



# Impact of rice-fish/shrimp co-culture on the N<sub>2</sub>O emission and NH<sub>3</sub> volatilization in intensive aquaculture ponds

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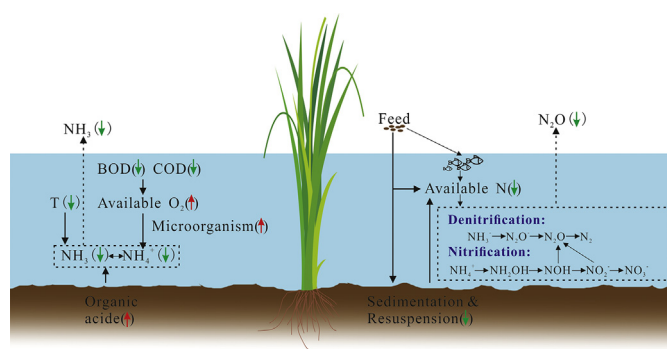
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## HIGHLIGHTS

- We investigated the effect of a new rice-fish/shrimp system on gaseous N loss from pond.
- Gaseous N flux rate significantly correlated with available N and water parameters.
- Rice-fish/shrimp system respectively reduced the N<sub>2</sub>O and NH<sub>3</sub> by 96.9% and 24.3%.
- Co-culture system was more effective in catfish than shrimp ponds.

## GRAPHICAL ABSTRACT



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## ABSTRACT

How to reduce the gaseous nitrogen (N) pollution (N<sub>2</sub>O and NH<sub>3</sub>) of intensive aquaculture ponds to atmosphere has gained increasing attention for the sustainable development of aquaculture. In this study, we constructed a new rice-fish/shrimp co-culture system in aquaculture ponds by using a specially developed high-stalk rice variety, and performed a 2-year field experiment to investigate the effect of this system on the N<sub>2</sub>O and NH<sub>3</sub> emissions from yellow catfish and freshwater shrimp ponds. The results showed that the mean emission factors of N<sub>2</sub>O and NH<sub>3</sub> to the total N input in feed was 0.18% and 0.89% for catfish monoculture pond, and 2.46% and 13.45% for shrimp monoculture pond, respectively. Rice-fish/shrimp co-culture not only reduced the N<sub>2</sub>O and NH<sub>3</sub> emission from rice platform of catfish and shrimp ponds, but also mitigated the N<sub>2</sub>O and NH<sub>3</sub> emission from the ditch without rice planted. The total amount of N<sub>2</sub>O and NH<sub>3</sub> were respectively mitigated by 85.6% and 26.0% for catfish pond, and by 108.3% and 22.6% for shrimp pond, as compared with that of monoculture ponds. Co-culture system was more effective on the mitigation of gaseous N loss in the catfish than shrimp ponds.

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## 1. Introduction

Aquaculture, accounting for nearly half of the world's fish food supply, plays an important role in ensuring food safety and farmer

livelihoods in developing countries (FAO, 2017). Aquaculture pond is a dominant component of aquaculture system and distributes widely around the world. The water surface area of global aquaculture pond was estimated to be 16.7 million ha in 2011 (Jescovitch et al., 2014). In order to improve the fish yield and profit, the culture method in fish pond has been transferred from traditional natural culture to intensive culture during the last three decades (Edwards, 2015). However, the rapidly development of intensive culture, characterized by high stocking density of fish with high input of pelleted feed, has led to

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serious environmental concerns, such as eutrophication, greenhouse gas emission and hypoxia (Bosma and Verdegem, 2011; Z. Hu et al., 2013).

Excessive nitrogen (N) loss to the environment is one of the major concerns in intensive aquaculture. The protein-rich feed is widely used in intensive aquaculture to meet the high protein requirement for fish, which is about two to three times higher than that of mammals (Walsh and Wright, 1995). However, the digestibility of fish is limited due to its short gut (Crab et al., 2007). The N budgets of various fish (snakehead, shrimp, crab, etc.) culture ponds showed that fish animals only absorbed 11.6%–46.5% of the N input in feed; more than half of the N remained undigested and excreted to the water and bottom soil of pond (Casillashernandez et al., 2006; Dai et al., 2010; Hargreaves, 1998; Jackson et al., 2003; Zhang et al., 2018). Abundant residue N in pond could induce serious N pollution to the natural water bodies through the discharge of effluents. Furthermore, the gaseous N loss ( $\text{N}_2\text{O}$  emission and  $\text{NH}_3$  volatilization) from aquaculture to atmosphere has gained increasing attention in recent years. Aquaculture has been considered as an important anthropogenic source of  $\text{N}_2\text{O}$  emission, which is a potent greenhouse gas that contributes to ozone layer depletion and global climate change. The total amount of  $\text{N}_2\text{O}$  emission from aquaculture has been estimated to be  $3.83 \times 10^{11}$  g in 2030, accounting for 5.72% of total anthropogenic  $\text{N}_2\text{O}$  emission (Hu et al., 2012).  $\text{NH}_3$ , an important atmosphere pollutant influencing the environmental and public health as well as climate change (Behera et al., 2013), volatilized from aquaculture was estimated to account for 8%–65.7% of total N loss in culture pond depended on the temperature and water pH (Gross et al., 2000; Hargreaves, 1998; Paez-Osuna et al., 1999). How to reduce these gaseous N loss to atmosphere, especially  $\text{N}_2\text{O}$  emission, is a new challenge for the sustainable development of aquaculture.

The integration of aquaculture with crops has showed great potential to remediate the N pollution because of its environmental and economic advantages (Enduta et al., 2011; Ghaly et al., 2005). A wide varieties of crops, such as lettuce, tomato, and barley, have been investigated to remediate the N pollution in the effluents of aquaculture by constructed hydroponics system in previous studies (Akinbile and Yusoff, 2011; Graber and Junge, 2009; Snow and Ghaly, 2008). However, little study was conducted to investigate their efficiency on the mitigation of the gaseous N loss from aquaculture. Additionally, a key factor determining the feasibility is the crop species used in the integration culture system, which regulates the system design, N reuse, and economic benefits. Most of previous studies focused on vegetable or triticeae crops (Enduta et al., 2011; Graber and Junge, 2009; Snow et al., 2008), but paid little attention to rice. Rice is the only cereal crop grown well in flooded soil. And rice-fish co-culture has been practiced in paddy field over 2000 years in Asian countries (Saiful Islam et al., 2015). The results from paddy field have showed that rice-fish co-culture could enhance nutrients use efficiency and reduce nutrients loss to environment because of the complementary use of nutrients between fish and rice (L. Hu et al., 2013; Li et al., 2008; Oehme et al., 2007; Xie et al., 2011). Therefore, rice-fish co-culture may have the inherent advantage and great potential in the mitigation of gaseous N loss from aquaculture.

Rice-fish co-culture is traditionally conducted in paddy field, whereas rarely conducted in aquaculture pond; because the rice varieties for paddy field could not grow well in the pond with deep water. In this study, we developed a new high-stalk rice variety; the height of which is up to 1.85 m (Fig. 1(b)), and can be directly cultivated in the bottom soil and grow well in fish pond. We hypothesized that additional rice cultivation in pond would uptake the residual N in the water and bottom soil and mitigate the gaseous N loss. We conducted a field experiment to investigate the effect of this rice-fish/shrimp co-culture system on: 1) seasonal changes of  $\text{N}_2\text{O}$  emission and  $\text{NH}_3$  volatilization; 2) the main factors regulated the  $\text{N}_2\text{O}$  and  $\text{NH}_3$  emissions; 3) N budget and use efficiency.

## 2. Materials and methods

### 2.1. Experiment design

This experiment was conducted in the experimental farm of China National Rice Research Institute ( $30^{\circ}05'N$ ,  $119^{\circ}95'E$ ) located in Zhejiang province, which is one of the major pond aquaculture regions in South China. Pond aquaculture has been practiced over 20 years in this farm. The fish species cultured in pond include yellow catfish (*Pelteobagrus fulvidraco*), freshwater shrimp (*Macrobrachium nipponense*), grass carp (*Ctenopharyngodon idellus*), crucian carp (*Carassius auratus*), and flat fish (*Pampus argenteus*). Rice-fish/shrimp co-culture has been practiced in aquaculture pond in this farm since 2010.

Two fish species (yellow catfish and freshwater shrimp) were selected to construct the rice-fish/shrimp co-culture system in this study. Four treatments, including yellow catfish-rice co-culture (YC-R), yellow catfish monoculture (YC), freshwater shrimp-rice co-culture (FS-R) and freshwater shrimp monoculture (FS), with three replications were arranged in twelve experiment plots. Each plot was 19 m long and 12 m wide. The plots were separated by the embankment in a pond used for fish culture over 15 years. The central platform for rice growing occupied 60% of the total area of pond (Fig. 1(a)). The depth of surrounding ditch was 0.5 m. The initial concentrations of total nitrogen in the bottom soil were  $1.23 \text{ g kg}^{-1}$  for YC-R,  $1.27 \text{ g kg}^{-1}$  for YC,  $1.20 \text{ g kg}^{-1}$  for FS-R, and  $1.24 \text{ g kg}^{-1}$  for FS, respectively. The layout and images of these plots were showed in Fig. 1.

The rice variety (namely Yudao No.1) used in this experiment was a new high-stalk rice variety (*Oryza sativa* L.) developed for constructing rice-fish/shrimp co-culture system in pond. The height of this rice variety can reach up to 1.85 m and it can be planted into pond with the water depth below 1.5 m. The water in fish pond was drained off before planting rice, and the bottom soil was kept moist but no water flooded. The rice seed was respectively sown on a nursery bed on June 11th in 2016 and on May 19th in 2017, and transplanted to the fish pond at a spacing of  $0.6 \text{ m} \times 0.6 \text{ m}$  in the center platform when the age of seedling reached up to 30 days. The water level was 0.1 m when rice seedlings was transplanted and rose gradually with the growth of rice. The rice was harvested on November 3rd in 2016 and October 25th in 2017, respectively. No chemical fertilizer, pesticide and herbicide were used for rice cultivation.

The fingerlings of yellow catfish were stocked into co-culture and monoculture ponds at a density of  $150,000 \text{ fingerlings ha}^{-1}$  ( $560 \text{ fingerlings kg}^{-1}$ ) on August 12th in 2016 and on July 11th in 2017, respectively. The freshwater shrimp was stocked at a density of  $450,000 \text{ fingerlings ha}^{-1}$  ( $6000 \text{ fingerlings kg}^{-1}$ ) on August 8th in 2016 and on July 20th in 2017, respectively. Yellow catfish and freshwater shrimp were respectively fed commercial formulated feed two times and one time per day, with 35%–45% protein commercial aquatic feed pellets. The total nitrogen input via feed was  $18.10 \text{ g m}^{-2}$  and  $25.93 \text{ g m}^{-2}$  for catfish pond and  $0.83 \text{ g m}^{-2}$  and  $1.99 \text{ g m}^{-2}$  for shrimp pond in 2016 and 2017, respectively. The water was added with the height of rice plant increased. The maximum water depth was 1 m at rice planting region. During the co-culture period, no water was discharged. The fingerlings of catfish and shrimp were stocked in the ditch before the jointing stage of rice, and began to move to the rice growing region when the water depth of platform exceeding 0.3 m. The management methods for yellow catfish and freshwater shrimp were similar in the co-culture and monoculture ponds.

### 2.2. Measurement of nitrous oxide emission

$\text{N}_2\text{O}$  flux rate was measured using static chamber method (Liu et al., 2016). The size of the chamber was 60 cm (length)  $\times$  60 cm (width)  $\times$  60 cm (height). Two sites (platform and ditch, Fig. 1(c)) were measured in each plot. Steel support was fixed as the base of chamber at each sampling site before rice transplantation, and the height of support was

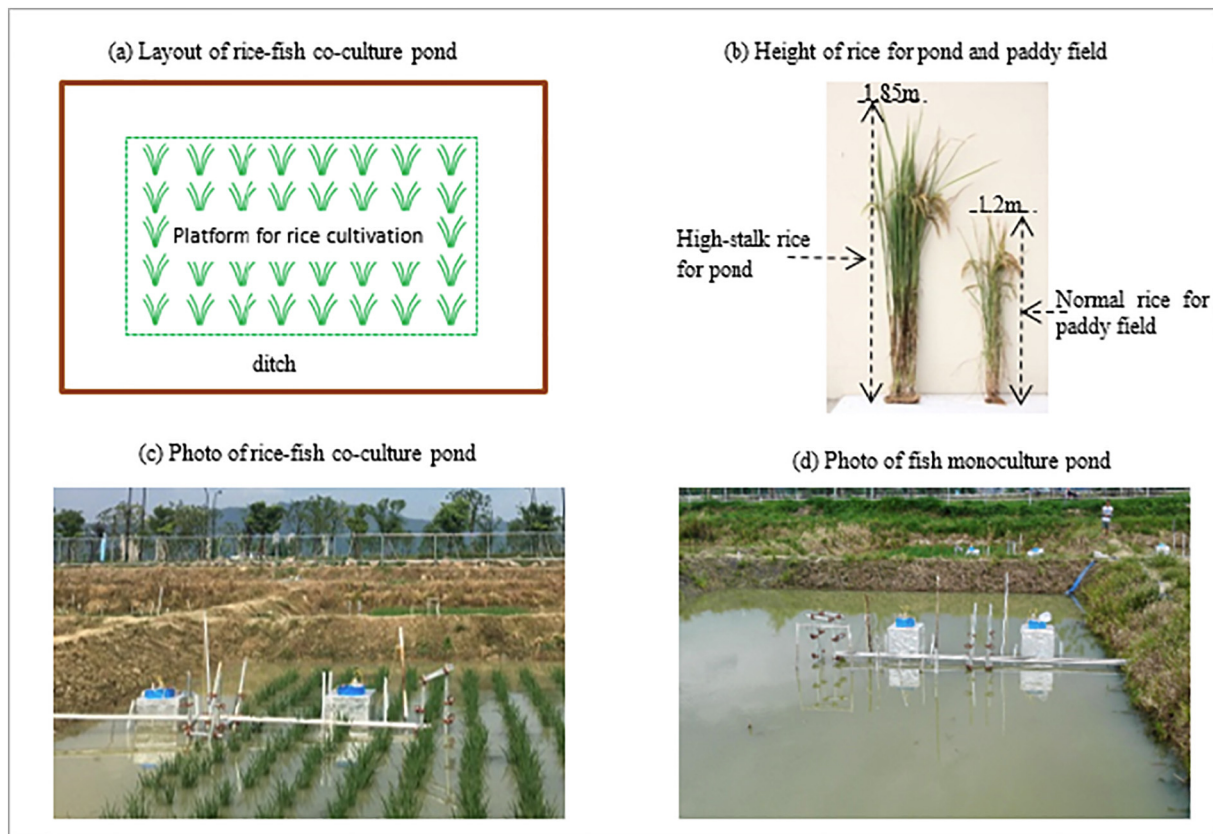


Fig. 1. Layout, rice variety and photos of rice-fish/shrimp co-culture system.

adjusted with the increase of water depth.  $N_2O$  samples were collected every 7 days between 8:30 AM and 10:30 AM during rice growing season. Four gas samples from each chamber were collected using 60 mL injection syringe and placed in pre-evacuated vacuum tubes with a capacity of 40 mL at 10 min intervals (0, 10, 20, and 30 min after chamber closure). The  $N_2O$  samples were analyzed using gas chromatography (GC 2010, Shimadzu, Kyoto, Japan) equipped with an electron capture detector. The  $N_2O$  flux rate was calculated by the following equation:

$$F = \rho \times \frac{V}{A} \times \frac{P}{P^0} \times \frac{dC}{dt} \times \frac{273}{273+T} \times 60 \quad (1)$$

where  $F$  represents the  $N_2O$  flux rate ( $\mu\text{g m}^{-2} \text{h}^{-1}$ );  $\rho$  represents the  $N_2O$  density at the standard state ( $\mu\text{g m}^{-3}$ );  $V$  is the volume of the static chamber ( $\text{m}^3$ );  $A$  is the basal area of the chamber ( $\text{m}^2$ );  $P$  is the atmospheric pressure in the chamber;  $P^0$  is the standard atmospheric pressure.  $dC/dt$  is the slope of  $N_2O$  content changing with time in the chamber ( $10^{-6} \text{ min}^{-1}$ );  $T$  is the mean temperature in the static chamber during gas sampling ( $^{\circ}\text{C}$ ).

### 2.3. Measurement of ammonia volatilization

Ammonia volatilization was measured with a modified continuous airflow enclosure method provided by Chen et al. (2014). Ammonia volatilization measurement device contained a chamber, vent pipe, two flasks and vacuum pump. The device was connected by pipes to form a confined space. The size and location of the chamber was the same as  $N_2O$  sampling chamber. Two 1 L flasks were chained and placed on the top of the chamber. One flask filled with 250 mL 0.01 M  $H_2SO_4$  was used to absorb the ammonia; the other one was used as safety bottle. The  $NH_3$  flux was measured once a week from 13:30 to 15:30 at the

same day of  $N_2O$  sampling. The sampling duration was 45 min. The content of absorbed ammonia in the flask was determined by indophenol blue colorimetric method. The ammonia volatilization rate was calculated by the following formula:

$$F_{NH_3} = C_{NH_3} \times V_{H_2SO_4} \times \frac{1}{t} \times \frac{1}{A} \quad (2)$$

where  $F_{NH_3}$  represents the  $NH_3$  flux rate ( $\text{mg m}^{-2} \text{h}^{-1}$ );  $C_{NH_3}$  represents the content of absorbed ammonia in the flask ( $\text{mg L}^{-1}$ );  $V_{H_2SO_4}$  is the volume of  $H_2SO_4$  (L);  $t$  is the sampling duration (h).  $A$  is area of the chamber ( $\text{m}^2$ ).

### 2.4. Measurement of water and bottom soil

Water samples were collected simultaneously with gaseous N sampling. Composite water samples were taken at 0.1 m depth with five replicates. Soil samples were collected at a depth of 0–15 cm every month after rice planted. Fish and rice samples were collected at the harvest time.

The concentrations of total nitrogen (TN), total phosphorus (TP), ammonia nitrogen ( $NH_4^+$ -N), nitrate nitrogen ( $NO_3^-$ -N), nitrite nitrogen ( $NO_2^-$ -N) and dissolved inorganic phosphorous (DIP) in water was measured using automatic flow analyser (SKALAR Sans Plus Systems, Netherlands). The contents of water turbidity, total suspended solid (TSS), total potassium (TK), chemical oxygen demand (COD) and biological oxygen demand (BOD) were analyzed using standard methods (SEPA, 2002). The contents of TN in the rice, bottom soil, fish, and fish feed samples were determined by the Kjeldahl method. The concentration of dissolved oxygen (DO), water temperature (T) and value of alkalinity (pH) were measured by portable apparatus (Mettler Toledo, Seven2Go Pro S9; Mettler Toledo, SG2) in situ.

**Table 1**

The mean contents of TN, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, and NO<sub>2</sub><sup>-</sup>-N in the water (mg L<sup>-1</sup>) and bottom soil (g kg<sup>-1</sup>) of co-culture and monoculture ponds in 2016 and 2017.

		TN		NH <sub>4</sub> <sup>+</sup> -N		NO <sub>3</sub> <sup>-</sup> -N		NO <sub>2</sub> <sup>-</sup> -N	
		2016	2017	2016	2017	2016	2017	2016	2017
Water	YC-R	2.50c	0.85a	0.47b	0.22a	1.32a	0.69a	0.03a	0.05a
	YC	3.64d	1.42b	0.73c	0.33b	1.58b	0.90b	0.05b	0.12b
	FS-R	1.63a	0.80a	0.34a	0.20a	1.30a	0.55a	0.03a	0.03a
	FS	1.93b	1.80b	0.56b	0.28b	1.47ab	0.64a	0.05b	0.03a
Bottom soil	YC-R	1.40a	1.22a	10.99a	35.22a	6.56a	7.01a	/	/
	YC	1.44a	1.27a	20.06c	52.97b	6.41a	6.21a	/	/
	FS-R	1.40a	1.28a	9.12a	39.30a	7.86a	5.85a	/	/
	FS	1.29a	1.23a	16.45b	52.36b	8.01a	6.29a	/	/

Different letters indicate statistical significance at the 0.05 level.

### 3. Results

#### 3.1. Nitrogen in the pond water and bottom soil

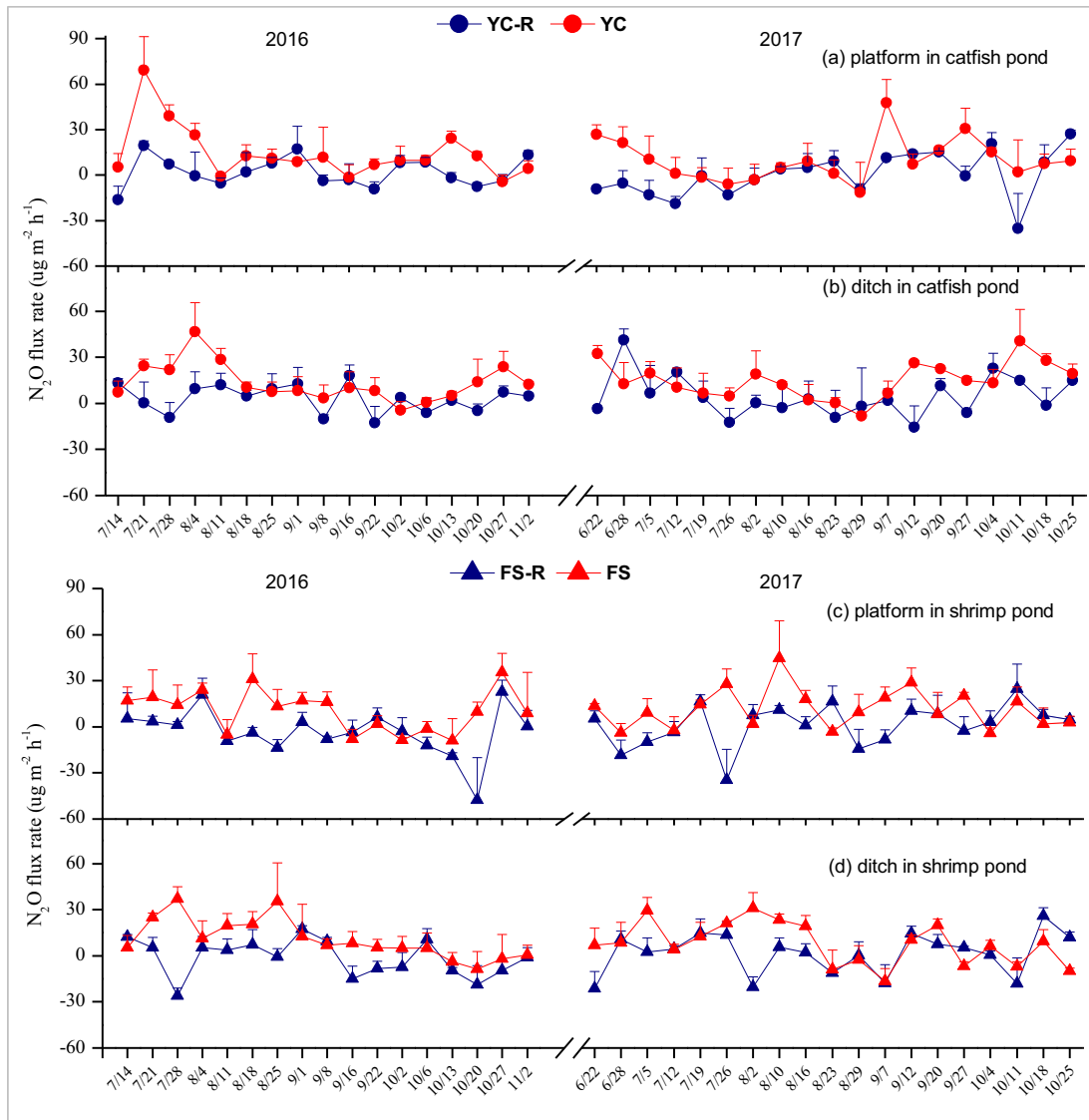
Table 1 showed the mean contents of total and inorganic N in the water and bottom soil of co-culture and monoculture ponds. The seasonal changes of N content in the water and bottom soil were illustrated in

Figs. A.1–A.4 in the Appendix. Co-culture with rice reduced the N accumulation in the water of catfish and shrimp ponds (Table 1). The contents of TN, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, and NO<sub>2</sub><sup>-</sup>-N in the water of YC-R co-culture pond were significantly reduced by 31.4%, 35.5%, 16.1%, and 43.2% in 2016, and by 40.1%, 34.2%, 22.9%, and 58.1% in 2017 by the co-culture of rice with catfish, respectively. The contents of TN, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N in the water of FS-R co-culture pond were also significantly lower than that in FS monoculture pond both in 2016 and 2017 (Table 1). The content of NO<sub>2</sub><sup>-</sup>-N only showed significant reduction in 2016 (Table 1).

Co-culture with rice only significantly reduced the content of NH<sub>4</sub><sup>+</sup>-N in the bottom soil in catfish and shrimp ponds (Table 1). The mean content of NH<sub>4</sub><sup>+</sup>-N was 45.2% and 33.5% lower in YC-R than YC in 2016 and 2017, and 44.6% and 24.9% lower in FS-R than FS in 2016 and 2017, respectively. The contents of TN and NO<sub>3</sub><sup>-</sup>-N in the bottom soil did not differ between the co-culture and monoculture ponds.

#### 3.2. N<sub>2</sub>O emission

The dynamics of N<sub>2</sub>O flux rate during rice-fish/shrimp co-culture season in 2016 and 2017 were showed in Fig. 2, respectively. The N<sub>2</sub>O emission from the platform fluctuated in the range of -16.18–19.35 μg m<sup>-2</sup> h<sup>-1</sup> for YC-R and -4.27–69.16 μg m<sup>-2</sup> h<sup>-1</sup> for YC in 2016,



**Fig. 2.** Seasonal changes of N<sub>2</sub>O flux rate in the co-culture and monoculture ponds in 2016 and 2017.



**Table 2**  
Total amounts of N<sub>2</sub>O and NH<sub>3</sub> in the co-culture and monoculture ponds in 2016 and 2017.

		2016 (kg ha <sup>-1</sup> )			2017 (kg ha <sup>-1</sup> )		
		Platform	Ditch	Total	Platform	Ditch	Total
N <sub>2</sub> O	YC-R	0.040a	0.064a	0.060a	-0.012a	0.135a	0.047a
	YC	0.405c	0.347b	0.382b	0.308b	0.434b	0.358b
	FS-R	-0.109a	-0.047a	-0.074a	0.026a	0.021a	0.024a
	FS	0.246b	0.306b	0.270b	0.376b	0.259b	0.329b
NH <sub>3</sub>	YC-R	1.02a	1.32b	1.14a	1.63ab	1.84b	1.71b
	YC	1.77c	1.76c	1.77c	2.21c	1.88b	2.08c
	FS-R	1.04a	1.04a	1.04a	1.48a	1.52a	1.50a
	FS	1.51b	1.44b	1.48b	1.87bc	1.69a	1.80bc

Different letters indicate statistical significance at the 0.05 level.

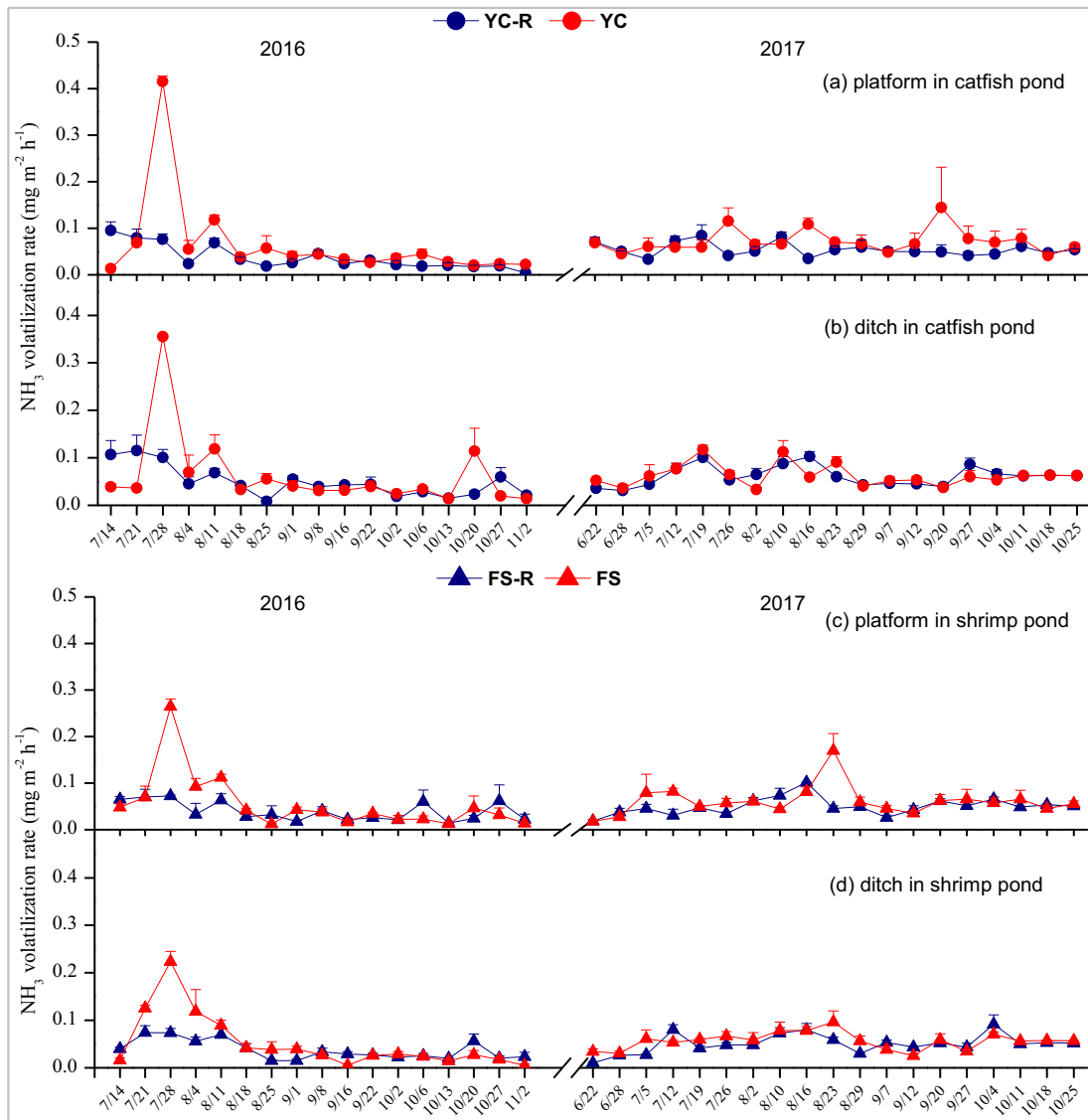
and  $-35.26$ – $27.01 \mu\text{g m}^{-2} \text{h}^{-1}$  for YC-R and  $-11.60$ – $47.80 \mu\text{g m}^{-2} \text{h}^{-1}$  for YC in 2017, respectively (Fig. 2(a)). A peak of N<sub>2</sub>O emission existed around July 21st in YC in 2016; while three peaks of N<sub>2</sub>O emission were observed around June 22nd, September 7th, and September 27th in YC in 2017 (Fig. 2(a)). The platform of YC-R showed significantly lower N<sub>2</sub>O flux rate than that of YC at early and late stages of co-culture season. The total amount of N<sub>2</sub>O emission from the platform of YC-R was  $0.040 \text{ kg ha}^{-1}$  in 2016 and  $-0.012 \text{ kg ha}^{-1}$  in 2017 (Table 2), which

was respectively reduced 90.1% and 103.9% as compared with that of YC. The ditch of YC-R also showed significantly lower N<sub>2</sub>O flux rate than that of YC in the two years. The overall N<sub>2</sub>O emission from the platform and ditch of YC-R was significantly reduced by 84.4% in 2016 and 86.9% in 2017 as compared with that of YC, respectively.

As in the shrimp pond, the platform and ditch of co-culture system (FS-R) also showed lower N<sub>2</sub>O flux rate than that of monoculture system (FS) (Fig. 2(c, d)). Significant reduction of N<sub>2</sub>O flux from the platform of FS-R than FS was mostly observed in the middle and late stages of rice growing season in 2016 and 2017 (Fig. 2(c)). The N<sub>2</sub>O emission from the platform was significantly reduced by 144.3% in 2016 and 93.1% in 2017 for FS-R than FS (Table 2), respectively. The overall N<sub>2</sub>O emission from the platform and ditch was significantly reduced by 127.4% and 92.7% for FS-R than FS in 2016 and 2017, respectively.

### 3.3. NH<sub>3</sub> volatilization

Fig. 3 illustrated the seasonal change of NH<sub>3</sub> volatilization rate in the co-culture and monoculture ponds. NH<sub>3</sub> volatilization from the platform of YC showed flux peaks around July 28th of 2016, and around July 26th, August 16th, and September 20th of 2017. While in YC-R, significantly lower or no flux peak were observed around these days (Fig. 2(a)).



**Fig. 3.** Seasonal changes of NH<sub>3</sub> volatilization rate in the co-culture and monoculture ponds in 2016 and 2017.

YC-R significantly reduced not only the accumulated amount of NH<sub>3</sub> volatilization from the platform, but also from the ditch in 2016 (Table 2). But the reduction of NH<sub>3</sub> volatilization from the ditch was not significant in 2017. The overall amount of NH<sub>3</sub> volatilization of the platform and ditch was significantly reduced by 35.6% in 2016 and 17.8% in 2017 for YC-R than YC, respectively.

Similar as in the catfish pond, significantly lower flux peaks of NH<sub>3</sub> were observed in FS-R than FS. Additional rice cultivation in FS-R significantly reduced the NH<sub>3</sub> volatilization from the platform and ditch in 2016; but did not reduce the NH<sub>3</sub> volatilization from the ditch in 2017 (Fig. 2(c, d)). The overall amount of NH<sub>3</sub> volatilization from the platform and ditch was significantly reduced by 29.7% in 2016 and 16.7% in 2017 by rice cultivation, respectively.

3.4. Correlation of N<sub>2</sub>O and NH<sub>3</sub> with water parameters

The N<sub>2</sub>O flux rate of catfish pond significantly correlated with the contents of TN, NH<sub>4</sub><sup>+</sup>-N, and NO<sub>2</sub><sup>-</sup>-N in pond water (Table 3). While in the shrimp pond, the N<sub>2</sub>O flux rate showed significantly positive correlation with inorganic N (NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, and NO<sub>2</sub><sup>-</sup>-N) in the water, and negative correlation with TSS. More water parameters showed significant correlation with NH<sub>3</sub> volatilization than N<sub>2</sub>O in catfish and shrimp ponds. The NH<sub>3</sub> volatilization rate of catfish pond showed significantly positive correlation with water temperature, and the contents of COD, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, and NO<sub>2</sub><sup>-</sup>-N in pond water; but significantly negative correlation with TSS. The NH<sub>3</sub> volatilization rate of shrimp pond showed significantly positive correlation with water temperature, and the contents of COD, water turbidity, TN, NH<sub>4</sub><sup>+</sup>-N, and NO<sub>3</sub><sup>-</sup>-N in pond water; but significantly negative correlation with TSS and DO.

3.5. Harvest and loss of feed N

Catfish and shrimp only absorbed 16.0%–18.4% of the total N input in the feed (Table 4). The utilization efficiency of catfish and shrimp did not show significant difference between co-culture pond and monoculture pond. The low utilization efficiency of shrimp in 2016 was attributed to the low survival rate caused by the high temperature after shrimp stocking. The growth of rice differed between YC-R and FS-R in 2016 (Fig. A5). The biomass of rice leaf and the N contents of rice stem, leaf and grain were significantly lower for FS-R than YC-R. The N accumulated in rice plants was significantly higher in catfish pond (5.85 g m<sup>-2</sup>) than shrimp pond (3.39 g m<sup>-2</sup>) in 2016, due to the higher N contents in the water of catfish pond (Table 1); but did not show significantly difference between catfish pond (5.96 g m<sup>-2</sup>) and shrimp pond (6.28 g m<sup>-2</sup>) in 2017. The N accumulated in rice was partly from the bottom soil especially in shrimp pond, because the N harvest

Table 4  
The percentage of N harvest and loss in co-culture and monoculture ponds.

		N input Feed (g m <sup>-2</sup> )	N harvest		N loss		
			Fish/shrimp	Rice	Residue in water	N <sub>2</sub> O	NH <sub>3</sub>
2016	YC-R	18.10	18.0%	32.3%	8.2%	0.03%	0.63%
	YC	18.10	18.2%		18.5%	0.21%	0.98%
	FS-R	0.83	5.4%	408.4%	41.7%	-0.90%	12.54%
	FS	0.83	6.3%		49.4%	3.26%	17.84%
2017	YC-R	25.93	16.5%	23.0%	5.3%	0.02%	0.66%
	YC	25.93	18.6%		10.8%	0.14%	0.80%
	FS-R	1.99	26.6%	315.6%	29.0%	0.12%	7.53%
	FS	1.99	27.2%		63.1%	1.66%	9.06%
Average	YC-R	22.01	17.3%	27.4%	6.8%	0.03%	0.65%
	YC	22.01	18.4%		12.5%	0.18%	0.89%
	FS-R	1.41	16.0%	361.9%	44.4%	-0.39%	10.03%
	FS	1.41	16.7%		56.3%	2.46%	13.45%

in rice was far higher than the total N input in the feed. The N residue in water was significantly lower in co-culture than monoculture pond in 2017 (Table 4). The percentage of N residue in the water of shrimp pond was higher than that of catfish pond due to the lower total feed N input in shrimp pond.

The emission factor of N<sub>2</sub>O and NH<sub>3</sub> to total N input in feed ranged from -0.90% to 3.26% and from 0.63% to 17.84% during the two experiment seasons, respectively. Additional rice cultivation significantly reduced the emission factor of N<sub>2</sub>O both in 2016 and 2017. On average, the emission factor of N<sub>2</sub>O was significantly reduced by 83.3% and 115.9% for catfish and shrimp ponds by co-culture with rice, respectively. The mean emission factor of NH<sub>3</sub> was significantly reduced by 27.0% and 25.4% for catfish and shrimp ponds by rice cultivation.

4. Discussion

4.1. Effect of rice-fish/shrimp co-culture on N<sub>2</sub>O emission

N<sub>2</sub>O emission from aquaculture system has gained increasing attention in recent years. However, the direct measurement of N<sub>2</sub>O emission from aquaculture system was still very limited. This study reported the N<sub>2</sub>O flux rate from catfish and shrimp ponds based on two years' measurement by using chamber-gas chromatography method. The mean N<sub>2</sub>O flux rates of catfish (12.14 μg m<sup>-2</sup> h<sup>-1</sup>) and shrimp monoculture ponds (9.67 μg m<sup>-2</sup> h<sup>-1</sup>) in this study were close to the results of coastal shrimp monoculture (10.74 μg m<sup>-2</sup> h<sup>-1</sup>) and shrimp-fish co-culture (11.80 μg m<sup>-2</sup> h<sup>-1</sup>) ponds (Yang et al., 2015); but lower than those of inland crucian carps pond (54.78 μg m<sup>-2</sup> h<sup>-1</sup>) (Wu et al., 2018) and crab-fish pond (48.10 μg m<sup>-2</sup> h<sup>-1</sup>) (Liu et al., 2016). The variety of N<sub>2</sub>O flux rate among different fish ponds was possibly attributed to the discrepancy in assimilation efficiency of fish species, culture practices (such as feeding rate, water depth, and water drainage), and environmental factors (Hu et al., 2012; Wu et al., 2018). The N<sub>2</sub>O flux rates of this study were far lower than that measured by using N<sub>2</sub>O-sensor (458.3–2358.3 μg m<sup>-2</sup> h<sup>-1</sup>) (Z. Hu et al., 2013) and that estimated by model approaches (116.7 × 10<sup>3</sup>–191.7 × 10<sup>3</sup> μg m<sup>-2</sup> h<sup>-1</sup>) (Paudel et al., 2015). The emission factor of N<sub>2</sub>O emission to total N input in feed was 0.18% for catfish pond in this study (Table 4), which was lower than that of crucian carps (0.46%) (Wu et al., 2018) and crab-fish ponds (0.66%) (Liu et al., 2016). However, the emission factor of N<sub>2</sub>O emission from shrimp pond (2.46%) (Table 4) was higher than the results of crucian carps and crab-fish ponds (Liu et al., 2016; Wu et al., 2018). As for the rice-fish/shrimp co-culture ponds, the mean N<sub>2</sub>O flux rates of YC-R (2.14 μg m<sup>-2</sup> h<sup>-1</sup>) and FS-R (-0.48 μg m<sup>-2</sup> h<sup>-1</sup>) in this study were lower than that of rice-fish co-culture system in paddy field (80.46 μg m<sup>-2</sup> h<sup>-1</sup>) (Li et al., 2008), which was possibly attributed to the high inorganic N application and low water level in paddy field.

Table 3  
Pearson's correlation coefficient between gaseous nitrogen loss and water parameters.

	N <sub>2</sub> O		NH <sub>3</sub>	
	Catfish pond	Shrimp pond	Catfish pond	Shrimp pond
Chl- <i>a</i>	-0.180	0.152	-0.144	-0.010
pH	0.158	0.034	-0.001	0.103
BOD	0.086	0.068	0.013	0.049
COD	0.136	0.092	<b>0.481**</b>	<b>0.372**</b>
Turbidity	0.019	0.199	0.122	<b>0.248*</b>
TSS	-0.041	<b>-0.251*</b>	<b>-0.351**</b>	<b>-0.268*</b>
DO	0.222	-0.079	-0.134	<b>-0.346**</b>
Temperature	0.175	-0.132	<b>0.349**</b>	<b>0.404**</b>
TN	<b>0.235*</b>	<b>0.291*</b>	0.178	<b>0.299*</b>
NH <sub>4</sub> -N	<b>0.325**</b>	<b>0.340**</b>	<b>0.312**</b>	<b>0.371**</b>
NO <sub>3</sub> -N	0.220	<b>0.237*</b>	<b>0.491**</b>	<b>0.448**</b>
NO <sub>2</sub> -N	<b>0.339**</b>	<b>0.376**</b>	<b>0.470**</b>	0.226
TP	-0.038	0.198	-0.168	0.133
DIP	0.039	-0.083	-0.125	-0.027
TK	0.002	-0.004	0.023	0.053

\* and \*\* indicate significant correlations at the 0.05 and 0.01 levels, respectively.

The bioremediation of aquaculture wastes was mostly focused on N pollution to the water and bottom soils, but rarely on N<sub>2</sub>O emission to atmosphere in previous studies (Crab et al., 2007; Hu et al., 2014; Wongkiew et al., 2017). The results of this study showed that rice-fish/shrimp co-culture in aquaculture pond not only reduced the N accumulation in the water and bottom soil, but also showed great potential on the mitigation of N<sub>2</sub>O emission from aquaculture pond. The N<sub>2</sub>O emission was respectively reduced 85.5% in catfish pond and 108.3% in shrimp pond by additional rice cultivation. The effect of rice on N<sub>2</sub>O mitigation can be attributed to two possible pathways. Firstly, the Pearson correlation coefficient showed that the N<sub>2</sub>O flux rate showed significantly positive correlation with available N content in water both in catfish and shrimp ponds (Table 3). The contents of available N in water determined the substrate supply for the nitrification and denitrification processes, which were the main pathway producing N<sub>2</sub>O in the aquaculture pond (Hu et al., 2012). The growth of rice planted in pond could directly absorb the inorganic N in the water and bottom soil, thus reduced the substrate N for N<sub>2</sub>O production. Additionally, rice plant adsorbed the suspend solid in pond by stem and water roots, and then inhibited the N release from the deposition and resuspension of suspend solid (Zhu et al., 2015). Secondly, rice can transfer the O<sub>2</sub> from atmosphere to root zone in pond mud through aerenchyma (Armstrong, 1971; Colmer et al., 2006). And the waving of rice plants induced by wind promoted the diffusion of the O<sub>2</sub> from surface water into deep water (Fostermartinez and Variano, 2016). Therefore, rice could improve the aerobic condition in the water and bottom soil, thus decrease the denitrification-driven N<sub>2</sub>O production.

Whereas, rice also has positive effect on the production and emission of N<sub>2</sub>O in pond. The N<sub>2</sub>O flux rate showed significantly negative correlation with the content of TSS (Table 3) in the shrimp pond. The population of nitrifying bacteria in suspend solid was much higher than that in water phase (Xia et al., 2004). Increasing TSS could accelerate the nitrification process and possibly reduce the intermediate (NO<sub>2</sub><sup>-</sup>) for N<sub>2</sub>O production. Thus, the N<sub>2</sub>O flux rate showed the negative correlation with TSS. Rice–shrimp co-culture reduced the TSS in pond water due the adsorption of stem and water root, thus may prompt the N<sub>2</sub>O production in pond. However, the TSS did not showed significant correlation with N<sub>2</sub>O in the catfish pond (Table 3). This was possibly due to the relative higher N contents in the water of catfish pond than shrimp pond. Available N contents were the dominant factors determining the N<sub>2</sub>O production in the catfish pond. Additionally, the results from paddy field showed that the ventilation system of rice plant facilitate the transport of N<sub>2</sub>O from bottom soil or water to atmosphere (Yan et al., 2000), thus benefit the N<sub>2</sub>O emission from aquaculture pond. These effects may weaken the efficiency of rice on the mitigation of N<sub>2</sub>O emission from aquaculture pond.

#### 4.2. Effect of rice-fish/shrimp co-culture on NH<sub>3</sub> volatilization

The NH<sub>3</sub> volatilization from aquaculture ponds showed a wide range (0.01–36.75 mg m<sup>-2</sup> h<sup>-1</sup>) in previous studies depended on environmental parameters, especially the pH, temperature, and wind speed (Gross et al., 2000; Hargreaves, 1998; Hou et al., 2018). The NH<sub>3</sub> volatilization rates of catfish and shrimp ponds in this study were in the range of previous results. The emission factor of NH<sub>3</sub> volatilization to total N input in feed for catfish pond in this study was close to that of the snake head and bighead carp polyculture pond (0.6%–1.06%) in previous study (Hou et al., 2018), but lower than the shrimp ponds in this and previous studies (10%–65.7%) (Dien et al., 2018; Lorenzen and Struve, 1997; Paez-Osuna et al., 1999). This was possibly attributed to the relative higher pH in the water of shrimp pond than fish pond. As for the co-culture ponds, the average NH<sub>3</sub> volatilization rates of YC-R (0.49 mg m<sup>-2</sup> h<sup>-1</sup>) and FS-R (0.45 mg m<sup>-2</sup> h<sup>-1</sup>) were similar to the result (0.52 mg m<sup>-2</sup> h<sup>-1</sup>) of rice-fish co-culture in paddy field (Li et al., 2008).

NH<sub>3</sub> volatilization from pond was determined by the equilibrium between unionized ammonia (NH<sub>3</sub>) and ionized ammonium (NH<sub>4</sub><sup>+</sup>) (Hargreaves, 1998). More water parameters showed significant correlation with NH<sub>3</sub> volatilization than N<sub>2</sub>O emission (Table 3). This is possible because that the generating process of N<sub>2</sub>O is more complicated than that of NH<sub>3</sub> (Hargreaves, 1998; Hu et al., 2012). Thus, the relative contribution of each environmental factor to the variation of N<sub>2</sub>O is lower; most of the factors did not show significant correlation. Co-culture with rice significantly reduced the NH<sub>3</sub> volatilization from catfish and shrimp ponds (Table 2). Rice planted in pond could directly absorb the NH<sub>4</sub><sup>+</sup> in the water and bottom soil, and reduced the available NH<sub>4</sub><sup>+</sup> for the production of NH<sub>3</sub>. The NH<sub>3</sub> volatilization rate also showed significant correlation with water environmental factors, such as positive correlation with COD, water turbidity and temperature, and negative correlation with TSS and DO, especially in shrimp pond (Table 3), indicating that several indirect pathways may existed for rice to reduce the NH<sub>3</sub> volatilization. Initially, rice-fish/shrimp co-culture significantly reduced the water temperature of pond by the shading of rice leaves especially in summer, thus benefit to reduce the NH<sub>3</sub> volatilization. Furthermore, rice reduced the COD and BOD, which may reduce the oxygen competition with nitrification process and prompt the nitrification of NH<sub>4</sub><sup>+</sup> in pond. Additionally, though the NH<sub>3</sub> volatilization rate did not showed significant correlation with pH; rice significantly reduced the pH both in catfish and shrimp ponds (Table A1) because of the organic acids in rice root exudates (Bacilio-Jiménez et al., 2003), which may inhibit the production of unionized ammonia in pond water.

## 5. Conclusions

The results showed that intensive aquaculture pond was an important source of N<sub>2</sub>O and NH<sub>3</sub> emissions. The flux rate of N<sub>2</sub>O and NH<sub>3</sub> showed significant correlation with the content of available N and water environment factors, such as COD, TSS and temperature, especially in shrimp pond. Rice-fish/shrimp co-culture significantly reduced the contents of available N in pond water and bottom soil. The total amount of N<sub>2</sub>O and NH<sub>3</sub> emission from co-culture pond were also significantly lower than that of monoculture ponds. These results indicated that rice-fish/shrimp co-culture was an efficient measure to mitigate the gaseous N loss from intensive aquaculture ponds.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.10.440>.

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