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Full Length Research Paper

Laboratory study of the effects of nitrification inhibitors on the abundance of ammonia-oxidizing bacteria and archaea in a Hap-Ustic Luvisol

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Nitrification inhibitors (NIs) could slow down nitrification by suppressing ammonia oxidizers. Responses of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) to NIs were studied in grassland soil and acidic agricultural soil, but we know little about effects of NIs and NIs *per se* on AOA and AOB abundance in neutral agricultural soil. We aimed to investigate the effects of NIs, dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP), on the dynamics of AOA and AOB populations in a Hap-Ustic Luvisol. Laboratory studies were installed to observe the effects of DCD and DMPP on the abundance of AOA and AOB. We determined the dynamics of AOA and AOB populations during 112 days by using real-time PCR targeting *amoA* genes. Soil mineral nitrogen contents and potential ammonia oxidation were measured. AOB growth was stimulated by ammonium addition and reached peak value at day 14 in treatment N. When ammonium was applied with DCD or DMPP, AOB *amoA* copies decreased significantly. In contrast to AOB, AOA showed no response to the addition of ammonium and the two NIs during 91 days, even when DCD and DMPP applied were double dose. Nitrification inhibition rates of DCD and DMPP were highest at day 14. Although potential ammonia oxidation was suppreseed at day 14, AOA populations were still not affected by DCD or DMPP *per se*. Our results suggest that AOA were not stimulated by large amounts of inorganic ammonium exogenous and were tolerant to DCD and DMPP in a neutral agricultural soil.

Key words: 3,4-dimethylpyrazole phosphate (DMPP), dicyandiamide (DCD), mineral nitrogen, real-time PCR.

INTRODUCTION

Nitrification in soil, one of the key processes of nitrogen cycle, oxidizes ammonia to nitrite and then to nitrate, enhances losses of ammonium-based fertilizers applied to soil by leaching and denitrification of nitrate, and is a main source of N_2O and NO to the atmosphere (Zerulla et al., 2001; Macadam et al., 2003). Nitrification inhibitors have been applied to improve nitrogen use efficiency via suppressing nitrification for several decades. Dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) have been proven to be effective in reducing nitrification rates and nitrate leaching in agricultural soils. In the past, most studies focused on the effects of nitrification inhibitors on soil nitrogen transformation and crop yield. With development of molecular technology, many researches focused on the study of effects of nitrification inhibitors on soil ammonia oxidizers.

Ammonia-oxidizing bacteria (AOB) were traditionally considered to dominate ammonia oxidation, which is the first and rate-limiting step in soil(Barraclough and Puri, 1995, Boer and Kowalchuk, 2001). However, contribution of ammonia-oxidizing archaea (AOA) to ammonia oxidation was expected with discovery of large numbers of AOA (Leininger et al., 2006; He et al., 2007). As

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expected, AOA were found to dominate soil ammonia oxidation in several studies (Offre et al., 2009; Gubry-Rangin et al., 2010). In contrast, Di et al. (2009) suggested that AOB rather than AOA dominate nitrification in nitrogen-rich grassland soils by application of DCD. In addition, AOA growth was not suppressed by DCD (O'Callaghan et al., 2010) and even increased in root-rhizosphere complex (pH 5.5) of cauliflower when DMPP was applied (Kleineidam et al., 2011). Liquid formulation of DMPP was as effective as DCD on inhibiting AOB amoA copies in six New Zealand grassland soils (Di and Cameron, 2011).

As soil pH is a key factor affecting abundance and diversity of ammonia oxidizers (Nicol et al., 2008) and ammonium availability decreased with decreasing soil pH (Boer and Kowalchuk, 2001), and land use manners influenced distribution of ammonia oxidizers (Keil et al., 2011), limited information is available concerning the effects of DCD and DMPP on the AOB and AOA abundance in neutral agricultural soil. Therefore, we investigated in a laboratory study, the effects of DCD and DMPP on the dynamic changes of AOA and AOB abundance in a neutra l agricultural soil. Additionally, in order to eliminate impact of nitrogen exogenous on AOA, we determined the effects of DCD and DMPP *per se* on AOA abundance. The abundance of AOA and AOB was quantified by real-time PCR targeting *amoA* genes.

MATERIALS AND METHODS

Surface (0-20 cm) cinnamon soil (Hap-Ustic Luvisol, WRB 1998) was collected from the Experimental Station of Chaoyang (41°49'N, 122°48'E) in Liaoning Province of Northeast China. Soil pH (soil:water was 1:2.5) was 7.3. Particle size distribution was sand = 43%, silt = 38% and clay = 19%. Total carbon and nitrogen were 11.5 g kg^{-1} and 1.1 g kg^{-1}, respectively. Initial potential ammonia oxidation (PAO) was 1.98 mg NO_2^--N kg^{-1} soil 5 h^{-1}.

After the field moist was determined, soil was crushed to pass through a 2-mm sieve. Then, it was further thoroughly mixed and pre-incubated at 40% water filled pore space (WFPS) for 2 weeks at 25°C in the dark.

Experiment 1

Six treatments: (1) Control (no nitrogen added); (2) N (ammonium sulphate); (3) N+DCD1 (ammonium sulphate + DCD1); (4) N+DCD2 (ammonium sulphate + DCD2);(5) N+DMPP1 (ammonium sulphate + DMPP1) and (6) N+DMPP2 (ammonium sulphate + DMPP2) were replicated thrice.

In each triplicate per treatment, equivalent to 50 g air-dried soil was placed in 150 ml centrifuge tubes with lids. Ammonium sulphate was applied at a rate of 0.5 g N kg^{-1} air-dried soil (almost equivalent to 1300 kg N ha^{-1}), and that of DCD and DMPP was 1 and 2% N in treatments N+DCD1 and N+DMPP1, respectively. The amounts of DCD and DMPP applied were double in treatments N+DCD2 and N+DMPP2. DCD and DMPP were supplied by Shanghai Chemical Institute, with a purity of 99.5 and 97%, respectively.

Ammonium sulphate and nitrification inhibitors were applied in liquid solution. A vacuum method was chosen to mix the solution with the soil well (Cavagnaro et al., 2008) and make soil water content reach 60% WFPS. To maintain soil water content, sample tubes were covered with lids and weighed at 2 day intervals by dripping water to the initial weight. All samples were placed in a temperature controlled incubator (LTI- 1001SD, EYELA, Japan) at 25°C in the dark.

At day 1, 3, 5, 7, 14, 28, 49, 70, 91 and 112 after incubation, three replicates for each treatment were destructively sampled to determine soil mineral nitrogen contents and abundance of AOA and AOB.

Experiment 2

In order to eliminate impact of nitrogen exogenous on AOA, we determined the effects of DCD and DMPP *per se* on AOA abundance. Ten treatments with three replicates were installed. Other four treatments were added besides the six treatments in Experiment 1. The four treatments (DCD1, DCD2, DMPP1 and DMPP2) represent single and double dose DCD and DMPP without application of ammonium sulphate, respectively. The incubation was the same way as that in Experiment 1. Due to nitrification inhibition, rates of DCD and DMPP were highest at day 14 in Experiment 1; we sampled at day 14 after incubation to determine mineral nitrogen contents, PAO and AOA populations.

About 10 g soil was taken from each replicate in both experiments and immediately stored at -60°C. The remaining soil was applied to determine the mineral nitrogen contents and PAO. Soil NH_4^+-N and NO_3^--N concentrations were immediately analyzed by AutoAnalyzer III Continuous Flow Analyzer (Bran+Luebbe, Norderstedt, Germany) after extraction with 2 M KCl solution. Soil PAO was obtained by a chlorate inhibition method (Hart et al., 1994).

Based on previous studies (McCarty and Bremner, 1989), nitrification inhibition rate (%) was calculated as follows: (C-T)/C × 100, where T is the amount of (NO_3^-+NO_2^-)-N produced in the soil sample treated with nitrification inhibitor and C is the amount of (NO_3^-+NO_2^-)-N produced by the control sample. Only NO_3^--N was calculated because the NO_2^--N produced was easily oxidized in the soil.

Soil DNA was extracted from 0.5 g soil by using a FastDNA® Spin kit for soil (Qbiogene, Inc., Irvine, CA). The purity of DNA was checked by using Nanodrop® ND-1000 UV-Vis Spectrophotometer. The extracted soil DNA was then stored at -20°C for subsequent molecular analysis.

According to the protocol described in previous studies (Zhang et al., 2009), the copy numbers of archaeal and bacterial amoA genes were determined by real-time PCR, using an iCycler iQ 5 Thermocycler (Bio-Rad, USA) with the fluorescent dye SYBR-Green I. The DNA extracts were ten-fold diluted, and used as template with a final content of 1-9 ng in each reaction mixture. The primer pairs (amoA 1F/amoA 2R and Arch-amoA AF/Arch-amoA AR) targeted the amoA gene of the AOB and AOA DNA (Rotthauwe et al., 1997; Francis et al., 2005) Amplifications were carried out in 25 µl reaction mixtures, including 12.5 µl SYBR® Premix Ex TaqTM (TaKaRa, Japan), 1 µl bovine serum albumin (25mg ml-1), 0.5 µl each primer (10 µM) and 1 µl DNA template. Cycling conditions were 95°C for 1 min, followed by 40 cycles of 10 s at 95°C, 30 s at 55°C for AOB or 53°C for AOA, 1 min at 72 and plate read at 83°C. A melting curve analysis was performed to check PCR products specificity after amplification, and PCR products were confirmed by standard 1% agarose gel electrophoresis. PCR products were gel-purified and ligated into the pGEM-T Easy Vector (Promega, Madison), and the resulting ligation products were transformed into *Escherichia coli* JM109 competent cells. The positive clones were selected to extract plasmid DNA after re-amplification with the vector-specific primers T7 and SP6. The concentration of plasmid was determined on a Nanodrops ND-1000 UV-Vis Spectrophotometer and the copy numbers of amoA genes were

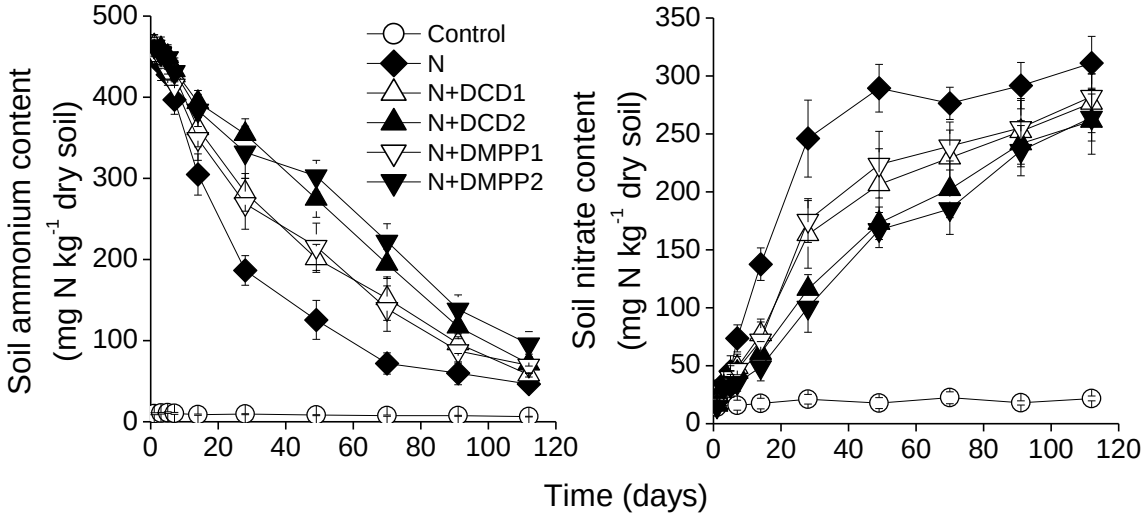

Figure 1. Dynamics of soil NH_4^+-N and NO_3^--N contents during 112 days. Control, no nitrogen added; N, ammonium sulphate; N+DCD1, N+DCD2, N+DMPP1 and N+DMPP2 represent ammonium sulphate combined with single and double dose DCD and DMPP, respectively. Points are average values from three replications. Vertical bars indicate standard deviations of the mean.

Table 1. Nitrification inhibition rate of DCD and DMPP (%).

Treatment[a]	Incubation time (day)								
	3	**5**	**7**	**14**	**28**	**49**	**70**	**91**	**112**
N+DCD1	29±4^{ab}	35±3^{a}	44±4^{ab}	50±3^{a}	36±3^{a}	30±2^{ab}	18±3^{ab}	14±1^{ab}	11±1^{a}
N+DCD2	34±3^{a}	36±2^{a}	56±5^{ab}	70±2^{b}	69±4^{b}	53±2^{c}	38±2^{c}	31±3^{b}	26±3^{b}
N+DMPP1	33±3^{a}	37±3^{a}	40±2^{a}	51±2^{a}	28±1^{a}	22±3^{a}	12±3^{a}	11±1^{a}	8±1^{a}
N+DMPP2	31±2^{a}	40±3^{a}	63±2^{b}	71±4^{b}	63±4^{b}	44±2^{bc}	34±1^{bc}	22±1^{ab}	15±1^{a}

[a]Treatments N+DCD1, N+DCD2, N+DMPP1 and N+DMPP2 represent ammonium sulphate combined with single and double dose DCD and DMPP combined with ammonium sulphate, respectively. [b]Mean ± SD (n=3). Values within the same column followed by the same letter do not differ at $P < 0.05$.

calculated directly from the contribution of the extracted plasmid DNA. A ten-fold dilution series of a known copy number of the plasmid DNA in triplicate was used as a standard. AOB amoA amplification efficiencies of 89 to 103% were obtained with R values of 0.967 and 0.999, and AOA *amoA* amplification efficiencies of 87 to 101% were obtained with R values of 0.978 and 0.996.

ANOVA combined with Duncan-test was used to estimate the significant differences ($P < 0.05$) between treatments. All values were mean values of three independent replicates expressed on an oven-dried soil weight (105°C, 24 h). Data analysis was calculated using SPSS version 16.0.

RESULTS

Experiment 1

Soil NH_4^+-N and NO_3^--N contents

Soil ammonium content in treatment N decreased sharply and was significantly lower than that in treatments N+DCD1, N+DCD2, N+DMPP1 and N+DMPP2 at day 14 till day 91 (Figure 1).

The applied double dose DCD or DMPP (treatment N+DCD2 and N+DMPP2) could maintain higher soil ammonium contents than single dose DCD or DMPP (treatment N+DCD1 and N+DMPP1) from day 28 to day 70.

In contrast to the soil ammonium contents, soil nitrate contents increased with increasing incubation period under the application of ammonium sulphate (Figure 1). From day 14, soil nitrate contents in treatment N were substantially more than those in other treatments. Compared to N+DCD1 and N+DMPP1, the double dose DCD and DMPP applied slowed the rate of nitrate produced significantly from day 49 to 91.

Table 1 shows that the nitrification inhibition rates of the two nitrification inhibitors varied with the incubation

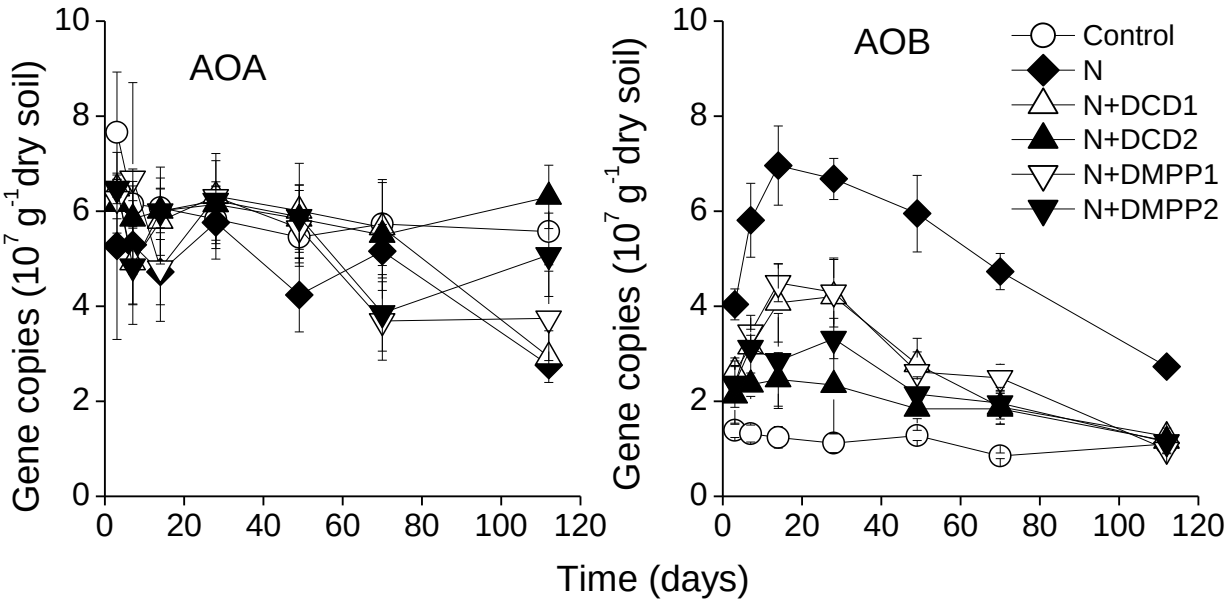

Figure 2. Dynamics of AOA and AOB *amoA* copies in the soil during 112 days. Points are average values from three replications. Vertical bars indicate standard deviations of the mean.

period. From day 3, the nitrification inhibition rates increased and had maximum values at day 14, and then dropped gradually. Based on the nitrification inhibition rates, double dose DCD and DMPP were more effective than single dose from day 14 to day 70, respectively.

During the first 91 day incubation, no significant difference on mineral nitrogen contents and nitrification inhibition rates was found between treatments N+DCD1 and N+DMPP1, and similar phenomenon was observed between N+DCD2 and N+DMPP2.

Abundance of soil AOB and AOA

Figure 2 shows the different responses of AOA and AOB abundance to ammonium addition. Among all the treatments, AOA *amoA* gene copy numbers showed no significant differences during the whole incubation period except at day 112.

AOB *amoA* gene copy numbers increased rapidly in treatment N and were significantly larger than those in the control treatment (Figure 2). Ratios of AOB abundance in treatment N to that in control treatment ranged from 2.48 to 5.96 during the whole incubation period. However, when DCD or DMPP were applied with ammonium sulphate, the growth of AOB was suppressed significantly compared to that in treatment N. It was the same as DCD, no matter the DMPP applied was single dose or double dose, there were no significant differences on suppressing the growth of AOB. Between treatment N+DCD1 and N+DMPP1, the inhibition effects on the growth of AOB were not significant, and similar results were found between treatment N+DCD2 and N+DMPP2.

During the whole incubation period, ratios of AOA to AOB ranged from 4.26 to 6.73 in the control treatment, from 0.68 to 1.30 in treatment N and from 1.07 to 5.38 in the other treatments. The results show that AOA were more abundant than AOB in the soil, except for rapid growth of AOB in treatment N.

Experiment 2

After 14 days incubation, the mineral nitrogen contents were not changed by the application of the two nitrification inhibitors only, compared to that in control treatment (Table 2). DCD and DMPP were as effective as they showed in Experiment 1.

The PAO was suppressed by application of DCD and DMPP alone except that in treatment DMPP1, compared to that in control treatment (Table 2). Addition of ammonium sulphate alone stimulated the PAO, whereas, the PAO was suppressed when ammonium was applied with DCD and DMPP. Among treatments N+DCD1, N+DCD2, N+DMPP1 and N+DMPP2, there were no significant differences on PAO.

Compared to AOA abundance in control treatment, AOA abundance in the other treatments remained largely unchanged after 14 days incubation (Table 2). This indicated that DCD and DMPP had no inhibitory effect on AOA in the soil.

Table 2 The mineral nitrogen contents, PAO and AOA *amoA* gene copies in the soil after 14 day incubation

Treatments[a]	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})	PAO (mg NO_2^--N kg^{-1} soil $5h^{-1}$)	AOA *amoA* copies (10^7 g^{-1} dry soil)
Control	8.51±0.70^{ab}	14.63±0.95^{a}	1.87±0.10^{de}	5.17±0.36^{ab}
DCD1	9.52±0.37^{a}	12.37±0.33^{a}	1.64±0.07^{bc}	5.17±0.19^{ab}
DCD2	9.75±0.49^{a}	12.50±0.26^{a}	1.04±0.04^{a}	5.35±0.41^{ab}
DMPP1	9.28±0.48^{a}	13.65±1.03^{a}	1.74±0.07^{cd}	4.89±0.66^{ab}
DMPP2	10.23±0.41^{a}	13.39±0.90^{a}	1.53±0.12^{b}	5.73±0.73^{b}
N	300.08±15.12^{b}	151.39±13.62^{d}	2.49±0.12^{f}	5.01±1.48^{ab}
N+DCD1	342.26±16.70^{cd}	100.85±5.30^{c}	1.97±0.20^{e}	4.32±0.52^{a}
N+DCD2	383.64±15.80^{e}	88.09±7.51^{b}	1.81±0.19^{cde}	4.53±0.96^{a}
N+DMPP1	329.91±8.89^{c}	95.33±7.95^{bc}	1.95±0.05^{de}	5.29±0.39^{ab}
N+DMPP2	361.74±22.44^{d}	89.52±6.78^{b}	1.82±0.05^{cde}	4.87±0.82^{ab}

[a]DCD1, DCD2, DMPP1 and DMPP2 represent single and double dose DCD and DMPP alone, respectively. [b]Mean ± SD (n=3). Values within the same column followed by the same letter do not differ at $P < 0.05$.

DISCUSSION

In Experiment 1, AOB grew rapidly when ammonium sulphate was added, which offered ammonia for AOB as substrate, as observed in microcosm and field conditions (Okano et al., 2004; Jia and Conrad, 2009). DCD and DMPP could suppress the growth of AOB significantly and maintain higher ammonium contents in the soil. In contrast, AOA showed no response to the addition of ammonium and/or the two nitrification inhibitors, even if the amount of DCD and DMPP applied doubled. Similar results were reported in previous studies, which found that AOA were more stable than AOB under the addition of DCD and DMPP (O'Callaghan et al., 2010, Di and Cameron, 2011). An ammonia oxidizing archaeon (*Nitrososphaera viennensis*) from soil was isolated by Tourna et al. (2011), who reported strain EN76 could convert ammonia to nitrite and grow well under 15 mM ammonium concentrations but was inhibited under 20 mM ammonium concentrations. Because the application rate of ammonium sulphate (0.5 g N kg^{-1} air-dried soil, almost equivalent to 1300 kg N ha^{-1}) in the study was far higher than 20 mM ammonium concentrations, so AOA could not grow well with high ammonium contents. In agricultural and grassland soils, it has been reported that AOB prefer high nitrogen conditions and AOA may be adapted to low nitrogen conditions (Mahmood and Prosser, 2006; Schauss et al., 2009; Di et al., 2010).

In order to eliminate the effects of high ammonium exogenous, AOA abundance was determined after incubation for 14 days with the two nitrification inhibitors alone in Experiment 2. Whereas, although no ammonium sulphate was added, AOA growth was not inhibited by DCD or DMPP even if the PAO was suppressed. This indicated that AOA was more tolerant than AOB to DCD and DMPP.

Different effects of the ammonium sulphate and nitrification inhibitors on AOB and AOA showed they adapted to different habitats due to their distinct physiological property (Valentine, 2007; Kleineidam et al., 2011).

Our results indicate that AOB rather than AOA made major contribution to ammonia oxidation in the agricultural soil with large amount inorganic ammonium inputs. This is in accordance with previous studies (Di et al., 2009; Jia and Conrad, 2009; Xia et al., 2011). However, the fact that AOA controlled ammonia oxidation in the soils was obtained by other researches (Offre et al., 2009; Stopnisek et al., 2010; Zhang et al., 2010). The tested soil in their studies was either acidic or high potential of mineralization, which could release ammonia for AOA utilization. In contrast, large amount of inorganic ammonium was added to the non-acidic soil in the experiment. Different soil physical and chemical characteristics resulted from different soil types, which determine different ecophysiological diversity of AOA (Chen et al., 2010). Hence, ammonia source, pH and species of AOA influenced contribution of AOA in soil.

As reported in previous studies (Leininger et al., 2006, He et al., 2007), ratios of AOA to AOB in all treatments except that in treatment N during all sampling dates showed AOA were more abundant than AOB in the soil. If the *amoA* copy numbers were converted to AOB and AOA cells by dividing by 2.5 and 1 (Norton et al., 2002, Wu et al., 2011), respectively, the phenomenon will be more obvious. Ammonium sulphate combined with DMPP or DCD mixed well with the soil in our study. This was different with field soil with plants, especially in the rhizosphere, where the ratio of AOA to AOB might differ.

Although some previous studies have shown that DMPP was more effective than DCD in reducing ammonium oxidation (Macadam et al., 2003; Chaves et al., 2006; Pereira et al., 2010), DMPP and DCD showed no significant differences in inhibiting the growth of AOB populations in the study. Similar results were obtained in six New Zealand grazed pastured soils (Di and Cameron,

2011). Soil properties, temperature and application form are factors affecting the effectiveness of nitrification inhibitors (Barth et al., 2001, Irigoyen et al., 2003, Di and Cameron, 2011), so the effects of nitrification inhibitors are variable in different soils under different conditions. In addition, although DMPP and DCD showed no significant differences in inhibiting AOB populations, AOB DNA from recently dead cells could not be avoided in the study.

RNA extraction will be applied to determine the effects of DMPP and DCD on activities of AOA and AOB in further study.

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REFERENCES

Barraclough D, Puri G (1995). The use of 15N pool dilution and enrichment to separate the heterotrophic and autotrophic pathways of nitrification. Soil Biol. Biochem. 27(1):17-22.

Barth G, von Tucher S, Schmidhalter U (2001). Influence of soil parameters on the effect of 3,4-dimethylpyrazole-phosphate as a nitrification inhibitor. Biol. Fert. Soils 34(2):98-102.

Boer WD, Kowalchuk GA (2001). Nitrification in acid soils: micro-organisms and mechanisms. Soil Biol. Biochem. 33:853-866.

Cavagnaro TR, Jackson LE, Hristova K, Scow KM (2008). Short-term population dynamics of ammonia oxidizing bacteria in an agricultural soil. Appl. Soil Ecol. 40(1):13-18.

Chaves B, Opoku A, De Neve S, Boeckx P, Van Cleemput O, Hofman G (2006). Influence of DCD and DMPP on soil N dynamics after incorporation of vegetable crop residues. Biol. Fert. Soils 43(1):62-68.

Chen X, Zhang LM, Shen JP, Xu ZH, He JZ (2010). Soil type determines the abundance and community structure of ammonia-oxidizing bacteria and archaea in flooded paddy soils. J. Soils Sed. 10(8):1510-1516.

Di HJ, Cameron KC (2011). Inhibition of ammonium oxidation by a liquid formulation of 3,4-Dimethylpyrazole phosphate (DMPP) compared with a dicyandiamide (DCD) solution in six new Zealand grazed grassland soils. J. Soils Sed. 11(6):1032-1039.

Di HJ, Cameron KC, Shen JP, Winefield CS, O'Callaghan M, Bowatte S, He JZ (2009). Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. Nat. Geosci. 2(9):621-624.

Di HJ, Cameron KC, Shen JP, Winefield CS, O'Callaghan M, Bowatte S, He JZ (2010). Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. FEMS Microbiol. Ecol. 72(3):386-394.

Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB (2005). Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. P. Natl. Acad. Sci. USA, 102(41):14683-14688.

Hart SS, J. Davidson E, Firestone MK (1994). Nitrogen, mineralization, immobilisation and nitrification. In: Methods of soil Analyses, part 2: Microbiological and Biochemical Properties Book Series, no. 5, pp. 1011-1015.

He J, Shen J, Zhang L, Zhu Y, Zheng Y, Xu M, Di HJ (2007). Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. Environ. Microbiol. 9(9):2364-2374.

Irigoyen I, Muro J, Azpilikueta M, Aparicio-Tejo P, Lamsfus C (2003). Ammonium oxidation kinetics in the presence of nitrification inhibitors DCD and DMPP at various temperatures. Aust. J. Soil Res. 41(6):1177-1183.

Jia ZJ, Conrad R (2009). Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. Environ. Microbiol. 11(7):1658-1671.

Keil D, Meyer A, Berner D, Poll C, Schutzenmeister A, Piepho HP, Vlasenko A, Philippot L, Schloter M, Kandeler E, Marhan S (2011). Influence of land-use intensity on the spatial distribution of N-cycling microorganisms in grassland soils. FEMS Microbiol. Ecol. 77(1):95-106.

Kleineidam K, Kosmrlj K, Kublik S, Palmer I, Pfab H, Ruser R, Fiedler S, Schloter M (2011). Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on ammonia-oxidizing bacteria and archaea in rhizosphere and bulk soil. Chemosphere 84(1):182-186.

Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C (2006). Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806-809.

Macadam XMB, del Prado A, Merino P, Estavillo JM, Pinto M, Gonzalez-Murua C (2003). Dicyandiamide and 3,4-dimethyl pyrazole phosphate decrease N_2O emissions from grassland but dicyandiamide produces deleterious effects in clover. J. Plant Physiol. 160(12):1517-1523.

Mahmood S, Prosser JI (2006). The influence of synthetic sheep urine on ammonia oxidizing bacterial communities in grassland soil. FEMS Microbiol. Ecol. 56(3):444-454.

McCarty GW, Bremner JM (1989). Inhibition of nitrification in soil by heterocyclic nitrogen-compounds. Biol. Fert. Soils 8(3):204-211.

Nicol GW, Leininger S, Schleper C, Prosser JI (2008). The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. Environ. Microbiol. 10(11):2966-2978.

Norton JM, Alzerreca JJ, Suwa Y, Klotz MG (2002). Diversity of ammonia monooxygenase operon in autotrophic ammonia-oxidizing bacteria. Arch. Microbiol. 177(2):139-149.

O'Callaghan M, Gerard EM, Carter PE, 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 Biol. Biochem. 42(9):425-1436.

Offre P, Prosser JI, Nicol GW (2009). Growth of ammonia-oxidizing archaea in soil microcosms is inhibited by acetylene. FEMS Microbiol. Ecol. 70(1):99-108.

Okano Y, Hristova KR, Leutenegger CM, Jackson LE, Denison RF, Gebreyesus B, Lebauer D, Scow KM (2004). Application of real-time PCR to study effects of ammonium on population size of ammonia-oxidizing bacteria in soil. Appl. Environ. Microb. 70(2):1008-1016.

Pereira J, Fangueiro D, Chadwick DR, Misselbrook TH, Coutinho J, Trindade H (2010). Effect of cattle slurry pre-treatment by separation and addition of nitrification inhibitors on gaseous emissions and N dynamics: A laboratory study. Chemosphere 79(6):620-627.

Rotthauwe JH, Witzel KP, Liesack W (1997). The ammonia monooxygenase structural gene amoA as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microb. 63(12):4704-4712.

Schauss K, Focks A, Leininger S, Kotzerke A, Heuer H, Thiele-Bruhn S, Sharma S, Wilke BM, Matthies M, Smalla K, Munch JC, Amelung W, Kaupenjohann M, Schloter M, Schleper C (2009). Dynamics and functional relevance of ammonia-oxidizing archaea in two agricultural soils. Environ. Microbiol. 11(2):446-456.

Stopnisek N, Gubry-Rangin C, Hofferle S, Nicol GW, Mandic-Mulec I, Prosser JI (2010). Thaumarchaeal ammonia oxidation in an acidic forest peat soil is not influenced by ammonium amendment. Appl. Environ. Microb. 76(22):7626-7634.

Tourna M, Stieglmeier M, Spang A, Konneke M, Schintlmeister A, Urich T, Engel M, Schloter M, Wagner M, Richter A, Schleper C (2011).

Nitrososphaera viennensis, an ammonia oxidizing archaeon from soil. P. Natl. Acad. Sci. USA, 108(20):8420-8425.
Valentine DL (2007). Adaptations to energy stress dictate the ecology and evolution of the Archaea. Nat. Rev. Microbiol. 5:316-323.
Wu YC, Lu L, Wang BZ, Lin XG, Zhu JG, Cai ZC, Yan XY, Jia ZJ (2011). Long-term field fertilization significantly alters community structure of ammonia-oxidizing bacteria rather than Archaea in a paddy soil. Soil Sci. Soc. Am. J. 75(4):1431-1439.
Xia W, Zhang C, Zeng X, Feng Y, Weng J, Lin X, Zhu J, Xiong Z, Xu J, Cai Z, Jia Z (2011). Autotrophic growth of nitrifying community in an agricultural soil. ISME J. 5:1226-1236.
Zerulla W, Barth T, Dressel J, Erhardt K, von Locquenghien KH, Pasda G, Radle M, Wissemeier AH (2001). 3,4-Dimethylpyrazole phosphate (DMPP) - a new nitrification inhibitor for agriculture and horticulture - An introduction. Biol. Fert. Soils 34(2):79-84.
Zhang LM, Offre PR, He JZ, Verhamme DT, Nicol GW, Prosser JI (2010). Autotrophic ammonia oxidation by soil thaumarchaea. P. Natl. Acad. Sci. USA, 107(40):17240-17245.
Zhang LM, Wang M, Prosser JI, Zheng YM, He JZ (2009). Altitude ammonia-oxidizing bacteria and archaea in soils of Mount Everest. FEMS Microbiol. Ecol. 70(2):208-217.