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Stimulatory effect of exogenous nitrate on soil denitrifiers and denitrifying activities in submerged paddy soil

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ABSTRACT

Paddy soil is submerged under water for most of the rice growing season, but our understanding of the driving mechanisms of nitrogen cycling, N_2O production, and N_2O consumption under submerged conditions is limited. In this study, intact paddy soil cores were sampled and an incubation experiment was conducted with nitrate amendments under flooding. N_2O concentrations in the soil profile and N_2O flux rates were measured by gas chromatography. The community compositions and abundances of *narG*- and *nosZ*-containing denitrifiers were analyzed by terminal restriction fragment length polymorphism (T-RFLP) and real-time quantitative polymerase chain reactions (qPCR), respectively. The results showed that N_2O emissions and N_2O concentrations in the submerged soil profile were significantly affected by the nitrate inputs. Higher NO_3-N additions stimulated a sharp increase in N_2O flux rate, which suggested an obvious stimulation caused by the increasing nitrate input. This effect was closely related to the significant increases in the population size of *narG*-containing denitrifiers and obvious alterations in its community composition. Therefore, high nitrate concentrations in submerged paddy soil can stimulate much higher N_2O production and emissions, and in this process, *narG*- rather than *nosZ*-containing denitrifiers are the important drivers. We also observed that N_2O concentrations in the 0–5 cm soil layer were clearly lower than those in the 5–10 cm layer, but the nitrate contents in these layers were reversed, indicating large N_2O losses occur from the 0–5 cm layer. The emitted N_2O is derived mainly from the uppermost soil layer (0–5 cm).

1. Introduction

Nitrous oxide (N_2O), an important greenhouse gas, greatly contributes to global warming and stratospheric ozone depletion (Ravishankara et al., 2009). Agricultural land emits a large amount of N_2O to the atmosphere and accounts for about 84% of global anthropogenic N_2O emissions (Smith et al., 2008). In agricultural ecosystems, rice paddy fields are a major land use, with >135 million ha utilized worldwide for rice cultivation (Li et al., 2014). Unlike upland soils, paddy soils are submerged during most of the rice growing season and they maintain a high denitrifying potential (Ferre et al., 2012; Wang et al., 2015). Although previous studies have demonstrated that N_2O fluxes in paddy fields occur during flooding-drying cycles (Chen et al., 1997; Hua et al., 1997; Ruser et al., 2006), under flooded conditions, this greenhouse gas is clearly emitted after nitrogen (N) fertilizer applications (Weitz et al., 2001; Yao et al., 2012; Das and Adhya, 2014), and low emission rates are also maintained consistently (Cai et al., 1997; Zheng et al., 2000; Hansen et al., 2014). The cumulative amount of emitted N_2O during paddy soil submergence is considerable, however, the mechanisms of N transformation and N_2O emission of paddy soils under submerged conditions have barely been investigated.

Long term submergence induces a strict anaerobic environment in paddy soils, and under such a situation O_2 availability is very limited, thus, denitrification is the dominant N_2O-producing process (Mathieu et al., 2006). It has been reported that paddy soil clearly harbors lower copy numbers of denitrifiers under constant flooded conditions than under drying conditions (Liu et al., 2012; Yang et al., 2016), which implies that submerged soils might have low denitrifying activities. On the contrary, other studies have reported that water saturated soils possess much higher denitrification potentials than unsaturated soils (Bettez and Groffman, 2012; Song et al., 2014; Brauer et al., 2015). Since the determination of denitrification potential is conducted using exogenous nitrate as a substrate, it is obvious that nitrate addition can stimulate the denitrifying reactions, however, the microbial mechanisms by which nitrate amendments influence denitrification potentials remains unknown. It has also been reported that submerged paddy soils normally contain very low NO_3^- concentrations (Wang et al., 2011; Liu

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et al., 2012; Pittelkow et al., 2013). Therefore, the fact that anaerobic paddy soils host low denitrifying populations might be strongly linked to the low nitrate concentrations. Previous studies have demonstrated that nitrate amendments induce increases in N_2O emissions from sediment slurries and low marsh soils (Royer et al., 2004; Hernandez and Mitsch, 2007; Hu et al., 2015), and different amounts of added NO_3^- induce different patterns of N_2O flux (Lalissegrundmann et al., 1988; Gillam et al., 2008; Wang et al., 2013). However, other studies have indicated that high nitrate concentrations in soil solution can significantly inhibit denitrification (Senbayram et al., 2012). Thus, it is imperative to explore the mechanisms by which denitrifiers respond to variations in soil nitrate contents.

An additional factor influencing microbes is soil heterogeneity, which is a common feature in both cultivated and natural soils (Franzluebbers, 2002). This phenomenon also exists within the 0–5 cm topsoil layer, and it has been demonstrated that the distributions of soil nutrients and denitrifying microorganisms in the uppermost soil exhibits strong variation (Uchida et al., 2014; Yang et al., 2016). The surface soil normally retains higher amounts of soil nutrients and obviously harbors a higher density of microorganisms compared to the subsoil (Fierer et al., 2003; Minamikawa et al., 2013; Gao et al., 2014). It has also been shown that this heterogeneity includes the denitrifying microbial communities and denitrification enzyme activities existing within the topsoil (plough layer) (Mergel et al., 2001; Dhondt et al., 2004). Although N_2O emissions are measured directly at the soil surface, the correlations between N_2O fluxes and N_2O production and consumption within the soil profile are still not well understood.

In the present study, we collected intact soil cores (0–20 cm depth) from a paddy field and conducted laboratory incubation experiments with applications of nitrate under flooding conditions. Intact soil cores maintain in situ soil properties and structure, and show more sensitive responses to waterlogging events and higher N_2O emission than sieved soil (Li et al., 2014; Uchida et al., 2014). We hypothesized that the heterogeneities of the biogeochemical properties in the top layers of the paddy soil profiles would result in differential denitrifying activities for coping with nitrate inputs under submerged conditions. The objectives of this study were to explore (1) the responses of N_2O production and emissions to nitrate additions in submerged paddy soil, (2) the links between the dynamics of N_2O concentrations and the shifts in denitrifying bacterial communities as influenced by NO_3^- amendments, and (3) the relationships between N_2O emissions and N_2O concentrations at various soil depths.

2. Materials and methods

The sampling field is located in Changsha, China (28°14′08″N; 113°13′05″E), and has grown double rice crops for >100 years. The paddy soil, derived from quaternary red clay and classified as loamy clay (Hydragric Anthrosols) (Soil Survey Staff, 2010), was sampled in February 2014 during the winter fallow period. Soil properties were as follows: Gravimetric water content, 26.14 g H_2O per 100 g dry soil; soil pH (H_2O) 5.11; NH_4^+-N, 2.57 mg kg^{-1}; NO_3^--N, 2.73 mg kg^{-1}; organic matter content, 30.50 g kg^{-1}; organic carbon content, 1.77 g kg^{-1}. The soil bulk densities of the 0–5, 5–10, 10–15 and 15–20 cm layers were 1.00, 1.04, 1.07 and 1.30 g cm^{-3} respectively.

2.1. Intact soil column collection

PVC cylinders (15 cm diameter, 25 cm high) and bottom circular PVC plates (18 cm diameter) were prepared before soil sampling, two adjacent holes (1.6 cm diameter) were drilled in the cylinder wall at positions of 2.5, 7.5, 12.5 and 17.5 cm from the bottom of cylinder, respectively. In the field, plant residues were manually removed from soil surface and soil columns were dug. PVC cylinders were fitted to the soil columns by trimming off extra soil with a spade. Afterwards, the intact soil column (0–20 cm) was removed from the field, the bottom was covered with a plate and the cylinder was wrapped with film to prevent water loss. In total, 40 intact soil columns were collected and transported to the laboratory. For each column, after the bottom plate was sealed to the cylinder with glue, two horizontal tunnels at each level were made across the soil column with a stainless tube (1.6 cm diameter) through the holes in the PVC cylinder. Each tunnel was filled with a gas sampler, which was constructed as follows. Silicon tubes (each 14 cm long, 1.2 cm internal diameter, 0.2 cm wall thickness) were closed with silicone septa at both ends, and twin parallel tubes connected with a U type stainless steel tube at one end were inserted into a pair of tunnels. The stainless steel U tubes (2 mm) were inserted into the silicon tubes through the septa and three-way stopcocks were fixed to the steel tubes outside the cylinders (Fig. 1). The holes in PVC wall were then sealed with glue and the space between the soil columns and PVC cylinders was filled with soil slurry.

2.2. Soil incubation

Three treatments were used with nitrate (KNO_3) applications of 0 mg N per pot (N0), 100 mg N per pot (N100) and 350 mg N per pot (N350), which amounts to the N-fertilizer applications of 0, 60 and 200 kg N per hectare in the field practice. Each treatment consisted of 12 pots, 3 for gas sampling and 9 for soil sampling at 3 different times. In effect there were 3 experimental replicates for each treatment. All the pots were randomly arranged. The nitrate solutions of 0, 1.50,

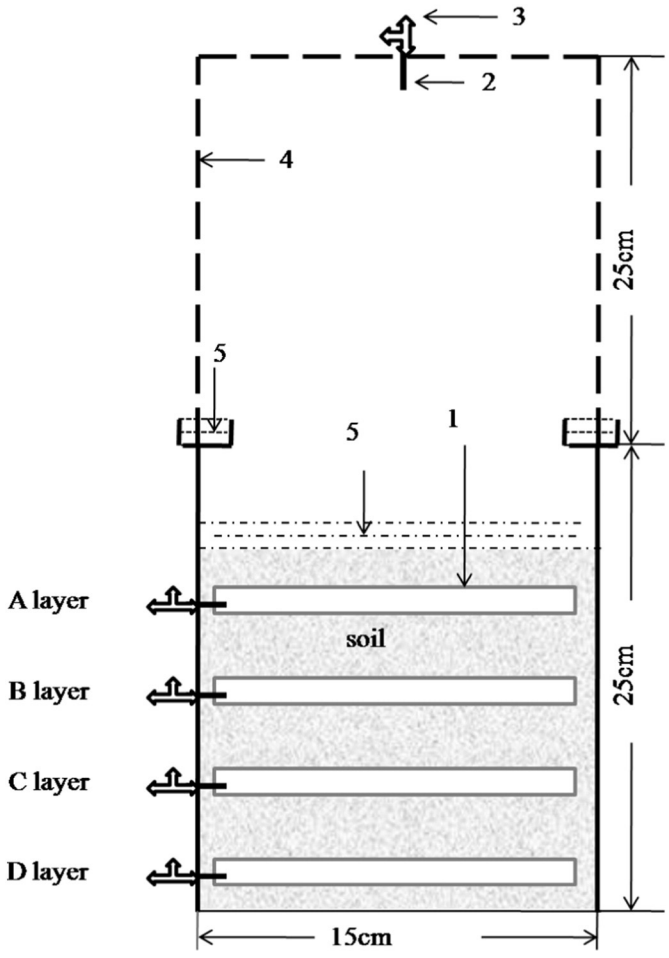

Fig. 1. Schematic diagram of the pot for gas sampling. 1: silicon tubes, 2: stainless steel tube, 3: three-way stopcock, 4: gas sampling static chamber, 5: water. A layer represented 0–5 cm soil depth; B layer represented 5–10 cm soil depth; C layer represented 10–15 cm soil depth; and D layer represented 15–20 cm soil depth.