Influence of Long-Term Application of Organic Fertilization on the Effects of DCD in Brown Soil

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Abstract. Nitrification (DCD) inhibitor has been proved to be effective in retarding nitrification process of nitrogen in the soil. Application of nitrification inhibitors to the field is considered to be a major method in controlling nonpoint pollution induced by nitrogen fertilizer in agricultural production. Thus a simulating experiment was carried out to study the Influence of long-term application of organic fertilization on the effects of different concentrations of Dicyandiamide in brown soil, and the best application rate was screened to provide references for agricultural practice. Soil samples (0-20cm) were taken from two treatments in October 2017: (1) no fertilization (CK); (2) pig manure input (M). The samples were sieved while still fresh and incubated at a constant temperature (25℃) and soil moisture in different treatments remained 60 percent of field water capacity for 42 days with periodic subsamplings. The experiment included unfertilized control, soil appended with urea nitrogen of 0.4g/kg alone, soil appended with urea nitrogen of 0.4g/kg and 1%DCD, soil appended with urea nitrogen of 0.4g/kg and 2%DCD and soil appended with urea nitrogen of 0.4g/kg and 5%DCD (The Percents represents the percentage of DCD depended on the amount of applied pure N). During the experimental period, the contents of ammonium nitrogen (NH₄⁺-N), nitrite nitrogen (NO₂⁻-N), pH and nitrification potential were measured. Results of laboratory incubations indicated that DCD effectively inhibited the transformation from ammonium nitrogen to nitrate nitrogen in brown soil and the trends were N+5%DCD > N+2%DCD > N+1%DCD>N. It was found that nitrification was always greater in long-term application of organic fertilizer soil than in long-term unfertilized soil. Long-term application of organic fertilizer reduced the inhibitory effect of DCD.

1. Introduction

Nitrogen is one of the necessary nutrition elements in plant growth, and it can increase grain yield. Therefore urea is widely used in agricultural production as a convenient source of nitrogen. However, when applied to soil, urea has been reported to have low N response efficiencies due to volatilization, nitrification, denitrification, erosion, runoff and leaching. Raun and Johnson's study found that the rate of nitrogen loss in agricultural ecosystems was nearly 70 percent [1]. Previous studies show that
nitrification inhibitors are useful in improving N use efficiency which can inhibit the oxidation of ammonium [2]. Nitrification inhibitors have also been shown to be effective in reducing nitrate leaching and nitrous oxide emissions. It can reduce by inhibition of nitrification of ammonium nitrogen fertilization of nitrogen leaching and N₂O emissions, in order to improve the utilization rate of nitrogen fertilizer. Nitrification inhibitors have been applied to practical production in many countries [3]. However, the effect of nitrification inhibitors is affected by many factors such as soil type, organic matter, pH, soil microecological environment, and ecological conditions of crop growth, and the effect of the same nitrification inhibitor on soil with different physical and chemical properties is also different. DCD is one type of nitrification inhibitors widely used throughout the world and it can play an important role in reducing N losses from agricultural fields. Compared with other nitrification inhibitors, DCD has better nitrification inhibition, its physical and chemical properties are stable, and has the advantages of water solubility, weak volatility, complete degradation, economic efficiency and so on [4, 5]. A large number of studies have been carried out on DCD focusing on its inhibitory effects. However, very limited information is available on effects of DCD on long-term application of organic fertilizer in brown soil. Therefore, this study aims to investigate the effects of DCD after a long-term of organic fertilization for 39 years in a brown soil and screen out the best application amount of DCD to provide the basis for the rational application of nitrification inhibitors in brown soil.

2. Materials and methods

2.1. Experiment design
The selected soil samples were collected from shenyang agricultural university in October, 2017, under a long-term fertilization for 39 years in a brown soil (40°48′N and 123°33′E). Basic chemical properties of experimental soil in 1979 and the application rates of fertilizer are described by Luo et al [6]. In this laboratory incubation experiment, two fertilization treatments were selected: no fertilization (CK) and pig manure input (M). The organic manure was pig manure, which was applied as base fertilizer before seeding. The soil samples were taken from 0 ~ 20 cm top layer of soil. After fresh soil samples were collected, pass through 2 mm sieve, fully mixed, removed the gravel, plant roots organic residue and other sundries. The soil moisture content was determined and remained 60 percent of field water capacity. Thermostatic incubation indoor at 25℃ for advance 15 days, during incubation, 60% of soil moisture in the field was controlled by gravimetric method.

The experimental treatments for DCD with different dosages were as follows: (1) no fertilization (CK); (2) single urea treatment (N); (3) urea+1%DCD (N+1%DCD); (4) urea+2%DCD (N+2%DCD); (5) urea +5%DCD (N+5%DCD). Three replicates were set for each treatment, the same amount of urea was applied with 0.4 g N kg⁻¹ soil, and the dosage of inhibitor was the percentage of urea pure nitrogen. The amount of soil tested is equivalent to the dry soil weight of 100g. Before the experiment, urea mixed with inhibitor was mixed with soil and transferred to culture containers. Soil moisture was controlled by gravimetric methods to 60 percent of field water capacity, replenished every two days, and containers were randomly arranged. The small holes were punched in the containers to prevent water loss and to ensure air permeability.

The containers were placed in a room with a constant temperature of 25℃ and cultured in a dark place. Destructive sampling was conducted on days 1, 3, 7, 14, 21, 28, 35 and 42, respectively.

2.2. Soil physicochemical property analysis
Soil mineralized nitrogen was determined by 5.00g fresh soil, which was extracted with 0.01mol·l⁻¹ CaCl₂ (liquid-soil ratio=10:1), and soil was oscillated for 1h in a water-bath thermostatic oscillation machine (temperature 25, frequency 180Hz). The filtrate is filtered and determined by the AA3 multi-channel flow analyzer produced in Germany. Soil nitrification potential was determined by alpha naphthylamine colorimetry. Soil pH was determined with a glass electrode using a soil/water ratio of 1:2.5.
2.3. Statistical analysis
The charts were made by Origin 2017 and Variance Analysis adopts SPSS 19.00.

3. Results and discussion

![Figure 1](image1.png)

Figure 1. The NH$_4^+$-N of different fertilization treatments.  
(Note: a stands for long-term unfertilized soil and b stands for long-term application of organic fertilizer soil.)

The NH$_4^+$-N of different fertilization treatments was shown in figure 1. In the whole process of cultivation, the change of NH$_4^+$-N in different treatments in long-term unfertilized soil first increased and then gradually decreased, while in long-term application of organic fertilizer soil, the change showed a gradual downward trend. There was no significant change in the content of ammonium nitrogen of CK in two different treatments soil. In the long-term unfertilized soil and long-term application of organic fertilizer soil, the contents of ammonium nitrogen in DCD treatments were significantly higher than that in N treatment ($p<0.05$), and the trends were N+5%DCD > N+2%DCD > N+1%DCD>N. The results showed that compared to long-term application of organic fertilizer soil, the contents of ammonium nitrogen in DCD treatments in long-term unfertilized soil were higher, while nitrate nitrogen concentrations were significantly lower (Fig.1, Fig.2). Compared with long-term application of organic fertilizer soil, use of N-based fertilizers in combination with DCD significantly lengthens N presence in the ammonium form in long-term unfertilized soil (NH$_4^+$-N), with beneficial effects for agriculture and related ecosystems [7].

![Figure 2](image2.png)

Figure 2. The NO$_3^-$-N of different fertilization treatments.
From figure 2, it can be clearly seen that, except CK treatment, the contents of NO$_3^-$-N had the same change tendency in the long-term unfertilized soil and long-term application of organic fertilizer soil—gradually rising, but the change in the long-term application of organic fertilizer soil rise faster. The contents of NO$_3^-$-N in N treatment were significantly higher than that in other treatments with DCD, and the trends were N > N+1%DCD > N+2%DCD > N+5%DCD.

Combining the contents of NH$_4^+$-N and NO$_3^-$-N, it can be seen that long-term application of organic fertilizer promotes the nitrification of soil. Adding DCD to the soil can make the fertilizer urea N more maintained in NH$_4^+$-N form and less accumulated in NO$_3^-$-N form, this trend is even more pronounced in long-term unfertilized soil. It could be because long-term application of organic fertilizer increased the number of soil ammonia-oxidizing bacteria (AOB) and AOB was likely more metabolically active in organic manure-fertilized soils than in long-term unfertilized soil after long-term application [7].

**Figure 3.** The nitrification potential of different fertilization treatments.

In the two different treatments soil, the change trend of nitrification potential was very different. It was found that nitrification potentials in treatments with DCD were lower than that in N treatment and nitrification inhibitory rate in N+5%DCD treatment was the highest than that in other treatments.

**Figure 4.** The pH of different fertilization treatments.

The soil pH was shown in figure 4 in different treatments. Overall, soil pH showed a general trend of gradual decline. Within 14 to 42 days of cultivation, compared with the treatments in long-term application of organic fertilizer soil, the treatments in long-term unfertilized soil showed a significant lower in pH value and this may be because it has a higher ammonium nitrogen content. In the addition
of urea treatments, soil pH was lowest in the N treatment and highest in the N+5%DCD treatment. The CK treatment in long-term application of organic fertilizer soil had a higher pH (ranged from 6.40 to 6.88) than that in long-term unfertilized soil (ranged from 5.70 to 6.86), that is because long-term application of organic fertilizer can enrich cations, thus increasing the pH value of soil [8].

After long-term experiment for 39 years, Long-term application of organic fertilization resulted in significant differences in most soil physicochemical properties whichare clearly major factors influencing niche separation of Ammonia-oxidizing microorganism [9] and thus affecting the nitrification inhibition of DCD. Those parts needs further study.

4. Conclusion
1) This study has demonstrated that after 39-y long-term application of organic fertilization, the long-term application of organic fertilizers increased pH value. Compared with long-term application of no fertilizer, long-term application of organic fertilizer promoted the nitrification of soil. Long-term application of organic fertilizer reduced the inhibitory effect of DCD.
2) It was found that DCD effectively inhibited the transformation from ammonium nitrogen to nitrate nitrogen in brown soil and the inhibit effect ranked as N+5%DCD > N+2%DCD > N+1%DCD>N.

Acknowledgments
This work is supported by The National Key Research and Development Program of China (Grant Number: 2017YFD0200707).

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