Soil Biology and Biochemistry 129 (2019) 48–59

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

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

Effects of the biological nitrification inhibitor 1,9-decanediol on nitrification and ammonia oxidizers in three agricultural soils

Yufang Lu[a], Xiaonan Zhang[a], Jiafeng Jiang[b], Herbert J. Kronzucker[c], Weishou Shen[d], Weiming Shi[a,*]

[a] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, 210008, China
[b] Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing, 210014, China
[c] School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, VIC, 3010, Australia
[d] School of Environmental Science and Engineering, Nanjing University of Information Science and Technology, Nanjing, 210044, China

ARTICLE INFO

Keywords:
Biological nitrification inhibition/inhibitor (BNI)
1,9-Decanediol
Ammonia oxidizer
Abundance
Community composition
Nitrous oxide

ABSTRACT

The application of biological nitrification inhibitors (BNIs) is considered an important new strategy to mitigate nitrogen losses from agricultural soils. 1,9-decanediol was recently identified as a new BNI in rice root exudates and was shown to inhibit nitrification in bioassays using *Nitrosomonas*. However, the effect of this compound on nitrification and ammonia oxidizers in soils remained unknown. In this study, three typical agriculture soils were collected to investigate the impact of 1,9-decanediol on nitrification and ammonia oxidizers in a 14-day microcosm incubation. High doses of 1,9-decanediol showed strong soil nitrification inhibition in all three agricultural soils, with the highest inhibition of 58.1% achieved in the acidic red soil, 37.0% in the alkaline fluvo-aquic soil, and 35.7% in the neutral paddy soil following 14 days of incubation. Moreover, the inhibition of 1,9-decanediol was superior to the widely used synthetic nitrification inhibitor, dicyandiamide (DCD) and two other BNIs, methyl 3-(4-hydroxyphenyl) propionate (MHPP) and α-linolenic acid (LN), in all three soils. The abundance of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) was significantly inhibited by 1,9-decanediol addition across the three soils. All AOB sequences fell within the *Nitrosospira* group, and the dominant AOA sequences belonged to the *Nitrososphaera* cluster in all three soils. Changes in the community composition of AOB were more pronounced than AOA after the application of 1,9-decanediol. The AOB community structure shifted from *Nitrosospira* cluster 2 and cluster 3a toward *Nitrosospira* clusters 8a and 8b. As for AOA, no significant impact on the proportion of the dominant *Nitrososphaera* cluster was observed in the fluvo-aquic soil and paddy soil while only the *Nitrosopumilus* cluster decreased in the red soil. 1,9-decanediol could also significantly reduce soil N_2O emissions, especially in acidic red soil. Our results provide evidence that 1,9-decanediol is capable of suppressing nitrification in agricultural soils through impeding both AOA and AOB rather than affecting soil NH_4^+ availability. 1,9-decanediol holds promise as an effective biological nitrification inhibitor for soil ammonia-oxidizing bacteria and archaea.

1. Introduction

Nitrogen (N)-use efficiency (NUE) in agricultural crops is notoriously poor. Globally, about 50% of the N fertilizer applied to cropping systems is lost to the environment as ammonia (NH_3), nitrate (NO_3^-), and nitrous oxide (N_2O, a greenhouse gas), raising agricultural production costs and contributing to environmental pollution and climate change (Galloway et al., 2008; Coskun et al., 2017a, 2017b). These losses are driven by nitrification and subsequent denitrification processes catalyzed by soil microorganisms. Nitrification is traditionally considered to be a two-step process where ammonia (NH_3) is first oxidized to nitrite (NO_2^-) by ammonia oxidizers, and subsequently to nitrate (NO_3^-) by nitrite-oxidizing bacteria (NOB); however, recent evidence indicates complete nitrification by *Nitrospira* bacteria that are present in natural soil environments (Daims et al., 2015). Efforts to understand and mitigate nitrification in agricultural systems have recently enjoyed renewed research focus, with the goal of improving NUE in crops and of reducing environmental impact (Coskun et al., 2017a).

Synthetic nitrification inhibitors (NIs), e.g. nitrapyrin, dicyandiamide (DCD), and 3,4-dimethylpyrazole phosphate (DMPP), have been widely used to reduce soil nitrification in agricultural production systems, but they have many drawbacks, including difficulties in

* Corresponding author.
E-mail address: wmshi@issas.ac.cn (W. Shi).

https://doi.org/10.1016/j.soilbio.2018.11.008
Received 31 May 2018; Received in revised form 5 November 2018; Accepted 10 November 2018
Available online 14 November 2018
0038-0717/

application, high cost, environmental pollution in their own right, and food safety risks (Subbarao et al., 2006a). With the advent of new bioassay systems that enable the quantification of inhibitory compounds exuded from roots, new research into nitrification inhibitors from plant root exudates, termed biological nitrification inhibitors (BNIs), has emerged (Subbarao et al., 2006b, 2009). BNIs present a low-cost and environmentally friendly alternative to synthetic nitrification inhibitors. Field crops and pastures have been evaluated for BNI capacity. Several BNIs exuded from roots have been identified, such as sorgoleone and methyl 3-(4-hydroxyphenyl) propionate (MHPP) in sorghum (Subbarao et al., 2013; Zakir et al., 2008) and brachialactone in *Brachiaria humidicola* (Subbarao et al., 2009). However, relatively few studies have investigated biological nitrification inhibition in the 'big three' agricultural crops (rice, wheat, and maize; Coskun et al., 2017a; Coskun et al., 2017b). Root exudates from *Leymus racemosus*, a wild relative of wheat, showed signs of substantial BNI activity (Subbarao et al., 2007). In a study of root exudates from 36 rice genotypes, Tanaka et al. (2010) showed that half of the rice root exudates possessed nitrification inhibition ability. Sun et al. (2016) have found strong BNI potential in both indica and japonica genotypes, and identified a novel hydrophobic BNI, 1,9-decanediol, from rice root exudates.

Although the above BNIs show desirable inhibition activity on *Nitrosomonas* sp. in pure cultures, not all BNIs released from root systems of plants are expected to be effective in suppressing soil nitrification activity in the field. Certain BNIs, such as linoleic acid and linolenic acid (two fatty acids isolated from leaf tissues of *B.humidicola*), gradually lost their effectiveness in soil after 80 days (Subbarao et al., 2008). Sorgoleone (the most important component of hydrophobic BNI activity in sorghum) and MHPP (the compound isolated based on hydrophilic BNI activity in sorghum) are effective in suppressing soil nitrification, whereas sakuranetin, a hydrophilic BNI compound isolated from sorghum, has no inhibitory effect on soil nitrification (Subbarao et al., 2013; Nardi et al., 2013). Therefore, the nitrification inhibition of 1,9-decanediol in agricultural soils needs to be verified.

The efficiency of nitrification inhibitors (e.g. DMPP and DCD) to suppress soil nitrification is highly variable, since the abundance and composition of ammonia-oxidizing bacteria (AOB) and archaea (AOA) are largely controlled by soil types (Chen et al., 2010). In addition, these two ammonia-oxidizing groups tend to occupy different niches in terms of sensitivity to soil pH, soil temperature, organic matter, and substrate concentration (Nicol et al., 2008; Jia and Conrad, 2009). The test of BNI nitrification inhibition abilities is mostly limited to one type of soil, such as a volcanic ash soil (Subbarao et al., 2008; Nardi et al., 2013). Few studies have focused on different soil types.

Several studies have explored the abundance, diversity, and structure of ammonia-oxidizing microbial communities in soils in response to NI addition. The commercial NIs DCD and DMPP were found to inhibit nitrification through influencing the abundance and activity of AOB, with no significant inhibition of AOA (Shi et al., 2016; Chen et al., 2015; O'Callaghan et al., 2010). However, it has been suggested that AOA may be particularly dominant under unfavorable environmental conditions, for example, low-nutrient and strongly acidic environments (Zhang et al., 2012). AOA and AOB may exhibit distinctly different responses to NIs, owing to the different metabolic pathways they possess and the different ecological niches they occupy. In the specific context of BNIs, Nardi et al. (2013) showed that MHPP from sorghum is capable of suppressing nitrification in soil by reducing the abundance and activity of ammonia-oxidizing microorganisms, suggesting that both AOB and AOA are targets of this BNI. However, overall, very little is known about the interaction of ammonia-oxidizing microbial communities in soils with BNIs.

Many scientists have attempted to assess the potential of NIs in the mitigation of N_2O emissions in microcosms and from agricultural soils (Shi et al., 2016; Ding et al., 2011). However, the effect of BNIs on N_2O emission remains largely uninvestigated. Our previous studies demonstrated that 1,9-decanediol quantities in root exudates were positively correlated with plant ammonium-use efficiency and with ammonium preference in rice cultivars (Sun et al., 2016). In the present study, we designed a microcosm experiment wherein different amounts of 1,9-decanediol isolated from rice root exudates were used, to monitor the extent and variation of nitrification activity, and the abundance and structure of AOB and AOA, in three agricultural soils. The objectives of our study were as follows: to investigate the nitrification inhibitory effect of 1,9-decanediol on different soil types; to compare the nitrification inhibitory activity of 1,9-decanediol with the synthetic NI DCD and other BNIs; to examine the effect of 1,9-decanediol on the abundance and community structure of AOA and AOB; and to determine the potential of 1,9-decanediol to reduce soil N_2O emissions.

2. Methods and materials

2.1. Site description and soil sampling

Soil samples were collected in May 2017 from three sites: a typical fluvo-aquic soil at Fengqiu Agro-ecological Experimental Station (35°00′N, 114°24′E) of the Chinese Academy of Sciences, Henan Province of China, a paddy soil at Yixing (31°17′ N, 119°54′ E), Jiangsu Province of China, and a red soil at Yingtan National Agro-ecosystem Field Experiment Station (28°15′N, 116°55′E) of the Chinese Academy of Sciences in Jiangxi Province, China. The three sites are located in the typical agricultural areas in China, with average annual rainfall of 615 mm (Fengqiu), 1198 mm (Yixing) and 1795 mm (Yingtan), and mean annual temperatures of 13.9 °C (Fengqiu), 15.7 °C (Yixing) and 17.6 °C (Yingtan). The fluvo-aquic soil at Fengqiu is classified as sandy loam with a pH value of 7.92, and the paddy soil at Yixing is classified as silt loam with a pH value of 6.25, while the red soil at Yingtan is classified as loamy clay with a pH value of 4.26. At each site, surface soil (0–20 cm) was collected, thoroughly mixed, and transported on ice to the laboratory. The soils were then air-dried, sieved (< 2 mm), and stored at room temperature before use.

2.2. Soil physicochemical analysis

Soil pH was determined using a glass electrode in 1:2.5 soil:water solution (w/v). Soil organic matter was measured using the $K_2Cr_2O_7$ wet oxidation method. Soil NH_4^+-N and NO_3^--N were extracted with 2 M KCl and determined on a continuous-flow analyzer (Skalar, Breda, Netherlands). Total N was measured using a Vario Max elemental analyzer (Elementar, Hanau, Germany). Particle size analysis was performed using sieve and hydrometer procedures. Details of the soil physical and chemical properties are shown in Table 1.

Table 1
Basic properties of the three soils at 0–20 cm.

Property	Agricultural soil		
Soil tested	Fluvo-aquic soil	Paddy soil	Red soil
Soil pH	7.92	6.25	4.26
Cation-exchange capacity (cmol kg^{-1})	11.6	11.3	10.0
Organic matter (%)	0.729	2.37	1.51
NH_4^+-N (mg kg^{-1})	3.62	3.23	28.37
NO_3^--N (mg kg^{-1})	19.72	6.26	46.75
Total N (%)	0.060	0.135	0.091
Texture	Sandy loam	Silt loam	Loamy clay
Particle size (%)			
Sand (0.02–2 mm)	56.9	40.7	24.4
Salt (0.002–0.02 mm)	34.9	41.9	24.0
Clay (< 0.002 mm)	8.2	17.4	51.6

2.3. Soil incubation experiments

Soil microcosms consisted of 100-mL glass bottles (diameter = 48 mm; height = 62 mm), containing 6 g of soil (oven dry equivalent, with the thickness of the soil layer at about 3.5 mm). 1,9-decanediol ($C_{10}H_{22}O_2$; MW:174) was synthesized by WuXi AppTec (Shanghai, China), methyl 3-(4-hydroxyphenyl) propionate (MHPP), α-linolenic acid (LN), and dicyandiamide (DCD) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Treatments were: (1) Nitrogen 200 mg kg^{-1} soil, added as ammonium sulfate ($(NH_4)_2SO_4$ considered as the control sample); (2) Nitrogen plus 1,9-decanediol at a concentration of 1000 mg kg^{-1} soil (equivalent to 700 mg C kg^{-1} soil; 1,9-decanediol-high dose); (3) Nitrogen plus 1,9-decanediol at a concentration of 500 mg kg^{-1} soil (equivalent of 350 mg C kg^{-1} soil; 1,9-decanediol-medium dose); (4) Nitrogen plus 1,9-decanediol at a concentration of 100 mg kg^{-1} soil (70 mg C kg^{-1} soil, 1,9-decanediol-low dose); (5) Nitrogen plus DCD at a concentration of 20 mg kg^{-1} soil (recommended dose, 10% of applied NH_4^+-N). If hydrophobic 1,9-decanediol is added to the stock soil by dissolution in methanol/DMSO, it is absorbed by soil particles. Although the stock soil will be uniformly distributed into the dilution soil series, 1,9-decanediol does not readily dissolve in such a system due to its hydrophobicity, leading to separation in space compared to NH_4^+. Thus, 1,9-decanediol was dispersed as a powder in $(NH_4)_2SO_4$ solutions by ultrasound exposure in our present study, the oil-in-water emulsion was then added to soils, for uniform distribution. This also avoids the use of methanol or DMSO solvent and prevents BNI-$(NH_4)_2SO_4$ separation in space in soils. The doses of 100, 500, and 1000 mg MHPP or LN kg^{-1} soil were also added in the same manner for comparison of the inhibitory activity with 1,9-decanediol. The microcosms were incubated at 25 °C in the dark for 14 days and maintained at 60% waterfilled pore space (WFPS). The bottles were aerated every three days. To maintain the moisture level throughout the incubation period, sterilized distilled water was added every three days. Three replicates for each treatment were performed.

2.4. Soil and gas sampling

Destructive samples were collected on days 0, 7, 14 for determination of soil NH_4^+-N and NO_3^--N. Samples for molecular analysis were collected on days 14. Nitrification inhibition (%) based on the amount of NO_3^--N produced was calculated using the following equation (McCarty and Bremner, 1989):

Nitrification inhibition (%) = (C-T)/C *100%

Where C is the amount of NO_3^- produced in the $(NH_4)_2SO_4$ control between day 0 and day 7 or day 14 (mg kg^{-1} soil) and T is the amount of NO_3^- produced in the BNI and DCD treatments between day 0 and day 7 or day 14 (mg kg^{-1} soil).

Gas samples (2 mL) were taken from the headspace with a syringe at 1, 2, 3, 5, 7, 10, and 14 days and were injected into 20-mL pre-evacuated Exetainers. All bottles were ventilated for 5 min after gas sampling and then sealed again. N_2O concentrations were measured with a gas chromatograph (HP7820A, Agilent Technologies, CA, USA) using an electron capture detector (ECD).

2.5. Soil microbial activity

Soil microbial activity was analyzed by the substrate-induced respiration (SIR) method, which was measured as CO_2 production (Anderson and Domsch, 1978). Soil samples (5 g dried soil) were placed in an airtight flask in a 21% O_2 atmosphere, supplemented with a nutrient solution including glucose (1.2 mg of C g^{-1} of dried soil), to establish 60% of water holding capacity. Soil was incubated at 25 °C, and the CO_2 levels were measured each hour for 8 h. The slope of the linear CO_2-time regression was used to estimate aerobic respiration as the CO_2-C produced (mg g^{-1} of dried soil h^{-1}).

2.6. Potential nitrification activity

The shaken slurry method (Hart et al., 1994) was used to determine potential nitrification activity (PNA). The procedure is briefly described as follows. Stock solutions of 0.2 M KH_2PO_4 (27.22 g L^{-1}), 0.2 M of K_2HPO_4 (34.83 g L^{-1}), and 50 mM of $(NH_4)_2SO_4$ (6.60 g L^{-1}) were prepared by using sterilized distilled water. A phosphorous-nitrogen (PN) working solution having a final concentration of 50 mM PO_4^{3-} and 75 mM NH_4^+ at pH 7.2 was obtained. At 14 days of soil incubation, a set of three soil microcosms for each treatment was collected, and 15 g of soil were transferred into Erlenmeyer flasks and supplemented with 100 mL of working solution. Slurries were incubated for 24 h under continuous shaking at 180 rpm on a shaker at 25 °C. Aliquots of 10 mL were taken at 2, 4, 22, and 24 h intervals and centrifuged at 8000 g for 10 min at 4 °C. The NO_3^--N concentration was determined by Continuous Flow Analysis (Skalar, Breda, Netherlands). PNA was calculated by regression analysis of NO_3^--N concentration vs. time.

2.7. Soil DNA extraction and quantification by real-time PCR

Total genomic DNA was isolated from 0.25 g of soil using MoBio PowerSoil DNA-isolation kits (MoBio Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. The concentration and quality of the extracted DNA were assessed using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA). Bacterial/archaeal *amoA* genes were determined by real-time quantitative PCR (qPCR) on a LightCycler 480 real-time PCR system (Roche Diagnostics, Mannheim, Germany) using the primer sets shown in Table S1. The 10-μL reaction mixture contained 5 μL of SYBR Premix Ex Taq (TaKaRa, Tokyo, Japan), 0.4 μL of each primer (10 μM), and 0.5 μL of template DNA. Standard curves were generated using 10-fold serial dilutions of plasmids containing correct inserts of the target genes. Melting curve analysis was performed between 72 and 95 °C at the end of each amplification assay to evaluate the specificity of quantitative PCR (qPCR) products. Real-time PCR was performed in triplicate and amplification efficiencies of 92.2–105.3% were obtained, with R^2 values > 0.99.

2.8. Pyrosequencing and bioinformatic analysis

The sequencing of the amplicon libraries was carried out on an Illumina MiSeq platform with 300-bp paired-end reads. Pyrosequencing of bacterial and archaeal *amoA* genes in the total DNA extract was performed using the *amoA* primer pair amoA-1F/amoA-2R and Arch-amoA26F/Arch-amoA417R (Park et al., 2008). The sequencing data has been deposited in the NCBI Sequence Read Archive (SRA) database under accession number SRP148613. Pairs of reads from the raw data were first merged with FLASH version 1.2.7 (Magoc and Salzberg, 2011), in which forward and reverse reads had an overlapping base length > 10 bp and did not allow a base mismatch. Sequencing reads were processed with Mothur version 1.31.1 (Schloss et al., 2009). The low-quality sequences that had a quality score < 20, contained ambiguous nucleotides, or did not match the primer and barcode were removed. After the sequences of samples were sorted according to the barcodes, the barcode and primer sequences were deleted. The remaining sequences were aligned against the *amoA* gene database, and the failed and chimeric sequences were also removed.

Sequences were classed into operational taxonomic units (OTUs), using a sequence similarity threshold of 97%. A representative sequence of the *amoA* gene within each OTU was retrieved by selecting the most abundant sequence in that OTU. All OTUs in each soil were taxonomically classified by the construction of neighbour-joining phylogenetic trees in MEGA 7.0 using representative sequences of the *amoA* genes, together with taxonomically-determined reference sequences

from GenBank. The nomenclature for bacterial *amoA* clusters was defined following the studies of Avrahami and colleagues (Avrahami et al., 2002; Avrahami and Conrad, 2003) and the nomenclature for archaeal *amoA* clusters was defined by Nicol et al. (2008) and Pester et al. (2012).

2.9. Statistical analysis

Statistical analyses were carried out using the SPSS 18.0 software package for Windows. Normality of data distribution (Shapiro-Wilk test) and homogeneity of variances (Levene test) assumptions were satisfied. Statistically significant differences among treatments were determined by one-way ANOVA and least significant difference (LSD) calculations at a 5% confidence level. The relationships between the amount of NO_3^--N produced, and bacterial or archaeal *amoA* gene copy numbers were assessed using linear regression analysis of Origin 8.

3. Results

3.1. NO_3^--N and NH_4^+−N concentrations

During the 14-d incubation, nitrogen dynamics differed between all treatments of the three agricultural soils. In the fluvo-aquic soil, NH_4^+-N decreased to 69.5 mg NH_4^+-N kg^{-1} after 7 days of incubation, with a further reduction to 8.7 mg NH_4^+-N kg^{-1} soil at day 14 (Fig. 1B). This was accompanied by a growing trend in NO_3^--N from 20 mg at day 0–134 mg (day 7) and 187 mg kg^{-1} soil (day 14) (Fig. 1A), indicating that nitrification was rapid in the $(NH_4)_2SO_4$ control. A dose-response relationship was found between the 1,9-decanediol addition and nitrification inhibition. The high-dose and medium-dose 1,9-decanediol could significantly suppress nitrification, while the low-dose treatment had no effect. At day 7, more than 70% of NH_4^+-N remained (121 mg NH_{4+}-N kg^{-1}), and after 14 days about 20% of the ammonium remained (48 mg NH_4^+-N kg^{-1}) in the high-dose 1,9-decanediol treatment, compared to less than 5% in the control until the end of the incubation. These slower decreases in ammonium concentrations were consistent with low nitrate concentrations that only reached 122 mg NO_3^--N kg^{-1} after 14 days for the high-dose 1,9-decanediol treatment, compared to more than 180 mg NO_3^--N kg^{-1} after 7 days in the control and low dose 1,9-decanediol treatment. However, inhibition by the synthetic inhibitor DCD was significantly stronger than that by the high-dose 1,9-decanediol, with about 40% of NH_4^+-N remaining at day 14.

In the paddy soil, the nitrification rate was slower than in the alkaline fluvo-aquic soil, and about 20% of the ammonium was converted to nitrate in the $(NH_4)_2SO_4$ control. Similarly, the medium-dose and high-dose 1,9-decanediol significantly slowed down the reduction of NH_4^+-N levels, whereas the low-dose treatment showed no activity (Fig. 1E). Although DCD produced a 20% inhibition of nitrification, the ammonium concentration was higher in the high-dose 1,9-decanediol treatment (182.9 mg kg^{-1}) than in the treatment with DCD (179.2 mg kg^{-1}) at day 7, which is in line with the lower nitrite level with high-dose 1,9-decanediol compared to DCD (Fig. 1B), indicating a stronger nitrification inhibition of 1,9-decanediol than DCD in the neutral paddy soil.

It is well known that nitrification in natural red soil is extremely weak due to low pH and soil texture (e.g. forest soils), but nitrification has been accelerated in many such soils following tillage in recent decades. In the present study, we chose a typical agricultural red soil with some nitrification activity, which we considered eminently suitable for evaluation of the nitrification inhibition effect of 1,9-decanediol. Although the nitrification rate appeared to be the slowest in the acidic red soil (Fig. 1C), low-dose, medium-dose, and high-dose 1,9-decanediol all significantly inhibited nitrate production. Moreover, the inhibition by high-dose 1,9-decanediol was higher in the red soil (58.1%) than the fluvo-aquic (37.0%) and paddy soil (35.7%) after 14-d incubation (Table 2). No significant inhibition was observed in the DCD treatment at day 7 and day 14.

Moreover, total inorganic nitrogen in the 1,9-decanediol high-dose treatment remained unchanged at day 7 in the three soils (Fig. S1), suggesting that NH_4^+ immobilization did not occur. Between days 7 and 14, some NH_4^+ immobilization probably occurred in the high 1,9-decanediol treatment, since total inorganic N was about 20 mg lower than in other treatments in the fluvo-aquic soil while no significant difference was found in the paddy and red soils. However, NH_4^+ concentrations in the soil remained significantly higher compared to the N treatments indicating that the suppression of nitrification was also responsible for the low nitrate level observed during the incubation.

In addition to DCD, we also compared the effect of 1,9-decanediol with LN and MHPP, two BNIs that had exhibited significant inhibition activity in soils (Table 2). Similar to 1,9-decanediol, nitrification inhibition activity progressively increased with the addition of LN and MHPP in all three soils. In addition, stronger inhibition of 1,9-decanediol was found than with LN and MHPP. At day 7, the inhibition rates in the high-dose 1,9-decanediol, LN, and MHPP treatments were 64.8%, 36.0%, and 50.1% in the fluvo-aquic soil, 29.7%, 18.3%, and 14.4% in the paddy soil, and 46.9%, 23.5%, and 25.1% in the red soil, respectively. After 14-d incubation, the inhibition rates showed a decreasing trend among all the BNIs treatments in the fluvo-aquic soil whereas the inhibition of 1,9-decanediol and LN was still persistent in red and paddy soils. Of particular interest, the nitrification inhibition by 1,9-decanediol increased to 58.1% at day 14, while it decreased to 21.2% by MHPP in the red soil, suggesting a more stable and effective role of 1,9-decanediol than MHPP in acidic red soil.

3.2. Potential nitrification activity (PNA)

At the end of soil incubation experiments, no significant effect of 1,9-decanediol was observed on CO_2 emissions revealed by SIR (Fig. S1), indicating that 1,9-decanediol may not affect soil microbial activity more generally. To further evaluate whether 1,9-decanediol specifically affected nitrification, potential nitrification activity (PNA) in soil samples taken after the 14-d incubation period was determined (Fig. 2). PNA was 2.0 mg NO_3^--N kg^{-1} h^{-1} in the N-amended fluvo-aquic soil, higher than in paddy (1.4 mg NO_3^--N kg^{-1} h^{-1}) and red soil (0.6 mg NO_3^--N kg^{-1} h^{-1}). It decreased to 1.6, 1.5, and 1.1 mg NO_3^--N kg^{-1} h^{-1} in the low-dose, medium-dose, and high-dose 1,9-decanediol treatments, respectively, while DCD showed a much higher activity, of about 65%, than 1,9-decanediol in the fluvo-aquic soil. No significant difference was found between the medium- and high-dose 1,9-decanediol and DCD in paddy soil. PNA was 1.4 mg NO_3^--N kg^{-1} h^{-1} in the control treatment of paddy soil, and it declined by about 50% in soil samples treated with both 1,9-decanediol levels and DCD. Although PNA of red soil was slower than that of the other two soils, all the 1,9-decanediol treatments showed a significant inhibition by 60–78%, whereas DCD had no significant effect, indicating superior efficacy of 1.9-decanediol in regulating nitrification in red soil compared to DCD.

3.3. Abundance of AOB and AOA

A quantitative PCR assay was used to estimate the population sizes of soil AOB and AOA populations following the application of nitrification inhibitors 1,9-decanediol and DCD in the three selected agricultural soils at day 14. Both AOA and AOB *amoA* genes were detected in large numbers but their abundance varied widely in the three soils. The AOA population size was greater than that of AOB in all the soils (Fig. 3). A dose-response relationship was also found between 1,9-decanediol levels and AOB and AOA populations.

After 14 days of incubation, the AOB *amoA* gene copy numbers in the control treatments were 6.6×10^8 copies g^{-1} soil in fluvo-aquic, 9.1×10^6 copies g^{-1} soil in paddy, and 1.2×10^6 copies g^{-1} soil in red soil, respectively (Fig. 3). When the nitrification inhibitors 1,9-

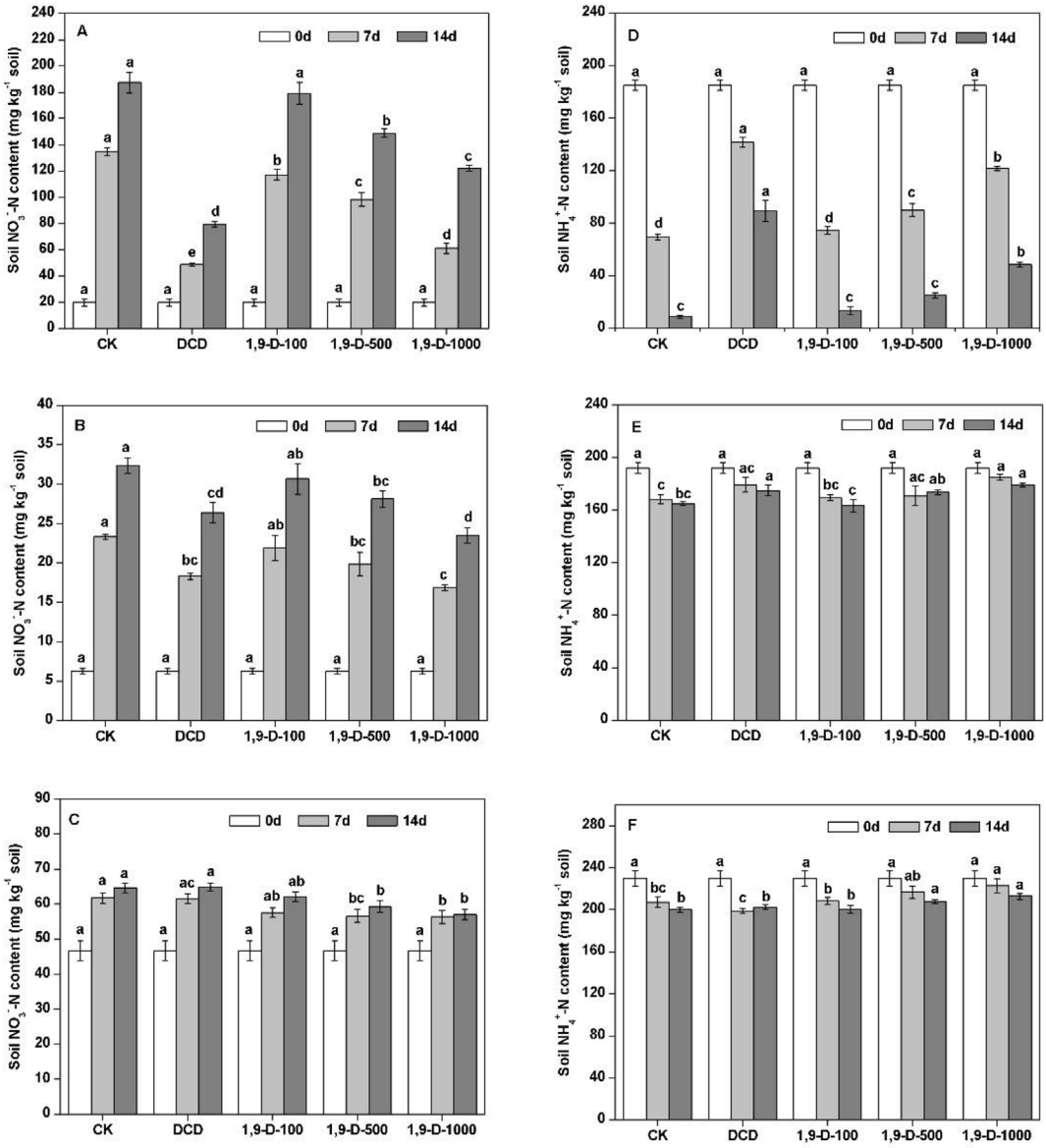

Fig. 1. NO_3^--N and NH_4^+-N contents in fluvo-aquic (A and D), paddy (B and E), and red soil (C and F) at 0, 7, and 14 days of incubation. Additions to CK (Control) were as follows: 200 mg NH_4^+-N kg^{-1} soil, DCD (20 mg kg^{-1} soil), 1,9-D-100 (100 mg 1,9-decanediol kg^{-1} soil), 1,9-D-500 (500 mg 1,9-decanediol kg^{-1} soil), 1,9-D-1000 (1000 mg 1,9-decanediol kg^{-1} soil). Mean values and standard errors are shown (n = 3). Different letters indicate significant differences between different treatments at $P < 0.05$ by LSD test at each incubation time.

Table 2
Nitrification inhibition by 1,9-decanediol and two other prominent BNIs (LN, MHPP) in three agricultural soils after 7-d and 14-d incubation.

BNI_S	Nitrification inhibition %					
	Fluvo-aquic soil		Paddy soil		Red soil	
	7d	14d	7d	14d	7d	14d
1,9-D-100	15.5 ± 3.5 f	5.6 ± 1.4 d	8.3 ± 2.6 cd	8.0 ± 3.1 f	36.8 ± 2.4 b	24.3 ± 0.5 cd
1,9-D-500	31.9 ± 1.0 cd	25.6 ± 0.3 b	20.3 ± 2.2 b	20.1 ± 2.2 bc	45.1 ± 3.2 a	43.0 ± 2.5 b
1,9-D-1000	64.8 ± 1.5 a	37.0 ± 2.0 a	38.3 ± 1.5 a	35.7 ± 3.8 a	46.9 ± 3.3 a	58.1 ± 3.0 a
LN-100	2.6 ± 0.9 g	8.6 ± 1.3 d	1.5 ± 0.4 de	2.7 ± 1.0 f	8.8 ± 2.6 e	26.0 ± 2.3 cd
LN-500	23.4 ± 2.7 e	17.2 ± 4.6 c	6.8 ± 1.3 de	5.3 ± 1.8 f	18.9 ± 1.2 cd	25.2 ± 2.3 cd
LN-1000	36.0 ± 1.6 c	19.2 ± 2.6 bc	16.6 ± 3.9 bc	16.8 ± 3.9 cd	23.5 ± 3.5 c	29.5 ± 1.1 c
MHPP-100	1.6 ± 0.3 g	2.0 ± 1.8 d	0.6 ± 0.2 e	1.2 ± 0.2 f	6.4 ± 0.9 e	12.9 ± 2.7 f
MHPP-500	28.8 ± 4.0 de	18.7 ± 1.0 bc	10.8 ± 2.7 cd	10.1 ± 2.4 d	11.3 ± 2.1 de	16.3 ± 3.4 ef
MHPP-1000	50.1 ± 1.7 b	26.0 ± 4.5 b	22.1 ± 2.2 b	25.7 ± 3.8 b	25.1 ± 2.3 c	21.2 ± 2.2 de

Different letters indicate significant differences between different treatments at $P < 0.05$ by LSD test at each incubation time for each soil type.

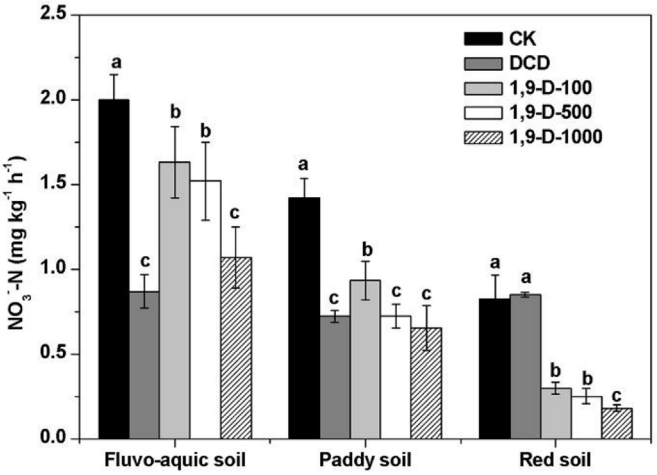

Fig. 2. Potential nitrification activity in fluvo-aquic, paddy, and red soil, sampled after 14-d incubation. Mean values and standard errors are shown (n = 3). Different letters indicate significant differences between different treatments in each soil type at $P < 0.05$ by LSD test.

decanediol and DCD were applied, AOB population growth was significantly inhibited (Fig. 3). AOB *amoA* gene abundance, significantly inhibited by DCD (72%, $P < 0.05$), was 1.86×10^8 copies g^{-1} soil in the fluvo-aquic soil, whereas 1,9-decanediol had a relatively lower inhibitory activity of 21%–50% (Fig. 3A). By contrast, high-dose 1,9-decanediol exhibited a strong inhibition of about 81% in paddy soil (1.8×10^6 copies g^{-1} soil) and 94% in red soil (7.5×10^4 copies g^{-1} soil), respectively, both significantly higher than the activity of DCD (Fig. 3B and C).

Unlike AOB, AOA *amoA* gene abundance remained largely unchanged in all DCD-treated soils (Fig. 3), indicating a lack of significant inhibition by DCD of AOA populations. However, AOA abundance was greater in the control than in the low-, medium-, and high-dose 1,9-decanediol treatments in all three soils. In particular, the inhibition by medium- and high-dose of 1,9-decanediol of AOA abundance reached 72% and 93% in the red soil (Fig. 3C, $P < 0.05$).

Pearson's correlation analysis revealed that AOB abundance showed significant correlations with NO_3^--N concentrations produced in fluvo-aquic soil ($R^2 = 0.67$, $P < 0.001$; Fig. S3A), paddy soil ($R^2 = 0.68$, $P < 0.001$; Fig. S3B), and red soil ($R^2 = 0.40$, $P = 0.005$; Fig. S3C). However, a significant correlation between AOA abundance and NO_3^--N was only observed in red soil ($R^2 = 0.61$, $P < 0.001$; Fig. S3F) and paddy soil ($R^2 = 0.35$, $P = 0.01$; Fig. S3E), but with no quantitative relationship found in the fluvo-aquic soil (Fig. S3D).

3.4. Community structure of AOB and AOA

Pyrosequencing of AOB and AOA partial *amoA* gene fragments was conducted using samples obtained on day 14 of incubation. The neighbour-joining phylogenetic trees for AOA and AOB were constructed using the representative sequence under each group's operational taxonomic units (OTUs) and related sequences in GenBank, respectively. The results suggest all the AOB sequences in the three soils belonged to the *Nitrosospira* group (Fig. S4). The clone sequences were mainly grouped into *Nitrosospira* cluster 8b, cluster 8a, cluster 3a, cluster 3b, cluster 2, and cluster 1 in paddy soil while *Nitrosospira* cluster 3a and cluster 3b were the most abundant in fluvo-aquic soil. Similarly, most sequences in the red soil were affiliated to clusters 8a and 3a. A few remaining sequences were affiliated with clusters 8b, 2. Sequences falling into cluster 3a were further divided into 3a.1 and 3a.2 due to their unstable affiliations. Based on the result of phylogenetic trees, OTUs under the same phylogenetic classification were merged and used for the calculation of relative percentage of individual

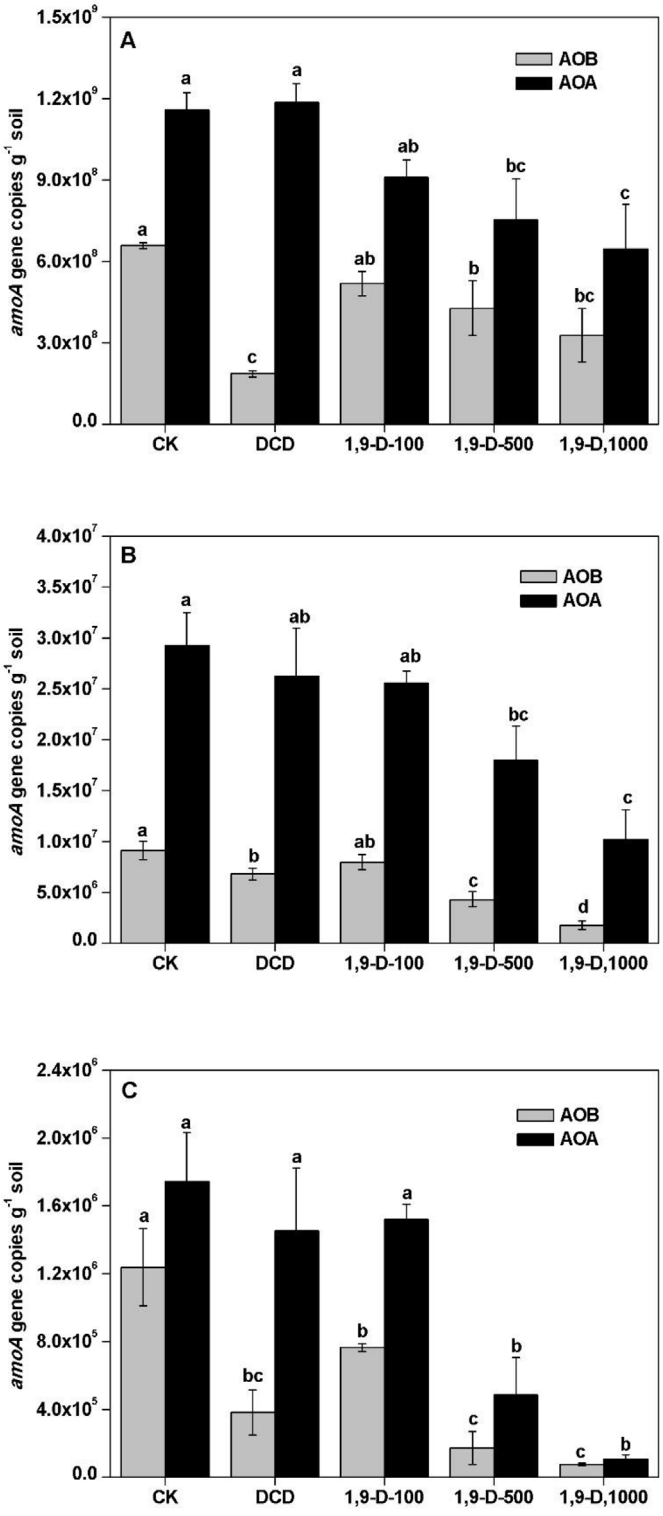

Fig. 3. *AmoA* gene copies of ammonia-oxidizing bacteria (AOB) and archaea (AOA) in fluvo-aquic soil (A), paddy soil (B), and red soil (C) in different treatments after 14 days of soil incubation. Mean values and standard errors are shown (n = 3). Different letters indicate significant differences between different treatments at $P < 0.05$ by LSD test.

phylogenetic lineages for each soil. The results show that 1,9-decanediol slightly shifted *Nitrosospira* cluster 3b to cluster 3a.2 in the fluvo-aquic soil (Fig. 4A), but significantly decreased the relative percentage of *Nitrosospira* cluster 3a, cluster 3b, cluster 1, and cluster 2 in paddy soil (Fig. 4B), and *Nitrosospira* cluster 3a.2 and cluster 2 in red soil

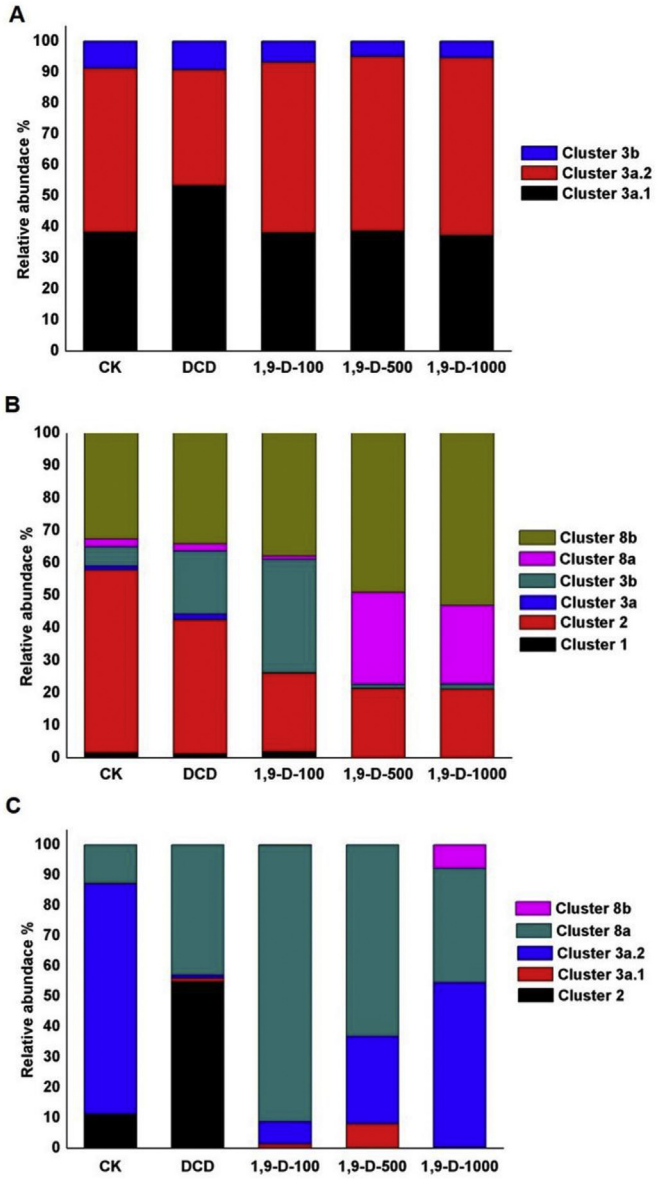

Fig. 4. Phylogenetic affiliation and relative abundance of bacterial *amoA* gene sequences retrieved from fluvo-aquic soil (A), paddy soil (B), and red soil (C) under five treatments on day 14. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Fig. 4C). Of particular interest was that the addition of 1,9-decanediol increased the relative percentage of *Nitrosospira* clusters 8a and 8b in both paddy soil and red soil.

Classification of the archaeal *amoA* gene according to Pester et al. (2012) reveals that the dominant OTUs in fluvo-aquic soil all belonged to the *Nitrososphaera* cluster (also known as Group 1.1b thaumarchaeota) (Fig. S5). In paddy and red soil, most AOA *amoA* gene sequences were also grouped into the *Nitrososphaera* cluster, whereas a few remaining sequences were affiliated with the *Nitrosotalea* lineage (also known as Group 1.1a associated) and the *Nitrosopumilus* lineage (also known as Group 1.1a), respectively. The results show that 1,9-decanediol had no impact on the proportion of the AOA *Nitrososphaera* in fluvo-aquic soil, but significantly decreased the relative abundance of *Nitrososphaera* cluster 9 in paddy soil (Fig. 5A), and the *Nitrosopumilus* cluster and *Nitrososphaera* cluster 7 in red soil (Fig. 5B). By contrast, the relative abundance of *Nitrososphaera* cluster 10 increased after the application of 1,9-decanediol.

A dose-effect relationship was observed between 1,9-decanediol and both AOB and AOA community structures. For example, in paddy soil, high-dose 1,9-decanediol not only inhibited *Nitrosospira* clusters 1, 3b, and 3a, but also significantly increased the relative percentage of *Nitrosospira* clusters 8a and 8b; however, low-dose 1,9-decanediol only slightly shifted *Nitrosospira* cluster 2 to cluster 3b (Fig. 4B). In regard to AOA, high-dose 1,9-decanediol significantly decreased the relative abundance of *Nitrososphaera* cluster 9 whereas low-dose 1,9-decanediol showed no significant effect (Fig. 5A). Moreover, DCD had a less pronounced effect on AOB and AOA community structures than 1,9-decanediol.

3.5. N_2O emission rate

The dynamics of N_2O emission rates varied significantly, with large differences among soils and treatments (Fig. 6). The N_2O emissions in fluvo-aquic soil reached a peak of 1053 μg N_2O kg^{-1} soil day^{-1}, and then declined sharply in the $(NH_4)_2SO_4$ control. N_2O emissions from treatments with 1,9-decanediol and DCD were lower than from CK, with the inhibition of DCD stronger than 1,9-decanediol (Fig. 6A). Likewise, 1,9-decanediol significantly suppressed N_2O production from paddy soil but did not show any difference to DCD (Fig. 6B). In the acidic red soil, 1,9-decanediol completely inhibited N_2O during the 14-d incubation; however, DCD showed no significant effect (Fig. 6C).

4. Discussion

Nitrification inhibition can increase NUE in crops and substantially reduce N loss from agroecosystems. Plant-derived BNIs are cost-effective, environmentally friendly, and can be functionally highly effective in regulating soil nitrification (Subbarao et al., 2008). However, not all BNIs released from plant root systems under laboratory conditions are effective in suppressing soil nitrifier activity in the field. As a newly identified BNI from rice, one of the big three crops in the world, 1,9-decanediol has been shown to inhibit nitrification of *Nitrosomonas europaea* and to increase NUE of rice (Sun et al., 2016). Thus, the elucidation of the mechanism of soil nitrification inhibition by 1,9-decanediol may facilitate the potential of specific BNI selection and application in the field, with the goal of increasing NUE in crops.

4.1. Contrasting effects of 1,9-decanediol on nitrification in three agricultural soils

Soil pH has been considered as one of the most important factors controlling NI efficacy, because pH has potential to impact the mobility and degradation rate of the NIs in soils (Zhang et al., 2004). In our study, a more long-term, stable effect of 1,9-decanediol in the acidic red and the neutral paddy soil, as opposed to the alkaline fluvo-aquic soil, was found (Table 2). Similarly, the half-life of nitrapyrin is longer in acidic than in alkaline soil (Wolt, 2000) and DMPP performs better in neutral than in alkaline soil (Liu et al., 2015). As known BNIs are mainly derived from *Brachiaria humidicola*, sorghum, and rice, all of which are widely grown in acidic and neutral soils, and their exudation is enhanced by low pH and the presence of NH_4^+ (Subbarao et al., 2007; Di et al., 2018), they are expected to be more stable under acidic conditions. Several studies have confirmed that fatty acids and derivatives (BNIs from *Brachiaria humidicola* shoot tissues) and phenolic acids (BNIs from *Brachiaria humidicola* root tissues) are more stable at low pH levels (Brzozowska et al., 2013; Jang et al., 2014). The virucidal activities of fatty alcohols (BNIs from rice root exudates) are more active at pH 4.2 than at pH 7 (Hilmarsson et al., 2007). Accordingly, the degradation of 1,9-decanediol in acidic soil might be slower than the alkaline soil used in our present study. The fate of 1,9-decandiol and other BNIs in different soils needs to be further investigated. Moreover, the nitrification rate measured in our study was relatively lower in the neutral and acidic soil than the alkaline soil, hence resulting in a greater co-location of NH_4^+ and 1,9-decanediol.

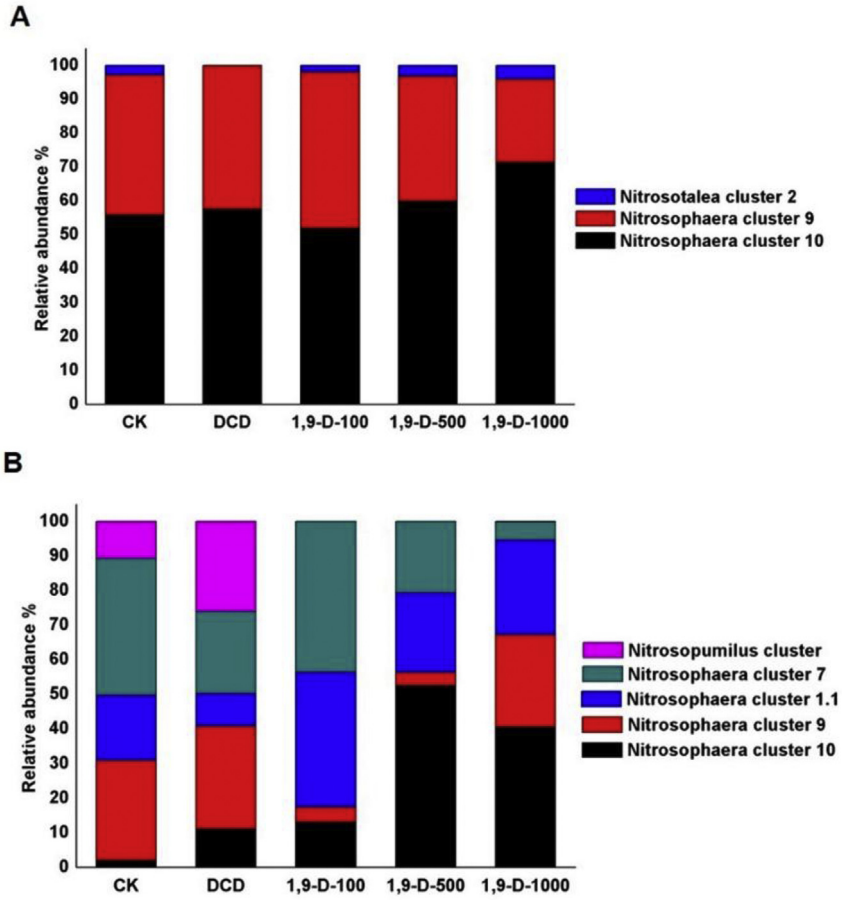

Fig. 5. Phylogenetic affiliation and relative abundance of archaeal *amoA* gene sequences retrieved from paddy soil (A), and red soil (B) under five treatments on day 14. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Aside from soil pH, NI effectiveness can be affected by other soil properties such as soil texture. As a fatty alcohol compound, 1,9-decanediol can be adsorbed onto soil particles with high levels of clay, silt, and organic matter, thereby leading to high absorption of 1,9-decanediol in soils and improved binding of 1,9-decandiol to soil ammonia-oxidizing microorganisms. Levels of the organic matter, clay, and silt components in our study were much higher in the red soil than in the fluvo-aquic soil (Table 1). Similar to the case for 1,9-decanediol, the adsorption of other NIs, such as DCD, DMPP and nitrapyrin, to soil organic matter has been recognized as an important factor influencing inhibitory efficacy (Zhang et al., 2004; Fisk et al., 2015; Shi et al., 2016).

The sensitivity of dominant ammonia-oxidizing microorganisms in soils to 1,9-decanediol may also result in efficacy differences. The dose of 90 μg mL^{-1},9-decanediol has been reported to possess an 80% nitrification inhibition of *Nitrosomonas europaea* (Sun et al., 2016) while it showed a much smaller inhibition of *Nitrosomonas stercoris* in pure bacterial culture systems (data not shown). It should be noted that the main ammonia oxidizer targeted by 1,9-decanediol in soils in this study was quite different, with the AOB *Nitrosospira* cluster 3b being suppressed in the fluvo-aquic soil, as opposed to the AOA *Nitrospumlus* cluster in the red soil, respectively (Figs. 4 and 5). Taken together, this provides an explanation for the differential effects of 1,9-decanediol in the three agriculture soils.

4.2. Contrasting effects of 1,9-decanediol and other NIs on nitrification

Differences of 1,9-decanediol and DCD in nitrification inhibitory efficacy may be attributed to a lower mobility of 1,9-decanediol in comparison to DCD, due to a greater sorption of 1,9-decanediol, especially in acidic soils. 1,9-decanediol is hydrophobic, making it relatively immobile in aqueous media and soil, which results in it remaining close to the site of application. By contrast, due to its higher hydrophilicity and mobility, a greater spatial separation of DCD away from the NH_4^+ point sources (Li et al., 2016) and from nitrifying microorganisms (Ruser and Schulz, 2015) can be achieved, and DCD can, therefore, also be easily leached away from N-application zones in the crop rhizosphere. However, rainfall has not been considered in the present soil-incubation systems, and it is possible that 1,9-decanediol may display a superior inhibition profile than DCD in the field, even in alkaline soils. As a plant-derived BNI, 1,9-decanediol is easily available and more environmentally friendly than DCD.

It is well known that DCD is bacteriostatic rather than bactericidal (bacteria are only inhibited in their activities but not killed) (Amberger, 1989). By contrast, it is possible that 1,9-decanediol changes may be bactericidal at elevated concentrations. This hypothesis is supported by the observation that AOB and AOA *amoA* gene abundance was greatly inhibited by 1,9-decanediol at high concentrations, especially in the acidic red soil (Fig. 3C). In addition, Togashi et al. (2007) demonstrated that 1-nonanol, 1-decanol, and 1-undecanol have bactericidal activity and membrane-damaging activities against *Staphylococcus aureus*. Mukherjee et al. (2013) revealed that the bactericidal activity of fatty alcohols can partly be attributed to their ability to damage the robust and complex cell envelope of Mycobacteria. The best bactericidal activity was found at a C-10 chain length. As a fatty alcohol with a C-10 chain length, 1,9-decanediol may, therefore, have bactericidal activity toward nitrifiers. Overall, this renders 1,9-decanediol a potentially better nitrification inhibitor than DCD due to its specific chemical

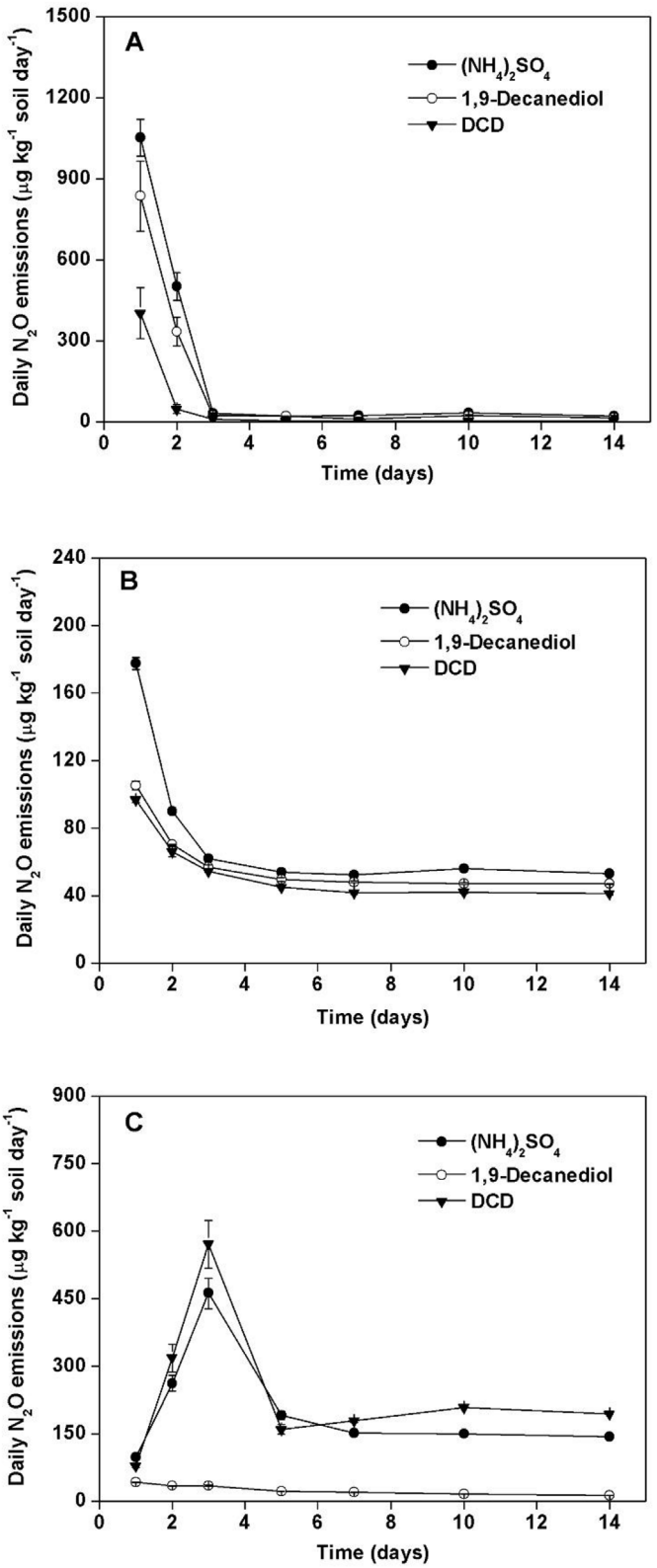

Fig. 6. Daily N_2O emissions during the 14-d microcosm study in fluvo-aquic soil (A), paddy soil (B), and red soil (C). Error bars represent standard errors from three replicates.

structure.

It has been found that the inhibition by 1,9-decanediol and the two BNIs in soils differs from that in culture bioassays (Sun et al., 2016). Similarly, Sakuranetin, a hydrophilic flavan-on BNI exuded from sorghum showed strong inhibitory activity in culture assays, but its inhibitory function was lost in soil incubation experiments (Subbarao et al., 2013). Therefore, not all compounds with BNI activity detected in an *in vitro* culture bioassay are consistently effective in soil, and BNI activity should be confirmed using a soil system as part of the characterization of BNI function.

4.3. Effects of 1,9-decanediol on the abundance of ammonia oxidizers

Ammonia oxidizers play important roles in the nitrification processes of most terrestrial ecosystems. We observed that both AOB and AOA *amoA* gene abundance were significantly decreased after 1,9-decanediol application, whereas DCD only targeted AOB (Fig. 3). This is consistent with previous studies that showed that DCD could only inhibit nitrification through influencing the abundance and activity of AOB rather than AOA (Chen et al., 2015; O'Callaghan et al., 2010). Another BNI, MHPP, was also found to be capable of suppressing soil nitrification by reducing the abundance and activity of AOA and AOB (Nardi et al., 2013). These results suggest that both AOB and AOA can be the targets of BNIs, which might be another advantage of BNIs compared to synthetic NIs. Moreover, the inhibition of 1,9-decanediol on the abundance was particularly effective in red soil, which is in line with the inhibitory effect on PNA in this acidic soil, indicating the great potential of 1,9-decanediol for regulating ammonia oxidizers in acidic soils.

It is believed that the enzyme AMO for ammonia oxidation is involved in the 1,9-decanediol inhibitory mechanism of nitrification (Sun et al., 2016). Unlike bacterial ammonia oxidation, archaeal ammonia oxidation engages an electron transfer mechanism that heavily depends on copper due to a lack of cytochrome-c proteins (Walker et al., 2010). It is worth noting that many polyphenols can act as copper chelators, due to their typically abundant hydroxyl group (Fremont et al., 1999). It is not unreasonable to postulate a specific role for two hydroxyl groups of 1,9-decanediol in the enzyme activities involved in nitrification. Therefore, we suggest that 1,9-decanediol may lower the ability of ammonia oxidizers to obtain energy, inhibiting their growth, and thus decreasing the abundance of the products of the archaeal *amoA* gene. Further study is necessary to fully understand the molecular mechanism underlying the inhibitory effect of 1,9-decanediol on ammonia-oxidizing archaea.

4.4. Effects of 1,9-decanediol on the community composition of ammonia oxidizers

Since the dominant ammonia oxidizers among different soils are different, it is difficult for nitrification inhibitors to display consistently effective roles in different habitats. However, we found that the community composition of both AOB and AOA in different soil types can respond to 1,9-decanediol (Figs. 4 and 5). Specifically, the ammonia oxidizing bacteria targeted by 1,9-decanediol may be *Nitrosospira* cluster 3 and cluster 2. This is in agreement with previous findings that nitrification is mainly driven by *Nitrosospira* cluster 3 and cluster 2 in neutral and alkaline soils (Phillips et al., 2000; Chu et al., 2007; Ouyang et al., 2016). It is also interesting to note that the predominant AOB communities shifted to *Nitrosospira* clusters 8a and 8b after the addition of 1,9-decanediol in the paddy and red soils. These *Nitrosospira* clusters have been reported to be favored by the application of chemical nitrogen fertilizer (Zhong et al., 2016; Guo et al., 2017).

AOA showed a less sensitive response to 1,9-decanediol than AOB, with no significant impact on the proportion of the dominant *Nitrososphaera* cluster (also known as Group 1.1b thaumarchaeota) in the fluvo-aquic soil and paddy soil. However, the *Nitrosopumilus* cluster (also known as Group 1.1a) disappeared after the application of 1,9-decanediol in the acidic red soil (Fig. 5B), suggesting the strong potential of 1,9-decanediol in regulating AOA in the acidic soils. More specifically, we found that the AOA target for 1,9-decanediol could be

Nitrososphaera cluster 7 and the genus *Nitrosopumilus*. All widely applied commercial nitrification inhibitors, e.g. dicyandiamide (DCD), 3,4-dimethylpyrazole phosphate (DMPP) and nitrapyrin were only found to inhibit nitrifying bacteria while having little impact on AOA communities (Wang et al., 2016; Chen et al., 2015). Since soil AOA are the dominant nitrification contributors in many terrestrial ecosystems, particularly in acidic soils (Zhang et al., 2012), it is necessary to understand the influence and efficacy of nitrification inhibitors on nitrifying archaea (Li et al., 2018). Taking the response of ammonia oxidizers, in terms of abundance, to 1,9-decanediol, our results indicate that 1,9-decanediol possesses great potential to be developed as a new effective biological nitrification inhibitor for nitrifying archaea in acidic soils.

4.5. Effects of 1,9-decanediol on N_2O emissions

To our knowledge, this is the first study focusing on the effect of specific BNIs on greenhouse gas emissions. Of particular interest, we found that 1,9-decanediol completely suppresses N_2O emissions while DCD exhibited no significant effect in the AOA-dominated acidic soil (Fig. 6C); AOA abundance was affected only by 1,9-decanediol whereas AOB abundance was suppressed by both DCD and 1,9-decanediol (Fig. 3). These results provide evidence that AOA may play a more important role than AOB in controlling soil N_2O production in acidic soil. Physiological analyses of the first pure culture of the AOA *Nitrososphaera viennensis* demonstrated that AOA can produce N_2O at a similar rate and yield to the AOB *Nitrosospira multiformis* under oxic conditions (Stieglmeier et al., 2014). Therefore, N_2O production resulting from the activity of AOA and AOB in soil should be clearly distinguished in future studies. Since acidic soils (defined as soils with pH $<$ 5.5) occupy 30% of the world's ice-free lands, it is of great significance to develop effective nitrification inhibitors for ammonia archaea in acidic soils. Our observations here highlight the role of the novel BNI 1,9-decanediol in reducing N_2O emissions in terrestrial ecosystems, especially in acidic soils. It is also important to point out that nitrification in acidic soils can lead to further acidification and consequent aluminum toxicity (He et al., 2012), indicating the potential of 1,9-decanediol in controlling acidification and ensuing metal toxicities of soils more generally.

4.6. The possible mechanism of 1,9-decanediol inhibition

Low nitrification rates have been attributed to a decline in NH_4^+ availability rather than to the toxicity to nitrifiers (Schimel et al., 1996). It has therefore been argued that addition of organic compounds may represent a carbon source for soil microorganisms, which may favor heterotrophs that are better competitors for NH_4^+ than autotrophs. As a result, ammonium oxidizers would have limited NH_4^+ supply (NH_4^+ immobilization), and soil nitrification could be reduced indirectly by addition of a carbon source such as 1,9-decanediol.

The possibility of NH_4^+ immobilization was investigated and several lines of evidence suggest that it was of minor importance and that 1,9-decanediol exerted a more direct effect on ammonia oxidizers. First, nitrogen dynamics of the high-dose 1,9-decanediol treatment mirrored those seen for the commercially available nitrification inhibitor DCD and was distinctly different from the N-control treatments, both for nitrate and ammonium (Fig. 1). Second, the sum of both N forms at day 7 indicates that NH_4^+ immobilization did not occur in the 1,9-decanediol treatments in the three soils (NO_3^- concentration increased concomitant with the decrease in NH_4^+) (Fig. S1), while it was a factor for the glucose treatment where rapid disappearance of NH_4^+ was not followed by NO_3^- production (Nardi et al., 2013). Thus, lower nitrate concentrations on day 7 in the high-dose 1,9-decanediol and DCD treatments can be attributed to reduced nitrification and not to any change in NH_4^+ availability. This was further confirmed by the analysis of PNA using the soil-slurry method (Fig. 2). The ammonia-oxidizing activity was high in the N control treatment but inhibited (around 50–80% inhibition compared to the N control) at both 1,9-decanediol concentrations and in the DCD treatment (not in the red soil). Since PNA was measured in soil samples supplemented with an excess of NH_4^+ and the assay was continuously shaken to ensure an aerobic condition, the possibility that substrate unavailability or denitrification played a role in the reduced nitrification rates in the 1,9-decanediol and DCD treatments can be ruled out. The last line of evidence for a direct effect of 1,9-decanediol was provided by the analysis of soil microbial respiration using the substrate-induced respiration (SIR) method. No significant effect of 1,9-decanediol was observed on CO_2 emissions among three soils (Fig. S2), suggesting that the observed inhibition of potential nitrification was probably due to a direct effect of 1,9-decanediol targeting ammonia oxidizers. Similar results showed that procyanidins, biological denitrification inhibitors released by the invasive species *Fallopia spp.*, inhibited denitrification enzyme activity but not SIR in soil (Bardon et al., 2017).

4.7. Concentration dependence and possible modes of application

The BNI effect of 1,9-decanediol appears to be stable and dose-dependent in soil incubation experiments. However, in the fluvo-aquic soil examined here, both the decreased inhibition rate and NH_4^+ immobilization in the high 1,9-decanediol treatment between days 7 and 14 days, suggest microbial degradation and uptake after 7 days (Marsden et al., 2016). This loss of activity may be recovered in rice fields by a continuous or pulsed release of 1,9-decanediol during the growing season. In addition, the spatio-temporal characteristics of BNIs, due to their continuous release, could provide a better match for the dynamics of ammonia oxidizers and the NH_4^+ substrate itself than commercial NIs. It is challenging to quantify BNIs in soil culture due to difficulties inherent in the extraction process (Tesfamariam et al., 2014). We therefore estimated 1,9-decanediol concentrations in the rhizosphere (60 mg kg^{-1} soil day^{-1}, Supplementary Text 1) based on the concentrations found in the water phase of the rice hydroponic culture (Sun et al., 2016). Given that a large portion of 1,9-decanediol exuded from rice roots may be confined to the root surface due to its hydrophobicity rather than freely diffusing in the water phase, the total 1,9-decanediol amount released by rice roots may be substantially underestimated. Therefore, one may speculate that our low and high doses of 1,9-decanediol added in the soil incubation experiments can indeed occur in rice fields.

In addition, BNI concentrations in soil incubation experiments conducted by others (Subbarao et al., 2008, 2013; Nardi et al., 2013; Tesfamariam et al., 2014) range from 0 to 2000 mg BNI kg^{-1} soil, and we note that our high dose falls into the middle of this range. We had also designed the 1,9-decanediol doses in part on C equivalents used in previous soil incubation studies where other allelochemicals were added (Vivanco et al., 2004). The high dose of 1,9-decanediol (equivalent to 680 mg C·kg^{-1} soil) falls into the range of such C additions. Moreover, previous studies have highlighted that allelochemicals can be released into the soil in unexpectedly large quantities in nature. Concentrations of catechin, a phenolic compound exuded from the roots of *Centaurea maculosa*, have varied from 0 to 800 mg C·kg^{-1} soil over time (Perry et al., 2007), suggesting the possibility that secondary metabolites may be released in pulses.

Regarding the high effective dose of 1,9-decanediol in soil incubation experiments, it is important to consider practical application methods to reduce the amount of 1,9-decanediol applied while maintaining its inhibition role. First, the examination of whether planting of high-BNI-releasing cultivars alone is sufficient to inhibit nitrification, instead of adding BNI chemicals, is important. For example, Karwat et al. (2017) found a residual effect of BNIs exuded by *Brachiaria humidicola* in a pasture on nitrogen recovery and grain yield of subsequently planted maize. Additionally, the examination of exudate synergisms deserves scrutiny. Given that rice root exudates are a mixture

of compounds, and that 1,9-decanediol could affect nitrification synergistically with other exudates having BNI activity, this is an important further step of exploration. Our ongoing work shows that a hydrophilic BNI from rice root exudate, SA (a phenolic acid), can enhance the nitrification inhibitory activity of 1,9-decanediol (data not shown). That is to say, the concentration of 1,9-decanediol required for an inhibitory effect on nitrification may be significantly reduced by SA, which might point to another means for BNIs (BNI combinations) to be applied to soils, at lower concentrations. Furthermore, with the advent of material technology, BNIs could be modified using novel materials (e.g. nanomaterials) to reduce the effective dose and to produce higher persistence in soils.

5. Conclusions

In this study, we demonstrated for the first time that a compound exuded from rice root exudates, 1,9-decanediol, can act as biological nitrification inhibitor in soils, most likely through inhibiting the growth and structures of both AOB and AOA rather than affecting soil NH_4^+ availability. Moreover, our findings showed that 1,9-decanediol has the ability to reduce soil N_2O emissions, especially in acidic soils. Given the excellent role of 1,9-decanediol in regulating AOA, the present findings represent a significant step toward the design and development of new effective inhibitors for ammonia archaea in acidic soils.

Acknowledgements

This work was supported by funded by grants from the Natural Science Foundation of Jiangsu Province (BK20151053), the Strategic Priority Research Program (B)-'Soil-microbial system function and regulation' of the Chinese Academy of Sciences (XDB15030100), the National Natural Science Foundation of China (31501836 and 31761143015), the Leading Project of the Institute of Soil Science, Chinese Academy of Sciences (ISSASIP1606).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2018.11.008.

References

Amberger, A., 1989. Research on dicyandiamide as a nitrification inhibitor and future outlook. Communications in Soil Science and Plant Analysis 20, 1933–1955.
Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry 10, 215–221.
Avrahami, S., Conrad, R., 2003. Patterns of community change among ammonia oxidizers in meadow soils upon long-term incubation at different temperatures. Applied and Environmental Microbiology 69, 6152–6164.
Avrahami, S., Conrad, R., Braker, G., 2002. Effect of soil ammonium concentration on N_2O release and on the community structure of ammonia oxidizers and denitrifiers. Applied and Environmental Microbiology 68, 5685–5692.
Bardon, C., Poly, F., Haichar, F.E., Le Roux, X., Simon, L., Meiffren, G., Comte, G., Rouifed, S., Piola, F., 2017. Biological denitrification inhibition (BDI) with procyanidins induces modification of root traits, growth and N status in Fallopia x bohemica. Soil Biology and Biochemistry 107, 41–49.
Brzozowska, A.M., Mugele, F., Duits, M.H.G., 2013. Stability and interactions in mixed monolayers of fatty acid derivatives on Artificial Sea Water. Colloids and Surfaces A: Physicochemical and Engineering Aspects 433, 200–211.
Chen, X., Zhang, L.M., Shen, J.P., Xu, Z., He, J.Z., 2010. Soil type determines the abundance and community structure of ammonia-oxidizing bacteria and archaea in flooded paddy soils. Journal of Soils and Sediments 10, 1510–1516.
Chen, Q.H., Qi, L.Y., Bi, Q.F., Dai, P.B., Sun, D.S., Sun, C.L., Liu, W.J., Lu, L.L., Ni, W.Z., Lin, X.Y., 2015. Comparative effects of 3,4-dimethylpyrazole phosphate (DMPP) and dicyandiamide (DCD) on ammonia-oxidizing bacteria and archaea in a vegetable soil. Applied Microbiology and Biotechnology 99, 477–487.
Chu, H.Y., Fuji, T., Morimoto, S., Lin, X.G., Yagi, K., Hu, J.L., Zhang, J.B., 2007. Community structure of ammonia-oxidizing bacteria under longterm application of mineral fertilizer and organic manure in a sandy loam soil. Applied and Environmental Microbiology 73, 485–491.
Coskun, D., Britto, D.T., Shi, W.M., Kronzucker, H.J., 2017a. Nitrogen transformations in modern agriculture and the role of biological nitrification inhibition. Nature Plants 3, 17074.
Coskun, D., Britto, D.T., Shi, W.M., Kronzucker, H.J., 2017b. How plant root exudates shape the nitrogen cycle. Trends in Plant Science 22, 661.
Daims, H., Lebedeva, E.V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R.H., von Bergen, M., Rattei, T., Bendinger, B., Nielsen, P.H., Wagner, M., 2015. Complete nitrification by *Nitrospira* bacteria. Nature 528, 504–509.
Di, T.J., Afzal, M.R., Yoshihashi, T., Deshpande, S., Zhu, Y.Y., Subbarao, G.V., 2018. Further insights into underlying mechanisms for the release of biological nitrification inhibitors from sorghum roots. Plant and Soil 423, 99–110.
Ding, W.X., Yu, H.Y., Cai, Z.C., 2011. Impact of urease and nitrification inhibitors on nitrous oxide emissions from fluvo-aquic soil in the North China Plain. Biology and Fertility of Soils 47, 91–99.
Fisk, L.M., Maccarone, L.D., Barton, L., Murphy, D.V., 2015. Nitrapyrin decreased nitrification of nitrogen released from soil organic matter but not *amoA* gene abundance at high soil temperature. Soil Biology and Biochemistry 88, 214–223.
Fremont, L., Belguendouz, L., Delpal, S., 1999. Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. Life Sciences 64, 2511–2521.
Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z.C., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320, 889–892.
Guo, J.J., Ling, N., Chen, H., Zhu, C., Kong, Y.L., Wang, M., Shen, Q.R., Guo, S.W., 2017. Distinct drivers of activity, abundance, diversity and composition of ammonia-oxidizers: evidence from a long-term field experiment. Soil Biology and Biochemistry 115, 403–414.
Hart, S.C., Stark, J.M., Davidson, E.A., Firestone, M.K., 1994. Nitrogen mineralization, immobilization, and nitrification. In: Weaver, R.W., Angle, J.S., Bottomley, B.S. (Eds.), Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties. Soil Science Society of America, Madison, pp. 985–1018.
He, J.Z., Hu, H.W., Zhang, L.M., 2012. Current insights into the autotrophic thaumarchaeal ammonia oxidation in acidic soils. Soil Biology and Biochemistry 55, 146–154.
Hilmarsson, H., Traustason, B.S., Kristmundsdottir, T., Thormar, H., 2007. Virucidal activities of medium- and long-chain fatty alcohols and lipids against respiratory syncytial virus and parainfluenza virus type 2: comparison at different pH levels. Archives of Virology 152, 2225–2236.
Jang, J.H., Park, Y.D., Ahn, H.K., Kim, S.J., Lee, J.Y., Kim, E.C., Chang, Y.S., Song, Y.J., Kwon, H.J., 2014. Analysis of green tea compounds and their stability in dentifrices of different pH levels. Chemical and Pharmaceutical Bulletin 62, 328–335.
Jia, Z.J., Conrad, R., 2009. Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. Environmental Microbiology 11, 1658–1671.
Karwat, H., Moreta, D., Arango, J., Nunez, J., Rao, I., Rincon, A., Rasche, F., Cadisch, G., 2017. Residual effect of BNI by *Brachiaria humidicola* pasture on nitrogen recovery and grain yield of subsequent maize. Plant and Soil 420, 389–406.
Li, Y.L., Kronzucker, H.J., Shi, W.M., 2016. Microprofiling of nitrogen patches in paddy soil: analysis of spatiotemporal nutrient heterogeneity at the microscale. Scientific Reports 6, 27064.
Li, Y.Y., Chapman, S.J., Nicol, G.W., Yao, H.Y., 2018. Nitrification and nitrifiers in acidic soils. Soil Biology and Biochemistry 116, 290–301.
Liu, R., Hayden, H., Suter, H., He, J.Z., Chen, D.L., 2015. The effect of nitrification inhibitors in reducing nitrification and the ammonia oxidizer population in three contrasting soils. Journal of Soils and Sediments 15, 1113–1118.
Magoc, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963.
Marsden, K.A., Marín-Martínez, A.J., Vallejo, A., Hill, P.W., Jones, D.L., Chadwick, D.R., 2016. The mobility of nitrification inhibitors under simulated ruminant urine deposition and rainfall: a comparison between DCD and DMPP. Biology and Fertility of Soils 52, 491–503.
McCarty, G.W., Bremner, J.M., 1989. Laboratory evaluation of dicyandiamide as a soil nitrification inhibitor. Communications in Soil Science and Plant Analysis 20, 2049–2065.
Mukherjee, K., Tribedi, P., Mukhopadhyay, B., Sil, A.K., 2013. Antibacterial activity of long-chain fatty alcohols against mycobacteria. FEMS Microbiology Letters 338, 177–183.
Nardi, P., Akutsu, M., Pariasca-Tanaka, J., Wissuwa, M., 2013. Effect of methyl 3-4-hydroxyphenyl propionate, a Sorghum root exudate, on N dynamic, potential nitrification activity and abundance of ammonia-oxidizing bacteria and archaea. Plant and Soil 367, 627–637.
Nicol, G.W., Leininger, S., Schleper, C., Prosser, J.I., 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. Environmental Microbiology 10, 2966–2978.
O'Callaghan, M., Gerard, E.M., Carter, P.E., Lardner, R., Sarathchandra, U., Burch, G., Ghani, A., Bell, N., 2010. Effect of the nitrification inhibitor dicyandiamide (DCD) on microbial communities in a pasture soil amended with bovine urine. Soil Biology and Biochemistry 42, 1425–1436.
Ouyang, Y., Norton, J.M., Stark, J.M., Reeve, J.R., Habteselassie, M.Y., 2016. Ammonia-oxidizing bacteria are more responsive than archaea to nitrogen source in an agricultural soil. Soil Biology and Biochemistry 96, 4–15.
Park, S.J., Park, B.J., Rhee, S.K., 2008. Comparative analysis of archaeal 16S rRNA and *amoA* genes to estimate the abundance and diversity of ammonia-oxidizing archaea in marine sediments. Extremophiles 12, 605–615.
Perry, L.G., Thelen, G.C., Ridenourm, W.M., Callaway, R.M., Paschke, M.W., 2007. Concentrations of the allelochemical (±)- catechin in Centaurea maculosa soils. Journal of Chemical Ecology 33, 2337–2344.
Pester, M., Rattei, T., Flechl, S., Gröngröft, A., Richter, A., Overmann, J., Reinhold-Hurek,

B., Loy, A., Wagner, M., 2012. *amoA*-based consensus phylogeny of ammonia-oxidizing archaea and deep sequencing of *amoA* genes from soils of four different geographic regions. Environmental Microbiology 14, 525–539.
Phillips, C.J., Harris, D., Dollhopf, S.L., Gross, K.L., Prosser, J.I., Paul, E.A., 2000. Effects of agronomic treatments on structure and function of ammonia-oxidizing communities. Applied and Environmental Microbiology 66, 5410–5418.
Ruser, R., Schulz, R., 2015. The effect of nitrification inhibitors on the nitrous oxide (N_2O) release from agricultural soils—a review. Journal of Plant Nutrition and Soil Science 178, 171–188.
Schimel, J.P., Van Cleve, K., Cates, R.G., Clausen, T.P., Reichardt, P.B., 1996. Effects of balsam poplar (Populus balsamifera) tannins and low molecular weight phenolics on microbial activity in taiga floodplain soil: implications for changes in N cycling during succession. Canadian Journal of Botany 74, 84–90.
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75, 7537–7541.
Shi, X.Z., Hu, H.W., Muller, C., He, J.Z., Chen, D.L., Suter, H.C., 2016. Effects of the nitrification inhibitor 3,4-dimethylpyrazole phosphate on nitrification and nitrifiers in two contrasting agricultural soils. Applied and Environmental Microbiology 82, 5236–5248.
Stieglmeier, M., Mooshammer, M., Kitzler, B., Wanek, W., Zechmeister-Boltenstern, S., Richter, A., Schleper, C., 2014. Aerobic nitrous oxide production through N-nitrosating hybrid formation in ammonia-oxidizing archaea. The ISME Journal 8, 1135–1146.
Subbarao, G.V., Ito, O., Sahrawat, K.L., Berry, W.L., Nakahara, K., Ishikawa, T., Watanabe, T., Suenaga, K., Rondon, M., Rao, I.M., 2006a. Scope and strategies for regulation of nitrification in agricultural systems — challenges and opportunities. Critical Reviews in Plant Sciences 25, 303–335.
Subbarao, G.V., Ishikawa, T., Ito, O., Nakahara, K., Wang, H.Y., Berry, W.L., 2006b. A bioluminescence assay to detect nitrification inhibitors released from plant roots: a case study with *Brachiaria humidicola*. Plant and Soil 288, 101–112.
Subbarao, G.V., Tomohiro, B., Masahiro, K., Osamu, I., Samejima, H., Wang, H.Y., Pearse, S.J., Gopalakrishnan, S., Nakahara, K., Hossain, A.K.M.Z., Tsujimoto, H., Berry, W.L., 2007. Can biological nitrification inhibition (BNI) genes from perennial *Leymus racemosus* (Triticeae) combat nitrification in wheat farming? Plant and Soil 299, 55–64.
Subbarao, G.V., Nakahara, K., Ishikawa, T., Yoshihashi, T., Ito, O., Ono, H., Ohnishi-Kameyama, M., Yoshida, M., Kawano, N., Berry, W.L., 2008. Free fatty acids from the pasture grass *Brachiaria humidicola* and one of their methyl esters as inhibitors of nitrification. Plant and Soil 313, 89–99.
Subbarao, G.V., Nakahara, K., Hurtado, M.P., Ono, H., Moreta, D.E., Salcedo, A.F., Yoshihashi, A.T., Ishikawa, T., Ishitani, M., Ohnishi-Kameyama, M., Yoshida, M., Rondon, M., Rao, I.M., Lascano, C.E., Berry, W.L., Ito, O., 2009. Evidence for biological nitrification inhibition in *Brachiaria* pastures. Proceedings of the National Academy of Sciences, USA 106, 17302–17307.
Subbarao, G.V., Nakahara, K., Ishikawa, T., Ono, H., Yoshida, M., Yoshihashi, T., Zhu, Y.Y., Zakir, H.A.K.M., Deshpande, S.P., Hash, C.T., Sahrawat, K.L., 2013. Biological nitrification inhibition (BNI) activity in sorghum and its characterization. Plant and Soil 366, 243–259.
Sun, L., Lu, Y.F., Yu, F.W., Kronzucker, H.J., Shi, W.M., 2016. Biological nitrification inhibition by rice root exudates and its relationship with nitrogen-use efficiency. New Phytologist 212, 646–656.
Tanaka, J.P., Nardi, P., Wissuwa, M., 2010. Nitrification inhibition activity, a novel trait in root exudates of rice. AoB Plants 2010, plq014.
Tesfamariam, T., Yoshinaga, H., Deshpande, S.P., Rao, P.S., Sahrawat, K.L., Ando, Y., Nakahara, K., Hash, C.T., Subbarao, G.V., 2014. Biological nitrification inhibition in sorghum: the role of sorgoleone production. Plant and Soil 379, 325–335.
Togashi, N., Shiraishi, A., Nishizaka, M., Matsuoka, K., Endo, K., Hamashima, H., Inoue, Y., 2007. Antibacterial activity of long-chain fatty alcohols against *Staphylococcus aureus*. Molecules 12, 139–148.
Vivanco, J.M., Bais, H.P., Stermitz, F.R., Thelen, G., Callaway, R., 2004. Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. Ecological Letters 7, 285–292.
Walker, C.B., De La Torre, J.R., Klotz, M.G., Urakawa, H., Pinel, N., Arp, D.J., Brochier, A.C., Chain, P.S.G., Chan, P.P., Gollabgir, A., 2010. *Nitrosopumilus maritimus* genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaea. Proceedings of the National Academy of Sciences, USA 107, 8818–8823.
Wang, Q., Zhang, L.M., Shen, J.P., Du, S., Han, L.L., He, J.Z., 2016. Effects of dicyandiamide and acetylene on N_2O emissions and ammonia oxidizers in a fluvo-aquic soil applied with urea. Environmental Science & Pollution Research 23, 23023–23033.
Wolt, J.D., 2000. Nitrapyrin behavior in soils and environmental considerations. Journal of Environmental Quality 29, 367–379.
Zakir, H.A.K.M., Subbarao, G.V., Pearse, S.J., Gopalakrishnan, S., Ito, O., Ishikawa, T., Kawano, N., Nakahara, K., Yoshihashi, T., Ono, H., Yoshida, M., 2008. Detection, isolation and characterization of a root-exuded compound, methyl 3-(4-hydroxyphenyl) propionate, responsible for biological nitrification inhibition by sorghum (*Sorghum bicolor*). New Phytologist 180, 442–451.
Zhang, H.J., Wu, Z.J., Zhou, Q.X., 2004. Dicyandiamide sorption-desorption behaviour on soils and peat humus. Pedosphere 14, 395–399.
Zhang, L.M., Hu, H.W., Shen, J.P., He, J.Z., 2012. Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils. The ISME Journal 6, 1032–1045.
Zhong, W., Bian, B., Gao, N., Min, J., Shi, W., Lin, X., Shen, W., 2016. Nitrogen fertilization induced changes in ammonia oxidation are attributable mostly to bacteria rather than archaea in greenhouse-based high N input vegetable soil. Soil Biology and Biochemistry 93, 150–159.