The effect of nitrification inhibitor on N_2O, NO and N_2 emissions under different soil moisture levels in a permanent grassland soil

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A B S T R A C T

Emissions of gaseous forms of nitrogen from soil, such as nitrous oxide (N_2O) and nitric oxide (NO), have shown great impact on global warming and atmospheric chemistry. Although in soil both nitrification and denitrification could cause N_2O and NO emissions, most studies demonstrated that denitrification is the dominant process responsible for the increase of atmospheric N_2O, while nitrification produces mostly NO. The use of nitrification inhibitors (NIs) has repeatedly been shown to reduce both N_2O and NO emissions from agricultural soils; nevertheless, the efficiency of the mitigation effect varies greatly. It is generally assumed that nitrification inhibitors have no direct effect on denitrification. However, the indirect impact, due to the reduced substrate (nitrate) delivery to microsites where denitrification occurs, may have significant effects on denitrification product stoichiometry that may significantly lower soil-borne N_2O emissions. Soil-water status is considered to have a remarkable effect on the relative fluxes of nitrogen gases. The effect and mechanism of NI on N_2O, NO and N_2 emission under different soil water-filled pore space (WFPS) is still not well explored. In the present study, we conducted a soil incubation experiment in an automated continuous-flow incubation system under a He/O_2 atmosphere. Ammonium sulfate was applied with and without NI (DMPP) to a permanent UK grassland soil under three different soil moisture conditions (50, 65, and 80% WFPS). With every treatment, glucose was applied to supply enough available carbon for denitrification. Emissions of CO_2, N_2O, NO and N_2 were investigated. Additionally, isotopic signatures of soil-emitted N_2O were analyzed. Generally, higher WFPS led to higher N_2O and NO emissions, while N_2 emissions were only detected at high soil moisture condition (80% WFPS). Different processes were responsible for N_2O and NO emission in different phases of the incubation period. The application of DMPP did significantly reduce both N_2O and NO emissions at all three soil moisture conditions. Furthermore, DMPP application increased N_2 emissions and decreased the $N_2O/(N_2O + N_2)$ product ratio at 80% WFPS.

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1. Introduction

Emissions of nitrogenous gases from agricultural soil, such as nitrous oxide (N_2O), nitric oxide (NO) and dinitrogen (N_2), represent a loss of N fertilizer and a reduction of plants N use efficiency (Bouwman et al., 2013). Grasslands, which are the dominant global ecosystem and cover 17% world surface, are also one of the main sources of N_2O and NO emissions (Cárdenas et al., 2007; Stehfest and Bouwman, 2006). Both N_2O and NO have great impact on global environmental change and atmospheric chemistry. Nitrous oxide has a global warming potential of about 300 times that of CO_2 and is considered as the major cause of ozone layer depletion in the 21st century (Bouwman et al., 2002; Myhre et al., 2013). Global anthropogenic N_2O emissions are estimated as approx. 6.5 Tg N yr^{-1} in 2010 (IPCC, 2013), of which soils are the largest source (Ciais et al., 2014). Although both nitrification and denitrification could produce N_2O in soil, recent studies suggested that denitrification is the dominant process responsible for the increase in atmospheric N_2O (Baggs, 2008). Denitrifying activity could be exhibited by both

bacteria and fungi. However, fungal denitrification pathway, which recently has been found to be a major process in the nitrogen cycle, is not capable of reducing N_2O to N_2 (Laughlin and Stevens, 2002; Shoun et al., 2012). Anthropogenic nitrogen oxide ($NO_x = NO + NO_2$) emissions were estimated as approx. 43 TgN yr^{-1} in 2010 globally (IPCC, 2013). The atmospheric lifetime of NO_x is relatively short (1–2 days), but as they are readily deposited on land and water surfaces (soil, plants, open waters), they lead to eutrophication and acidification of ecosystems (Crutzen, 1979). A recent study indicates that NO also plays an important role in haze formation of urban air pollution (Guo et al., 2014). In soil, NO can be produced by both nitrification and denitrification, as NO is not only a facultative by-product of the nitrification pathway, but also an obligatory intermediate of the denitrification pathway (Skiba et al., 1997). Nevertheless, nitrification is believed to be the main source of NO, as the diffusion of NO is restricted at high soil moisture contents and NO produced from denitrification is reduced to N_2O before it escapes to the soil surface (Davidson, 1992; Firestone and Davidson, 1989; Skiba et al., 1997). Yet some studies showed that denitrification could also be a major source of NO emission from soils (Cárdenas et al., 1993; Loick et al., 2016; Pereira et al., 2010; Sanhueza et al., 1990).

Nitrification inhibitors (NIs) have been widely tested and studied for the purpose of decreasing nitrate leaching and mitigating greenhouse gas (GHG) emissions. Nitrification inhibitors are a group of chemical compounds that can reduce the bacterial oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) in the soil by inhibiting the activity of ammonia-oxidizing bacteria, e.g., of the genus *Nitrosomas*, in the soil (Zerulla et al., 2001). Most of NIs inhibit the first enzymatic step of nitrification, which is catalyzed by the enzyme ammonia monooxygenase (AMO) (Subbarao et al., 2006). A large number of NIs are known, but only a few of them, such as dicyandiamide (DCD) and 3, 4-Dimethylpyrazole phosphate (DMPP), have been widely and commercially used (Ruser and Schulz, 2015). The addition of NIs has been frequently reported to reduce both N_2O and NO emissions from agricultural soils, although their efficiency varies greatly in different environments (Pereira et al., 2010; Ruser and Schulz, 2015). Interestingly, some authors reported that the use of the NI reduced N_2O emission more effectively under higher soil moisture level, which is more favoured by denitrification (Di et al., 2014; Menendez et al., 2012). Although previous studies showed that most NIs did not have a direct effect on denitrification (Bremner and Yeomans, 1986; Müller et al., 2002), other studies suggested that denitrification-derived N_2O emission may also be affected by NIs indirectly via altering the product stoichiometry of denitrification (Hatch et al., 2005; Wu et al., 2017). As a key process of the global N cycle, denitrification leads to significant N losses from agricultural systems by converting NO_3^- and NO_2^- into NO, N_2O and N_2 (Bouwman et al., 2013). However, the product stoichiometry of denitrification, which is usually studied as $N_2O/(N_2O + N_2)$ product ratio, is affected by factors such as soil NO_3^- concentration, water-filled pore space (WFPS), and soil available carbon (C) (Weier et al., 1993). The effects of these factors on the product ratio are still not well understood, as the direct and precise measurements of N_2 production via denitrification in soils are challenging due to the high N_2 abundance in the atmosphere.

The difference between ^{15}N at the central (α position) and the terminal N atom (β position) in the asymmetric N_2O molecule (^{15}N site preference, SP) has been shown as useful indicators of N_2O production and consumption processes in soils (bacterial nitrification: 34–37‰, bacterial denitrification: -10-0‰) (Sutka et al., 2008, 2006; Toyoda et al., 2005). The advantages of this isotopic technique are that it is a non-invasive, source-process tracking method, enabling convenient low-cost gaseous sampling, which facilitates investigation of both laboratory incubation and field-

scale experiments (Decock and Six, 2013). The limitations of this technique have also been demonstrated, e.g., the uncertainties of N_2O source partitioning due to the overlapping or unknown SP signature of various pathways (Baggs, 2008; Decock and Six, 2013).

The first objective of this study was to examine the effectiveness of NI on mitigating N_2O and NO emissions at different soil moisture conditions in a UK grassland soil, as NIs have been widely used in grazed grassland. Furthermore, as the same soil was used in previous studies to investigate the sources and fate of N pools involving nitrogenous gas emissions (Loick et al., 2016), we further explored the effect of different soil moisture conditions on the fluxes, with and without the presence of NI, and sources of N_2O, NO and N_2, in order to gain a better understanding of the different processes involved, thereby helping to develop better management strategies to mitigate N_2O and NO emissions.

2. Material and methods

2.1. Soil

The soil was collected from a permanent grassland in North Wyke, Devon, UK (50° 46′ 10″ N, 3° 54′ 05″ E) to a depth of 15 cm in November 2013. The soil was classified as clayey pelostagnogley soil (Clayden and Hollis, 1985) (44% clay, 40% silt, 15% sand) and contained 0.5% total N and 11.7% organic matter, with a pH of 5.6. Root and plant residues were removed and the soil was sieved to <2 mm and stored at 4° C since 7 days before rewetting.

2.2. Automated soil incubation experiment

The incubation experiment was carried out at Rothamsted Research, North Wyke, UK, in a denitrification incubation system using a He/O_2 atmosphere (Cárdenas et al., 2003; Loick et al., 2016). Soils were packed into 12 stainless steel vessels of 140 mm diameter at a bulk density of 0.8 g cm^{-3}, which is similar to previous studies (Loick et al., 2016; Meijide et al., 2010). The atmospheric N_2 was removed by flushing the soil core with a mixture of He:O_2 (80:20) in order to measure N_2 fluxes. The experiment consisted of 6 treatments in total, i.e. soil amended with mineral N fertilizer (ammonium sulfate) and glucose (AS), or NI (DMPP) mixed with ammonium sulfate and glucose, at 50, 65, and 80% WFPS, respectively (AS50, DMPP50, AS65, DMPP65, AS80, DMPP80). The incubation experiment was conducted in two consecutive runs due to limited numbers of vessels. Prior to incubation, the soil was pre-incubated for 7 days at a soil moisture level that after taking the later amendment into account would achieve the final required WFPS. Ammonium sulfate was applied at a rate of 150 kg N ha^{-1} and glucose was applied at a rate providing 400 kg C ha^{-1}. DMPP was added at rate of 1.5 kg ha^{-1}. The amendment was dissolved in 50 ml water and added to each vessel. The temperature of the incubation cabinet was set at 22 °C.

2.3. Measurement of trace gases

For online trace gas concentration analysis of N_2O and CO_2, gas samples from each incubation vessel were measured every two hours and quantified using a gas chromatograph (Clarus 500, Perkin Elmer Instruments, Beaconsfield, UK), fitted with a flame ionization detector (FID) and methanizer for the quantification of CO_2, and an electron capture detector (ECD) for N_2O. Nitric oxide (NO) emissions were quantified using a chemiluminescence analyzer (Sievers NOA280I. GE Instruments, Colorado, USA). Dinitrogen (N_2) emissions were measured by using a gas chromatograph fitted with a helium ionization detector (VICI AG International, Schenkon, Switzerland) and are presented as average fluxes per day. The flow