

# Impact of *Litopenaeus vannamei* bioturbation on nitrogen dynamics and benthic fluxes at the sediment–water interface in pond aquaculture

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**Abstract** We investigated the effects of the bioturbation of *Litopenaeus vannamei* at the sediment–water interface through field experiments and ex situ incubation. Sediment oxygen consumption (SOC), benthic nitrogen dynamics (nitrification, denitrification, and nitrate ammonification), and the benthic fluxes of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ , and soluble reactive phosphorus were measured monthly from April to September. The results showed that *L. vannamei* promoted SOC and decreased the concentration of dissolved oxygen in the bottom water. Enhanced nutrient release from the sediments to the overlying water due to *L. vannamei* was observed, which consequently enriched the bottom water. The presence of *L. vannamei* inhibited nitrification later in the cultivation period and hardly had any significant effects on denitrification and nitrate ammonification throughout the study. The results suggest that farmers should more thoroughly consider changes in the benthic fluxes of oxygen and nutrients, as these aspects could be influenced by shrimp bioturbation, whereas benthic nitrogen dynamics in aquaculture ponds were comparatively less influenced.

**Keywords** *Litopenaeus vannamei* · Bioturbation · Benthic fluxes · Nitrogen dynamics · Sediment–water interface · Pond aquaculture

## Abbreviations

DO Dissolved oxygen  
SOC Sediment oxygen consumption  
SRP Soluble reactive phosphorus

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## Introduction

*Litopenaeus vannamei* is one of the most prolific shrimp species in the world and is naturally distributed in the Eastern Pacific Ocean from off Northern Mexico to off Northern Peru, between the 20 °C isotherms (Holthuis 1980). After being introduced into China in the 1980s, the shrimp soon became widely popular (Wyban 2002). The output of *L. vannamei* in 2012 reached 1.45 million tons, accounting for 85 % of the total yield of shrimp culture in China (National Bureau of Statistics 2012). As *L. vannamei* can tolerate a wide salinity range, from brackish water of 1–2 ppt to hypersaline water of 40 ppt (Bray et al. 1994), the output of this shrimp in the inland brackish ponds of China has risen in recent years and nearly equaled its mariculture output in 2012 (National Bureau of Statistics 2012). In light of ecological and economic concerns, *L. vannamei* in inland ponds is usually mixed with fishes (e.g., *Ctenopharyngodon idellus*, *Hypophthalmichthys molitrix*; Zhang et al. 2011a, b; Zhu et al. 2011). However, the shrimp possesses benthic behavior, which inevitably imposes bioturbation upon the sediment–water interface.

As the boundary between water and sediment, the sediment–water interface shows significant variation in its physical, chemical, and biological characteristics and is involved in material transportation and exchange in natural water bodies (Nixon 1986); it therefore plays an important role in material circulation, transfer, and storage in aquatic environments (Santschi et al. 1990). The migration and transformation of nutrients at the sediment–water interface can greatly impact the budget and circulation dynamics of nutrients (Thibodeau et al. 2010) and is also related to eutrophication (Hou et al. 2013). Inorganic nitrogen ( $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and  $\text{NO}_2^-\text{-N}$ ) and soluble reactive phosphorus (SRP) are important nutrients used to measure water quality in aquaculture.  $\text{NH}_4^+\text{-N}$  is the final product of protein breakdown and is present in large quantities (Walsh and Wright 1995); in water,  $\text{NH}_4^+\text{-N}$  and  $\text{NH}_3\text{-N}$  reach an equilibrium dependent on the pH and water temperature (Emerson et al. 1975), and high levels of  $\text{NH}_3$  accumulation are toxic to aquatic organisms (Randall and Tsui 2002).  $\text{NO}_x^-\text{-N}$  ( $\text{NO}_3^-\text{-N}$  and  $\text{NO}_2^-\text{-N}$ ) is an important form in the aquatic nitrogen cycle, representing not only the product of nitrification but also the substrate of denitrification and nitrate ammonification.  $\text{NO}_3^-\text{-N}$  and  $\text{NO}_2^-\text{-N}$  can be transformed reversibly, but the latter inorganic compound is potentially toxic to aquatic organisms (Jensen 2003). SRP is an important link in the circulation of materials in ponds: as a limiting nutrient for the growth of phytoplankton, it can promote or restrict the transformation of materials and energy in aquaculture ecosystems (Hecky and Kilham 1988). The nitrogen dynamics (nitrification, denitrification, and nitrate ammonification) and benthic fluxes of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ , and SRP can directly affect the form and content of nitrogen and phosphate in the sediment and water, determining the budget of nitrogen and phosphate in aquaculture water and affecting water quality (Eyre et al. 2011).

Bioturbation refers to the modification of sediment texture and the dispersal of solid particles that result from the burrowing, feeding, bioirrigation, excretion, and ventilation of benthic animals at the sediment–water interface (Aller 2001; Meysman et al. 2006). Many previous studies have shown that the bioturbation of benthic animals can affect benthic fluxes and N cycling at the sediment–water interface (Michaud et al. 2006; Nizzoli et al. 2007; Adámek and Maršálek 2013), and species-specific effects have been found due to different irrigation behaviors (Banta et al. 1999), feeding strategies (Christensen et al. 2000), and burrowing depths (Mermillod-Blondin et al. 2004). However, no detailed report exists regarding the bioturbation of *L. vannamei* in polyculture ponds.

The purpose of the present study was to investigate the effects of *L. vannamei* bio-turbation on nitrogen dynamics and benthic fluxes at the sediment–water interface in pond aquaculture. This study will improve the understanding of the nitrogen and phosphate nutrient cycles in different aquaculture modes and provide a reference for pond water quality management.

## Materials and methods

### Experimental site and setup

The study was conducted in aquaculture ponds at Zhaodian village, Gaoqing City, Shandong Province, P. R. China, from April to September 2012. The ponds were rectangular in size with an area of approximately 2,333.33 m<sup>2</sup>, a length to width ratio of approximately 2:1, and an average water depth of approximately 1.5 m. Two treatments were set with three replicates each: treatment CH was set with *C. idellus* and *H. molitrix*; treatment CHL was set with *C. idellus*, *H. molitrix* and *Litopenaeus vannamei*. The stocking densities and specifications of each treatment are listed in Table 1. The aquatic organisms were successively put into the ponds from early to mid-April, and feeding was started simultaneously. Each pond was equipped with a feeding machine. The same amount of factitious combinative feed for fishes was provided to each pond four times a day. The feeding quantity was decided by the specifications of the fishes and the weather conditions. One 2,000-W aerator, situated at the center of each pond, was activated from 0000 to 0600 hours and 1100 to 1300 hours every day. The aquatic organisms were harvested in October.

### Sample collection

Sampling was conducted from April to September. Water and sediment samples were collected for the determination of oxygen and nutrient fluxes on the 20th of each month, and samples for the determination of nitrification, denitrification, and nitrate reduction rates were collected on the 21st of each month. In each pond, three sampling sites were selected (Fig. 1). Sediment samples were collected with a core sampler, which minimizes disturbances to the sediment surface (Wang and Xu 2004). The chambers ( $d = 5$  cm,  $h = 33$  cm) containing sediment cores were removed from the core sampler and sealed with rubber lids. The samples were then transferred to the laboratory within 1 h. In the laboratory, the cores in the chambers were adjusted to a depth of 8–10 cm and equilibrated for 2 h. Bottom water samples were collected with a water sampler. The measurements of dissolved oxygen (DO) concentration and water temperature were conducted instantly with a digital dissolved oxygen meter (YSI). A 3 L sample of bottom water from each sampling site was brought to the laboratory, where 100 mL of the water was filtered through 0.45- $\mu$ m cellulose acetate membranes and frozen at  $-20$  °C until the  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N,  $\text{NO}_2^-$ -N, and SRP analyses; the remainders of the samples were used for the incubation experiments.

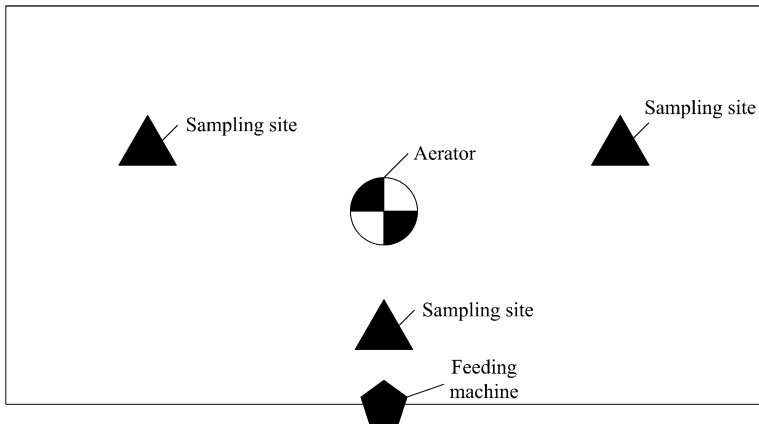
### Determination of oxygen and nutrient fluxes

Oxygen and nutrient fluxes were determined by ex situ incubation. After equilibration, the overlying water retained in each chamber was carefully replaced by bottom water, and the final water height in each chamber was maintained at approximately 20 cm. Each chamber

**Table 1** Description of the studied treatments

Treatment	Stocking density (ind m <sup>-2</sup> )	Initial specifications
CH	<i>C. idellus</i> , 1.84; <i>H. molitrix</i> , 0.92	<i>C. idellus</i> , 6–8 ind kg <sup>-1</sup>
CHL	<i>C. idellus</i> , 1.84; <i>H. molitrix</i> , 0.92; <i>L. vannamei</i> , 55.51	<i>H. molitrix</i> , 4–6 ind kg <sup>-1</sup> <i>L. vannamei</i> , 0.8–1.2 cm

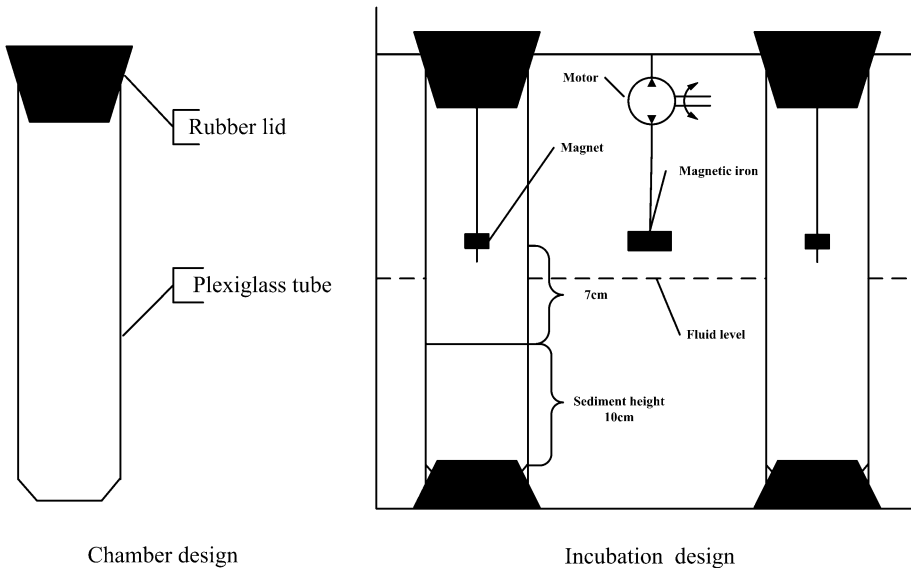
*C. idellus*, *Ctenopharyngodon idellus*; *H. molitrix*, *Hypophthalmichthys molitrix*; *L. vannamei*, *Litopenaeus vannamei*

**Fig. 1** Sampling sites in each pond

was sealed with rubber lids, and the upper lid was equipped with a magnet that was stirred by a central magnet at 50 rpm (Fig. 2). Incubations were conducted in a water bath in an incubator for 4 h in darkness at the in situ temperature. Initial and final overlying water samples (30 mL) were taken from each chamber. These overlying water samples were first measured for DO concentration using a digital dissolved oxygen meter (YSI), then filtered through 0.45- $\mu$ m cellulose acetate membranes and frozen at  $-20$  °C until later analyses of  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N,  $\text{NO}_2^-$ -N, and SRP.

#### Determination of nitrification, denitrification, and nitrate ammonification rates

Nitrification, denitrification, and nitrate ammonification rates were determined using a modified acetylene ( $\text{C}_2\text{H}_2$ ) inhibition method (Kim et al. 1997). Two sediment cores were collected at each sampling site: one sediment was inhibited by  $\text{C}_2\text{H}_2$ , and the other was established as a control with no  $\text{C}_2\text{H}_2$ . After equilibration, the overlying water in each chamber was replaced carefully by bottom water, and an extra 30 mL of  $\text{C}_2\text{H}_2$ -saturated water was added to the  $\text{C}_2\text{H}_2$  inhibition treatment chambers; the final height of the water in each chamber reached approximately 20 cm. The incubation equipment and procedure were the same as those previously described. Initial and final overlying water samples were collected from each chamber. A 0.3 mL aliquot of saturated  $\text{HgCl}_2$  solution was added to 60 mL of water in glass bottles for the measurement of  $\text{N}_2\text{O}$ . The other 30 mL of water was filtered through 0.45- $\mu$ m cellulose acetate membranes and frozen at  $-20$  °C until later analyses of  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, and  $\text{NO}_2^-$ -N.



**Fig. 2** A sketch of the plexiglass tubes for the coring device and sediment incubation

Measurements and calculation of data

NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, NO<sub>2</sub><sup>-</sup>-N, and SRP contents in the bottom water were determined by the spectrophotometric method according to Lei (2006). The N<sub>2</sub>O content was determined by headspace gas chromatography.

Sediment oxygen consumption (SOC), nutrient fluxes, and the rates of nitrification, denitrification, and nitrate ammonification were calculated by the following equation (Kim et al. 1997; Zheng et al. 2009):

$$F = \frac{\Delta C \cdot V}{A \cdot \Delta t}$$

where *F* (μmol m<sup>-2</sup> day<sup>-1</sup> for denitrification rate and mmol m<sup>-2</sup> day<sup>-1</sup> for the other variables) represents SOC, nutrient fluxes, and the rates of nitrification, denitrification, and nitrate ammonification; *V* (m<sup>3</sup>) is the volume of the incubator; *A* (m<sup>2</sup>) is the bottom area of the incubator; Δ*t* (days) is the incubation duration; and Δ*C* (mmol m<sup>-3</sup>) is the change in the concentrations of DO and nutrients before and after incubation. Δ*C* (mmol m<sup>-3</sup> or μmol m<sup>-3</sup>) represents the difference in the concentrations of ammonia (mmol m<sup>-3</sup>) or N<sub>2</sub>O (μmol m<sup>-3</sup>) between the C<sub>2</sub>H<sub>2</sub> inhibition treatment and the control after incubation for calculating the nitrification and denitrification rates, whereas for calculating the nitrate ammonification rate, Δ*C* (mmol m<sup>-3</sup>) represents the change in the concentration of nitrate (mmol m<sup>-3</sup>) after incubation in the C<sub>2</sub>H<sub>2</sub> inhibition treatment chambers.

Statistical analysis

Statistical analysis of the data was performed using a statistical package (SPSS 13.0 for Windows, SPSS Inc., Richmond, CA, USA). Concentrations of ammonium, nitrate, nitrite, and SRP; SOC; nutrient fluxes; and the rates of nitrification, denitrification, and nitrate

ammonification among the months and treatments were compared using analysis of variance (ANOVA) followed by Duncan's multiple range tests for post hoc comparisons. The normal distribution of the data and homogeneity of variances among the treatments were verified before ANOVA. The significance level was defined as 0.05 when Levene's tests were violated to further reduce the chance of a type I error (falsely identifying a significant difference).

## Results

### Bottom water quality

The parameters measured in the two treatments are presented in Table 2. The water temperature varied between 21.5 °C in April and 29.2 °C in July. The DO concentrations in April and May were higher in treatment CH than in treatment CHL, while the opposite pattern was observed in the following 4 months. In April, no significant differences were observed in the concentrations of the nutrients ( $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ , and SRP) between the two treatments ( $P > 0.05$ ), but the concentrations of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  in treatment CHL were significantly higher than in treatment CH from June onward ( $P < 0.05$ ). As the experiment progressed, the  $\text{NO}_2^-\text{-N}$  concentrations varied little in treatment CH but fluctuated in treatment CHL. The  $\text{NO}_2^-\text{-N}$  concentrations in treatment CHL were significantly higher than in treatment CH from July to September ( $P < 0.05$ ). The SRP concentrations in treatment CH were comparatively low, except in April, and from the month of May onward, the SRP concentrations in treatment CHL were significantly higher than in treatment CH ( $P < 0.05$ ).

### Sediment nitrification, denitrification, and nitrate ammonification rates

The nitrification rates in the two treatments ranged from  $5.74 \pm 1.35$  to  $113.56 \pm 11.51$   $\text{mmol m}^{-2} \text{day}^{-1}$  (Fig. 3). No significant differences were observed between the nitrification rates of the two treatments from April to June, but from July onward, the nitrification rates in treatment CH were significantly higher than in treatment CHL ( $P < 0.05$ ). The denitrification rates in the two treatments ranged from  $0.71 \pm 6.02$  to  $9.99 \pm 1.52$   $\mu\text{mol m}^{-2} \text{day}^{-1}$  (Fig. 4). The nitrate ammonification rates ranged from  $0.062 \pm 0.002$  to  $1.572 \pm 0.076$   $\text{mmol m}^{-2} \text{day}^{-1}$ , two to three orders of magnitude greater than the denitrification rates (Fig. 5). No significant differences were observed in the denitrification rates between the two treatments except in September ( $P > 0.05$ ). Significant differences in the nitrate ammonification rates between the two treatments were observed in August and September, but only in August was the nitrate ammonification rate in treatment CHL significantly higher than in treatment CH ( $P < 0.05$ ).

### Sediment oxygen consumption and benthic nutrient fluxes

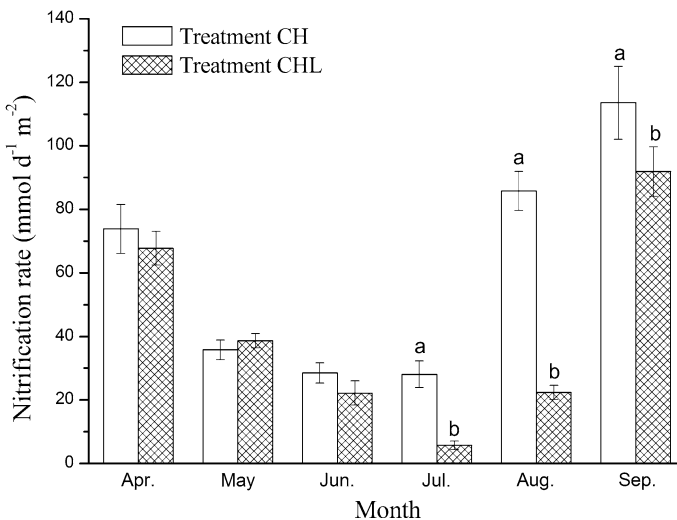
The SOC rates in the two treatments ranged from  $24.18 \pm 2.43$  to  $202.69 \pm 8.76$   $\text{mmol m}^{-2} \text{day}^{-1}$ , and both treatments reached their maximum rate in July, when the water temperature was highest (Fig. 6a). The SOC in treatment CHL was significantly higher than in treatment CH from April to September ( $P < 0.05$ ). The fluxes of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_x^-\text{-N}$ , and SRP in the two treatments were negative in the month of April, indicating that

**Table 2** Parameters measured in bottom water in the experimental treatments

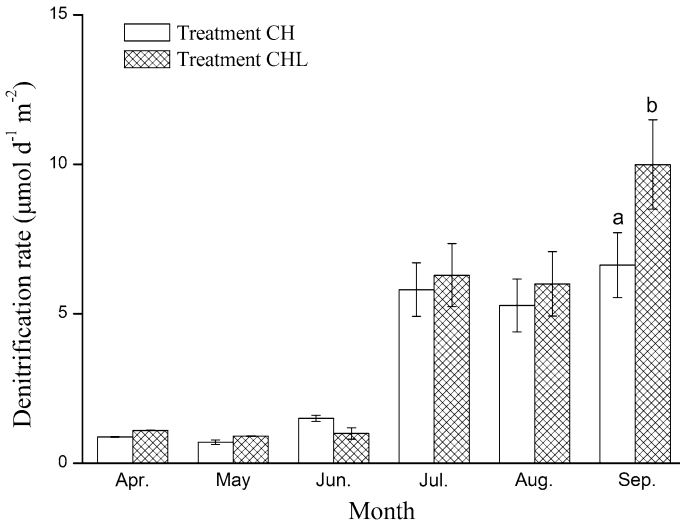
Parameters	NH <sub>4</sub> <sup>+</sup> -N (mg L <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> N (mg L <sup>-1</sup> )	NO <sub>2</sub> <sup>-</sup> N (mg L <sup>-1</sup> )	SRP (mg L <sup>-1</sup> )	DO (mg L <sup>-1</sup> )	T (°C)
April						
CH	3.118 ± 0.086	0.385 ± 0.021	0.013 ± 0.0021	0.105 ± 0.0042	8.73	21.5
CHL	3.283 ± 0.222	0.357 ± 0.025	0.016 ± 0.0013	0.109 ± 0.0151	9.88	
May						
CH	0.280 ± 0.031	0.539 ± 0.017	0.011 ± 0.0015	0.007 ± 0.0002 <sup>a</sup>	6.4	25.4
CHL	0.551 ± 0.074	0.600 ± 0.036	0.036 ± 0.0079	0.121 ± 0.0207 <sup>b</sup>	7.81	
June						
CH	1.642 ± 0.117 <sup>a</sup>	0.463 ± 0.014 <sup>a</sup>	0.012 ± 0.0008	0.003 ± 0.0002 <sup>a</sup>	3.85	27.7
CHL	2.564 ± 0.143 <sup>b</sup>	0.976 ± 0.014 <sup>b</sup>	0.021 ± 0.0006	0.187 ± 0.0254 <sup>b</sup>	2.98	
July						
CH	0.397 ± 0.06 <sup>a</sup>	0.663 ± 0.027	0.027 ± 0.0099 <sup>a</sup>	0 <sup>a</sup>	2.74	29.2
CHL	1.493 ± 0.052 <sup>b</sup>	0.702 ± 0.053	0.131 ± 0.0033 <sup>b</sup>	1.031 ± 0.2014 <sup>b</sup>	1.06	
August						
CH	0.885 ± 0.045 <sup>a</sup>	0.207 ± 0.053 <sup>a</sup>	0.062 ± 0.0067 <sup>a</sup>	0.003 ± 0.0002 <sup>a</sup>	2.31	27.2
CHL	2.992 ± 0.101 <sup>b</sup>	1.165 ± 0.092 <sup>b</sup>	0.406 ± 0.0166 <sup>b</sup>	1.154 ± 0.0149 <sup>b</sup>	1.56	
September						
CH	1.275 ± 0.067	0.783 ± 0.042 <sup>a</sup>	0.012 ± 0.0025 <sup>a</sup>	0.002 ± 0.0003 <sup>a</sup>	5.13	22.7
CHL	1.637 ± 0.207	1.336 ± 0.345 <sup>b</sup>	0.316 ± 0.0101 <sup>b</sup>	0.963 ± 0.0071 <sup>b</sup>	3.33	

Values are given as means or mean ± SD (*n* = 3)

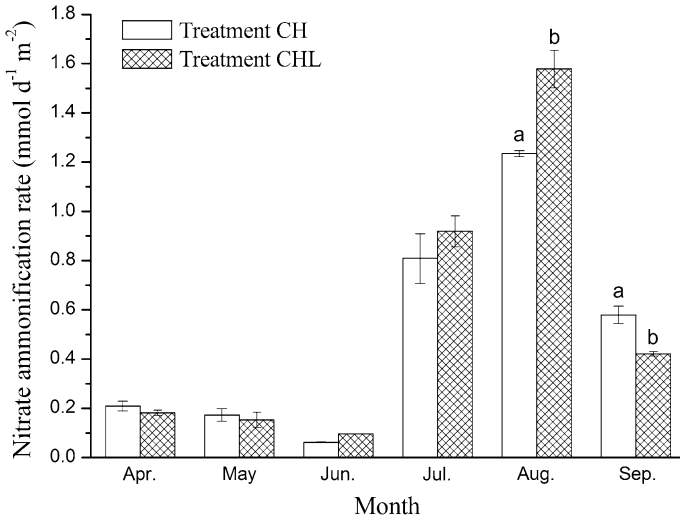
<sup>a, b</sup> Different letters in the same column and sampling month indicate significant differences (*P* < 0.05)



**Fig. 3** Nitrification rate at the sediment–water interface in different treatments. Bars denote standard deviation (*n* = 3). Different letters indicate differences between treatments within the same month. Means that do not share a common letter are significantly different (*P* < 0.05). Unlabeled columns are not different at the significance level of *P* = 0.05



**Fig. 4** Denitrification rate at the sediment–water interface in different treatments. Bars denote standard deviation ( $n = 3$ ). Different letters indicate differences between treatments within the same month. Means that do not share a common letter are significantly different ( $P < 0.05$ ). Unlabeled columns are not different at the significance level of  $P = 0.05$



**Fig. 5** Nitrate ammonification rate at the sediment–water interface in different treatments. Bars denote standard deviation ( $n = 3$ ). Different letters indicate differences between treatments within the same month. Means that do not share a common letter are significantly different ( $P < 0.05$ ). Unlabeled columns are not different at the significance level of  $P = 0.05$

these nutrients were absorbed by the sediment from the water at the beginning of the study, but became positive in the succeeding months (Fig. 6b–d). No significant differences were found in the nutrient fluxes between the treatments in April ( $P > 0.05$ ), while the fluxes in



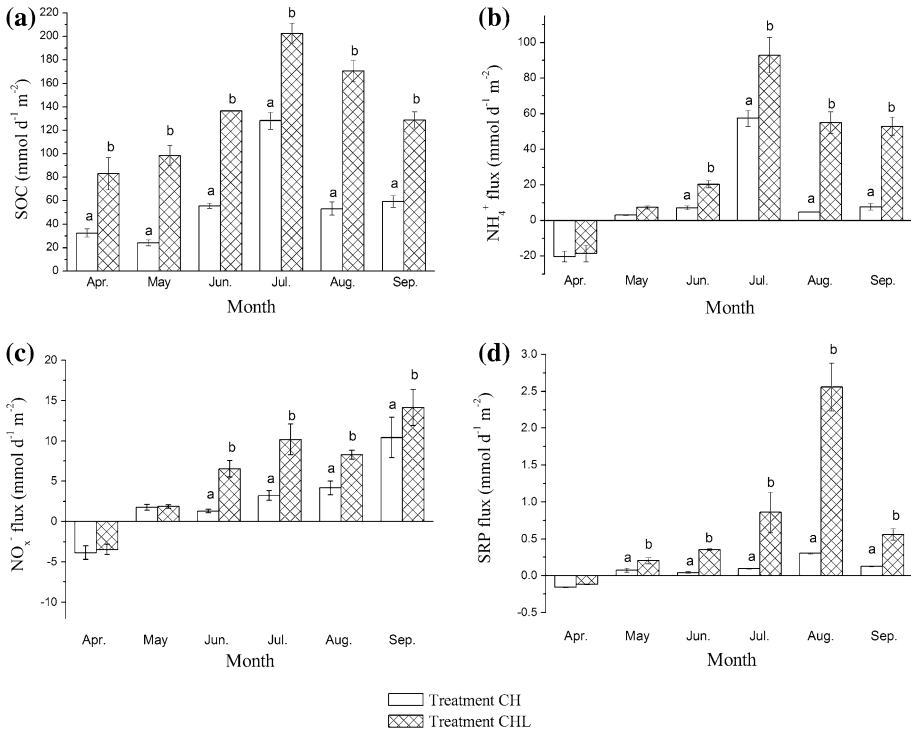
treatment CHL were significantly higher than in treatment CH from May (SRP flux) and June ( $\text{NH}_4^+$ -N and  $\text{NO}_x^-$ -N flux) onward ( $P < 0.05$ ). The significant differences ( $P < 0.05$ ) in the fluxes between the two treatments ranged from 13.09 to 50.24  $\text{mmol m}^{-2} \text{day}^{-1}$  ( $\text{NH}_4^+$ -N flux), from 3.73 to 6.95  $\text{mmol m}^{-2} \text{day}^{-1}$  ( $\text{NO}_x^-$ -N flux), and from 0.13 to 2.26  $\text{mmol m}^{-2} \text{day}^{-1}$  (SRP flux).

## Discussion

### Sediment nitrification, denitrification, and nitrate ammonification

Nitrification is a process in which ammonia is oxidized to nitrate by microorganisms. Nitrate can in turn be reduced into gaseous nitrogen by denitrification or into  $\text{NH}_4^+$ -N again by nitrate ammonification, and these procedures can be influenced by factors such as dissolved oxygen, water temperature, substrate concentration, pH, microbe activity, and available surface (Hargreaves 1998). Many studies have shown that the bioturbation caused by some macrobenthos can modify sediment textures and change the oxygen supply through burrowing or bioirrigating in sediments (Welsh 2003; Meysman et al. 2006), as well as by stimulating the nitrifying bacteria in the sediment located immediately below the penetration depth of oxygen and in burrow linings (Henriksen et al. 1983), thereby promoting nitrification rates at the sediment–water interface. Previous studies have also found that bioturbation activity can increase the nitrate consumption and denitrification rates in the sediment (Henriksen et al. 1983; Michaud et al. 2006; Nizzoli et al. 2007). This phenomenon is primarily due to the introduction of nitrate from the overlying water into the burrows (Svensson and Leonardson 1996), the extended oxic/anoxic interface created by burrow structures, and the occasional enhancement of nitrification (Nielsen et al. 2004).

In the present study, the bioturbation of *L. vannamei* inhibited rather than promoted nitrification in the later stages of cultivation and hardly had any significant promoting effect on denitrification and nitrate ammonification. These differences may be explained by the diversity of bioturbating functional groups. On the basis of sediment mixing modes, five functional groups can be distinguished: biodiffusers, upward-conveyors, downward-conveyors, regenerators, and gallery-diffusers (François et al. 1997; Gerino et al. 2003). Most previously studied macrobenthos belong to the latter four functional groups, burrowing or bioirrigating deep into sediments and promoting biogeochemical processes [e.g., nitrification, denitrification, and oxidation of Fe(II) by SRP]. In contrast, *L. vannamei* does not burrow or creates only small burrows, with a maximum depth of 2 cm, on the surface of sediments (Boddeke 1983; Guo 2011). According to the observations of previous studies and the results of the present study, we speculate that the shrimp belongs to the group of biodiffusers. Mermillod-Blondin et al. (2004) reported that the mode of sediment mixing, as associated with irrigation behavior and burrowing depth, is sufficient for determining the effects of different macrobenthos on microbial communities and biogeochemical processes at the sediment–water interface. The present study revealed that the bioturbation of biodiffusers such as *L. vannamei* on the surface of sediments does not promote nitrogen dynamics at the sediment–water interface. As previous studies have mainly focused on the bioturbation of burrowing or bioirrigating macrobenthos, and few reports exist regarding biodiffusers, the present study provides a scientific basis for understanding the effects of biodiffusers on biogeochemical processes at the sediment–water interface.



**Fig. 6** Sediment oxygen and nutrient fluxes of the experimental treatments over 6 months. **a** SOC sediment oxygen consumption; **b** NH<sub>4</sub><sup>+</sup>-N flux; **c** NO<sub>3</sub><sup>-</sup>-N + NO<sub>2</sub><sup>-</sup>-N flux; **d** SRP soluble reactive phosphorus flux. Bars denote standard deviation ( $n = 3$ ). Positive fluxes indicate nutrient migration from the sediment to the water column, and negative fluxes indicate the opposite. Different letters indicate differences between treatments within the same month. Means that do not share a common letter are significantly different ( $P < 0.05$ ). Unlabeled columns are not different at the significance level of  $P = 0.05$

### Benthic fluxes and water quality

The water temperature changed with the season over the study, but the two treatments used had the same area and depth, and so their water temperatures were essentially the same in each month. Many studies have been conducted on SOC in aquaculture water. Nicholas and Zheng (2014) reported that the bioturbation activity of *Cyclina sinensis* could enhance SOC through the oxidation of anoxic sediment and increased microbial activity. Zheng et al. (2009) suggested that the enriched sediments made by benthic macrofauna also led to increased SOC. In the present study, the SOC in the treatment with *L. vannamei* was significantly higher for the duration of the experiment. As *L. vannamei* only disturbed the surface of the sediments and did not promote biogeochemical processes (e.g., nitrification, denitrification, and nitrate ammonification), SOC was not increased by the promotion of oxidation reactions deep in the sediments. As *L. vannamei* live close to the sediments, we hypothesized that the feces of *L. vannamei* directly increase the organic matter content the sediment surface; rates of SOC generally increase with the enrichment of the sediment (Boyd 1995). Regarding water quality, the content of DO in the bottom water of treatment CHL was initially higher than in treatment CH, but this situation was reversed from June

onward, which indicated that the increased SOC caused by *L. vannamei* decreased the concentration of DO in the bottom water. Therefore, greater attention should be paid to the management of DO in the bottom water of ponds cultured with *L. vannamei*.

In this study, the fluxes of  $\text{NH}_4^+\text{-N}$  were promoted in the treatment with *L. vannamei* from June onward. This finding demonstrated that, as with other macrobenthos, the bioturbation effect of *L. vannamei* enhanced the release of  $\text{NH}_4^+\text{-N}$  from the sediment and increased the concentration of  $\text{NH}_4^+\text{-N}$  in the bottom water (Svensson 1997; Michaud et al. 2006; Zhang et al. 2011a, b). Several reports have suggested that the enhancement of  $\text{NH}_4^+\text{-N}$  release may be caused primarily by animal excretion and the transport of accumulated  $\text{NH}_4^+\text{-N}$  in the anoxic sediment (Svensson 1997; Zhang et al. 2011a, b). The ammonia excretion rate of *L. vannamei* is very low ( $1.77\text{--}7.04\text{ mmol m}^{-2}\text{ day}^{-1}$ ; Jiang et al. 2000), while the increase in the  $\text{NH}_4^+\text{-N}$  flux caused by *L. vannamei* was relatively high ( $13.09\text{--}50.24\text{ mmol m}^{-2}\text{ day}^{-1}$ ) in this experiment. Therefore, we can infer that the ammonia excretion of *L. vannamei*, which increased the concentration of  $\text{NH}_4^+\text{-N}$  in the sediments, was not the most important contributor to the enhancement of  $\text{NH}_4^+\text{-N}$  release. We hypothesized that the physical disturbance of the surface of the sediments by *L. vannamei*, which increased the suspension of nutrients, may be the major cause. As *L. vannamei* could not make deep burrows or disrupt the anoxic zone, which would accelerate  $\text{NH}_4^+\text{-N}$  release to the water (Svensson 1997; Jørgensen et al. 2005), the results of the present study show that the bioturbation of the sediment surface can also significantly enhance the release of  $\text{NH}_4^+\text{-N}$  from sediments to the overlying water in aquaculture ponds.

Previous studies have found that the bioturbation of some macrobenthos can promote the transport of  $\text{NO}_x^-\text{-N}$  from the overlying water deep into the sediment, where the  $\text{NO}_x^-\text{-N}$  is consumed by denitrification and nitrate ammonification, thereby decreasing the concentration of  $\text{NO}_x^-\text{-N}$  in the overlying water (Svensson et al. 2001; Shang et al. 2013). Other studies have found that burrowing activity can increase sediment water content and sediment mixing, causing a greater consumption of SRP by the oxidation of Fe(II) in the sediment and inhibiting SRP release (Zhang et al. 2010). In the present study, however, *L. vannamei* enhanced the release of both  $\text{NO}_x^-\text{-N}$  and SRP from the sediment and increased the concentrations of  $\text{NO}_x^-\text{-N}$  and SRP in the bottom water. As can be seen from the results, *L. vannamei* made no or shallow burrows and did not promote biogeochemical processes (e.g., nitrification, denitrification, and nitrate ammonification); we therefore hypothesized that the species did not promote the oxidation of Fe(II) either. In contrast with the findings of previous studies, biogeochemical processes were not determining factors in the fluxes of  $\text{NO}_x^-\text{-N}$  and SRP at the sediment–water interface in the present study. In addition, Zhang et al. (2011a, b) reported that the excretion of SRP by *Corbicula fluminea* may play a major role in SRP release from sediments. In the present study, the increases in the  $\text{NO}_x^-\text{-N}$  and SRP fluxes caused by *L. vannamei* were  $3.73\text{--}6.95$  and  $0.13\text{--}2.26\text{ mmol m}^{-2}\text{ day}^{-1}$  during the incubation, respectively, which were much higher than previously reported shrimp excretion rates of  $\text{NO}_x^-\text{-N}$  ( $0.01\text{--}0.04\text{ mmol m}^{-2}\text{ day}^{-1}$ ; Jiang et al. 2000) and SRP ( $0.007\text{--}0.081\text{ mmol m}^{-2}\text{ day}^{-1}$ ; Li et al. 2006). These results implied that the excretion of *L. vannamei*, which could enrich the sediments, contributed little to the enhancement of  $\text{NO}_x^-\text{-N}$  and SRP release from the sediment. We hypothesized that, as with the  $\text{NH}_4^+\text{-N}$  release, disturbance to the sediment surface may have been the major cause of the enhanced release of  $\text{NO}_x^-\text{-N}$  and SRP. In light of the results of previous studies, we found that the bioturbation of different functional groups may have opposite effects on the fluxes of  $\text{NO}_x^-\text{-N}$  and SRP at the sediment–water interface.

## Conclusion

Biodiffusers such as *L. vannamei* engage in bioturbation only at the sediment surface, but this action is sufficient to promote SOC and the release of nitrogen and phosphorus nutrients from the sediments; in contrast, the bioturbation of some burrowing macrobenthos may inhibit these processes. The bioturbation of *L. vannamei* did not promote the nitrogen dynamics at the sediment–water interface, which indicated that a certain bioturbating depth is required to significantly affect biogeochemical processes and that different bioturbating depths may have different effects. Therefore, *L. vannamei* cannot enhance nitrogen removal from water bodies in aquaculture; conversely, the species may induce the degradation of bottom water, which should be taken into consideration in monitoring water quality during cultivation.

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