Soil & Tillage Research 180 (2018) 82–90

Contents lists available at ScienceDirect

Soil & Tillage Research

journal homepage: www.elsevier.com/locate/still

Effects of urease and nitrification inhibitors on nitrous oxide emissions and nitrifying/denitrifying microbial communities in a rainfed maize soil: A 6-year field observation

Dan Dong[a,b,c], Yongping Kou[d], Weichao Yang[a,e], Guanxiong Chen[a], Hui Xu[a,e,*]

[a] Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] National Field Observation and Research Station of Shenyang Agro-Ecosystems, Shenyang 110016, China
[d] Key Laboratory of Environmental and Applied Microbiology, CAS, Environmental Microbiology Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China
[e] Key Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China

ARTICLE INFO

Keywords:
Precipitation
DCD
Ammonia oxidizing bacteria

ABSTRACT

Application of the inhibitor-amended nitrogen fertilizers is a recommended method for reducing agricultural nitrous oxide (N_2O) emission. However, the potential impacts of these inhibitors on soil environments still needs to be evaluated using long-term experiments. Through a 6-year field observation, the effects of combined application of a nitrification inhibitor (dicyandiamide, DCD) and a urease inhibitor (hydroquinone, HQ) on N_2O emission, as well as soil ammonia oxidizers and denitrifiers in a maize (*Zea mays* L.) field in Northeast China were investigated. The results showed that annual soil N_2O emissions were 0.466, 1.021 and 0.874 kg N_2O-N ha^{-1} for N0 fertilizer treatment (CK), Urea treatment (U) and Urea + DCD + HQ treatment (UDH), respectively. A significant linear correlation was found between the N_2O accumulation in the first month after fertilizer application and the short-term precipitation (*i.e.*, a period from 10 days before to 20 days after fertilizer application). The N_2O emissions in the freeze-thaw period accounted for up to 42.5% of the year-round N_2O emissions. The remarkable fluctuations of annual N_2O emissions were observed (their coefficients of variation were 68.3%, 77.7% and 71.2% for CK, U and UDH treatments, respectively); these fluctuations were mainly attributed to the precipitation. The averaged N_2O emission factors (EF) (0.308% and 0.227% for U and UDH treatments, respectively) were far less than the default mean EF of 1% proposed by (IPCC, 2006). An averaged N_2O mitigation of 26.4% was fulfilled by UDH application. The results of quantitative PCR for soil nitrification and denitrification gene copy numbers measurement showed that UDH treatment significantly decreased the ammonia oxidation bacteria (AOB) *amoA* gene copy numbers by 74% on the 10th day after UDH application. No significant effects of combined application of DCD and HQ on microbial denitrification functional gene abundance were observed.

1. Introduction

Nitrous oxide (N_2O), which is an important greenhouse gas, has a global warming potential 310 times greater than carbon dioxide over a 100-year time horizon (IPCC, 2014). N_2O is also involved in stratospheric ozone depletion (Ravishankara et al., 2009). Agricultural soils, as the major source of atmospheric N_2O, account for approximately 60% of the global anthropogenic N_2O emissions (IPCC, 2007). Furthermore, it was confirmed by nitrogen stable isotope measurement that fertilized soils are primarily responsible for the historic increase in atmospheric N_2O (Park et al., 2012). However, Stehfest and Bouwman (2006) found that despite the rapid increase in the number of N_2O measurements for agriculture fields, large uncertainties existed in the estimates of global N_2O emissions from agro-systems. Therefore, more field studies on N_2O emission and its mitigation from agricultural soils are required.

The application of inhibitor-amended nitrogen fertilizer is an effective method for reducing agricultural N_2O emission. In a pot experiment, a combination of nitrification inhibitors (NI) dicyandiamide (DCD) and urease inhibitor (UI) hydroquinone (HQ) was shown to be an efficient method to improve urea-N efficiency and crop quality and to decrease N_2O emission (Xu et al., 2000). However, only limited field

* Corresponding author at: Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China.
E-mail address: xuhui@iae.ac.cn (H. Xu).

https://doi.org/10.1016/j.still.2018.02.010
Received 27 September 2017; Received in revised form 24 January 2018; Accepted 22 February 2018

measurements on N_2O emission under HQ + DCD application were reported (Chen et al., 1998; Boeckx et al., 2005; Li et al., 2009; Wang et al., 2015).

Moreover, in *in situ* N_2O flux measurements, large seasonal and inter-annual fluctuations of N_2O emissions were usually found. For example, a 71.6% coefficient of variation of N_2O emissions over a 5-year period from the semi-arid grassland in Inner Mongolia, China (Du et al., 2006), and a 51% variation coefficient of N_2O emissions in a maize-wheat field during 4 successive years observation in Hebei province, China were reported (Zhang et al., 2014). In addition, N_2O emissions from N-fertilized agricultural fields have been found to vary between 0.001 and 6.8% of the applied N (Bouwman, 1990; Minami, 2000). Although the emission factor (EF) of 1% of the N applied was recommended by IPCC as a 'default value' to calculate the direct emissions of N_2O from fertilized soils (IPCC, 2006), more precise national or regional EFs were required. Hence, long-term successive field N_2O fluxes monitoring was necessary to obtain an accurate estimation of the N_2O budget as well as the influence of inhibitors on N_2O budget in a certain region.

N_2O is mainly produced through nitrification and denitrification processes in soils. Additionally, it has been estimated that ammonia oxidation, a rate-limiting step of nitrification, may contribute up to 80% of the soil N_2O flux, depending on soil types, temperature, and water content (Gödde and Conrad, 1999). Ammonia oxidation is thought to occur *via* the ammonia oxidizing bacteria (AOB) and the ammonia oxidizing archaea (AOA) (Xiao et al., 2014). DCD inhibits nitrification in soil by deactivating the ammonia monooxygenase enzyme, which is responsible for ammonia oxidation (Di et al., 2009). In addition, it was also reported that DCD significantly reduced AOB abundance in nitrogen-rich soils (Dai et al., 2013), whereas, hydroquinone (HQ), a form of UI, delays urea hydrolysis and subsequently affects nitrification and denitrification (Zaman et al., 2008). However, there are few reports on the effects of DCD and HQ on the soil nitrification and denitrification microbial community after a long-term application.

Cambisol covers approximately 10.2% of the agricultural land in China (Dong et al., 2013). To the best of our knowledge, few studies have considered Cambisol on N_2O emissions under long-term field observation in Northeast China; as a result, considerable uncertainty exists considering the arable N_2O budget on this type of soil.

Therefore, we present here a long-term and year-round observation of N_2O emission from Cambisol cropped with maize (widely cropped in Northeast China) in Northeast China to elucidate that the following: (1) the seasonal and inter-annual variations of N_2O fluxes; (2) the magnitude of N_2O emission reduction by inhibitors; (3) the key factors controlling inter-annual N_2O emission; (4) the influence of long-term combined use of DCD and HQ with urea on soil microbial functional genes involved in soil nitrification and denitrification.

2. Materials and methods

2.1. Study sites and soil properties

The experiments were conducted at the National Field Observation and Research Station of Shenyang Agro-ecosystems Chinese Academy of Sciences (41°31′N, 123°22′E). According to the FAO taxonomy (FAO soil classification), the soil type is a Cambisol (Dong et al., 2013). The mean annual air temperature is 7.5 °C, the mean annual rainfall is approximately 680 mm. The soil has a pH (1:1 water) of 5.8, organic carbon content of 8.52 g kg^{-1}, total N of 0.95 g kg^{-1}, and a soil bulk density of 1.25 g cm^{-3}.

2.2. Experimental design and field management

Plots of three treatments, *i.e.*, control (CK, without urea), urea (U, urea applied), and urea combined with DCD and HQ (UDH, urea applied with DCD + HQ), were established on 14 May 2010. Three replicated plots (4 m × 5 m) of each treatment were established based on a randomized complete block design. Urea was applied at rate of 180 kg N ha^{-1}. All treatments received a basal amount of calcium superphosphate (P) and potassium chloride (K) at rates of 90 kg P_2O_5/K_2O ha^{-1}. In the UDH treatment, the HQ and DCD were applied at rates of 1.8 kg $ha^{-1}$and 5.4 kg ha^{-1}, respectively; they were thoroughly mixed and then applied manually into soil using band application at the day when the maize was sown. Then, different cultivars of Maize (*Zea mays* L., cultivars of fuyou #1 (2010–2011) and fuyou #9 (2012–2015)) were planted. Maize was sown at the end of April or the beginning of May, and harvested at the end of September. Maize was seeded with row spacing of 60 cm (45,000 plants ha^{-1}). Before sowing, a chamber for N_2O fluxes measurement was installed in each plot. The growing season considered here was from May to September, the non-growing season was from October to April.

2.3. N_2O flux measurements

A static closed-chamber method was used to monitor the soil-to-atmosphere N_2O fluxes (Jiang et al., 2017). The chamber size was 28 cm × 56 cm × 20 cm, with its short edge parallel to the maize rows. Gas samples in chamber were taken between 9:00–11:00 a.m. The gas samples were collected with a 50-ml polypropylene syringe equipped with a 3-way stopcock at 0, 20 and 40 min after the chambers were closed and then injected into 12-ml vacuum vials fitted with butyl rubber stoppers. The N_2O concentrations of gas samples were analysed with a gas chromatograph (Agilent 7890A, USA) equipped with an electron capture detector (ECD). When each gas flux was measured, the air temperature and soil temperatures at 0 cm and 5 cm of soil depth were synchronously recorded with bent stem thermometers and volumetric soil moistures (%, m^3 H_2O m^{-3} soil) in the 0–5 cm soil layer were monitored using time domain reflectometry.

2.4. Soil sampling and analysis

Soil samples from 0 to 20 cm soil layer in each plot were collected using a 5-cm diameter stainless steel soil sampler. Each soil sample was a composite of five subsamples, which were collected randomly from different sites of each plot (we did not sample the soil on the fertilizer bands). After visible roots were picked up, the soil samples were sieved through a 2-mm mesh. Concentrations of NO_3^--N, and NH_4^+-N in the soil samples was extracted with 2 M KCL solution. Extracted samples were analysed with a continuous flow injection analyser (Alliance, Futura, France). Soil water content was measured gravimetrically, after drying at 105 °C for 24 h; the corresponding volumetric water content was switched to percentage of water-filled pore space (WFPS) and was calculated by the following equation:

$$\text{WFPS} = \text{volumetric water content}/(1 - \text{bulk density}/2.65) \quad (1)$$

where 2.65 g cm^{-3} was the assumed particle density of the soil (Ding et al., 2015).

The precipitation and air temperature data were acquired from the meteorological station of the National Field Observation and Research Station of Shenyang Agro-Ecosystems.

2.5. Quantitative PCR

The soil samples at a depth of 0–20 cm in each plot for measuring the abundance of soil microbial were collected on May 20, 2015 (seeding time) and September 29, 2015 (harvest time). The total DNA contents of soil sample were extracted using Omega DNA extraction kits for soil (E.Z.N.A. Soil DNA Kit; Omega Bio-Tek Inc., GA, USA) according to the manufacturer's protocol. The quality and the purity of DNA were verified by 1% agarose gel electrophoresis and NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, DE, USA).

The abundance of functional genes of nitrifiers and denitrifiers in soil samples (taken in May and September of 2015) was determined by