

Benthic nutrient fluxes influenced by the common carp *Cyprinus carpio* in pond polyculture with special reference to nitrification, denitrification and nitrate ammonification

DASEN ZHONG, FANG WANG, SHUANGLIN DONG AND SACHIN ONKAR KHAIRNAR*

The Key Laboratory of Mariculture, Ministry of Education, Fisheries College, Ocean University of China
Qingdao – 266 003, People's Republic of China

*ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar - 752 104, Odisha, India
e-mail: wangfang249@163.com

ABSTRACT

Cyprinus carpio is an important species in pond aquaculture as it can effect bioturbation of sediments which might influence benthic fluxes and biogeochemical processes at the sediment-water interface. In this study, the influence of common carp *Cyprinus carpio* on nutrient fluxes, specifically nitrification, denitrification and nitrate ammonification rates at the sediment-water interface in pond polyculture was investigated through field experiments and *ex situ* incubation. *C. carpio* enhanced the sediment oxygen consumption (SOC) from April to September (except in July). The fish did not influence the $\text{NH}_4^+\text{-N}$ flux, but promoted $\text{NO}_x^-\text{-N}$ ($\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$) release from the sediment to the water from May to September and it also promoted soluble reactive phosphorus (SRP) release from July to September. Nitrification, denitrification and nitrate ammonification were also significantly promoted by *C. carpio*. The results suggest that *C. carpio* can play a very important role in the migration and transformation of nitrogen and phosphorus at sediment-water interface which could also help nitrogen removal in pond water.

Keywords: *Cyprinus carpio*, Denitrification, Nitrate ammonification, Nitrification, Nutrient fluxes, Polyculture, Pond aquaculture, Sediment-water interface

Introduction

Pond culture is the most important aquaculture practice in China since thousands of years and the production has touched 1.94 million t (National Bureau of Statistics, 2012). Pond aquaculture plays an important role especially in the integrated polyculture sector, which has become the main mode of pond aquaculture in China. Polyculture incorporating different fish species is a means of species diversification, which can also lead to improvement of the aquaculture environment and ecology (Soto, 2009). *Cyprinus carpio* is a popular carp species used in pond polyculture (Naylor *et al.*, 2000).

The sediment-water interface is where, the most significant variations in physical, chemical and biological characteristics takes place and has an important role in material transport and exchange in natural water bodies (Nixon, 1986). The migration and transformation of nutrients at the sediment-water interface affect the budget and circulation dynamics of nutrients (Thibodeau *et al.*, 2010) and is also related to the eutrophication in water bodies (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 the prime limiting nutrients in water and these

are also the key factors responsible for the quality of aquaculture waters (Paerl, 2009; Xu *et al.*, 2010). The main processes in the migration and transformation of nitrogen and phosphate at sediment-water interface are the diffusion of nitrogen and phosphate across sediments and the overlying water as well as nitrification, denitrification and nitrate ammonification mediated by microorganisms. These processes can directly decide the budget of nitrogen and phosphate in aquaculture water and finally affect the water quality (Eyre *et al.*, 2011).

Macrobenthic organisms are important components at sediment-water interface as these can change sediment grain size, porosity, permeability and other physical properties by digging, feeding, respiration, excretion and other activities and also change the original biological and chemical properties of sediment which affect the migration and transformation of nutrients at sediment-water interface (Meysman *et al.*, 2006). There are few reports on the effects of bioturbation on the migration and transformation of nitrogen and phosphate at sediment-water interface, especially due to *Apostichopus japonicus* (Zheng *et al.*, 2009), *Ruditapes philippinarum* (Nizzoli *et al.*, 2006), *Neohelice granulata* (Fanjul *et al.*, 2011) and

Nereis spp. (Nizzoli *et al.*, 2007). However, there is no such detailed report on the common carp *Cyprinus carpio*, which is an important species in pond polyculture.

In this study, sediment oxygen consumption (SOC), nitrogen ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and $\text{NO}_2^-\text{-N}$) and soluble reactive phosphorus (SRP) fluxes and the rates of nitrification, denitrification and nitrate ammonification were measured in order to study the effect of *C. carpio* on the migration and transformation of nitrogen and phosphate at the sediment-water interface.

Materials and methods

Experimental site and setup

The study was conducted in aquaculture ponds at Zhaodian Village, Gaoqing City, Shandong Province in the Peoples Republic of China from April to September, 2012. The ponds were rectangular with an area of 2333.33 m², length to width ratio of 2:1 and an average water depth of 1.5 m. Two treatments were set with three replicates each: treatment GS was set with grass carp (*Ctenopharyngodon idella*) and silver carp (*Hypophthalmichthys molitrix*) and treatment GSC with grass carp, silver carp and common carp (*Cyprinus carpio*). The details of stocking in each treatment is listed in Table 1. The stocking and feeding started from early to mid-April. Each pond was equipped with a feeding machine. The same amount of factitious composite feed was provided to each pond four times a day. The feeding quantity was decided as per the requirement of each species/fishes and the weather conditions. One 2000-W impeller type aerator, situated at the center of each pond, was activated from 0:00 to 6:00 hrs and 11:00 to 13:00 hrs every day. The fishes were harvested in October.

Table 1. Details of stocking

| Treatment | Stocking density (No. m ⁻²) | Size range (kg per individual) |
|-----------|--|-----------------------------------|
| GS | G - 1.84; S - 0.92 | G - 6 - 8 S - 4 - 6 |
| GSC | G - 1.84; S - 0.92; C - 0.55 | C - 8 - 10 |

GS: Grass carp and silver carp; GSC: Grass carp, silver carp and common carp

Monthly 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) and two samples were collected from each site. Sediment samples were collected using a core sampler, with minimum disturbances to the sediment surface (Wang and Xu, 2004). The chamber

(d=5 cm, h=33 cm) containing sediment was removed from the core sampler, sealed with rubber lids and transported to the laboratory within 1 h. In the laboratory, the sediments in the chambers were adjusted to a depth of 8 to 10 cm and kept for stabilising for 2 h. Bottom water samples were collected with a water sampler. The dissolved oxygen (DO) concentration and water temperature were measured with a digital dissolved oxygen meter (YSI). Bottom water samples (3 l) from each sampling site was brought to the laboratory, where 100 ml of the water was filtered through 0.45 μm cellulose acetate membranes and frozen at -20°C for analyses of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$, and SRP; and the rest of the samples were used for the *ex situ* incubation experiments.

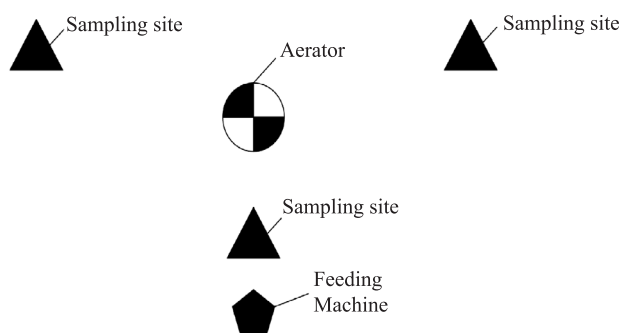


Fig. 1. Diagrammatic representation of the sampling sites in each pond

Determination of oxygen and nutrient fluxes

Oxygen and nutrient fluxes were determined by *ex situ* incubation. After stabilising the sediment, 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 was sealed with rubber lids and the upper lid was equipped with a magnet that was stirred using a central magnet at 50 rpm (Fig. 2). Incubation was conducted in a water bath incubator for 4 h in darkness at ambient temperature. Initial and final overlying water samples (30 ml each) were taken from each chamber, DO concentration was measured using a digital dissolved oxygen meter (YSI), and then filtered through 0.45 μm cellulose acetate membrane and frozen at -20°C for later analyses of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$ and SRP.

Determination of nitrification, denitrification and nitrate ammonification rates

Nitrification, denitrification and nitrate ammonification rates were determined using modified acetylene (C_2H_2) inhibition method (Kim *et al.*, 1997). After stabilising the sediment and water, one sample from each site was inhibited by C_2H_2 , by carefully replacing 30 ml of the overlying

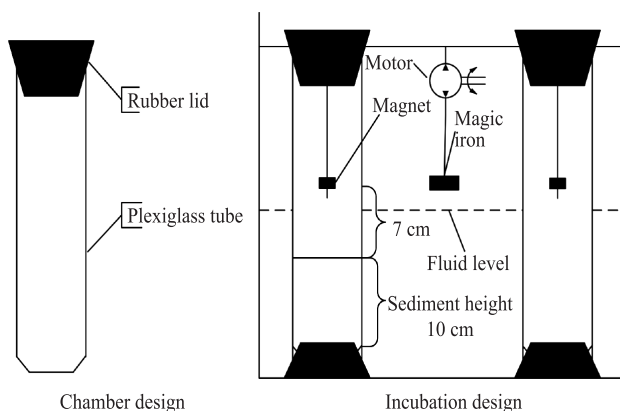


Fig. 2. Sketch showing sediment incubation set up using the plexiglass chambers

water with 30 ml of C_2H_2 -saturated water. Initial and final overlying water samples were collected from each chamber and 0.3 ml aliquot of saturated $HgCl_2$ solution was added to 60 ml of water in glass bottles for the measurement of N_2O . The remaining 30 ml of water was filtered through 0.45 μm cellulose acetate membrane and frozen at $-20^\circ C$ until used for analyses of NH_4^+-N , $NO_3^- -N$ and $NO_2^- -N$.

NH_4^+-N , $NO_3^- -N$, $NO_2^- -N$, and SRP contents in the bottom water were determined by spectrophotometric method according to Lei (2006). The N_2O content was determined by headspace gas chromatography.

Sediment oxygen consumption (SOC), nutrient fluxes and the rates of nitrification, denitrification and nitrate ammonification were calculated using the following equation (Zhong *et al.*, 2013):

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

where F ($\mu mol m^{-2} d^{-1}$ for denitrification rate and $mmol m^{-2} d^{-1}$ for the other variables) represents SOC, nutrient fluxes and the rates of nitrification, denitrification and nitrate ammonification; V (m^3) is the volume of the incubator; A (m^2) is the bottom area of the incubator; Δt (d) is the incubation duration; and ΔC ($mmol m^{-3}$) is the change in the concentrations of DO, ammonia, N_2O , and nitrate before and after incubation in respective cases.

Statistical analysis

Statistical analysis of the data was performed using statistical package SPSS 13.0 for Windows (SPSS Inc., Richmond, CA, USA). Concentrations of ammonia, 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 further to reduce the chance of a type I error (falsely identifying a significant difference).

Results and discussion

Sediment oxygen consumption and nutrient fluxes

The parameters measured in the two treatments are presented in Table 2. The water temperature changed with season during the experiment which ranged between $21.5^\circ C$ in April and $29.2^\circ C$ in July. There are many factors that influence bottom water DO in ponds, SOC being one of the key factors. The DO concentrations varied between 2.31 and 10.78 $mg l^{-1}$, the highest DO concentrations were observed in April and the lowest in August, in the two treatments. In the present study, the SOC rates in treatment with *C. carpio* was significantly higher ($p < 0.05$) all through the study, except in July (Fig. 3a). *C. carpio* prefers to live at the bottom of the water body and their activities would dig and stir the sediments, which would make more organic particles exposed to water which in turn consume more oxygen (Avnimelech *et al.*, 1999). The excretion of *C. carpio* could also contribute to the content of organic matter in the sediments, and the rates of SOC generally increase with the enrichment of sediments (Boyd, 1995).

The fluxes of NH_4^+-N , $NO_x^- -N$ and SRP in the two treatments were negative in the month of April, indicating that these nutrients were absorbed by the sediment from the water at the beginning of the study, but became positive ($p > 0.05$) in the succeeding months (Fig. 3b - d).

No significant difference was found in the NH_4^+-N fluxes between the two treatments except in the month of August ($p > 0.05$), while the $NO_x^- -N$ fluxes in treatment GSC were significantly higher than in treatment GS from May onwards ($p < 0.05$). During the first three months of the experiment, the SRP fluxes in the two treatments showed no significant difference, however from the month of July onwards, the SRP fluxes in treatment GSC were significantly higher than in treatment GS ($p < 0.05$).

Sediment nitrification, denitrification and nitrate ammonification rates

Nitrification is the process by which ammonia is oxidised to nitrate by microorganisms, and can be influenced by many factors such as dissolved oxygen, water temperature, substrate concentration, pH, the number of nitrifying bacteria and available surface (Hargreaves, 1998). The nitrification rates in the two treatments ranged from 4.32 ± 1.12 to 113.56 ± 11.51 $mmol m^{-2} d^{-1}$ (Fig. 4). There was no significant difference between the nitrification rates of the two treatments in April and May, but the situation changed since June with the rates in treatment

Table 2. Bottom water parameters recorded in the experimental treatments

| Parameters | NH ₄ ⁺ -N (mg l ⁻¹) | NO ₃ ⁻ -N (mg l ⁻¹) | NO ₂ ⁻ -N (mg l ⁻¹) | SRP (mg l ⁻¹) | DO (mg l ⁻¹) | T (°C) |
|---------------|--|--|--|------------------------------|-----------------------------|-----------|
| April | | | | | | |
| Treatment GS | 3.118±0.086 | 0.385 ± 0.021 | 0.013±0.0018 | 0.105±0.004 | 8.73 | 21.5 |
| Treatment GSC | 3.003±0.271 | 0.374±0.056 | 0.014±0.0007 | 0.099±0.014 | 10.78 | |
| May | | | | | | |
| Treatment GS | 0.280±0.031 | 0.539±0.017 | 0.011±0.0015 ^a | 0.007±0.0002 | 6.41 | 25.4 |
| Treatment GSC | 0.242±0.089 | 0.490±0.022 | 0.028±0.0017 ^b | 0.009±0.0003 | 6.02 | |
| June | | | | | | |
| Treatment GS | 1.642±0.117 | 0.463±0.014 ^a | 0.012±0.0008 ^a | 0.003±0.0002 | 3.85 | 27.7 |
| Treatment GSC | 1.469±0.143 | 0.530±0.028 ^b | 0.003±0.0006 ^b | 0.004±0.0003 | 2.33 | |
| July | | | | | | |
| Treatment GS | 0.397±0.06 | 0.663±0.027 ^a | 0.027±0.0099 ^a | 0 ^a | 2.74 | 29.2 |
| Treatment GSC | 0.504±0.09 | 1.329±0.089 ^b | 0.743±0.2260 ^b | 0.072±0.0001 ^b | 1.16 | |
| August | | | | | | |
| Treatment GS | 0.885±0.045 ^a | 0.207±0.051 ^a | 0.062±0.0067 ^a | 0.003±0.0002 | 2.31 | 27.2 |
| Treatment GSC | 1.090±0.045 ^b | 0.942±0.016 ^b | 0.497±0.0060 ^b | 0.005±0.0004 | 1.63 | |
| September | | | | | | |
| Treatment GS | 1.275±0.067 | 0.783±0.042 ^a | 0.012±0.0025 ^a | 0.002±0.0003 | 5.13 | 22.7 |
| Treatment GSC | 1.158±0.443 | 1.625±0.161 ^b | 0.172±0.0149 ^b | 0.003±0.0001 | 4.66 | |

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

^{a,b} Different superscripts in the same column and sampling month indicate significant differences (p < 0.05)

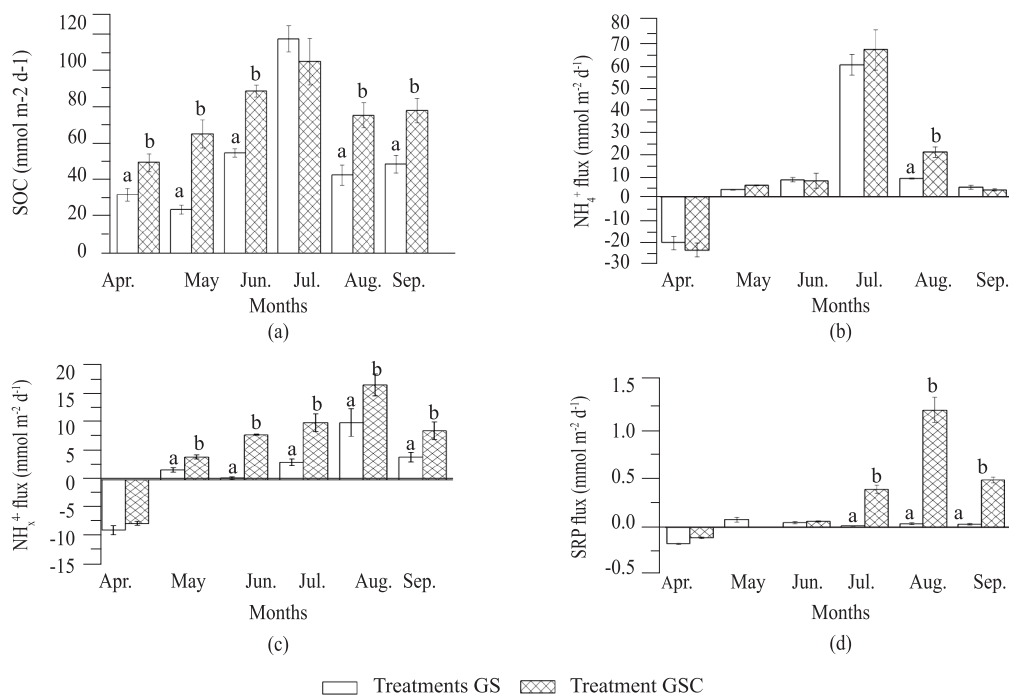


Fig. 3. Sediment oxygen and nutrient fluxes of the experimental treatments over 6 months. (a) SOC (sediment oxygen consumption); (b) NH₄⁺-N flux; (c) NO₃⁻-N + NO₂⁻-N flux; (d) SRP (soluble reactive phosphorus) flux. Bars denote standard deviation (n = 3). Positive fluxes indicate nutrient migration from 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 p = 0.05

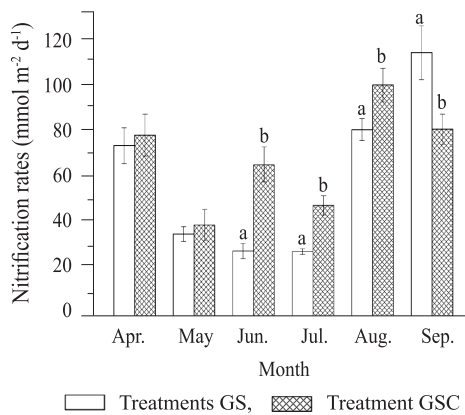


Fig. 4. Nitrification rate at the sediment-water interface in different treatments Bars denote standard deviation ($n=3$). Different letters indicate differences between treatments within the same month. Means that do not share a common letter are significantly different ($p<0.05$). Unlabeled columns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

GSC being significantly higher than in treatment GS from June to August ($p<0.05$). The rates of denitrification and nitrate ammonification in treatment GS were found to be low and showed little variation with time (Fig. 5 and 6). At the start of the experiment, the rates of denitrification and nitrate ammonification in treatment GSC were low too, but increased significantly ($p<0.05$) in July and were significantly higher than in treatment GS from July to September ($p<0.05$). The highest denitrification rate in treatment GSC ($161.79 \pm 6.02 \mu\text{mol m}^{-2} \text{d}^{-1}$) was about 24 times higher than in treatment GS and the highest nitrate ammonification rate in GSC ($14.23 \pm 0.16 \text{mmol m}^{-2} \text{d}^{-1}$) was about 18 times higher than in GS. It is inferred that, nitrification was promoted in treatment GSC from June to August because of the irrigation and sediment mixing by

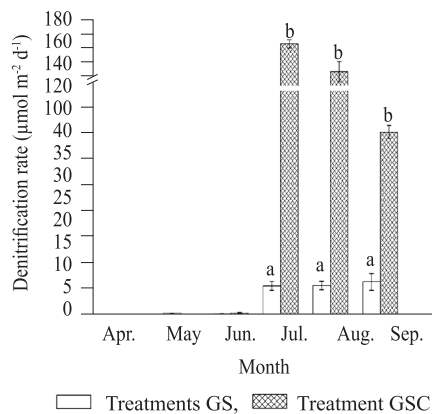


Fig. 5. Denitrification rate at the sediment-water interface in different treatments Bars denote standard deviation ($n = 3$). Different letters indicate differences between treatments within the same month. Means that do not share a common letter are significantly different ($p < 0.05$). Unlabeled columns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

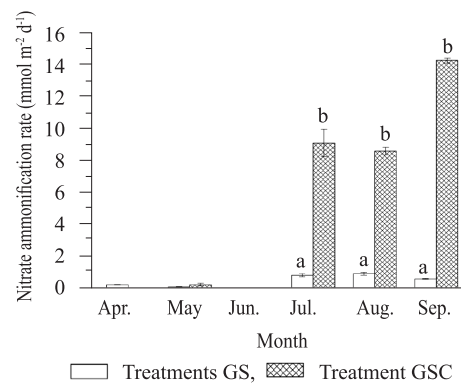


Fig. 6. Nitrate ammonification rate at the sediment-water interface in different treatments Bars denote standard deviation ($n = 3$). Different letters indicate differences between treatments within the same month. Means that do not share a common letter are significantly different ($p<0.05$). Unlabeled columns are not different at $p = 0.05$

C. carpio which flushed the sediments with oxygenated water (Welsh, 2003) and stimulated nitrifying bacteria in the sediment located immediately below the penetration depth of oxygen as well as in burrow linings (Henriksen *et al.*, 1983; Kristensen, 1985) and there was much $\text{NH}_4^+\text{-N}$ in the excretion of *C. carpio* (Kaushik *et al.*, 1982) which would provide substrate for nitrification.

During denitrification, microbes use organic compounds as carbon source and electron donor to reduce nitrite and nitrate into gaseous nitrogen (Hargreaves, 1998). Denitrification plays an important role in reducing N load and alleviating eutrophication in ponds. Olah *et al.* (1994) studied the nitrogen balance in Hungarian carp pond for 20 years and found that denitrification accounted for 34% of the total nitrogen removal. In the present study, the denitrification rates in both treatments were almost undetectable in the first three months, which could be attributed to the low concentration of DO. Studies have shown that denitrification takes place only at $\text{DO} < 0.2 \text{mg l}^{-1}$ (Hynes and Knowles, 1984). Initial water temperature in both the treatments was low while DO was relatively high, which was not suitable for denitrification before July. The denitrification rates in both treatments increased in July, especially in treatment GSC which stayed significantly higher since July with the biggest difference of 24 times. Michaud *et al.* (2006) observed that only when the bioturbation reached the denitrification zone, it could influence the denitrification rate. Zhong *et al.* (2013) found that *C. carpio* could effect bioturbation up to 10 cm depth, which is the denitrification zone. The physical mixing of sediment during burrow building and maintenance activities by *C. carpio* can serve to enhance the microbial communities in the sediment and introduce the nitrate into sediments (Papaspyrou *et al.*, 2006; Shang *et al.*, 2013),

thereby enhancing denitrification. At the same time, the enhanced nitrification could provide much more nitrate for denitrification.

Nitrate in water can be transformed into N_2O and N_2 by denitrification and then discharged into air. It can be reduced into NH_4^+-N again by nitrate ammonification, and the quantity of nitrate reduced *via* this way can be much higher than that formed through denitrification (Kaspar, 1982). Therefore it is evident that nitrate ammonification plays an important role in nitrogen cycle in ponds. The factors influencing nitrate ammonification rates are similar to that of denitrification. In this experiment, the changing trend of nitrate ammonification rates in the two treatments was very similar to that of denitrification rates. *C. carpio* could also promote nitrate ammonification later in the study, and the reason should be the same as mentioned with denitrification.

Bottom water quality and benthic fluxes

NH_4^+-N is the final product of protein breakdown (Walsh and Wright, 1995) and in water, NH_4^+-N (ammonium) and NH_3 (ammonia) are in equilibrium depending on the pH and water temperature (Emerson *et al.*, 1975), while it will be toxic to aquatic organisms when NH_3 accumulates to a certain amount (Randall and Tsui, 2002). NH_4^+-N flux across sediment-water interface is an important link of nitrogen cycle in water environment and it can affect the content and existence form of nitrogen in water directly. Zhang *et al.* (2011) considered that the excretion of benthic organisms could increase NH_4^+-N content in sediments, and subsequently it could promote the NH_4^+-N flux from the sediment to the water, but this was not found to be consistent in the present study as there was no significant difference found in the NH_4^+-N fluxes and the NH_4^+-N concentrations between the two treatments throughout the study (except in August). It can be presumed that there were many factors that influenced NH_4^+-N flux and the effect of each factor was not independent.

NO_x^-N comprises NO_3^-N and NO_2^-N and it is not only the product of nitrification but also the substrate of denitrification and nitrate ammonification. At the same time, the two forms can be transformed into each other and NO_2^-N is a potentially toxic inorganic compound to aquatic organisms (Jensen, 2003). *C. carpio* promoted NO_x^-N release from sediments to overlying water (Kristensen *et al.*, 2012) from May onwards. This may be due to the fact that the excreta of *C. carpio* accumulated in the sediments, which led to increase in the nitrate content of surface sediments and the benthic phytoplankton absorb inorganic nitrogen (NO_x^-N) only when NH_4^+-N was exhausted (Eviner and Chapin, 1997). Phytoplankton had a less significant effect on the content of NO_x^-N than on NH_4^+-N in the present study. The nitrification rates in treatment GSC were much higher than the sum of the

denitrification and nitrate ammonification rates which led to the accumulation of NH_4^+-N in the sediment (Fig. 5 and 6). From the water quality aspect, the variation of the content of NO_3^-N and NO_2^-N was consistent with that of NO_x^-N fluxes in the two treatments. This reveals that *C. carpio* could significantly increase the NO_3^-N and NO_2^-N concentrations in bottom water during later stages of culture and it was directly related to the release of NO_x^-N from the sediments to the water, caused by bioturbation of *C. carpio*.

In this study, the SRP flux remained at extremely low levels from April to June, while the SRP flux in treatment with *C. carpio* increased in July and was significantly higher in the last three months (Fig. 3d). It is inferred that *C. carpio* could also promote SRP migrating from sediments to water in the later stage of culture, as the excretion of SRP by benthic organisms played a major role in SRP release from sediment (Zhang *et al.*, 2011).

The stocking of *C. carpio* significantly enhanced NO_x^-N as well as SRP fluxes, nitrification, denitrification and nitrate ammonification. *C. carpio* also helped to promote the migration and transformation of nitrogen and phosphorus at sediment-water interface simultaneously. *C. carpio* also contributed towards increase of oxygen consumption in sediments and concentrations of NO_2^-N in bottom water later during the cultivation period which could contribute to pond productivity.

Acknowledgements

This work was supported by Nature Science Foundation of Shandong Province (Z.R2014 CM 042) and open Reserach Fund Program of Guangxi Key Laboratory of Marine Biotechnology (GLMBT-201402)

References

- Avnimelech, Y., Kochva, M. and Hargreaves, J. A. 1999. Sedimentation and resuspension in earthen fish ponds. *J. World Aquacult. Soc.*, 30(4): 401-409.
- Boyd, C. E. 1995. *Bottom soils, sediment, and pond aquaculture*. Springer, US, New York, 348 pp.
- Emerson, K., Russo, R. C., Lund, R. E. and Thurston, R. V. 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. *J. Fish. Res. Board Can.*, 32: 2379-2383.
- Eviner, V. T. and Chapin, T. S. 1997. Plant-microbial interactions. *Nature*, 385: 26-27.
- Eyre, B. D., Ferguson, A. J. P., Webb, A., Maher, D. and Oakes, J. M. 2011. Denitrification, N-fixation and nitrogen and phosphorus fluxes in different benthic habitats and their contribution to the nitrogen and phosphorus budgets of a shallow oligotrophic sub-tropical coastal system (southern Moreton Bay, Australia). *Biogeochemistry*, 102: 111-133.

- Fanjul, E., Bazterrica, M. C., Escapa, M., María, A. G. and Oscar, I. 2011. Impact of crab bioturbation on benthic flux and nitrogen dynamics of South-west Atlantic intertidal marshes and mudflats. *Estuar. Coast. Shelf Sci.*, 92: 629-638.
- Hargreaves, J. A. 1998. Nitrogen biogeochemistry of aquaculture ponds. *Aquaculture*, 166: 181-212.
- Henriksen, K., Rasmussen, M. B. and Jensen, A. 1983. Effect of bioturbation on microbial nitrogen transformations in the sediment and fluxes of ammonium and nitrate to the overlying water. *Ecol. Bull.*, 35: 193-205.
- Hou, D., He, J., Lu, C., Sun, Y., Zhang, F. and Otgonbayar, K. 2013. Effects of environmental factors on nutrients release at sediment-water interface and assessment of trophic status for a typical shallow lake, North-west China. *Sci. World J.*, 2013: 1-16.
- Hynes, R. K. and Knowles, R. 1984. Production of nitrous oxide by *Nitrosomonas europaea*: effects of acetylene, pH, and oxygen. *Can. J. Microbiol.*, 30: 1397-1404.
- Jensen, F. B. 2003. Nitrite disrupts multiple physiological functions in aquatic animals. *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.*, 135: 9-24.
- Kaspar, H. F. 1982. Denitrification in marine sediment: measurement of capacity and estimate of *in situ* rate. *Appl. Environ. Microbiol.*, 43: 522-527.
- Kaushik, S. J., Dabrowski, K. and Luquet, P. 1982. Patterns of nitrogen excretion and oxygen consumption during ontogenesis of common carp (*Cyprinus carpio*). *Can. J. Fish. Aquat. Sci.*, 39(8): 1095-1105.
- Kim, D. H., Matsuda, O. and Yamamoto, T. 1997. Nitrification, denitrification and nitrate reduction rates in the sediment of Hiroshima Bay, Japan. *J. Oceanogr.*, 53: 317-324.
- Kristensen, E. 1985. Oxygen and inorganic nitrogen exchange in a *Nereis virens* (Polychaeta) bioturbated sediment-water system. *J. Coast. Res.*, 1(2): 109-116.
- Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T., Quintana, C. O. and Banta, G. T. 2012. What is bioturbation? The need for a precise definition for fauna in aquatic sciences. *Mar. Ecol. Prog. Ser.*, 446: 285-302.
- Lei, Y. Z. 2006. *Water environmental chemistry for aquaculture experiment*. China Agriculture Press, China (in Chinese).
- Meysman, F. J. R., Middelburg, J. J. and Heip, C. H. R. 2006. Bioturbation: a fresh look at Darwin's last idea. *Trends Ecol. Evo.*, 21: 688-695.
- Michaud, E., Desrosiers, G. and Mermillod-Blondin, F. 2006. The functional group approach to bioturbation, II. The effects of the *Macoma balthica* community on fluxes of nutrients and dissolved organic carbon across the sediment-water interface. *J. Exp. Mar. Biol. Ecol.*, 337: 178-189.
- National Bureau of Statistics 2012. *Chinese Rural Statistical Yearbook 2012*. China Statistics Press, China.
- Naylor, R. L., Goldburg, R. J. and Primavera, J. H. 2000. Effect of aquaculture on world fish supplies. *Nature*, 405: 1017-1024.
- Nixon, S. W. 1986. Nutrient dynamics and the productivity of marine coastal waters. In: Halwagy, R., Clayton, D. and Behbehani, M. (Eds.), *Marine environment and pollution*. Alden Press, Oxford, UK, p. 97-115.
- Nizzoli, D., Bartoli, M. and Viaroli, P. 2006. Nitrogen and phosphorous budgets during a farming cycle of the Manila clam *Ruditapes philippinarum*: an *in situ* experiment. *Aquaculture*, 261: 98-108.
- Nizzoli, D., Bartoli, M. and Cooper, M. 2007. Implications for oxygen, nutrient fluxes and denitrification rates during the early stage of sediment colonisation by the polychaete *Nereis* spp. in four estuaries. *Estuar. Coast. Shelf Sci.*, 75: 125-134.
- Olah, J., Szabo, P., Esteky, A. A. and Nezami, S. A. 1994. Nitrogen processing and retention in Hungarian carp farms. *J. Appl. Ichthyol.*, 10: 335-340.
- Paerl, H. W. 2009. Controlling eutrophication along the freshwater-marine continuum: dual nutrient (N and P) reductions are essential. *Estuaries Coasts*, 32: 593-601.
- Papaspyrou, S., Gregersen, T., Kristensen, E., Bjarne, C. and Cox, R. P. 2006. Microbial reaction rates and bacterial communities in sediment surrounding burrows of two nereidid polychaetes (*Nereis diversicolor* and *N. virens*). *Mar. Biol.*, 148: 541-550.
- Randall, D. J. and Tsui, T. K. N. 2002. Ammonia toxicity in fish. *Mar. Pollut. Bull.*, 45(1): 17-23.
- Shang, J., Zhang, L., Shi, C. and Fan, C. 2013. Influence of chironomid larvae on oxygen and nitrogen fluxes across the sediment-water interface (Lake Taihu, China). *J. Environ. Sci.*, 25(5): 978-985.
- Soto, D. 2009. *Integrated mariculture: a global review*. FAO Fisheries and Aquaculture Technical Paper, 529, FAO, Rome, 183 pp.
- Thibodeau, B., Lehmann, M. F., Kowarzyk, J., Mucci, A., Gelinás, Y. and Gilbert, D. 2010. Benthic nutrient fluxes along the Laurentian Channel: Impacts on the N budget of the St. Lawrence marine system. *Estuar. Coast. Shelf Sci.*, 90: 195-205.
- Wang, Y. S. and Xu, J. R. 2004. *A single pipe sediment sampler with no boundary perturbation*, China patent 200410026847.3.
- Walsh, P. J. and Wright, P. A. 1995. *Nitrogen metabolism and excretion*. CRC Press, Boca Raton, Florida, 337 pp.
- Welsh, D. T. 2003. It's a dirty job but someone has to do it: the role of marine benthic macrofauna in organic matter turnover and nutrient recycling to the water column. *Chem. Ecol.*, 19(5): 321-342.
- Xu, H., Paerl, H. W., Qin, B., Zhu, G. and Gao, G. 2010. Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. *Limnol. Oceanogr.*, 55(1): 420-432.
- Zhang, L., Gu, X., Fan, C., Shang, J., Shen, Q., Wang, Z. and Shen, J. 2010. Impact of different benthic animals on phosphorus dynamics across the sediment-water interface. *J. Environ. Sci.*, 22(11): 1674-1682.

- Zhang, L., Shen, Q., Hu, H., Shao, S. and Fan, C. 2011. Impacts of *Corbicula fluminea* on oxygen uptake and nutrient fluxes across the sediment-water interface. *Water Air Soil Poll.*, 220: 399-411.
- Zheng, Z. M., Dong, S. L., Tian, X. L., Wang, F., Gao, Q. F. and Bai, P. F. 2009. Sediment-water fluxes of nutrients and dissolved organic carbon in extensive sea cucumber culture ponds. *Clean Soil Air Water*, 37: 218-224.
- Zhong, D. S., Wang, F., Wang, C. S. and Dong, S. L. 2013. Effect on the bioturbation of carps in different density on denitrification, nitrification and nitrate ammonification rates on sediment-water interface. *Acta Hydrobiol. Sin.*, 37: 1103-1111 (in Chinese with English abstract).

Date of Receipt : 09.08.2014

Date of Acceptance : 11.08.2015