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Bioturbation by the razor clam *Sinonovacula constricta* affects benthic nutrient fluxes in aquaculture wastewater treatment ecosystems

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ABSTRACT: The rapid development of the aquaculture industry has led to the growth of environmental problems. Bioremediation is an effective method for treating aquaculture effluents. A 30 d laboratory experiment was conducted to investigate the effects of *Sinonovacula constricta* bioturbation on sediment and nutrient fluxes across the sediment–water interface in aquaculture wastewater treatment ecosystems. The experiment was designed with 3 treatment groups and 1 control with 3 replicates each. The rates of sediment oxygen consumption (SOC) and the physical and chemical properties of the sediment and nutrient fluxes (NH_4^+, NO_3^- + NO_2^- and PO_4^{3-}) were determined. After the experiment, the levels of total organic nitrogen, total organic carbon, and total phosphorus in the surficial sediment of the treatment groups had declined significantly, and the alkaline phosphatase content and microbial activity had increased significantly ($p < 0.05$). SOC rate in all treatment groups was significantly higher than that in the control group before Day 24 and increased with clam density (no. of clams per unit area) ($p < 0.05$); in most treatment groups, nutrients (NH_4^+, NO_3^- + NO_2^- and PO_4^{3-}) were released from the sediment into the overlying water, and the rate of release increased with clam density and time. The results obtained in this study revealed that bioturbation by *S. constricta* reduced organic matter accumulation and accelerated the mineralization of organic matter in the sediments, which may promote nutrient recycling in aquaculture wastewater treatment ecosystems and eventually bioremediate aquaculture effluents.

KEY WORDS: *S. constricta* · Effluent · Bioremediation · Nitrate/nitrite · Sediment–water interface

1. INTRODUCTION

The rapid development of the aquaculture industry has been accompanied by an increase in environmental impacts. Intensive aquaculture production generates increasingly substantial amounts of polluted effluent (Ziemann et al. 1992, Hopkins et al. 1993), resulting in eutrophication and environmental deterioration of the receiving waters. As a consequence, many efforts have been made to find an effective treatment to minimize the negative effects of aquaculture activities (Marinho-Soriano et al. 2009, Jones et al. 2001). There are currently 3 main treatment methods for aquaculture wastewater: physical, chemical and bioremediation (Gupta & Ali 2012). Among them, bioremediation is thought to be the most effective and environmentally friendly method due to its advantages of low cost, simple operation

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and high security (MacDonald & Rittman 1993). At present, one of the popular effluent treatments in China is an integrated method in which bioremediation is crucial (Bu et al. 2003). The treatment process includes natural sedimentation, aeration, shellfish filtering and macroalgae adsorption (Jones et al. 2001, Qin et al. 2017, Lukwambe et al. 2018). The organic matter of aquaculture effluent can be transformed into inorganic nutrients that can be utilized as a rich source of nutrients for phytoplankton under the action of aeration (Granéli et al. 1999). Phytoplankton is recognized as a rapidly growing plant that can quickly absorb nutrients. In the shellfish filtering area, bioturbation by benthic shellfish may enhance the mineralization of organic matter; the release of nutrients from the sediment significantly improves nutrient recycling in the effluent treatment ecosystem because the feeding, burrowing and other activities of the shellfish alters the physical, chemical and ecological properties of the sediment (Thayer 1979, Meysman et al. 2006, Creed et al. 2010, Nicholaus & Zheng 2014, Zheng et al. 2017). Shellfish, such as razor clams *Sinonovacula constricta* and *Tagelus plebeius* (Klerks et al. 2018), may then feed on the phytoplankton that is promoted by the released nutrients and suspended organic particulates in the water. Thus, organically enriched sediment may be bioremediated by benthic shellfish, and the released nutrients may further promote the growth of phytoplankton, which could be reabsorbed by the shellfish (Meysman et al. 2006, Tian et al. 2016, Zheng et al. 2017). Therefore, razor clams play a key role in this ecosystem, and it is important to understand the extent and mechanisms of their effects.

The razor clam *S. constricta* has become one of the most commercially important mariculture species in China in recent years, with annual production of razor clams reaching about 78 000 t (Du 2016). The clams live in muddy or sandy tidal flats along the coastal intertidal zone with a buried and burrowed habitat (Wang & Wang 2008). The polyculture of *S. constricta* with other animals is now widely implemented in the coastal areas of China and is also widely used in aquaculture wastewater treatment systems (Tian et al. 2001, Bu et al. 2003, Zheng et al. 2017, Lukwambe et al. 2018). Studies on the impact of bioturbation on nutrient flux at the sediment–water interface have been well reported thus far; e.g. Nicholaus & Zheng (2014) found that *Cyclina sinensis* could increase the fluxes of inorganic nutrients (NH_4^+, NO_3^- + NO_2^-, PO_4^{3-}) and sediment oxygen consumption (SOC) across the sediment–water interface. *Chironomus plumosus* can promote the formation of Fe/Al-P and Ca-P in sediment (Cai et al. 2017). Wrede et al. (2017) found that *Echinocardium cordatum* enhanced the fluxes of ammonium and nitrite at the sediment–water interface. There are also reports of shellfish such as *Anodonta woodiana*, zebra mussels and *Ruditapes philippinarum* being used to remediate the eutrophication in shallow lakes (Wu et al. 2018, Bagdanavičiūtė et al. 2018, Breda et al. 2018). However, there are few studies on the role of bioturbators in the ecological treatment systems for aquaculture wastewater.

The primary aims of this study were to determine the effects of bioturbation by *S. constricta* on sediment physicochemical characteristics and benthic nutrient fluxes in aquaculture wastewater treatment ecosystems. The approach included establishing a mesocosm with different *S. constricta* stocking densities in indoor areas and measuring sediment biogeochemical characteristics, such as organic N, C and P contents, alkaline phosphatase content and microbial activities. Oxygen and nutrient fluxes were investigated across the sediment–water interface under dark incubations. The results of this study will increase the knowledge of the management of aquaculture wastewater treatment and improve understanding of how clams affect nutrient cycling in wastewater treatment systems.

2. MATERIALS AND METHODS

2.1. Experimental materials

The sediment and aquaculture wastewater for this study were collected from Chun Lin Aquaculture Farm, an intensive shrimp farm (raising *Penaeus vannamei* Boone) in Ningbo, Zhejiang Province, China. The NH_4^+, NO_3^- + NO_2^- and PO_4^{3-} contents of the wastewater were 1.05, 186.8 and 0.58 mg l^{-1}, respectively. The sediment comprised 71.2% silt and clay, 25% sand and 3.8% gravel, with an average particle size of 10.77 µm. The organic matter content of the sediment was 7.37 ± 0.54%, which was measured as loss upon ignition (500°C). The sediments were sieved with 1 mm mesh prior to analysis to remove all heavy gravel, impurities and macrobiotics. Razor clam *Sinonovacula constricta* individuals (average wet weight: 5.092 g) with integrated shells and high vitality were selected from the mudflat near the farm and acclimated for 10 d before being used in the experiment. During acclimation, the clams were fed every day with 2 laboratory-cultured microalgae: *Nannochloropsis* and *Chlorella*.

2.2. Mesocosm

In the Aquatic-Ecological Laboratory of Ningbo University, a mesocosm was established using a 0.55 m^3 plastic bucket with ca. 200 l of the shrimp farm wastewater. Sieved sediments were added to 12 transparent cylindrical polypropylene (PP) chambers (14.2 cm diameter; 18.6 cm height) to form an 8 cm thick sediment layer, and each chamber was equipped with 2 sampling ports, as described in Dollar et al. (1991) and Nicholaus & Zheng (2014). The experiment was designed with 3 treatment groups and 1 control with 3 replicates each: (1) low density: 1 clam chamber^{-1} (equivalent to 63.1 ind. m^{-2}); (2) medium density: 2 clams chamber^{-1} (126.3 ind. m^{-2}); (3) high density: 4 clams chamber^{-1} (252.6 ind. m^{-2}); and (4) control: chambers with sediment and without clams. The treatment densities were set according to productive stocking density (the medium density treatment is the average stocking density on production scale). All chambers (without the lids) were placed into the mesocosm. Approximately 25 % of the total volume of wastewater in the mesocosm was replaced with fresh wastewater every day. Aeration was provided to the mesocosm every 24 h. The experiment lasted for 30 d.

Water quality parameters, including water temperature (WT), salinity (S), pH and dissolved oxygen (DO), were measured daily using a YSI-550A oxygen meter before fresh wastewater was added to the mesocosm.

2.3. Sampling and nutrient flux measurements

The rates of nutrient fluxes and SOC were determined every 6 d through dark incubation in the laboratory, as previously described by Nicholaus & Zheng (2014). Prior to dark incubations at each incubation time, all the PP-chambers were sealed with lids and carefully transferred from the mesocosm to the incubation water bath, and magnetic stirrers were used to keep the overlying water molecules evenly distributed (Fig. 1). The incubations lasted for 3 h in the water baths in darkness at *in situ* temperatures (Nicholaus & Zheng 2014). Three chambers with clams and without sediment were also incubated as controls of incubation to correct the nutrient and DO contents. The DO of the overlying water was measured using a YSI-550A oxygen meter at the beginning and end of the 3 h incubation period. For nutrient flux (NH_4^+, PO_4^{3-}, NO_3^- + NO_2^-) measurements, 45 ml of water samples were collected from a distance of 1 cm

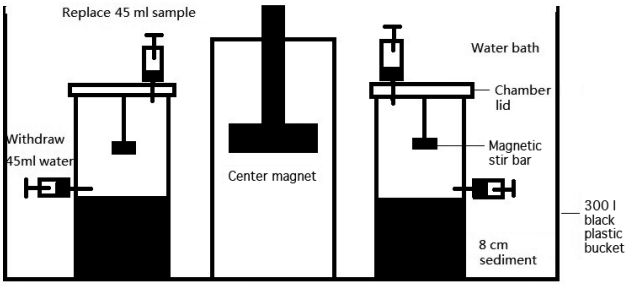

Fig. 1. Dark incubation device specifically designed for the present study

above the sediment using a syringe and needle at 1 h intervals (0, 1, 2 and 3 h). The volume of water that was withdrawn was replaced with 45 ml of fresh wastewater, to correct the concentration of nutrients in the overlying water of every chamber, which was measured simultaneously (Michaud et al. 2006). The extracted samples were immediately filtered through a 0.45 µm GF/F Whatman glass fibre filter and frozen at −20°C in HCl-washed PP cryovial tubes for later analyses of NH_4^+, PO_4^{3-} and NO_3^- + NO_2^-. After incubation, all chambers were placed back into the mesocosm, and the experiment continued. In the analysis, the indophenol blue method was used to determine ammonia, nitrate was measured with the cadmium-copper reduction method and the phosphate concentration was determined using the ammonium molybdate method. All nutrients were measured with a WESTCO SmartChem discrete analyser.

2.4. Sediment characteristics

Initial and final sediment samples were taken from chambers with sterile Plexiglas tubes. Each final sediment sample (8 cm in depth) was divided into 3 layers (0–2, 2–5 and 5–8 cm) and frozen for later analyses of total organic nitrogen (TON), total phosphorus (TP), total organic carbon (TOC), alkaline phosphatase (APA) and microbial activity (MBA). The TON and TOC contents were determined using a vario EL Cube element analyser. TON and TOC were acidified by the gas phase method before determination. The sediments were placed in a dryer with concentrated hydrochloric acid overnight and then placed in a conventional laboratory oven at 60°C to remove the excess hydrochloric acid. TP was measured with the European Commission's SMT Programme-approved method (Murphy & Riley 1962).

The sediment APA was analysed by means of p-nitrophenyl phosphate disodium (p-NPP) (Sayler et al. 1979): APA was determined spectrophotometri-

cally as the release of p-nitrophenol from the model substrate p-NPP. The reaction mixture for sediments contained 1 g sediment, 2.6 ml 0.05 M Tris-buffer pH 8.4, 0.03 ml 0.1 M $MgCl_2$ and 0.1 ml 10 mM p-NPP. Samples were incubated in a water bath at 37°C for 1 h. After that, the reaction was immediately terminated by the addition of 0.3 ml 1 M NaOH. Finally, the samples were centrifuged, the clear liquid was collected and the samples were analysed spectrophotometrically at 418 nm. The results of specific APA are reported in mg p-nitrophenol (μg g^{-1} h^{-1}). All samples were executed in triplicate.

Sediment MBA was analysed by means of fluorescein diacetate (FDA) (Battin 1997): 2 g wet sediment sample was placed into the 50 ml reaction tube; 15 ml of 60 mM phosphate buffer reagent (pH = 7.6), and 0.2 ml FDA reagent were added, at which point the reaction started. The sample was mixed uniformly at 30°C, and then shaken (100 RPM) for 20 min. No FDA substrate was added to the blank. Then, 15 ml chloroform/methanol (volume ratio 2:1) was added to the solution and it was immediately shaken uniformly to stop the reaction. The tubes were centrifuged at ~1500 × *g* for 3 min; the clear liquid was collected and put under 490 nm to measure absorbance. The units of the measured results were converted into dry weight of sediment.

2.5. Data analyses

Fluxes of dissolved inorganic nutrients (NH_4^+, NO_3^- + NO_2^- and PO_4^{3-}) were calculated from the slope of a linear regression of concentration against time (Michaud et al. 2006). The SOC rate was calculated according to the equation in Dollar et al. (1991):

$$F = \frac{\Delta C \cdot V}{A \cdot t} \quad (1)$$

where F is the SOC flux (mg m^{-2} d^{-1}), ΔC is the change in the concentration of oxygen (mg l^{-1}) before and after incubation, V is the volume of overlying water (m^3), A is the cross-sectional area (m^2) of the incubation chamber and t is the experiment duration (days).

APA and MBA were calculated using the following formulae:

$$R_{APA} = C \times V / (M \times T) \quad (2)$$

$$R_{MBA} = C_f \times V / (M \times T) \quad (3)$$

where R_{APA} represents sediment alkaline phosphatase activity (μg g^{-1} h^{-1}), R_{MBA} represents sediment microbial activity (μg g^{-1} min^{-1}), C represents nitrophenol concentration (g ml^{-1}), C_f (fluorescein concentration) represents MBA activity (g ml^{-1}), V is the volume of the reaction system (ml), M is the reaction mass of the sediment samples (g, as benchmark calculated by dry weight) and T is the incubation time (min).

All data were analysed using SPSS v.18.0 software. Data were compared with 1-way ANOVA, followed by Tukey's multiple range tests for post hoc comparisons at a significance level of 0.05. Prior to statistical analyses, raw data were first processed in Microsoft Excel 2003, and the percentage data (including TOC, TON, TP content) were arcsine-transformed before comparison analysis. Normal distribution of the data and homogeneity of variances among treatments were verified before the ANOVA was carried out. The figures were created with OriginPro v.8.0 software.

3. RESULTS

3.1. Visual observations and water quality in the mesocosm

When razor clams were added to the chambers, they began to plunge into the sediment quickly and destroyed the initial sediment surface, leaving tubular channels. During the experiment, the clams seldom occurred on the sediment surface, but their siphons occasionally extended above the sediment surface. The siphon holes were sometimes covered by sediment, making them invisible. At the end of the experiment, the sediment surface of the control group remained smooth and had a thin oxidation zone that was approximately 0.85 cm deep, as revealed by the brown colour of the sediment. The clams extended the brown-black oxidation zone to a depth of approximately 5–8 cm, which suggests that bioturbation stimulated the formation of the oxidized zone due to dissolved oxygen from the overlying water being brought into the sediments by the clams.

The temperature and salinity in the mesocosm ranged from 23.4 to 29.0°C and from 23.1 to 26.9 ppt, respectively. Both increased with the experiment time. The pH was 8.60 ± 0.13, and the DO was greater than 4.90 mg l^{-1}. The NH_4^+-N, (NO_3^- + NO_2^-)-N and PO_4^{3-}-P were 1.05 ± 0.25, 18.68 ± 6.72 and 0.58 ± 0.11 mg l^{-1}, respectively.

3.2. Sediment characteristics

TON and TOC contents in the sediment ranged from 0.358 to 0.416% dry weight (DW) and 1.028 to

1.204 % DW, respectively, throughout the 30 d experiment. ANOVA results showed that TON and TOC in the surface sediment (0–2 cm layer) were significantly affected by clam density (ANOVA, $F_{3,8}$ = 10.554, p = 0.004 [TON]; $F_{3,8}$ = 4.863, p = 0.033 [TOC]). However, there were no clear differences in sediment in the 2–5 or 5–8 cm layers among the treatment groups (p = 0.086 and 0.068 for TON at 2–5 and 5–8 cm, respectively; p = 0.591 and 0.710 for TOC at 2–5 and 5–8 cm, respectively). The mean values of TOC and TON in the 3 treatments with clams (low, medium and high densities) were significantly lower than those in the control groups on the sediment surface (0–2 cm layer) (Tukey's HSD, p < 0.05; Table 1). The values of TP in the surface and bottom sediments were more variable with significant differences among groups, and these values decreased significantly with increasing clam density (ANOVA, F = 5.77, p = 0.021; Table 1). The final MBA and APA contents of the sediment were about 3–4 times those values before the trial, and these contents in the surface and bottom sediments were significantly higher in the high-density treatment than those in the low-density treatment and control group (ANOVA, $F_{3,8}$ = 112.198, p < 0.001 [0–2 cm], $F_{3,8}$ = 5.617, p = 0.023 [5–8 cm] for MBA; ANOVA, $F_{3,8}$ = 14.508, p = 0.01 [0–2 cm], $F_{3,8}$ = 4.570, p = 0.038 [5–8 cm] for APA; Tukey's HSD, p < 0.05); however, the values in the middle sediments (2–5 cm) of all groups did not change significantly (ANOVA, $F_{3,8}$ = 0.750, p = 0.552 for MBA; ANOVA, $F_{3,8}$ = 3.529, p = 0.068 for APA).

3.3. SOC

SOC rates ranged from 41.43 to 79.23 mmol m^{-2} d^{-1}. There were significant differences in the SOC among treatments on all sampling days (2-way ANOVA, $F_{3,40}$ = 100.99, p < 0.001). Oxygen fluxes across the sediment–water interface increased with increasing density from the beginning to Day 24. However, at the end of the experiment (on Day 30), the SOC in the medium-density treatment was significantly lower than that in the control and other treatments (Fig. 2).

3.4. Nutrient fluxes

During the experiment, ammonium showed a release flux in all chambers, with the exception of the low-density group on Day 6 (Fig. 3A). The efflux of ammonium increased significantly with increases in density and from Day 6 to Day 24 (2-way ANOVA, $F_{3,40}$ = 23.672, p < 0.001 for density; $F_{4,40}$ = 37.430, p < 0.001 for time; Fig. 3A). The fluxes changed significantly with time; e.g. the nutrient fluxes of (NO_3^- + NO_2^-)-N and PO_4^{3-}-P in the control groups changed significantly and most of them showed an increasing trend with time. There was a decreasing trend in the fluxes of all nutrients after Day 18 in the high-density group; the NH_4^+-N fluxes of all treatments increased with time before Day12. On Day 6, the nitrate + nitrite was absorbed by the sediments of each cham-

Table 1. Total organic nitrogen (TON), total organic carbon (TOC), total phosphorus (TP), microbial activity (MBA) and alkaline phosphatase (APA) in the sediments of different treatments (razor clam density levels) at different sediment depths. Data are means ± SD (n = 3). Different superscript letters indicate differences between treatments at the same depth (Tukey's HSD, p < 0.05); no letters indicate no significant difference between treatments at the same depth

Sediment layer (cm)	Treatment	TON (%)	TOC (%)	TP (%)	MBA (μg g^{-1} min^{-1})	APA (μg g^{-1} h^{-1})
Beginning of experiment						
Fresh sediment		0.358 ± 0.005	1.151 ± 0.016	0.128 ± 0.001	4.946 ± 0.211	23.242 ± 2.039
End of experiment						
0–2	Control	0.409 ± 0.004^{b}	1.204 ± 0.017^{b}	0.112 ± 0.002^{b}	13.372 ± 0.690^{a}	103.993 ± 7.703^{a}
	Low	0.380 ± 0.011^{ab}	1.150 ± 0.047^{ab}	0.108 ± 0.009^{ab}	17.318 ± 0.755^{b}	94.900 ± 4.191^{a}
	Medium	0.358 ± 0.009^{a}	1.081 ± 0.056^{a}	0.086 ± 0.023^{ab}	17.504 ± 0.381^{b}	94.762 ± 4.142^{a}
	High	0.382 ± 0.048^{a}	1.097 ± 0.046^{ab}	0.071 ± 0.014^{a}	23.815 ± 0.896^{c}	117.468 ± 0.830^{b}
2–5	Control	0.383 ± 0.009	1.049 ± 0.028	0.113 ± 0.032	10.973 ± 4.906	78.881 ± 4.807
	Low	0.376 ± 0.005	1.096 ±0.025	0.090 ± 0.009	12.248 ± 3.736	65.875 ± 8.630
	Medium	0.416 ± 0.032	1.060 ± 0.053	0.093 ± 0.010	11.547 ± 0.493	81.819 ± 9.519
	High	0.423 ± 0.031	1.118 ± 0.112	0.094 ± 0.034	15.530 ± 5.306	84.893 ± 7.043
5–8	Control	0.367 ± 0.009	1.079 ±0.040	0.132 ± 0.031^{b}	9.076 ± 2.783^{a}	64.901 ± 2.821^{a}
	Low	0.370 ± 0.009	1.100 ± 0.022	0.097 ± 0.005^{ab}	11.992 ± 1.943^{a}	75.132 ± 5.454^{ab}
	Medium	0.415 ± 0.016	1.028 ± 0.024	0.096 ± 0.004^{ab}	10.844 ± 0.403^{a}	76.855 ± 2.479^{ab}
	High	0.395 ± 0.036	0.996 ± 0.057	0.085 ± 0.014^{a}	17.615 ± 4.170^{b}	79.316 ± 7.848^{b}

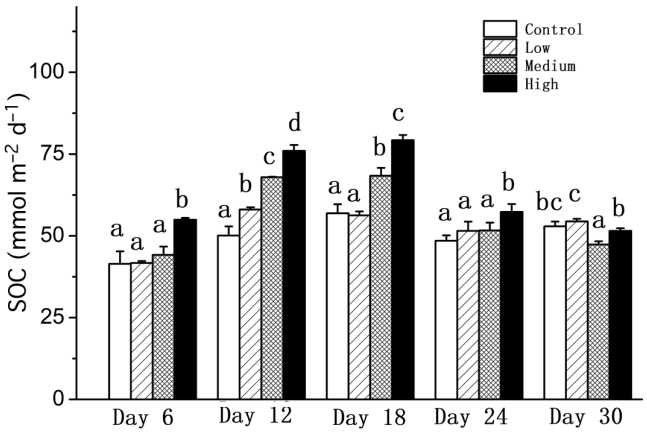

Fig. 2. Sediment oxygen consumption (SOC) rates determined in the razor clam experimental chambers. Data are means + SD (n = 3). Different letters indicate significant differences between treatments on the same experimental days (Tukey's HSD, $p < 0.05$)

ber; the nitrite + nitrate fluxes of the different density treatments began to release from the sediment to the overlying water beginning on Day 12, whereas this occurred in the control group after Day 24 (Fig. 3B). The efflux rate of nitrite + nitrate increased significantly with the increase in clam density in the chambers and showed higher effluxes in the 3 density treatments than those in the control from Day 12 to Day 24 (2-way ANOVA, $F_{3,40} = 182.353$, $p < 0.001$ [density]; Tukey's HSD, $p < 0.05$; Fig. 3B). Phosphate levels of the control group showed uptake at all incubation times, and the influx rates presented a declining trend with experimental time. However, the phosphate fluxes of all 3 density treatments were released from the sediments on all days except Day 6 (Fig. 3C). The effluxes from the treatment chambers were higher than those in the control (2-way ANOVA, $F_{3,40} = 108.054$, $p < 0.001$ [density]; Tukey's HSD, $p < 0.05$; Fig. 3C). The efflux rates in the treatment chambers were higher than those in the control chambers from Day 6 to Day 24 (Tukey's HSD, $p < 0.05$). The efflux rates of the medium-density treatment were significantly higher than those of the other treatments on Day 30 (Tukey's HSD, $p < 0.05$).

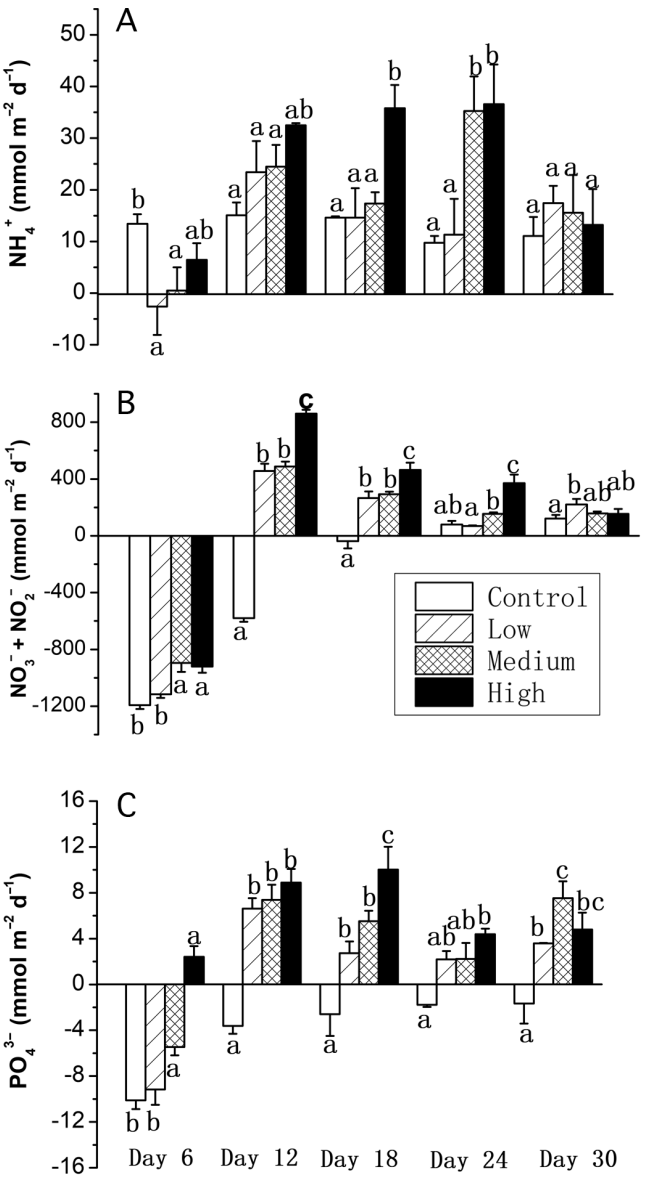

Fig. 3. Nutrient fluxes across the sediment–water interface for control, low-density, medium-density and high-density razor clam treatments. (A) Ammonium; (B) nitrate + nitrite; (C) phosphate. Data are means +/– SD (n = 3). Letters indicate differences between treatments on the same experimental days; means not sharing a common letter are significantly different (Tukey's HSD, $p < 0.05$)

4. DISCUSSION

In the present study, the effects of *Sinonovacula constricta* on sediment properties (TOC, TP, TON, APA, MBA) and nutrient fluxes (NH_4^+, $NO_3^- + NO_2^-$, PO_4^{3-}) varied significantly among different treatments during the experiment, indicating that *S. constricta* not only plays a key role in remediation of effluents by consuming algae but also evidently impacts nutrient recycling and enhances the mineralization process in aquaculture wastewater treatment ecosystems. The levels of TOC, TON and TOP in the surface sediments of the treatment groups were significantly lower than those in the control (Table 1), which may be because bioturbation promotes the entry of fresh organic particles from the surface of

the sediment along the burrow into the sediment, and microbial-mediated mineralization accelerates the decomposition of organic matter, suggesting that bioturbation by the clams promotes the reduction of organic substances in the sediment. MBA and APA are indices of microorganism activity and, theoretically, positively related to the number of microbes and organic matter (Jiao et al. 2011). However, in our study the increased MBA and APA seems conflictive to the decreased TOC, TON and TOP on the surface in clam groups compared with that in the control (Table 1). This result is likely due to the pseudofeces and feces produced by the clams on the surface of the sediment, which contain a higher number of microbes with less organic matter (Cognie & Barille 1999, Novais et al. 2016) and are very diffuse (Baker et al. 1998).

SOC is the most widely used indicator for measuring the total mineralization of benthic organisms. The SOC value is usually proportional to the organic matter content in the sediment and can be approximately equal to the total mineralization of direct or indirect biochemical oxidation of organic matter (Thamdrup & Dalsgaard 2000). As a filter-feeding shellfish, *S. constricta* ingests and assimilates organic particulate matter from the overlying water and reduces the accumulation of organic matter on the sediment surface. On the other hand, the metabolic waste of the clam and its own mucus increase the enrichment of the sediment to a certain degree. In present study, the upward trend of SOC fluxes with increasing density and culture time at the beginning of the experiment and the downward trend later (after Day 18; Fig. 2) is probably due to the fact that the microbes proliferated rapidly and decomposed large amounts of organic matter in the sediment during the early stage of the experiment (Ye et al. 2018). The mineralization of sediment organic matter and the sedimentation of organic matter are a dynamic equilibrium process, and Day 18 may have been the turning point. Nicholaus & Zheng (2014) also found that the SOC flux in their experimental group was significantly greater than that in the control. However, the sedimentation rate of the treatment group in the late stage of this experiment showed a downward trend, which may be due to an increase in feeding and bio-assimilation of the clams, leading to the decrease in organic matter in the sediment. These results further indicate that the long-term bioturbation activities of *S. constricta* would reduce the accumulation of organic matter in the sediment of the aquaculture wastewater treatment systems.

Nitrification is the microbial-mediated process of converting ammonia (NH_4^+) into nitrate, in which the nitrate is turned into molecular nitrogen (N_2) or nitrous oxide (N_2O) through denitrification, or into NH_4^+ again by ammonization (Zhong et al. 2015). The results obtained in the present study showed that the release of NH_4^+-N and (NO_3^- + NO_2^-)-N increased with incubation time, which implies that clam bioturbation may break the dynamic balance of nitrification and denitrification and speed up N cycling across the sediment–water interface. Several reports have suggested that increased animal excretion and the accumulation of NH_4^+-N in the anoxic sediment may enhance the release of NH_4^+-N from the sediment into the overlying water (Svensson 1997, Zhang et al. 2011). The shellfish introduce fresh organic matter into the burrow linings and promote oxygen and nitrate influxes from the overlying water into the anoxic sediment (Zhong et al. 2015), which may stimulate the mineralization of organic matter. In this study, the NH_4^+ fluxes were significantly improved in the treatment with *S. constricta*, and the TON contents of different density treatments on the surface sediment (0–2 cm) were significantly lower than those in the control group at the end of the experiment. These findings indicate that the bioturbation of *S. constricta* enhanced the release of NH_4^+-N and N element mineralization, especially on the sediment surface. The flux measurements in this study indicate that *S. constricta* favoured an influx of (NO_3^- + NO_2^-)-N from the overlying water into the sediment at the initial stage, which may be because the nitrification rate in the burrows of *S. constricta* did not support a net flux of nitrate out of the sediment. Denitrification at the initial stages consumed more nitrate than was produced during ventilation. Bertics & Ziebis (2009) found that burrowing activity may enhance the surface area available for microbial colonization and chemical reactions, resulting in the diffusion of nutrients from the sediment. The increase in the nitrate concentration in the bottom water and the sediment–water interface stimulates the release of NO_3^- + NO_2^- fluxes from the sediment. From Day 12, the study showed the release of nitrate and nitrite from the sediments in all treatments, and the NO_3^- + NO_2^- fluxes at different *S. constricta* densities were higher than those in the control group. Increasing trends of nutrient fluxes in the control and decreasing trends in the clam groups with time (Fig. 3) suggests that organic matter accumulated during the experiment and could be remediated by the bioturbation of the clams. These results indicate that the influence of the high level of burrowing of

S. constricta stimulates additional oxygen uptake by the sediment and accelerates the nitrification process in the sediments. For the eutrophication of water bodies, nitrification and denitrification are indispensable processes for eliminating N pollution in sediments (Sun et al. 2010). Our previous research found that bioturbation of *S. constricta* increased the abundances of the bacterial phyla *Betaproteobacteria*, *Actinobacteria* and *Nitrospirae* in the sediment, which oxidize nitrite to nitrate (Lukwambe et al. 2018). Thus, enhanced release of N nutrients in the present study has important implications for the positive effects of *S. constricta* bioturbation on accelerating nitrification and bioremediation of wastewater treatment systems.

Similarly, the oxygen and redox potentials supplied during ventilation of benthic animals in burrows might influence the mineralization of organic phosphate matter in sediments (Waldbusser et al. 2004). The influx of oxygen during irrigation of benthic animals may have mobilized the organic phosphate of sediments and released the dissolved inorganic phosphate into the overlying water. Bates & Neafus (1980) also found that bioturbation increased the rate of phosphate release from the sediment by 2 times under the same conditions (such as DO and pH). In our study, the results showed the release of phosphate in different density treatments and the uptake of phosphate in the control groups (Fig. 3C), and the TP contents in the surface (0–2 cm) and bottom (5–8 cm) sediments of different density treatments (Table 1) were significantly lower than those in the control group at the end of the experiment, suggesting bioturbation of the clams accelerated the release of P. In addition, the burrowing activity of *S. constricta* makes the diffusive flux of phosphate break the oxide layer of the surface sediment. The results of the present study show an increase in the release of nitrogen and phosphate from the sediment into the overlying water (Fig. 3). These nutrients can be a rich source of food for microalgae in wastewater treatment ecosystems. *S. constricta* feed on microalgae and reduce the concentrations of inorganic nutrients in the overlying water. This cycle can reduce the excessive amounts of organic matter in pond effluents and convert them into *S. constricta* biomass. Ultimately, the purpose of purifying the intensive aquaculture wastewater pond was fulfilled.

As the clams need food to eat and may produce waste themselves, they should be applied with other components in bioremediation systems. The reasonable combination and area ratio of different components (or sections) are expected in the ecological treatment system of aquaculture wastewater. Lukwambe et al. (2018) conducted an experiment on a commercial scale with high treatment efficiency, which included 4 sequential areas: the physical settlement (1/5 of the total area), biofilms and aeration (1/5 of the total area), the clam area (2/5 of the total area), and the wetlands (1/5 of the total area). However, further investigations are needed to develop better or more optimized models of the treatment system.

5. CONCLUSIONS

This study showed that the clam *S. constricta* can change the physical and chemical properties of aquaculture wastewater ecosystem sediment. Bioturbation accelerates the benthic release of N and P nutrient fluxes and the mineralization of organic matter particles in the sediment, slowing the accumulation of organic matter on the sediment surface in aquaculture wastewater ecosystems. At the same time, benthic bioturbation increased the aerobic rates and microbial activities in the sediment. The increased reproduction of microorganisms is beneficial to various biochemical reactions and enhances the self-purification ability in aquaculture wastewater treatment systems. Therefore, bioturbations of *S. constricta* have ecological benefits for the remediation of aquaculture wastewater.

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