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ORIGINAL PAPER

Responses of soil nitrous oxide production and abundances and composition of associated microbial communities to nitrogen and water amendment

Qing Wang[1,2] · Yu-Rong Liu[1,2] · Cui-Jing Zhang[1,2] · Li-Mei Zhang[1,2] · Li-Li Han[1,2] · Ju-Pei Shen[1,2] · Ji-Zheng He[1,2,3]

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Abstract Soil moisture and nitrogen (N) are two important factors influencing N_2O emissions and the growth of microorganisms. Here, we carried out a microcosm experiment to evaluate effects of soil moisture level and N fertilizer type on N_2O emissions and abundances and composition of associated microbial communities in the two typical arable soils. The abundances and community composition of functional microbes involved in nitrification and denitrification were determined via quantitative PCR (qPCR) and terminal restriction length fragment polymorphism (T-RFLP), respectively. Results showed that N_2O production was higher at 90% water-filled pore (WFPS) than at 50% WFPS. The N_2O emissions in the two soils amended with ammonium were higher than those amended with nitrate, especially at relatively high moisture level. In both soils, increased soil moisture stimulated the growth of ammonia-oxidizing bacteria (AOB) and nitrite reducer (*nirK*). Ammonium fertilizer treatment increased the population size of AOB and *nirK* genes in the alluvial soil, while reduced the abundances of ammonia-oxidizing archaea (AOA) and denitrifiers (*nirK* and *nosZ*) in the red soil. Nitrate addition had a negative effect on AOA abundance in the red soil. Total N_2O emissions were positively correlated to AOB abundance, but not to other functional genes in the two soils. Changed soil moisture significantly affected AOA rather than AOB community composition in both soils. The way and extent of N fertilizers impacted on nitrifier and denitrifier community composition varied with N form and soil type. These results indicate that N_2O emissions and the succession of nitrifying and denitrifying communities are selectively affected by soil moisture and N fertilizer form in the two contrasting types of soil.

Keywords Nitrification · Denitrification · Nitrous oxide · Arable soils · Soil moisture · Nitrogen amendment · Nitrifier · Denitrifier

Introduction

Nitrous oxide (N_2O) is one of the most important greenhouse gases that contribute to global warming and ozone destruction in the stratosphere (Ravishankara et al. 2009). Globally, soils are major sources of N_2O emissions, and 60% (3.5 Tg N $year^{-1}$) of N_2O emissions are derived from arable soils (Goldberg and Gebauer 2009; IPCC 2013). There is little doubt that increasing atmospheric concentration of N_2O is primarily caused by the excessive use of nitrogen (N) fertilizer (Davidson 2009; Shcherbak et al. 2014; Zhu et al. 2015). However, N_2O production from N fertilizer depends mainly on soil properties but also by soil moisture and the type of N fertilizer (Zhu et al. 2013b; Cheng et al. 2014; Wang et al. 2016a; Zhang et al. 2016).

N_2O emissions in soils mainly occur through microbial-mediated nitrification and denitrification (Wrage et al. 2001; Liu et al. 2016). Nitrification-associated pathways are performed by ammonia oxidizers through oxidizing ammonium

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✉ Yu-Rong Liu
yrliu@rcees.ac.cn

[1] State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

[2] University of Chinese Academy of Sciences, Beijing 100049, China

[3] Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria 3010, Australia

(NH_4^+) to nitrite (NO_2^-) and emit N_2O under aerobic conditions, but this can happen via the reduction of NO_2^- by ammonia-oxidizing bacteria (AOB) (Shaw et al. 2006; Kim et al. 2010; Hu et al. 2015a). Under anaerobic conditions, N_2O is the intermediate product of denitrification where denitrifiers reduce nitrate (NO_3^-) to dinitrogen (N_2) through NO_2^-, nitric oxide (NO), and N_2O (Baggs 2011). Soil moisture, usually measured by the water-filled pore space (WFPS), regulates oxygen (O_2) concentration and controls the aerobic and anaerobic conditions in the soil, which can affect the relative contribution of nitrification and denitrification to N_2O emissions (Stevens et al. 1997; Bateman and Baggs 2005; Kool et al. 2011; Cheng et al. 2014; Liu et al. 2017). Nitrification is believed to be the primary pathway of N_2O production in well-aerated soils with 30% < WFPS < 60%, while denitrification is the major source in wet soils with WFPS of >60% (Bateman and Baggs 2005; Kool et al. 2011). Besides, soil moisture is a key factor influencing diffusion and transport of nutrients, such as dissolved organic C and N availability, which in turn affects microbial community composition and activities in soils (Gleeson et al. 2010; Hu et al. 2015b; Banerjee et al. 2016). Although efforts have been devoted to revealing the impact of soil moisture on N_2O emission pathways, it is still unclear how soil moisture affects the growth of nitrifiers and denitrifiers and their roles in N_2O emissions from the contrasting arable soils.

N fertilizer application (ammonium and nitrate fertilizers) has been recognized as another important factor influencing N_2O emissions from arable soils (Zhu et al. 2013a; Rosa et al. 2016; Zhang et al. 2016; Krauss et al. 2017) because it provides substrates for driving soil nitrification and denitrification processes. Application of ammonium fertilizer can produce N_2O by nitrification, be converted to NO_3^-, while NO_3^- in soils can produce N_2O by denitrification (Del Prado et al. 2006). However, the effects of different forms of N addition on nitrifier and denitrifier communities and subsequent N_2O emissions in response to altered soil moisture conditions have not been well explored.

Beyond the significant impact of soil moisture and N fertilizer type, soil type determines soil physicochemical characteristics (e.g., soil texture and pH), which is important in shaping microbial community composition and regulating N_2O production (Wang et al. 2015). Soil texture regulates soil N_2O emissions through influencing O_2 availability (Corre et al. 1999), because soil texture can affect the size and distribution of soil pores and therefore influence soil aeration and water content (Singurindy et al. 2006; Chen et al. 2013a). Soil pH has been identified as another key regulator of soil N_2O emissions (Butterbach-Bahl et al. 2013), and product ratios of $N_2O/(N_2 + N_2O)$ have a significantly negative relationship with soil pH within the normal range from pH 5 to 8 in agricultural soils (Chapuis-lardy et al. 2007). Further, the activity of AMO, NIRK, or NOS is impacted by pH and O_2 availability (Giles et al. 2012; Banerjee et al. 2016). Thus, the effects of water and N fertilizer amendment on N_2O emissions and community composition of functional guilds might depend on soil type.

The mechanisms of global change (e.g., water and N amendment) impact on N_2O emissions remain not well completely understood. Therefore, we explored the role of associated functional guilds in N_2O emissions from the two typical arable soils. Therefore, two typical arable soils with different physicochemical properties were collected from northeast and southern China, respectively. Soils were amended with different types of N fertilizer under two different soil moisture levels, and the abundances and community composition of nitrifiers and denitrifiers were measured to investigate their responses to short-term changes in soil N amendment at different soil moisture levels. We hypothesized that (1) N fertilizer type and soil water content determined N_2O emissions and predominant N cycling processes in the arable soils and (2) the responses of N_2O emissions and the abundances and composition of associated microbial community to water and N amendment depended on distinct soil characteristics.

Materials and methods

Soil sampling and basic properties

Soil samples were collected from two upland fields in the summer of 2014. The first site is located in Luancheng City, Hebei Province, China (40° 7′ 34″ N, 119° 11′ 27″ E), and the second one is in Qiyang County, Hunan Province, China (26° 24′ 26″ N, 112° 00′ 45″ E). The soil in Luancheng is classified as Ustochrept (alluvial soil) according to the USDA Soil Taxonomy (USDA 1994), and the soil in Qiyang belongs to Paleudults (red soil). Soil samples were taken from the upper 15 cm depth, and sieved (<2 mm) and stored. Soil pH was determined in a 1:2.5 dilution with deionized water using a pH meter (Mettler-Toledo Instruments Co., Shanghai, China). Total N was determined using an Element Analyzer (Elementar, Germany). Soil organic C (SOC) was determined by wet digestion using H_2SO_4 and $K_2Cr_2O_7$. Exchangeable NH_4^+-N and NO_3^--N concentration was determined using a continuous flow analyzer (SAN++, Skalar, Breda, Holland) after extraction with 0.01 M $CaCl_2$. Particle size analysis was measured using the sieve and hydrometer procedures. The main soil properties were for alluvial soil: pH, 7.8; SOC, 9.5 g kg^{-1}; TN, 0.89 g kg^{-1}; exchangeable NH_4^+-N, 2.4 mg kg^{-1}; NO_3^--N, 117.8 mg kg^{-1}; and particle size, 58.0% sand, 28.1% silt, and 13.9% clay and for the red soil: pH, 6.2; SOC, 22.4 g kg^{-1}; TN, 1.51 g kg^{-1}; exchangeable NH_4^+-N, 3.0 mg kg^{-1}; NO_3^--N, 27.8 mg kg^{-1}; and particle size, 4.3% sand, 53.2% silt, and 42.5% clay.

Soil microcosm incubation

Soil microcosm experiment was conducted in 250-ml glass bottles containing 30 g of fresh soil. Soils for the experiment were pre-conditioned at 25 °C in the dark for 1 week. Two different types of N fertilizer were applied, $(NH_4)_2SO_4$ (NH_4^+) and KNO_3 (NO_3^-), and then, no fertilizer addition was set up as a control. N fertilizer treatments received a dose of 200 mg N kg^{-1} dry soil. In order to assure uniform distribution, N fertilizer was added as N solution. Soil moisture was adjusted to either 50 or 90% WFPS with sterile deionized water. The control received deionized water to reproduce the moisture contents of the treatment samples. Each bottle was covered with Parafilm which was poked with three or four small holes to facilitate gas exchange. Three replicates were set up for each treatment. All samples were incubated for 20 days in the dark at 25 °C.

N_2O emission measurement and soil sampling

Gas samples were collected at different time intervals (1, 2, 4, 7, 10, and 20 days) of 20-day incubation. Before gas collection, bottles were closed with a rubber stopper for 12 h. Gas samples were taken from the headspace by a 20-ml syringe. N_2O concentration was determined using a Shimadzu GC14B gas chromatograph (Shimadzu GC 14B, Tokyo, Japan). The total N_2O emissions were evaluated using the following equation according to Ma et al. (2009):

$$TN_2O = \sum_{i=1}^{n} (Ri \times Di)$$

where Ri is the N_2O emission rate in the ith sampling interval, Di is the number of days, and n is the number of sampling intervals.

Soil moisture was checked every 3–4 days and water was supplemented if necessary. Soil samples were collected for the measurement of soil pH and inorganic N (exchangeable NH_4^+-N and NO_3^--N) concentration at the end of incubation. The remaining soil samples were stored at −20 °C for molecular analysis.

DNA extraction and real-time PCR (qPCR) analysis

Total genomic DNA was extracted from 0.5 g fresh soil using MoBio Powersoil™ DNA Isolation Kit (Mobio Laboratories, USA). DNA extraction yields were in the range of 20.5–40.0 ng/μl and an A260/280 ratio of 1.75–1.90.

The abundance of ammonia oxidizers (AOA and AOB *amoA*) and denitrifiers (*nirK* and *nosZ*) was estimated on iQ5 Real-Time PCR Detection System (Bio-Rad, USA). The PCR primers and thermal cycling conditions of all functional genes were listed in Table S1. The 20 μl PCR mixture included 10 μl of 2× SYBR Premix Ex Taq™, 0.5 μM of each primer, and 1–10 ng of template DNA. Melting curve analysis (65–95 °C) was conducted to assess the qPCR product specificity at the end of each amplification process. The amplification efficiencies of all qPCR reactions were 90–100% and R^2 was between 0.993 and 0.999.

Community profiling of the nitrifiers and denitrifiers by T-RFLP assay

The community structure of AOA *amoA*, AOB *amoA*, *nirK*, and *nosZ* was determined by T-RFLP analysis using the fluorescently labeled (6-FAM) forward primers. PCR procedure for each gene was the same as described above. PCR products were purified (QiAGEN Gel Extraction Kit, Hilden, Germany) firstly and then were digested with the restriction enzyme listed in Table S1. Terminal restriction fragments (TRFs) were determined with an ABI 3730XL DNA Analyzer and analyzed using Peak Scanner software v1.0 (Applied Biosystems).

Statistical analyses

The *amoA* gene copy numbers were log-transformed to meet normality assumptions. Two-way ANOVA was conducted to evaluate effects of soil moisture, N amendment and their interactions on pH; inorganic N concentration; total N_2O emissions; and *amoA*, *nirK*, and *nosZ* gene abundance in SPSS 17.0 (IBM, USA). P value <0.05 was believed to be statistically significant. Best of fit modeling of regression between total N_2O emissions and the abundance of AOB *amoA* were performed in Sigmaplot (Version 10) using exponential growth equation. Principal coordinate analysis (PCoA) was used to visualize the Bray-Curtis dissimilarity matrices based on the T-RFLP data from soils collected on day 20 of the incubation. Per-mutational multivariate ANOVA (PERMANOVA) was conducted to test the effects of factors and their interactions on the abundances and composition of associated microbial communities, by using the *adonis* function of the *vegan* package in R 3.3 software.

Results

The effects of water and N amendment on soil properties and total N_2O emissions

We observed different impacts of water and N amendment on soil properties in the two soils (Table 1, Fig. 1). In the alluvial soil, pH remained unchanged around 7.8 after the incubation across all the treatments (Fig. 1a), whereas it was significantly influenced by the addition of water, nitrogen, and their interactions in the red soil (Table 1). In the red soil, pH at 90% WFPS was higher than that at 50% WFPS under ammonium treatment ($P < 0.001$). Ammonium amendment significantly

Table 1 Summary for the two-way ANOVA on the soil pH, exchangeable NH_4^+-N and NO_3^--N, and total N_2O emissions for the two factors (soil moisture and N amendment) and their interactions

	pH	Exchangeable NH_4^+-N	NO_3^--N	Total N_2O emissions
Alluvial soil				
Water	0.951	0.498	0.741	*<0.001*
Nitrogen	0.413	0.18	*<0.001*	*<0.001*
Water × nitrogen	0.376	0.523	0.563	*<0.001*
Red soil				
Water	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Nitrogen	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Water × nitrogen	*<0.001*	*<0.001*	*0.028*	*<0.001*

P values ($P < 0.05$) are indicated in italics

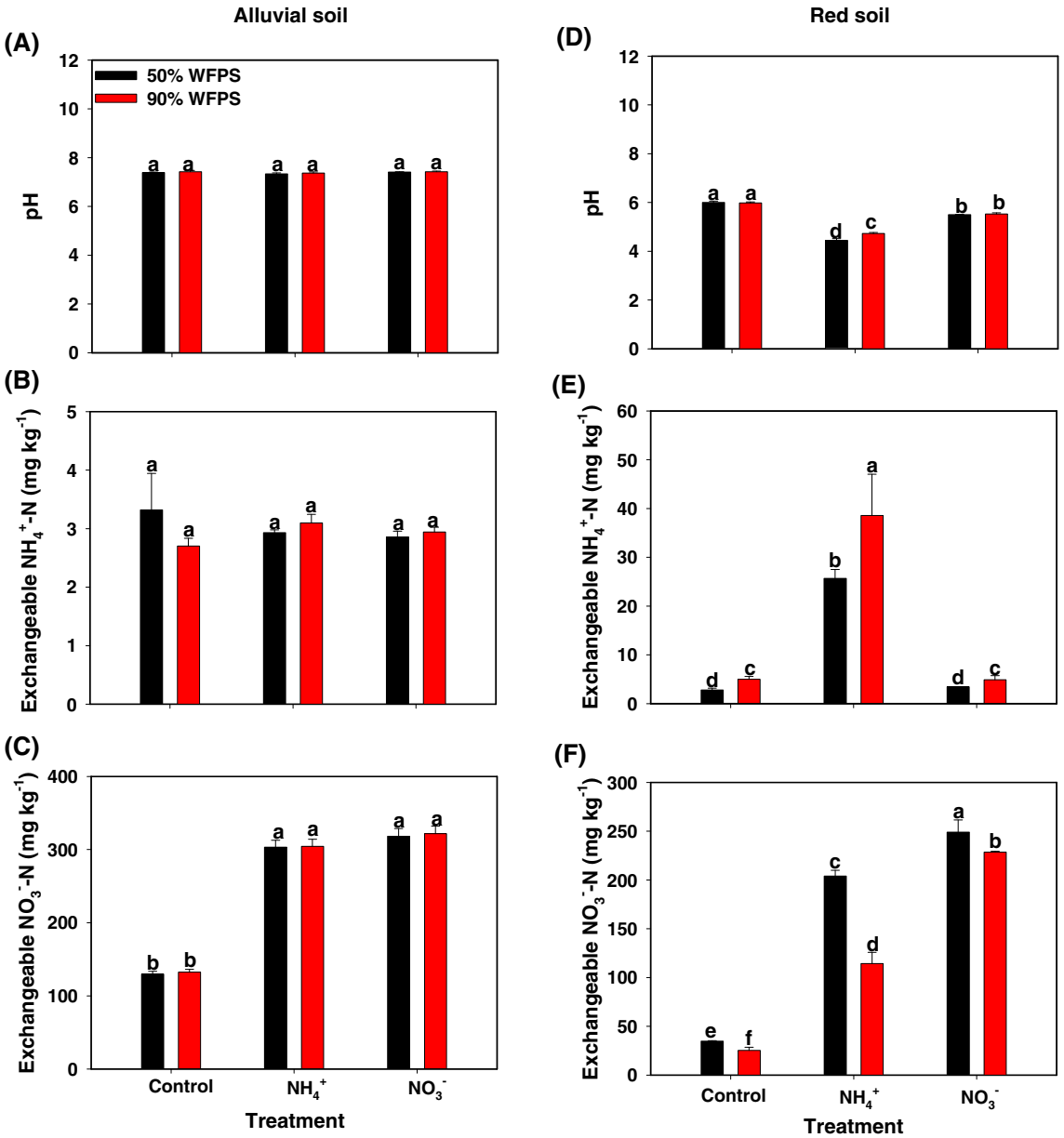

Fig. 1 Effects of soil moisture and N amendment on soil pH and exchangeable NH_4^+-N and NO_3^--N content in the alluvial soil (**a**–**c**) and the red soil (**d**–**f**). *Error bars* are standard errors ($n = 3$). Statistically significant differences among treatments are represented by *different lowercase letters* ($P < 0.05$)

reduced pH ($P < 0.001$), which was much lower than that in the NO_3^--treated soils ($P < 0.001$) (Fig. 1d).

Soil moisture showed different effects on exchangeable NH_4^+-N and NO_3^--N concentration in the two soils (Table 1, Fig. 1). In the alluvial soil, soil moisture change did not affect exchangeable NH_4^+-N and NO_3^--N concentration (Fig. 1b, c). However, NO_3^--N concentration was markedly influenced by N amendment ($P < 0.001$) (Table 1, Fig. 1c). In the red soil, high soil moisture (90% WFPS) led to higher exchangeable NH_4^+-N concentration compared to low moisture level (50% WFPS) (Fig. 1e), while NO_3^--N concentration was lower at 90% WFPS than at 50% WFPS (Fig. 1f).

Total N_2O emissions were significantly affected by soil moisture, N fertilization, and their interactions (Table 1). In Fig. 2, we found that N_2O emissions were higher at 90% WFPS than that at 50% WFPS in the two soils. There were also significant differences in total N_2O production in the two soils under N treatments though soil moisture was kept for the same, with higher values in NH_4^+-treated soils than in NO_3^--treated soils.

The effects of water and N amendment on abundance of nitrifiers and denitrifiers

For nitrifiers in the two soils, AOA *amoA* abundance was not affected by the changed soil water content (Table 2, Fig. 3a). Soil moisture significantly affected AOB *amoA* abundance with 90% WFPS soil containing 1.5–2 times as many AOB *amoA* copies as 50% WFPS in both soils (Fig. 3b, f). AOA *amoA* abundance was not strongly altered by N addition in the alluvial soil, whereas N amendment reduced it in both NH_4^+ and NO_3^- treatments in red soil (Fig. 3e). The population size of AOB was higher in the NH_4^+ treatment than in other treatments in the two soils (Fig. 3b, f).

For denitrifiers, enhanced soil moisture markedly increased *nirK* abundance but not *nosZ* abundance in the alluvial soil (Fig. 3c, d). Ammonium addition significantly increased the *nirK* abundance while NO_3^- amendment did not influence it compared to the control (Fig. 3c). *NosZ* abundance did not respond to NH_4^+ or NO_3^- fertilizers in this soil (Fig. 3d). In the red soil, *nirK* abundance greatly increased with the soil moisture increased from 50 to 90% WFPS (Fig. 3g). The application of NH_4^+ significantly reduced *nirK* and *nosZ* abundance, which was unchanged in the NO_3^--related treatments (Fig. 3g, h).

The regression analysis revealed that total N_2O emissions were significantly correlated with AOB *amoA* abundance in the alluvial soil ($R^2 = 0.98$, $P < 0.001$) (Fig. 4a) and red soil ($R^2 = 0.65$, $P < 0.001$) (Fig. 4b). However, other functional genes were not correlated with total N_2O emissions in the two soils.

The effects of water and N amendment on nitrifier and denitrifier community composition

Soil moisture affected AOA *amoA* community composition in the alluvial soil, whereas there was no effect of N treatment on AOA *amoA* community composition (Table 3, Fig. 5a). In the red soil, PERMANOVA analysis revealed that AOA *amoA* community composition was influenced by soil moisture, N amendment, and their interactions (Table 3), which was further supported by PCoA (Fig. 5e). AOB *amoA* community composition was significantly affected by NH_4^+ addition and a pair-wise comparison revealed a significant difference between control and NH_4^+ treatment in both soils (Table 3; Fig. 5b, f). A pair-wise PERMANOVA test, however, delivered no significant difference between control and NO_3^--treated AOB *amoA* community composition in both soils (Table 3).

Soil moisture and N amendment did not influence denitrifier (*nirK* and *nosZ*) community composition in the alluvial soil (Table 3; Fig. 5c, d). In contrary, soil moisture and N

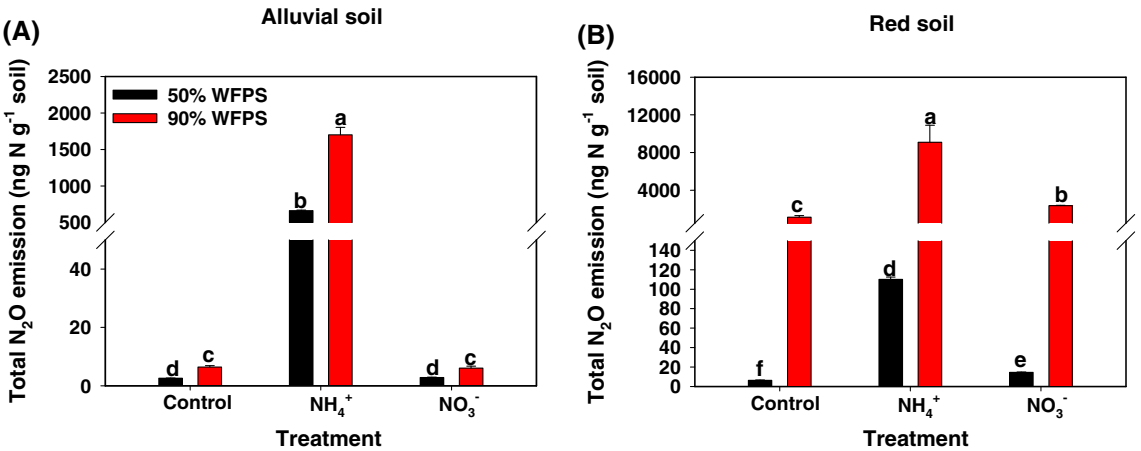

Fig. 2 Effects of soil moisture and N amendment on total N_2O emissions for the entire experiment (20 days) in the alluvial soil (**a**) and the red soil (**b**). *Error bars* are standard errors ($n = 3$). Statistically significant differences among treatments are represented by *different lowercase letters* ($P < 0.05$)

Table 2 Summary for the two-way ANOVA on AOA *amoA*, AOB *amoA*, *nirK*, and *nosZ* gene abundance for the two factors (soil moisture and N amendment) and their interactions

	Abundance of AOA *amoA*	Abundance of AOB *amoA*	Abundance of *nirK*	Abundance of *nosZ*
Alluvial soil				
Water	0.751	*<0.001*	*<0.001*	0.529
Nitrogen	0.793	*<0.001*	*0.007*	0.918
Water × nitrogen	0.554	0.059	*0.016*	0.698
Red soil				
Water	0.354	*<0.001*	*<0.001*	0.076
Nitrogen	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Water × nitrogen	0.692	0.751	*0.002*	0.822

P values ($P < 0.05$) are indicated in italics

addition and their interactions significantly affected *nirK* and *nosZ* community composition in the red soil and a pair-wise comparison showed obvious difference among the treatments (Table 3; Fig. 5g, h).

Discussion

Our results showed that soil moisture was a key factor for N_2O emissions from N-treated soils, and higher N_2O emissions were observed at high soil moisture than at low soil moisture in both soils. Normally, the soil becomes more anaerobic as soil water content increased, leading to high N_2O emissions from denitrification. Obviously, the higher N_2O production at 90% WFPS indicated that both soils did not become completely anaerobic at high soil water content because complete denitrification can lead to the conversion of N_2O to N_2, decreasing N_2O production (Smith et al. 1998; Zhu et al. 2013a). We observed higher N_2O emissions in soils treated with ammonium than in soils treated with nitrate, especially under high soil water content level (90% WFPS). These results indicate that N_2O emission process is regulated by soil moisture and N fertilizer type (Zhu et al. 2013b; Minick et al. 2016). In addition, we also observed AOB abundance was significantly correlated to total N_2O emissions (Fig. 4). These findings together emphasized that ammonia oxidation (nitrifier nitrification and nitrifier denitrification) was the predominant pathway for N_2O emissions in this study. In accordance, Zhu et al. (2013a, b) demonstrated that nitrifier denitrification is a significant source of N_2O at high soil moisture or low O_2 availability in soils. The nitrifier denitrification is conducted by AOB, which used NO_2^- rather than O_2 as a terminal electron acceptor, reducing NO_2^- to N_2O (Wrage et al. 2001). Nitrification and denitrification have optima under different environmental conditions, and they can appear simultaneously in different microsites (Butterbach-Bahl et al. 2013). The sandy loam in the alluvial soil, being well aerated, poor in SOC content, and with relatively high pH, is likely to provide more favorable conditions for nitrification (Wan et al. 2009; Huang et al. 2014a). However, the silty clay loam in the red soil might become more anoxic at higher soil water content than sandy loam soil in the alluvial soil (Szukics et al. 2010). Therefore, higher N_2O emissions in high soil moisture that depended on ammonium addition in the red soil might derive from nitrification and denitrification because ammonia oxidation could produce large amount of nitrate, which could be used as substrate for denitrification.

The abundances of nitrifiers in the alluvial soil and red soil responded distinctively to soil moisture and N addition, suggesting N transformation processes varied with different soil types. In both soils, increased soil moisture obviously stimulated the growth of AOB rather than AOA, indicating that soil moisture was an important factor influencing AOB abundance. Similarly, Chen et al. (2013b) showed that precipitation significantly increased the abundance of AOB, while AOA abundance kept stable in the Inner Mongolia grassland. Di et al. (2014) also observed that the population size of AOB increased with increasing soil water content (from 60 to 130% WHC) in a grassland soil. We speculated that increased soil moisture could promote diffusion and transport of nutrients in soils, providing microorganisms with key substrates such as NH_3, NO_3^-, and soluble organic C (Blagodatsky and Smith 2012). In the N treatments, ammonium amendment significantly enhanced AOB abundance in the alluvial soil and red soil. However, ammonium addition did not have obvious effect on the abundance of AOA in the alluvial soil, but reduced it in the red soil. The AOA abundance might have been reduced by low pH caused by the application of ammonium fertilizer in the red soil, since it was reported previously that AOA abundance was positively correlated with soil pH (3.7–8.7) at 47 sites across the UK (Gubry-Rangin et al. 2011). Although the negative effect of N fertilization on the AOA abundance has been frequently reported before (Di et al. 2014; Wang et al. 2016b), the effect of N form on it is still equivocal and has rarely been attributed to nitrate application. In the study, the strongly negative effect of nitrate addition on AOA abundance in the red soil indicated that nitrate was also the effective N form that inhibited the growth of AOA in this

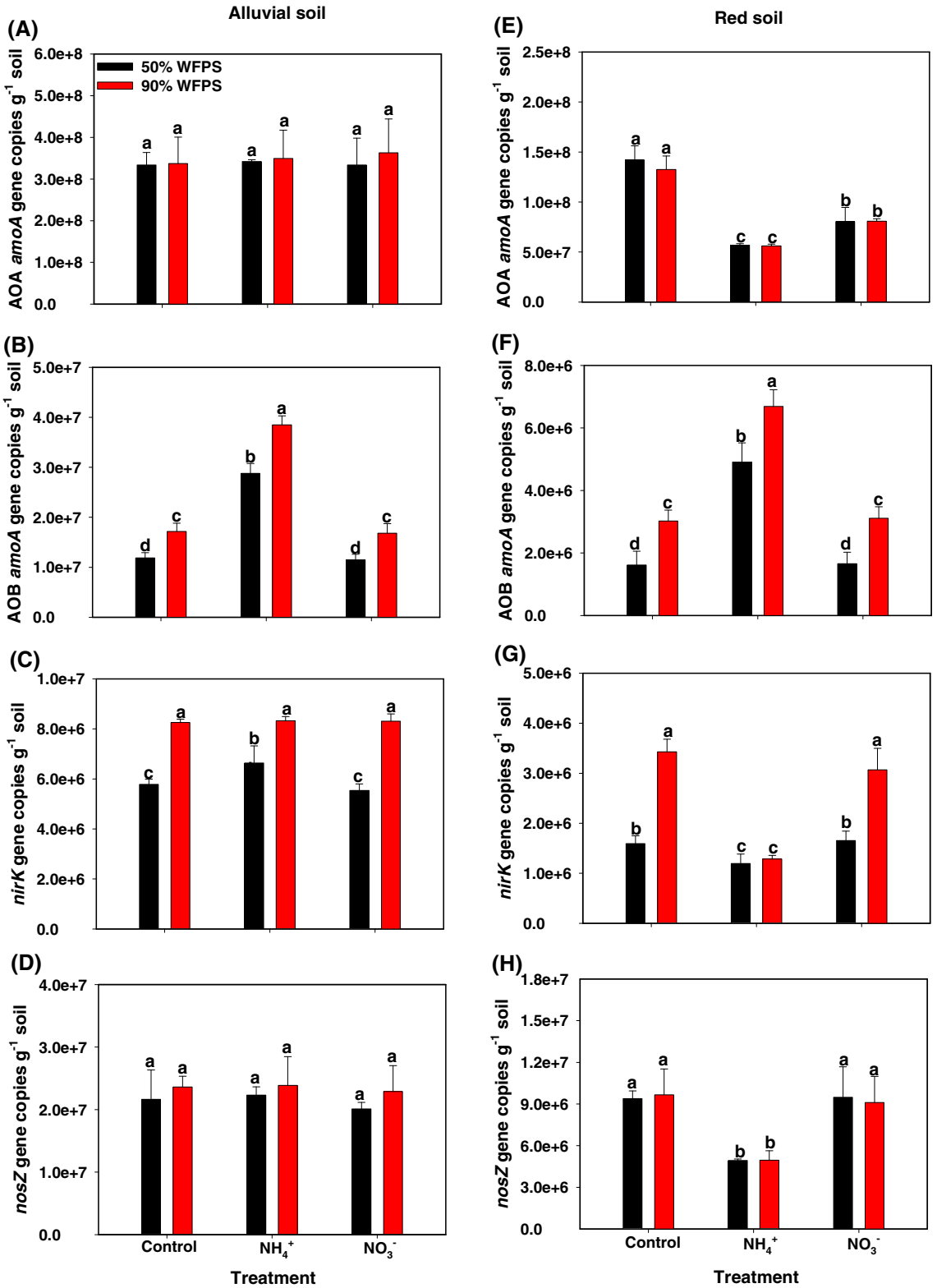

Fig. 3 Effects of soil moisture and N amendment on the abundance of AOA *amoA*, AOB *amoA*, *nirK*, and *nosZ* genes in the alluvial soil (**a**–**d**) and the red soil (**e**–**h**). *Error bars* are standard errors ($n = 3$). Statistically significant differences among treatments are represented by *different lowercase letters* ($P < 0.05$)

soil. Similar result was reported recently in Inner Mongolia grassland soil (Ying et al. 2017). Further study needs to be carried out to unravel inhibitive mechanisms of nitrate on AOA growth.

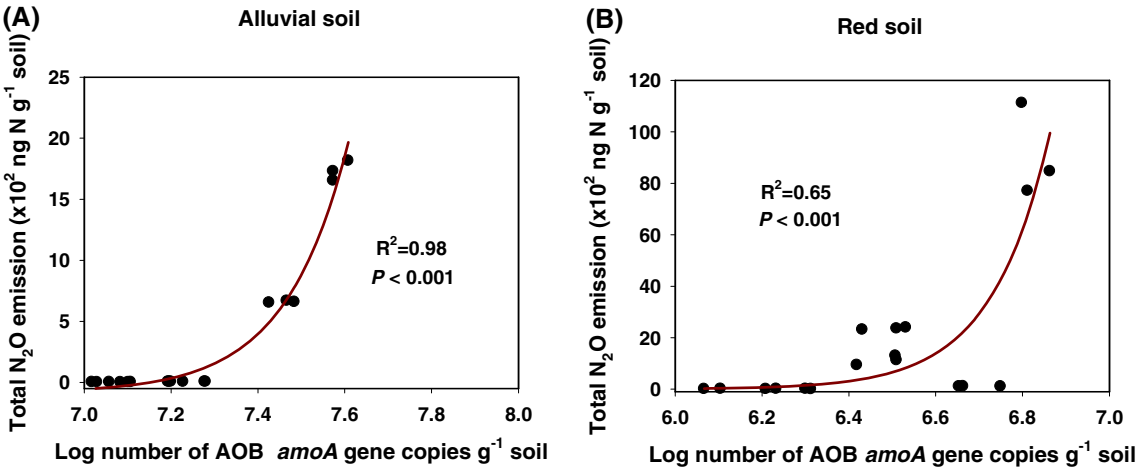

Fig. 4 Relationships of total N_2O emissions with AOB *amoA* abundance in the alluvial soil (**a**) and the red soil (**b**)

In the present study, soil moisture significantly changed AOA community composition in the alluvial soil and red soil, while no similar effect was observed on AOB community composition, showing that soil moisture was a crucial determinant of AOA community composition. The significant response in AOA community composition to soil moisture was similar as other studies (Gleeson et al. 2010; Szukics et al. 2012). Both groups of AOA and AOB have distinct physiological characteristics and ecological niches in response to soil water availability. There appeared to be two dissimilar populations of AOA in the soils, one grew under oxic conditions and the other grew under sub-oxic conditions. In the N treatment, the AOB community composition was significantly altered by ammonium addition in both soils. However, our result was inconsistent with previous study of Avrahami et al. (2002) who reported that agricultural soils did not show any AOB community shifts under ammonium treatment with 4–6 weeks of incubation. This inconsistency might be attributed to higher ammonium level in our study, which could promote the growth of AOB in soils (Jia and Conrad 2009; Cui et al. 2013). AOA community composition remained unchanged after N amendment in the alluvial soil, while was altered by it in the red soil. AOB is more sensitive than AOA in response to N fertilization in alkaline soil while AOA is more sensitive to N fertilization than AOB in acidic red soil. This is in agreement with Chen et al. (2010) suggesting that soil type is most likely another key factor in affecting ammonia oxidizer community composition under the N treatment.

Water and N amendment have a significant impact on the abundances of denitrifiers (*nirK* and *nosZ*) in the tested soils. Increasing soil water content reduces gaseous diffusion rates, limiting oxygen which is favorable for the growth of denitrifiers (Blagodatsky and Smith 2012), as the *nirK* abundance increased with enhanced soil moisture. However, the *nosZ* abundance remained stable between the soil water treatments which implied that the soil condition was less anaerobic because N_2O reductase (NOS) was the most sensitive to O_2 inhibition (Knowles 1982; Morley et al. 2008). The increased *nirK* abundance in the alluvial soil after the addition of ammonium was in line with earlier reports suggesting that application of urine increased the population size of *nirK* (Hamonts

Table 3 Results of PERMANOVA for testing the effects of soil moisture, N amendment, and their interactions on ammonia oxidizer and denitrifier community composition in the alluvial soil and red soil

	AOA *amoA*	AOB *amoA*	*nirK*	*nosZ*
Alluvial soil				
Water	*<0.001*	0.144	0.103	0.254
Nitrogen	0.477	*<0.001*	0.544	0.114
Water × nitrogen	0.243	0.404	0.058	0.329
50% WFPS				
Control × NH_4^+	0.451	*<0.001*	0.403	0.531
Control × NO_3^-	0.226	0.129	0.676	0.244
NH_4^+ × NO_3^-	0.821	*<0.001*	0.658	0.561
90% WFPS				
Control × NH_4^+	0.601	*0.004*	0.062	0.852
Control × NO_3^-	0.221	0.297	0.182	0.632
NH_4^+ × NO_3^-	0.168	*0.005*	0.83	0.302
Red soil				
Water	*<0.001*	0.124	*<0.001*	*<0.001*
Nitrogen	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Water × nitrogen	*<0.001*	0.113	*<0.001*	*<0.001*
50%WFPS				
Control × NH_4^+	*<0.001*	*<0.001*	*0.002*	*0.003*
Control × NO_3^-	*<0.001*	0.335	*<0.001*	*0.004*
NH_4^+ × NO_3^-	*0.003*	*<0.001*	*0.004*	*0.004*
90%WFPS				
Control × NH_4^+	*<0.001*	*<0.001*	*0.005*	*0.005*
Control × NO_3^-	*0.009*	0.405	*0.012*	*0.003*
NH_4^+ × NO_3^-	*<0.001*	*<0.001*	*0.004*	*0.007*

P values ($P < 0.05$) are indicated in italics

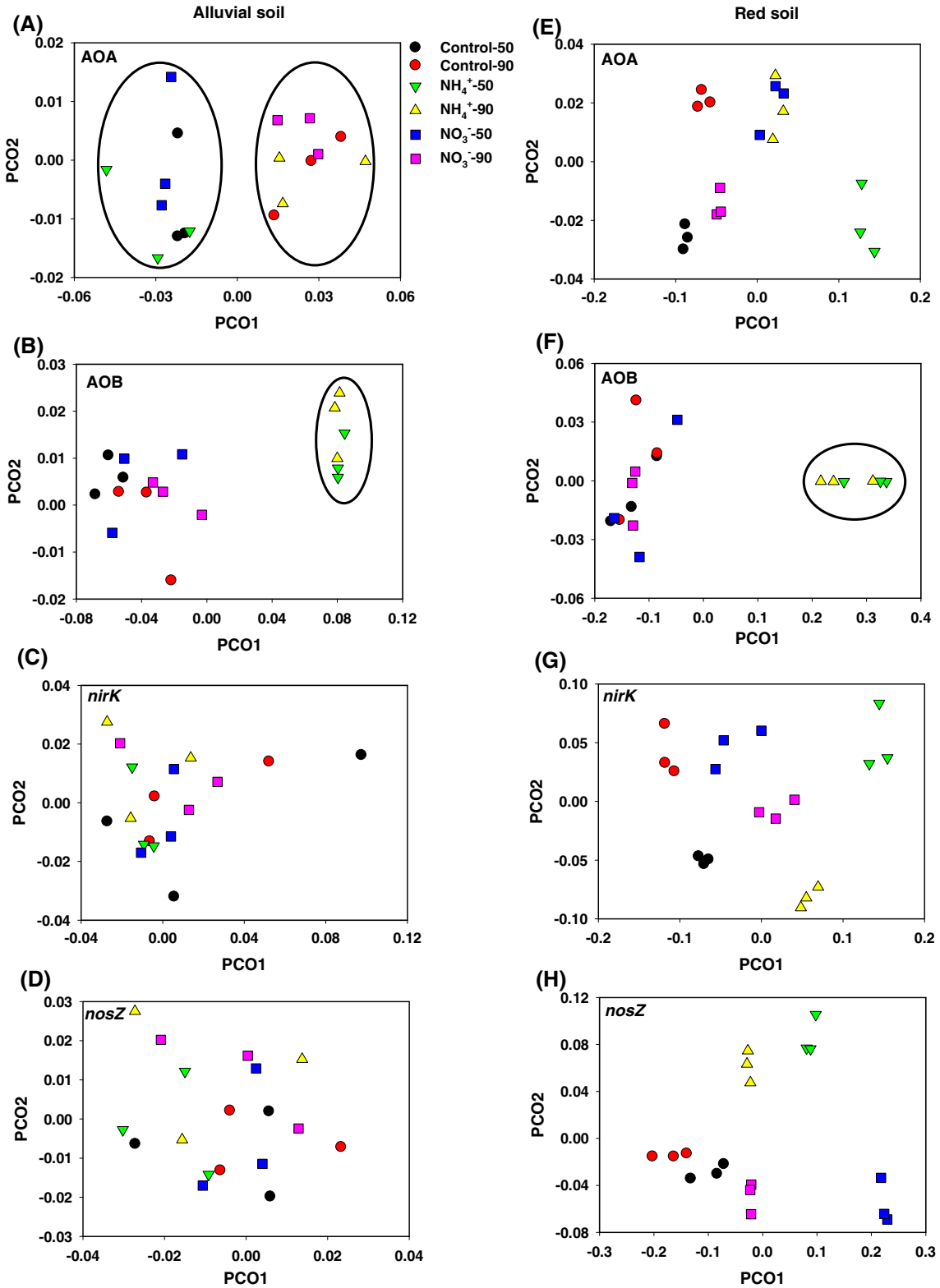

Fig. 5 Principal coordinate analysis (PCoA) of AOA *amoA*, AOB *amoA*, *nirK*, and *nosZ* gene community profiles in response to NH_4^+ and NO_3^- and without N addition in the alluvial soil (**a**–**d**) and the red soil (**e**–**h**)

et al. 2013; Di et al. 2014). Overall, the changes of the *nirK* abundance were similar to that of AOB in the alluvial soil (Fig. 3b, c). Genome analysis suggested that most of AOB communities contained the *nirK* gene which was related to the pathway known as nitrifier denitrification (Shaw et al. 2006; Kim et al. 2010). However, in the red soil, the

abundances of *nirK* and *nosZ* genes decreased under the ammonium treatment, implying the response of denitrifying genes to ammonium addition varied depending on soil properties. Soil pH was obviously reduced in the ammonium-treated soils in comparison with the control after incubation which could significantly inhibit the growth of denitrifiers (Čuhel et al. 2010; Bakken et al. 2012; Huang et al. 2014b). A notable discovery was that the community composition of *nirK* and *nosZ* responded differently to the water and N amendment in the two soils. Denitrifier community composition was less affected in response to water and N treatments in the alluvial soil, whereas clearly changed in the red soil. The distinct responses of denitrifier community composition to water and N amendment in the alluvial soil and red soil could be ascribed to soil type.

Conclusion

In conclusion, our study demonstrates dissimilar responses of N_2O emissions and associated microbes to N addition at altered soil moisture levels in the two soils. Total N_2O emissions induced by increased soil water content and N addition in the red soil were much higher than that in the alluvial soil. Increased soil moisture increased AOB and *nirK* abundance in the two soils. Soil NH_4^+ addition increased AOB and *nirK* abundance in the alluvial soil, but decreased *nirK* and *nosZ* abundance in the red soil. Different soil types could result in differences in soil SOC, soil texture, and pH, which may influence N_2O emissions and the growth of associated functional guilds in soils. Our results suggest that caution should be taken when applying N fertilization into high-moisture soils for mitigating N_2O emissions in the agricultural management.

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