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A novel amended nitrification inhibitor confers an enhanced suppression role in the nitrification of ammonium in soil

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Abstract

Purpose Nitrification inhibitor plays an important regulatory role in inhibiting the nitrification of ammonium in soils. However, most of nitrification inhibitors lack the sustainable effects in suppressing the nitrification of ammonium. In this study, a novel DMS nitrification inhibitor was prepared and tested to explore its lasting effect of nitrification suppression in black soil.

Materials and methods Both culture experiments and field trial were performed in black soils. Three kinds of nitrification inhibitors (NIs), dicyandiamide (DCD) with low bioactivity, 3,4-dimethylpyrazole phosphate (DMPP) with high bioactivity, and a novel 3,4-dimethylpyrazole sulfate zinc (DMS) with long half-life, were applied into soils, respectively, and the abundance changes of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) were investigated; then, the accumulation changes of inorganic nitrogen, nitrogen use efficiency, and crop yields were furtherly evaluated.

Results and discussions A novel DMS nitrification inhibitor with high activity and long half-life maintained a persistent effect of nitrification suppression, and remarkably increased the accumulation of ammonium nitrogen in soil, thus improving nitrogen use efficiency and crop yields. This study implies that lowering the nitrogen loss of nitrification-triggered in soil is of great importance for improving nitrogen use efficiency.

Conclusions This study provided an insight into the sustainable nitrification suppression of a novel DMS nitrification inhibitor under excessive application of nitrogen fertilizer in black soils. Compared with improving the activity, reasonably prolonging the validity of nitrification inhibitors in soil is a more important strategy increasing the sustainable effects of nitrification inhibition, and the survival period of nitrification inhibitors in soil should be a crucial factor improving nitrogen use efficiency.

Keywords Ammonia-oxidizing bacteria · DMS · Nitrification inhibitors · Nitrification of ammonium · Black soil

1 Introduction

Nitrogen fertilizer plays an important role in maintaining the requirement of crop nutrient and has been a major nutrition source supplying food protein and energy (Ti and Yan 2020). Statistics shows that a large nitrogen fertilizer input resulted in an increase of more than 40% in crop yields in the world (Li et al. 2009), while the increased grain yields could meet the food requirement of nearly 50% of the population (Erisman et al. 2008). However, the global average nitrogen fertilizer utilization rate is still maintained at a lower level, and usually ranged from 42 to 47% (Mueller et al. 2017; Zhang et al. 2015), indicating that more than 50% of nitrogen fertilizer are lost or wasted by multiple pathways such as NO^{3-}-N leaching, nitrous oxide (N_2O) emissions, and ammonia volatilization (Sutton et al. 2011; Wang et al. 2018; Zhang et al. 2019). Lower utilization rate of nitrogen fertilizer not only results in economic waste, but also triggers serious environmental problems such as water pollution, eutrophication, climate anomalies, loss of biodiversity, air pollution, and soil degradation (Galloway et al. 2004; Zhang et al. 2015; Yu et al. 2019). From 1980 to 2010, although

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the amounts of nitrogen absorption in crops have doubly increased in agricultural production in China, the input of nitrogen fertilizer was increased by threefold (Liu et al. 2016; Ti and Yan 2020). For improving the utilization rate of nitrogen fertilizer, the Ministry of Agriculture of China issued the "Zero Growth Plan 2020" in 2015 to reduce nitrogen fertilizer loss and ensure food security. Therefore, reasonable fertilization practice is of great importance for alleviating the environmental stress and maintaining agricultural sustainable development. Especially, with the increase of intensive agriculture production, excess application of nitrogen fertilizer has obviously lowered the soil quality. For example, as a major fertile soil resource, the black soil in the northeast of China has been facing a problem of severe soil degradation (Liang et al. 2016), both the intensive cultivation and a large input of chemical fertilizers have aggravated the loss of nitrogen in soils, and thus lowering the soil productivity in this region (Gu et al. 2018; Tong and Xu 2012; Zhu et al. 2018).

Loss pathway of nitrogen is usually related to the nitrification in soil, while nitrification is a process involving in a circle of soil nitrogen and represents an oxidation process converting ammonia into nitrite, thus eventually producing nitrates (Musiani et al. 2020). Excessive accumulation of nitrates in soil subsequently triggers nitrogen loss by leaching pathway of nitrous nitrate and nitrogen oxide emissions (Ding et al. 2021). Therefore, how to improve the utilization efficiency of nitrogen fertilizer is of great importance for reducing the loss of nitrogen in soils (Chen et al. 2015). As additive of fertilizer, nitrification inhibitors could remarkably delay the nitrification process of ammonium nitrogen in soil, and widely have been applied as an effective means lowering the loss of nitrogen and improving the utilization rate of nitrogen fertilizer as well as increasing crop yields (Di and Cameron 2012; Shi et al. 2016a, b; Gao et al. 2021).

In this study, to systematically observe the sustainable effects of a novel DMS nitrification inhibitor in soil, two traditional nitrification inhibitors, DCD with low activity and DMPP with high activity, were used as references, and applied in the soil culture and field experiment, respectively. In incubation experiment and pot culture, all of tested soils come from the same black soil as the field experiment in Jilin province in the northeast of China. Under the presence of three nitrification inhibitors, both the accumulation of inorganic nitrogen and abundance of ammonium-oxidizing bacteria (AOB) in soils were investigated; then, nitrogen use efficiency and maize yields were evaluated. This study confirmed a sustainable regulatory role of a novel nitrification inhibitor DMS in improving nitrogen fertilizer use efficiency in black soil, and provided insight into the development of novel nitrification inhibitor with persistence of nitrification inhibition, indicating that reasonable application of a novel DMS nitrification inhibitor and traditional nitrification inhibitors is of great importance for lowering the loss of nitrogen fertilizer in this region.

2 Materials and methods

2.1 Preparation and identification of nitrification inhibitor

DMS was synthesized by the methods of Adams et al. (2010) with a minor modification. Approximately 9.6 g of 3,4-dimethylpyrazole (DMP) was dissolved into 30 mL of absolute ethyl alcohol. The generating solution was slowly transferred into a beaker of 500 mL containing 8 g of anhydrous zinc sulfate of 10% to obtain a clear solution. A pH value of the solution was adjusted to 6.5 by using ammonium hydroxide, and the solid sediments with light yellow were produced. After stirring for 30 min, suction filtration was conducted, and the filtrated product was washed and sedimented alternatively by using distilled water and ethyl alcohol for three times, respectively, then the generating target compound DMS was obtained by drying process. The elemental components of DMS were determined by using an elemental analyzer (240C PerkinElmer Inc., USA) and mainly composed of 32.86% of C, 4.25% of H, and 15.36% of N. The related structural formula of the compound is shown in Fig. 1.

For determining the content of DMS, this compound was dissociated by diluting with sulfuric acid; then, the DMS content in the target compound was determined by high-performance liquid chromatography (LC-20AT, Shimadzu Inc., Japan). A C18 silica gel chromatographic column (Shiseido, 250×4 mm) was used for the detection, while the liquid phase was composed of methanol solution and high-purity water with a ratio of 4:6 of methanol/water, the flow velocity was set by 1 mL of per minute, and the detection was performed under wavelength of 220 nm. The effective components in DMS (i.e., DMP) accounted for 41.8%, and the purity of the target compound reaches to 95.0%. DCD with the purity of 99.5% and DMPP with a purity of 97% were supplied by the Chemical Institute of Jilin University, China. DMPP or DMS or DCD was mixed with ammonium sulfate (AS) of 21%, and was applied in both soil culture and field experiments; all fertilizer nitrogen were applied by 1%, 1%, and 5% of NH_4^+-N in soils, respectively (O'Callaghan et al. 2010).

2.2 Soil collection, characterization, and preparation

Soil samples were collected from the layer of 0–20-cm soil of farmlands in Dehui city, Jilin province, China (44° 31′ N, 125° 43′ E). The soil type was classified into black soil; the basic properties of soils are shown in Table 1.

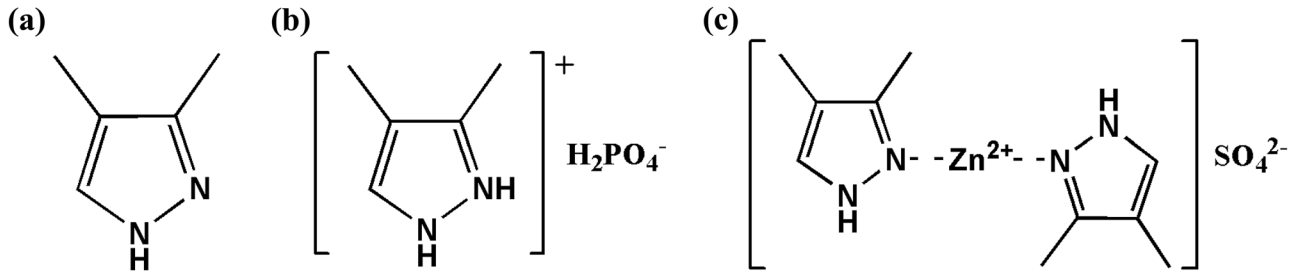

Fig. 1 Chemical structural formulas of DMP (**a**), DMPP (**b**), and DMS (**c**)

Natural dried soils were sieved through a sieve less than 2 mm and used for property analysis, and the remained soils were used for soil incubation and pot culture experiments. For both soil incubation and pot culture, the moisture in air-dry soil was maintained at the field levels, and the field moisture capacity was determined by using the column method described previously (Asher et al. 2002).

2.3 Incubation experiment

A soil incubation experiment was carried out in laboratory to investigate the nitrification inhibition effect of three nitrification inhibitors. The experiment was composed of four treatments including the control with NH_4^+-N of 200 mg kg^{-1} dry soil, DMPP treatment with NH_4^+-N of 200 mg kg^{-1} dry soil + DMPP (1% of NH_4^+-N), DMS treatment with NH_4^+-N of 200 mg kg^{-1} dry soil + DMS (1% of NH_4^+-N), and DCD with NH_4^+-N of 200 mg kg^{-1} dry soil + DCD (5% of NH_4^+-N). Each treatment was replicated by three times at least.

A total of 200.0 g of the air dried soil was thoroughly mixed with a composite of AS, DCD, DMPP, and DMS, respectively, and placed into a covered plastic pot of 500 mL, and the water capacity was maintained at a level of 60% by supplement of deionized water and was similar to the field moisture contents, and the holes on the cover were punched to ensure sufficient oxygen during the incubation at 25 °C, and the amounts of water loss were supplemented in an interval of 2 days by weighing method. For soil sampling at the different periods of culture, each treatment was composed of 20 pots to ensure three replication's requirements at least. The samples were collected by four replicates after 3, 6, 12, 24, and 48 days of beginning incubation, respectively, and soils in each pot were blended evenly. About 100 g of collected soil samples were divided into two groups, then, one group was stored at –20 °C and another was stored at −80 °C, and used to detect the contents of ammonium nitrogen or nitrate nitrogen as well as AOA/AOB abundance, respectively.

2.4 Pot culture experiment

The soil samples were sieved by a sieve of 5 mm and put into pots with a diameter of 25 cm and a height of 33 cm, and each pot was added by a mass weight of 8 kg dry soil. Similar to soil incubation, pot experiment was composed of four treatments, and each treatment was composed of 12 pots, and 0.15 g of NH_4^+-N kg^{-1} dry soil of per pot was applied. The fertilizers and inhibitors were thoroughly mixed with soil samples; then, 0.1 g of P_2O_5 kg^{-1} dry soil in the form of monocalcium phosphate and 0.15 g of K_2O kg^{-1} dry soil in the form of potassium chloride were applied into each pot, respectively. Quantitative supplement of water was performed to maintain a moisture content of 60% which is basically identical with the field water capacity during wheat growth period. Total of 15 seeds of spring wheat (*Triticum aestivum* L. cv. Shenmian#26) were sown in each pot, and the seedlings of 6 plants of per pot were fixed after 20 days of germination and seedling establishment.

A destructive sampling of soils and plants was finished at the wheat seedling period, stem elongation period, pustulation period, and maturity period, which are represented by 25, 58, 85, and 125 days after sowing, respectively. The fresh soil samples were packed with ice pack and transported to the laboratory, and then passed through a sieve of 2 mm after removing debris. A part of the samples was stored at −20 °C for chemical analysis, and the remaining part was stored at −80 °C for DNA extraction. For molecular biological analyses, about 10 g of fresh soil was collected from each sample.

Table 1 Basic chemical properties of the tested soils

Soil type	Total N (g kg^{-1})	Available N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Olsen-P (mg kg^{-1})	Available K (mg kg^{-1})	pH (H_2O)	Organic matter (g kg^{-1})
Black soil	1.05	134.56	8.17	21.97	39.45	178.56	6.52	23.21

Plant samples were dried at 65 °C for 48 h and used for biomass determination. The harvested stems, leaves, and grains of plants at the maturity period were dried and separately weighted. Plant samples were homogeneously ground and used for determining N contents by the Kjeldahl method (Tan 2005).

2.5 Field experiment

The field experiment was conducted by using the same soils as the incubation or pot experiment. The testing site is located at the temperate region with an average annual precipitation of 516 mm and average air temperature of 4.9 °C. The crop growth period is mainly gathered in the period from April to September, which annually has an average temperature of 16 °C and average precipitation of 180.5 mm.

Field trial was composed of three treatments including the control with only addition of urea, DMPP with urea (1% of urea-N), and DMS with urea (1% of urea-N). The application doses of N, P, and K in each treatment were performed by N of 210 kg ha^{-1}, P_2O_5 of 90 kg ha^{-1}, and K_2O of 90 kg ha^{-1}, respectively. Urea, monocalcium phosphate, and potassium chloride were used as testing fertilizers.

Both fertilizers and nitrification inhibitors were ground into powder and completely mixed as described in the culture experiments, then evenly applied into the position of 10 cm apart from the row ridge by incorporating a depth of about 5 cm. Each treatment was composed of three independent plots, and each plot was designed by 4 m × 10 m, and corn was sown by a seeding density of 4500 plants of per ha on May 7, 2015. The corn harvesting was performed by hand-cutting on October 5, 2015.

2.6 Soil parameter analysis

To determine the concentrations of ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) in soil, fresh soil samples were extracted with 0.01 mol·L^{-1} $CaCl_2$ solution; then, the concentrations of ammonium- and nitrate-nitrogen in leaching liquor were measured by an analyzer of AA3 continuous-flow (Bran Luebbe, Germany) according to the described method in Ghani et al. (2007).

Soil pH in the solution of 1:2.5 ratio of water versus soil was determined by a glass electrode. Dried soil samples were digested by the standard procedures and used for nitrogen measurement by a Kjeldahl apparatus (Tan 2005). Soil available K was extracted with ammonium acetate solution and determined through flame photometry (Isaac and Kerber 1971). Soil Olsen P was extracted by using $NaHCO_3$ (pH 8.5) of 0.5 mol and determined by molybdenum antimony blue colorimetry. Soil organic matter content was determined through the potassium dichromate digestion method (Tan 2005).

2.7 Quantitative PCR of amoA genes in soils

Total soil DNA extraction was implemented by using the PowerSoil® DNA Isolation Kit according to the manufacturer's instructions (MobioLaboratories, USA). Copy numbers of archaeal and bacterial genes were determined by using quantitative PCR SYBR Green-based chemistry and a real-time PCR system (Rotor-Gene Q, Qiagen, Inc., Germany) (Limpiyakorn et al. 2011).

In brief, archaeal *amoA* genes were quantified by qPCR with a pair of primers, Arch-amoAF and Arch-amoAR, and an amplification fragment of 635 bp was obtained. While a fragment of approximately 490 bp expressing the *amoA* gene was generated by using the specific primers of AmoA-1F and amoA-2R (Table 2). Cycling procedures used for quantitative PCR detection were performed. In brief, a total of 20 μL PCR reaction system contained 2 μL of template DNA, 10 μL of 2 × SYBR® Premix Ex Taq™, 0.8 μL of forward and 0.8 μL of reverse primers, and 6.4 μL of sterilized deionized water in each tube; then, the PCR thermal cycle was performed by the following steps: initial denaturation at 94 °C for 5 min and degeneration at 94 °C for 10 s with 40 cycles of 55 °C for 15 s, 72 °C for 45 s, and 72 °C for 10 min. The generated *amoA* gene plasmids representing the AOB and AOA in clone library were diluted by 10^5, 10^6, 10^7, 10^8, and 10^9 folds to generate standard curves by cascading dilution method. Specific amplification was checked by melting curve analysis and agarose gel electrophoresis.

2.8 Nitrogen use efficiency calculation

The components of N efficiency were determined by a previous method (Guillaumes and Villar 2004). For the pot experiment, nitrogen uptake (NUp) (g pot^{-1}) representing total amounts of N in plant were estimated by using the N concentrations in grain and straw, while nitrogen utilization efficiency was determined by a formula of NUE (%) = N $uptake_{fertilized\ crop}$ / $N_{applied}$ × 100%, and apparent use efficiency of N fertilizer was estimated by a formula of AUN (%) = (N $uptake_{fertilized\ crop}$ − N $uptake_{unfertilized\ crop}$) / $N_{applied}$ × 100%. For the field experiment, the following components of N efficiency were determined by using the crop yield. Nitrogen partial factor productivity was determined

Table 2 qRT-PCR primers used for amplification of functional target genes

Target gene	Primer	Primer sequence, 5′–3′
AOB amoA	amoA-lF	GGGGTTTCTACTGGTGGT
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC
AOA amoA	Airch-amoAF	STAATGGTCTGGCTTAGACG
	Airch-amoAR	GCGGCCATCCATCTGTATGT

by a formula of NFP ($kg \cdot kg^{-1}$) = $Yield_{fertilized\ crop}/N_{applied,}$ and nitrogen agronomic efficiency was estimated by a formula of NAE ($kg \cdot kg^{-1}$) = ($Yield_{fertilized\ crop} - Yield_{unfertilized\ crop}$) / $N_{applied.}$

2.9 Statistical analyses

Statistical analyses were carried out by using the SPSS 18.0 software package for Windows, and significant difference identification was performed by one-way ANOVA with the least significant difference (LSD) method at a level of less than 5%. The relationship between the accumulation amounts of NO_3^--N and the copy numbers of bacterial or archaeal *amoA* genes in soils was assessed by using linear regression analysis of Origin 8.

3 Results and discussions

3.1 Nitrification inhibitors remarkably inhibited the nitrification of ammonium in soil

Nitrification inhibitors generally function in suppressing the oxidation of ammonia monooxygenase (AMO) (Di and Cameron 2011), thus delaying the conversion of ammonium NH_4^+ to nitrite (NO_2^-) and reducing the accumulation of nitrate in soils (Florio et al. 2014). In this study, for investigating the suppression effect of nitrification inhibitors on the nitrification of ammonium, both soil incubation and pot experiment were performed to determine the accumulation profiles of ammonium and nitrate nitrogen in soils. As shown in Fig. 2a, after 48 days of short-term soil incubation or 125 days of long-term pot experiment, the ammonium nitrogen content in soil of the control treatment was obviously decreased, while three treatments with nitrification inhibitor demonstrated higher contents of the ammonium nitrogen within 48 days of soil incubation comparing to the control. However, in the case of pot experiment, the treatments with nitrification inhibitor still revealed an increase of the ammonium nitrogen after 83 days of culture comparing to the control (Fig. 2b). Data shows that the contents of ammonium nitrogen in the treatments with nitrification inhibitor were increased by tenfold after 12 days of soil incubation, while the ammonium nitrogen was only increased by threefold after 57 days of pot culture comparing to the control, respectively.

In the case of soil incubation experiment, three kinds of nitrification inhibitors demonstrated differential effects in suppressing the nitrification of ammonium. Both the DMPP and DMS treatments remarkably increased the accumulation of ammonium nitrogen in soils comparing to the DCD treatment. Particularly, when the incubation time was prolonged, this difference in ammonium accumulation became more significant after 24 days of soil incubation (Fig. 2a). Data shows that the DMPP treatment and DMS treatment, respectively, accumulated 140.40 mg kg^{-1} and 123.15 mg kg^{-1} of ammonium nitrogen ammonium nitrogen in soils, while the DCD treatment only showed an accumulation of 108.84 mg kg^{-1} of ammonium nitrogen ($P < 0.05$), but the accumulation of ammonium nitrogen in the treatments with nitrification inhibitor was significantly higher than 10.62 mg kg^{-1} dry soil of ammonium nitrogen in the control. After 48 days of soil incubation, the DMS treatment showed 74.52 mg kg^{-1} dry soil of ammonium nitrogen, which was higher than 72.36 mg kg^{-1} dry soil in the DMPP treatment and 42.35 mg kg^{-1} dry soil in the DCD treatment ($P < 0.05$). Similar differences were also observed in the pot experiment of 83 days (Fig. 2d). Especially, accumulation of ammonium nitrogen in the DMS treatment reached the highest level and was increased by 62.18% comparing to the DMPP treatment, but increased by 17.16% comparing to the DCD treatment ($P < 0.05$). In contrast, the control treatment showed an increasing trend of soil nitrate nitrogen after 12 days of early culture stage and then demonstrated a decrease after 57 days of the late culture stage. As shown in Fig. 2b, e, the nitrate nitrogen in the control treatment reached the highest peak of 185.75 mg kg^{-1} dry soil after 12 days of culture, and still was maintained at a level of 181.95 mg^{-1} dry soil even after 57 days of culture.

In this study, three nitrification inhibitors effectively inhibited the generation of nitrate in the cultured soil of 12 days. Although the contents of nitrate in the nitrification inhibitor treatments slowly increased, the overall level of nitrate was still significantly lower than that in the control after 12 days of culture, while the nitrate contents in the treatments with nitrification inhibitors were less than 10% of the highest peak value of nitrate in the control (Fig. 2b). Measurements show that nitrification inhibitor treatments revealed a significant difference in suppressing the nitrification after 24 days of culture, both the DMPP and DMS treatments demonstrated a similar effect, and the contents of nitrate in these two treatments were lowered by 37% comparing to the DCD treatment, and lowered by 74% comparing to the control after 48 days of culture. Data shows that the DMS treatment accumulated 90.34 mg kg^{-1} of nitrate in soil, which basically was identical with 92.90 mg kg^{-1} in the DMPP treatment, while the DCD treatment accumulated 110.43 mg kg^{-1} of nitrate in soil ($P < 0.05$) (Fig. 2b).

Pot experiment planting wheat showed that after 57 days of culture, the treatments with nitrification inhibitors significantly lowered the accumulation of nitrate than the control. The nitrification inhibitor treatments did not demonstrate differences in the accumulation of nitrate after 28 days of culture, but the accumulation of nitrate was lowered by 35% comparing to the control. Data shows that the DMS treatment demonstrated the best nitrification

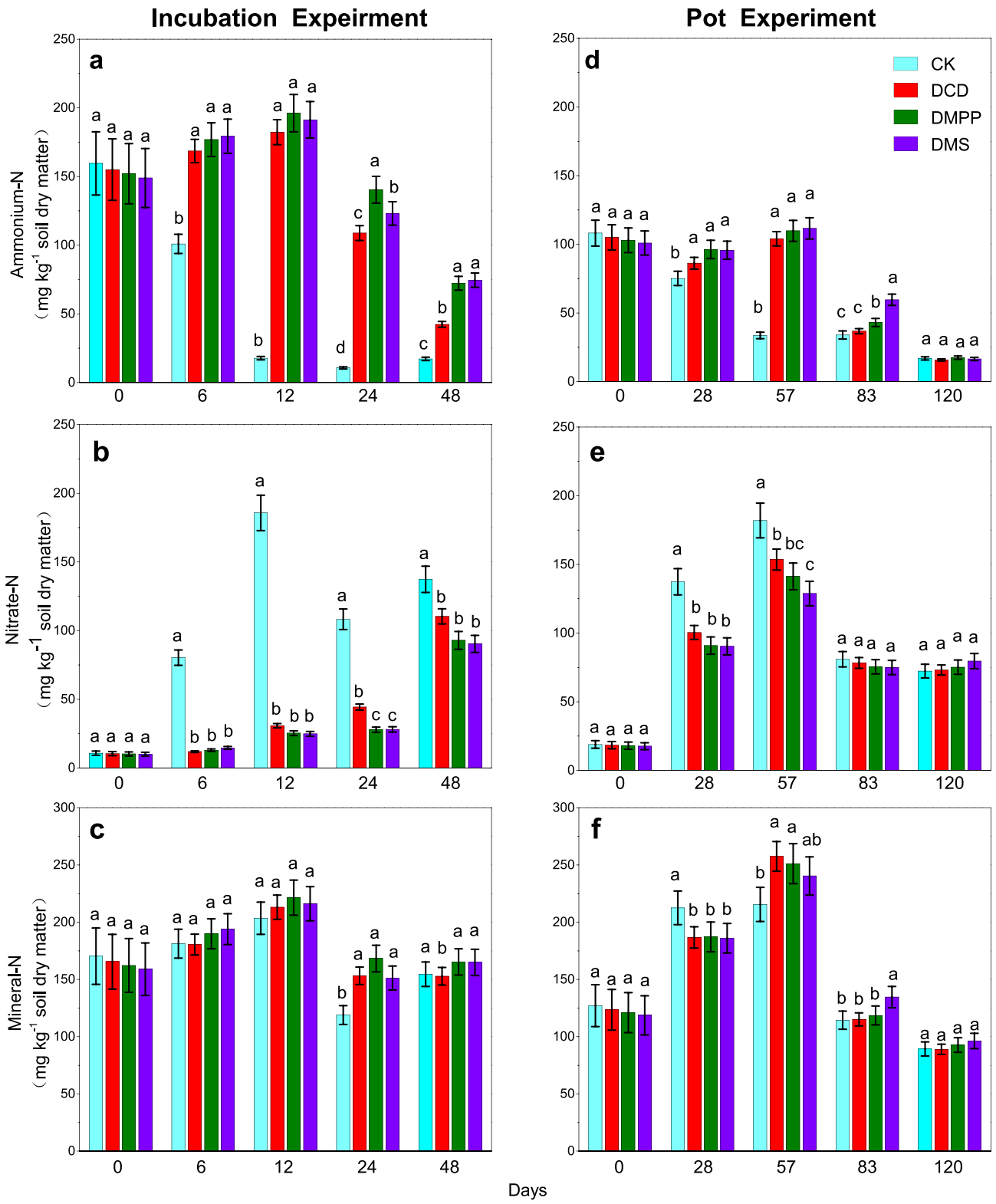

Fig. 2 The dynamic characteristics of soil nitrogen. (**a**, **d**) NH_4^+-N; (**b**, **e**) NO_3^--N; (**c**, **f**) mineral N (NO_3^--N + NH_4^+-N) in black soils from incubation experiment and pot culture. Bars represent the standard errors of the mean ($n = 3$). The different lowercase letters indicate significant difference at the level of less than 5%

inhibition effect ($P < 0.05$). The contents of nitrate in the DMS treatment were maintained at a lower level of 128.78 mg kg^{-1}, while the DMPP treatment demonstrated an accumulation of 141.29 mg kg^{-1} of nitrate, and the DCD treatment accumulated 153.54 mg kg^{-1} of nitrate in soils (Fig. 2). Comparing to the peak value of nitrate in the control, the DMS treatment lowered 29.22% of nitrate, the DMPP treatment lowered 22.35% of nitrate, and the DCD treatment lowered 15.62% at this stage, but all treatments basically accumulated identical levels of nitrate after 57 days of culture (Fig. 2).

Pot experiment showed that the treatment with DMS nitrification inhibitor revealed more accumulation of ammonium nitrogen in soil after 83 days, and significantly lowered the contents of nitrate after 57 days comparing to the DMPP treatment ($P < 0.05$). Generally, nitrification inhibitors could significantly increase the contents of ammonium in soils, thus triggering the evaporation of ammonium and lowering the contents of nitrate in soils (Davies and Williams 1995; Menéndez et al. 2006) despite of differences in soil texture and pH values (Zerulla et al. 2001).

Although total inorganic nitrogen in soil was not changed under the presence of nitrification inhibitor after 24 days or 48 days of culture (Fig. 2c, f), the nitrification inhibitor treatments increased the accumulation of inorganic nitrogen of more than 30% after 24 days or 48 days of soil culture comparing to the control. In the case of 48-day culture, the contents of inorganic nitrogen in the DMPP and DMS treatments were 165.26 mg kg^{-1} and 164.86 mg kg^{-1} dry soil, respectively, while the DCD treatment maintained at a level of 152.78 mg kg^{-1} of inorganic nitrogen which is similar to 154.57 mg kg^{-1} dry soil of inorganic nitrogen in the control. Inorganic nitrogen was increased by 8.07% in the DMS and by 7.81% in the DMPP treatment comparing to the control, respectively (Fig. 2c). Pot experiment with planting wheat showed that the treatments with nitrification inhibitor increased 34.09% of soil inorganic nitrogen after 57 days, and all treatments showed a peak value of inorganic nitrogen, but no significant differences in inorganic nitrogen between nitrification inhibitor treatments were observed. However, after 83 days of culture, the contents of soil inorganic nitrogen in both the DMPP and DCD treatments rapidly declined, and basically were maintained at a similar level to that in the control; only the DMS treatment increased accumulation of inorganic nitrogen of 17.68% comparing to the control, indicating that a novel DMS revealed long-lasting effect in inhibiting nitrification than DMPP (Fig. 2f).

In this study, a novel high-activity and long-lasting DMS nitrification inhibitor remarkably suppressed the nitrification of ammonium in black soil, thereby effectively inhibiting the production of nitrate nitrogen and increasing the accumulation of ammonium nitrogen in soils after 40 days of soil culture (Fig. 2a) or after 58 days of plant culture (Fig. 2d). This result is consistent with the previous reports that the nitrification inhibitors played an important role in inhibiting nitrification in different soils (Xu et al. 2001; Gong et al. 2013; Zhou et al. 2020). Our study shows that both the soil incubation and pot experiment accumulated more ammonium nitrogen and lowered the loss of nitrate in soils because of the presence of nitrification inhibitors, thus increasing the accumulation of ammonium- and nitrate-nitrogen in soils (Fig. 2c, f), which is the same as previously reported by Wu et al. (2007), indicating that application of a novel DMS nitrification inhibitor in black soils could effectively increase the accumulation of ammonium nitrogen.

3.2 Nitrification inhibitors increased the amounts of AOA and lowered abundance of AOB in soil

To evaluate the effects of a novel DMS on the key microorganisms involving in the nitrification reactions, the abundance changes of AOB and AOA in soils were investigated by the fluorescent quantitative PCR (Figs. 2 and 3). Data shows that the number of gene copies of ammonia oxide bacteria (AOA) was ranged from 2.3×10^7 to 1.15×10^8 g^{-1} dry soil, and initial value was tenfold of AOB in soil. Although the AOA abundance in the two trials was increased gradually after nitrogen fertilization, but not affected by the nitrification inhibitors (Fig. 2b, d). Statistics shows that more enrichment of AOB is an important factor affecting nitrate accumulation in soil. For example, the amounts of soil nitrate showed a significant positive linear correlation with soil bacterial *amoB* gene copy number ($P < 0.01$) (Fig. 3), but no significant correlation with copy number of archaeal *amoA* gene in soils although no data was shown, indicating that the AOB bacteria abundance is a key factor reflecting the nitrification process in soil.

Data shows that the number of *amoA* gene copies of AOB was ranged from 2.90 × 106 to 5 27 × 108 g^{-1} dry soil. In the case of soil incubation, the abundance of AOB in the control rapidly was increased by tenfold after fertilization and maintained at a level of 9.20 × 107 g^{-1} dry soil comparing to the abundance value of AOB after 6 days of soil incubation (Fig. 3a), indicating that nitrification inhibitor treatments obviously inhibited the growth of AOB in soils. Measurement showed that the DCD treatment revealed an AOB abundance of 51.54% comparing to the control after 24 days of culture, but after 48 days of soil incubation, the same treatment accounted for 70.11% of the abundance of AOB in the control. However, the DMS treatment only accounted for 16.72% of AOB abundance in the control, while AOB abundance in the DMPP treatment also was decreased and only accounted for 13.57% of that in the control after 24 days of soil incubation. With prolonging culture time, the AOB abundance in the DMS treatment accounted for 31.69% of that in the control and accounted for 32.34%

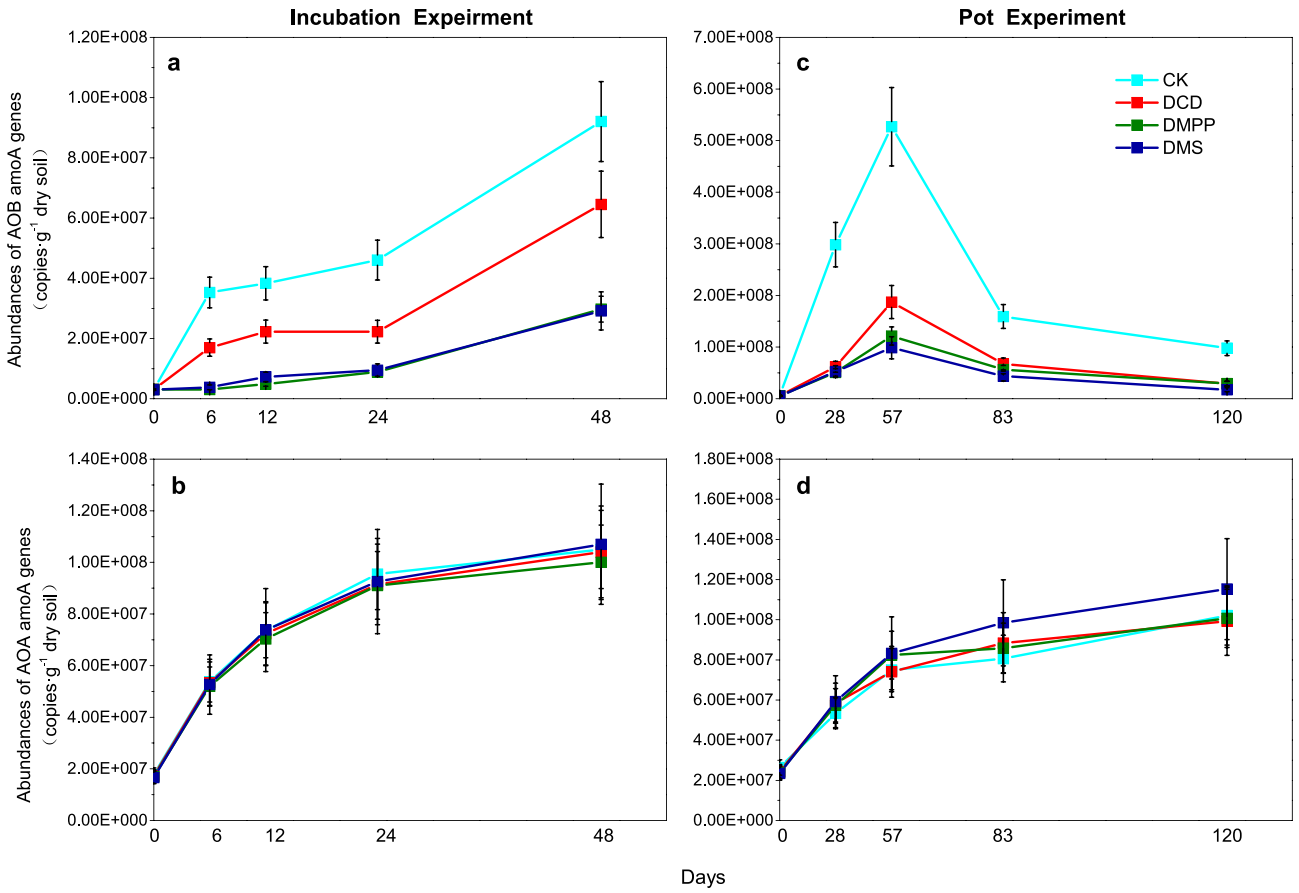

Fig. 3 Bacterial and archaeal *amoA* gene copies in black soil from both incubation (**a**, **c**) and pot experiment (**b**, **d**). Bars represent the standard errors of the mean ($n = 3$)

of that in the DMPP treatment after 48 days, showing that novel DMS nitrification inhibitor exhibited the best effect in suppressing the bacteriogenic growth (Fig. 3a).

Pot experiment showed that all treatments revealed a peak of AOB abundance between 28 and 57 days of culture, then demonstrated a rapid decease. Data shows that the control enriched an AOB abundance of 5.27×10^8 g^{-1} dry soil after 57 days of culture and resulted in an increase of 5.38-fold in the AOB abundance comparing to the measurement value after 120 days of culture. All of nitrification inhibitors could effectively inhibit the growth of AOB, while the DMS treatment demonstrated the lowest abundance of AOB, and only accounted for 20.41% of average abundance value of AOB in the control. Average abundance value of AOB in both the DMPP and DCD treatments accounted for 26.35% and 32.08% of that in the control, respectively, and a significant AOB peak appeared at the 57th day ($P < 0.05$). Using the abundance value of AOB in the control as 100%, the DMS treatment showed the lowest abundance value of 18.73% AOB, which was significantly lower than 23.05% in the DMPP and 35.55% in the DCD treatments (Fig. 3c). This suggests that nitrification inhibitors affected the abundance of ammonia-oxidizing bacteria and ammonia-oxidizing archaea in soils. Studies have shown that nitrification inhibitors could effectively inhibit the growth of soil AOB and mainly function by regulating soil N transformation (Shi et al. 2017; Chen et al. 2019; Fan et al. 2019). In this study, the novel DMS nitrification inhibitor significantly inhibited the abundance of AOB and revealed a stable-lasting nitrification suppression effect in black soil, indicating that DMS plays an important regulatory role in effectively inhibiting the rapid growth of AOB bacteria (Fig. 3a, c) (Florio et al. 2014; Shi et al. 2017).

In our study, the increasing growth of AOA of fertilization-triggered was not affected by nitrification inhibitors (Fig. 3b, d), and confirmed by previous reports (Kleineidam et al. 2011; Gong et al. 2013; Chen et al. 2015) because AOA possibly demonstrates the insensitivity to the nitrification inhibitors (He et al. 2007). Report also showed that the abundance of AOB was positively linearly correlated

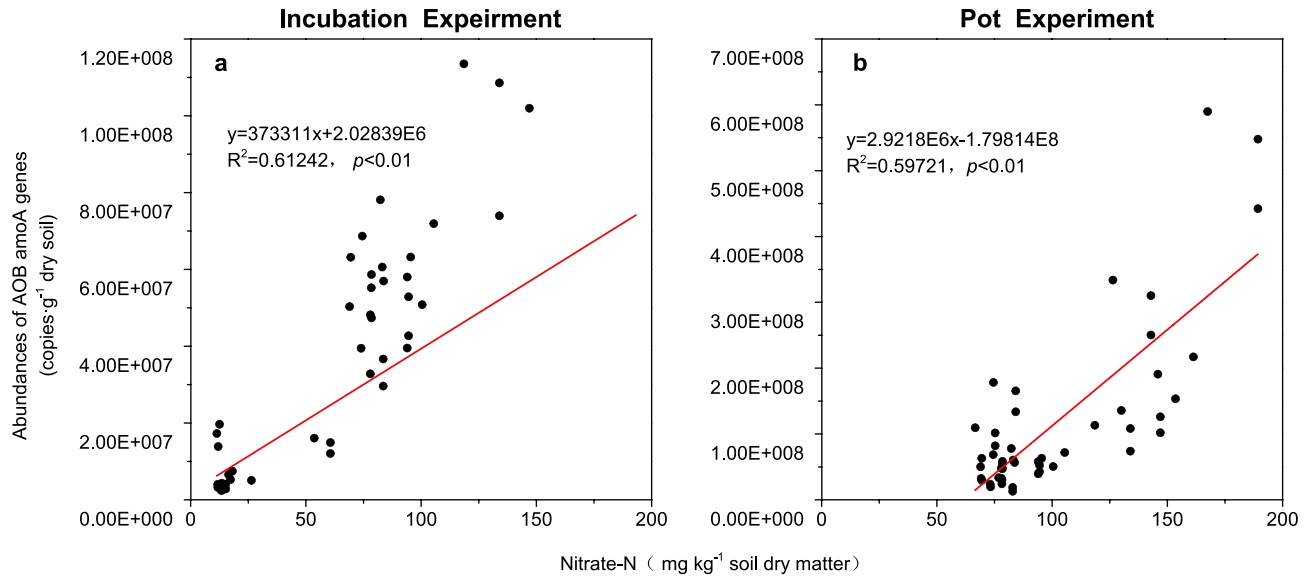

Fig. 4 Relationships between the soil NO_3^--N contents and the abundance of ammonia oxidizing bacteria (AOB). (**a**) An incubation experiment and (**b**) a pot experiment ($n = 3$)

with the accumulation of nitrate nitrogen in soil, while the abundance of AOA has no correlation with nitrate nitrogen (Gong et al. 2013). Studies have shown that the nitrification of ammonium nitrogen in soil is affected by the AOB abundance (Shen et al. 2008; Xia et al. 2011), and that the loss of N_2O of AOA growth-triggered is significantly lower than that of AOB (Hink et al. 2017), indicating that the nitrification process is mainly controlled by the abundance of AOB. Wang et al. (2019) found that AOB revealed a great potential in suppressing the nitrification by investigating the soils of six types from different regions in China. In this study, both soil incubation and pot experiments showed that the abundance of *amoA* genes reflecting the number of AOB and the contents of nitrate nitrogen revealed a significant positive correlation at the level of less than 0.01 (Fig. 4), suggesting that the nitrification process in black soil is majorly regulated by the abundance of AOB, and the application of nitrification inhibitors on black soil can effectively function by regulating soil nitrogen conversion.

3.3 Nitrification inhibitors are beneficial to increasing crop yield by promoting nitrogen use efficiency

For determining whether the nitrification plays regulatory role in improving nitrogen use efficiency, nitrogen use efficiency in wheat or maize was investigated by pot experiment or field experiment. Measurements showed that nitrification inhibitor treatments remarkably increased the crop yield, nitrogen absorption, and nitrogen use efficiency. Particularly, fertilizer apparent use efficiency was increased by 42–66%, while nitrogen fertilizer use efficiency was increased by 12.5–17.5% comparing to the control (Table 4). The DMS treatment revealed the highest grain yield of 11.38 g, biomass of 32.29 g, and nitrogen absorption of 0.75 g of per pot, while the grain yield in the DMS treatment was increased by 32.63% comparing to the control and by 14.26% comparing to the DCD treatment, respectively (Table 3). Apparent utilization rate of nitrogen fertilizer in the DMS treatment

Table 3 Nitrogen use efficiency and yield responses of Nis-mediated in pot experiment

Treatment	Grain yield (g·pot^{-1})	Biomass yields (g·pot^{-1})	The rate of grain increase (%)	NUA (g·pot^{-1})	Apparent use efficiency of N fertilizer (%)	Nitrogen use efficiency (%)
DMPP	11.16 ± 0.44 ab	31.33 ± 2.98 a	30.07%	0.74 ± 0.02 a	19.00 ± 0.58 ab	46.33 ± 1.45 a
DMS	11.38 ± 0.46 a	32.29 ± 2.19 a	32.63%	0.75 ± 0.03 a	20.16 ± 0.67 a	47.00 ± 1.53 a
DCD	9.96 ± 0.29 b	31.26 ± 2.53 a	16.08%	0.72 ± 0.03 ab	17.46 ± 0.67 b	44.67 ± 1.76 ab
CK	8.58 ± 0.34 c	30.30 ± 2.59 a	-	0.64 ± 0.02 b	12.23 ± 0.33 c	40.00 ± 1.53 b

The different lowercase letters indicate a significant difference between different treatments at a level of less than 5% for each time point

Table 4 Nitrogen efficiency and yield responses of Nis-mediated in the field trial

Treatment	Grain yield (t·hm^{-2})	Biomass yield (t·hm^{-2})	Nitrogen partial factor productivity (kg·kg^{-1})	Nitrogen agronomic efficiency (kg·kg^{-1})	The rate of yield increase (%)
DMPP	12.14 ± 0.31 a	22.58 ± 0.57 a	57.81 ± 4.16 a	15.05 ± 1.08 a	23.75%
DMS	12.25 ± 0.26 a	22.82 ± 0.47 a	58.33 ± 4.98 a	15.57 ± 1.33 a	24.87%
CK	9.81 ± 0.26 b	19.85 ± 0.51 b	46.71 ± 3.78 b	3.95 ± 0.32 b	

The different lowercase letters indicate a significant difference between different treatments at a level of less than 5% for each time point

reached to a level of 20.67%, and significantly higher than those in the DCD and the control ($P < 0.05$). Field experiment showed that the DMPP and DMS treatments separately showed a grain yield level of 12.14 t ha^{-1} and 12.25 t ha^{-1}, which revealed an increase of more than 20% comparing to the control. In addition, compared with the control, the partial productivity and agronomy utilization rate of nitrogen fertilizer in the DMS treatment were increased by more than 24% and 300%, respectively, indicating that the DMS treatment overall revealed the best effects in improving nitrogen use efficiency (Table 4).

Studies showed that nitrification inhibitors demonstrate different nitrification suppression effects in diverse soils (Chen et al. 2008; Shi et al. 2016a, b). As typical nitrification inhibitors, DMPP and DCD have been widely applied to improve nitrogen use efficiency, thus increasing crop yields (Pasda et al. 2001; Guillaumes and Villar 2004; Fangueiro et al. 2009; Wang et al. 2016; Souza et al. 2020; Wallace et al. 2020). In this study, both the DMPP and DCD treatments significantly increased the accumulation of ammonium nitrogen and raised wheat or maize yields because of an increasing nitrogen use efficiency comparing to the control. However, the novel DMS nitrification inhibitor demonstrated the long-lasting effects in improving nitrogen use efficiency and crop yield, indicating that a novel synthesized DMS nitrification inhibitor could function in suppressing nitrification of ammonium and be suitable for application in black soil of northeast China.

Usually, the effect of nitrification inhibitor is mainly controlled by the biological activity and survival period of itself in the soil, while the biological activity and survival period are affected by soil temperature, humidity, and microbial community, thus resulting in unstable and variable effects of nitrification inhibitors (McGeough et al. 2016; Vilas et al. 2019). As previously reported, the DCD nitrification inhibitor demonstrated a lower biological activity and survivability than the DMPP (Supplemental material Fig. S1). Azam et al. (2001) found that maintaining the same effects of nitrification inhibition requires higher dosages of nitrification inhibitor as demonstrated in both this trial and the previous report (Chen et al. 2015). Additionally, DCD is a kind of nitrification inhibitor with vegetable toxic; overuse of DCD easily triggers the damage to the plants, thus limiting its application in the agricultural production (Macadam et al. 2003).

Although DMPP is an ideal nitrification inhibitor and presently has been applied in agricultural production because of an advantage in suppressing nitrification, its unstable physical and chemical properties still limit its wide application. Particularly, high-dosage application of more than100 mg kg^{-1} in soil usually triggers toxicity to the plants (Rodrigues et al. 2018). Importantly, a large application of DMPP results in an increase in cost-effectiveness because of more than tenfold price comparing to the DCD. In this study, DMS is a novel nitrification inhibitor which was amended by improving DMPP; both DMS and DMPP contain the same core group 3,4-dimethylpyrazole nitrification suppression, not increasing negative effect and costs because DMS was produced by a reaction between zinc sulfate and DMP, while DMPP is produced by DMP reacting with phosphoric acid (Chen et al. 2015). Therefore, both DMPP and DMS have a similar production process, while zinc sulfate and phosphoric acid have no cost differences basically. Our study indicates that a novel DMS nitrification inhibitor revealed more strong effect in suppressing nitrification of ammonium and maintained a long-lasting alleviation role in releasing rates in soil because of its insoluble in water comparing to the DMPP (Supplementary material Table S1). Compared to DMPP, DMS with a neutral pH has better stability in soil and is slightly soluble in water. Importantly, DMS reveals a sustainable nitrification inhibition effect, and no potential side effect was observed in soil. Therefore, we believe that DMS is a reasonable optimization and improvement of DMPP without increasing cost. Both soil incubation and field experiment showed that DMS exhibited stronger bactericidal effects than DMPP after 58 days or 83 days, indicating that high accumulation of soil ammonium nitrogen in soils demonstrated a strong nitrification suppression role of DMS, thus increasing nitrogen use efficiency and crop yield by reducing the loss of nitrogen.

4 Conclusion

In summary, this study confirms that a novel amended DMS nitrification inhibitor revealed an important regulatory role in suppressing the conversion of ammonium nitrogen to nitrate nitrogen, and significantly improved nitrogen fertilizer use efficiency by inhibiting the activity of AOB in soils. DMS nitrification inhibitor is suitable for application in the black soil region in northeast of China. This study provided an insight into the regulatory role of a novel DMS nitrification inhibitor in enhancing the efficiency of nitrogen fertilizer, directly proving that high biological activity and long survival period of DMS in soil are two important factors maintaining sustainable nitrification inhibition effects, while prolonging the survival of nitrification inhibitor in soil is required for effectively lowering the loss of nitrogen in soil.

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Author contribution G.C., Z.D., and H.X. conceived and designed the experiment; G.C., Z.D., and W.Y. analyzed the data, and G.C., Z.D., and H.X wrote the paper. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare no competing interests.

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