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ORIGINAL PAPER

Nitrogen fertiliser-induced changes in N_2O emissions are attributed more to ammonia-oxidising bacteria rather than archaea as revealed using 1-octyne and acetylene inhibitors in two arable soils

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Abstract Nitrification is believed to be one of the major sources of N_2O production emitted from soil. Previous studies showed that both ammonia-oxidising bacteria (AOB) and archaea (AOA) can produce N_2O via nitrification but their relative contributions are still poorly defined. Here, we used acetylene, an inhibitor of AOB and AOA ammonia monooxygenase (AMO), and 1-octyne, a selective inhibitor that specifically inhibits AOB AMO, to investigate how AOB versus AOA contribute to N_2O emissions in two distinct arable soils. Soil amended with ammonium (NH_4^+) increased N_2O emissions to a greater extent than nitrate (NO_3^-), and acetylene had a greater impact on N_2O emissions in NH_4^+-treated soils than that in NO_3^--amended soils, which indicated that nitrification was the dominant N_2O emitting process in these two arable soils. In the alluvial and red soil, the percentage of evolved N_2O after application of NH_4^+ by AOB were 70.5 ~ 78.1 % and 18.7 ~ 19.7 % by AOA, respectively. Quantitative PCR revealed that NH_4^+ addition stimulated AOB growth, and the growth could be significantly inhibited by acetylene or 1-octyne in the two soils. The stimulation of N_2O emissions by NH_4^+ and the relative suppression by inhibitors paralleled fluctuations in the AOB growth. In addition, cumulative N_2O emissions were not correlated with AOA abundance in the two soils. Our results revealed that AOB could contribute more to soil N_2O production than AOA in the NH_4^+-amended arable soils.

Keywords Nitrous oxide (N_2O) emission · Nitrification inhibitors · Quantitative PCR · *amoA* gene · Arable soil

Introduction

Nitrous oxide (N_2O) is a powerful greenhouse gas that results in global warming and stratospheric ozone depletion. Increasing application of mineral N fertiliser and the expansion of arable soils contributes to over half of anthropogenic N_2O production over the past few decades (Davidson 2009; Smith et al. 2012; Shcherbak et al. 2014). Therefore, it is necessary to understand the microbial mechanism of N_2O emission from soil in order to develop future mitigation strategies and achieve sustainable agriculture practices.

Nitrification is an important N cycling process in agricultural ecosystems, which oxidises ammonia (NH_3) to nitrate (NO_3^-) via intermediate products, i.e. hydroxylamine (NH_2OH) and nitrite (NO_2^-) in a two-step process, and can consequently emit N_2O as a by-product under certain soil conditions (Wrage et al. 2001). Ammonia oxidation, the oxidation of NH_3 to NO_2^- via NH_2OH, is the rate-limiting step in nitrification and can be carried out by both ammonia-oxidising bacteria (AOB) and ammonia-oxidising archaea (AOA). Nitrification-related processes (the oxidation of NH_2OH and NO_2^- reduction) mediated by AOB are recognised to be a main pathway of N_2O emission from arable soils (G dde and Conrad 1999; Zhu et al. 2013; Huang et al. 2014a). The traditional viewpoint that soil ammonia oxidation and associated N_2O emission is exclusively carried out by

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AOB has been challenged by the discovery of *amoA* and *nirK* genes in AOA strains (Venter et al. 2004; Lund et al. 2012) and by the demonstration of the N_2O production capacity of AOA enriched or isolated from arable soils and marine ecosystem (Jung et al. 2011, 2014; Santoro et al. 2011; Loscher et al. 2012). In most soils, AOA outnumbers AOB abundance, a common feature in multiple ecosystems (Leininger et al. 2006; He et al. 2007; Hu et al. 2013), and a high AOA ammonia oxidation activity has been assessed in certain soils (Yao et al. 2011; Zhang et al. 2012; Hu et al. 2015). These results suggested that AOA could play a greater role than AOB in ammonia oxidation and N_2O production in some soils (Nicol et al. 2008; Stieglmeier et al. 2014; Hink et al. 2016). However, by characterising the conserved *amoA* gene, which encodes a subunit of the ammonia monooxygenase (AMO), it has been observed that the populations of ammonia oxidisers are largely controlled by soil type, NH_4^+ concentration, pH and water content (Nicol et al. 2008; Di et al. 2009; Chen et al. 2010; Norton and Stark 2011). In ammonium-rich soils, AOB abundance and activity increased whereas AOA abudance is unaffected or inhibited in response to a high concentration of ammonium fertilisers (Jia and Conrad 2009; Di et al. 2009; Di and Cameron 2011; Sterngren et al. 2015; Ouyang et al. 2016). In unfertilised or acidic soils, AOA abundance and metabolic activity are much higher than those of AOB (Offre et al. 2009; Gubry-Rangin et al. 2010; Zhang et al. 2012). Thus, the role of AOB and AOA in overall ammonia oxidation and N_2O emission is not very well understood across different soils.

A key functional enzyme in both bacterial and archaeal ammonia oxidisers is AMO. Acetylene (C_2H_2) is a suicide substrate for AMO (Hynes and Knowles 1978), and thus inhibits ammonia oxidation by autotrophic nitrifiers (Jia and Conrad 2009) at low concentration (10–100 Pa) and inhibits N_2O reductase during denitrification at high concentration (10 KPa) (Huang et al. 2014b; Cui et al. 2016). Inhibition of soil ammonia oxidation by C_2H_2 is also used in routine methods to distinguish N_2O production by nitrifiers and denitrifiers in soils (Bateman and Baggs 2005; Zhu et al. 2013). Recently, it has been shown that AOB is more sensitive to 1-octyne, a C8 alkine inhibitor, compared with AOA (Taylor et al. 2013, 2015). Moreover, Lu et al. (2015) and Ouyang et al. (2016) distinguished AOB and AOA contribution to nitrification by using 1-octyne in soil microcosm incubation and found that 1-octyne selectively inhibited AOB growth rather than AOA in forest and arable soils. Therefore, we could use 1-octyne to assess the relative contribution of AOB and AOA to N_2O emission from two different arable soils. We hypothesised that nitrification was the main process of N_2O emission in arable soils and AOB rather than AOA was the major contributor to soil N_2O production in ammonium-rich arable soils.

Material and methods

Soil samples

Two arable soils from Luancheng county (40°7′34″N, 119°11′27″E), Hebei Province in northern China and Qiyang county (26°24′26″N, 112°00′45″E), Hunan Province in southern China were collected from the 0 to 20 cm layer: an upland alluvial soil and an upland red soil, respectively. The alluvial soil from Luancheng is classified as an aquic inceptisol according to US soil taxonomy (USDA 1994); its main properties are as follows: total N, 0.42 g kg^{-1}; dissolved organic C (DOC) 26 mg kg^{-1}; pH 8.0 (1:2.5 H_2O); exchangeable NH_4^+-N 2.4 mg kg^{-1}; exchangeable NO_3^--N 117.8 mg kg^{-1}. The red soil from Qiyang is classified as paleudults in the USDA Soil Taxonomy; its main properties are as follows: total N, 1.51 g kg^{-1}; dissolved organic C (DOC) 33 mg kg^{-1}; pH 6.0 (1:2.5 H_2O); exchangeable NH_4^+-N and NO_3^--N concentrations were 3.0 and 27.8 mg kg^{-1}, respectively.

Microcosm incubation

To evaluate the contribution of different microbial groups to N_2O emission, soils were subjected to microcosms incubation with acetylene and 1-octyne as selective inhibitors. The soil samples were sieved (<2 mm) and stored at 4 °C. The incubation experiments were conducted in 120-ml serum bottles containing 20 g of dry weight soil. The soil samples were pre-incubated for 7 days at 28 °C to stabilize the microbial population at 40 % water filled pore space (WFPS). After pre-incubation, soil was adjusted to 55 % WFPS following amendment with sterilised water (control, no N fertiliser or inhibitors) or a solution of 150 mg N as $(NH_4)_2SO_4$ or KNO_3 kg^{-1} soil in the presence or absence of 1-octyne (Oct) (5 μM aqueous) as described by Taylor et al. (2013) or acetylene (Ace) (0.1 % *v*/*v*). In total, nine treatments were described as follows: control, Oct, Ace, NH_4^+, NH_4^+ + oct, NH_4^+ + ace, NO_3^-, NO_3^- + oct and NO_3^- + ace. Each treatment was replicated three times. As acetylene was able to completely inhibit ammonia oxidation by autotrophic nitrifiers (AOA and AOB), N_2O emisison from AOA and AOB (AOA + AOB) was calculated by substracting N_2O emisison in the NH_4^+ + ace treatment from values measured in the NH_4^+ only treatment. N_2O production by AOA was determined by substracting N_2O production in the NH_4^+ + ace treatment from that detected in the NH_4^+ + oct treatment, since 1-Octyne specifically inhibits AOB ativity but not AOA. N_2O production from AOA plus AOB (AOA + AOB) minus N_2O emission by AOA was attributed to AOB. The relative contribution of N_2O emission by AOA and AOB was therefore deduced.

N_2O and soil sampling

Gas samples (10 ml) were collected from the headspace with syringe at 1, 2, 3, 4, 7, 10, 14 and 21 days after the start of incubation. N_2O concentration was analysed by a gas chromatograph (HP7890A, Agilent Technologies, CA, USA). Cumulative N_2O emission during the incubation period was estimated according to Ma et al. (2009). Aerobic conditions were maintained by opening bottles for 20 min at intervals of 2–3 days when water content and 1-octyne or acetylene were supplemented (Offre et al. 2009; Taylor et al. 2013; Hink et al. 2016). Three bottles per treatment were destructively sampled after incubation for 0, 7, 14 and 21 days. Exchangeable NH_4^+-N and NO_3^--N were extracted with 0.01 M $CaCl_2$, under shaking for 1 h, and then, soil suspensions were filtered through Whatman no. 42 filter papers. Extracts were analysed colorimetrically by flow injection analyser (Skalar SAN++). The soil samples for molecular analysis were immediately stored at −80 °C.

DNA extraction and quantitive PCR (qPCR) analyses

Soil DNA was extracted from 0.4 g dry soil with MoBio Powersoil™ DNA isolation kit (MoBio Laboratories Inc, Carlsbad, CA) according to the manufacturer's protocol. Concentration and quality of the extracted DNA were measured using a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Soil DNA extracts were stored at −80 °C.

Quantification of *amoA* for AOB and AOA were determined using amoA1f/amoA2r (Rotthauwe et al. 1997) and crenamoA23f/crenamoA616r (Hu et al. 2015), respectively. PCR reactions were conducted on a CFX96 (Bio-rad, USA). Each 25-μl reaction mixture included 12.5 μl 2 × Supermix (Bio-rad, USA), 0.5 μl BSA, 0.5 μl of each primer (10 mM) and 2 μl 10-fold diluted DNA and 9 μl sterilised deionised water. The thermocycling conditions for AOB and AOA were as follows: denaturation at 95 °C for 3 min, 40 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 40 s, extension at 72 °C for 50 s and data collection at 83 °C for 15 s. Products' specificity was checked by agarose gel electrophoresis and melting curve analysis. Plasmids as prepared as previously (He et al. 2007) were used as standards for AOB and AOA qPCR, respectively. The efficiencies for AOB and AOA *amoA* amplification ranged from 90 to 98 %, and the coorelation coefficient (R^2) was between 0.993 and 0.999 for both genes.

Statistical analysis

The statistical analysis was performed using SPSS 17.0 software (IBM Co., Armonk, NY, USA). ANOVA was performed to assess the significance of different treatments on N_2O production, *amoA* gene abundance and exchangeable NH_4^+-N and NO_3^--N concentrations. Correlations of cumulative N_2O emissions with AOB or AOA *amoA* gene abundance were assessed by Pearson's correlation procedure.

Results

N_2O emission

In the two soils, there was no clear difference in N_2O production among control, Oct and Ace treatments after 21 days of incubation (Fig. 1). In the alluvial soil, N_2O emission significantly increased with NH_4^+ addition and reached 287.9 ng N g^{-1} soil ($P < 0.01$). When NH_4^+ was applied with 1-octyne or acetylene, cumulative N_2O production was dramatically reduced by 78.1 and 97 %, respectively. Nitrate-associated treatments did not significantly affect N_2O emission (relative to the control) ($P > 0.05$). In the red soil, N_2O emission was stimulated with the application of NH_4^+, but to a lesser extent, i.e. reaching 84.9 ng N g^{-1} soil ($P < 0.01$). N_2O emissions in the NH_4^+ + oct or NH_4^+ + ace treatments were reduced by 70.5 and 90 %, respectively. No significant difference in N_2O emissions in acetylene-treated soil samples with or without NH_4^+ addition ($P > 0.05$) were observed. Amendment with NO_3^- slightly stimulated N_2O emission compared to the control. In the NO_3^--amended soil, acetylene addition inhibited N_2O emission, but 1-octyne had no signficant inhibitory effect on it. In addition, the cumulative N_2O emission from the NO_3^- treatment was 36.4 and 4.7 times lower than that from the NH_4^+ treatment in the alluvial and red soils, respectively. These results indicated that nitrification rather than heterotrophic denitrification might be the main sources of N_2O emssion in the two soils.

Exchangeable NH_4^+-N and NO_3^--N concentration

In the two soils, exchangeable NH_4^+-N concentration was consistently low in the control, and 1-octyne alone had no significant effect on it ($P > 0.05$) (Fig. 2). In all acetylene-treated soils, nitrification was completely blocked and exchangeable NH_4^+-N concentration strongly increased due to accumulation of mineralisation-derived NH_3 after 21 days of incubation. In the alluvial soil, exchangeable NH_4^+-N concentration in the NH_4^+ treatment decreased from 143.7 to 2.7 mg N kg^{-1} soil and was almost completely consumed due to continued oxidation by the ammonia oxidisers in the first 7 days. After that, the exchangeable NH_4^+-N concentration showed no significant difference from the control ($P > 0.05$). In the red soil, the exchangeable NH_4^+-N concentration gradually decreased to approach that of the control at day 21. After application of 1-octyne with NH_4^+, exchangeable NH_4^+-N concentrations declined more slowly in the two

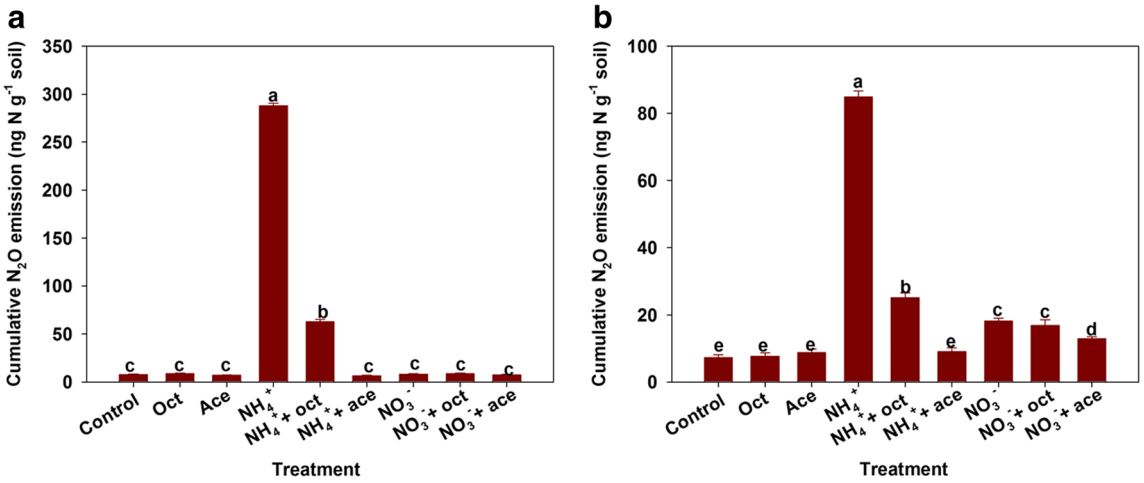

Fig. 1 Cumulative N_2O emissions after 21 days of laboratory incubation in an alluvial soil (**a**) and a red soil (**b**) which were amended with distilled sterilised water, NH_4^+ or NO_3^- (control, NH_4^+ or NO_3^-), with 1-octyne (Oct, NH_4^+ + oct or NO_3^- + oct) or with acetylene (Ace, NH_4^+ + ace or NO_3^- + ace). Data are means with standard deviations ($n = 3$). *Different letters above the bars* denote significant difference and the *same letters* denote no significant difference

soils. Amendment with NO_3^- alone did not significantly affect the change in exchangeable NH_4^+-N concentration. No obvious difference in exchangeable NH_4^+-N concentration was observed between NO_3^- and NO_3^- + oct treatments ($P > 0.05$).

In the two soils, the exchangeable NO_3^--N concentration in the control slightly increased due to nitrification of NH_3 derived from organic N mineralisation during the incubation (Fig. 2). 1-Octyne addition alone had no significant effect on soil exchangeable NO_3^--N concentration, but acetylene

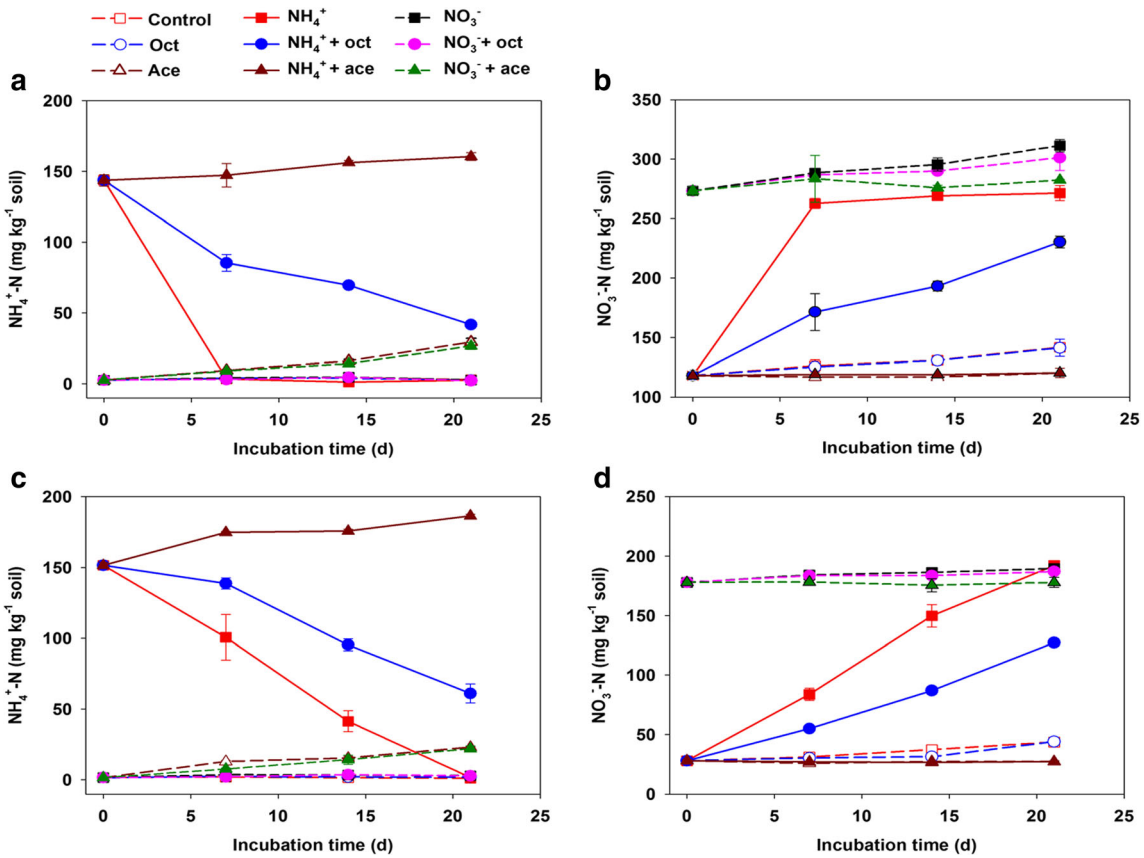

Fig. 2 The dynamics of NH_4^+ and NO_3^- during incubation for 21 days in an alluvial soil (**a**, **b**) and a red soil (**c**, **d**) which were amended with distilled sterilised water, NH_4^+ or NO_3^- (control, NH_4^+ or NO_3^-), with 1-octyne (Oct, NH_4^+ + oct or NO_3^- + oct) or with acetylene (Ace, NH_4^+ + ace or NO_3^- + ace). Data are means with standard deviations ($n = 3$)

completely suppressed exchangeable NO_3^--N production. Ammonium amendment substantially increased exchangeable NO_3^--N concentrations ($P < 0.01$), whereas 1-octyne partly slowed exchangeable NO_3^- formation in the NH_4^+ + oct treatment (Fig. 2). Exchangeable NO_3^--N concentrations in the NO_3^- and NO_3^- + oct treatments were similar after 21 days of incubation.

Abundance of AOB and AOA *amoA* genes

After 21 days, the AOB *amoA* gene abundance in the control was about 1.1×10^7 copies g^{-1} soil in the alluvial soil and 2.8×10^6 copies g^{-1} soil in the red soil, respectively (Fig. 3). No significant difference of AOB *amoA* gene abundance was observed among the control, Oct, Ace, NO_3^-, NO_3^- + oct and NO_3^- + ace treatments in the two soils ($P > 0.05$). The application of NH_4^+ significantly increased AOB *amoA* gene abundance, reaching 3.1×10^7 copies g^{-1} soil in the alluvial soil and 7.2×10^6 copies g^{-1} soil in the red soil, respectively ($P < 0.01$). However, AOB *amoA* gene abundance in the NH_4^+ + oct and NH_4^+ + ace treatments was obviously lower than those in the NH_4^+ treatment. No significant variation of AOB *amoA* gene abundance was observed between NH_4^+ + oct and NH_4^+ + ace treatments ($P > 0.05$).

In the alluvial soil, AOA *amoA* gene abundance ranged from 1.7×10^8 to 1.9×10^8 copies g^{-1} soil, showing no significant difference among all the treatment groups ($P > 0.05$). In the red soil, AOA *amoA* gene abundance between the control and Oct treatments was similar, at around 3.1×10^7 copies g^{-1} dry soil. However, NH_4^+ or NO_3^- addition significantly reduced AOA *amoA* gene abundance, showing a possible inhibitory effect of inorganic N on the AOA populations in the acidic red soil.

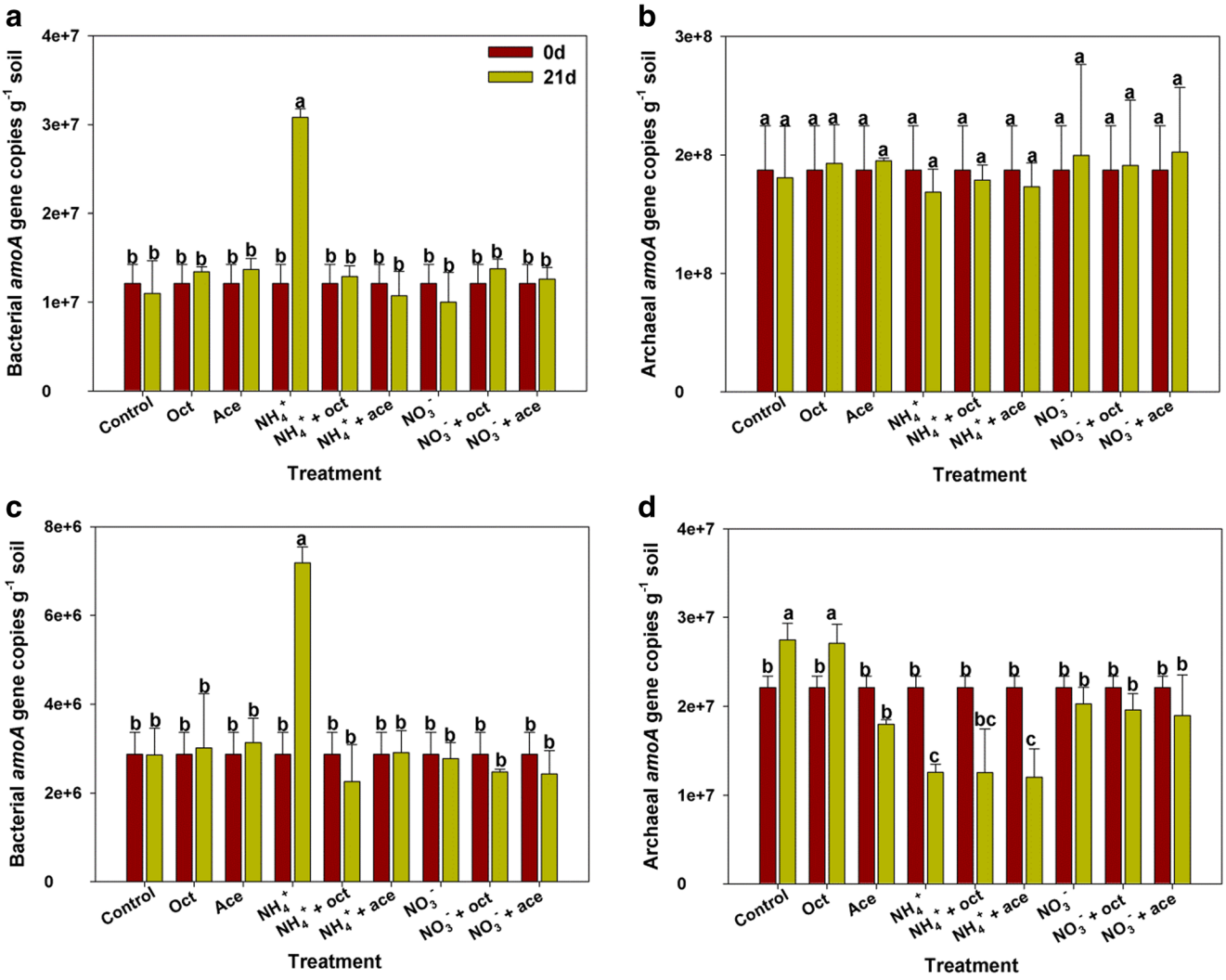

Fig. 3 AOB and AOA *amoA* genes abundance after 0 and 21 days of laboratory incubation in an alluvial soil (**a**, **b**) and a red soil (**c**, **d**) which were amended with distilled sterilised water, NH_4^+ or NO_3^- (control, NH_4^+ or NO_3^-), with 1-octyne (Oct, NH_4^+ + oct or NO_3^- + oct) or with acetylene (Ace, NH_4^+ + ace or NO_3^- + ace). Data are means with standard deviations ($n = 3$). *Different letters above the bars* denote significant difference and the *same letters* denote no significant difference

The regression analysis between the AOB and AOA *amoA* gene abundance and cumulative N_2O emission from different treatments is illustrated in Fig. 4. Cumulative N_2O emissions were positively correlated with AOB abundance in the alluvial soil ($y = 1.46e^{0.17x}$, $R^2 = 0.98$, $P < 0.001$) and the red soil ($y = 3.06e^{0.048x}$, $R^2 = 0.85$, $P < 0.001$). However, AOA abundance was not correlated with cumulative N_2O emissions in either of the two soils.

The contribution of AOB and AOA to N_2O production in two arable soils

Octyne-sensitive (AOB) N_2O emissions varied significantly between soil types and NH_4^+ amendment (Fig. 5). In the NH_4^+ treatment, the fraction of octyne-sensitive (AOB) N_2O emission was much higher than that of octyne-resistent (AOA) N_2O emission in the two soils, and this effect depended on soil type. For example, octyne-sensitive (AOB) N_2O emission in the alluvial soil was 78.1 %, higher than in the red soil (70.5 %). The percentage of octyne-resistent (AOA) N_2O emission was 19.7 % in the alluvial soil and 18.7 % in the red soil, respectively. In unamended soils, no significant difference between the percentage of octyne-resisitent (AOA) N_2O emission and the fraction of octyne-sensitive (AOB) N_2O emission was observed (data not shown).

Discussion

Nitrification and denitrification are considered to be the two primary processes of N_2O production in arable soils (Wrage et al. 2001). It is commonly suggested that nitrification was the major process of N_2O emission in soils below 60–70 % WFPS, whereas denitrification would be the main source N_2O production above 70 % WFPS (Bateman and Baggs 2005; Braker and Conrad 2011; Ma et al. 2015). Here, we postulated that nitrification was the major source of N_2O emission because: (1) soil treated NH_4^+ resulted in much higher N_2O production compared to that in NO_3^--treated soils; (2) applications of acetylene, which inhibits autotrophic ammonia oxidisers (AOB or AOA), reduced N_2O emission by 90–97 % in our soils, highlighting the importance of nitrifiers to N_2O emission; (3) all experiments were performed under aerobic conditions, and soil water content was kept at 55 % WFPS, which was more conductive to nitrification than denitrification (Bateman and Baggs 2005). Hence, these

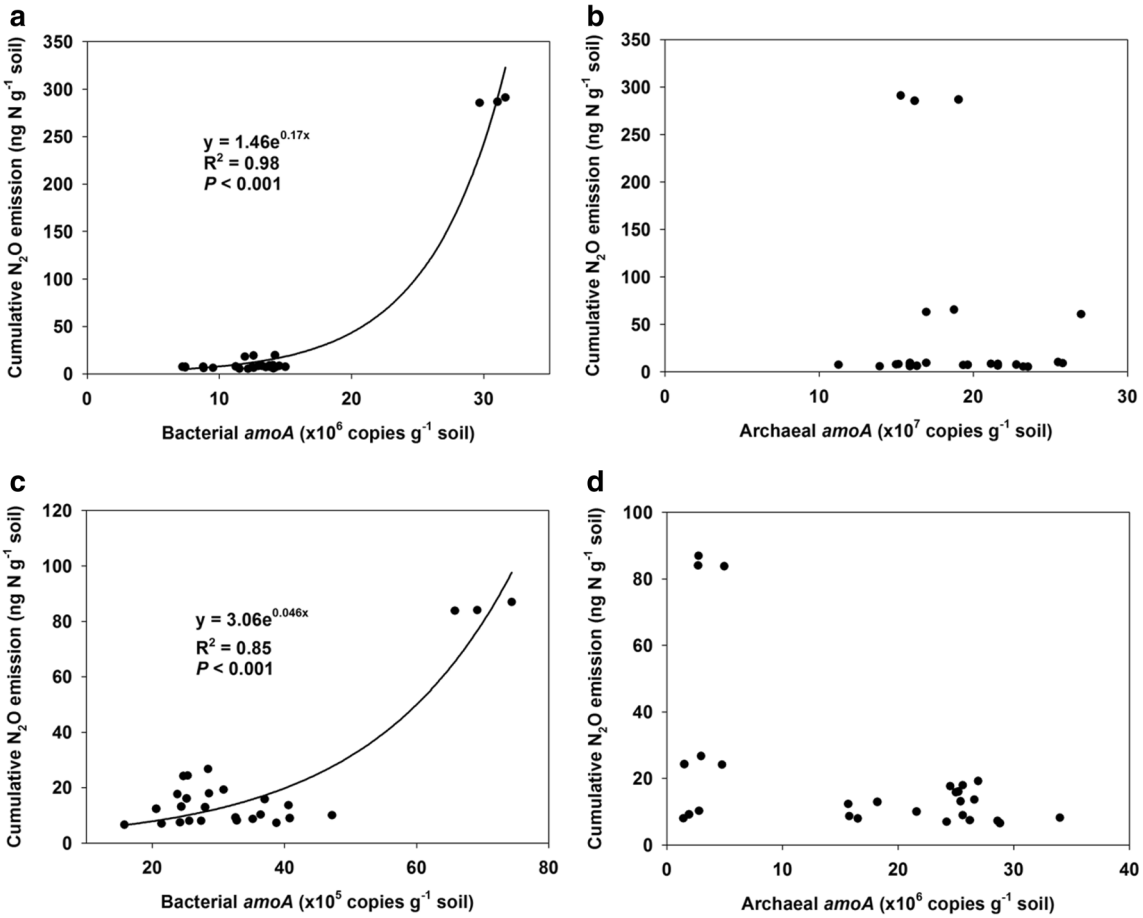

Fig. 4 Relationship of cumulative N_2O emissions with AOB (**a**, **c**) and AOA (**b**, **d**) *amoA* genes abundance in an alluvial soil (**a**, **b**) and a red soil (**c**, **d**)

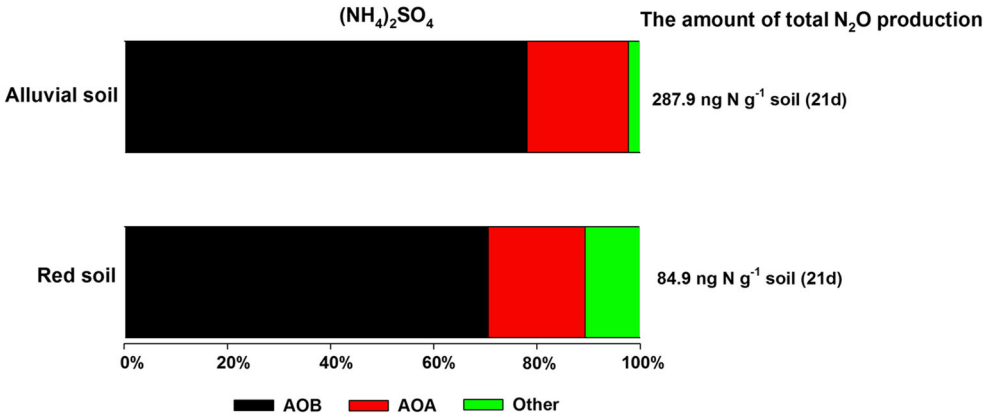

Fig. 5 Relative contributions of AOB and AOA to N_2O emissions in the NH_4^+ treatment after 21 days of incubation in an alluvial soil and a red soil. The contributions related to AOB (*black*), AOA (*red*) and other microbes and processes (*green*) are shown as percentages of total N_2O production

results indicated that N_2O was mainly produced from nitrification in these two agricultural soils.

Clearly, soil treated with NH_4^+ strongly stimulated AOB growth rather than AOA in the two soils. AOA did not respond to the application of NH_4^+ in the alluvial soil, while it was inhibited with respect to the control in the red soil, thus confirming what it was obtained in agricultural soils (Wu et al. 2011; Ai et al. 2013; Habteselassie et al. 2013; Di et al. 2014, 2014; Chen et al. 2015). Generally, AOB growth is favored by high NH_4^+-N soil conditions (Jia and Conrad 2009; Verhamme et al. 2011; Taylor et al. 2012), whereas AOA growth is not affected or suppressed (Di et al. 2009, 2010; Di and Cameron 2011; Di et al. 2014). Actually, the AOA population only grew in the control in the red soil, where NH_4^+ was generated from mineralised organic N, suggesting that AOA growth is favored in low fertility status soils (Nicol et al. 2008; Di et al. 2009, 2010; Zhang et al. 2012). Here, the stimulation and inhibition of AOB growth by NH_4^+ and inhibitors, respectively, occurred. Consequently, AOB *amoA* gene abundance was significantly correlated with cumulative N_2O emissions in the two soils. Similar studies also showed that AOB must play a critical role in N_2O production in high urea-N soils (Cui et al. 2013; Dai et al. 2013).

1-Octyne is a potentially selective inhibitor of AOB activity in agricultural soils (Taylor et al. 2013), and by using this selective inhibition, we showed that AOB had a great potential of contributing to soil N_2O production in high NH_4^+-N agricultural soils despite the presence of abundant AOA in these soils. Two mainly N_2O-yielding can explain the reason for the higher N_2O production by AOB than AOA, NH_2OH oxidation and nitrifier denitrification (Shaw et al. 2006; Stein 2011; Schreiber et al. 2012). It was reported that N_2O emission from the oxidation of NH_2OH contributed very little to total N_2O production and nitrifier denitrification was believed to be the predominant process for N_2O production in soils with $50\ \% < WFPS < 70\ \%$ (Kool et al. 2010, 2011). Recently, Vajrala et al. (2013) found that NH_2OH was an intermediate of ammonia oxidation in AOA, but they would not be able to produce N_2O like AOB probably due to lack of genes for a homologue of hydroxylamine oxidoreductase (HAO) known to be responsible for N_2O formation in AOB (Hooper and Terry 1979). In addition, AOA also lack genes encoding a potential NO-reductase (NOR), which is involved in nitrifier-denitrification and thus N_2O production in AOB (Stein 2011; Tourna et al. 2011). Therefore, we suggested that N_2O emission from the two tested soil probably depended on denitrification by AOB.

In addition, NI addition has limitations and the effectiveness of inhibitors in N_2O emission, and asssociated microbe studies may depend on soil conditions (Hatch et al. 2005). Stable isotope enrichment approaches should be applied to identify N_2O production during different processes following application of ^{15}N-labelled NH_4^+ or NO_3^- and ^{18}O-labelled H_2O and NO_3^- in short-term experiments (Cheng et al. 2014; Li and Lang 2014; Zhu et al. 2013). Zhu et al. (2013) carried out a dual labelling approach that employed an ^{18}O-, ^{15}N-enrichment method for distinguishing among nitrous oxide (N_2O) production from nitrifier nitrification, nitrifier denitrification and heterotrophic denitrification. A dual labelling method may improve our ability to discrimate between the contributions of nitrification and denitrification to total N_2O production. A combination of the stable isotopic enrichment approach coupled with molecular method may be required to estimate the contributions of all known N_2O-genic processes in the future.

In conclusion, we compared relative bacterial and archaeal contribution to soil N_2O production from two contrasting arable soils. By using inhibitors for specific micobial groups and metablic pathways, we demonstrated that nitrification was the predominant driving process of N_2O emission and AOB dominated in N_2O production when NH_4^+ was applied in the alluvial and red soils. The relative contribution of N_2O emission from AOA is lower than that from AOB under this condition. Moreover, we also observed that the changes of N_2O emissions among all treatments were correlated with variation of AOB abundance but not with AOA abundance in the two tested arable soils. Therefore, AOB may play a more important role than AOA in N_2O emission in a high NH_4^+-N concentration arable soils.

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