

# Effects of key species mud snail *Bullacta exarata* (Gastropoda) on oxygen and nutrient fluxes at the sediment-water interface in the Huanghe River Delta, China

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## Abstract

Since the mud snail *Bullacta exarata* was introduced for economic aquaculture in the Huanghe River (Yellow River) Delta in 2001, its quick population growth and expanded distribution make it a key-species in the intertidal zone of this area. This significantly contributed to the economic income of the local people, but its potential ecological impact on the benthic ecosystem remains unknown. A mesocosm study was conducted to test whether its bioturbation activities affect the microphytobenthos (MPBs; i.e., sedimentary microbes and unicellular algae) productivity and the nutrient exchange between the sediment-water interface. Our results show that the mud snail significantly impacted the dissolved oxygen (DO) flux across the sediment-water interface on the condition of normal sediment and light treatment, and significantly increased the ammonium efflux during recovery period in the defaunated sediment and dark treatment. The presence of micro- and meiofauna significantly increased the NH<sub>4</sub>-N flux in dark treatment. Whereas, in light treatment, these small animals had less effects on the DO and NH<sub>4</sub>-N flux between sediment-water interface. Our results provide better insight into the effect of the mud snail *B. exarata* on the ecosystem functioning by benthic fluxes.

**Key words:** bioturbation, mud snail *Bullacta exarata*, oxygen flux, nutrient flux, benthic metabolism, Huanghe River (Yellow River) Delta

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## 1 Introduction

The mud snail *Bullacta exarata* (Philippi, 1848; belonging to Atyidae, Gastropoda, Mollusca) is widely distributed in the intertidal zone along the coasts of the West Pacific Ocean in China, Japan and DPRK. As a surface deposit-feeder, *B. exarata* has no clear preference for specific food types, consuming sources ranging from benthic diatoms, organic detritus to small crustaceans and invertebrate eggs (Yu et al., 2003). As an economically important fishery species, the farming area of mud snail has been expanding in China since the 1980s. *Bullacta exarata* was first introduced to the Huanghe River (Yellow River) Delta (YRD) in 2001 for aquaculture in the intertidal zone. After that, the snail quickly spread along this area, with harvest up to a staggering 50 thousand ton in 2005, with a value of 300 million Renminbi

(RMB) and net profit of 280 million RMB (Yuan et al., 2006). Given this economic importance, researchers have focused on its behavior characteristics (Zheng, 2003), reproductive biology and development (You et al., 1994), ecotoxicology (Bao et al., 2007), population characters (Ye and Lu, 2001b) and spatial distribution (Ye and Lu, 2001a). Surprisingly, at least to our knowledge, research about the effect of *B. exarata* on the intertidal ecosystems remains lacking.

Bioturbation at the water-sediment interface can have a major influence on the ecology and biogeochemistry of soft sediment habitats (Mermillod-Blondin and Rosenber, 2006; Mermillod-Blondin, 2011). Generally, marine benthic communities can be discriminated into different ecological units by size, from microbenthos (<42 μm) through meiobenthos (42 μm–0.5 mm) to

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macrobenthos (>0.5 mm), which play different roles at the water-sediment interface (Eleftheriou, 2013). Macrobenthic invertebrates are a very important group of bioturbators in soft substrate benthic ecosystems where they exert a structuring influence on the habitat and sediment particle distribution (Gerino, 1990). Their activities of grazing, predation and burrowing can influence the soft sediment habitats in a variety of ways, e.g., changing the permeability and water content of sediment, affecting chemical gradients in pore water, subducting organic matter, and altering rates of remineralization and inorganic nutrient efflux (Widdicombe and Austen, 1998; Lohrer et al., 2004). This kind of activity can significantly affect the fluxes of dissolved oxygen (DO) and thereby fluxes of dissolved inorganic nutrients (DIN). By allowing oxygen to penetrate more deeply to the sediment, anoxic processes such as denitrification may be inhibited while aerobic processes such as nitrification of ammonium to nitrate may increase (Widdicombe and Austen, 1998). Moreover, the bioturbation of benthic macrofauna can also control rates of organic matter degradation and carbon burial (Lohrer et al., 2004).

Microphytobenthos (MPB) are the main primary producers in most unvegetated shallow-water sediments, usually dominated by epipellic diatoms (Smith and Underwood, 2000). MPBs and macrofauna are considered the two key groups affecting benthic metabolism in shallow-water sediments, given their high abundance and high level of activity in benthic ecosystems (e.g., Lohrer et al., 2004). The macrofauna impact sediment in many ways, which could affect MPB both directly and indirectly. These include their grazing activities on MPB directly and physical impacts indirectly, such as burial, dispersal and resuspension and biogeochemical impacts such as irrigation (oxygenation) and nitrification (e.g., ammonia excretion). It is, however, not yet clear to which extent the bioturbation would affect the MPB production in shallow coastal sediments (Tang and Kristensen, 2007). The heterotrophic microbial oxygen consumption also contribute greatly to the oxygen flux at the sediment-water interface, which is an important and dynamic compartment in the benthic systems (Flindt and Nielsen, 1992).

In the Huanghe River Delta, the extreme increase of the snail

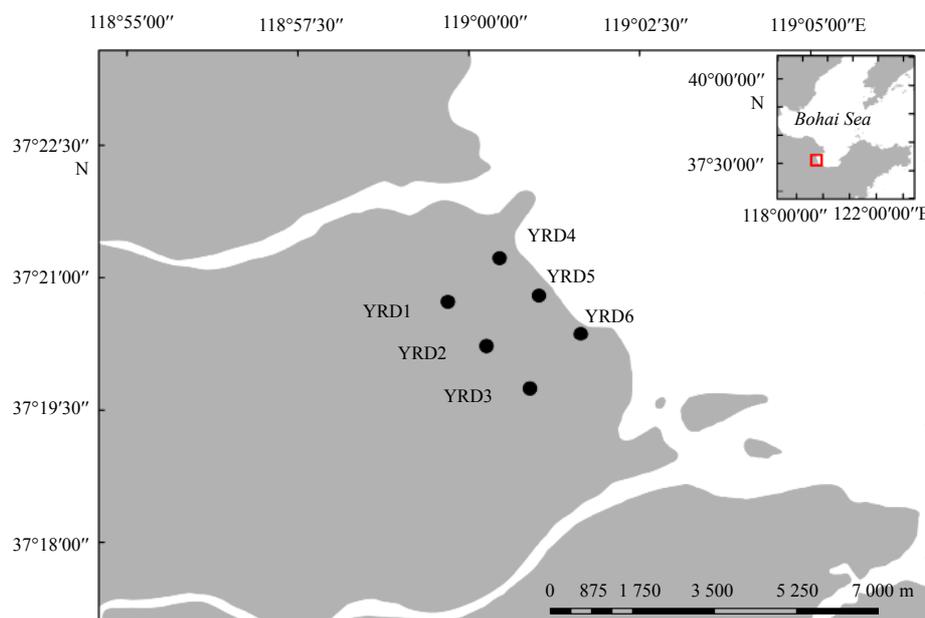
*B. exarata* has raised the question if this key species may influence the productivity or photosynthetic rate of the microphytobenthos (MPB). In the present paper, by means of mesocosm studies, we test the hypothesis that the key species *B. exarata* has major impact upon (1) MPBs productivity, (2) nutrient exchange between sediment-water interface and (3) sediment-related metabolic rates.

## 2 Materials and methods

### 2.1 Collection and acclimation

To investigate the current status of mud snails around YRD, we selected six sites to determine the density of mud snails in the following experiment (Fig. 1). Two treatments of sediment, defaunated sediment and normal sediment, were set in the experiment to understand the bioturbation effect of different groups of organisms on sediment, e.g., micro/meiofauna, microphytobenthos and mud snail. Plots of 2 m<sup>2</sup> sediment was defaunated by covering the sediment surface with thick plastic sheeting (Siebert and Branch, 2006; Montserrat et al., 2009). The plots were covered for a period of 40 d to impose a long anoxic condition and to get rid of the micro/meiofauna and microphytobenthos. When the defaunation period was finished, defaunated and untreated sediment, seawater and mud snails were collected from one site of the intertidal zone of YRD (Fig. 1, YRD1). Intact sediment cores were collected carefully by shovel and then passed through a 1 mm mesh to remove macrofauna and larger particle. The collected sediments were transferred directly to the lab within 3 h. The sediments were set in the tanks for 2 d for its settling into some sort of steady state, i.e., recover the biogeochemical zonation within the sediment. The mud content (<63 μm; Montserrat et al., 2009) and the median grain size were measured by Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK), showing that 86.7% muddy sediment in the silt and was named as cohesive sediment (median grain-size  $D_{50}$ =158.8 μm)

Active mud snails were collected at the same time by picking the individuals from the surface of sediment. Seawater was collected from the same area. As the laboratory temperature and



**Fig. 1.** Sampling sites of mud snail and sediment in the Huanghe River Delta.

benthic environmental factors were same to that of *in situ*, the acclimation duration for animals was 2 d prior to the experiment. Two tanks were filled to a depth of 15 cm sediment and the remaining volume (20 cm deep) was gently flooded with seawater. Upon introduction of the healthy active mud snails into the tanks, they burrowed rapidly (<10 min) into the sediment.

## 2.2 Experimental measurements

The experiments were conducted in two tanks filled with 15 cm of two kinds of sediment: defaunated sediment and normal

sediment (containing MPBs and micro and meiofauna). Each tank had four respiration chambers equipped with a magnetic stirring bar (20 cm in diameter, 33 cm in height). During the experimental period, the chambers were sealed with a lid. The experimental setting and process were followed as explained by Li et al. (2013). Pairs of 12-h experiments were conducted in two consecutive days, the first one in the light and the second in the dark with the same sediment used for each light/dark pair of experiments. The light intensity was  $\sim 450 \mu\text{mol}/(\text{m}^2\cdot\text{s})$  during the simulated daylight experimental periods (Fig. 2).

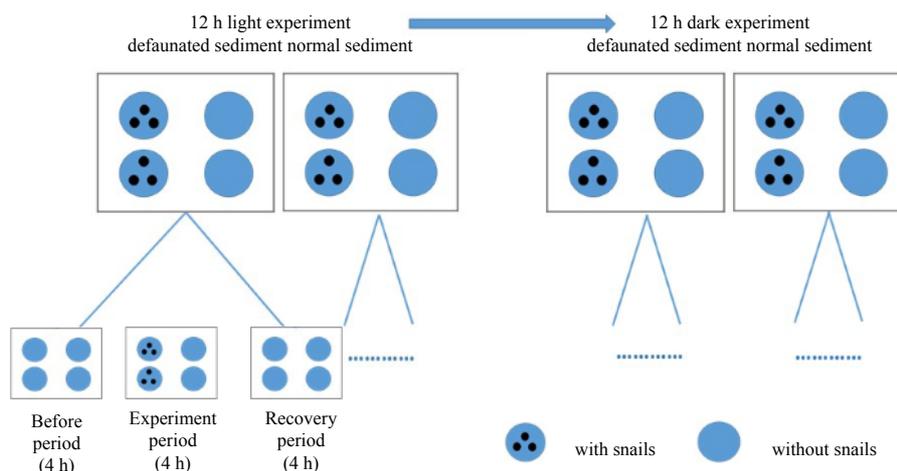


Fig. 2. The experimental set-up used for measuring the oxygen and nutrient fluxes.

In each 12-h experiment, there were three 4-h periods: “Before” period (background measurement), “Experiment” period and the “Recovery” period. In “Before” period, empty respiration chambers were carefully inserted into the sediment so as not to disturb the MPB controls and used to measure the background oxygen and nutrient fluxes of sediment and MPB. In the second 4-h period (“Experiment”), three active mud snails were introduced into two of the chambers in each tank to measure the effect of the interaction between the animals and MPB, and the other two chambers in each tank serve as controls. The mud snail density is equivalent to  $(100 \pm 51) \text{ ind.}/\text{m}^2$ , which is close to the average densities observed in the study area<sup>①</sup>. The control chambers were lifted from the sediment so as to be treated in the same manner as the experimental chambers which had to be lifted from the sediment to insert the animals. In the final 4-h “Recovery” period, animals were removed to measure the ongoing effect of the bioturbation. Again, the same level of disturbance from handling the chambers was applied to both treatments and controls. During each 12-h experiment, hourly measurements of oxygen consumption rate and ammonia-N excretion rate were conducted by taking 20 mL water sample with syringe from each chamber. Dissolved oxygen concentrations were measured using YSI 5100 dissolved oxygen meter (Cole-Parmer, YSI 5100 OUR/SOUR, USA). Duplicate samples of incubation chamber were taken for analyzing ammonia and nitrate which were frozen immediately and kept at  $-20^\circ\text{C}$  until analysis. Nutrient concentrations, including nitrate ( $\text{NO}_3\text{-N}$ ), nitrite ( $\text{NO}_2\text{-N}$ ), ammonium ( $\text{NH}_4\text{-N}$ ) and soluble reactive phosphorus ( $\text{PO}_4\text{-P}$ ), were measured by flow injection analysis (AA3, Bran+Luebbe, Germany). The analysis procedures followed the WOCE (World Ocean Circulation Experiment) Methods Manual WHPO 91-1 (Gordon et

al., 1993; JGOFS Protocols, June 1994). The limits of detection were  $0.015 \mu\text{mol}/\text{L}$  for  $\text{NO}_3\text{-N}$ ,  $0.003 \mu\text{mol}/\text{L}$  for  $\text{NO}_2\text{-N}$ ,  $0.04 \mu\text{mol}/\text{L}$  for  $\text{NH}_4\text{-N}$ , and  $0.02 \mu\text{mol}/\text{L}$  for  $\text{PO}_4\text{-P}$ . The fluxes were from the slopes of linear regressions of oxygen concentration against time (Michaud et al., 2005, 2006).

## 2.3 Data analysis

The relationships between mud snail respiration, normal sediment/defaunated sediment and dark/light were analyzed by independent samples *T* test and Kruskal-Wallis test in the case of heterogeneity of variance, to determine the significance of variation among means of all measured variables. A significance level of 5% was used in all analyses. All the variables were  $\lg(X+10)$  transformed before proceeding significance test.

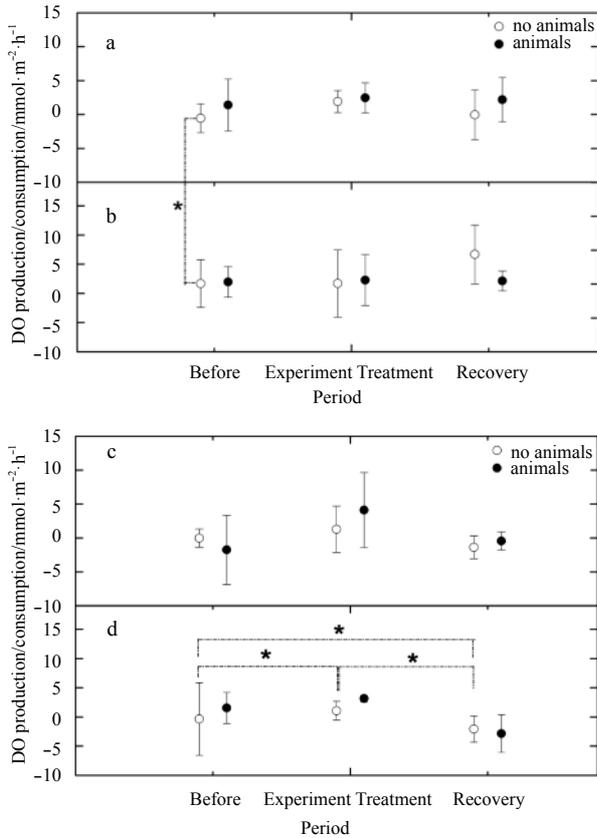
## 3 Results

### 3.1 Benthic respiration and primary production

Normally, the presence of snails could increase the oxygen production indicated by the oxygen flux values of the “Experiment” period in the groups with snails which was higher than that of the groups without snails (Fig. 3). In the light and normal sediment during experiment period with snails, the oxygen flux value was significantly higher ( $3.14 \text{ mmol}/(\text{m}^2\cdot\text{h})$ ) than that in the before period ( $1.53 \text{ mmol}/(\text{m}^2\cdot\text{h})$ ) and the recovery period ( $-2.85 \text{ mmol}/(\text{m}^2\cdot\text{h})$ ) (Chi-sq=6,  $P<0.05$ ; Kruskal-Wallis test) (Fig. 3d). There was no significant difference among the three periods for the groups without snails (Chi-sq=2.9,  $P>0.05$ ; Kruskal-Wallis test) (Figs 3c and d).

In dark, the oxygen flux of the defaunated sediment was signi-

<sup>①</sup> Li Baoquan, Wang Quanchao, Li Xiaojing, Chen Linlin. Population characters of mud snail *Bullacta exarata* in the Huanghe River Delta (in preparation).



**Fig. 3.** The oxygen production and consumption interaction between mud snail activities and MPB production response from experiments in August 2012 at different conditions, respectively, namely, dark and defaunated sediment (a), dark and normal sediment (b), light and defaunated sediment (c), and light and normal sediment (d). The oxygen production and consumption were the average of four repeated experiments with standard deviation. Positive values indicate efflux of materials out of the sediment; and negative values influx into the sediment. “Before”, “Experiment Treatment” and “Recovery” were the three periods of each 4 h set up in sequence. In “Before” period, without animals; in “Experiment Treatment”, three individuals were introduced in two of the four chambers; and in “Recovery” period, the three individuals were removed, without animals again. \* indicates the significant difference ( $P < 0.05$ ) between/among the treatments.

ificantly lower than that of normal sediment during the “Before” period with a negative value, which indicated defaunated sediment consumes more oxygen from the water and the normal sediment with the presence of micro/meiofauna and MPB can significantly decrease the oxygen consumption on these conditions (normal sediment vs. defaunated sediment,  $P < 0.05$ , independent samples  $T$  test) (Figs 3a and b). However, on other conditions, the effect of snails on oxygen production and consumption between water-sediment interface were not significant ( $P > 0.05$ ; Kruskal-Wallis test).

### 3.2 Nutrient recycling and fluxes

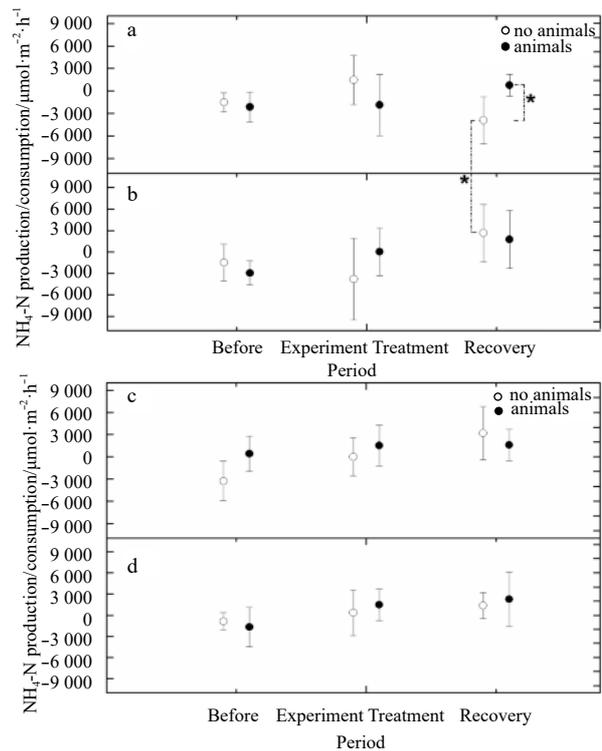
#### 3.2.1 Ammonia

Most of the average ammonia flux values in the “Recovery”

periods of both groups with and without snails were higher than those in the experiment and before periods. During recovery period in the defaunated and dark condition, the ongoing effect after removing snails showed that ammonia production had happened in the chambers with snails rather than ammonia consumption in the chambers without snails ( $P < 0.05$ , independent samples  $T$  test) (Fig. 4a). During the recovery period in dark, the presence of micro and meiofauna (normal sediment vs. defaunated sediment) can also significantly increase the ammonia production under these conditions ( $P < 0.05$ , independent samples  $T$  test) (Figs 4a and b). However, the effect of mud snail on ammonia flux between water-sediment interface under the other conditions were not significant ( $P > 0.05$ , Kruskal-Wallis test).

#### 3.2.2 $NO_3 + NO_2$ efflux

During recovery period in the defaunated sediment and dark condition, the ongoing effect after removing snails showed  $NO_x$ -N production was happened in the chambers previously with snail rather than  $NO_x$ -N consumption in the chambers previously without snails ( $P < 0.05$ , independent samples  $T$  test). And, in dark during the recovery period, the presence of micro and meiofauna (normal sediment vs. defaunated sediment) can also significantly increase the  $NO_x$ -N production on these conditions



**Fig. 4.** Ammonium production and consumption interaction between the activity of mud snails and microphytobenthic production response from experiments in August 2012 at different conditions, respectively, namely, dark and defaunated sediment (a), dark and normal sediment (b), light and defaunated sediment (c), and light and normal sediment (d). The Ammonium production/consumption were the average of four repeated experiments with standard deviation. Positive values indicate efflux of materials out of the sediment; and negative values influx into sediment. “Before”, “Experiment Treatment” and “Recovery” were the same to that in Fig. 3. \* indicates the significant difference ( $P < 0.05$ ) between/among the treatments.

( $P < 0.05$ , independent samples  $T$  test) (Figs 5 and 6).

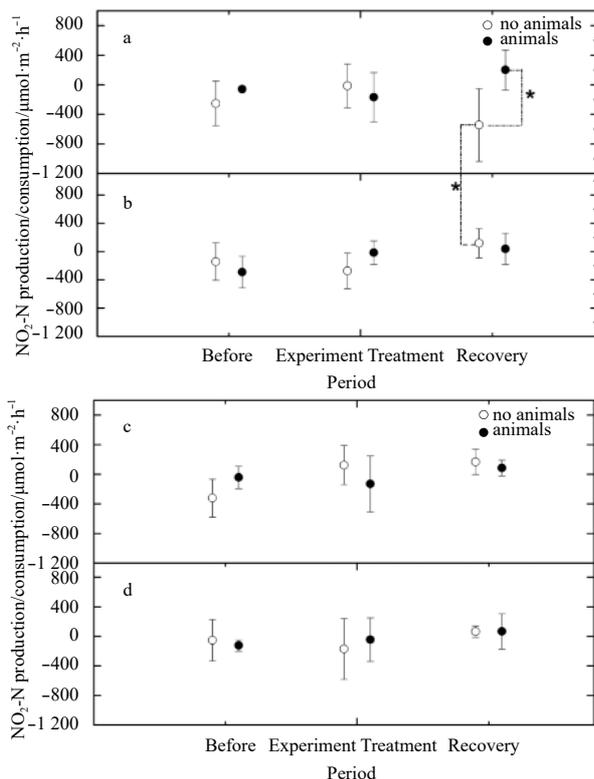
### 3.2.3 Phosphate flux

During the “Recovery” period in the defaunated and dark condition, the effect of removing snails showed that phosphate production happened in the chambers with snail rather than phosphate consumption in the chambers without snails ( $P < 0.05$ , independent samples  $T$  test). In addition, in light condition during the recovery period, the presence of micro and meiofauna (normal sediment vs. defaunated sediment) can result in the phosphate consumption ( $P < 0.05$ , independent samples  $T$  test). However, the effect of mud snail on phosphate flux between water-sediment interface under the other conditions were not significant ( $P > 0.05$ , Kruskal-Wallis test) (Fig. 7).

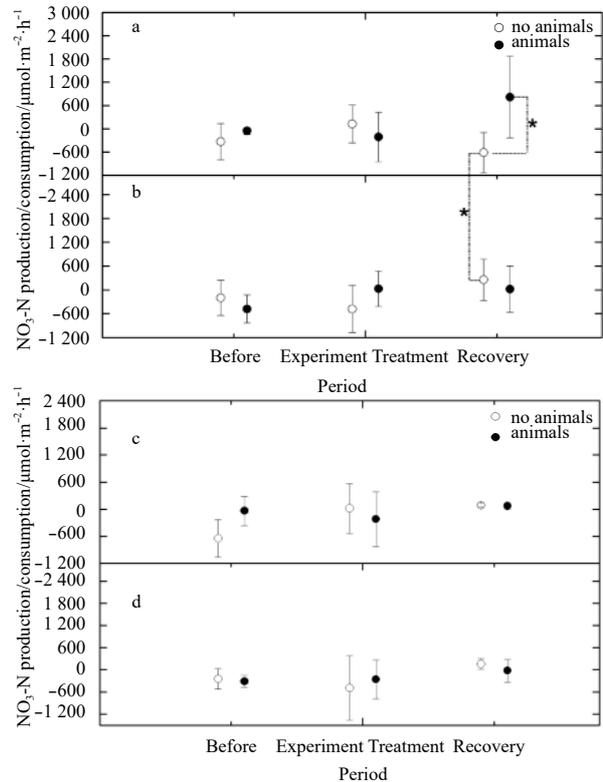
## 4 Discussion

### 4.1 Effect of the presence of mud snails, micro- and meiofauna on the oxygen flux

In the present paper, the presence of mud snail had significantly impacted the DO flux across the sediment-water interface in the condition of normal sediment and light treatment.



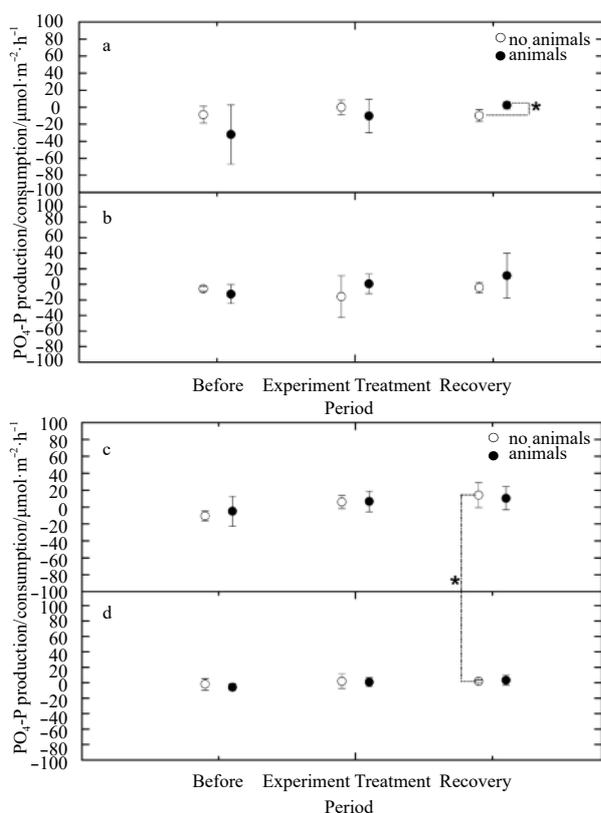
**Fig. 5.** Effect of mud snail *Bullacta exarata* on nitrite fluxes from experiments in August 2012 at different conditions, respectively, namely, dark and defaunated sediment (a), dark and normal sediment (b), light and defaunated sediment (c), and light and normal sediment (d). The nitrite production and consumption were the average value of four repeated experiments with standard deviation. Positive values indicate efflux of materials out of the sediment; and negative values influx into sediment. “Before”, “Experiment Treatment” and “Recovery” were the same to that in Fig. 3. \* indicates the significant difference ( $P < 0.05$ ) between/among the treatments.



**Fig. 6.** Effect of mud snail *Bullacta exarata* on nitrate fluxes from experiments in August 2012 at different conditions, respectively, namely, dark and defaunated sediment (a), dark and normal sediment (b), light and defaunated sediment (c), and light and normal sediment (d). The nitrate production and consumption were the average of four repeated experiments with standard deviation. Positive values indicate efflux of materials out of the sediment; and negative values influx into sediment. “Before”, “Experiment Treatment” and “Recovery” were the same to that in Fig. 3. \* indicates the significant difference ( $P < 0.05$ ) between/among the treatments.

However, the effect of mud snail was not significant in other conditions, which indicates the mud snail with natural average density ( $(100 \pm 51)$  ind./ $m^2$ ) had less effect on the DO flux except for the conditions in the daytime. In fact, the mud snail usually prefers to gather together in shallow puddles of intertidal zone, leading its density up to  $262$  ind./ $m^2$  (Li et al., 2019), which is much higher than the density we set in the present work. Normally, the higher strength of bioturbation (density), the more disturbance effects on sediment. The presence of macrofauna could affect the distribution of solutes and microbial population through biogenic changes in material transport, relative reaction rates and indirect/direct interactions (such as feeding activity) (Aller and Yingst, 1985). Moreover, the benthic sediment mineralization also plays an important ecological role on primary producers in overlying water with supply of a significant fraction of the nutrients in the water column (Callender and Hammond, 1982).

The normal sediment was sieved through a 1 mm mesh, for both micro- and meiofauna (Tita et al., 2002). The presence of these animals can change the transport and reaction rates at the sediment-water interface and oxic processes in the bioturbated zone (Rysgaard et al., 2000; Røy et al., 2002). While, the bioturbation should have less effects on those processes in defaunated



**Fig. 7.** Effect of mud snail *Bullacta exarata* on phosphate fluxes from experiments in August 2012 at different conditions, respectively, namely, dark and defaunated sediment (a), dark and normal sediment (b), light and defaunated sediment (c), and light and normal sediment (d). The phosphate production and consumption were the average of four repeated experiments with standard deviation. Positive values indicate efflux of materials out of the sediment; and negative values influx into sediment. “Before”, “Experiment Treatment” and “Recovery” were the same to that in Fig. 3. \* indicates the significant difference ( $P < 0.05$ ) between/among the treatments.

sediment. Our finding in the present work also agrees with that, namely, the presence of micro and meiofauna significantly decrease the oxygen consumption in dark treatment comparing to that of defaunated sediment. Another important reason is that the defaunated sediment changed the heterotrophic microbial structure and elevated the organic matter content within the sediments (Freitag et al., 2003), which improves the heterotrophic microbial oxygen consumption in the sediment, and changes the oxygen flux in the sediment (Flindt and Nielsen, 1992).

However, these small animals had less effects on the DO flux between sediment-water interface in light treatment, which was mostly due to the net primary production by the MPBs. The MPBs produce enough oxygen to compensate for the oxygen consumed by the heterotrophs in the sediment. The benthic microalgae use nutrients and regulate benthic processes at the sediment-water interface, which are probably due to their photosynthetic activity, oxygen production and enhancement of aerobic processes (Zilius et al., 2012). Another reason was that the key species in the benthic community were often responsible for most of the bioturbation (Davey, 1993). In the present work, the mud snail was the key species and should contribute more to the

bioturbation effect than micro and meiofauna.

#### 4.2 Effect of mud snails, micro and meiofauna on the nutrient recycling and fluxes

Ammonium ( $\text{NH}_4\text{-N}$ ) was produced by animal excretory activities which could be nutrient source for MPBs at the sediment surface. Some animals like *Echinocardium* has positive effect on ammonium efflux from sediments in dark chambers and supply a utilizable form of inorganic nitrogen to the overlying waters (Lohrer et al., 2004). The mud snail also had positive effect on the ammonium efflux during recovery period in the defaunated sediment and dark treatment. The mechanisms might be complicated. The activities of mud snail might not directly produce  $\text{NH}_4\text{-N}$ , but they can excrete the mucus and feces pellets on the sediment surface, which are a very good source for heterotrophic mineralization and thus  $\text{NH}_4\text{-N}$  is produced during this process. However, the effect of mud snail on ammonia flux during experiment period was not significant. The reason can be explained by the effect of “time lag” on the release of  $\text{NH}_4\text{-N}$ . It takes time for the heterotrophic mineralization on mucus and feces pellets produced by mud snail during experiment period to produce  $\text{NH}_4\text{-N}$  and release them from the sediment into the overlying waters during the recovery period.

The presence of micro and meiofauna also significantly increased the  $\text{NH}_4\text{-N}$  flux in dark treatment during recovery period. Whereas, in light treatment, these small animals had less effect on the  $\text{NH}_4\text{-N}$  flux between sediment-water interface, which was mostly due to the  $\text{NH}_4\text{-N}$  consumption by MPBs during the process of photosynthesis. Macrofauna could increase the net  $\text{NH}_4\text{-N}$  production rate by 20%–30% via lowering of inhibitory metabolite concentrations, favorable oxygen, injection of new substrate into sediment such as mucus secretions, and stimulation of bacteria during grazing (Aller and Yingst, 1985). In the treatment group, the  $\text{NH}_4\text{-N}$  efflux in the experiment period was higher than that of the before period suggesting that ammonia excretion by the animal was greater than uptake of  $\text{NH}_4\text{-N}$  by the microphyte community and nitrification by bacteria. However, in the control group with defaunated sediment, the  $\text{NH}_4\text{-N}$  efflux in the experiment period was also higher than the before period suggesting it is an experimental artifact, which is hard to interpret here.

Mud snail also affected the  $\text{NO}_x\text{-N}$  and phosphate flux between sediment-water layer during recovery period in the defaunated and dark condition, without showing any significant difference on other conditions, which are most likely due to the complex process of nitrification and denitrification happened inside the sediment. The factors involved in this process were comprehensive, including N availability, oxic condition, microbial processes, presence of animals and MPBs. The process of nitrification and denitrification can be either stimulated or depressed by MPBs activity, depends on the N availability (Bartoli et al., 2003; Nils, 2003; Sundbäck and McGlathery, 2005). Further study shows that the daytime denitrification rates exceeded nighttime rates because the oxygen concentrations decreased at night, then the benthic microalgae enhanced the oxygen production rates, coupling between the rate of nitrification and denitrification when ammonium was not limited (An and Joye, 2001). Besides the photosynthesis by MPB can expand the oxic sediment horizon with a potential positive effect on nitrifiers (Revsbech et al., 1981). We also found that the presence of micro and meiofauna produced  $\text{NO}_x\text{-N}$  during the recovery period in dark. Another fact is, the benthic macrofauna can enhance solute regeneration and stimulate both aerobic and anaerobic microbial processes,

e.g., nitrification and denitrification by metabolic processes (e.g., excretion) and behavioral activities (e.g., bioturbation) (Nizzoli et al., 2007). The active transport of particulate matter, including biodeposition and bioresuspension, can affect mineralization dynamics as well (Graf and Rosenberg, 1997).

Mud snail *B. exarata* has proven to be a very successfully introduced species for aquaculture in the intertidal zone of the Huanghe River Delta and brought substantial economic benefits for local people and government. However, the ecological consequences of the mud snail spread is poorly known and becomes an urgent issue to be solved by both the local government and scientists.

## 5 Conclusions

We investigated the effect of the presence of mud snail on oxygen and nutrient fluxes between sediment-water interface in this work and concluded as the followings: (1) Mud snail significantly impacted the DO flux across the sediment-water interface under the condition of normal and light treatment, and the ammonium flux as well as nutrients during recovery period in the defaunated sediment and dark treatment. (2) The presence of micro and meiofauna significantly increased the  $\text{NH}_4\text{-N}$  flux in dark treatment during recovery period but had less effect on the  $\text{NH}_4\text{-N}$  flux in light treatment, which was mostly due to the utilization of  $\text{NH}_4\text{-N}$  by MPBs in photosynthesis.

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