Applied Soil Ecology 120 (2017) 111–120

Contents lists available at ScienceDirect

Applied Soil Ecology

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

Effects of land-use change and fertilization on N_2O and NO fluxes, the abundance of nitrifying and denitrifying microbial communities in a hilly red soil region of southern China

Xing Wu[a,b,*], Huifeng Liu[a,c], Bojie Fu[a,b], Qing Wang[a,c], Ming Xu[d], Huiming Wang[d], Fengting Yang[d], Guohua Liu[a,c,*]

[a] State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[b] Joint Center for Global Change Studies, Beijing 100875, China
[c] University of Chinese Academy of Science, Beijing 100049, China
[d] Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Nature Resources Research, Chinese Academy of Science, Beijing 100101, China

ARTICLE INFO

Keywords:
Land-use change
Fertilization
Nitrous oxide
Nitric oxide
Ammonia oxidizers
Denitrifying communities

ABSTRACT

Nitrous oxide (N_2O) and nitric oxide (NO) are generally interrelated in soil nitrogen biogeochemical cycles. However, the effects of land-use change and fertilization on N_2O and NO fluxes and the underlying mechanisms are not well understood, especially in fields converted from rice paddies (RPs) to upland cultivation. This study investigated the effects of land-use change from RPs to a citrus orchard (OR) as well as fertilization on N_2O and NO fluxes, and the gene abundance of nitrifying and denitrifying microbial communities by using static chamber-gas chromatography and quantitative real-time PCR (qPCR). Structural equation modeling was performed to determine whether soil properties and the abundances of nitrifiers and denitrifiers influenced *in situ* N_2O and NO fluxes. Land-use change from RPs to an OR and fertilization significantly increased N_2O and NO fluxes. Land-use change increased AOA abundance, did not affect AOB abundance, and decreased *nir*K, *nir*S, and *nos*Z abundances. The growth of AOA and AOB abundance in response to fertilization was generally stimulated by providing more NH_4^+, but sometimes was inhibited by lower pH. Fertilization did not significantly affect the abundance of denitrifiers. Soil properties and functional microbial groups were explanatory variables, while land-use change altered their role in predicting *in situ* N_2O and NO fluxes. For N_2O fluxes, dissolved organic carbon was the strongest predictor in the RPs, whereas the primary explanatory factor was *nir*K abundance in the OR. For NO fluxes, soil temperature was the most important explanatory variable. Our results demonstrate the need for comprehensive approaches incorporating nitrifying and denitrifying processes to improve our understanding of biogeochemical cycles.

1. Introduction

It is of great concern worldwide that nitrogenous gases contribute to regional and global-scale environmental issues (IPCC, 2013). Nitrous oxide (N_2O) is one of the main potent long-living greenhouse gases that contributes to global warming and participates in the destruction of stratospheric ozone (Ravishankara et al., 2009). Nitric oxide (NO) is not only a precursor for tropospheric ozone but is also involved in the regulation of the oxidation balance of the atmosphere (Bouwman et al., 2002). In most agricultural soils, N_2O and NO emissions strongly depend on the activity of the microbiological nitrogen processes of nitrification and denitrification, in which both gases are produced as byproducts or intermediate products (Bouwman et al., 2002).

Land-use change, which is regarded as the second largest anthropogenic source of greenhouse gas emissions, can significantly alter the dynamics of the global nitrogen cycle (IPCC, 2013; van Lent et al., 2015). Although the effects of land-use change on greenhouse gas emissions have been widely reported, few *in situ* studies have been conducted after land-use change from conventional rice paddies (RPs) to upland cultivation, especially including year-round continuous measurements of both N_2O and NO fluxes (Yao et al., 2015; Zhang et al., 2016). Several previous studies have demonstrated relatively

* Corresponding authors at: State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China.
E-mail addresses: xingwu@rcees.ac.cn (X. Wu), ghliu@rcees.ac.cn (G. Liu).

http://dx.doi.org/10.1016/j.apsoil.2017.08.004
Received 22 March 2017; Received in revised form 8 July 2017; Accepted 8 August 2017
Available online 30 August 2017
0929-1393/

lower N_2O emissions from RPs than from upland soils because anaerobic conditions limit nitrate availability and strict anaerobiosis favors complete denitrification to N_2 (Zou et al., 2005; Liu et al., 2016). However, by conducting two years of field measurements of N_2O flux from four different types of land uses in subtropical red soil, Lin et al. (2012) reported that N_2O emissions from rice paddies were approximately 54.4% higher than those from orchard soil. This discrepancy might be partly attributed to the differences in the land-use legacies and fertilization managements (Scheer et al., 2008; Shao et al., 2017). Fertilization is the management practice most frequently used for agricultural soils and has long been identified as an important factor in regulating N_2O and NO fluxes (Bouwman et al., 2002; Dai et al., 2013; Liu et al., 2015). Fertilization is widely accepted to cause a significant increase in N_2O and NO emissions by providing more inorganic N contents (Bouwman et al., 2002; Rowlings et al., 2013). However, few year-round field studies have investigated the response of N_2O and NO emissions to fertilization simultaneously from the red soil region of China, resulting in uncertainties about the emission inventory of nitrogenous gases (Bouwman et al., 2002; Akiyama et al., 2005).

Nitrification is the biological oxidation of ammonium (NH_4^+) to nitrite (NO_2^-), which is performed by ammonia-oxidizing archaea (AOA) and bacteria (AOB), followed by the oxidation of the NO_2^- to nitrate (NO_3^-) (Rotthauwe et al., 1997). Denitrification is the stepwise reduction of NO_3^- to N_2 *via* NO_2^-, NO and N_2O by denitrifiers, in particular the NO_2^- reductase encoded by either *nir*K or *nir*S gene and the N_2O reductase encoded by *nos*Z gene (Throbäck et al., 2004). Land-use change from paddies to uplands involves niche specialization with environmental factors (*e.g.,* oxygen, ammonia and pH) that are key factors influencing nitrifiers and denitrifiers (Alam et al., 2013; Yang et al., 2016). However, detailed measurements of the abundances of nitrifying and denitrifying microbial communities with simultaneous N_2O and NO flux determination are still limited, especially for the red soil regions in China. Moreover, though a large number of studies have documented nitrifiers and denitrifiers in response to fertilization (Szukics et al., 2009; Alam et al., 2013), some discrepancy remains, resulting from different land-use types (He et al., 2007; Di et al., 2014; Long et al., 2016). Furthermore, because microbial communities play an essential role in the production and consumption of N_2O and NO (Bergaust et al., 2010; Medinets et al., 2015), conceptual models have been used to predict the dynamics of trace gas fluxes (Petersen et al., 2012; Lammel et al., 2015; Martins et al., 2015). Therefore, to improve our understanding of the dynamics of N_2O and NO fluxes and the underlying mechanisms, detailed studies of the abundances of soil nitrifiers and denitrifiers following land-use change and fertilization are needed.

Red soil, one of the typical agricultural soils in southern China, covers approximately 11.8% of the country's land surface, produces 80% of the rice, and supports 22.5% of the population of China (Liu et al., 2016). In recent decades, the red soil regions have been undergoing remarkable land-use change due to increasing socio-economic developments and demands for livestock products. In particular, changing rice paddies to upland cultivation for growing vegetables, fruits and economic forests have been locally recommended to meet the increasing market demands and obtain higher economic returns in these regions (Lin et al., 2012). This process has led to a diverse range of land uses with different cultivation, irrigation and fertilizer rates, which could result in significant differences in soil nitrifiers and denitrifiers and subsequent N_2O and NO fluxes. Therefore, understanding the effects of land-use change from paddies to orchards on N_2O and NO fluxes as well as the abundances of nitrifying and denitrifying microbial communities is critical for elucidating the mechanisms and processes of nitrogenous gas emissions. However, few studies have simultaneously paid attention to the changes in N_2O/NO fluxes and the abundances of nitrifying and denitrifying microbial communities in these regions. The present study thus aimed to (1) assess the effects of land-use change and fertilization on *in situ* N_2O and NO fluxes; (2) examine how land-use change and fertilization affect the abundances of nitrifying and denitrifying microbial communities; and (3) evaluate the existing relationships among N_2O/NO fluxes, soil properties, and functional gene abundance.

2. Materials and methods

2.1. Field site and experimental setup

The study site is located at the Qianyanzhou Experimental Station (26°44′N, 115°04′E), Chinese Academy of Science (CAS), in Jiangxi Province, southern China. This region experiences a subtropical warm and humid monsoon climate. During the period 1989–2010, the mean annual air temperature and precipitation were 18.0 °C and 1509.0 mm, respectively (Liu et al., 2016). The soil is typical red soil found in middle-subtropical China and classified as Cambisol according to the Ultisol classification. The soil texture is sandy loam, consisting of 58% sand, 31% silt, and 11% clay. Double-cropping rice is the main cropping pattern in this region.

The two most common land-use types in the hilly red soil regions of southern China were selected for the present study, namely, rice paddy (*Oryza sativa* L.) and citrus orchard (*Citrus reticulate* L.). Conventional rice paddies had been continuously cultivated for approximately 10 years following the regime of local field managements, and a portion of the fields was converted to orchard in June 2012. Under each land-use type, two fertilizer treatments (*i.e.,* conventional fertilization and no fertilization) were established. The fertilization treatment followed the local cropping regimes and farmer fertilization practices. The fertilizers used were compound fertilizer (15% N) and urea (46% N). The other was treated as a control without fertilization, with additional management practices being the same as in the fertilization treatment. Therefore, four treatments—rice paddies with fertilization (RP-F) and without fertilization (RP-NF) and citrus orchards with fertilization (OR-F) and without fertilization (OR-NF) —were arranged in a completely randomized block design with four replicates (12 × 14 m). Detailed descriptions of the experimental site, management practices and experimental design can be found in previous publications (Liu et al., 2016, 2017). To ensure survival and yield, a floodwater layer of 5–7 cm was maintained in the RPs until the mid-season drainage, from July 29 to Sep 15, 2013 for the late rice and from April 18 to Jun 10, 2014 for the early rice. The cultivations and fertilization practices in the RP and OR are shown in Table 1.

2.2. Measurement of N_2O and NO fluxes

In-situ fluxes of N_2O and NO were simultaneously measured from July 2013 to August 2014 using a static chamber-based method as described by Mei et al. (2009) and Yao et al. (2015). A stainless steel collar (diameter = 40 cm) was pre-installed in the centre of each plot before rice transplanting or orchard planting. The top edge of the collar contains a groove (5 cm in depth) filled with water to seal the rim of a chamber during gas collection. Cylindrical sampling chambers with a diameter of 40 cm and height of 0.39 or 0.69 m (according to the plant height) were covered with a layer of thermal insulation to minimize air temperature changes inside the chamber and equipped with a circulating fan to ensure complete gas mixing during the gas sampling period. The base frames were kept in the same location throughout the entire measurement period in the orchard plots, whereas those in the paddy fields were removed before tillage and placed (24 h before the measurement) in the location marked for subsequent measurements.

Generally, the flux measurements were carried out daily within duration of 5–7 days after each fertilization event, and performed once or twice per week for the remaining period. On each sampling day, gas samples were collected between 09:00 and11:00 am. Five samples for the N_2O analysis were taken from the headspace of chamber using 20 mL polypropylene syringes at an interval of 10 min after chamber