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Reduction in soil N_2O emissions by pH manipulation and enhanced *nosZ* gene transcription under different water regimes☆

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ABSTRACT

Several studies have been carried out to examine nitrous oxide (N_2O) emissions from agricultural soils in the past. However, the emissions of N_2O particularly during amelioration of acidic soils have been rarely studied. We carried out the present study using a rice-rapeseed rotation soil (pH 5.44) that was amended with dolomite (0, 1 and 2 g kg^{-1} soil) under 60% water filled pore space (WFPS) and flooding. N_2O emissions and several soil properties (pH, NH_4^+–N, NO_3^--N, and *nosZ* gene transcripts) were measured throughout the study. The increase in soil pH with dolomite application triggered soil N transformation and transcripts of *nosZ* gene controlling N_2O emissions under both water regimes (60% WFPS and flooding). The 60% WFPS produced higher soil N_2O emissions than that of flooding, and dolomite largely reduced N_2O emissions at higher pH under both water regimes through enhanced transcription of *nosZ* gene. The results suggest that ameliorating soil acidity with dolomite can substantially mitigate N_2O emissions through promoting *nosZ* gene transcription.

1. Introduction

Nitrous oxide (N_2O) is one of the major gases contributing to global warming and causing destruction of the stratospheric ozone layer. N_2O gas has 265 times higher global warming potential than CO_2 on a 100-year time scale (IPCC, 2013). Agricultural activities are primary source of soil N_2O emissions accounting for about 60% of total anthropogenic N_2O released into the atmosphere (IPCC, 2013). Several factors regulate N_2O production and emission that include soil structure and texture (Skiba et al., 1998), moisture (Khalil et al., 2004), nitrogen (N) fertilization and soil pH (Firestone et al., 1980; Shaaban et al., 2014).

Soil N_2O emission is generally increased with N fertilizer application to arable lands. High doses of N-based fertilizers are used for obtaining high crop yield in the intensified agricultural systems (Guo et al., 2010). Excess application of N fertilizers can also drive soil acidification (Guo et al., 2010). The pH is measure of H^+ concentration and soil acidity denotes its relevant severity. Soils having pH less than 7 are termed as acidic soils, and are generally problematic for growth and development of agronomic crops if pH declines over 5.5 (Thomas et al., 1996). To ameliorate acidic soils, farmers adopt liming practice in acidic soils which normally leads to significant changes in the chemical and biochemical reactions and microbiological processes and thereby regulates N_2O production (Page et al., 2010; Kunhikrishnan et al., 2016). Previous studies have shown contradictory results of adjusting soil pH on N_2O emissions (Clough et al., 2004; Kunhikrishnan et al., 2016). Baggs et al. (2010) demonstrated that lime application to acid soils caused an increase in soil mineral N contents, and at high soil pH levels increased N_2O emission. Higher N_2O emissions in lime treated soils were resulted from elevated mineral N concentrations caused by varied biochemical processes in the soils (Feng et al., 2003).

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Liming could at least transiently enhance (rather than reduce) the N_2O emissions by evoking the phenomenon of accelerated mineralization of soil organic carbon and nitrogen (SOC; SON), which would enhance oxygen consumption and nitrification, hence N_2O emission (Baggs et al., 2010). In contrast, Šimek and Cooper (2002) documented that amending acidic soils with lime lowered N_2O emissions having a negative relationship with soil pH. Weslien et al. (2009) observed that soil N_2O emissions were diminished when pH increased by liming. Shaaban et al. (2014, 2015) recently reported that N_2O emissions from acidic soils were lowered by dolomitic lime addition due to increase in soil pH. Therefore, soil pH is a main factor affecting N_2O production.

Soil pH can regulate N_2O production directly through affecting microflora particularly N transforming bacteria. The bacteria encoded *nosZ* gene can reduce N_2O emissions through assembling and synthesizing N_2O reducing enzymes i.e. N_2O-reductase (Bakken et al., 2012). The N_2O-reductase is a key enzyme of denitrification pathway which is the only identified enzyme for reducing N_2O to N_2 (Bakken et al., 2012). The transcription of *nosZ* gene and synthesis and functionality of N_2O reductase is inhibited at acidic pH (Qu et al., 2014) because the enzyme is located in a weakly buffered area in the periplasm of cell (Bergaust et al., 2010). In acidic soils N_2O-reductase cannot perform well and thereby leading to higher N_2O emissions (Bergaust et al., 2010). Liu et al. (2010) proved similar results of N_2O production at low pH levels in the transcription study of *nosZ* gene. They used the soil that was underwent a long-term liming experiment and reported that soil pH has an omnipresent control on N_2O production, even though transcripts of *nosZ* gene were not markedly influenced by pH. The findings of Čuhel and Šimek (2011) divulged that the functionality of N_2O-reductase was refurbished instantaneously by raising the soil pH following lime application and thus lowered N_2O emissions in pasture lands. Qu et al. (2014) supported these findings and stated that high N_2O emission from acidic soils is mainly because of limited activity of N_2O-reductase at low soil pH. Although the experimental evidences have shown negative relationship between soil N_2O emission and pH, but the underlying enzymatic and genetic mechanisms of N_2O reduction are not well studied.

In addition to soil pH, water regimes such as drying-rewetting cycle, drainage, the extent and time of water residence in soil and flooding are also important factors affecting N_2O production in soils (Bateman and Baggs, 2005; Hernandez and Mitsch, 2006; Kjellin et al., 2007; Wu et al., 2017). The increased emission of N_2O from agricultural soils have been documented with increasing soil water content (from 30 to 90% WFPS) (Simojoki and Jaakkola, 2000; Dobbie and Smith, 2001). Generally nitrification has been identified as the main process of N_2O production at 60% WFPS, while denitrification dominates when soil moisture content exceeds 60% WFPS (Linn and Doran, 1984). Water regimes can determine the availability and exchange of O_2 between soil and atmosphere controlling denitrification rate and N_2O emissions (Chapuis-Lardy et al., 2007).

Flooded soil conditions greatly vary for N_2O production compared with upland soils due to changes in oxygen (O_2) concentrations. Previous studies have shown relatively low N_2O emissions (and even N_2O uptake) from rice paddy with large N stores due to submerged condition (Wu et al., 2017). Flooding of soil creates anaerobic environment restricting mineralization and nitrification but favoring complete denitrification (Wu et al., 2017). The N_2O-reductase has been identified rigorously sensitive to O_2 in soil (Thomson et al., 2012). Long term flooding conditions induce an austere anoxic environment (limited availability of O_2) in soils, leading to complete denitrification and low N_2O emissions. Several studies have revealed the effects of water regimes on soil N_2O emissions in the past, but the effects of water regimes during ameliorating soil acidity are still not well studied.

To stringently validate liming effects on soil N_2O emissions, in the present study an acidic soil was amended with dolomite to manipulate soil pH under different water regimes (60% WFPS and flooding). We hypothesized that manipulation of soil pH and the water regime will affect the N_2O-reductase enzyme and *nosZ* gene, hence N_2O emission. Therefore, the aim of the present study was to investigate the influence of soil pH manipulation on the activity of N_2O-reductase enzyme encoded by *nosZ* gene and soil N_2O emissions under different water regimes (60% WFPS and flooding).

2. Material and methods

2.1. Soil characteristics

Soil used in the present study was obtained from an arable field (29°88′209N, 114°39′416E; Xian-ning city, Hubei province, China) that underwent rapeseed-rice rotation for ~30 years. Soil samples (0–20 cm) were randomly collected from 10 points from selected field after rice harvest (October 2015). Soil samples were composited in a plastic bucket. Plant roots, visible debris and stones were manually removed from the soil. After drying in open air, soil was crumbled to sieve 2 mm and analyzed for physicochemical properties. The $pH_{(H2O)}$, total organic C, total N, bulk density and texture of soil were 5.44, 11.88 g kg^{-1}, 1.35 g kg^{-1}, 1.46 g cm^{-3} and silt loam, respectively. Soil is Ultisol according to Soil Survey Staff (2010).

2.2. Treatments and experimental set-up

Air dried soil was first incubated at 40% WFPS at 25 ± 1 °C for 7 days to initiate and stabilize the microbial activity prior to imposing treatments. After 7 days, 100 g soil (oven dry basis equivalent) was treated with/without dolomite (<0.3 mm size) under two water regimes [60% water filled pore space (WFPS), and flooding]. Treatments were as following: (i) soil without dolomite at 60% WFPS, (ii) soil with low dose dolomite (LD) at 60% WFPS, (iii) soil with high dose dolomite (HD) with 60% WFPS, (iv) soil without dolomite under flooding, (v) soil with low dose dolomite under flooding and (vi) soil with high dose dolomite under flooding (HDF). Each treatment contained three replicates. Low and high doses of dolomites were 1 and 2 g dolomite kg^{-1} soil which is 2920 and 5840 kg ha^{-1}, respectively. After adding and thoroughly mixing dolomite in the soil, moisture was increased to either 60% WFPS or flooding conditions (1:1, V/W, 0.5 cm above soil surface). The experiment was conducted in 1000 ml glass jars at 25 ± 1 °C in the dark for 35 days. Soil water content was kept constant at flooding (1:1, V/W) or 60% WFPS by weighing the jars and adding distilled water at alternate days. Two separate sets of treatments were prepared for taking gas and soil samples.

2.3. Gas sampling and analysis

Gas samples were collected from jars after imposing treatments at every 24 h until 15-day, and then at every 48 and 72 h until 25 and 35 day, respectively. The jar tops were covered with thin plastic sheet to minimize moisture loss, but 80 pin-holes were done in the sheet for aeration. Gas sampling method was adopted as described by Shaaban et al. (2016). Briefly, the jars were closed using air tight lids containing a rubber septum for gas sampling. The gas in the headspace of jars during the closure period (60 min) was taken at time T_0 (immediately after closure) and T_{60} (after 60 min). An air-tight three way stop-cock syringe was used for collecting gas samples. The headspace samples of gas were analyzed for the determination of N_2O gas concentration using a gas chromatograph (Agilent 7890-A, USA) equipped with electron capture detector