NITROUS OXIDE EMISSION AND PRODUCTION PATHWAYS UNDER ALTERNATE WETTING-DRYING CONDITIONS IN RICE PADDY SOILS

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Abstract. The aim of this study was to evaluate the relative contributions of nitrification and denitrification in rice paddy soils under various water events. A laboratory incubation study was conducted in China to quantify N_2O production during alternate wetting and drying cycle (AWD) versus permanent flooding (PF). The soils were treated with long-term chemical fertilizer (CF); chemical fertilizer plus pig manure (PMCF); and chemical fertilizer plus rice straw (SRCF) for 5 years. The results showed that N_2O flux during AWD was consistently higher than PF. The highest N_2O flux during AWD was 1.94 mg m^{-2} h^{-1}. The PMCF and SRCF soils had higher N_2O emissions compared to CF and CK soils. Ammonia oxidizer community peaks were found at 60% field capacity (FC) ($p < 0.0001$), while, for denitrifier, this increase was maintained for a certain period of time (10d) and then started to decrease. Autotrophic nitrification appeared to be an important and dominant process of N_2O emissions during AWD and PF, contributed 79.03% of N_2O emissions during AWD, while 36.53% during PF. Thus, the results concluded that under AWD event the addition of pig manure and rice straw plus chemical fertilizer significantly increased the N_2O flux, by stimulating the growth of microbial communities.
Keywords: *N_2O, flux, water events, pig manure, rice straw, ^{15}N stable isotopes*

Introduction

Ecosystems are adversely affected by the global warming if actions to mitigate it are not taken. Besides industrial emissions, biological processes can also contribute to the greenhouse gas emissions (GHG), especially nitrous oxide (N_2O). N_2O is an important greenhouse gas and its concentration in the atmosphere is comparatively lower than CO_2 concentration but its global warming potentials (GWP) is relatively 265 times higher over 100 years than that of CO_2 (IPCC, 2013). Agricultural soils contribute about 60% to anthropogenic N_2O emissions which is mainly due to fertilizer application (Charles et al., 2017; Liu et al., 2017). Fertilization of agricultural soils is considered to be an important source of N_2O emissions, which contributes about 13-24% of annual emissions (IPCC, 2007). Among these agricultural soils, rice paddy soils are considered to be a main source of N_2O emissions. Rice is a staple food for half of the world's population and approximately 155 million ha. is grown annually, around the globe (Abid et al., 2018).

Alternating wetting and drying (AWD) practice is a common practice which is being used to save water in rice fields. AWD reduce water use by 23-33% in rice paddy soils (Carrijo et al., 2017). It has been used in many countries such as Philippines (Belder et

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al., 2004), China (Cabangon et al., 2004) and Japan (Chapagain and Yamaji, 2010). The AWD practice has been found to give higher or equal rice yield (Zhang et al., 2009) compared to conventional practice, i.e. continuous flooding practice, where surplus of N_2O is produced during this process (Peng et al., 2011). However, the underlying causes, e.g. microbial mechanisms of N_2O emissions are generally unknown.

Nitrous oxide is mainly emitted by nitrification and denitrification processes (Wrage et al., 2001), and both processes may occur in soil simultaneously (Abbasi and Adams, 2000; Garrido et al., 2002; Webster and Hopkins, 1996). To what extent each of these processes contributes to the N_2O emissions in the rice during AWD and permanent flooding (PF) are not fully understood. This is mainly attributed to oxygen content of the soil, and further estimation of water filled pore space (WFPS) has been considered as a major influencing factor. Nitrogen stable isotopes with acetylene (C_2H_2) can offer a precise information about nitrogen cycle in ecosystems (Ostrom et al., 2002). It is of interest to use ^{15}N as a tracer because a negligible isotope fractionation is being used as a tracer during biological processes. Acetylene (C_2H_2) inhibits NH_3^+ oxidation at low concentration (10 Pa) and start to inhibit N_2O reductase at high concentration (10 kPa) during denitrification (Berg et al., 1982). Also, C_2H_2 may inhibit NH_4^+ oxidation by autotrophs but inhibition by heterotrophic nitrifiers is not documented (Moir et al., 1996; Hynes and Knowles, 1982; Daum et al., 1998). Recently, stable isotope signatures coupled with C_2H_2 (10 Pa; 0.01% v/v) are being used to estimate N_2O source and relative contributions of denitrification, heterotrophic nitrification and autotrophic nitrification processes in soil (Baggs et al., 2003; Stevens et al., 1997).

The obvious benefits of implementing AWD strategy are to save irrigation water and reduce CH_4 emissions. On the other hand, the combined effect of long term fertilization and AWD on N_2O is still inconclusive. Also, the N_2O production source during AWD and PF events is still poorly understood. Therefore, the objectives of the present study were: (1) to determine the potential of AWD to produce N_2O from rice paddies as compared to the normal practice of rice (PF); (2) to determine the effect of long term organic (PMCF, SRCF) and inorganic (CF) fertilizers along with different water events (AWD, PF) on N_2O gas emissions; and (3) to quantify the contribution of different processes (nitrification, denitrification) to N_2O emissions under AWD and PF related to long term fertilization, using ^{15}N stable isotopes in combination with C_2H_2.

Materials and methods

Soil sampling site

A long-term fertilized experiment (LTFE) was conducted from 2010 to 2014 and soil samples were collected. This site was located in Jintan county, Jiangsu province, China (120° 0’ 41” East, 29° 57’ 9” North). The annual mean precipitation and temperature of study area are 1452.5 mm and 16.27 °C, respectively. A rice paddy field was selected with 5-yr canola-rice rotation history. The soil samples were grouped into four treatment groups, including no fertilization (CK), 100% chemical fertilization (CF: N 314 kg ha^{-1}, P_2O_5 31.5 kg ha^{-1}, K_2O 153 kg ha^{-1}), pig manure compost plus 50% chemical fertilization (PMCF: Pig manure 6000 kg ha^{-1} + N 157 kg hm^{-2}, P_2O_5 31.5 kg ha^{-1}, K_2O 153 kg ha^{-1}) and rice straw plus 50% chemical fertilization (SRCF: rice straw 6000 kg ha^{-1} + N 157 kg ha^{-1}, P_2O_5 31.5 kg ha^{-1}, K_2O 153 kg ha^{-1}). Just after the rice harvested in 2014, five soil cores were collected at a depth of 15 cm from three replicates of each treatment and five hundred grams of soils were packed. The soil cores

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were put in sterile plastic bags, zipped and transported to the laboratory and stored at 4 °C. Each replicated sample was divided and one sub sample was air-dried and sieved through a 2.0 mm for subsequent chemical analysis and another sub sample was incubated under different dry and wet conditions.

Incubation design

The incubation was carried out at 25 °C with two water events including: (a) alternating with 7 days continuous flooding followed by 7 days of air-drying (AWD); (b) permanent flooding for 14 days (PF). The detailed methodology of the incubation experiment was as follows (*Fig. 1*). Two hundred g of soil collected from CK, CF, PMCF and SRCF treatments were put in 1000 mL glass pots and to make flooding conditions additional water was added. For AWD, soils were incubated as flooded for 7 days, with gas and soil samples were collected after 1 and 7 days of flooding incubation (100%FC, 1 d; 100%FC, 7 d). Then the water was removed to reach 80% field capacity (80%FC, 10 d) and 60% field capacity (60%FC, 14 d), samples were also collected at 80%FC, 10 d and 60%FC, 14 d). For PF, the same treatments were incubated with flooded conditions for 14 d and gas and soil samples were collected after 1, 7, 10 and 14 d. Headspace gas samples were collected after 1, 7, 10 and 14 d immediately after closing the jars and 30 min later using a hypodermic needle and a polypropylene syringe. These gas samples were used to analyze the isotopic ^{15}N-N_2O. N_2O was calculated using GC 2010 plus (Shimadzu).

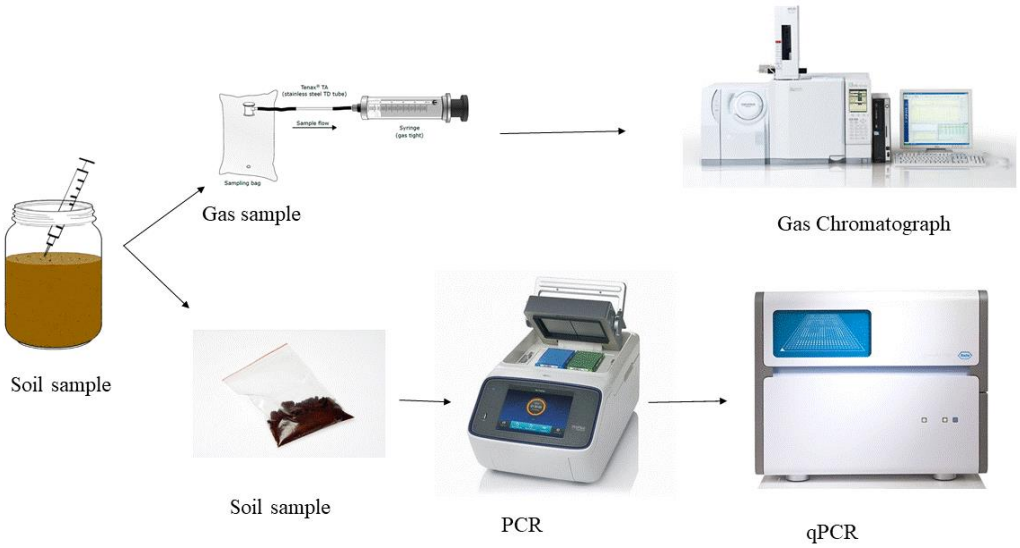

Figure 1. *Schematic diagram of whole study plan*

After 14 days of incubation, all soils were treated with (a) $^{14}NH_4{}^{15}NO_3$, (b) $^{15}NH_4{}^{15}NO_3$, (c) $^{15}NH_4{}^{15}NO_3 + C_2H_2$ (0.01% v/v), and (d) no N addition. $^{14+15}N$ was applied at 200 mg total N m^{-2} (20% atom % excess ^{15}N) combined with C_2H_2 (10 Pa) (*Table 1*). Additional water was added to achieve the desired moisture contents (flooding). Also, three replicates of each treatment was created for gas analyses. To create gas-tight incubations, the lids of the jars were then closed. Five ml (1% v/v in air) of C_2H_2 gas was injected to the headspace of a further three replicates of the $^{15}NH_4{}^{15}NO_3$ fertilizer treatments (treatment c) (*Table 1*) to make a final concentration of

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0.01% (v/v) which was considered sufficient to inhibit oxidation of NH_3^+ (Bateman and Baggs, 2005). Five days after fertilizer additions, the jars were opened because inhibition continued for about 3 days after 0.01% (v/v) C_2H_2 addition, and there was no effect of jars opening on trace gas production (Bateman and Baggs, 2005). Seven days after fertilizer addition, 20 ml gas samples were collected at 0 and 30 min after closing the glass jars from each treatment using poly-pyrine syringe. The ^{15}N was analyzed using isotope ratio mass spectrometer (Europa PDZ 20:20).

Table 1. *Outline of the ^{15}N fertilizer and C_2H_2 inhibition treatments used to estimate the contribution of denitrification and autotrophic and heterotrophic nitrification to ^{15}N-N_2O emissions*

Treatment	Source of ^{15}N-N_2O
(a) $^{14}NH_4{}^{15}NO_3$	Denitrification
(b) $^{15}NH_4{}^{15}NO_3$	Denitrification and nitrification (autotrophic and heterotrophic)
(c) $^{15}NH_4{}^{15}NO_3$ + C_2H_2(0.01% v/v)	Denitrification and heterotrophic nitrification
(d) (c) minus (a)	Heterotrophic nitrification

q-PCR to determine the abundance of nitrifiers and denitrifiers

After 1, 7, 10 and 14 d of different water application, soil samples were collected and 0.5 g sampled soil was used to extract DNA using TAKARA DNA standard protocol. Nano drop technology was also used to quantify DNA (Nano Drop Technologies, Wilmington, DE, USA). Real time quantitative PCR was used to quantify the abundance of denitrifier (*nirS, nir*K) and nitrifier (AOA *amo*A, AOB *amo*A) communities. Serial dilutions of linearized plasmids were used to produce standard curves by using cloned AOA, AOB, *nirS* and *nir*K genes amplified from denitrifying and nitrifying strains. The cloning kit (V007 TsingKe China) was used to prepare standard (www.tsingke.net). The qPCR primers sets used to target desired genes are indicated in *Table 2*. A reaction mixture of 20 μL containing 1 μL target gene primer, SYBR Premix 10 μL, total DNA template 1 μL, and deionized distilled water to make final volume 20 μL. The copy number of these plasmids was directly calculated based on the concentrations and lengths (base pairs) of them. The copy number of the target genes in unknown soil DNA were analyzed by comparing with their reactive standard curves.

Table 2. *Real-time PCR primer sets, conditions of the assay*

Target gene base pairs		Primer	Nucleotide sequence (5'-3')	Annealing temperature and time	Reference
Bacterial *amo*A	491 bp	amoA-1F amoA-2R	GGGGTTTCTACTGGTGGT CCCCTCKGSAAAGCCTTCTTC	55 °C for 45 s	Rotthauwe et al., 1997
Archaeal *amo*A	635 bp	amoAF amoAR	STAATGGTCTGGCTTAGACG GCGGCCATCCATCTGTATGT	53 °C for 45 s	Francis et al., 2005
nirK	472 bp	F1aCu R3Cu	ATCATGGTSCTGCCGCG GCCTCGATCAGRTTGTGGTT	63 °C for 30 s	Hallin and Lindgren, 1999
nirS	425 bp	Cd3aF R3 Cd	GTSAACGTSAAGGARACSGG GASTTCGGRTGSGTCTCTTGA	57 °C for 30 s	Throbäck et al., 2004

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Statistical analysis

SPSS 16.0 package was used to perform all statistical (SPSS, Chicago, IL, USA). One way analysis of variance (ANOVA) test was used to analyze significant differences among different treatments. ANOVA, followed by the least significant difference (LSD) test, in which $P < 0.05$ was considered statistically significant. Also, Duncan's Multiple Range Test was used to determine significant mean differences. Correlation analysis was performed to analyze the correlation between variables. All results were accepted at significant probability 0.05.

Results

N_2O gas emission

Nitrous oxide (N_2O) fluxes of the incubated soils showed different trends during AWD and PF events (*Fig. 2*). N_2O flux increased with reducing moisture contents over time during AWD. N_2O emitted over 14 days (60% FC) was significantly higher ($P < 0.001$) throughout the experiment during AWD than PF. While comparing fertilizer treatments, the highest emissions were recorded from SRCF (1.88 mg m^{-2} h^{-1}) and PMCF (1.34 mg m^{-2} h^{-1}) over 14 days, which were 12 and 20 times greater than those over 10 days and 7 days of AWD, respectively. N_2O emissions from fertilized soils decreased after one day throughout trial period but were greater than unfertilized controls. The highest fluxes were measured at 60% FC over 14 days. In the CK and CF treatments, N_2O fluxes were minimal over 1 day which was 0.02 and 0.01 mg m^{-2} h^{-1}, while the treatments of PMCF and SRCF showed slightly higher N_2O emissions, ranging from 0.12 to 0.28 mg m^{-2} h^{-1} with same moisture contents. No significant differences were observed in total N_2O produced over 1 day and 7 days of flooded conditions. During AWD, the emissions of N_2O increased when exposed to air-drying conditions after continuous flooding over one week. While During PF, the fluxes significantly decreased with time and the lowest fluxes were measured over 14 days of continuous flooding ($P < 0.0001$).

Sources of N_2O

Labelling of ^{15}N is the only way to differentiate N pool, as a possible substrate for N_2O production. N_2O emitted from C_2H_2 treatments could be produced either by heterotrophic nitrification or denitrification (treatment c), and ^{15}N-N_2O production from $^{14}NH_4{}^{15}NO_3$ (treatment a) could be produced by denitrification (*Table 1*). The difference in the emissions of ^{15}N-N_2O between the $^{14}NH_4{}^{15}NO_3$ (treatment a) and $^{15}NH_4{}^{15}NO_3$ (treatment b) was attributed to nitrification, and the difference in the emissions of ^{15}N-N_2O between $^{14}NH_4{}^{15}NO_3$ (treatment a) and $^{15}NH_4{}^{15}NO_3 + C_2H_2$ (treatment c) was used to quantify heterotrophic nitrification. Results of the present study showed relatively higher ^{15}N-N_2O emissions were measured during AWD than PF (*Fig. 3*) but this relation was not significant. The total ^{15}N-N_2O emissions were recorded during AWD condition in PMCF and SRCF treatments (0.23, 0.24 mg m^{-2} h^{-1}), which were significantly higher than CK and CF treatments. ^{15}N-N_2O emissions during 60% FC in the presence of C_2H_2 were significantly lower than the other treatments, indicating autotrophic nitrification as main contributor to N_2O emissions (*Table 2*). In the presence of C_2H_2, N_2O produced was accredited to denitrification and/or heterotrophic nitrification. A strong significant positive correlation was found between autotrophic nitrification nitrous oxide production,

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indicating autotrophic nitrification as a predominant ^{15}N-N_2O source during both AWD and PF ($P < 0.01$, $R^2 = 0.77$) (*Table 3; Fig. 4*). The maximum N_2O production from autotrophic nitrification was 0.19 mg N m^{-2} h^{-1} during AWD. Between 57 and 72% of N_2O production during both AWD and PF emitted from autotrophic nitrification in all fertilized treatments and significantly higher compared to CK. The mean ^{15}N-N_2O emissions during AWD were 0.89 mg N m^{-2} h^{-1}, contributing 79% by autotrophic nitrification, while during PF, the N_2O emissions were 0.13 mg N m^{-2} h^{-1}, and 52% was contributed by denitrification and 36% by autotrophic nitrification (*Table 4*).

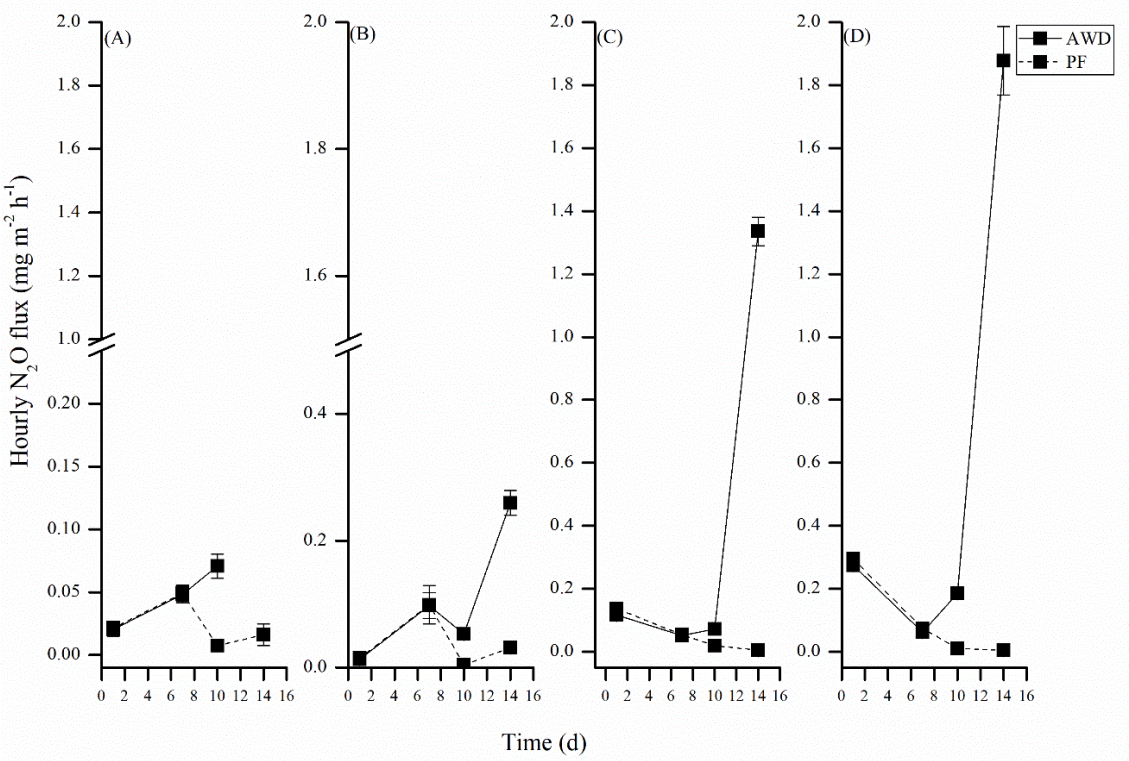

Figure 2. *Hourly N_2O emission (mg m^{-2} h^{-1}) from (A) CK, (B) CF, (C) PMCF and (D) SRCF treatments after 1, 7, 10 and 14 days of different water treatment incubation. AWD represents alternate drying and flooding conditions while PF represents permanent flooding conditions. Arrow bar represents standard deviation. CK: control, CF: Chemical fertilization, PMCF: Pig manure plus chemical fertilizer, SRCF: Rice straw plus chemical fertilizer*

Table 3. *Correlation of total isotopic $^{15}N_2O$ with denitrification, heterotrophic nitrification and autotrophic nitrification (n = 8)*

	Total $^{15}N_2O$ flux	**Denitrification**	**Heterotrophic nitrification**	**Autotrophic nitrification**
Total $^{15}N_2O$ flux	1.00	0.03	0.36	0.88**
Denitrification	0.03	1.00	0.50*	-0.44
Heterotrophic nitrification	0.36	0.50*	1.00	0.05
Autotrophic nitrification	0.88**	-0.44	0.05	1.00

*Correlation is significant at level 0.05
**Correlation is significant at level 0.01

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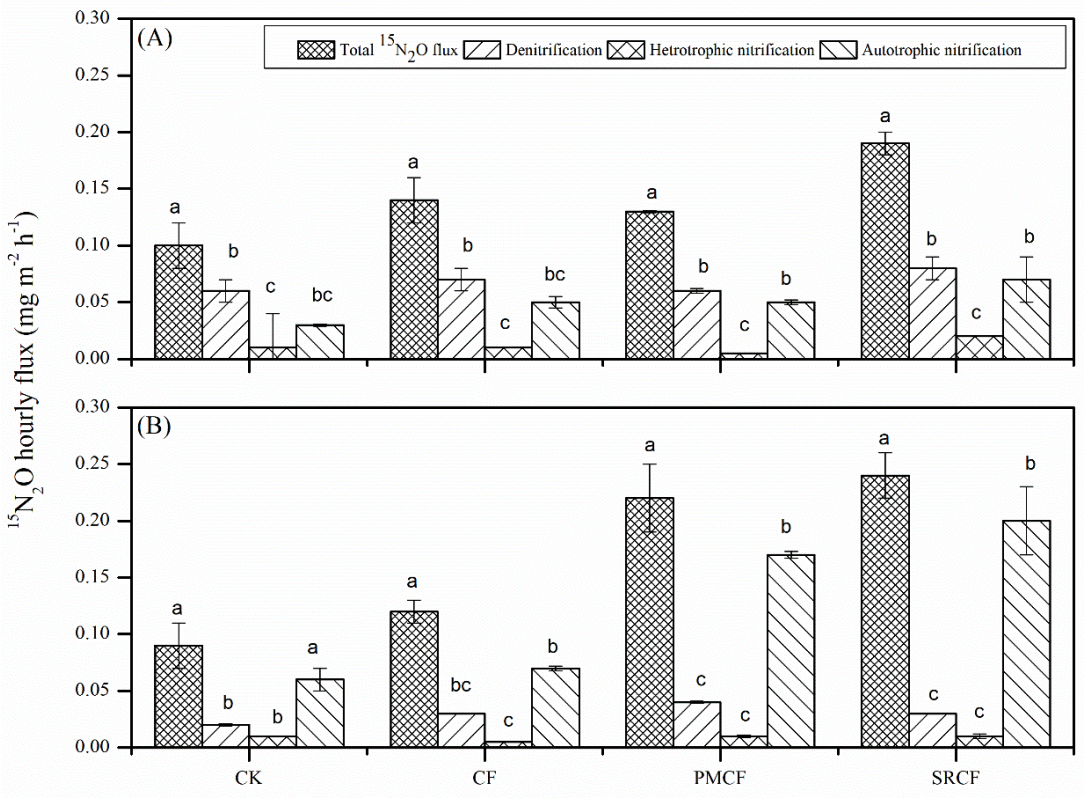

Figure 3. *Hourly isotopic N_2O emission (mg m^{-2} h^{-1}) and the emission come from denitrification, heterotrophic and autotrophic nitrification from (A) PF and (B) AWD conditions. Arrow bar represents standard deviation (n = 3). Different letters show significant differences among treatments (P < 0.05). CK: control, CF: Chemical fertilization, PMCF: Pig manure plus chemical fertilizer, SRCF: Rice straw plus chemical fertilizer*

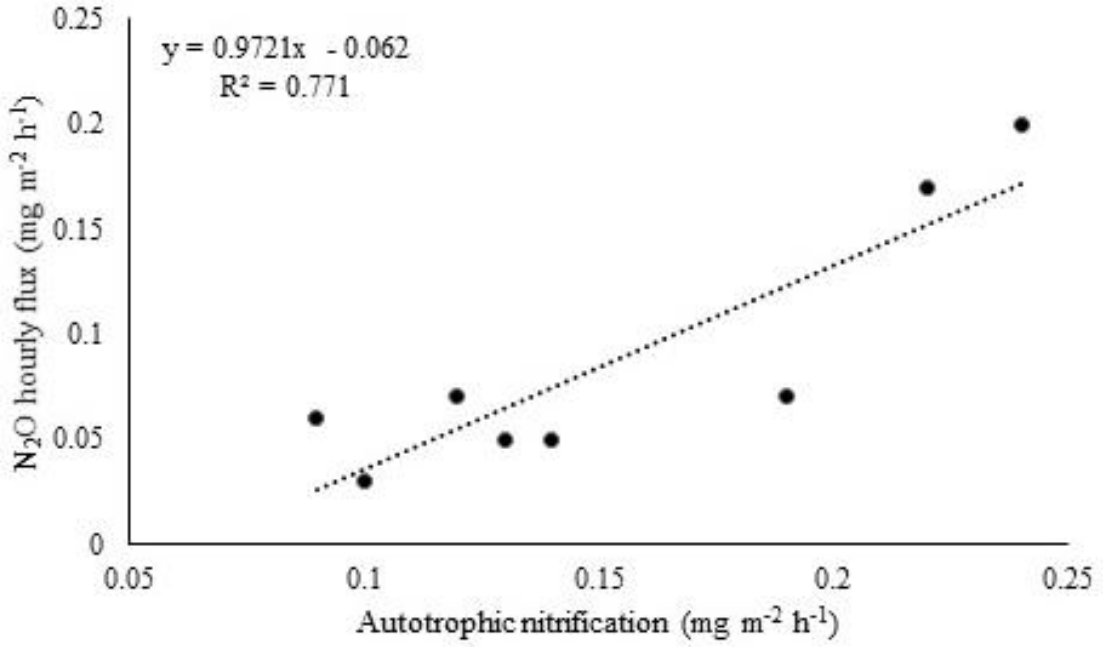

Figure 4. *Correlation between total $^{15}N_2O$ emission and autotrophic nitrification*

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Table 4. *Mean hourly emission of $^{14+15}N_2O$ emission (mean ± S.D), total and percentage (%) contribution of denitrification, heterotrophic and autotrophic nitrification during AWD and PF events (n = 4)*

	AWD	**PF**
	60% FC, 14 d	**Flooding, 14 d**
Total $^{14+15}N_2O$ emission (mg m^{-2} h^{-1})		
$^{14}N_2O$ hourly flux	0.89 ± 0.22	0.15 ± 0.01
$^{15}N_2O$ hourly flux	0.16 ± 0.06	0.13 ± 0.03
Contributing processes (mg m^{-2} h^{-1})		
Denitrification	0.03 ± 0.01 (16.12%)	0.07 ± 0.01 (52.88%)
Heterotrophic nitrification	0.01 ± 0.00 (5.16%)	0.01 ± 0.01 (10.57%)
Autotrophic nitrification	0.12 ± 0.06 (79.03%)	0.05 ± 0.01 (36.53%)

AWD: alternate wetting and drying, PF: permanent flooding

Abundance of nitrifier and denitrifier

The AOA *amo*A gene abundance was found in the range of 2.16E + 06 - 1.61E + 08 copies g^{-1} of the soil (*Fig. 5*). The copy numbers increased with time during both AWD and PF events. The AOA *amo*A gene abundance was found significantly higher at 60% FC (14 days) in the CK, PMCF and SRCF ($P < 0.0001$). While, during PF, PMCF only increased AOA *amo*A gene abundance, especially after 10 days and 14 days.

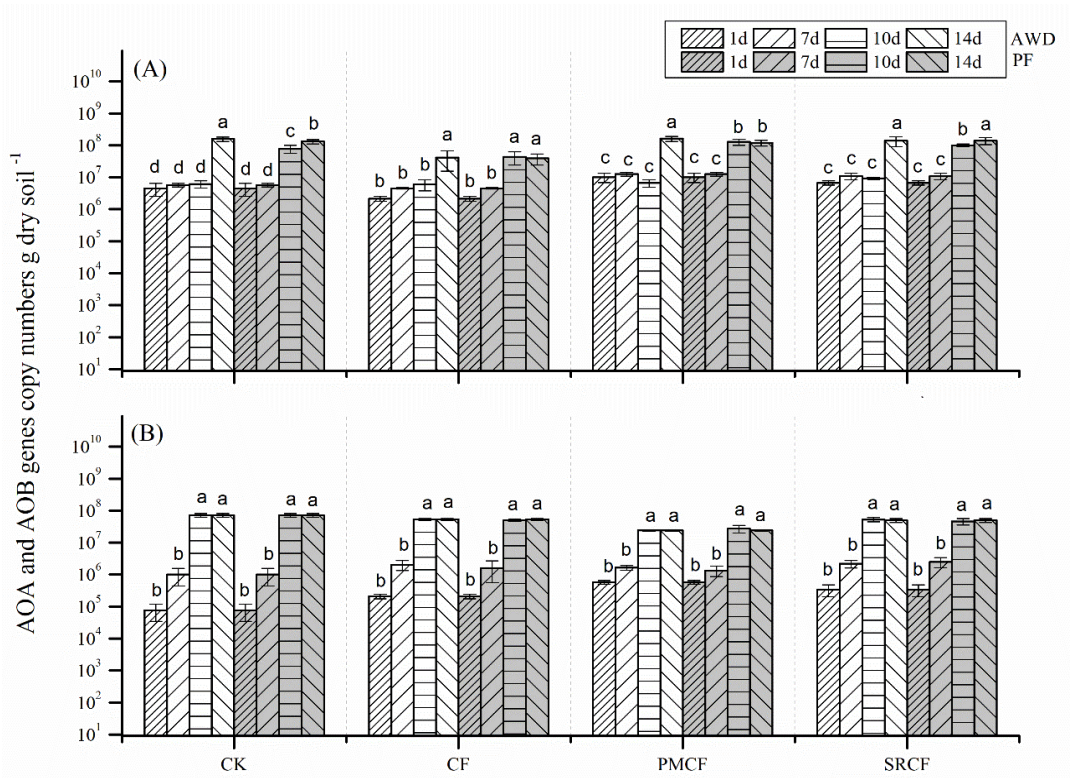

Figure 5. *Abundance of (A) AOA and (B) AOB gene (copy numbers g dry $soil^{-1}$) after 1, 7, 10 and 14 days of incubation during AWD and PF events. AWD represents alternate drying and flooding conditions while PF represents permanent flooding conditions. Arrow bar above the line shows standard deviation (n = 3, P < 0.0001). CK: control, CF: Chemical fertilization, PMCF: Pig manure plus chemical fertilizer, SRCF: Rice straw plus chemical fertilizer*

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The AOB *amo*A gene abundance was found in the range of 7.68E + 04 - 7.13E + 07 copy numbers g^{-1} of dry soil, with the highest value at 60% FC (14 days) in CK and CF treatments (*Fig. 5*). While, during AWD, a gradual increase in copy numbers were noted with time and relatively high at 80% FC and 60% FC. Also, during PF, this increase in copy numbers were recorded with time in all treatments. It was interesting that significant increases in copy numbers were found in CK and CF treatments during early stages ($P < 0.0001$). After 14 d, significant differences were noted between treatments.

The abundance of the *nir*S gene significantly increased with time during both water events. Significant differences were found among treatments during the later stage of experiment ($P < 0.0001$). During PF event, this increase in copy numbers were in certain limit and after that it started to decrease or remain constant. Moreover, relatively higher gene abundances were found in PMCF and SRCF during the whole trial period during both water events.

Although the abundance of *nir*K gene varied with time under all moisture conditions i.e. 60% FC and 100% FC. The high genes abundance was found during flooded condition in all treatments but relatively higher abundance was in PMCF treatment (*Fig. 6*). The highest value (6.94E + 06) of *nir*K was observed in the PMCF treatment after 14 days of PF.

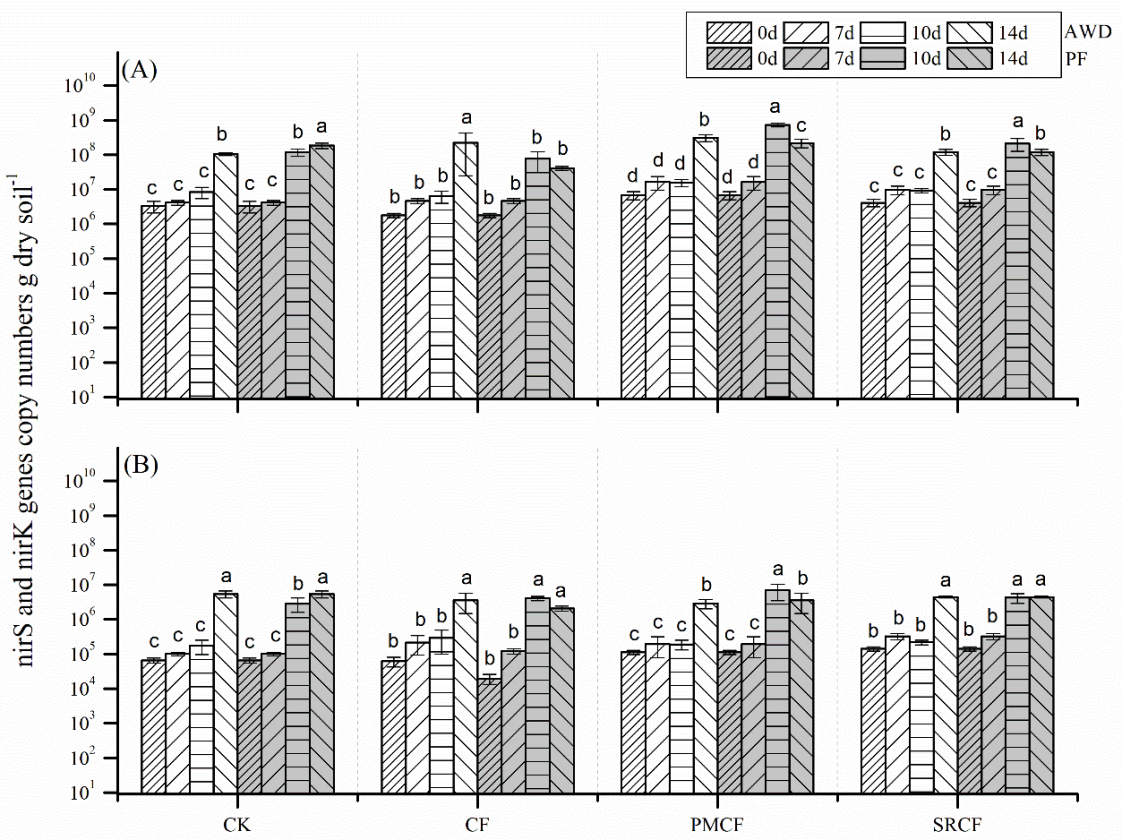

Figure 6. *Abundance of (A) nirS and (B) nirK genes (copy numbers g dry soil^{-1}) after 1, 7, 10 and 14 days of incubation during AWD and PF events. AWD represents alternate drying and flooding conditions while PF represents permanent flooding conditions. Arrow bar above the line shows standard deviation (n = 3, P < 0.0001). CK: control, CF: Chemical fertilization, PMCF: Pig manure plus chemical fertilizer, SRCF: Rice straw plus chemical fertilizer*

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Proportion of greenhouse gases emission associated with microbial biomass and water condition

The relationship of nitrifiers and denitrifiers with N_2O emissions under AWD and PF conditions are given in *Table 5*. A significant correlation was observed between AOB and N_2O emissions during both AWD and PF, suggesting that AOB is responsible for N_2O emissions whatever the water events are (AWD or PF). The correlation between N_2O and nitrifiers and denitrifiers suggested that N_2O emissions is mainly affected by AOB.

Table 5. *Correlation between N_2O, AOA, AOB and nirK genes (n = 24)*

	N_2O	**AOA**	**AOB**	***nir*S**	***nir*K**
N_2O	1.00	-0.96**	0.54*	0.11	0.92**
AOA	-0.96**	1.00	-0.36	-0.29	-0.81**
AOB	0.54*	-0.36	1.00	0.01	0.81**
*nir*S	0.11	-0.29	0.01	1.00	0.01
*nir*K	0.92*	-0.81**	0.81**	0.01	1.00

*Correlation is significant at level 0.05 (two tail test)
**Correlation is significant at level 0.01 (two tail test)

Discussion

Effect of moisture contents on N_2O emissions

A plethora of previous literature have described the significant changes in N_2O emissions during alternate flooding and air drying events in paddy fields (Toyoda et al., 2011; Nishimura et al., 2011). AWD generally promotes N_2O emissions compared to PF in most of studies (Prieme and Christensen, 2001; Wu et al., 2017; Zou et al., 2005; Hou et al., 2012). Although, significant higher N_2O fluxes were observed when the water was removed from the all treatments (10d, 14d), confirmed by earlier findings (Toyoda et al., 2011; Nishimura et al., 2004, 2011). Jorgensen et al. (1998) and Scholes et al. (1997) have reported a rise in N_2O flux during AWD after submerged conditions, and these results were due to the combined effect of high microbial activity and escape of entrapped N_2O (Rice and Smith, 1982). Moreover, the N_2O emissions in PMCF, SRCF and CF treatments were greater than control (CK) suggesting that the application of long-term inorganic or organic fertilizers increase N_2O productions during soil drying-rewetting events (*Fig. 2*). This high N_2O emission in the PMCF and SRCF might be due to the high concentration of organic N fertilizer in the PMCF and SRCF. In this study, N_2O fluxes were positively affected by fertilization (CF, PMCF, and SRCF). Similar results were reported in previous studies conducted in rice paddies (Hou et al., 2012; Zou et al., 2005) presenting that fertilization had a noticeable effect on N_2O emissions because majority of inorganic N is supplied by fertilization in the form of NH_4^+-N and NO_3^--N, which promotes N_2O production (Liu et al., 2015). Harrison-Kirk et al. (2013) revealed N_2O fluxes increased exponentially by increasing C up to 50 mg g^{-1} soil by organic fertilizer application. It is interesting that N_2O fluxes always showed the following order: CK < CF < PMCF < SRCF during whole trial period.

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Effect of water conditions on nitrifier and denitrifier abundance based on long term fertilization

AOA abundance in our results was more dominant compared to AOB, which was consistent with previous studies (He et al., 2007; Alam et al., 2013). This increase in AOA compared to the abundance of AOB might be due to high AOA affinity for oxygen (Szukics et al., 2009). However, Soil pH is another sole factor which may increase AOA abundance, producing favorable conditions for AOA in the paddy fields. Meanwhile, AOB abundance was mainly increased by pH (Nicol et al., 2008). In addition, some previous studies revealed that fertilization rate is the main driver of AOB and AOA abundances to decrease or remain unaffected (Zhong et al., 2016; Di et al., 2014). Also, the influence of fertilizer application on AOA and AOB were reported in some other filed studies (Dai et al., 2013; Alam et al., 2013) and laboratory incubation (Di et al., 2009). NH_4^+ availability produced by organic nitrogen mineralization or either by added inorganic NH_4^+ is the sole factor responsible for the abundance of AOA and AOB (Tourna et al., 2008). However, the pH might influence NH_4^+availability because under low pH it would be ionized to NH_4^+ (Nicol et al., 2008; He et al., 2007). Therefore, in the rice paddies, the drop of soil pH by fertilization, was a more significant factor to increase the availability of substrate for AOA.

The abundances of *nirK* and *nirS* genes had dramatically increased with time after 7 days in AWD and in PF until 10 days and then maintained or decreased (after 14 days). These results were in agreement with previous study of Uchida et al. (2014), indicated that under the absolute anaerobic conditions, the denitrifier abundance was reduced. In addition, the denitrifier abundance was influenced by flooding-drying pattern because denitrifier growth usually stimulate near anaerobic conditions (Uchida et al., 2014; Di et al., 2014). Alternatively, Cui et al. (2016) recognized a notable increase in denitrifiers abundance (*nirK*, *nirS*) after long-term fertilization which is similar to our results in which the abundance of denitrifiers were found high in PMCF and SRCF treatments. Hamonts et al. (2013) reported that dissolved organic carbon (DOC), NO_3^- and NO_2^- might increase denitrifiers copy numbers in fertilized soils. Moreover, low pH is recognized to reduce the assembly and turnover of N_2O reductase (Bergaust et al., 2010). In a recent study, DOC and NO_3^- contents increased by fertilization could decrease soil pH (data not shown). Together, these factors might have led to a neutral effect on the denitrifiers abundances.

Contribution of nitrification and denitrification on N_2O emissions with respect to water conditions

Nitrous oxide in soil are produced by various N transformation processes, i.e. nitrification (heterotrophic or autotrophic) and denitrification (Ruser et al., 2006; Beare et al., 2009; Hayakawa et al., 2009). However, in rice paddy soil, the water contents and fertilization rate are the main drivers for particular N transformation process. According to our results, the main contributor to N_2O production was autotrophic nitrification at 60% FC while denitrification was the predominant source during PF, consistent with Bateman and Baggs (2005). It is interesting that denitrification and autotrophic nitrification produce equally in PMCF and SRCF treatments during PF events and this relation is not significant however in CK and CF this relation was significant. The predominant contribution of autotrophic nitrification might be due to favorable conditions for substrate and O_2 diffusion. Similarly, autotrophic nitrification contributed

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equally during PF because it proceeds under limited short-term O_2 during process of nitrifier denitrification (Poth and Focht, 1985; Goreau et al., 1980; Wrage et al., 2001 Bollmann and Conrad, 1998). In addition, nitrifier denitrification at higher water level might be another reason of high autotrophic nitrification contribution (Bollmann and Conrad, 1998). Alternatively, at high concentration of 200 kg N ha^{-1} NH_4NO_3 (>10 μg g^{-1}) inhibited the conversion of N_2O to N_2 for a limited time because NO_3^- is more suitable electron acceptor over N_2O (Blackmer and Bremner, 1978). Although, a very low heterotrophic nitrification contribution was observed during both water events, and this might be due to a limited C and O_2 availability for heterotrophic nitrification or that N_2O emission during heterotrophic nitrification process was unable to be detected (Bateman and Baggs, 2005). I has been assumed that both denitrification and nitrification processes had contribution to N_2O emission at 60% FC. In addition, the contribution of nitrification in fertilization treatments was higher than denitrification because fertilization always promotes nitrification in the soil.

Conclusions

Rewetting and drying conditions had an important impact on N_2O emissions, growth of denitrifier and nitrifier communities along the experiment. However, denitrifier and ammonia oxidizer growth (AOA, AOB, *nir*S, *nir*K) were significantly affected by soil moisture contents. Meanwhile, AWD event promoted N_2O fluxes compared to PF. PMCF and SRCF increased N_2O emissions during both water events (AWD, PF). After finding out the dominant contributor it had been evaluated that autotrophic nitrification had a dominant role in both AWD and PF conditions. Moreover, a significant correlation between the N_2O emission and AOB copy numbers were found in different wetting-drying conditions, showing that AOB has dominant role in N_2O emission by the process of nitrification. Thus present study emphasizes that the PF water conditions are the better option to mitigate nitrous oxide form terrestrial environment to atmospheric environment. However, further study needed to emphasize the underlying process of nitrous oxide emissions.

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