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Nitrous oxide fluxes and nitrifier and denitrifier communites as affected by dry-wet cycles in long term fertilized paddy soils

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ABSTRACT

Drying and rewetting events of soil represents a common physiological stress for soil microbial communities. We investigated the effect of alternate drying-rewetting cycles on N_2O emissions and soil microbial communities in jar experiments with soil samples. The results showed that instant flooding and air-drying moments are two important steps of soil drying and rewetting periods influencing soil microbial communities and N_2O emissions. N_2O fluxes in the air dry (AD) steps were always higher than those in the instant flooding (IF) steps, especially in the early stages of AD and IF. The soil treated with long-term organic matter and chemical fertilizer promoted N_2O emissions but inhibited the N_2O release from newly applied urea when soil went through drying and rewetting events. Soil moisture content(s) also significantly affected the growth of ammonia oxidiser and denitrifier communities, with the functional gene abundance increasing with increasing soil moisture content. While comparing first and second cycles, N_2O fluxes were six times higher in the first cycle than in the second cycle. It is concluded that sudden changes in moisture condition influenced the N_2O flux, and nitrifier and denitrifier functional genes by affecting the growth of ammonia oxidiser and denitrifier communities.

1. Introduction

The consecutive events of prolonged drying with subsequent rewetting is a common feature of many surface soils, particularly agricultural soils. For example, alternate wetting and drying (AWD) of rice paddies are common, where the paddy fields are drained and re-flooded once or more times during the growing season. It has been adapted as a strategy to decrease the use of irrigation water and reduce greenhouse gas (GHG) emissions from rice cultivation while maintaining or improving yields (Richards and Sander, 2014). A plethora of studies showed that AWD reduced CH_4 emissions by 48–93% (Linquist et al., 2015; Pandey et al., 2014; Qin et al. 2010; Xu et al., 2015), but it resulted in increased nitrous oxide (N_2O) emissions. As N_2O is a potent greenhouse gas with a high global warming potential (IPCC, 2007), this trade-off between CH_4 and N_2O does not always result in an overall reduction in global warming potential (GWP) associated with AWD (Linquist et al., 2015; Pandey et al., 2014; Xu et al., 2015). However, how and why does AWD promotes N_2O are still known.

The temporal dynamic change in water content of soils may have direct influences on many soil properties. Soil dwelling microbes undergo considerable stress under this rapidly changing soil condition. For instance, the rewetting of a soil after a prolonged period of dryness, can lead to microbial cell lysis and possibly the release of intracellular solutes as a result of the osmotic shock (Halverson et al., 2000; Magid et al., 1999). It has been reported that consecutive anaerobic and aerobic cycling has a significant positive influence on the release of N_2O compared with constant aerobic or anaerobic states (Fierer et al., 2003). A wide range of heterotrophic bacteria are able to reduce NO_3^- and NO_2^- to N_2O or N_2 during denitrification under anaerobic conditions (Knowles, 1982; Shoun et al., 1992). This process is often considered as the main N_2O production process in soils, as many studies have shown that emissions of N_2O increase with increasing soil water content and most rapidly above 60% WFPS (Water Filled Pore Space) (Dobbie et al., 1999; Abbasi and Adams, 2000; Skiba and Ball, 2002; Di et al., 2014). However, still little is known about the effect of AWD on N_2O emission, nitrifying and denitrifying communities on weekly bases by completing more than one cycle in long term fertilized paddy soils.

Rice is one of the most important food crops in the world and is grown on approximately 155 million ha around the globe. It is consumed by more than 50% of the world's population. The yield of rice grain has also increased noticeably over the last few decades, with the rapid increase of nitrogen (N) fertilizer use (Nicolaisen et al., 2004).

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Rice always prefers NH_4^+ as N source than NO_3^- especially, under anarerobic condition. In term of efficiency of fertilizer utilization NH_4^+ is superior to NO_3^- in rice paddy soil (Guo et al., 2007). Use of large amounts of fertilizers combined with flood irrigation have also resulted in considerable amounts of N losses through NH_3^+ volatilization, N_2O emission and nitrate (NO_3^-) leaching (Xing and Zhu, 2000). However, N_2O emission from the soil in presence of growing plants is always less than the plant free soil system, because more than 170% of soil N is taken up by the plants (Silabaev, 1986).

Soil hydrological cycles are very important to increase the risk of waterlogging and flooding in paddy soil due to intensified precipitation regimes (Knapp et al., 2008). It can be expected that altered soil moisture dynamics will have significant effect on nitrous oxide (N_2O) emissions (Castellano et al., 2010). However, it is a big challenge to address the N_2O in response to sudden changes in moisture contents (ADW). Still less is known about the impact of AWD on N_2O fluxes (Hussain et al., 2015). It is generally recognized that anaerobic-aerobic cycling promotes N_2O emissions (Granli and Bockman, 1994; Cai et al., 1997). N_2O is a by-product of nitrification and an intermediate product of denitrification. The processes of nitrification, denitrification and possible shifts in the abundance of nitrifiers and denitrifiers during the drying and rewetting (DW) cycles for one and two weeks are still poorly understood. Therefore, two incubation experiments were conducted to determine the effect of different periods of drying and rewetting: (1) one incubation experiment to measure N_2O flux and its microbial communities for one week and two weeks flooding and subsequent air-drying (ADW-7&ADW-14). (2) Incubation experiment to measure N_2O emissions on the basis of instant flooding and air-drying conditions (IF&AD).

2. Materials and methods

2.1. Site background and soil samples

Soil samples were collected from a long-term fertilizer experiment (LTFE) field, located in Fuyang County, Zhejiang Province, China (29° 57′ 9″ North, 120° 0′ 41″ East). The soil type of Fuyang County is clay red earth (Zhang et al., 2009). The annual mean temperature and precipitation of Fuyang County are 16.27 °C and 1452.5 mm, respectively. Soil samples were collected from silt loam paddy field with history of 5 yr canola-rice rotation. The experiments had a completely randomized block design with three treatments and three replicates, including no fertilization (CK), 100 % chemical NPK fertilization (CF: N 314 kg ha^{-1}, P 13.73 kg ha^{-1}, K 153 kg ha^{-1}), and pig manure compost plus 50% chemical fertilization (OMCF: Pig manure 6000 kg ha^{-1} + N 157 kg ha^{-1}, P 13.73 kg ha^{-1}, K_2O 153 kg ha^{-1}). Five soil cores were collected from each replicate of the treatments to make a composite sample (three replicates) after the harvest of rice crop in December 2014. The soil cores were packed in sterile plastic bags, sealed and transported to the laboratory. The samples were stored at −4 °C. Each replicate sample was divided into two samples, with one sub sample used for incubation studies and the other sieved through a 2.0 mm screen for analysis of soil properties.

2.2. Soil chemical properties

Physical and chemical properties (soil pH, organic matter, organic N, total N, total P) of the soil samples were determined before the start of the incubation experiment. Soil pH was measured using soil to deionized water ratio of 1:2.5. Soil available P was extracted with Bray-2 solution and determined by the molybdate blue colorimetric method. Soils samples were ground to such an extent to pass through a sieve of 60 mesh for analyzing the total N (TN) and organic matter. Total N concentration and organic N were determined by micro-Kjeldahl digestion method followed by salicylate-nitroprusside colorimetric determination. NH_4^+-N and NO_3^--N were determined by colorimetric methods after the extraction with 1 M KCl (25 g soil to 100 mL KCl solution).

2.3. Incubation experiments

The main rationale for conducting experiments in laboratory was to have a control over variables like temperature and moisture, which would not have been possible in the field. To overcome this problem, we tested the suitability of varying incubation conditions (anaerobic versus aerobic conditions) and incubation temperature (25 °C) in the laboratory. All the conditions in laboratory incubation experiment were same as in field so the results can be justified with the trends as in the field. Incubation experiment 1 was designed to study hourly N_2O emissions from instant flooding (IF) to air drying (AD) stage. Two hundred g of soil were added into a 1000 mL jar and distilled water was added to the soil until flooded condition was obtained and jars were exposed to light. The dry soil samples were incubated at 25 °C and gas samples were collected 0, 1, 3, 5, 7 and 24 h after water addition and then again at the same intervals after water was removed from the jars. Gas samples from each treatment were collected at 0 and 30 min after enclosing the jars with the syringe and was analyzed for N_2O.

Incubation experiment 2 was designed for two different cycles of flooding and drying: (a) flooded for 7 days (DW-7) and (b) flooded for 14 days (DW-14) after urea application. Water was removed from DW-7 and DW-14 soils after 7 and 14 days to achieve field capacities (FC). In brief, two hundred g of soil were put in glass pots and water was added to obtain 40% field capacity. Then, urea (total conc. 200 mg N kg^{-1} of soil) was added to the soil and mixed thoroughly. Water was added again into the pots until flooded. For DW-7, the soil was incubated under flooded conditions for 7 days. Gas samples were collected with polypropylene syringe after 1 day of flooding. After 7 days of continuous flooding conditions, water was removed from the jars and gas and soil samples were sampled after 1–2 h after removing water from the jars (100% FC, 7 d), also at 60% after 10 days (60% FC, 10 d) and 40% field capacity after 13 days (40% FC,13 d), respectively. Cycle 2 was repeated as per procedure described above. Also, for DW-14, soil samples with the same treatments DW-7 were incubated under flooded conditions for 14 days and gas and soil samples were collected after 1 day (100 %FC, 1 d), 7 days (100% FC, 7 d), and water was then removed and sampled at 14 days (100% FC, 14 d), 17 days (60% FC, 17 d) and 20 days (40%FC, 20 d) after water removal. Headspace gas samples were collected immediately after closing the jars and 30 min later using a hypodermic needle and a polypropylene syringe and was analyzed for N_2O. N_2O was quantified using GC 2010 plus made by Shimadzu.

2.4. Quantitative real-time PCR

DNA was extracted from 0.5 g of soil by using TAKARA DNA standard protocol. DNA was also quantified by Neno drop. The population abundance of the nitrifiers and denitrifiers was determined by quantitative PCR (qPCR) for genes encoding the catalytic subunit of the key enzymes of the nitrification and denitrification pathways, i.e. ammonia-oxidizing bacterial ammonium monooxygenase (AOB *amo*A), ammonia-oxidizing archaeal ammonium monooxygenase (AOA *amo*A) and copper-nitrite reductase (*nir*K). Standard curves were obtained using serial dilutions of linearized plasmids containing cloned AOA, AOB and *nir*K genes amplified from nitrifying and denitrifying strains. The qPCR primers used to amplify these target genes are shown in Table 1. Twenty mL reaction mixture containing 1 mL of each primer for a target gene, 10 mL of SYBR Premix, 1 mL of total DNA template, and sterile distilled water to complete the volume of 20 mL was used to perform Real-time PCR. The copy number of the target genes in unknown soil DNA extracts were calculated using the individual standard curves. All data were analyzed (comparing means by one-way ANOVA and Pearson correlation) using the SPSS statistical package (16.0).