



Potential dual effect of nitrification inhibitor 3,4-dimethylpyrazole phosphate on nitrifier denitrification in the mitigation of peak N₂O emission events in North China Plain cropping systems

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ABSTRACT

The winter wheat–summer maize rotation system in the North China Plain is a major source of nitrous oxide (N₂O) emissions due to high nitrogen (N) fertilizer and irrigation water inputs. However, a detailed understanding of the contribution of N₂O production sources is still limited because of the complexity of N₂O generation in soils and a lack of relevant field studies. Moreover, the efficiency and mechanisms of N₂O mitigation approaches in this area, i.e. the use of nitrification inhibitors, remains poorly understood. To elucidate the N₂O production pathways from this rotation system and to evaluate the effect of a widely used nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on mitigating N₂O emissions, we monitored N₂O fluxes and analyzed isotopomer ratios of soil-emitted N₂O during one rotation year. Results indicate that the application of DMPP significantly reduced N₂O emissions by 67% in the winter wheat season and 47% in the summer maize season. Isotopomer analysis revealed that in the N-fertilized treatment, nitrification and/or fungal denitrification accounted for up to 36% of the N₂O emission peaks observed after fertilization and irrigation events, whereas the nitrifier denitrification pathway was likely to be the major source, accounting for the remaining N₂O emissions. The high effectiveness of the nitrification inhibitor on mitigating N₂O emissions at high soil moisture may be attributed to the dual inhibitory effect on nitrifier denitrification, i.e. reducing the supply of nitrite, which is the substrate of nitrifier denitrification and inhibiting ammonia-oxidizing bacteria activities, which carry nitrifier denitrification.

1. Introduction

Emissions of nitrous oxide (N₂O) have had a great impact on global warming and stratospheric ozone depletion (Bouwman et al., 2002; Ravishankara et al., 2009). Agricultural soils are the major source of atmospheric N₂O (IPCC, 2014). High nitrogen (N) application levels generally lead to large N₂O emissions (Bouwman et al., 2002). However, the sustainability of high agricultural productivity strongly depends on the use of synthetic N fertilizers (Sutton et al., 2011). To secure crop production, the winter wheat–summer maize rotation system in the North China Plain, which accounts for about 40% of wheat and maize yield in China (National Bureau of Statistics of China, 2016), has been amended with large amounts of N fertilizer, leading to a number

of environmental problems, such as groundwater pollution and greenhouse gas emissions (Ju et al., 2004; Shi et al., 2013). Nitrous oxide emissions represent an important pathway for gaseous N loss in the North China Plain (Meng et al., 2005). In soil, N₂O production is mainly related to the type and activity of the microbial processes involved. Nitrification and denitrification have been found to be the key sources of N₂O emissions (Baggs, 2011; Butterbach-Bahl et al., 2013). Previous studies have suggested that nitrification accounted for 80–90% of N₂O emissions in the North China Plain due to the large supply of ammonium-based N fertilizers and weak denitrification potential in the soil (Ju et al., 2004; Wan et al., 2009; Ding et al., 2010).

The natural abundance of ¹⁵N and ¹⁸O of N₂O can provide an indication of the N₂O production processes, since N₂O produced during

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nitrification is more depleted in ^{15}N and ^{18}O relative to substrates than that produced during denitrification (Yoshida, 1988). However, this approach is unreliable for source partitioning of N_2O emissions as both $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ are affected by precursors, which can vary temporally and spatially (Baggs, 2011). The difference between ^{15}N at the central (α position) and the terminal N atom (β position) in the asymmetric N_2O molecule has been found to differ among different N_2O source pathways (bacterial nitrification and fungal denitrification: 34–37‰, nitrifier denitrification and bacterial denitrification: –11 to 2‰) (Sutka et al., 2006; Frame and Casciotti, 2010; Toyoda et al., 2015), and it is assumed to be independent of the $\delta^{15}\text{N}$ value of the precursor species (Toyoda et al., 2011; Decock and Six, 2013). Thus, it has the potential to be used to gain information about the underlying N_2O source processes. Compared to commonly used acetylene inhibition and isotope labeling techniques, the main advantages of N_2O site preference source partition technique are as follows: it is non-invasive, low-cost gaseous sampling and facilitates investigation of both incubation and field-scale experiments (Lewicka-Szczebak et al., 2017). In addition to the conventional laboratory-based isotope-ratio mass-spectrometry in combination with a flask-sampling approach, newly developed laser spectroscopic techniques, such as quantum cascade laser absorption spectroscopy, have enabled real-time analysis of N_2O isotope signatures in both laboratory and field studies (e.g. Heil et al., 2014; Yamamoto et al., 2017). Nevertheless, deploying N_2O site preference (SP) values for N_2O source partitioning to nitrification and denitrification processes is complicated by the similar SP values for fungal denitrification and nitrification and for nitrifier denitrification and bacterial denitrification (Sutka et al., 2006; Rohe et al., 2014). Furthermore, the isotopic fractionation during N_2O reduction to nitrogen gas (N_2) tends to enrich ^{15}N at the α position of the N_2O molecule, thereby increasing SP values (Sutka et al., 2006). Therefore, the nitrification process as a source of N_2O could be overestimated if the fractionation effect of N_2O reduction is not considered. Moreover, there are other microbial N_2O production pathways, such as archaeal nitrification, anammox (anaerobic ammonium oxidation) or DNRA (dissimilatory nitrate reduction to ammonium), for which hardly any characteristic isotopic N_2O signatures have yet been identified. Considering the limitations mentioned above, at this moment the N_2O site preference source partition approach should be treated as numerical rather than analytical models for rough estimation of N_2O emission sources (Decock and Six, 2013).

Nitrifier denitrification carried out by ammonia-oxidizing bacteria (AOB) has been identified as another key process responsible for N_2O emissions in soil, which is supported by an increasing number of studies based on a multi-isotope tracing approach (Kool et al., 2011; Zhu et al., 2013; Huang et al., 2014). However, the contribution of nitrifier denitrification to soil N_2O emissions remains unclear due to a lack of field measurements. Indeed, several recent studies pointed to the overlooked major role of nitrifier denitrification on N_2O emissions in the North China Plain (Zhang et al., 2016; Huang et al., 2014), thereby suggesting that the contribution of ammonia oxidation on N_2O emissions has been overestimated, since most previous studies did not distinguish between the contribution of ammonia oxidation and nitrifier denitrification.

Nitrification inhibitors (NI) are a group of compounds that can decrease the bacterial oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) by inhibiting the activity of *Nitrosomonas* sp. in the soil (Zerulla et al., 2001). Different inhibition mechanisms were believed to be involved for various NIs. Nevertheless, commercially utilized NIs such as nitrin, dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) were found to inhibit the first enzymatic step of nitrification through the removal of co-factors by chelating compounds like Cu (Subbarao et al., 2006; McCarty, 1999; Ruser and Schulz, 2015). The use of NIs has repeatedly been shown to effectively reduce N_2O emissions from cropland soils, such as in the North China Plain, with mitigation efficiency of 38–44% as suggested by recent meta-analysis studies (Qiao et al., 2015). Different factors, e.g. soil moisture (Menendez et al., 2012), atmospheric oxygen content (Hatch et al., 2005), soil

available C (Wu et al., 2017), have been found to affect the mitigation effect of an NI on N_2O emissions. This indicated that the extent to which the NI inhibits N_2O emissions might depend on the pathway of N_2O production. Among the various types of NI, only a few have been widely tested and used commercially, e.g. DCD and DMPP. Compared with DCD, DMPP has been shown to have several distinct advantages, including higher N_2O mitigation effect, longer active duration and lower application rates (Weiske et al., 2001; Zerulla et al., 2001).

The objectives of our study were (a) to evaluate the effect of application of DMPP with urea to reduce N_2O emissions during one winter wheat–summer maize rotation in the North China Plain, and (b) to illustrate the main processes contributing to N_2O emissions by investigating the isotopic signature of N_2O during peak emission events.

2. Materials and methods

2.1. Study site and field management

The experiment was conducted in Huantai County, Shandong province, North China (36°57.75'N; 117°59.21'E). Wheat (*Triticum aestivum* L.) was planted on 10th October 2015 and harvested on 13th June 2016, while maize (*Zea mays* L.) was planted on 20th June 2016 and harvested on 2nd October 2016. The average air temperature and precipitation for winter wheat season and summer maize season was 10.3 °C/234 mm and 26.4 °C/481 mm, respectively. The soil was classified as aquic inceptisol (a calcareous, fluvo-aquic clay loam) and consisted of 38% clay (< 0.002 mm), 32% silt (0.002–0.02 mm) and 30% sand (0.02–2 mm). The soil had a bulk density of 1.4 g cm⁻³, pH in water of 7.7, soil organic carbon (C) of 10.0 g kg⁻¹ and total N content of 1.1 g kg⁻¹ (0–20 cm).

A randomized block design was employed with three replicates (8 × 7.5 m²) for each of three treatments: CK (no fertilizer N input), U (urea), and DMPP (urea plus DMPP). Urea was applied at a rate of 300 kg N ha⁻¹ season⁻¹ (50% as basal fertilization and 50% as top-dressing fertilization) for both wheat season and maize season. The NI (DMPP) was thoroughly mixed with urea and then spread onto the soil surface at a rate of 1% of the applied urea N. The straw of wheat and maize were both returned to the field after harvest. Irrigation was carried out immediately after fertilization twice in the wheat season and once in the maize season (75 mm each).

2.2. Gas sampling and flux measurement

The static chamber-gas chromatography technique was used for measuring N_2O fluxes (Shi et al., 2013; Zhou et al., 2014). The N_2O emissions were measured once every day for one week immediately following fertilization and irrigation events, and then twice a week afterwards. At 0, 15, 30, 45 and 60 min after chamber closure (chamber area: 0.5 × 0.5 m², the height was adjusted according to the plant growth), five gas samples from the chamber headspace were obtained using a 60-mL polypropylene syringe, and relevant information (including specific sampling time, air temperature and chamber temperature) was recorded. Gas sampling for flux measurements were performed between 9:00 and 11:00 a.m. local time and analyzed on a gas chromatograph (7820 A, Agilent, Shanghai, China) within 24 h. The flux was calculated as a linear slope of the concentration evolution over the chamber closure time (Shi et al., 2013). Gas samples for isotopomer analyses were collected from the static flux chamber after 90 min closure time.

2.3. Isotope analysis

The N_2O $\delta^{15}\text{N}_{\text{bulk}}$ (i.e. the average $\delta^{15}\text{N}$ over the N_2O molecule), $\delta^{15}\text{N}_{\alpha}$ (i.e. $\delta^{15}\text{N}$ at the central position of the N_2O molecule), and $\delta^{18}\text{O}$ isotope signatures were determined by analyzing m/z 44, 45, and 46 of intact N_2O^+ molecular ions, and m/z 30 and 31 of NO^+ fragment ions

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