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Nitrogen transformations in intensive aquaculture system and its implication to climate change through nitrous oxide emission

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HIGHLIGHTS

- About 1.3% of the nitrogen input was emitted as nitrous oxide (N_2O) in laboratory-scale aquaculture system.
- Nitrification and denitrification processes were equally responsible for the emissions of N_2O from aquaculture systems.
- Dissolved oxygen (DO) concentrations and feeding rates had significant effects on N_2O emissions.

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ABSTRACT

The rapid development of aquaculture could result in significant environmental concerns such as eutrophication and climate change. However, to date, very few studies have been conducted to investigate nitrogen transformations in aquaculture systems; and specifically the emission of nitrous oxide (N_2O), which is an important greenhouse gas and ozone-depleting substance. In this study, nitrogen transformations in intensive laboratory-scale Chinese catfish (*Clarias fuscus*) aquaculture systems were investigated by identifying and quantifying N_2O emissions. Results indicated that about 1.3% of the nitrogen input was emitted as N_2O gas. Dissolved oxygen (DO) concentrations and feeding rates had significant effects on N_2O emissions. Higher N_2O emissions were obtained in aquaculture systems with lower DO concentrations and higher feeding rates. Both nitrification and denitrification appeared to be responsible for the emissions of N_2O. Key factors which correlated with the N_2O emission rate in aquaculture systems were NO_2^-, DO and total ammonia nitrogen concentrations.

1. Introduction

Aquaculture is among the fastest-growing segments of the food economy in modern times. Since the mid-1970s, aquaculture production has been increasing at an average growth rate of 8.3% per year and recently, it was estimated that the total aquaculture production in 2011 was 63.6 million metric tons, accounting for nearly 48.6% of all the fish consumed by human beings (FAO, 2012). In contrast, production from capture fisheries has leveled off, and most ocean fisheries stocks are now recognized as fully or overfished. The Food and Agriculture Organization (FAO) estimated that the global food fish production would increase from 72.1 million metric tons in 2006 to approximately 150 million metric tons by 2030, to meet human demands for protein. In the United States, an aquaculture policy to increase domestic aquaculture production fivefold before 2025, has been underway since 1999 (Naylor et al., 2001). Intensive aquaculture, in which fish are raised at very high densities, may be the most promising solution to meet the rapidly growing demand.

In aquaculture systems, microbial nitrogen transformations control overall nitrogen turnover. Several studies on aquaculture systems using protein-rich fish feed indicated that on average only 25% (range: 11–36%) of the nitrogen digested by fish is converted to fish biomass (Hargreaves, 1998). The other digested nitrogen is mainly excreted by fish as unionized ammonia, a byproduct of protein metabolism. In aquaculture systems, ammonia is present in ionized (NH_4^+) or unionized (NH_3) form. The relative proportion of the two forms is affected by pH, temperature and the salinity of water (Stumm and Morgan, 1995). Ammonia is toxic to fish, even at very low concentrations. It can be oxidized to nitrite and nitrate by ammonia oxidizing bacteria and nitrate oxidizing bacteria,

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respectively, predominantly under aerobic conditions. Nitrate can further be reduced to nitrogen gas through denitrification, predominantly under anoxic conditions (Rassamee et al., 2011). However, to date, no detailed and precise data on different forms of nitrogen exist for an aquaculture system, mainly due to a lack of information on gaseous forms of nitrogen, e.g., nitrogen gas (N_2), nitrous oxide (N_2O), and NH_3.

N_2O is the third most important greenhouse gas after carbon dioxide (CO_2) and methane (CH_4), with a 100-year global warming potential 310 times higher than that of CO_2 (IPCC, 2007). Although atmospheric N_2O accounts for only 6% of the greenhouse effect, its high increase rate (0.25–0.30% per year) has been of great concern (He et al., 2001). In addition, N_2O has been identified as the dominant ozone-depleting substance (Ravishankara et al., 2009). Agriculture is considered to be the primary anthropogenic source of N_2O emission (Reay et al., 2012). However, most of the studies are focused on natural or fertilized soil and very few studies have been conducted on N_2O emissions from aquaculture systems. Williams and Crutzen (2010) tentatively estimated that the global N_2O-N emissions from aquaculture systems in 2009 were 9.0×10^{10} g, representing 0.5% of global N_2O-N emissions. And our preliminary results showed that aquaculture industries may account for 5.7% of anthropogenic N_2O-N emissions by 2030 if they continue to increase at the current annual growth rate of 7.1% (Hu et al., 2012). However, no published data based on actual experimental measurement of N_2O emissions from aquaculture systems currently exists.

In aquaculture systems, N_2O can be produced during both microbial nitrification and denitrification processes. During denitrification, N_2O is an obligate intermediate, and is produced as a result of oxygen or nitrite inhibition or biodegradable carbon limitation (Kampschreur et al., 2009). The type of carbon source also influences N_2O emission from denitrification (Lu and Chandran, 2010). During nitrification, N_2O can be produced through three pathways: (1) aerobic hydroxylamine oxidation, where hydroxylamine generated during ammonia oxidation is oxidized to NO directly by hydroxylamine oxidoreductase, and then reduced to N_2O by different nitrite reductases (Stein, 2011); (2) nitrifier denitrification by ammonia oxidizing bacteria (AOB), which is a sequential reduction of NO_2^- to NO and N_2O via nitrite reductase and nitric oxide reductase, respectively (Chandran et al., 2011); and (3) chemical decomposition of intermediates (e.g., nitroxyl) from the oxidation of NH_4^+ to NO_2^- or NO_2^- itself with organic (e.g., amines) or inorganic (e.g., Fe^{2+} or Cu^{2+}) compounds (Wrage et al., 2001). This pathway is also called chemodenitrification.

The exact mechanisms of N_2O production are related to the specific operating parameters and environmental conditions. In aquaculture systems, operational parameters which affect nitrification and denitrification, such as feeding rate, dissolved oxygen (DO) concentration, pH, water exchange rate, etc., may also have an effect on N_2O production. For example, higher feeding rate, which can lead to higher total ammonia nitrogen (TAN) concentration, may increase nitrification-driven N_2O production (Yu et al., 2010); and low DO concentration caused by insufficient aeration, may enhance denitrification-driven N_2O production, which could be mediated by both nitrifying and denitrifying bacteria (Chandran et al., 2011). Further research, however, is needed to examine the effects of operating parameters on N_2O emissions from aquaculture systems.

Therefore, the overarching goal of this study was to evaluate N_2O emissions from intensive aquaculture systems cultured with Chinese catfish (*Clarias fuscus*). The specific objectives were (i) to investigate the nitrogen transformations in intensive aquaculture system by focusing on quantification of N_2O emissions; (ii) to identify the key factors responsible for N_2O emissions from aquaculture systems.

2. Methods

2.1. Fish stocking and tank management

Chinese catfish (*C. fuscus*), imported to Hawaii from Asia over 100 years ago, is a popular aquaculture species in Hawaii due to its high market value (Qin et al., 1998). Fish stockings (5-months old) were obtained from Windward Community College, Honolulu, Hawaii. Plastic tanks (KMT85, Tuff Stuff, Terra Bella, CA), with a working volume of 200 L, were stocked with 16 fish (individual wet weight: 235.5 ± 48.5 g). The fish were fed with 42% protein commercial aquatic feed pellets (Silver Cup Trout Feed, Tooele, UT) and were fed once daily at 5:00 PM (Casillas-Hernández et al., 2006). To minimize un-consumed feed, the feed pellets remaining above water for ten minutes after feeding were collected, dried and weighed. The feeding rate was adjusted in the subsequent day so that the leftover (un-consumed) feed 10 min after each feeding was no more than 5% of the total added feed. The feed consumption was recorded daily and the feed conversion rate (FCR) was calculated as the ratio of feed consumption to fish biomass gain.

Duplicate tanks were operated side by side. The desired DO concentration was obtained by adjusting flow rate of air which was supplied through three diffusers placed at the bottom of each tank. The air flow also provided adequate mixing for the tank water. Biofilter (with volume of 8.0 L) was composed of mesh nylon biofilter media bag filled with 1.5 kg of biomedia (Kaldnes @ media, Aquatic EcoSystem, Apopka, FL). In each tank, one biofilter was placed adjacent to air diffusers to facilitate the growth of nitrifying bacteria. For the first four weeks, the tanks were operated without water exchange, followed by a 2% daily water exchange of the total water volume, using tap water to maintain a good water quality.

The tanks were kept in an air-conditioned room, and the water temperature was maintained at around 24.7 ± 1.1 °C. The pH was kept around 7.1 ± 0.8 by periodic manual dosing of $NaHCO_3$. Each tank was covered with a semi-transparent acrylic plastic lid to minimize both water evaporation and algal growth. Water samples from each tank were obtained every other day at 10:00 AM and immediately analyzed for TAN, NO_2^-, NO_3^-, chemical oxygen demand (COD), and total suspended solids (TSS). DO concentrations, temperature, pH and salinity were monitored *in situ* daily.

2.2. Experimental design

Usually, it takes approximately 4 weeks for the aquaculture system to establish the required microbial community (Avnimelech, 2009). In the present study, fish were transferred to the tanks 6 weeks before the start of the experiment, to ensure full acclimation prior to the experiments. During the acclimation period, the feeding rate was 15.8 ± 4.6 g/d (dry weight). Following the acclimation period, fish were weighed and then subjected to an 8-week study. It was observed that weighing disturbed fish appetite. Therefore, the feeding rate was increased gradually to avoid leftover feed.

The study period was divided into four stages. During the first two weeks, the feeding rate was maintained at around 10.0 g/d, and was subsequently increased to around 20.0 g/d in the following 2 weeks. Then, the feeding rate was increased to around 30.0 g/d for 1 week, which was observed to be in excess. It was then adjusted back to around 20.0 g/d during the last 3 weeks of the study period. During the first three stages, the aeration rate was adjusted with the feeding rate to maintain consistent DO concentrations of 3.0–4.0 mg/L. During the last stage, higher DO concentrations of 5.0–6.0 mg/L were maintained in the tanks. The various parameters of the four different stages of aquaculture operation are presented in Table 1.

Table 1
Operational parameters during different stages of intensive aquaculture system.

Parameters	Stages			
	Stage I	Stage II	Stage III	Stage IV
Feeding rate (g/d) [a]	~10.0	~20.0	~30.0	~20.0
DO concentration (mg/L)	3.0–4.0	3.0–4.0	3.0–4.0	5.0–6.0
Aeration rate (L/min)	0.7	1.1	1.7	1.7
Time (week)	2	2	1	3

[a] "~" means around.

2.3. Quantification of N_2O emissions

The daily N_2O emissions were monitored after the target feeding rate was achieved during each stage. The intensive monitoring of N_2O emissions was conducted on day 10–13, day 24–27, day 34–36, and day 50–53. A Clark type electrode N_2O sensor (Unisense, Aarhus N, Denmark) was used to measure the dissolved N_2O concentrations in aqueous phase (Andersen et al., 2001). N_2O concentrations in the gas phase were estimated using the following equation (Rassamee et al., 2011);

$$C_{N_2O,gas} = (K_La * S_{N_2O} * V_r)/(Q_{air} + V_r * K_La/H) \quad (1)$$

where, $C_{N_2O,gas}$ is the gas phase N_2O concentration (μmol/L); K_La is the volumetric mass transfer coefficient (1/h), which varied with different air flow rates and is determined according to equation provided by Foley et al. (2010); $S_{N_2O,}$ is the concentration of N_2O in the liquid phase (μmol/L); V_r is the working volume of the tank (L); Q_{air} is the air flow rate (L/h); H is the Henry's constant, which is 1.303 for N_2O at 25 °C (Sander, 1999). Compared with the gas phase measurement of N_2O using gas chromatography with electron capture detectors (GC-ECD), the direct and *in situ* continuous measurement of N_2O production by using N_2O sensor can provide more reliable results (Andersen et al., 2001).

Dissolved N_2O concentration was monitored once every minute. To simplify the calculations, and to minimize large variations in dissolved N_2O concentrations, the average N_2O emissions rate was calculated at hourly interval using the following equation;

$$M_{N_2O,gas,HA} = \sum_{i=1}^{60} C_{i,N_2O,gas} * M_{N_2O} * Q_{gas}/60 \quad (2)$$

where, $M_{N_2O,gas,HA}$ is the average N_2O emission rate in an hour (μg/min); $C_{i,N_2O,gas}$ is the gas phase N_2O concentration at i minute (μmol/L), and summing up to 60 min; M_{N_2O} is the molecular weight of N_2O (44.02 g/mol); Q_{gas} is the flow rate of outlet gas (L/min). The daily N_2O emissions were then calculated using Eq. (3);

$$m_{N_2O} = \sum_{n=1}^{24} M_{N_2O,gas,HA,n} * 0.038 \quad (3)$$

where, $m_{N_2O}$is the daily N_2O-N emissions from the aquaculture system (mgN/d).

2.4. Analytical methods

TAN was measured using the reaction kit Ammonia TNTplus, ULR (TNT 830, Hach, Loveland, CO). Nitrite was measured using the reaction kit Nitrite TNTplus, LR (TNT 839, Hach, Loveland, CO). Nitrate was measured using the reaction kit Nitrate TNTplus, HR (TNT 836, Hach, Loveland, CO). COD was determined using the reaction kit COD Reagent, TNTplus, LR (TNT 821, Hach, Loveland, CO). Biomass concentrations were estimated by determining TSS according to Standard Methods (APHA, 2005). DO concentration, temperature, and pH were measured routinely using the HQ40d Portable Water Quality Lab Package (Hach, Loveland, CO). Salinity was measured using a waterproof SaltTestr (OAKTON Instruments, Vernon Hills, IL). A LECO TruSpec CN analyzer (LECO Corp., St. Joseph, MI) was used to measure the total nitrogen (TN) content of fish, fish feed and biofloc. Ammonia volatilization was measured *in situ* according to the method of Valero and Mara (2007). Outlet gases were collected and passed through 2% boric acid solutions, and TAN concentrations of the boric solutions were measured before and after the 24 h absorption experiment. The recovered ammonia represented ammonia volatilization of the system.

2.5. Statistical analysis

Statistical analyses were performed using SPSS v16.0 for Windows software (SPSS v16.0 IBM Corporation, Somers, NY). All data were expressed as mean ± standard deviation (SD). Significant difference between the treatments was determined by using one-way analysis of variance (ANOVA). The significance for all p values was 0.05 unless otherwise stated.

3. Results and discussion

3.1. Variation of fish performance and water quality

During the 8 weeks study period, no fish mortality occurred. Moreover, no fin erosion and histopathological signs was observed in all fish. No algal growth was found throughout the study period.

The variation of feeding rate in both tanks during the 8 weeks study period is shown in Fig. 1. During all four stages divided based on feeding rate, the feed consumption and fish mass increase in both tanks were fairly similar. After 8 weeks of operation, the total feed consumption and net wet weight gain of fish biomass in Tank 1 were 888.2 and 627.1 g, respectively. In Tank 2, the total feed consumption and net wet weight gain of fish biomass were 891.4 and 629.5 g, respectively. Both tanks had the same FCR of 1.4.

The COD concentrations, which represent the concentration of organic matter in the tank, were stable at around 40.5 ± 2.9 mg/L, and the TSS concentrations were around 118.7 ± 10.0 mg/L. These values were consistent with the results of Nootong et al. (2011). In their study, the TSS concentrations in the tanks that received around 18.0 g feed per day remained fairly constant at around 99.0 ± 17.0 mg/L. Fig. 2 shows the variations of TAN and NO_3^- concentrations. Both TAN and NO_3^- concentrations increased with the feeding rate. The highest TAN and NO_3^- concentrations were observed in stage III on day 34, which were 0.2 ± 0.0 and 158.4 ± 1.5 mgN/L, respectively. The concentrations of NO_2^- were fairly

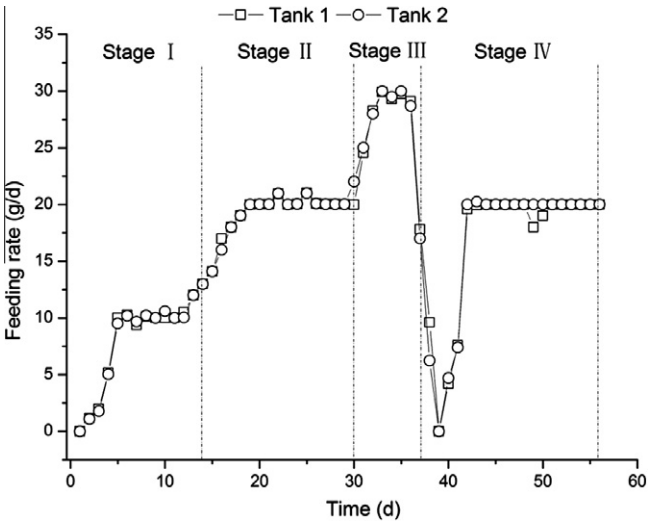

Fig. 1. Variation of feeding rates during the study period.

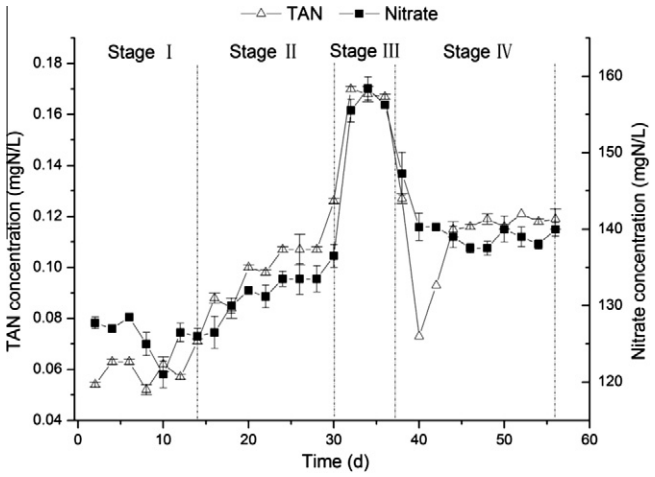

Fig. 2. Variation of TAN and nitrate concentrations during the study period. Each data point is the mean of at least three measurements.

stable during the study period, except for a sharp increase when the feeding rate was increased from 20.0 to 30.0 g/d, subsequent to which NO_2^--N concentrations returned to below 0.1 mgN/L within two days of operation (data not shown).

3.2. Nitrous oxide emissions from intensive aquaculture system

Fig. 3 shows the diurnal variations of N_2O emission rates at different stages during the study period. The time-weighted N_2O-N emissions and conversion rates are reported in Table 2.

In aquaculture systems, persistent N_2O emissions were observed during the study period. At higher N_2O emissions in stage II and stage III, N_2O emission rate initially increased and then decreased. The highest N_2O emission rate occurred at around 7–9 h. However, there were no significant variations of N_2O emission rates in stage I and stage IV, where N_2O emission rates were comparatively low.

Statistically, feeding rate significantly influenced the N_2O emissions from intensive aquaculture system ($p < 0.01$). N_2O-N emissions increased with the feeding rate under the same DO concentration. When the feeding rate increased from 10.0 to

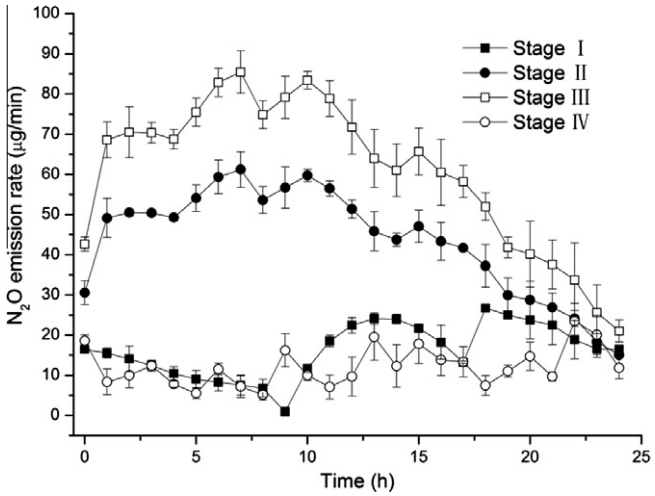

Fig. 3. Diurnal variation of N_2O emission rates at different stages. Error bars correspond to the SD calculated from three repeated experiments.

Table 2
N_2O-N emission and conversion rate of intensive aquaculture system.

Parameters	Stages			
	I	II	III	IV
N_2O-N emission (mgN/d)	14.8 ± 1.8	40.5 ± 2.8	56.6 ± 4.6	11.0 ± 2.7
N_2O-N conversion rate (%)*	2.1	2.9	2.7	0.8

* N_2O-N conversion rate, % = (N_2O-N emission/Nitrogen input of the tank) * 100%.

30.0 g/d, the daily N_2O-N emissions increased from 14.8 ± 1.8 to 56.6 ± 4.6 mgN/d. However, the N_2O-N conversion rate at a feeding rate of 20.0 g/d was 7.4% higher than that at 30.0 g/d. This is likely due to high nitrogen input in aquaculture system at the higher feeding rate. It is apparent from Fig. 1 and Fig. 2 that the concentrations of TAN and NO_3^- were directly correlated with feeding rate. Pedersen et al. (2012) reported a similar correlation between nitrate concentration and feeding rate in an intensive rainbow trout (*Oncorhynchus mykiss*) aquaculture system, with initial mean fish weight of 89.0 ± 7.0 g. At constant DO concentrations, the increase in feeding rate resulted in higher TAN and NO_3^- concentrations in the system. High ammonium concentration facilitates nitrification-driven N_2O emission, while high NO_3^- concentration can enhance denitrification-driven N_2O emission. Furthermore, increasing the air supply to maintain the desired DO concentration in aquaculture system at higher feeding rate also resulted in higher N_2O emission due to stripping.

DO concentration also plays a pivotal role in controlling N_2O emissions from aquaculture systems. Theoretically, DO concentration should be maintained above 5.0 mg/L for optimum fish growth in most aquaculture systems, which is achieved by intensive and continuous aeration. However, studies showed that intense aeration for maximum fish production is less profitable than moderate aeration in improving water quality and enhancing feed conversion efficiency (Boyd, 1998). Normally, DO concentration higher than 3.0 mg/L is good for fish growth.

Significant N_2O emissions differences were found in aquaculture systems under different DO concentrations. At the same feeding rate of 20.0 g/d, daily N_2O emissions increased by nearly 268% when DO concentration was decreased from 5.0–6.0 to 3.0–4.0 mg/L. N_2O was generated during nitrification and denitrification processes. During nitrification, the autotrophic ammonia oxidizing bacteria can use NO_2^- as the terminal electron acceptor for the oxidation of ammonia under oxygen limitation, which results in N_2O emission as a byproduct. During denitrification, the inhibition effect of low oxygen concentration on the activity of N_2O reductase is more prominent than that on other denitrification enzymes, thereby resulting in a higher amount of N_2O emissions (Kampschreur et al., 2009).

When the DO concentrations were maintained between 3.0 and 4.0 mg/L, the N_2O-N conversion rate of the aquaculture system varied from 2.1% to 2.9%. Zheng et al. (1994) conducted a series of laboratory-scale experiments to examine N_2O emissions from nitrifying activated sludge. The results showed that N_2O conversion rates were around 2.5% when the DO concentrations were above 1.7 mg/L. Park et al. (2000) found that biofilm reactor showed a N_2O conversion rate of around 3% when DO concentrations were between 3.0 and 4.0 mg/L. Higher DO concentration (above 5.0 mg/L) is uncommon in wastewater treatment processes, but the decrease of N_2O emission with the increase of DO concentration has been reported by others (Kampschreur et al., 2009; Foley et al., 2010).

3.3. The mass balance of nitrogen in intensive aquaculture system

By assuming that the N_2O-N conversion rates in Table 2 could represent the average N_2O-N conversion rates of each stage, the

nitrogen input and output of the laboratory-scale intensive aquaculture system during the study period were calculated, and are presented in Fig. 4. Although the daily water exchange rate of the system was only 2%, around 40% of the nitrogen input was discharged as effluent due to its high NO_3^- concentration. About 27.4% of the nitrogen input was converted to fish biomass, which was very close to the theoretical value of 25% (Hargreaves, 1998). The concentration of total nitrogen, which was calculated as the sum of TAN, NO_2^- and NO_3^- concentrations, increased slightly following 8 weeks of operation. Only 4% of the nitrogen input was retained in the tank water. About 3.8% of the nitrogen input was distributed as microbial biomass in the tank, and another 3.6% was discharged from the tank as suspended solids. This was consistent with the results of Schneider et al. (2005) who reported that about 7% of feed nitrogen could be converted into bacterial biomass in intensive aquaculture systems.

In aquaculture systems, part of the nitrogen input was emitted to the atmosphere in the form of NH_3, N_2O and N_2. No ammonia volatilization was detected during the study period. The TAN concentrations, pH and salinities in the system were 0.1 ± 0.0 mgN/L, 7.1 ± 0.1, and 0.1 ± 0.1 ppt, respectively. Under such conditions, ammonia volatilization was considered to be negligible. Results showed that about 0.8 ± 0.1 g N_2O-N was emitted to the atmosphere during the 8 weeks study period, accounting for 1.3% of the total nitrogen input. This is much lower than the N_2O emission factor used in the estimation of Williams and Crutzen (2010), which was 5%.

If the present study can be extrapolated linearly, according to the estimation protocol of Hu et al. (2012), the estimated world aquaculture production in 2030 would be 2.3×10^8 metric tons. Since the N_2O emission factor based on the present study was 1.3 gN_2O-N/kg fish, the global N_2O-N emissions from aquaculture in 2030 would be around 2.9×10^{11} g, accounting for 4.3% of anthropogenic N_2O-N emissions. The estimated global distribution of N_2O emissions from aquaculture across the regions and countries are presented in Fig. 6. Based on the present study, Asia accounted for nearly 89% of global N_2O emissions from aquaculture in 2010. China, followed by India and Vietnam, was the largest contributor to global N_2O emissions from aquaculture, with estimated N_2O-N emissions of 4.7×10^{10} g in 2010, equivalent to 2.3×10^7 - tons of CO_2 emission. The top ten aquaculture producing countries were responsible for about 88% of global N_2O emissions from aquaculture systems.

Studies on other aquatic ecosystems, such as rivers and estuaries, showed that about 1% of the nitrogen input was converted to N_2O, while about 0.8% of nitrogen input to streams was emitted to the atmosphere as N_2O (Seitzinger et al., 2000; Beaulieu et al., 2011). Although N_2O conversion rates in different aquatic ecosystems are quite similar, there is no prior study examining N_2O emissions from aquaculture systems. Because of the high nitrogen input and rapid growth rate of aquaculture industry, more attention should be paid to the quantification and mitigation of N_2O emissions from aquaculture systems.

The rest of unknown nitrogen loss, which was calculated to be 13.2 gN, accounting for 20.9% of the total nitrogen input, was attributed to be N_2 emission. N_2 is the final product of denitrification. Although the DO concentrations in the system were high throughout the study period, there were likely micro-anoxic zones at the inner region of bioflocs. High NO_3^- concentrations and the availability of dissolved organic carbon in the system could facilitate the occurrence of denitrification in anoxic zones.

3.4. Possible links between N_2O emission rates and water quality parameters

In a short time scale (24 h), pH, temperature, abundance of nitrifiers and denitrifiers, and NO_3^- concentrations were relatively stable (data not shown). The possible links between diurnal N_2O emission rates and DO, NO_2^-, TAN and COD concentrations in stage II were then investigated (Fig. 5).

Fish were fed at time (t) = 1 h. Feeding had a significant impact on DO concentration. Following feeding, DO concentration started to decrease from the steady state of 4.0 mg/L, and the lowest DO concentration of 2.6 ± 0.2 mg/L was obtained at 7 h. After 7 h, DO concentrations began to increase and reached the steady state concentration again at 18 h. The NO_2^- and TAN concentrations increased after feeding and the peak concentrations of 0.1 ± 0.0 and 0.2 ± 0.0 mgN/L, were obtained at 10 and 8 h, respectively. NO_2^- and TAN concentrations then decreased gradually to steady state concentrations of around 0.0 and 0.1 mgN/L, respectively. The COD concentrations were fairly stable at around 41.6 ± 1.1 mg/L throughout the study period.

The variation of N_2O emission rates, DO, NO_2^-, TAN and COD concentrations were associated with a normal distribution, which enabled the comparison of Pearson's rank correlations between them. Significant positive correlations were found between both N_2O emission rates and NO_2^- ($r = 0.73$, $p < 0.05$) and TAN ($r = 0.63$, $p < 0.05$) concentrations, while N_2O emission rates were significant negatively correlated with DO concentrations ($r = -0.83$, $p < 0.05$). No significant correlation was observed between N_2O emission rates and COD concentrations.

During the study period, DO concentrations in aquaculture systems were maintained at a high value (above 3.0 mg/L) to ensure optimal fish growth. Thus, nitrogen transformations in the aquaculture system were most likely driven by nitrification. From a fundamental perspective, NO_2^- concentration is one of the most important factors contributing to nitrification-driven N_2O emission. The accumulated NO_2^- can be used as an electron acceptor to produce N_2O by ammonia oxidizing bacteria, including *Nitrosomonas europaea* and several *Nitrosospira* spp., which may be abundant in aquaculture systems. At lower DO concentrations, the competition between ammonia oxidizing bacteria and nitrite oxidizing bacteria for oxygen caused an accumulation of NO_2^-, which in turn resulted in N_2O generation. In addition, as a biologically active substrate, the magnitude of nitrification-driven N_2O emission also depends directly on NH_4^+ concentration. This was also the reason why higher N_2O emissions were observed in aquaculture system at higher feeding rates.

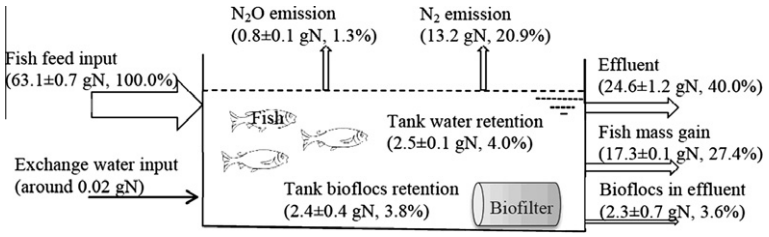

Fig. 4. Nitrogen balance in an intensive aquaculture system.

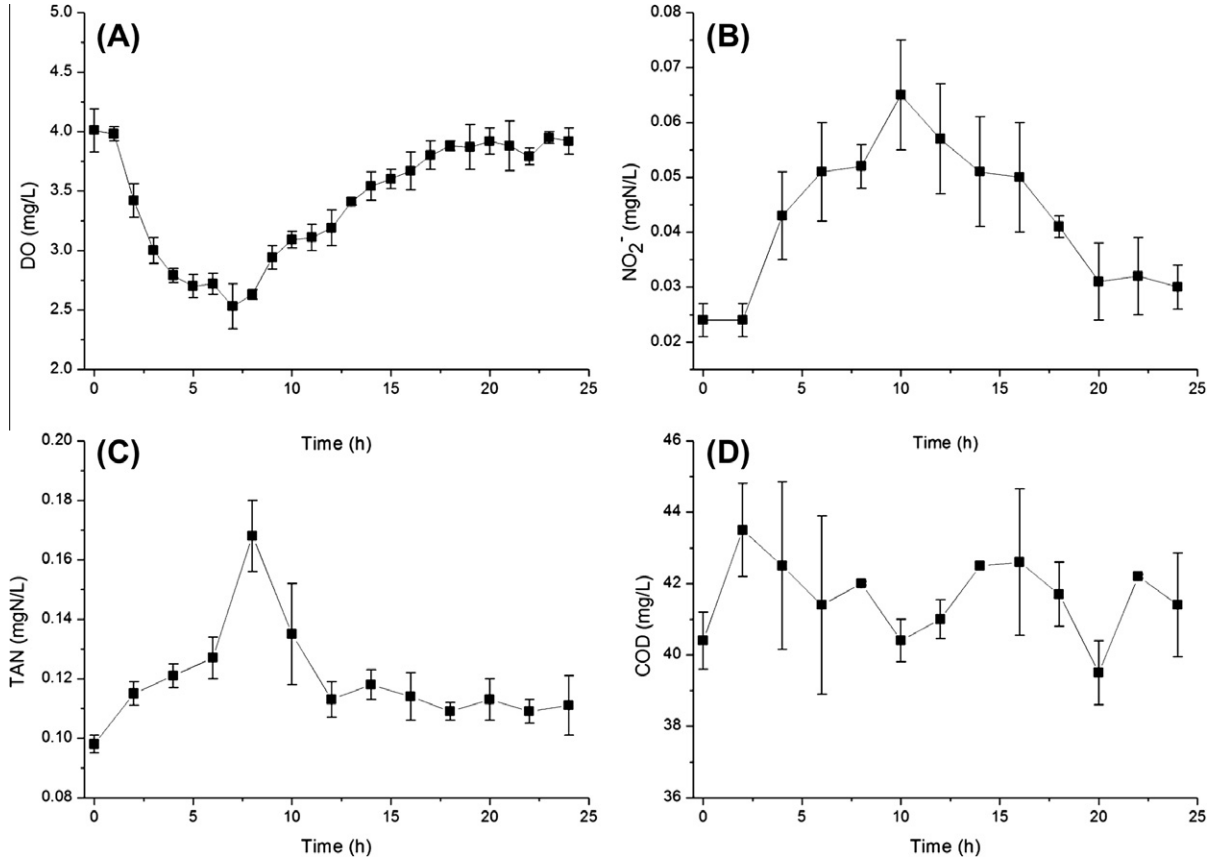

Fig. 5. Diurnal variation of DO, NO_2^-, TAN, and COD concentrations in Stage II. (A) DO; (B) NO_2^-; (C) TAN; (D) COD. Error bars correspond to the SD calculated from three repeated experiments.

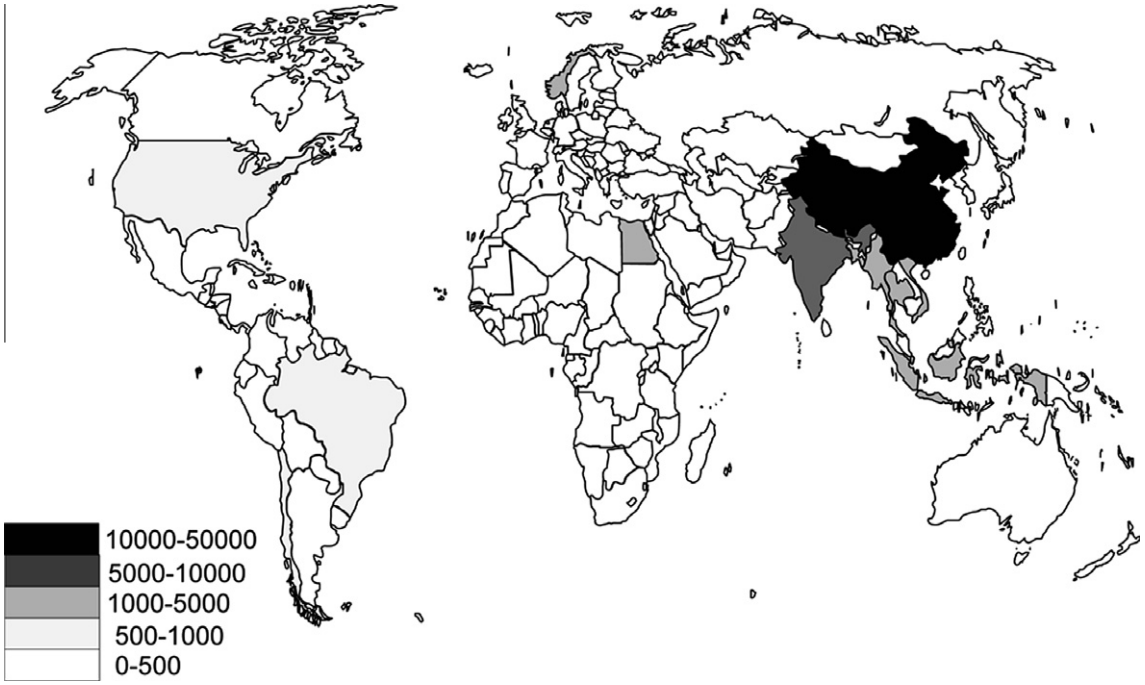

Fig. 6. Global mapping of N_2O emissions from aquaculture systems across the regions and countries.

It is important to note that although aquaculture system was dominated by oxic environment, the contribution of denitrification cannot be ignored. This was evident from the fact that a high quantity of nitrogen was lost as N_2. Denitrification occurred in the anoxic zone of the bioflocs. Denitrifiers are heterotrophs and need organic carbon for their metabolism. In the present study,

all the feed was consumed by fish and no external organic carbon source was provided; thus, organic carbon was a limiting factor for denitrification. Denitrification was fueled by the carbon compound released from the breakdown of endogenous carbon (van Rijn et al., 2006). Organic carbon limitation resulted in the accumulation of intermediate products of denitrification, such as NO and N_2O. Interestingly, as with nitrification, the denitrification-driven N_2O production was also influenced by low DO and high NO_2^- concentration. Higher DO concentration inhibits the activity of enzymes through denitrification and eliminates the inner anoxic zone of the bioflocs; thus reducing the denitrification driven N_2O production. High NO_2^- concentration during denitrification leads to the accumulation of N_2O (Lu and Chandran, 2010). Therefore, the N_2O emissions from aquaculture systems could be linked to both nitrification and denitrification.

In aquaculture, 80% of assimilated nitrogen is excreted to the surrounding water by fish, and 90% of the excreted nitrogen is TAN (Ebeling et al., 2006). During the 8 weeks study period, about 63.1 g nitrogen was fed to the system, and because all of the fish feed was digested by fish, nitrogen excreted as TAN would be 45.4 g. Assuming that a complete nitrification occurred in the system, most of the TAN would be oxidized to NO_3^-, except for 4.7 gN that was assimilated by microorganisms. Then about 13.6 gN would be removed through denitrification. Since the estimated N_2 emission was 13.2 gN, about 0.4 gN was emitted to the atmosphere as N_2O through denitrification pathway, the rest 0.4 g N_2O-N emission was ascribed to nitrification pathways. In order to mitigate the N_2O emissions from aquaculture systems, it is important to investigate the mechanisms of N_2O production in aquaculture systems. The present study does not precisely identify the contribution of each process to N_2O emissions. Further studies, with the combination use of stable isotope and molecular biology techniques, may be able to identify the sources of N_2O emissions from aquaculture systems.

4. Conclusions

Nitrogen transformations in intensive aquaculture system were studied by focusing on N_2O emission. Results showed that aquaculture was an important source of anthropogenic N_2O emissions. The N_2O emission rate was positively correlated with the concentrations of NO_2^-, DO and TAN within the system. Feeding rate and DO concentration could affect N_2O emissions from aquaculture systems significantly. Nitrification and denitrification processes were equally responsible for the emissions of N_2O from aquaculture systems.

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