Applied Soil Ecology 103 (2016) 101–109

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

# Responses of nitrifying and denitrifying bacteria to flooding-drying cycles in flooded rice soil

Huicui Yang[a,b], Rong Sheng[a], Zhenxing Zhang[a,b], Ling Wang[a,b], Qing Wang[a,b], Wenxue Wei[a,*]

[a] Key Laboratory of Agro-ecological Processes in Subtropical Regions and Taoyuan Station of Agro-Ecology Research, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China
[b] Graduate University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

*Article history:*
Received 7 September 2015
Received in revised form 9 March 2016
Accepted 15 March 2016
Available online 4 April 2016

*Keywords:*
Paddy soil
Flooding-drying
Micro-sublayer
Nitrifier
Denitrifier

ABSTRACT

The flooding-drying cycle can cause obvious increases of nitrous oxide ($N_2O$) emissions from paddy soil. However the relationships between $N_2O$ flux and $N_2O$ concentrations in soil and the microbial driving mechanisms during the flooding-drying process are unclear. In this study, a flooding-drying incubation experiment was carried out with a paddy soil. The topsoil (0–6 cm) was divided into 6 micro-sublayers each of 1 cm depth which were sampled independently. Terminal restriction fragment length polymorphism (T-RFLP) and real-time quantitative polymerase chain reaction (qPCR) were employed to determine the community composition and abundance of nitrifiers and denitrifiers, respectively. Results showed that the dynamics of $N_2O$ flux were more closely related to the $N_2O$ concentrations at 2–3 cm in comparison with that at 4–5 cm depth in the soil profile. During the peak period of $N_2O$ flux, the top three micro-sublayers (0–3 cm) simultaneously harbored significantly higher ammonia oxidizing bacteria (AOB) population sizes, and contained higher nitrate and lower ammonia concentrations. Therefore, the top soil (0–3 cm) possesses a strong ability to produce nitrate substrate for denitrification during the flooding-drying process, and the drying surface soil, with $O_2$ penetration, favoured $N_2O$ generation. In contrast, although the bottom soil (4–6 cm) contained abundant nitrate reductase gene (*narG*) copy numbers, it maintained low levels of AOB abundance, which could suggest that low nitrifying activity would be the major restriction limiting $N_2O$ production in this layer. In conclusion, the flooding-drying process induced significant $N_2O$ emissions from the paddy soil, which were closely related to the increasing nitrifying capability in the topsoil within 0–3 cm and the dynamics of $N_2O$ concentrations at 2–3 cm depth.



## 1. Introduction

Nitrous oxide ($N_2O$) is a potent greenhouse gas contributing to global warming with 298 times higher global warming potential than carbon dioxide ($CO_2$) on a 100-year time scale (Pachauri and Reisinger, 2007). $N_2O$ is also involved in the destruction of stratosphere ozone (Ravishankara et al., 2009). Agricultural systems are the main emission source of anthropogenic $N_2O$, and accounted for 58% of the global anthropogenic $N_2O$ emissions in 2005 (Metz et al., 2007). Paddy rice fields comprise about 10% of the world's cultivated land, and the properties of paddy soils are greatly affected by human activities. Among various management practices, flooding-drying is a common manipulation for rice cultivation, and large amounts of $N_2O$ are emitted during this process (Xu et al., 1997; Peng et al., 2011). However, the microbial driving mechanisms of $N_2O$ emissions during this process are unclear.

Soil $N_2O$ emissions are influenced by various environmental factors, such as temperature, moisture, substrate availability and pH and etc. (Skiba et al., 1998; Hou et al., 2000; Smith et al., 2003). Among them, soil moisture is considered as a key factor (Weitz et al., 2001; Jauhiainen et al., 2012). Soil moisture influences soil redox status (Xing et al., 2002) and also affects the activities of nitrifiers and denitrifiers (Jha et al., 1996; Amha and Bohne, 2011; Ruser et al., 2006; Scheer et al., 2008). It has been shown that substantial $N_2O$ fluxes occur when water filled porosity (WFPS) is between 70–90% (Dobbie et al., 1999; Hou et al., 2012; Xu et al., 2013). In paddy fields, $N_2O$ fluxes mainly occur during the flooding-drying process while they are at minimum levels under flooding (Minami, 1987; Chen et al., 1997; Tsuruta et al., 1997).

* Corresponding author.
*E-mail address:* wenxuewei@isa.ac.cn (W. Wei).

http://dx.doi.org/10.1016/j.apsoil.2016.03.008
0929-1393/

$N_2O$ fluxes from agricultural soils have been intensively studied in recent years (Freney, 1997; Bateman and Baggs, 2005; Lin et al., 2012), but the relationships between the $N_2O$ flux from soil and the $N_2O$ concentration produced in soil profile and the mechanisms involved have rarely been investigated. Previous research has demonstrated that soil $N_2O$ concentrations are largely related to the water table or water level, and $N_2O$ accumulates in the narrow depth just above water table (van Groenigen et al., 2005; Jørgensen et al., 2012; Minamikawa et al., 2013). However, $N_2O$ emissions may not always be directly related to the $N_2O$ concentration in the soil profile (Hosen et al., 2000, 2002; Gao et al., 2014), but are dependent on its location. Generally, $N_2O$ in the subsoil is barely emitted through the topsoil due to the microbial consumption of $N_2O$ during long distance diffusion (Arah et al., 1991; Hopkins et al., 1997). It is predicted that the $N_2O$ produced in surface soil might be the important source for $N_2O$ emissions (Gao et al., 2014; Denmead et al., 1979). Gradients of soil texture, chemical and biochemical properties within topsoil still exist, which could largely influence $N_2O$ production, consumption and emission (Weitz et al., 2001), especially during the flooding-drying process, but the mechanisms involved remain unknown.

To date, the community composition and abundance of nitrifiers and denitrifiers in upper soil layers (0–15 cm or 0–20 cm) in relation to $N_2O$ emissions have been well investigated. It has been demonstrated that the variations in ammonia oxidizing archaea (AOA) are not correlated to $N_2O$ fluxes in cultivated soils (Di et al., 2010a; Andert et al., 2011) except for acidic soils where AOA are the major functional nitrifiers (Lehtovirta-Morley et al., 2011; Yao et al., 2011; Jung et al., 2014). In contrast, ammonia oxidizing bacteria (AOB) are the important nitrifying community in most soils (Di et al., 2009; Xia et al., 2011) and have been linked to $N_2O$ emissions (Di et al., 2010b, 2014; Xia et al., 2013). Among the denitrifiers, the role of the nitrate reductase gene (*narG*), nitrite reductase genes (*nirK*, *nirS*) and nitrous oxide reductase gene (*nosZ*) have commonly been studied. It has been reported that shifts in *narG*-containing communities, including cell numbers and community composition, in the top layer (0–5 cm) of paddy soils were closely related to $N_2O$ emissions during flooding-drying cycles while *nosZ*-containing communities, although sensitive to water content changes, were not linked to $N_2O$ fluxes (Liu et al., 2012). Recently, Uchida et al. (2014) also found that the abundance of denitrifying gene transcripts (*nirK*, *nirS* and *nosZ*) in the top 1 cm layer of soil was more significantly related to $N_2O$ flux than those in the 1–3 cm layer. These results suggest that sublayers within the top soil might differentially produce and emit $N_2O$. Therefore, it is important to understand the differential microbial driving mechanisms of $N_2O$ production and emission of defined micro-sublayers within the top soil, which would be crucial for efficient nitrogen fertilization and manipulation of $N_2O$ emissions in paddy fields. We hypothesise that the differential responses of nitrifiers and denitrifiers to the flooding-drying process within the upper soil profile could trigger the variations of $N_2O$ production, consumption and emission.

In the present study, micro-sublayers of one centimeter thickness were sequentially sampled from soil cores. The objectives were to investigate the relationships between $N_2O$ concentrations at various soil depths and $N_2O$ emissions during the flooding-drying process, and the microbial driving mechanisms of $N_2O$ production and consumption.

## 2. Materials and methods

The soil sampling site is located in Changsha, China (28°14′08″N and 113°13′05″E), which had been under double rice cropping for over 100 years. The paddy soil (0–20 cm) was collected after late rice harvest in December 2012. After air-drying the soil was sieved through a 2 mm sieve while visible plant residues were removed manually. This soil was derived from quaternary red clay and classified as loamy clay (Hydragric Anthrosols) (Soil Survey Staff, 2010), its basic properties were as follows: Soil pH = 5.3, total C = 16 g C $kg^{-1}$, total N = 1.7 g N $kg^{-1}$, $NH_4^+$-N = 36 mg $kg^{-1}$, $NO_3^-$-N = 5.2 mg $kg^{-1}$, organic matter = 27 g $kg^{-1}$.

### 2.1. Soil incubation

The soil incubation experiment consisted of two groups, one for gas collection with 6 pots continuously flooded (CF) and flooding-drying (FD) treatments. The other was for soil sampling at four time points during the flooding-drying process with 12 pots. All the treatments had three replicates. Each pot (19 cm diameter, 20 cm high) contained 5 kg air-dried soil which was well mixed with 1.09 g urea (100 mg N $kg^{-1}$ dry soil). The pots were filled with distilled water leaving about 2 cm of free water on the soil surface and incubated at 28 °C. After 17 days under flooding, the pots for the flooding-drying treatment were drained (Liu et al., 2012) while the rests remained flooded.

For soil gas collection, twin silicon tubes (total 30 cm long, internal diameter 10 mm, wall thickness 2 mm) connected with a U

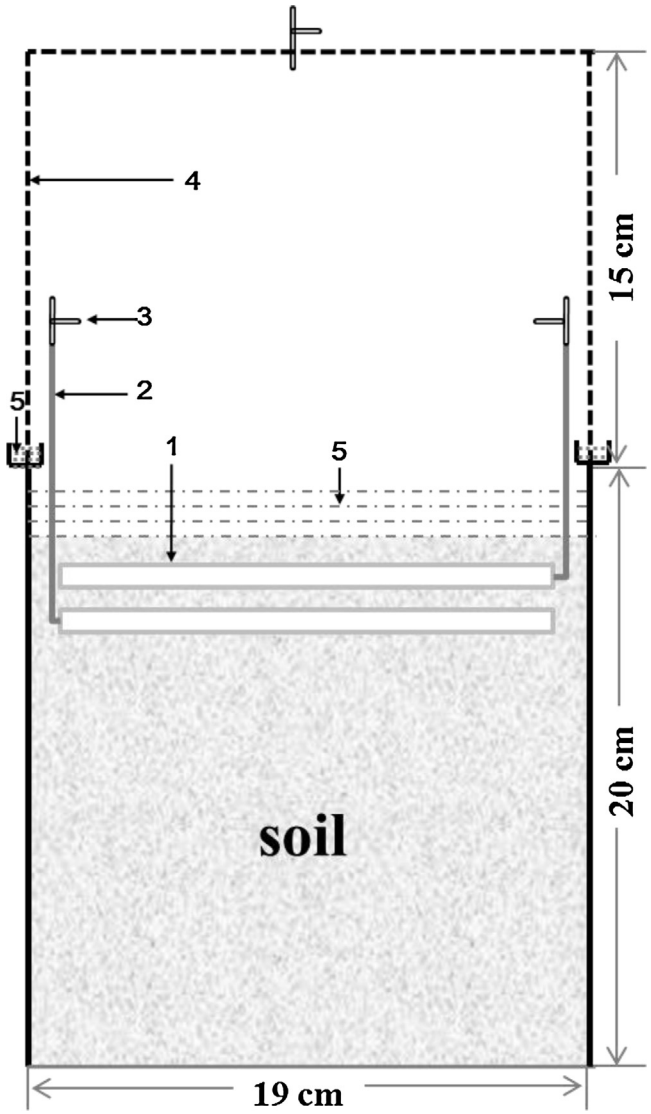


**Fig 1.** Schematic diagram of the pot for gas sampling. **1**: silicon tubes, **2**: U type stainless steel tube, **3**: three-way stopcock, **4**: gas sampling cover, **5**: water.