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Effects of different irrigation methods on nitrous oxide emissions and ammonia oxidizers microorganisms in greenhouse tomato fields

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ABSTRACT

Agricultural soils are strong sources of the potent greenhouse gas N_2O but soil N_2O emissions and its microbial mechanism in greenhouse field, especially ammonia-oxidizing microorganisms are unclear. We characterized a potential response in soil N_2O production and its influencing factors, such as soil temperature, moisture, pH, and inorganic nitrogen to different irrigation methods named drip irrigation (DI), subsurface irrigation (SI) and furrow irrigation (FI) in a long-term irrigation field in greenhouse. The abundance and metabolic activity of ammonia oxidizing archaea (AOA) and bacteria (AOB) in greenhouse soils were also investigated using *amoA* gene as a molecular biomarker by quantitative PCR and $^{13}CO_2$-DNA-stable isotope probing (SIP) methods. Results showed that N_2O flux peaks would obviously occur within 1–8 days after each irrigation. The soil N_2O flux in FI treatment was significantly higher than that in DI and SI treatments ($P < 0.05$). Correlation analysis between soil N_2O flux and its influencing factors indicated that soil moisture and nitrate nitrogen were substantially affecting soil N_2O emissions compared with soil temperature, pH and ammonium nitrogen. The copy numbers of AOA *amoA* gene in FI treatment were significantly higher than those in DI and SI treatments ($P < 0.05$), while there is no significant difference of AOB *amoA* gene among the three treatments. Also, the copy numbers of AOA *amoA* gene were significantly higher than those of AOB *amoA* gene. The $^{13}CO_2$-DNA-SIP and phylogenetic tree results indicated only AOB dominantly involved in *Nitrosospira* genera was active during the nitrification process in the three irrigation methods. Our findings provided direct evidence that drip irrigation and subsurface irrigation could effectively reduce soil N_2O emissions in greenhouse. AOA was dominant in abundance, while AOB played a key role in microbial community under the conditions of this experiment. Future characterization of the mechanisms for ammonia oxidation requires deeper studies in the greenhouse field.

1. Introduction

Global warming and climate change induced by greenhouse gases (GHG) have become the most noticeable environmental concern in modern society (Baruah et al., 2010; Cox et al., 2000). Nitrous oxide is a natural and efficient greenhouse gas, which presents a global warming potential 298 times larger than CO_2 in a 100 y horizon (Stocker et al., 2013). N_2O also participates in the destruction of the stratospheric ozone layer (Meade et al., 2011). Agricultural soils are the greatest sources of N_2O, accounting for approximately 60–80% of the total worldwide anthropogenic N_2O emitted to the atmosphere (Syakila and Kroeze, 2011). Nitrification and denitrification are supposed to the most important pathway for N_2O production (Baggs, 2008). The nitrification process is a crucial part of the N cycle and is required for the mobilization of ammonium in soils. Ammonium oxidation, which codes for the enzyme ammonia mono-oxygenase (AMO), is the first and rate-limiting step of nitrification, in which N_2O can be produced as a by-product (Kowalchuk and Stephen, 2001). Recent studies have shown that the AMO α subunit encoded by the *amoA* gene is present in both ammonia-oxidizing bacteria (AOB) and archaea (AOA) (Brochier-Armanet et al., 2008; Schleper et al., 2005). An emerging body of studies have also shown that both AOB and AOA are all key players in ammonia oxidation in agricultural soils (Jia and Conrad, 2009; Shen et al., 2008), which have been used as molecular markers for many studies (Andert et al., 2011; Gubryrangin et al., 2011; He et al., 2012). And the abundance of nitrifiers is also considered to play an important

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role in mediating soil N_2O emissions (Cavigelli and Robertson, 2000). However, little information is obtainable with respect to niche differentiation of AOA and AOB in response to different irrigation method. Therefore, there is a need to understand the community distribution, diversity and abundance of ammonia-oxidizing microorganism under different irrigation methods, which can provide insights and comprehensions of ammonia-oxidizing archaea and bacteria involved in nitrification of the N cycling in the agricultural ecosystems.

Soil N_2O emissions are affected by a variety of environmental factors, such as soil temperature, moisture, pH, NH_4^+-N, and NO_3^--N etc. (Čuhel et al., 2010; Khalil et al., 2002; Koponen et al., 2006; Zhang et al., 2016a), in which soil moisture is an important factor controlling N_2O emissions (Jauhiainen et al., 2011). At present, the researches on N_2O emission in agricultural soils are intensively focused on rice (Zhang et al., 2016b), wheat-maize rotation (Shan et al., 2016), grassland (Wolf et al., 2015) and other field crops, but the studies concentrated on N_2O emissions in greenhouse have rarely been investigated. In fact, the production areas of greenhouse vegetables have reached to 4.0 million hectares in 2015 in China, and the yields have nearly attained to 260 million tons (Yu and Zhou, 2016). Given the special characteristics of excellent sealing performance, frequent irrigation, high multiple cropping index and large application rate of nitrogen fertilizer, the soil N_2O emissions and its influencing factors in greenhouse vegetables are often differed with those in field crops (Zhao et al., 2010). Obviously, it is meaningful to clarify the characteristics of soil N_2O emissions from vegetable soil.

Drip, subsurface and furrow irrigation are widely used to accommodate sustainable growth demand for grains and vegetable (Ibrahim et al., 2016; Wang et al., 2007). Different irrigation methods caused big effects on crop growth, yield and water use efficiency (Amer, 2011; Cabangon et al., 2004; Xing et al., 2015). Previous researches on soil N_2O emissions in greenhouse primarily focus on nitrogen fertilization application (Cui et al., 2016; Li et al., 2015; Lou et al., 2012), and few studies have examined the soil N_2O emissions and characterized functional gene expression in microbial communities associated with nitrifier under different irrigation methods in greenhouse. Therefore, the objectives of this paper were to determine (i) the effect of different irrigation methods on soil N_2O emissions and influencing factors in the tomato greenhouse field (ii) the relationship between the abundance of ammonium oxidizers and soil N_2O emissions by real-time polymerase chain reaction (PCR) techniques (iii) the crucial effect and relative contribution of ammonia-oxidizing archaea and bacteria in nitrification by DNA-based stable isotope probing (DNA-SIP) technique.

2. Materials and methods

2.1. Description of field experiment

The experiment was performed at a long-term irrigation Experimental Station (began in 2010), Shen Yang Agricultural University, which is located in Shen Yang, Liaoning province, China (41°49′22″N, 123°33′55″E). The study area has a temperate sub-humid continental climate. The annual mean precipitation is approximately 721.9 mm. The experimental soil was a brown soil and classified as Mollic Gleysols (in the FAO-UNESCOsystem). The initial soil organic matter content is 12.58 g kg^{-1}, total N content 1.47 g kg^{-1}, and available N content 61.26 mg kg^{-1}, at pH 5.8. The tomato was planted on April 19th, and harvested on August 6th, 2015. Three irrigation methods as drip irrigation (DI), subsurface irrigation (SI), and furrow irrigation (FI) were performed in the present study. Plots (20 m^2) were interspersed in completely randomized blocks with three repetitions. The irrigation control upper and lower limits of three irrigation treatments were soil water suction 6 kPa and 30 kPa. The lower limits of all treatments were detected by tensiometers placed in 30 cm soil layer at 8:00 a.m. every day. The irrigation would begin during the time that the treated soil water suction value reached or exceeded the setting irrigation control lower limit (30 kPa) of soil water suction value, and when the irrigation and gas collection conflicted, the principle of gas collection was firstly taken. The soil water holding characteristic curve previously measured by the soil samples collected in the greenhouse was measured, and the soil water content corresponding to the upper and lower limits of soil water suction was calculated according to the Formula (1)

$$\theta = 0.5212[1 + (6.382h)^{11.5005}]^{-0.0094} \quad (1)$$

Where θ is soil volumetric water content ($cm^3\ cm^{-3}$); h is soil water suction (kPa). According to Formula (2) to calculate the single amount of irrigation

$$Q = (\theta_2 - \theta_1) \times H \times R \times S \quad (2)$$

Where Q is the single irrigation amount (m^3); H is plan wetting layer thickness; R is the wetting ratio; S is the plot area; θ_2 and θ_1 are the upper limits and lower limits of soil water suction irrigation control corresponding soil moisture content ($cm^3\ cm^{-3}$), respectively. According to the specific conditions of this experiment, H is 30 cm, S is 20 m^2. Due to the different wetting way in three irrigation methods, the wetting layer in FI treatment was received fully moistened, while the DI and SI treatments were partly moistened, and drainage holes in SI treatment were stronger around the root system of the underground crop. Hence, the wetting ratios (R) of FI, DI, and SI were set at 1.00, 0.50 and 0.33, respectively. The single irrigation amount of three irrigation methods based on above was calculated and shown in Table 1. In order to prevent the water from penetrating each other during the experiment process, all plots were separated by plastic film with a depth of 60 cm. The drip irrigation zone was placed on the surface, and the drainage holes were placed near the tomato plant (about 10 cm to the plant). The subsurface irrigation pipes were covered the depth of 30 cm, and the plastic film also employed to prevent the water from penetrating. Sawdust, as a filter layer of 1 cm thick on subsurface irrigation pipes, was used to prevent the wetting soil from plugging the drainage holes.

The type and amount of fertilizer applications among three treatments were consistent. Rotten cow dung (N = 1.84% and C/N = 13) and puffed chicken manure (N = 3.13% and C/N = 6) were applied before planting, and the application rates were 22.5 t ha^{-1} and 37.5 t ha^{-1}, respectively. At the time of colonization, urea (N = 46%), phosham (N = 17% and P_2O_5 = 45%), and potassium sulfate (K_2O = 51%) were applied when tomato planted, and the application rates were 0.15 t ha^{-1}, 0.6 t ha^{-1}, 0.6 t ha^{-1}, respectively. On the 9th and July 7th, the urea was applied twice with the corresponding irrigation method, and the application amount was 0.15 t ha^{-1}. Field management was similar to the local.

2.2. N_2O sample collection and measurement

The chamber was the 50 × 50 × 60 cm^3 rectangular box, a small fan was equipped with the interior of the box for mixing air to ensure uniform gas. The upper part of the chamber body was installed for the determination of the temperature thermometer. When the tomato plants grew to more than 60 cm, an additional box was added to ensure

Table 1
Text design of different irrigation methods.

Treatment	Irrigation control low limit		Wet proportion	Amount of each irrigation ($m^3\ ha^{-1}$)
	Soil water suction (kPa)	Volumetric water content ($cm^3\ cm^{-3}$)		
FI	30	0.2953	1.00	168.37
DI	30	0.2953	0.50	84.19
SI	30	0.2953	0.33	55.56

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