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Effects of the Combined Application of Organic and Inorganic Nitrogen Fertilizer with DMPP on the Soil Potential Nitrification Rate

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Abstract

A 64-day cultivation experiment was conducted to investigate the effects of the combined application of organic and inorganic nitrogen fertilizer with DMPP on the ammonia-oxidizers and potential nitrification rate in two different soils (yellow brown soil and silt soil). The experiment included five treatments as follows: control group (CK); inorganic nitrogen (N); combined application of organic and inorganic nitrogen (NM); inorganic with DMPP (N+NI); combined application of organic and inorganic nitrogen with DMPP (NM+NI). The results found that the nitrogen fertilizer treatments significantly decrease soil pH in yellow brown soil which treatments with DMPP were retard, but soil pH trend to stability in silt soil. NH_4^+-N decreased rapidly in two different soils, while the NO_3^--N was opposite. Potential nitrification rate (PNR) and the changes of nitrogen contents showed that DMPP had effect inhibition in two different soils. At the end of the experiment, DMPP had no significant effect on the PNR in yellow brown soil with the nitrogen fertilizer treatments, but the NM+NI treatments had a significant inhibitor effect on the PNR in silty soil. Therefore, the combined application of organic and inorganic nitrogen fertilizer will trend to an increase in the PNR in the soil.

Keywords

Nitrification inhibitor; Potential nitrification rate; Soil acidification.

1. INTRODUCTION

The nitrification process in soil refers to the transformation of ammonium nitrogen into nitrate nitrogen by nitrifying microorganisms. Among this, the ammonia oxidation process, performed by ammonia monooxygenase (AMO) in ammonia-oxidizing microorganisms, converting NH_3 to NO_2^-, is the rate-limiting step of the entire nitrification process. Soil nitrification affects the supply of nitrogen to plants and their utilization; excessively high rates of nitrification can decrease fertilizer usage efficiency, leading to soil acidification and increased greenhouse gas emissions[1]. Therefore, rational regulation of the nitrification process can effectively enhance nitrogen fertilizer utilization and mitigate environmental issues related to nitrogen fertilizer application.

Extensive research indicates that the combined application of organic and inorganic nitrogen fertilizers can improve nitrogen use efficiency. Field experiments in rice fields by Zhu Haijun et al. have demonstrated that replacing 40% of inorganic nitrogen with organic nitrogen can achieve optimal nitrogen utilization rates[2]; pot experiments by Yu Chunxiao et al. suggest that applying 20% organic with 80% inorganic fertilizer can improve soil nitrogen supply and fertilizer utilization[3] below. However, considering the significant variability in the effects of

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organic and inorganic nitrogen fertilizer application methods on improving nitrogen use efficiency, the actual outcomes of such applications require further investigation.

As a common fertilizer efficiency enhancer, nitrification inhibitors can effectively slow the conversion of ammonium nitrogen to nitrate nitrogen in the soil, thus enhancing nitrogen utilization and controlling soil acidification. 3,4-dimethylpyrazole phosphate (DMPP), a novel nitrification inhibitor, is known for its low application rate and high efficiency. Studies indicate that DMPP can maintain its inhibitory effect in the field for 4-10 weeks, with an application rate of 0.5-1.5 kg·ha^{-2} achieving optimal inhibition efficiency[5][6]. However, like other nitrification inhibitors, the efficiency of DMPP is also influenced by various factors such as soil texture, temperature, and microorganisms [7][8]. Thus, a comprehensive study on the effects and mechanisms of DMPP addition on nitrogen transformation in soil is needed.

Presently, numerous studies focus on enhancing nitrogen fertilizer utilization through different organic, inorganic, and combined fertilizer applications, yet experiments on the impact of combined application of organic-inorganic fertilizers and nitrification inhibitors on the ammonia oxidation process are scarce. Based on this, our study conducts indoor cultivation experiments to investigate the nitrogen transformation capacity of two types of soils under different fertilization modes with nitrification inhibitor addition. It aims to clarify the impact of combined organic-inorganic nitrogen fertilization and nitrification inhibitors on soil potential nitrification, providing a scientific basis for improving nitrogen fertilizer utilization schemes.

2. MATERIALS AND METHODS

2.1. Experimental sample

The soils selected for this experiment were yellow-brown soil and silty loam. The yellow-brown soil was collected from the Liuhe experimental base in Nanjing, Jiangsu province, and the silty loam was from Jiangjia Village, Erling Town, Danyang, Zhenjiang City, with a collection depth of 0-20 cm. After air-drying, the fresh soil samples were sieved through a 2 mm sieve, and stones and plants were removed before mixing. The basic physical and chemical properties were measured (Table 1). The soil humidity was adjusted to 60% of the soil's maximum water holding capacity (WHC) using deionized water. Then, the soil was placed in a constant temperature incubator and pre-cultured for 7 days at 25 ℃.

Table 1. Basic physicochemical properties of test soil

Agrotype	pH	WHC (%)	available nitrogen (mg·kg^{-1})	available phosphorus (mg·kg^{-1})	rapidly available potassium (mg·kg^{-1})	organic matter (g·kg^{-1})
yellow brown soil	6.48	64.5	106	8.40	115	13.3
silt soil	7.86	40.2	116	25.4	118	20.0

2.2. Experimental design

Immediately after pre-treatment, the soil samples were subjected to indoor cultivation experiments. Five treatments were set up for the cultivation experiments: ① without nitrogen addition (CK); ② inorganic nitrogen applied (N); ③ inorganic nitrogen and organic fertilizer applied, where the organic fertilizer replaced 20% of the nitrogen (NM); ④ inorganic nitrogen and nitrification inhibitor applied (N+NI); ⑤ inorganic nitrogen, organic fertilizer, and nitrification inhibitor applied (NM+NI). The amount of nitrogen added in each treatment was 150 mg N·kg^{-1}, with the nitrification inhibitor DMPP being used at 1% of the nitrogen application rate. The samples were cultured for 64 days at 25 ℃ under constant temperature

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and humidity, with soil samples collected on days 1, 2, 4, 8, 16, 24, 32, 40, 48, 56, and 64 to measure soil pH, ammonium nitrogen, and nitrate nitrogen content. At the end of the cultivation period, the soil nitrification potential was measured.

2.3. Experimental method

The determination of soil ammonium nitrogen was performed using the indophenol blue colorimetric method. Soil nitrate nitrogen was measured with a dual-wavelength ultraviolet spectrophotometer, and soil pH was determined by the electrode potential method (soil to water ratio of 1:2.5). The potential nitrification rate (PNR) was measured using a suspension culture method[9]. 5.00 g of fresh soil sample was taken, and 20 ml of 1 mmol·L^{-1} $(NH4)_2SO_4$ solution and 5 ml of 50 mg·ml^{-1} potassium chlorate solution was added to a 50 ml centrifuge tube for cultivation, with deionized water added to adjust the final concentration of potassium chlorate to 10 mg·ml^{-1} to inhibit the oxidation of produced nitrite. The suspension was shaken at 25 ℃ in the dark for 25 hours. 2 ml of the suspension was extracted at 1, 5, and 25 hours, and after centrifuging at 13000r for 3 minutes, the supernatant was collected. The nitrite nitrogen content in the supernatant was measured at a wavelength of 540 nm using the N-(1-Naphthyl) ethylenediamine dihydrochloride method. The soil nitrification potential is the linear accumulation rate of nitrite nitrogen (NO_2^--N) during the cultivation period. The calculation formula is as follows:

$$PNR = \frac{\Delta c_{NO_2^-} \times V}{m \times W \times T}$$

PNR [μg·NO_2^--N·g^{-1}·h^{-1}] is the soil nitrification potential; Δc is the difference in the nitrite nitrogen content in the suspension before and after cultivation [μg·ml^{-1}]; V is the volume of the buffer solution (ml); m is the weight of the fresh soil taken (g); W is the water content factor; T is the time of cultivation (h).

To extract microbial DNA from soil samples, 0.50 g of fresh soil was weighed and processed using the E.Z.N.A.® Soil DNA Kit (OmegaBio-tek, Norcross, GA, USA). The extracted DNA samples were then subjected to quantitative PCR (qPCR) analysis to determine the abundance of the *amoA* genes from ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB). The *amoA* genes of AOA and AOB were amplified using primers Arch-amoAF/Arch-amoAR and amoA1F/amoA2R, respectively (Table 2).

Table 2. Primers and sequences of this study

Gene	Primers	Sequence	Length bp
Arch-amoA	Arch-amoAF	5'- STAATGGTCTGGCTTAGACG -3‘	635 bp[10]
	Arch-amoAR	5'- GCGGCCATCCATCTGTATGT -3'	
Bac-amoA	amoA1F	5'- GGGGTTTCTACTGGTGGT -3'	491 bp[11]
	aomA2R	5'- CCCCTCKGSAAAGCCTTCTTC-3’	

The amplification reaction setup included the use of 2x Phanta Master Mix in a 30 μL reaction volume: 15 μL of 2x Phanta Master Mix, 1 μL of Bar-PCR primer (10μM), 1 μL of Primer R (10μM), 10-20 ng of Genomic DNA, and ddH_2O added to reach a total volume of 30 μL. The PCR amplification conditions were as follows: an initial denaturation at 95 ℃ for 5 minutes, followed by 30 cycles of denaturation at 95 ℃ for 30 seconds, annealing at 55 ℃ for 30 seconds, and extension at 72 ℃ for 45 seconds, with a final extension at 72 ℃ for 5 minutes. Each sample was also tagged with a 6-base barcode for identification purposes. The PCR reaction was carried out

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in a 30 μL mixture containing 15 μL of high-fidelity DNA polymerase, 1 μL of each F/R primer (10μM), and 20 ng of template DNA.

2.4. Data processing

Statistical analysis was performed using SPSS 25. One-way ANOVA with Duncan's test was used to analyze the significance of differences in nitrification potential among treatments, and Pearson's method was used for correlation analysis.

3. RESULTS AND ANALYSIS

3.1. Soil Physicochemical Properties under Different Treatments

The impact of different nitrogen application treatments on soil physicochemical properties showed variation (Table 3). Throughout the experiment, a significant decrease in pH was observed in the yellow-brown soil across all treatments, with the addition of DMPP effectively slowing down the decline of pH. Soil nitrate nitrogen content significantly increased with nitrogen application, and the addition of DMPP significantly increased the ammonium nitrogen content.

Table 3. Soil physic-chemical properties in yellow brown soil (a) and silt soil (b) under different fertilization treatments

Treatment	yellow brown soil				silt soil			
	pH	NH_4^+-N ($mg \cdot kg^{-1}$)	NO_3^--N ($mg \cdot kg^{-1}$)	SOM ($g \cdot kg^{-1}$)	pH	NH_4^+-N ($mg \cdot kg^{-1}$)	NO_3—N ($mg \cdot kg^{-1}$)	SOM ($g \cdot kg^{-1}$)
CK	6.40 a	3.11 b	36.1 c	13.3 b	7.87 a	2.46 b	59.8 c	20.0 c
N	6.09 c	4.44 b	151 a	14.1 a	7.94 a	2.23 b	173 a	23.5 b
NM	6.07 c	3.96 b	120 b	15.7 a	7.90 a	2.12 b	134 b	25.9 a
N+NI	6.21 b	15.84 a	117 b	15.0 a	7.84 a	2.72 b	125 b	24.2 ab
NM+NI	6.25 b	17.35 a	116 b	15.1 a	7.82 a	11.5 a	124 b	26.1 a

3.2. Soil Nitrification Potential under Different Treatments

Analysis of the changes in the nitrification potential of yellow-brown soil (Figure 1a) indicates that the input of nitrogen fertilizer significantly increased the soil's nitrification potential, with N, NM, and NM+NI treatments being significantly higher than the CK treatment (0.18 $\mu g \cdot NO_2^- \text{-} N \cdot g^{-1} \cdot h^{-1}$) at 0.24 $\mu g \cdot NO_2^- \text{-} N \cdot g^{-1} \cdot h^{-1}$, 0.35 $\mu g \cdot NO_2^- \text{-} N \cdot g^{-1} \cdot h^{-1}$, and 0.33 $\mu g \cdot NO_2^- \text{-} N \cdot g^{-1} \cdot h^{-1}$, respectively. The addition of DMPP had an inhibitory effect on the rise in nitrification potential caused by nitrogen application, though this effect was not significant.

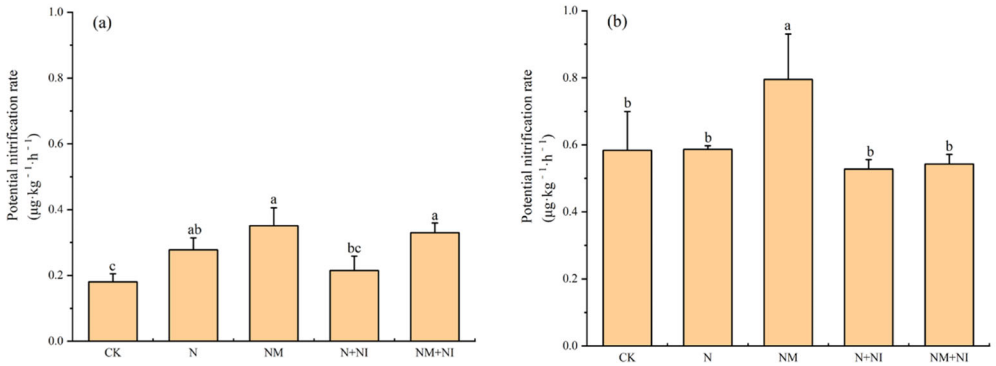

Figure. 1 Potential nitrification rate in yellow brown soil (a) and silt soil (b) under different fertilization treatments

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3.3. Abundance of Soil Ammonia-oxidizing Microorganisms

For yellow-brown soil, the abundance of *amoA* genes in soil, as illustrated in Figure 2a, showed that the combined application of organic and inorganic nitrogen fertilizers had no significant effect on the abundance of AOA and AOB *amoA* genes. Under nitrogen application conditions, the addition of DMPP significantly altered soil AOA and AOB. In yellow-brown soil, treatments with DMPP significantly increased soil AOA, and nitrogen fertilizer application significantly increased soil AOB, with N and NM treatments increasing soil AOB by 355.72% and 266.24% compared to CK, respectively. Moreover, the addition of DMPP significantly inhibited soil AOB under nitrogen application conditions, indicating that DMPP can suppress the increase in AOB caused by nitrogen application.

In silty loam, as depicted in Figure 2b, the fluctuation range of AOA and AOB across treatments was between 1.20 × 10^4 ~ 3.15 × 10^4 Copies·g^{-1} and 0.42 × 10^4 ~ 0.71 × 10^4 Copies·g^{-1}, respectively, with no significant response to nitrogen application. However, under the condition of combined organic and inorganic nitrogen fertilizer application, the addition of DMPP significantly increased AOA. Compared to NM, NM+NI increased soil AOB abundance by 160.70%.

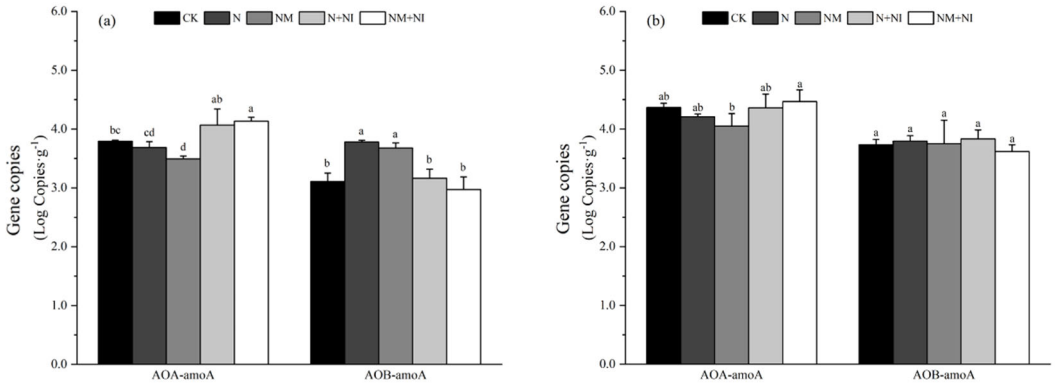

Figure 2. Copies of *aomA* gene in yellow brown soil (a) and silt soil (b) under different fertilization treatments

4. DISCUSS

The combined application of organic and inorganic fertilizers, as a common fertilization strategy, can effectively slow down soil degradation and improve nitrogen fertilizer utilization efficiency, thereby positively influencing crop yield. Analysis of soil nitrification potential at the end of the cultivation period showed that the method of combining organic and inorganic nitrogen significantly increased soil nitrification potential. The nitrification process, which influences the levels of nitrate nitrogen along with ammonium nitrogen in the soil affected by the application method of external nitrogen, can significantly increase the abundance of ammonia-oxidizing microorganisms in the soil [12][13], aligning with the findings of this study. However, the slow-release effect of organic nitrogen mineralization can impact crop growth; hence, improving nitrogen fertilizer utilization efficiency through the combined application of organic and inorganic nitrogen is vital.

The extensive application of chemical nitrogen fertilizers can lead to agricultural soil degradation, mainly associated with the large amounts of acids produced in the nitrogen cycle, such as the nitrification process of NH_4^+, especially evident in soils where long-term nitrogen application exceeds crop needs. Nitrification inhibitors, by inhibiting soil nitrification reactions, can enhance chemical fertilizer utilization and slow down soil degradation. In this study,

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significant variation was observed in the inhibitory effect of DMPP on soil pH decline across different soils; the application of DMPP significantly slowed down the decrease in pH value caused by nitrogen application in yellow-brown soil. However, the effect was not apparent in silty loam, possibly due to the inherent strong acid-buffering capacity of the silty loam tested. Meanwhile, throughout the cultivation process in different soils, DMPP significantly increased the content of ammonium nitrogen in the soil, indicating that DMPP exhibits evident nitrification inhibition capability in both soil types, with the strongest effect noted in NM+NI treatment. According to Figure 1 analysis, DMPP did not show significant inhibitory capability on nitrification potential at the end of cultivation in soils with low nitrification potential; in silty loam with higher nitrification potential, DMPP notably inhibited the increase in nitrification potential induced by organic fertilizer, indicating a shortcoming of DMPP in having a brief inhibitory period in some soils, consistent with Dong's findings on the inhibitory effects of DMPP [14].

The mechanism behind DMPP's inhibitory effect is yet to be conclusively determined, with some scholars suggesting it acts by inhibiting the transformation of NH3 to hydroxylamine in the ammonia oxidation process[15]. In this process, the ammonia monooxygenase (AMO), which catalyzes ammonia oxidation present in ammonia-oxidizing microorganisms, plays a decisive role. Analyzing the abundance of *amoA* genes in soil (Figure 2) reveals that in this study, the addition of DMPP significantly reduced the AOB in yellow-brown soil, while significantly increasing AOA in treatments with DMPP. Zhang's research indicates that DMPP mainly regulates the nitrification reaction by inhibiting AOB [16]. However, the inhibitory effect of DMPP varies across different soils, with previous studies indicating that soil pH and the type of ammonia-oxidizing microorganisms dominating the nitrification process under different pH environments are the main factors influencing DMPP's inhibitory capability[17][18],[19]. Consistent with the observed results in this study, DMPP mainly exhibits an inhibitory effect on AOB, aligning with Luchibia et al.'s findings[20] .

5. CONCLUSION

The combined application of organic and inorganic nitrogen can enhance soil nitrification potential by increasing the abundance of ammonia-oxidizing microorganisms, with significant effects observed in acidic soils with lower nitrification potential. DMPP exerts an inhibitory effect on soil nitrification potential by altering the abundance of AOB in acidic soils. Utilizing a combination of organic and inorganic nitrogen application along with the addition of DMPP can effectively extend the retention time of nitrogen in the soil and increase crop utilization efficiency of nitrogen fertilizers.

REFERENCES

[1] Guo J H, Liu X J, Zhang Y, et al,. Significant Acidification in Major Chinese Croplands[J]. Science, 2010, 327(5968):1008-1010.

[2] Zhu Haijun, Tang Zhou, et al,. Effects of Irrigation Patterns and Combined Application of Organic Fertilizer on Rice Yield, Photosynthetic Characteristics and Nitrogen Use Efficiency [J]. Soils, 2022,54(04):700-707.

[3] Yu Chun-xiao, Zhang Li-li, et. Combining N-inhibitor and chicken manure with reduced N fertilizer to improve the conversion and utilization of fertilizer N in a paddy soil[J]. Journal of Plant Nutrition and Fertilizers, 2021, 27(9): 1581-1591.

[4] Di H J, Cameron K C, Podolyan A, et al. Effect of soil moisture status and a nitrification inhibitor, dicyandiamide, on ammonia oxidizer and denitrifier growth and nitrous oxide emissions in a grassland soil[J]. Soil Biology & Biochemistry, 2014, 73:59-68.

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202406_10(6).0011

[5] Zerulla W, Barth T, Dressel J, et al. 3,4-Dimethylpyrazole phosphate (DMPP) – a new nitrification inhibitor for agriculture and horticulture[J]. Biology & Fertility of Soils, 2001, 34(2):79-84.

[6] Di H J, Cameron K C. How does the application of different nitrification inhibitors affect nitrous oxide emissions and nitrate leaching from cow urine in grazed pastures?[J]. Soil Use & Management, 2012, 28(1):54-61.

[7] Vitale, Ottaiano, Polimeno, et al. Effects of 3,4-dimethylphyrazole phosphate-added nitrogen fertilizers on crop growth and N2O emissions in Southern Italy[J]. PLANT SOIL ENVIRON, 2013, 2013,59(11)(-):517-523.

[8] Barth G, Von S, TUCHER, et al. Effectiveness of 3,4-Dimethylpyrazole Phosphate as Nitrification Inhibitor in Soil as Influenced by Inhibitor Concentration,Application Form, and Soil Matric Potential[J]. Pedosphere, 2008, 18(3):378-385.

[9] Hu H W, Macdonald C A, Trivedi P, et al. Effects of climate warming and elevated CO2 on autotrophic nitrification and nitrifiers in dryland ecosystems[J]. Soil Biology and Biochemistry, 2016, 92:1-15.

[10] Rotthauwe J H, Witzel K P, Liesack W. The Ammonia Monooxygenase Structural Gene amoA as a Functional Marker: Molecular Fine-Scale Analysis of Natural Ammonia-Oxidizing Populations[J]. Applied and Environmental Microbiology, 1998, 63(12):4704-4712.

[11] Francis C A, Roberts K J, Beman J M, et al. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean.[J]. Proceedings of the National Academy of Sciences of the United States of America, 2005, 102(41):P.14683-14688.

[12] J Liu, Shu A, Song W, et al. Long-term organic fertilizer substitution increases rice yield by improving soil properties and regulating soil bacteria[J]. Geoderma, 2021, 404:115287.

[13] Ren J H, Liu X L, Yang W P, et al. Rhizosphere soil properties, microbial community, and enzyme activities: Short-term responses to partial substitution of chemical fertilizer with organic manure, [J]. Journal of Environmental Management, 2021, 299: 113650.

[14] Dong D, Yang W, Sun H, et al. Nitrous oxide emissions in response to long-term application of the nitrification inhibitor DMPP in an acidic luvisol[J]. Applied Soil Ecology, 2020, 159.

[15] Irigoyen I, Muro J, Azpilikueta M, et al. Ammonium oxidation kinetics in the presence of nitrification inhibitors DCD and DMPP at various temperatures[J]. Soil Research, 2003, 41(6):1177-1183.

[16] Zhang M, Wang W, Bai S H, et al. Antagonistic effects of nitrification inhibitor 3,4-dimethylpyrazole phosphate and fungicide iprodione on net nitrification in an agricultural soil[J]. Soil Biology & Biochemistry, 2018, 116:167-170.

[17] Kaveney B, Condon J, et al. The distribution of soil nitrifiers and efficacy of 3,4-dimethylpyrazole phosphate changes with soil depth and calcium carbonate application[J]. Soil Biology & Biochemistry, 2020, 150:0038-0717.

[18] Kaveney B, Condon J, et al. Soil moisture impacts nitrification from nitrogen fertilisers treated with 3,4-dimethylpyrazole phosphate in acidic soils[J]. Soil research, 2021, 60(1):86-101.

[19] Bachtsevani E, Papalatani CV, et al. Effects of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on the activity and diversity of the soil microbial community under contrasting soil pH[J]. Biology and fertility of soils, 2021, 57(8):1117-1135

[20] Luchibia A O, Lam S K, Suter H, et al. Effects of repeated applications of urea with DMPP on ammonia oxidizers, denitrifiers, and non-targeted microbial communities of an agricultural soil in Queensland, Australia [J]. Applied