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Denitrifying communities differentially respond to flooding drying cycles in paddy soils

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ABSTRACT

Paddy soils are an important source of nitrous oxide (N_2O) emission, especially during frequent flooding and drying cycles. The N_2O flux from paddy soils is mainly driven by denitrifying microorganisms, but the response of denitrifying communities to flooding drying cycles has been little studied. N_2O emission was monitored under laboratory conditions in two paddy soils. Quantitative PCR (qPCR) and terminal restriction fragment length polymorphism (T-RFLP) were used to determine the abundance and community composition of *narG*- and *nosZ*-genes in denitrifiers. The N_2O emission was more significantly related to soil Eh than soil water content during the drying process. Significant increases in the copy number and obvious alterations in the community composition of both *narG*- and *nosZ*-containing denitrifiers were detected after only one day of drying. Among the two denitrifying communities, *narG* gene abundance was significantly correlated to both Eh and water content, whereas *nosZ* was only significantly correlated to water content during the flooding drying process, indicating that different responses among various denitrifiers occurred in flooding and drying cycles. Furthermore, *narG* copy number varied following the flooding drying cycles, where drying caused an obvious increase and flooding caused a decrease in numbers. However, despite the first drying phase resulting in a significant increase in *nosZ* copy number, further flooding and drying cycles did not cause any remarkable changes compared to the first drying. The *narG*-containing denitrifiers were much more closely correlated with the N_2O flux than *nosZ*-containing communities in the flooding drying cycles in the paddy soils studied here.

1. Introduction

Nitrous oxide (N_2O) is an important greenhouse gas whose global warming potential is about 298 times higher than that of carbon dioxide (Philippot et al., 2009). It is also involved in ozone depletion (Hou et al., 2000a) and has become a main factor affecting future climate changes and stratospheric ozone (Ravishankara et al., 2009; Wuebbles, 2009). Soil is considered to be one of the major contributors with 65% of the total global emission (Pathak, 1999). Of this, agricultural land occupies 37% of the earth's land surface and accounts for 84% of global anthropogenic N_2O emissions from soil (Smith et al., 2008).

Microbial denitrification is the main source of N_2O evolved from soil (Wrage et al., 2004). This process is mediated by physiologically diverse groups of microorganisms. The denitrifying bacteria belong to more than 50 genera (Zumft, 1997). However, few studies have examined the relationships between denitrifier communities and N_2O emissions. Early researches mainly used most probable number (MPN) counts and denitrifier enzyme activity (DEA) assay (Martin et al., 1988) to characterize denitrifier community and their activities. More recently, molecular methods have been used to examine the denitrifying community composition and diversity as well as the abundance of denitrifiers (Chen et al., 2012; Jung et al., 2011; Magalhães et al., 2011; Miller et al., 2012; Petersen et al., 2012) by focusing on the amplification of functional genes involved in denitrification. Denitrification includes the reduction of nitrate, nitrite, nitric oxide, and nitrous oxide which are transformed by the *narG* and *napA*, *nirS* and *nirK*, *qnorB* and *cnorB*, and *nosZ* genes of denitrifiers, respectively. In previous studies on denitrification in environmental samples (Jung et al., 2011; Kandeler et al., 2006; Morales et al., 2010), *narG* and *nosZ* were employed more frequently as genetic markers (Palmer et al., 2010).

Water availability is one of the key factors affecting N_2O emissions from soils (Huang et al., 2007). Wetting drying cycles can

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cause spatial and temporal variations in soil conditions as well as in denitrifying activity (Patrick, 1964; Reddy and Patrick, 1975). Denitrifying bacterial communities and their activities determine the denitrification rate and N_2O emission under anaerobic conditions (Cavigelli and Robertson, 2001, 2000; Philippot and Hallin, 2005; Rich et al., 2003). Drying and wetting cycles cause N_2O fluxes from soils (Huang et al., 2007). In upland soils the responses of bacterial communities to drying and rewetting are various and related to the supporting plant species. Thus, the bacteria community was relatively insensitive to drying and rewetting frequency in oak forests (Fierer et al., 2003), while in tall grass prairie soils there was a clear community shift in response to drying and rewetting cycles (Evans and Wallenstein, 2012). Also, in wetland soils, the variations in bacterial populations affected by drought were not consistent. For example, a 4 weeks drought caused a significant decline in 16S rRNA and *nirS* abundance in bog and fen, but not in riparian wetland (Kim et al., 2008). Wet and dry cycles significantly altered bacterial community structure and composition in a paddy soil and the relative abundance of predominant phyla (Somenahally et al., 2011). However, little is known about the relationships between the shifts of denitrifying communities and N_2O emission during wetting and drying cycles in paddy soils.

Rice fields are important agriculturally, comprise about 10% of the world's cultivated land. Flooding and drying cycles are an important management practice for achieving maximum rice yields, but unfortunately, this process can cause large N_2O fluxes (Xu et al., 1997). During the rice growing season, it was estimated that N_2O emissions comprised about 29.0 Gg N_2O–N, accounting for 7–11% of the annual total emission from croplands in China (Zou et al., 2007). Therefore it is crucial to understand the driving mechanisms of N_2O emission in rice paddy soil, especially during flooding and drying cycles. Wet and dry cycles alter the main pathway of N_2O emission (Yan et al., 2000b) by causing cracks to form in soil (Huang et al., 2008). This, in turn, may influence soil organic carbon (Baruah et al., 2010), NO_3^- (Linquist et al., 2011; Yan et al., 2000a), NH_4^+ (Lou et al., 2007), microbial biomass C concentrations (Lou et al., 2007) and soil redox potential (Eh) (Hou et al., 2000b; Xing et al., 2002; Xu et al., 1997). When paddy soils are flooded, NO_3^-, Mn^{4+}, Fe^{3+} and SO_4^{2-} are sequentially used as electron acceptors during microbial reduction processes, accompanied by release of trace gases including N_2O (Kögel-Knabner et al., 2010). The majority of previous studies have focused on the influence of soil physical and chemical conditions on N_2O emission, but the microbial mechanisms of N_2O emission influenced by flooding and drying are poorly understood in paddy soils.

Our aim was to test the hypothesis that the structure of the denitrifying community is the main factor influencing the rate of N_2O emission during flooding–drying alternates in paddy soils.

2. Materials and methods

2.1. Soils and their physical and chemical properties

Two paddy soils (A and B) were taken from the fields located in Changsha, China (A: 28°07′32.3″N and 113°06′31.2″E; B: 28°07′46.4″N and 113°08′46.0″E), which have grown double rice per annual for more than a hundred years. The upper layer (0–20 cm) was collected by randomly sampling twenty soil cores and mixing well to produce a single bulked soil sample in each field after late rice harvest in November 2009. The soils were then air-dried and sieved through 2 mm, obvious plant residues and soil organisms were manually removed. Both soils are loamy clay and their properties are shown in Table 1.

2.2. Soil incubation and sampling

2.2.1. Drainage experiment

Cylindrical polyethylene pots (20 cm high, diameter 19 cm), open at the top and sealed at the base, were specially designed with a circular groove on the top fringe in order to add water when gas sampling which can prevent gas exchange, and three drainage holes (diameter 1 cm) in the base. Each pot was filled with clean cobblestones (thickness 2 cm) and then 4 kg air-dried soil on top. Between the soils and stones was a piece of nylon cloth to prevent clogging of soil, and the holes in the base were sealed with rubber plugs. The pots were incubated at 30 °C after 3 L water was added to each pot to provide about 2 cm free water on the soil surface and the soil was thoroughly stirred with a glass rod. Both paddy soil A and B were used in this experiment. There were two treatments. Treatment 1 was continuous flooding (CF). Treatment 2 consisted of continuous flooding for 17 days followed by drying for 13 days (FD). Both treatments had three replicates. The drainage was performed by removing the rubber plugs in the holes in the base of pot to release the water naturally. The drying time was considered to have commenced when surface water disappeared. The drying continued till the end of the incubation. Redox electrodes (FJA-3, Nanjing, China) were inserted into the soil surface at 5 cm depth and Eh values were recorded after each gas sampling.

Gas samples for N_2O determination were taken on 1, 4, 7, 10, 13, 17, 18, 20, 22, 24, 27 and 30 day during the incubation, between 9 and 10 a.m. each sampling day. The gas sampling was carried out using a polyethylene cylinder lid (30 cm high, diameter 19 cm), open at one end. A septum was fitted at the top for gas collection and a small electric fan was fitted to mix the air inside. After it was covered on the incubation pot, the circular groove of the incubation pot was sealed with water. Gas was collected at 0, 30 and 60 min after running the fan for 5 min each time. Gas (30 mL) was removed with a 50 mL syringe and stored in pre-evacuated 12 mL vials (Labco limited high Wycombe UK) for N_2O determination.

Soil samples (0–5 cm), were collected immediately after gas collection with a small corer (diameter 1 cm) on 1, 7, 13, 18, 20, 22, 24 and 27 day during the incubation. One part of the soil sample was packed in foil and flash frozen in liquid nitrogen. After freeze-dried using a freeze-drier (NEOCOOLE, Yamato) the soil was ground to a fine powder, it was stored at −70 °C for molecular analysis. The other portion was used for chemical measurements.

2.2.2. Flooding drying cycles

This experiment was a supplementary validation for the drainage experiment, concerning the similar trends of the chemical and microbial parameters responded to flooding drying in the drainage experiment of the two soils, paddy soil B was selected for the experiment. Air-dried paddy soil B (100 g) was put in a 350 mL plastic box and 100 mL water was added to maintain about 2 cm free water level on the soil surface. Water content was maintained by daily adjustment based on the measurement of the total weight of the box. CF and flooding drying cycles (FDC) were designed with three replicates. There were three flooding and drying cycles, each with four days of flooding and four days of drying. The drying was conducted using a syringe to remove free surface water from the box, which was then maintained at 30 °C un-covered. In this experiment, 36 boxes were used for N_2O and soil sampling during the incubation and 6 boxes (3 for each treatment) were only used for continuous measurement of soil Eh during incubation. Gas sampling was conducted every day after the beginning of the incubation by randomly selecting three boxes of each treatment; the sampling procedures were as described above. Soil samples were taken on 4, 8, 12, 16, 20 and 24 days of incubation and the sampled boxes were discarded, the samples were treated as above.