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Impacts of bioturbation by Venus clam *Cyclina sinensis* (Gmelin, 1791) on benthic metabolism and sediment nutrient dynamics in a shrimp-clam polyculture pond

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ABSTRACT

Dynamics of benthic inorganic nutrients are key biogeochemical components of sediment metabolism and ecosystems. This study investigated the roles of the bivalve *Cyclina sinensis* (Gmelin, 1791) and its influence on benthic metabolism, nutrient fluxes and sediment oxygen consumption (SOC) in a shrimp-clam polyculture system in comparison with shrimp culture ponds without incorporating clams, in Ningbo Zhejiang China. The benthic inorganic nutrients fluxes (ammonium-NH_4^+, nitrate plus nitrite-NO_3^-+NO_2^- and phosphate-PO_4^{3-}) and SOC were measured across the sediment-water interface with dark incubation experiments. The results showed that there were significantly higher nutrient fluxes from the sediment into the overlying water ($p<0.05$) in the treatment ponds in the order of $NH_4^+ > PO_4^{3-} > NO_3^- + NO_2^-$. The SOC varied significantly ($p<0.05$) and was three times higher than that of the control ponds. Water quality (dissolved oxygen, pH, chlorophyll-*a* and salinity) recorded showed slight variations over time but were not significantly different ($p>0.05$) between the control and treatment ponds. Sediment organic matter and chlorophyll-*a* concentration measured in the shrimp-clam ponds varied significantly ($p<0.05$) as compared to control ponds. The results of this study suggest that the bioturbation activities by *C. sinensis* promoted the SOC, sediment organic matter degradation and mineralisation process that increased the exchange of nutrients and oxygen uptake between the sediment and the overlying water.

Keywords: Bioturbation, *Cyclina sinensis*, Nutrient flux, Sediment quality, Shellfish farming

Introduction

Aquatic macro-invertebrates have been used in water bodies to investigate water quality (Rosarie *et al.,* 2015). These animals include but are not limited to shrimp, crabs and bivalves. They play a key role in nutrient cycling, primary productivity and translocation of materials and decomposition of organic material within the aquatic environment (Wallace and Webster, 1996; Nicholaus *et al*., 2019a). Aquatic macro-invertebrates also form an important component in the sediment and water column of shallow lakes (Batzer *et al.,* 1993) and aquaculture ecosystems.

Benthic invertebrates contribute a vital part in oxygen uptake and nutrient cycling in the benthic boundary layers (Zhang *et al.,* 2013; Nicholaus *et al*., 2019a). They may significantly modify the exchange processes by bioturbation activities and enhance the benthic sediment organic matter through their biodeposits (Baudinet *et al.,* 1990). Interactions of the chemical, biological and physical processes created during bivalve bioturbations determine the extent to which nutrients are changed, released, or taken up by benthic communities. Burrowing activities alter the sediment pH (Zhu *et al.*, 2006), oxygen level (Pischedda *et al*., 2008; Nicholaus and Zheng, 2014; Zhao *et al*., 2019) and microbial activity (Kogure and Wada, 2005; Lukwambe *et al*., 2018). Marine bivalve molluscs impact nutrient dynamics through direct discharge and in an indirect way through microbial interceded remineralisation of their organic materials in the sediments (McKindsey *et al*., 2006a; Nicholaus *et al*., 2019b). The intensity of degradation and mineralisation of the deposited organic matter in the sediments determines the rates of nutrient release to the overlying water column. Episodes of sediment resuspension and particle mixing enhance nutrient regeneration and stimulate mineralisation of organic matter (Longhi *et al*., 2013). Demineralisation takes place and increases with the amount of labile organic matter present (Sloth *et al*., 1995) while benthic-pelagic fluxes are dependent on the nutrient concentrations in the water column. During inhabitation in the sediment, benthic invertebrates (*e.g.* clams), do respire, feed, excrete metabolites and renew their burrow water through bio-irrigation (Kristensen and Kostka, 2005).

In recent years, lot of information has been brought out on the importance of exchange of oxygen and nutrients across the sediment-water interface and the dynamics of interactions led by bivalves in estuarine and coastal ecosystems but limited to aquaculture ponds. Sediment oxygen consumption (SOC) can be an important sink for oxygen and sediment nutrient fluxes can be a large internal source of both nitrogen and phosphorus to the water column (Kemp and Boynton, 1992; Nicholaus and Zheng, 2014). These nutrients are of great importance for the growth of primary producers and small heterotrophs and benthic metabolism. Nutrient regeneration in the sediment through bioturbation supplies back a significant portion of phytoplanktonic nutrient demand (Cowan and Boynton, 1996). Benthic bivalves are important contributors of nitrogen in the form of NH_4^+-N in the aquatic systems (Zhao *et al*., 2019).

Scientific information related to seasonal changes in nutrient fluxes and water column concentrations in aquatic systems enables us to improve the ecosystem's productivity (Garcia-Robledo *et al*., 2016). Understanding the benthic-pelagic coupling of inorganic nutrient fluxes and oxygen distribution as related to bivalve aquaculture (Richard *et al*., 2007a) is very important to improve aquaculture productivity and ecosystem activities. Various studies have examined the effects of infaunal bivalves' bioturbation on dynamics of benthic nutrients including metabolisms and fluxes of ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (PO_4^{3-}) in sediments (Michaud *et al*., 2006; Zhang *et al*., 2011; Zheng *et al*., 2011; Zhao *et al*., 2019) as the most common nutrient limiting biological production in marine estuarine systems and ponds. However, very few studies have considered the potential effects of bivalve clam bioturbation in polyculture systems (*e.g.* shrimp-clam polyculture which is very popular in China) in relation to nutrient dynamics, fluxes and sediment quality.

The current study aims to quantify the nutrient fluxes released from sediments to the overlying water in a shrimp-clam polyculture pond and establish the potential roles of clam bioturbation in maintaining pond water and sediment quality and its effect on nutrients dynamics. We hypothesised that the sediment cores in the shrimp-clam cultured areas/ponds would have higher nutrient fluxes, improved nutrient efficiency and recycling and higher rates of SOC than the sediment cores in the non-clam cultured ponds. This study will help to strengthen the understanding of whether farmers who grow shrimp in conjunction with clams in polyculture systems are more likely to create sustainable, productive, sound and healthy ecosystems than those relying only on shrimp culture (monoculture). Also, it will shed light on the roles of clams on benthic nutrient metabolism, distribution and cycling. All such knowledge will help to design extension and outreach programs that meet the needs of shellfish farmers and help increase production, economic returns and improve resource utilisation.

Materials and methods

Study site and sampling

The experiments were conducetd in ponds (71x35 m) in a farming base located in Fenghua-Songao, Zhejiang Province, China. The treatment ponds cultured Venus clam *Cyclina sinensis* (Gmelin, 1791) along with shrimp *Penaeus vannamei* Boone, 1931 while ponds with shrimps alone without clams served as control. Three separate ponds were setup to serve as biological replicates for both treatment and control. Water was sourced from the adjacent sea (marine) near the experimental ponds. Throughout the study, clams were fed on plankton and benthos that are naturally and abundantly found in seawater. In the treatment ponds, Venus clam seeds were stocked at a density of 4000 individuals per m^2 and shrimp seeds were stocked at 90 per m^2. The study was carried out within a single culture season that lasted for 6 months and sediment samplings for analyses were done at bimonthly intervals (Samling I, II and III in June, August and October respectively).

The surface sediment layer (5 cm) of the pond (at the beginning) comprised 69.6% silt and clay and 30.4% sand particles and had a porosity of 74.3% on average. At each sampling time, the water parameters of the pond (temperature, pH, salinity, chlorophyll-*a* and dissolved oxygen concentrations) were measured *in situ*. The cores for measuring the sediment quality were extruded from the sampling device and the surface (10 cm layer) was sectioned into 0-2, 2-4, 4-6, 6-8 and 8-10 cm intervals for physico-chemical analysis.

Measurement of nutrient fluxes

Fluxes of NH_4^+, NO_3^-+NO_2^-, PO_4^{3-} and SOC of the sediment samples collected during the bimonthly samplings were estimated *via* dark laboratory incubation.

On each sampling date, 6 undisturbed sediment cores were collected with plexiglass tubes (sediment height: 10 cm, internal diameter: 4.5 cm) using a hand driven corer. The cores were closed with rubber stoppers and carefully brought to the laboratory within 1.5 h. In the laboratory, sediment height was adjusted to approximately 10 cm, giving a water height of approximately 15 cm before incubation (Zheng *et al*., 2011). Approximately 20 l of overlying water was collected from the site for core maintenance and use during the incubation experiment. The overlying water contained in the sediment core was carefully sucked out with a rubber tube without disturbing the sediment surface. Then the incubation tubes were refilled with fresh overlying water collected *in situ* during sampling after being aerated for 2 h to assume oxygen saturation prior to incubation.

Dark incubation experiment was conducted in a water bath (Fig. 1) with similar temperature conditions as *in situ* conditions, for 3 h. Teflon coated magnets (0.5 × 3 cm) were placed in the water column 5 cm above the sediment surface and driven by an external rotating magnet (60 rpm) to ensure appropriate stirring of the water column as previously described (Zheng *et al*., 2011). All cores were maintained for about 30 min before initiation of experiments. Three parallel incubation chambers with plexiglas tubes containing *in situ* water samples were used to correct nutrient fluxes. All cores were incubated for 3 h then rates of sediment oxygen consumption (SOC) and fluxes of ammonium (NH_4^+), nitrate plus nitrite (NO_3^- $+NO_2^-$) and phosphate (PO_4^{3-}) were measured for each sampling time.

Three parallel blank chambers (without sediment) were also incubated as control of incubation to correct for the nutrient fluxes and dissolved oxygen (DO) contents. At the end of the 3 h incubation period, DO was immediately measured using YSI-550A oxygen meter. Subsequently, samples for NH_4^+, NO_3^-+NO_2^- and PO_4^{3-} were collected as well. Water samples were filtered with a 0.45 µm pore size syringe filter (Song *et al*., 2013) and stored in polypropylene cryovial tubes at -20°C. The nutrient fluxes were measured using WESTCO SmartChem discrete analyser (Westcom Scientific Instrument Inc. Broockfield, USA). The indophenol blue method was used to measure ammonia, while nitrate plus nitrite was measured following cadmium-copper reduction method and phosphate was measured following the ammonium molybdate/ascorbic acid method (APHA, 2012). Water samples from incubation medium were analysed for inorganic nutrient fluxes and SOC rates as previously described by Nicholaus and Zheng (2014).

Sediment physico-chemical characteristics and pigment analysis

Sediment samples for physico-chemical analyses were kept in -20°C freezer prior to laboratory testing. A set of 6 sediment cores were sliced into 5 depth horizons (0-2, 2-4, 4-6, 6-8 and 8-10 cm), each sediment slice was immediately homogenised and samples were collected using a plexiglass tube. The samples were then analysed for sediment physico-chemical characteristics, including silt/clay fraction, sediment porosity, total organic matter (OM), total nitrogen (TN) and total phosphorus (TP) following the methods of Mudroch *et al*. (1997). Sediment Chl-*a* was determined following the methods of Bartoli *et al*. (2001) and Riaux-Globlin *et al*. (1987) for the fluorometric analysis of pigment. Sediments were freeze dried and homogenised before the pigments were extracted from about 1 g dry weight sample with 10 ml

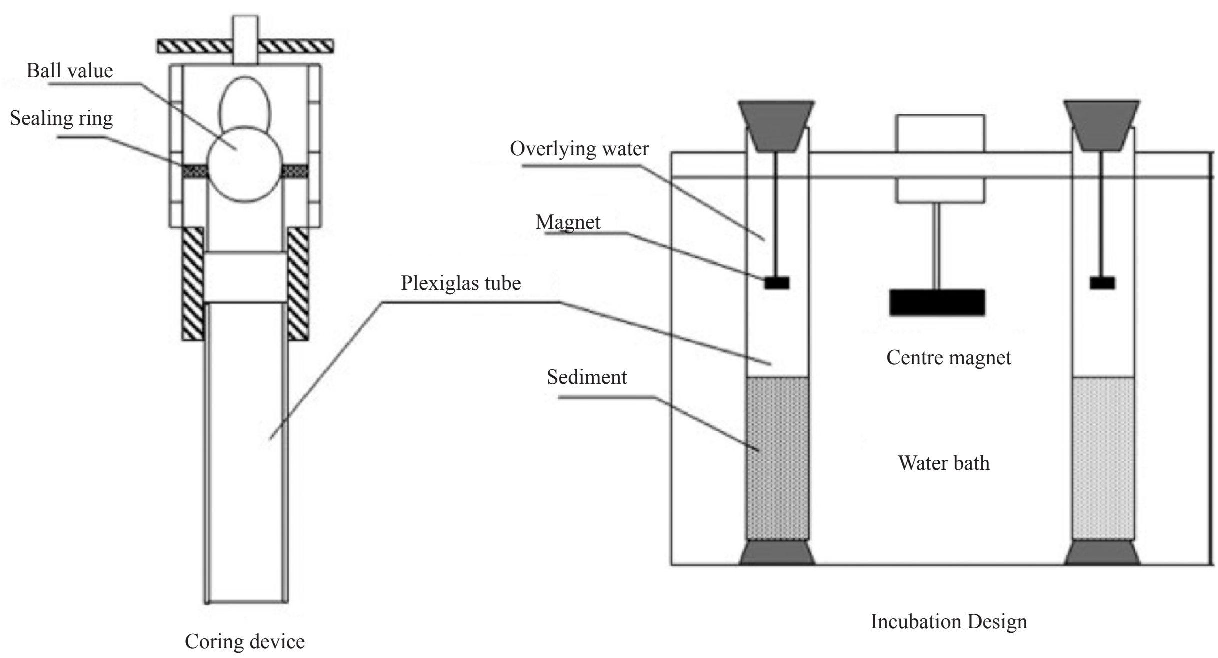


Fig. 1. The benthic chamber specially designed and built for the study. (As adopted from Zheng *et al*., 2011)

of 90% acetone. Complete pigment extraction was enhanced by allowing the samples to stay overnight at 4°C, then centrifuged at 400 rpm for 5 min (Volkenborn *et al*., 2007). For grain size analysis, sediment cores collected were dried to constant weight at 60°C and then analysed by dry sieving method. They were then sieved for sand, silt and clay fractions through 250, 100 and 63 mm mesh sizes respectively and weighed (Engelsen *et al*., 2008).

Statistical analysis

One-way ANOVA, was used to analyse and compare the inorganic nutrient fluxes, NH_4^+, NO_3^-+NO_2^-, PO_4^{3-} and SOC flux rates. When ANOVA indicated statistical significance among the treatments, Tukeys HSD analysis ($p<0.05$) was used as a *post hoc* test to determine pairwise comparison probabilities between treatment levels. Regression was used for correlation between the treatment and control. All statistical analyses were computed using the SPSS software, version 16.0 (SPSS Inc., Chicago, USA). Figures were drawn with Origin 8.0 software.

Results

Water quality parameters

The mean water quality parameters recorded during the experimental period are presented in Table 1, with an average of 2.7 µg ml^{-1} (dry weight) chlorophyll-*a* (Chl-*a*) and 5.2 mg l^{-1} dissolved oxygen (DO) according to *in situ* measurements. Water quality (DO, pH, Chl-*a* and salinity) showed variations over time but were not significantly different ($p>0.05$) between control and treatment ponds.

Sediment organic matter and pigment (Chl-a) characteristics

The sediment organic matter (OM) content (%OM±SE), measured as a loss on ignition (LOI), in the sediment between the treatment and the control group during the study, were 8.1±1.2 and 6.9±0.7% respectively and varied significantly (ANOVA, $p<0.05$; Fig. 2a). The highest OM content was measured in the 2 to 6 cm sediment layer and decreased sharply with depth drop from 9.3±0.5 to 3.7±0.4% dry weight over the 0-10 cm depth profile. The variations were much stronger in the treatment group and experienced a positive correlation with depth ($r^2=0.87$; $p<0.05$).

The concentration of sediment Chl-*a* in cores from the treatment and control groups were significantly different (One-way ANOVA, $p<0.05$) and decreased with depth (Fig. 2b). A different observation was recorded in October whereby neither the treatment nor the control group had significant variation in the Chl-*a* concentrations (ANOVA, $p<0.05$, Fig. 2b).

Total phosphorous and nitrogen in the sediments

Total phosphorus (TP) measured in June, August and October varied significantly between the treatment and control groups (ANOVA, $p<0.05$, Table 2). The mean TP was 9.0±3.0 and 13.7±30 mg g^{-1} for the treatment and the control ponds respectively. TP concentration in the treatment group decreased by 35% over the control during

Table 1. Water quality parameters in the treatment and control ponds during the six months study period

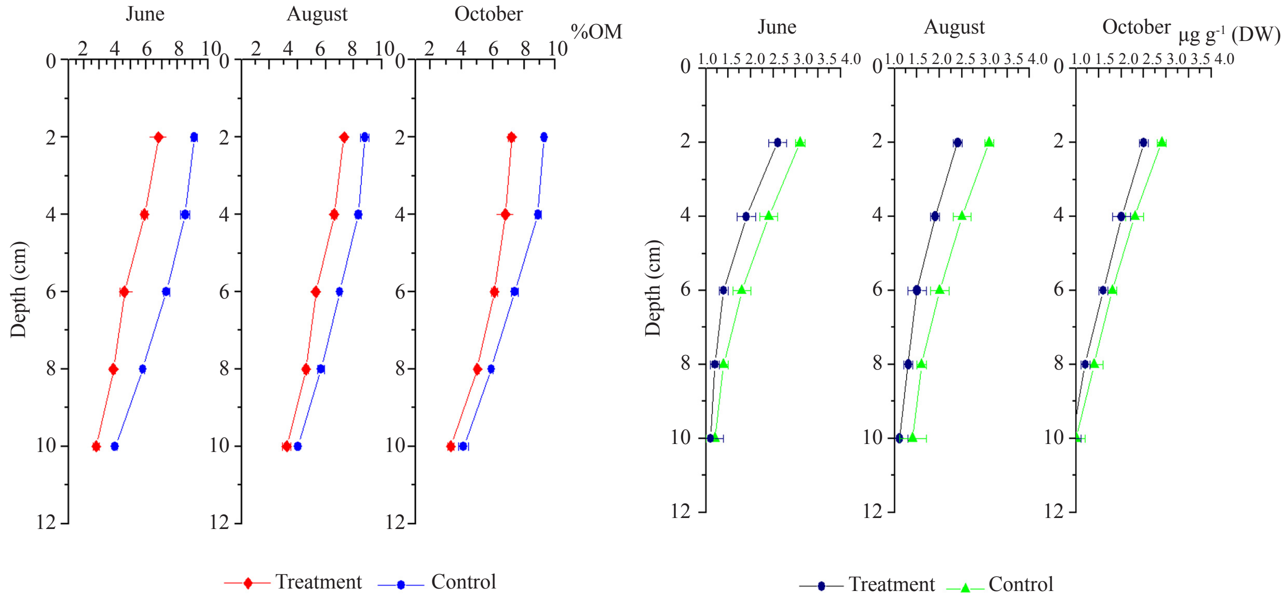
Sampling	Treatment	Temp. (°C)	DO (mg l^{-1})	pH	Salinity (‰)	Chlorophyll-a [µg ml^{-1} (DW)]
Sampling I	Control	27.2±1.5	5.5±0.7^a	7.7±0.6	22.3±1.0	2.7±0.3^a
	Treatment	27.2±1.3	4.9±0.9^b	7.5±0.1	22.4±0.3	3.2±0.7^b
Sampling II	Control	29.6±0.7	4.1±0.1^a	8.6±0.3	26.7±1.3	2.9±0.2^a
	Treatment	29.6±0.3	3.5±0.3^b	8.1±0.4	26.8±1.1	2.1±0.1^b
Sampling III	Control	25.8±0.3	5.9±0.4^a	8.6±0.4	23.6±1.0	2.4±0.1^a
	Treatment	25.5±0.6	5.2±0.3^b	8.6±0.5	22.8±0.9	1.8±0.3^b

Data are expressed as mean±SE, n=3. DW: Dry weight. Values bearing different superscripts differ significantly ($p<0.05$)

Table 2. Total phosphorus and total nitrogen in the sediment between the shrimp-clam cultured pond and non-clam cultured pond during the study period

Parameters	Month of collection	Control	Treatment
TP (mg g^{-1})	June	13.45±0.23^a	8.93±0.37^b
	August	13.72±0.29^a	8.84±0.16^b
	October	14.01±0.36^a	9.11±0.35^b
TN (mg g^{-1})	June	1.1±0.02^a	1.1±0.02^a
	August	1.2±0.02^a	1.2±0.01^a
	October	1.3±0.04^a	1.1±0.02^a

Data are presented as the means of three replicated experimental samples. Values bearing different superscript letters for the same month differed significantly ($p<0.05$)



(a) (b)

Fig. 2. Variation in (a) Organic matter-OM (%) and (b) Sediment chlorophyll-*a* (µg g^{-1} dry weight) at different depths of the sediment cores from shrimp-clam polyculture ponds in comparison with control ponds

the culture period. The total nitrogen (TN) did not show any statistically significant difference (Table 2).

Physico-chemical nutrient flux rates

The NH_4^+ fluxes in the treatment group were significantly higher (One-way ANOVA, $p<0.05$) and threefold higher than that in the control group (Fig. 3a). More precisely, ammonium flux rates ranged from 393.1±19.21 to 802.0±28.05 and from 107.2±16.4 to 311.6±29.1 µM m^{-2} h^{-1} for the treatment and the control ponds respectively. The PO_4^{3-} fluxes differed significantly between the treatments (One-way ANOVA, $p<0.05$) and fluxes ranged between 11.6±1.93 - 19.7±0.83 and 18.33±1.18 - 27.19±2.65 µM m^{-2} h^{-1} for the treatment and control groups respectively (Fig. 3b). The highest fluxes were measured in August which was three times higher than the control (Fig. 3b), equivalent to 52%. The nitrate plus nitrite (NO_3^-+NO_2^-) also differed significantly ($p<0.05$) and ranged from 15.05±1.04 - 23.38±1.06 and 8.56±0.52 - 13.64±1.3 µM m^{-2} h^{-1} for the treatment and the control ponds respectively (Fig. 3c). The highest NO_3^- + NO_2^- fluxes were recorded in August which was about two-fold higher in the treatment over the control (Fig. 3c). Furthermore, the NO_3^-+NO_2^- had the least efflux rate of all.

Sediment oxygen consumption (SOC)

The rate of sediment oxygen consumption (SOC) varied significantly (ANOVA, $p<0.05$, Tukey's *post hoc* test and ranged between 2.06 - 5.3 and 5.6 - 7.13 O_2 mM m^{-2} h^{-1} for control and treatment respectively (Fig. 4). During the study period, SOC showed an increasing trend in all the sampling time (June - August) and varied significantly. The SOC was 3 times higher in the treatment group than in the control. Moreover, the oxygen uptake was positively correlated with the sampling time and had the highest activity (upto 7.13 mM m^{-2} h^{-1}) recorded in August.

Discussion

Water quality and sediment characteristics

Oxygen is a major component for the overall nutrient cycling. Oxygen and organic matter (OM) are produced *via* photosynthesis and are consumed during respiration and remineralisation of OM by benthic macro-invertebrates in the water column and sediments (Bertics and Ziebis, 2009). Bivalves such as clams can influence their environment through bioturbation, feeding and biodeposits (Zhao *et al*., 2019; Nicholaus *et al*., 2019). These properties alter sediment silt, oxygen level, organic materials and water content. They can likewise influence sediment stability, change nutrient dynamics and modify the composition of benthic communities (Lukwambe *et al*., 2018). In this study, the water quality parameters measured showed typical characteristic of a healthy aquaculture pond ecosystem (Table 1) which could be attributed to the roles contributed by the clams in the sediments.

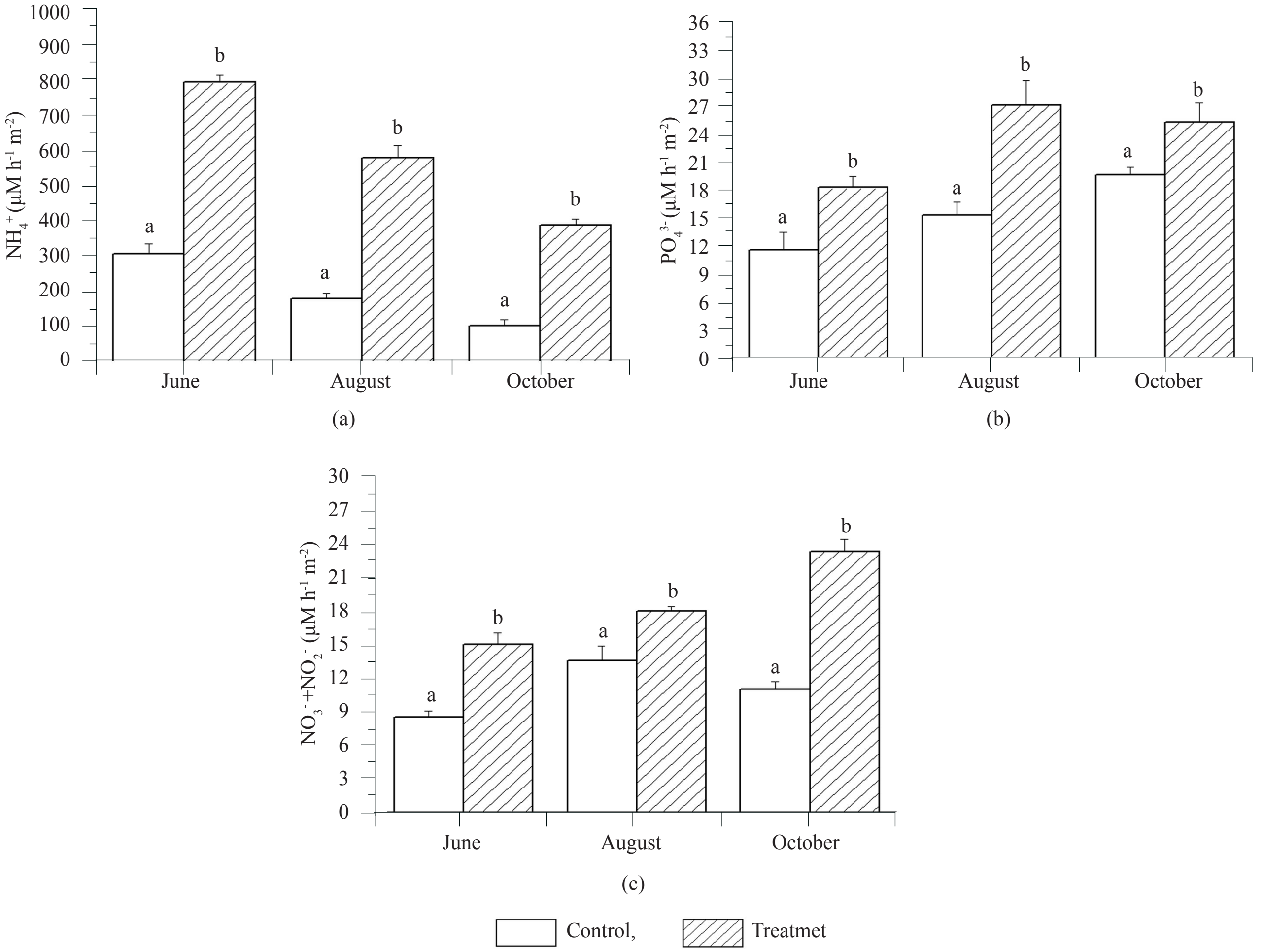


Fig. 3. Average nutrients fluxes (±SE, n=3) across the sediment water-interface between the shrimp-clam cultured pond and the shrimp (non-clam) cultured pond during the experiment. (a) Ammonium, (b) Phosphates and (c) Nitrate plus nitrite. Means not sharing a common alphabet are significantly different ($p<0.05$)

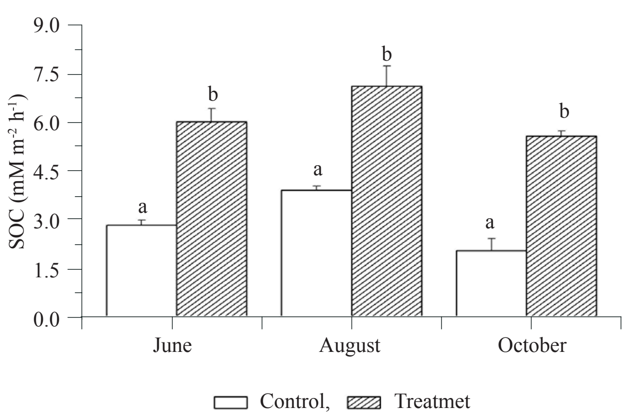


Fig. 4. Sediment oxygen consumption (SOC) rates recorded in the treatment and the control ponds during the experimental period. Data expressed as means±SE, n=3. Means that are not sharing a common alphabet are significantly different ($p<0.05$)

Sediment cores from the treatment group had significantly higher OM contents and Chl-*a* concentrations (Figs. 2a, b). Incorporation of *C. sinensis* in the culture system was found to increase the OM content three-times compared to the control (Fig. 2a). The increased sediment organic matter suggests that *C. sinensis*, probably improved the removal rate of suspended materials in the water column and increased burial of OM into the sediments. The intense filtration activity of the clams in the pond water column predicted by the sediment Chl-*a* (Fig. 2a) could count for that increase. As the filtration process tends to be intense, higher deposition rate by the clams into the sediments through their faeces and pseudofaeces might result in greater OM content. Similar observations on Asian clam *Corbicula fluminea* were reported showing an increased amount of organic matter in the sediments due to biodeposition *i.e.* faeces and pseudofaeces (Hakenkamp and Palmer, 1999). Similarly, accumulation of organic rich sediments had been previously reported for this kind of aquaculture (Christensen *et al*., 2003).

The concentration of sediment Chl-*a* was significantly influenced by the presence of *C. sinensis* and tended to decrease with depth, however, the degree of decrease was not consistent (Fig. 2b). In most cases, Chl-*a* content measured in the treatment sediment cores had high concentrations. Venus clam bioturbation activities are predicted to have accelerated and enhanced the biodeposition of Chl-*a* deep in the sediments. Bioturbation can increase the burial of sunken plankton particles down the sediment. Additionally, the greater surface area resulted from the clam burrows, would have created and improved biofilms in the benthic sediments. The study by Beutel (2006), reported burrowing in the sediment created greater space for Chl-*a* deposition into the sediment from the water column. In this study, *C. sinensis* increased OM input into the sediment and that algal pigment content may be an appropriate indicator of OM in sediments. Along with this, a substantial addition of phosphorus into the system was observed (Table 2), suggesting that continued clam farming in monoculture or polyculture pond system can be a solution for scarce phosphorous in the aquaculture pond bottom.

Benthic-pelagic nutrient flux

Present study suggests that clam bioturbation greatly influenced the physico-chemical properties and biogeochemistry of the benthic sediments. Sediment samples from the treated ponds had higher nutrient release than the control group suggesting that *C. sinensis* in all likelihood stimulated nutrients regeneration under improved oxygen levels *via* increased burrows and ventilation rates. NH_4^+ and PO_4^{3-} fluxes were generally higher except for nitrate plus nitrite (NO_3^-+NO_2^-) which was slightly low (Fig. 3 a-c). These observations may be explained by the enhanced mineralisation of clam bio-deposits which are rich in nitrogen and phosphorus. *C. sinensis* can cause pond soil perturbation and cracks that might have increased the transfer of oxygenated overlying water into the sediment and improved the efficient food web recycling and nutrient release. Similar results by Nicholaus and Zheng (2014) showed that *C. sinensis* constructed burrows and created bio-irrigation activities in the sediments, which improved oxygen flow.

Increased oxygen consumption as a result of resuspension may lead to the extension of anoxic/suboxic bottom waters with subsequently increased benthic flux of NH_4^+ and PO_4^{3-} (Almroth *et al*., 2009; Nicholaus and Zheng, 2014). Similarly, higher sediment NH_4^+ and PO_4^{3-} flux is generally observed *in situ* at bivalve farms (Christensen *et al*., 2003; Richard *et al*., 2007a). *C. sinensis* can cause bio-turbational resuspension of sediment into the water column that plays a vital role in mediating both physical and chemical processes near the sediment-water interface (Nicholaus and Zheng, 2014). Direct faeces and pseudo-faeces excreted by the clams tend to increase the OM content in the benthic sediment (Fig. 2a). Dalsgaard and Thamdrup (2002) reported that the addition of OM into the sediment promoted the rate of OM mineralisation and caused a significant increase in the rate of NH_4^+ production by 102%. Moreover, the overall increased NH_4^+ and PO_4^{3-} effluxes (Fig. 3a, b) is an indicative measure of improved oxygenation in the shrimp-clam treated ponds, which directly increased the nutrient interaction between the sediment and the overlying water. Furthermore, these data correspond to the results of Sundby *et al*. (1992), who found that the release rate of phosphate and total nitrogen from sediments had a sharp increase under anoxic conditions.

Benthic nitrogen fluxes are partly influenced by microbial processes like nitrification and denitrification among other factors (Jansen *et al*., 2012; Nicholaus *et al*., 2019a; Lukwambe *et al*., 2019). The effluxes of NO_3^- + NO_2^- in this study varied significantly and appeared marginal as compared to NH_4^+ and PO_4^{3-} fluxes. In similar patterns, the effluxes were higher in the cores collected from the pond that cultured both shrimp and clam. Contrary to other studies, the NO_3^-+NO_2^- results showed relatively higher effluxes. This suggests potential influences towards the nitrification process over denitrification due to the *C. sinensis* benthic-sediment reworking activities in the pond ecosystem. The bio-deposits from the clams *i.e.* faeces/pseudofaeces may elucidate the significant increase in sediment nitrate release to the overlying water (Fig. 3c). The nitrification activities associated with macrofauna contribute to overall benthic nitrification rates aided by the efficient solute exchange between the biofilms (Welsh and Castadelli, 2004). Previous studies have shown that burrows contain equal and at certain depths, greater numbers of nitrite oxidising bacteria compared to the sediment surface and extend the oxic-anoxic interface to deeper sediment layers (Laverock *et al*., 2011).

On the other hand, NO_3^-+NO_2^- fluxes are formed in sediment only under well oxygenated conditions by nitrifying bacteria through the nitrification process (Rittenberg *et al*., 1955). If the surficial sediments contain sufficient oxygen, then aerobic nitrifying bacteria can oxidise nitrogen compounds within the bio-deposits to NO_3^- and NO_2^- (Newell, 2004). The likely oxygen induced mineralisation due to *C. sinensis* sediment reworking may play a major role in controlling nutrient fluxes over the sediment-water interface in the treated zone. This result is also in accordance with the laboratory experiment by Nicholaus and Zheng (2014) that showed high NO_3^- flux rates in a highly oxygen improved sediments by *C. sinensis*. Moreover, the NO_3^-+NO_2^- effluxes could also be explained by the higher OM content in the clam sediments (Fig. 2a) which is considered to be the main source for nutrient supply in sediments.

Sediment oxygen consumption (SOC)

Sediment oxygen consumption (SOC) is widely studied to determine benthic metabolism and organic matter mineralisation (Glud, 2008) and can greatly exceed its consumption in the water column, especially in shallow waters (Eldridge and Morse, 2008; Sohma *et al*., 2008). Oxygen typically penetrates only a few millimeters into the aquatic sediments owing to rapid consumption during OM degradation (Gundersen and Jørgensen, 1990). The depth of oxygen penetration can be increased *via* bioturbation and bioirrigation (Bertics and Ziebis, 2009; Nicholaus and Zheng, 2014), sediment permeability and increased bottom water flow velocity. In this study, oxygen consumption varied significantly (Fig. 4) and about 3 times higher in the treatment group than the control group with peak activity of upto 7.13 mM m^{-2} h^{-1} being measured in August. The SOC results suggest the presence of improved sediment reworking activities and high organic matter degradation in the clam pond. Boucher *et al*. (1994) reported that higher OM, aerobic decomposition and mineralisation in the sediments led to high SOC. Nicholaus and Zheng (2014) showed that SOC increased greatly in the sediment cores treated with *C. sinensis*. Similarly, Zhang *et al*. (2011) reported that bioturbation by Asian clam *C. fluminea* increased oxygen uptake into the sediment. The enhanced sediment mixing by the deposit-feeding habit of *C. sinensis* could promote oxygen penetration into the sediments, a mechanism that may stimulate microbial metabolism and increase the oxygen demand. Increased NH_4^+ concentration (Fig. 3b) further fuel the declines in oxygen as ammonia gets transformed to NO_3^- in the water column by nitrification. Furthermore, higher OM content (Fig. 2a) implies that more SOC is required for its degradation by the microbes and microbial chemical processes created therein, thus high oxygen influxes.

Results of the present study suggest that *C. sinensis* significantly impacted the sediment characteristics, benthic nutrient fluxes and SOC. *C. sinensis* might have accelerated the inorganic nutrients mineralisation, improved nutrients recycling and stimulated primary production within the shrimp-clam cultured zone. Thus, the present study concludes that Venus clam (*C. sinensis*) plays an important functional role in the ecology of the pond ecosystem and could be used to counteract nutrients deficiency in the water column and benthic aquatic system of finfish and shellfish farms. Therefore it is suggested that shrimp-clam polyculture system may be encouraged for sustainable and environmentally responsible aquaculture.

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References

Almroth, E., Tengberg, A., Andersson, J. H., Pakhomova, S. and Hall, P. O. J. 2009. Effects of resuspension on benthic fluxes of oxygen, nutrients, dissolved inorganic carbon, iron and manganese in the Gulf of Finland, Baltic Sea. *Cont. Shelf Res*., 29(5-6): 807-818.

APHA 2012. *Standard methods for the examination of water and wastewater*, 22nd edn. American Public Health Association, American Water Works Association (AWWA) and Water Environment Federation (WEF), Washington, D.C., USA.

Bartoli, M., Nizzoli, D., Viaroli, P., Turolla, E., Castaldelli, G., Fano, E. A. and Rossi, R. 2001. Impact of *Tapes philippinarum* farming on nutrient dynamics and benthic respiration in the Sacca di Goro. *Hydrobiol*., 455: 203-212.

Batzer, D. P., McGee, M., Resh, V. H. and Smith, R. R. 1993. Characteristics of invertebrates consumed by mallards and prey response to wetland flooding schedules. *Wetlands*, 13: 41-49.

Baudinet, D., Alliot Berland, E., Grenz, B. C., Plante-Cuny, M. R., Plante, R. and Salen-Picard, C. 1990. Incidence of mussel culture on biogeochemical fluxes at the sediment-water interface. *Hydrobiol*., 207: 187-196.

Bertics, V. J. and Ziebis, W. 2009. Biodiversity of benthic microbial communities in bioturbated coastal sediments is controlled by geochemical microniches. *ISME J*., 3: 1269-1285. DOI: 10.1038/ismej.2009.62.

Beutel, M. W. 2006. Inhibition of ammonia release from anoxic profoundal sediments in lakes using hypolimnetic oxygenation. *Ecol. Engg.,* 28(3): 271-279. DOI: 10.1016/j.ecoleng.2006.05.009.

Boucher, G., Clavier, J. and Garrigue, C. 1994. Oxygen and carbon dioxide fluxes at the water-sediment interface of a tropical lagoon. *Mar. Ecol. Prog. Ser.,* 10: 185-193.

Christensen, P. B., Glud, R. N., Daalsgaard, T. and Gillespie, P. 2003. Impacts of longline mussel farming on oxygen and nitrogen dynamics and biological communities of coastal sediments. *Aquaculture,* 218: 567-588. https://doi.org/10.1016/S0044-8486(02)00587-2.

Cowan, J. M. and Boynton, W. R. 1996. Sediment-water oxygen and nutrient exchanges along the longitudinal axis of

Chesapeake Bay: seasonal patterns, controlling factors and ecological significance. *Estuaries*, 19(3): 562-580.

Dalsgaard, T. and Thamdrup, B. 2002. Factors controlling anaerobic ammonium oxidation with nitrite in marine sediments. *Appl. Environ. Microbiol*., 68: 3802-3808. doi: 10.1128/AEM.68.8.3802-3808.2002.

Eldridge, P. and Morse, J. W. 2008. Origins and temporal scales of hypoxia on the Louisiana shelf: Importance of benthic and subpycnocline water metabolism, *Mar. Chem*., 108: 159-171. DOI: 10.1016/j.marchem.2007.11.009.

Engelsen, A., Hulth, S., Pihl, L. and Sundbäck, K. 2008. Benthic trophic status and nutrient fluxes in shallow-water sediments. *Estuar. Coast. Shelf Sci*., 78: 783-795. DOI: 10.1016/j.ecss.2008.02.018.

Garcia-Robledo, E., Bohorquez, J., Corzo, A., Jimenez-Arias, J. L. and Papaspyrou, S. 2016. Dynamics of inorganic nutrients in intertidal sediments: Porewater, exchangeable and intracellular pools. *Front. Microbiol*., 7: 761. https://doi.org/10.3389/fmicb.2016.00761.

Glud, R. N. 2008. Oxygen dynamics of marine sediments, *Mar. Biol. Res*., 4: 243-289. https://doi.org/10.1080/17451000801888726.

Gundersen, J. K. and Jørgensen, B. B. 1990. Micro structure of diffusive boundary layer sand the oxygen uptake of the seafloor. *Nature,* 345: 604-607.

Hakenkamp, C. C. and Palmer M. A. 1999. Introduced bivalves in freshwater ecosystems: the impact of Corbicula on organic matter dynamics in a sandy stream, *Oecologia*, 119: 445-451.

Jansen, H. M., Verdegem, M. C. J., Strand, O. and Smaal, A. C. 2012. Seasonal variation in mineralisation rates (C-N-P-Si) of mussel *Mytilus edulis* biodeposits. *Mar. Biol*., 159: 1567-1580. doi: 10.1007/s00227-012-1944-3.

Kemp, W. M. and Boynton, W. R. 1992. Benthic-pelagic interactions: Nutrients and oxygen dynamics. In: Smith, D.E. and Leffler Mackiernan (Eds.), Oxygen dynamics in the Chesapeake Bay: A synthesis of recent research. Maryland Sea Grant, College Park, Maryland, USA, p. 149-209.

Kogure, K. and Wada, M. 2005. Impacts of macrobenthic bioturbation in marine sediment on bacterial metabolic activity. *Microbes Environ*., 20: 191-199. https://doi.org/10.1264/jsme2.20.191.

Kristensen, E. and Kostka, J. E. 2005. Macrofaunal burrows and irrigation in marine sediment: microbiological and biogeochemical interactions, In: Kristensen E., Haese, R. R. and Kostka, J. E. (Eds), *Interaction between macro- and microorganisms in marine sediments*. American Geophysical Union, Washington, DC, USA, p. 125-157.

Laverock, B., Gilbert, J. A., Tait, K., Osborn, A. M. and Widdicombe, S. 2011. Bioturbation: Impact on the marine nitrogen cycle, *Biochem. Soc. Trans.*, 39: 315-320. DOI: 10.1042/BST0390315.

Longhi, D., Bartoli, M., Nizzoli, D. and Viaroli, P. 2013. Benthic processes in freshwater fluffy sediments undergoing resuspension, *J. Limnol*., 72(1): 1-12. https://doi.org/10.4081/jlimnol.2013.e1.

Lukwambe, B., Yang, W., Zheng, Y., Nicholaus, R., Zhu, J. and Zheng, Z. 2018. Bioturbation by the razor clam (*Sinonovacula constricta*) on the microbial community and enzymatic activities in the sediment of an ecological aquaculture wastewater treatment system. *Sci. Total Environ.*, 643: 1098-1107. doi: 10.1016/j.scitotenv.2018.06.251.

Lukwambe, B., Zhao, L., Nicholaus, R., Yang, W., Zhu, J. and Zheng, Z. 2019. Bacterioplankton community in response to biological filters (clam, biofilm, and macrophytes) in an integrated aquaculture wastewater bioremediation system. *Environ. Pollut.*, 254: 113035.

McKindsey, C. W., Anderson, M. R., Barnes, P., Courtenay, S., Landry, T. and Skinner, M. 2006a. Effects of shellfish aquaculture on fish habitat. Canadian Science Advisory Secretariat, Fisheries and Oceans Canada, Mont-Joli, Quebec, Canada. *Ecology*, 82: 3463-3479.

Michaud, E., Desrosiers, G., Mermillod-Bondin, F., Sundby, B. and Stora, G. 2006. The functional group approach to bioturbation: II, The effects of the *Macoma balthica* community on fluxes of nutrients and dissolved organic carbon across the sediment-water interface. *J. Exp. Mar. Biol. Ecol*., 337: 178-189.

Mudroch, A., Azcue, J. M. and Mudroch, P. 1997. *Manual of physicochemical analysis of aquatic sediments*. CRC Press Inc., Boca Raton, Florida, USA.

Newell, R. I. E. 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. *J. Shellfish Res*., 23: 51-61.

Nicholaus, R. and Zheng Z. M. 2014. The effects of bioturbation by the Venus clam *Cyclina sinensis* on the fluxes of nutrients across the sediment-water interface in aquaculture ponds. *Aquac. Int*., 22(2): 913-924. DOI: 10.1007/s10499-013-9716-8.

Nicholaus, R., Lukwambe, B., Zhao, L., Yang, W., Zhu, J. and Zheng, Z. 2019a. Bioturbation of blood clam *Tegillarca granosa* on benthic nutrient fluxes and microbial community in an aquaculture wastewater treatment system. *Int. Biodeterior. Biodegr.,* 142**:** 73-82.

Nicholaus, R., Lukwambe, B., Lai, H., Yang, W. and Zheng, Z. 2019. Nutrients cycling in ecological aquaculture wastewater treatment systems: Vertical distribution of benthic phosphorus fractions due to bioturbation activity by *Tegillarca granosa. Aquac. Env. Inter.,* 11: 469-480. https://doi.org/10.3354/aei00328.

Riaux-Gobin, C., Lewellyn, C. A. and Klein, B. 1987. Microphytobenthos from two subtidal sediments from North Brittany. Variations of pigment compositions and concentrations determined by HPLC and conventional techniques. *Mar. Ecol. Prog. Ser*., 40: 275-283.

Richard, M., Archambault, P., Thouzeau, G. and Desrosiers, G. 2007a. Summer influence of 1- and 2-yr-old mussel cultures on benthic fluxes in Grande-Entree Lagoon, iles-de-la-Madeleine (Quebec, Canada). *Mar. Ecol. Prog. Ser.,* 338: 131-143. DOI: 10.3354/meps338131.

Rittenberg, S., Emery, K. and Wilson, L. 1955. Regeneration of nutrients in sediments of marine basins. *Deep Sea Res*., 3: 23-45.

Rosarie, D. M. F., Seronay, R. A. and Jumawan, J. C. 2015. Aquatic macroinvertebrate diversity and physico-chemical characteristics of freshwater bodies in Tubay, Agusan Del Norte, Philippines, *JEZS*, 3(5): 440-446.

Sloth, N. P., Blackburn, H., Hansen, L. S., Risgaard-Petersen, N. and Lomstein, B. A. 1995. Nitrogen cycling in sediments with different organic loading. *Mar. Ecol. Prog. Ser*., 116: 163-170.

Sohma, A., Sekiguchi, Y., Kuwae, T. and Nakamura, Y. 2008. A benthic pelagic coupled ecosystem model to estimate the hypoxic estuary including tidal flat - Model description and validation of seasonal/daily dynamics, *Ecol. Model*., 215: 10-39.

Song, G. D., Liu, S. M., Marchant, H., Kuypers, M. M. M. and Lavik, G. 2013. Anammox, denitrification and dissimilatory nitrate reduction to ammonium in the East China Sea sediment. *Biogeosci.,* 10: 6851-6864.

Sundby, B., Gobeil, C., Silverberg, N. and Muggi, A. 1992. The phosphorus cycle in coastal marine sediments. *Limnol. Oceanogr*., 37: 1129-1141.

Volkenborn, N., Hedtkamp, S. I. C., van Beusekom, J. E. E. and Reise, K. 2007. Effects of bioturbation and bioirrigation by lugworms (*Arenicola marina*) on physical and chemical sediment properties and implications for intertidal habitat succession. *Estuar. Coast. Shelf Sci*., 74(1-2): 331-343. https://doi.org/10.1016/j.ecss.2007.05.001.

Wallace, J. B. and Webster, J. R. 1996. The role of micro-invertebrates in stream ecosystem. *Annu. Rev. Entomol*., 41: 115-139.

Welsh, D. T. and Castadelli, G. 2004. Bacterial nitrification activity directly associated with isolated benthic marine animals, *Mar. Biol*., 144: 1029-1037. DOI: 10.1007/s00227-003-1252-z.

Zhang, L., Liao, Q., He, W., Shang, J. and Fan, C. 2013. The effects of temperature on oxygen uptake and nutrient flux in sediment inhabited by molluscs, *J. Limnol*., 72(1): e2 DOI:10.408/1jlimnol.e2.

Zhang, L., Shen, Q. S., Hu, H. Y., Shao, S. G. and Fan, C. X. 2011. Impacts of *Corbicula fluminea* on oxygen uptake and nutrient fluxes across the sediment-water interface. *Water Air Soil Pollut*., 220: 399-41.

Zhao, L., Zheng, Y., Nicholaus, R., Lukwambe, B., Zhu, J., Yang, W. and Zheng, Z. 2019 Bioturbation by the razor clam *Sinonovacula constricta* affects benthic nutrient fluxes in aquaculture wastewater treatment ecosystems: *Aquac. Env. Inter.,* 11: 87-96. DOI :10.3354/aei00298.

Zheng, Z. M., Lv, J., Lu, K., Jin, C., Zhu, J. and Liu, X. 2011. The impact of snail (*Bellamya aeruginosa*) bioturbation on sediment characteristics and organic carbon fluxes in a eutrophic pond. *Clean-Soil Air Water*, 0: 1-6.

Zhu, Q., Aller, R. C. and Fan, Y. 2006. Two-dimensional pH distributions and dynamics in bioturbated marine sediments. *Geochim. Cosmochim. Acta*, 70(19): 4933-4949. DOI: 10.1016/j.gca.2006.07.033.

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