3} d^{-1}) through the entire sediment depth, with the highest rate measured at 1 to 2 cm (Fig. 4a). In contrast, SR at the PB site increased from 36.4 nmol cm^{-3} d^{-1} near the surface layer (0–1 cm) to 262.6 nmol cm^{-3} d^{-1} at 1–2 cm depth and then decreased to 33.6 nmol cm^{-3} d^{-1} at 9–10 cm depth (Fig. 4d). SR was higher at the PB site than at the HB site with sediment depth (2-way ANOVA, $p = 0.002$), and depth-integrated (0–10 cm) SR was approximately 1.4 times higher at the PB site than at the HB site (Table 3).

FeR at the HB site was highest in the 0–2 cm interval (2172 nmol cm^{-3} d^{-1}) and then decreased consistently with depth to 1310, 840, and 504 nmol cm^{-3} d^{-1} at the 2–4, 4–6, and 6–10 cm intervals, respectively (Fig. 4b). FeR at the PB site was 2433 and 648 nmol cm^{-3} d^{-1} at the 0–2 and 2–4 cm depth intervals, respectively, and then decreased to 0–168 nmol cm^{-3} d^{-1} at the 4–10 cm interval (Fig. 4e). Depth-integrated (0–10 cm) FeR at the HB site was 1.8 times higher than at the PB site (*t*-tests, $p < 0.05$, Table 3).

3.4. Total anaerobic OC oxidation rate

Anaerobic respiration at the HB site decreased gradually from 704 to 215 nmol C cm^{-3} d^{-1} with depth, whereas it rapidly decreased with depth from 666 to 82 nmol

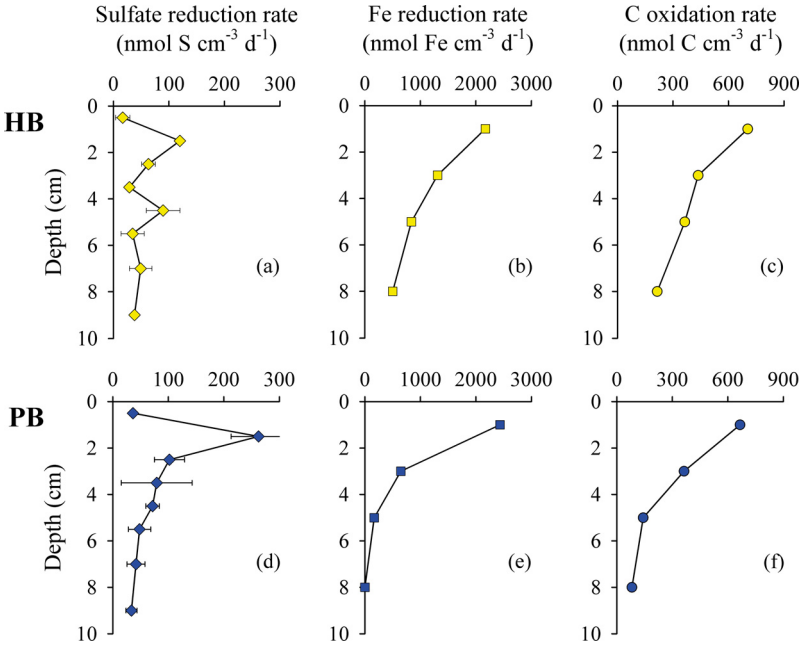

Fig. 4. Sulfate reduction from whole-core analyses, Fe reduction, and anaerobic carbon oxidation (dissolved inorganic carbon production rates) measured using anoxic bag incubation. Horizontal bars indicate SE. HB: highly bioturbated; PB: poorly bioturbated

cm^{-3} d^{-1} at the PB site (Fig. 4c,f). Depth-integrated (0–10 cm) anaerobic OC respiration rate at the HB site was significantly higher than at the PB site (*t*-tests, $p < 0.05$, Table 3).

4. DISCUSSION

4.1. Impacts of Manila clams on sediment geochemistry and OC oxidation

One of the distinguishing observations of this study was that anaerobic OC oxidation at the HB site exceeded that at the PB site to a depth of 10 cm (Fig. 4, Table 3). Anaerobic OC oxidation at both the HB and PB sites represented maximum values in surface sediment (0–2 cm); however, it decreased rapidly with depth at the PB site due to the lack of OC supply into the sediment. Suspension-feeding bivalves filter a large volume of suspended particles from the water

Table 3. Partitioning of sulfate and Fe(III) reduction in anaerobic organic carbon (OC) oxidation (mmol m^{-2} d^{-1}). HB: highly bioturbated; PB: poorly bioturbated; nd: not detected

Site	Depth (cm)	Anaerobic OC oxidation	Sulfate reduction	Total Fe(III) reduction	Fe(III) reduction by[a,b]		OC oxidation by[a,c]	
					Sulfide oxidation	Fe(III) reducer	Microbial sulfate reduction	Microbial Fe(III) reduction
HB	0–2	14.1	1.4	43.4	0.93 (2.1%)	42.51 (97.9%)	2.8 (19.9%)	10.63 (75.5%)
	2–4	8.8	0.9	26.2	0.60 (2.3%)	25.61 (97.7%)	1.8 (20.5%)	6.4 (72.8%)
	4–6	7.3	1.2	16.8	0.83 (4.9%)	15.97 (95.1%)	2.5 (34.1%)	4.0 (54.9%)
	6–10	8.6	1.7	20.2	1.16 (3.3%)	19.00 (96.7%)	3.5 (40.4%)	4.8 (55.2%)
Sum (0–10)		38.8	5.3	103.3	3.57 (3.5%)	99.63 (96.5%)	10.6 (29.1%)	25.8 (70.8%)
PB	0–2	13.3	1.2	48.7	0.83 (1.7%)	47.85 (98.3%)	2.4 (18.6%)	12.0 (89.7%)
	2–4	7.3	1.8	13.0	1.21 (9.3%)	11.75 (90.7%)	3.6 (49.8%)	2.9 (40.4%)
	4–6	2.9	1.0	3.4	0.67 (19.6%)	2.73 (80.4%)	2.0 (69.0%)	0.7 (23.6%)
	6–10	3.3	1.5	0.0	nd	nd	3.0 (91.8%)	nd
Sum (0–10)		26.8	7.4	56.2	2.78 (4.3%)	62.22 (95.7%)	11.0 (41.1%)	15.6 (58.3%)

[a]Stoichiometric equations were used to estimate the partitioning of OC mineralization; abiotic reduction of Fe(III) by sulfide oxidation: $3H_2S + 2FeOOH = 2FeS + S^0 + 4H_2O$; microbial Fe(III) reduction = total Fe(III) reduction – abiotic Fe(III) reduction; OC oxidation by sulfate reduction: $SO_4^{2-} + CH_3COO^- + 2H^+ = 2CO_2 + 2H_2O + HS^-$; OC mineralization by microbial Fe(III) reduction: $4FeOOH + CH_3COO^- + 8H^+ = CO_2 + 7H_2O + 4Fe^{2+}$

[b]Relative contribution (%) of abiotic and microbial Fe(III) reduction in total Fe(III) reduction

[c]Relative contribution (%) of sulfate reduction and Fe(III) reduction to total anaerobic OC respiration

column and eject uningested pseudo-feces and unassimilated feces into the sediment. Those feces are reactive and have low C:N molar ratios and are therefore suitable for stimulating benthic metabolism (Nedwell 2004, Varela et al. 2004). The difference in anaerobic OC oxidation rate between HB and PB sites was most distinct below 4 cm depth (Table 3). Studies on excavation and feeding behaviors of fiddler crabs have reported that bioturbation greatly modifies the distribution of organic matter (Botto & Iribarne 2000, Kristensen & Alongi 2006, Natálio et al. 2017), thereby stimulating benthic microbial remineralization in subsurface sediment (Kristensen 2000, Lohrer et al. 2005, Martinez-Garcia et al. 2015). In this study, depth distribution of TOC and rates of OC oxidation also showed a high linear correlation (r^2 = 0.722, $p < 0.05$), which suggests that macrofauna promote vertical transport of organic matter by redistributing particles along their burrows (Aller 1982, Aller & Aller 1998, Tang & Kristensen 2007, Bonaglia et al. 2014, Benelli et al. 2017).

Despite higher anaerobic OC oxidation at the HB site, accumulation of the reduced products of anaerobic metabolic activities (i.e. NH_4^+, PO_4^{3-}, and Fe^{2+}) in the pore water and reduced solid phase iron and sulfur [i.e. $Fe(II)_{(oxal)}$ and TRIS] in the sediment was lower at the HB site than at the PB site (Figs. 2 & 3, Tables 2 & 3). In contrast, $Fe(III)_{(oxal)}$ content was higher at the HB site. The results indicate that the reduced inorganic metabolites produced during OC oxidation were either actively re-oxidized or flushed out by bioirrigation because there was no difference in inundation frequency and sediment particle size composition of sediment that could affect the depth profiles of chemical constituents in pore water and the solid phase. The construction of ventilated burrows and irrigation stimulate an exchange of solutes between the sediment and overlying water and drive reduced metabolites out of and oxidized electron acceptors into the sediment (Kristensen & Kostka 2005, Timmermann et al. 2006, Quintana et al. 2015). Suspension feeders including the Manila clam ventilate large amounts of water to satisfy their demand for food (Kristensen et al. 2012). Furthermore, the biogenic microtopography termed bioroughness (e.g. burrow excavations, feeding traces, and fecal pellets) creates advective pore water flow and forces overlying water into the sediment (Huettel & Gust 1992). In addition, tidal water intrusion resulting from the large tidal ranges in the study area (average = 6 m, Eom et al. 2012) also affects the inventories of geochemical constituents in the pore water and sediment (Figs. 2 & 3, Table 2). Consequently, bioturbation by Manila clams and tidal inundation enhance the exchange of solutes between the pore water and surface water, thereby increasing re-oxidation and transport of reduced metabolites out of the sediment.

4.2. Partitioning of OC oxidation by SR and FeR

Ventilation by macrofauna promotes irrigation and thus re-oxidation of reduced compounds in sediment, which may stimulate degradation of organic matter by suboxic OC oxidation pathways (Aller 1994, Kristensen 2001). In the present study, microbial FeR dominated anaerobic OC oxidation at the HB site, accounting for 70.8% of the total anaerobic OC oxidation in the 0–10 cm sediment (Fig. 4, Table 3). FeR accounted for 54.9 to 55.2% of total anaerobic OC oxidation in the subsurface sediment layers (4–10 cm) at the HB site, which was still comparable to or higher than the contribution of SR, representing 34.1 to 40.4% of anaerobic OC oxidation (Fig. 4, Table 3). In contrast, SR was the dominant OC oxidation pathway below 2 cm of the sediment at the PB site, accounting for 49.8 to 91.8% of the total anaerobic OC oxidation (Fig. 4, Table 3). Our observations of high FeR under high Fe(III) conditions are similar to results of previous studies reported at highly bioturbated intertidal sediment (Kostka et al. 2002a,b, Gribsholt et al. 2003, Hyun et al. 2007, 2009). Oxygen can penetrate more deeply into highly bioturbated sediment through the burrows, which stimulates re-oxidation of Fe(II) to Fe(III) (Kristensen et al. 2012). Consequently, the dominance of FeR in the anoxic part of the sediment at the HB site was induced by the enhanced Fe(III) content, which ultimately suppresses SR (Hines & Jones 1985, Gribsholt & Kristensen 2002, Kristensen & Kostka 2005).

Stocking density is a significant factor for determining production rates in aquaculture (Hengsawat et al. 1997, Jones & Ruscoe 2000). Lower stocking densities eventually result in less competition and faster growth, but production cost per individual is higher (Rheault & Rice 1995, Verspecht et al. 2011). In contrast, high stocking densities cause stress from competition for food and space and pathogen infestation, which can lead to mass mortality of aquaculture organisms (Wilber & Wilber 1991, Dittel et al. 1995, Sheen 2000). Numerous studies have been carried out to determine the optimal stocking density in farm operations to maximize production rates (Wu et al. 1994, Azim et al. 2001, De Silva 2003, Biswas & Takii 2017). From a biogeochemical viewpoint, stocking density is also the main factor in controlling the envi-

ronmental impact of a Manila clam farm. For example, Welsh et al. (2015) reported that the flux of solutes (O_2, N_2, N_2O, NH_4^+, and NO_x), nitrification, and denitrification were significantly correlated with Manila clam density in the Po River Delta, Italy. Extremely high stock density (>1000 ind. m^{-2}) was associated with increased anaerobic metabolism, accumulation of reduced compounds, and even chronic hypoxia at a Manila clam farm in the Sacca di Goro Lagoon, Po River Delta (Bartoli et al. 2001, Meliá et al. 2003). In the present study, the dominance of FeR (>70%) in OC oxidation together with low TOC content (<1%) at the HB site (Tables 2 & 3) implies that a stock density of approximately 300 ind. m^{-2} provides environmentally sustainable conditions by lowering overall respiration rates and rapidly replenishing Fe oxides to support FeR, thereby suppressing SR. Because higher accumulation of OC resulting from extremely high stocking densities would increase SR, studies on the carrying capacity of Manila clam aquaculture that consider multiple environmental and eco-physiological aspects need to be conducted.

4.3. Dependence of FeR on Fe(III) concentration

FeR in the sediment is highly dependent on the availability of both reactive Fe(III) and labile OC (Thamdrup 2000). In organic-rich coastal sediment, re-oxidation of Fe(II) to Fe(III) mediated by bioturbation can sustain high rates of microbial FeR (Gribsholt & Kristensen 2002, Quintana et al. 2015, van de Velde & Meysman 2016). Indeed, more than 50% of total anaerobic OC oxidation was processed by FeR at the HB site, where $Fe(III)_{(oxal)}$ concentration was high (15–37 µmol cm^{-3}), whereas only 0 to 40% of total anaerobic OC oxidation was ascribed to FeR in sediment 2 to 10 cm deep at the PB site, where less $Fe(III)_{(oxal)}$ (2–14 µmol cm^{-3}) was available (Table 3). To further verify the dependence of FeR on the availability of Fe(III), we plotted the 2 variables using Eq. (2), which was proposed in a study of coastal sediment (Jensen et al. 2003, Kristensen et al. 2011):

$$\%FeR = 100\ (1 - e^{-a[Fe(III)]}) \quad (2)$$

where %FeR is OC oxidation through FeR as a percentage of total anaerobic OC oxidation, Fe(III) is the concentration of poorly crystalline Fe oxides, and a is the fitting parameter. The contribution of FeR to anaerobic respiration in sediment from Manila clam farms was significantly related to the concentration of reactive Fe(III) (Fig. 4). The relationship shows that more than 70% of the anaerobic OC oxidation was due to microbial FeR when the concentration of reactive Fe(III) exceeded about 30 µmol cm^{-3} (Fig. 5). The fitting parameter in this study was slightly lower (a = 0.042) than that for marine sediment reported from the Danish coastal area (a = 0.056, Jensen et al. 2003) and mangrove sediment (a = 0.053, Kristensen et al. 2011) but was higher than that from freshwater sediment (a = 0.029, Roden & Wetzel 2002). The variance may result from differences in estimating FeR and extraction methods of Fe(III). Jensen et al. (2003) calculated total anaerobic OC respiration as the sum of OC oxidation by SR and FeR assuming that the 2 pathways constitute most of the anaerobic OC oxidation. The OC oxidation by FeR was then estimated by subtracting the OC oxidation by SR from total anaerobic OC respiration. Considering that SR and FeR are the 2 major OC oxidation pathways (Jensen et al. 2003), the contribution of FeR to anaerobic respiration in this approach can be overestimated because other potentially significant OC oxidation pathways (e.g. denitrification) are neglected. On the other hand, solid phase Fe in mangrove and freshwater sediment is extracted by HCl according to Lovley & Phillips (1987) (Roden & Wetzel 2002, Kristensen et al. 2011). Oxalate and HCl extraction methods are

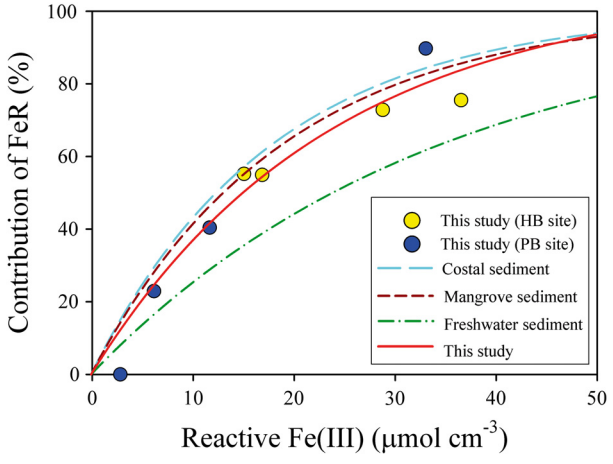

Fig. 5. Relative contribution of Fe(III) reduction (FeR) to anaerobic carbon oxidation as a function of reactive Fe(III) content in sediment. The red line shows the best fit of the data from Manila clam aquaculture sediment using Eq. (2) (Section 4.3) with a = 0.042. The blue line shows the general relationship of FeR to anaerobic carbon oxidation in coastal sediment using a = 0.056 as reported by Jensen et al. (2003). The brown line shows the relationship of FeR to anaerobic carbon oxidation in mangrove sediment using a = 0.053 (Kristensen et al. 2011). The green line represents the relationship of FeR to anaerobic carbon oxidation using a = 0.029 for freshwater wetlands (Roden & Wetzel 2002). HB: highly bioturbated; PB: poorly bioturbated

widely used to determine the poorly crystalline (i.e. reactive) Fe fraction in sediment (Thamdrup et al. 1994). The major difference between the oxalate and HCl methods is that HCl extraction does not capture magnetite, a type of poorly crystalline Fe (Poulton & Canfield 2005). Although the fitting parameter for Fe(III) concentration and FeR varies according to method of extraction and calculation of Fe concentration, the tight relationship between Fe(III) content and contribution of FeR to OC oxidation serves as an extensively adopted proxy to evaluate the significance of FeR and the redox conditions in the sediment of Manila clam aquaculture.

5. CONCLUSIONS

A combination of the analysis of sediment constituents as well as rates and partitioning of OC oxidation (i.e. relative significance of SR and FeR in anaerobic OC oxidation) was conducted to elucidate the effects of bioturbation by Manila clams on sediment biogeochemistry. Our results revealed that enhanced supply of reactive Fe(III) via increased solute exchange through sediment reworking and irrigation activities by Manila clams ultimately promoted OC oxidation coupled with FeR in the sediment of the aquaculture farms. In contrast to net cage and longline aquaculture, in which SR is the dominant OC oxidation process, Manila clam farms operated with an appropriate stock density in the intertidal sediment have a positive environmental effect in terms of expediting re-oxidation of reduced metabolic compounds, stimulation of FeR, and suppression of SR.

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Appendix.

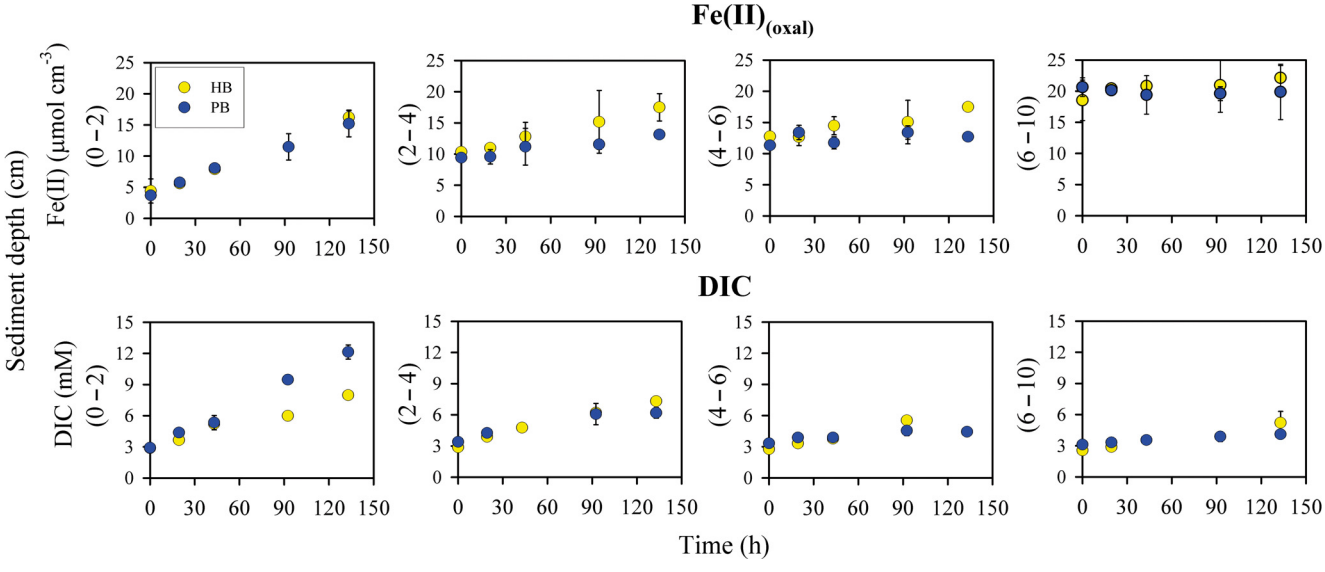

Fig. A1. Changes in $Fe(II)_{(oxal)}$ content in the sediment and changes in DIC concentrations in pore water during anoxic incubation of sediment at depths of 0–2, 2–4, 4–6, and 6–10 cm at highly bioturbated (HB) and poorly bioturbated (PB) sites. Vertical bars indicate SE

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