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Response of denitrification in paddy soils with different nitrification rates to soil moisture and glucose addition

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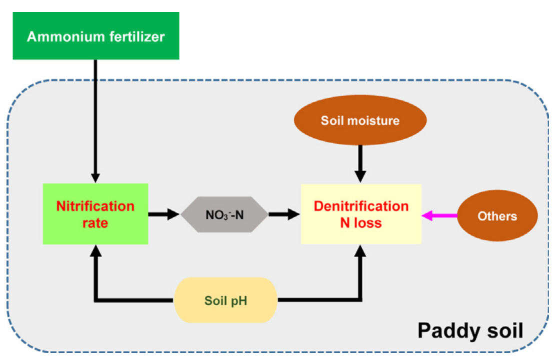
HIGHLIGHTS

- Nitrification rate significantly affected the recovered ^{15}N forms in paddy soils.
- Denitrification N losses varied significantly and increased with increasing net nitrification rates.
- Addition of glucose did not stimulate denitrification N losses.

GRAPHICAL ABSTRACT



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ABSTRACT

Denitrification is one of the most important N loss pathways in paddy soil. The nitrification rate is a key natural feature for controlling denitrification N loss in paddy soil. However, the relationship between nitrification and denitrification under different conditions in paddy soil remains unknown. By using ^{15}N tracing, we investigated the response of denitrification loss to soil moisture and glucose addition in six paddy soils, whose net nitrification rates ranged from 0.36 mg N kg^{-1} day^{-1} to 5.72 mg N kg^{-1} day^{-1}. The soils were amended with or without glucose to simulate root exudates at rates of 100 mg kg^{-1} of soil and incubated under either 60% water holding capacity (WHC) or flooded (2 cm depth) at 25 °C for 15 days. Denitrification loss was calculated by the unrecovered $^{15}NH_4^+$. The results showed that the soil nitrification rate significantly affected the N recovery form and denitrification loss of the applied ^{15}N. NH_4^+ was the main recovered N form of the applied ^{15}N in soil with a low nitrification rates. Denitrification losses were higher in the high nitrification rate soil than soil with low nitrification rate in all treatments. The correlation between denitrification and nitrification rates was well fit by Michaelis-Menten kinetics during the incubation, irrespective of soil moisture and glucose addition, and the R^2 ranged from 0.801 to 0.977 ($P < 0.05$). Glucose addition did not stimulate denitrification under either 60% WHC or flooded conditions. The results showed that nitrification rate, rather than labile organic supply, controlled denitrification in paddy soil.

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1. Introduction

Denitrification is one of the most important pathways of N loss in flooded paddy soil, accounting for more than 40% of the applied fertilizer N calculated by mass conservation laws (Zhu and Chen, 2002; Zhang et al., 2016). The denitrification process was believed not only to reduce rice plant N use efficiency but also to produce N_2O (Firestone and Tiedje, 1979; Firestone et al., 1980; Yang et al., 2017). Improved management can decrease N loss and increase N use efficiency in paddy field ecosystems (Subbarao et al., 2013).

Denitrification is often tightly associated with the concentration of nitrate (NO_3^-) in flooded paddy soil, as it is a substrate-dependent process under anaerobic conditions (Ishii et al., 2011; Li et al., 2014). Generally, ammonium is the most common N fertilizer used in paddy fields; thus, the concentration of NO_3^- in paddy soil is mainly determined by N transformation characteristics, especially the nitrification and nitrate consumption rates (Zhang et al., 2016). In soil with low nitrification ability and low nitrification/mineralization ratio, ammonium (NH_4^+) is the predominant inorganic N form, thus lowering the risk of N loss through denitrification, leaching and runoff (Zhang et al., 2013; Zhang et al., 2016). However, in soil with high nitrification ability or high nitrification/mineralization ratio, NO_3^- is the dominant inorganic N form, thus increasing the risk of N loss under flooded conditions. Therefore, the nitrification rate is one of the main factors controlling denitrification N loss in flooded paddy soil (Malla et al., 2005; Zhang et al., 2016; Yang et al., 2017). However, we have a poor understanding of the mechanisms involved in denitrification N loss for paddy soils with different nitrification rates under various environmental conditions.

Soil moisture and labile organic matter have also been identified as important factors affecting denitrification by controlling the dynamics of O_2 and the supply of easily decomposable organic carbon as denitrification is a heterotrophic process (Lan et al., 2015). Denitrification processes in paddy fields are generally stimulated by high nitrification ability, low O_2 partial pressure and abundant easily decomposable organic carbon (Carraso et al., 2004; Zhou et al., 2011). Soil moisture varies widely under natural conditions and the most common situations in paddy fields are continuous flooding or a flooding/drainage alternative (Zhou et al., 2012). The flooding status limits O_2 diffusion into soil, resulting in enhanced denitrification (Hernandez and Mitsch, 2007). Previous studies involving several agricultural soils demonstrated that the denitrification rate increased with increasing soil moisture (*e.g.*, Liu et al., 2012). Additionally, glucose is one of the most important root exudates with direct and indirect impacts on the denitrification process. Decomposition of glucose provides electrons for denitrification or inhibits autotrophic nitrification (Fisk et al., 2015; Haichar et al., 2014). Dendooven et al. (2010) found that the denitrification rate was stimulated when easily dissolved organic carbon was supplied by root exudates derived from rice plants under flooded conditions. Notably, previous studies often investigated the effects of soil moisture and root exudates on the denitrification rate in a given soil (Fisk et al., 2015; Dendooven et al., 2010). Greater efforts are needed to investigate denitrification in paddy soils with different nitrification rates, soil moisture levels and labile organic matter supplies.

Since denitrification is controlled by different factors in paddy soils with varied nitrification rates (Yang et al., 2017), we hypothesized that denitrification N loss in paddy soils with different nitrification rates would have different responses to soil moisture and labile organic supply. The objectives of this study were: (1) to compare the recovered form and proportion of the applied $^{15}NH_4^+$, denitrification in paddy soils with different nitrification rates under different soil moisture and with or without glucose, as a simulated labile organic matter and (2) to evaluate the influence of soil nitrification rate on denitrification N loss under different soil moisture conditions and with or without glucose addition.

2. Materials and methods

2.1. Sampling of paddy soils

Before the early rice was transplanted, six paddy soils with varied nitrification rates were collected from the A horizon (0–20 cm) in March 2014 from three main rice cultivation regions, *i.e.*, two acidic paddy soils site in Longhushan (JS) and Yingtang (JC) (28° 15′ N, 116° 55′ E) in Jiangxi province, China, two paddy soils were from sites in Yixing (YX) (31° 17′ N, 119° 54′ E) and Jurong (JR) (31° 56′ N, 119° 10′ E) in Jiangsu Province, China, and two alkaline paddy soils come from sites in Huaian (HA) (33° 43′ N, 118° 86′ E) in Jiangsu Province, China and Yanting (SC) (31° 16′ N, 105° 27′ E) in Sichuan Province, China. The soil properties were described previously (Yang et al., 2016, 2017). For convenience, the soil properties are shown in Table 1.

2.2. Column experiment

Denitrification loss was determined in a column experiment. For each soil, 90 g of soil (oven-dry basis) was mixed homogeneously and placed in PVC columns (10 cm depth and 5 cm diameter) with a PVC lid (10 cm depth and 6.0 cm diameter). The soil moisture content was adjusted to 40% WHC, followed by preincubation at 25 °C for 4 days.

Two milliliters of $(NH_4)_2SO_4$ solution containing 4.5 mg $(NH_4)_2SO_4$-N, equivalent to the N application rate of 50 mg N kg^{-1} soil, enriched with 10.32 atom% ^{15}N excess was added to the preincubated soil using a 5-needle injection in one dose, at a depth ranging from 3 to 5 cm. The soil columns were subsequently split into two equal aliquots. By adding deionized water, the moisture content of one portion was adjusted to 60% WHC, and the other was flooded with 2 cm surface water. Eleven days after $^{15}NH_4^+$ addition, two milliliters of 45% (m/m) glucose, equivalent to the application rate of 100 mg C kg^{-1} of soil, or 2 ml deionized water were injected into the soil by a 5-needle injection in one dose, as previously described. All treatments (Table 2) were incubated at 25 °C. Water lost through evaporation during the incubation was measured by weighing and replaced with deionized water. Six replicates were prepared for each soil.

2.3. Sampling and analyses

The NH_4^+-N, NO_3^--N, and organic N concentrations and their isotopic compositions were measured by randomly selecting three PVC columns from each paddy soil at 10 days (1 day before glucose addition) and 15 days after $^{15}NH_4^+$ addition. Soil from each PVC column with 60% WHC treatment was mixed thoroughly, and 20 g (oven-dry basis) was transferred to a 250-ml flask, with 100 ml of 2 M KCl added, and the mixture was shaken for 1 h at 250 rpm and 25 °C. The soil and water of each PVC column from the flooding treatments were thoroughly mixed to get a representative soil and flooded water sample, and 40 g of the soil slurry was transferred to a 250-ml flask, and added to 80 ml of 2.5 M KCl. The concentration of KCl after mixing with the soil slurry

Table 1
Characteristics of tested paddy soils collected various rice production regions in China.

Soil code	pH	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	TN (g kg^{-1})	SOC (g kg^{-1})	Clay (%) (<2 μm)	Net nitrification rate[a] (mg N kg^{-1} day^{-1})
JC	5.26	2.52	3.42	2.40	22.8	28.6	0.48
JS	5.23	3.62	8.77	0.80	7.90	7.13	0.36
JR	5.81	5.95	3.05	0.90	8.10	35.9	0.91
YX	5.92	4.07	1.00	1.30	11.2	8.62	2.25
HA	7.77	129	0.73	2.10	28.8	52.2	3.18
SC	7.83	7.03	2.00	2.00	25.9	18.1	5.72

[a] The net nitrification rate was calculated from the experiment reported previously (Yang et al., 2016).