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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.