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Inhibitory Effects of Different Types and Doses of Herbicides on Soil Nitrification Potentials

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Abstract To elucidate the inhibitory effects of different herbicides on soil nitrification, eight widely used herbicides, i.e., acetochlor, atrazine, dicamba, isoproturon, paraquat, puma, tribenuron-methyl, and 2,4-dichlorophenoxyacetic acid butyl ester (2,4-Dbe), which represent different chemical taxonomy were selected. Our results indicated that herbicide 2,4-Dbe displayed the best inhibitory effect on nitrification, followed by puma and tribenuron-methyl, whereas the remaining five herbicides exhibited less effect when 10 mg of active ingredient (A.I.) of every herbicide per kg of soil was applied in vegetable-planting soil. The inhibition appeared when 5–100 mg of A.I. 2,4-Dbe was employed, which was enhanced with an increment in its dose in both vegetable-planting and fluvo-aquic soils. However, the inhibitory effect of 10 mg of A. I. 2,4-Dbe exhibited obvious differences in these two types of soils, where the duration of inhibition was shorter as it only continued about a week in fluvo-aquic and calcic cambisols soils with strong nitrification activity but poorer effect as compared to 10 mg of dicyandiamide (DCD). In contrast, the duration of inhibition exceeded 2 months in dryland red and shajiang black soils with a weak nitrification activity which was equivalent to DCD. In addition, comparing with five nitrification inhibitors, 10 mg of 2,4-Dbe had better inhibition than the substituted pyrimidine (AM) and sulfocarbamide (SU), but was equivalent to DCD, nitrapirin, and 3,4-dimethylpyrazole phosphate (DMPP) at their recommended application rates in dryland red soil. These obtained data clearly indicated that 2,4-Dbe could play a stronger role as a nitrification inhibitor in soils.

Keywords Herbicide · Nitrification · 2,4-D · Soil types · Nitrification inhibitor

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1 Introduction

Decreasing the rate of soil nitrification is one of the important strategies to reduce runoff and leakage of nitrate nitrogen as well as nitrogen oxide emission from soils (Laura et al. 2013; Fisk et al. 2015; Yu et al. 2018). For this purpose, few nitrification inhibitors such as 2-chloro-6-trichloromethyl-pyridine (nitrapyrin), dicyandiamide (DCD), 3,4-dimethylpyrazole phosphate (DMPP), sulfocarbamide (SU), and substituted pyrimidine (AM) (Zerulla et al. 2001; Laura et al. 2013; Anjana et al. 2016) have been developed and frequently applied in agriculture, which displayed excellent inhibitory effects on soil nitrification. On this context, researchers continuously strive in finding out new nitrification inhibitors.

At this stage, different types of chemical herbicides are widely used to control harmful weed in agriculture. However, it has been confirmed from many studies that the herbicides have obvious impact on the activities of soil microorganisms and nitrogen transformation (Damin and Trivelin 2011). Moreover, these herbicides vary in their toxicity to microorganisms and also in terms of effectiveness in inhibiting nitrification. Thus, some of them are highly toxic, and others are poorly toxic to nitrifying organisms (Debona and Audus 1978). Martens (1987) reported that 10 of 46 herbicides retarded nitrification of urea nitrogen when 50 mg of active ingredient (A.I.) kg^{-1} was applied. The experimental results of Martens and Bremner (1993) indicated that 10 of 28 herbicides inhibited nitrification of urea nitrogen when 5 mg of A.I. kg^{-1} was applied and all of them retarded when more or less 50 mg kg^{-1} was employed.

2,4-Dichlorophenoxyacetic acid (2,4-D) is the first herbicide commercially released in 1946 (Robert 2007),which affects the microbial community (Fabio et al. 2007) and nitrogen transformation (Narain Rai 1992). In fact, the effect of 2,4-D on soil nitrification was conducted in early 1950s (Slepechy and Beck 1950). Olson and Lindwall (1991) considered that the application of 2,4-D at 2 and 100 times the field rate reduced nitrification under laboratory conditions by 11% and 79%, respectively.

However, very limited studies are focusing on the inhibitory effect of 2,4-D on nitrification. We hypothesized that the inhibitory impacts of 2,4-D was much stronger than those of recognized nitrification inhibitors. Here, in this study, the inhibitory effect of 2,4-D with other selected herbicides was compared comprehensively in terms of their dose-effect, differences in their performance in different types of soils, and comparing with recognized nitrification inhibitors to evaluate the level of influence of 2,4-D on nitrification as well checking if whether it could act as a nitrification inhibitor.

2 Materials and Methods

2.1 Experimental Herbicides, Nitrification Inhibitors, and Soils

In these experiments, eight herbicides which represent different chemical taxonomy were selected that include acetochlor, atrazine, dicamba, isoproturon, paraquat, puma, tribenuron-methyl, and 2,4-Dbe. Five different soils including upland red soil, vegetable-planting red soil, shajiang black soil, fluvo-aquic soil, and calcic cambisols soil and five recognized nitrification inhibitors including 2-chloro-6-trichloromethyl-pyridine (nitrapyrin), DCD, DMPP, SU, and AM were used. The details of the above materials have been shown in Tables 1, 2, and 3.

2.2 Experimental Design

All fresh soil samples from field sites were made to pass through a sieve of 2 mm. Then, 150 g (equal to the weight of dry soil) of each soil was placed into a 300 mL wide-mouth bottle sand each with four replications. Subsequently, the above samples were adjusted to 60%of water holding capacity using double deionized water in which herbicides or nitrification inhibitors and urea nitrogen (200 mg of N kg^{-1}) were dissolved. The control soils were prepared only with urea nitrogen solution, and the blank samples were prepared only with double deionized water. All the soil samples were incubated at 28 ± 1 °C, and 25 g of soil was sampled each time by destructive sampling from each bottle at certain time intervals after incubation.

2.3 Analysis of the Contents of Nitrate and Ammonium

The contents of NH_4^+-N andNO_3^--N in the soil samples were determined by extracting with 100 mL of KCl solution with a concentration of 2 mol L^{-1}, which was shaken for 1 h, filtered and then checked using a continuous-flow analyzer (Skalar, Breda, Netherlands).

2.4 Statistical Analysis

All statistical analyses were performed using SPSS13.0 (SPSS for Windows, Version 13.0). The differences in the contents of NH_4^+-N and NO_3^--N between treatment and control group were analyzed using ANOVA, and the differences were considered significant at $p < 0.05$.

3 Results

3.1 Effect of Different Types of Herbicides on Soil Nitrification

Figure 1a, b shows that the eight selected herbicides have different impact on nitrification in vegetable-

Table 1 Types of eight selected herbicides

Type	Active ingredient (A.I.) (%)	Form	Application rate (mg A.I. kg^{-1})	Manufacturing company
Paraquat	50	Particle	10	Shandong LubaChemical Co., Ltd.
Acetochlor	50	Emulsion	10	Shandong Qiaochan Chemical Co., Ltd.
2,4-Dbe	57	Emulsion	10	Dalian Songliao Chemical Co., Ltd.
Puma	6.9	Emulsion	10	Bayer Crop Science (China) Co., Ltd.
Tribenuron-methyl	10	Powder	10	Hefei Jiuyi Agro-development Co., Ltd.
Isoproturon	50	Powder	10	Zhejiang Mefront Agro-chemical Co., Ltd.
Dicamba	95	Emulsion	10	Jiangsu Tenglong Group
Atrazine	48	Powder	10	Qingdao Modern Agricultural Co., Ltd.

planting red soil with a relatively strong nitrification activity when 10 mg of A.I. per kg of dry soil was applied for every herbicide. The 2,4-Dbe displayed the most inhibitory effect among eight herbicides, followed by puma and tribenuron-methyl, whereas other five herbicides did not imply any obvious inhibiting action. The 2,4-Dbe significantly inhibited nitrification ($p < 0.05$) compared to control during incubation period from 1st to 17th days, and was significant compared to puma and tribenuron-methyl from 5th to 17th days; then, puma from 1st to 13th days and tribenuron-methyl from 1st to 9th days. These data elucidated that 2,4-Dbe had better inhibitory effect on soil nitrification.

3.2 Effect of Different Doses of 2,4-Dbeherbicide on Soil Nitrification

Based on the above experimental results, the application of different doses of 2,4-Dbein two soils with high nitrification potential was examined. Figure 2a, b indicated that the application of urea nitrogen into vegetable-planting red soil would be completely nitrified in 9 days. All the treatments with the application of 5–100 mg of A.I. 2,4-D retarded soil nitrification during 3–15 days of incubation, which enhanced with an increment in its dose. Especially, the effectiveness of inhibition was more apparent when applied doses were > 50 mg, and the residues of NH_4^+ contents in the soils were significantly higher than that of other treatments until over 32 days of incubation.

Fluvo-aquic soil is with a much stronger nitrifying capacity and the application of urea into this soil completely nitrified in 5 days (Fig. 2c, d). Although the pattern of inhibitory effectiveness of 2,4-Dbe on nitrification in this soil also was similar to that of vegetable-planting red soil where an enhancement was noted with an increment in doses. The duration of inhibition was significantly shorter (3–9 days) than in case of vegetable-planting red soil. However, when 10–100 mg of 2,4-Dbe was applied, the inhibitory effects still were outstanding in duration.

Table 2 Types of five selected nitrification inhibitors

Type	Active ingredient (A.I.) (%)	Form	Recommended application rate (mg A.I. kg^{-1})	Manufacturing company
DCD	100	Powder	10	Shanghai Macklin Biochemical Tech Co., Ltd.
Nitrapyrin	20	Aqua	2	Dow Agro-Sciences
DMPP	100	Powder	2	Shanghai Resun Trading Co., Ltd.
SU	98	Powder	4	Shanghai Macklin Biochemical Tech Co., Ltd.
AM	99	Crystal	2	Sinopharm Chemical Reagent Co., Ltd.

Table 3 Basic physicochemical properties of soils

Sites	Soil type	pH	Organic matter (g kg^{-1})	Total N (g kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})
Fujian Jiangle	Upland red soil	4.67	21.06	1.16	5.99	19.95
Henan Nunan	Shajiang black soil	5.91	16.09	1.25	10.18	25.46
Henan Fengqiu	Fluvo-aquic soil	8.34	10.60	0.97	1.02	28.46
Jiangxi Anfu	Vegetable-planting red soil	5.84	33.89	1.95	4.11	60.08
Shanxi Shenmu	Calcic cambisols soil	8.41	2.27	0.16	2.43	0.80

3.3 Comparison of the Inhibitory Effects of 2,4-Dbe on Soil Nitrification Among Different Types of Soils

DCD is commonly known as being a better nitrification inhibitor and thus has been widely applied in practice. In this study, differences in the inhibitory capacity of 2,4-Dbe in four types of soils and a comparison with DCD were studied. The adopted four soils could be divided into two groups: a stronger nitrifying activity in fluvo-aquic and calcic cambisols soils, whereas weak nitrifying activity in case of dryland red and Shajiang black soils.

When 10 mg of A.I. kg^{-1} was applied to four types of soils, the effect of 2,4-Dbe on nitrification showed considerable differences (Fig. 4a–e). The duration of inhibition of 2,4-Dbewas relatively shorter in the soil group with strong nitrifying activity, which only continued for about 1 week and significantly showed a poorer effect as compared to DCD (at an application rate of 10 mg) (Fig. 3a–d). However, better inhibitory effects were observed in the soil group with weak nitrifying activity similar to that of DCD (Fig. 3e–h), where the duration of inhibition could exceed 2 months. Moreover, the duration of inhibition of DCD also showed a difference in different types of soils, exceeding 22 days in Fluvo-aquic soil and 34 days in calcic cambisols soil.

3.4 Comparison of the Inhibitory Effects of 2,4-Dbe on Soil Nitrification with Different Nitrification Inhibitors

In order to compare the inhibitory effect of 2,4-Dbeon soil nitrification, five recognized nitrification inhibitors were applied. Figure 4a, b demonstrated that the inhibitory effect of 2,4-Dbe was better than AM and SU when applied with 10 mg, and no obvious differences were found out between AM and SU. But, the effects were equivalent to DCD, nitrapyrin, and DMPP at the recommended application rates and also did not show any obvious differences among DCD, nitrapyrin, and DMPP in dryland red soil, where the duration of inhibition exceeded 2 months. Notably, the nitrification rate of this soil was relatively slow, but the obtained data clearly implied that 2,4-Dbe played a more or less an inhibitor role.

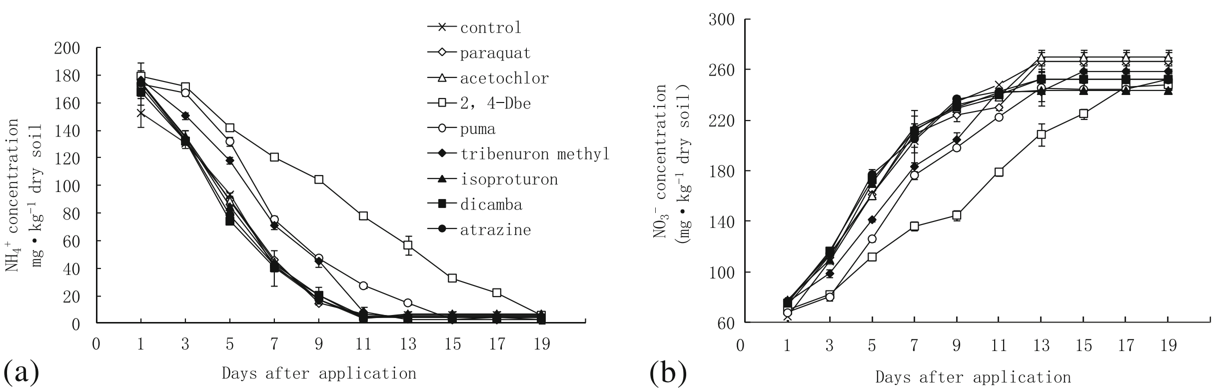

Fig. 1 Changes in the contents of ammonium (**a**) and nitrate (**b**) in vegetable-planting soil under different treatments of herbicide

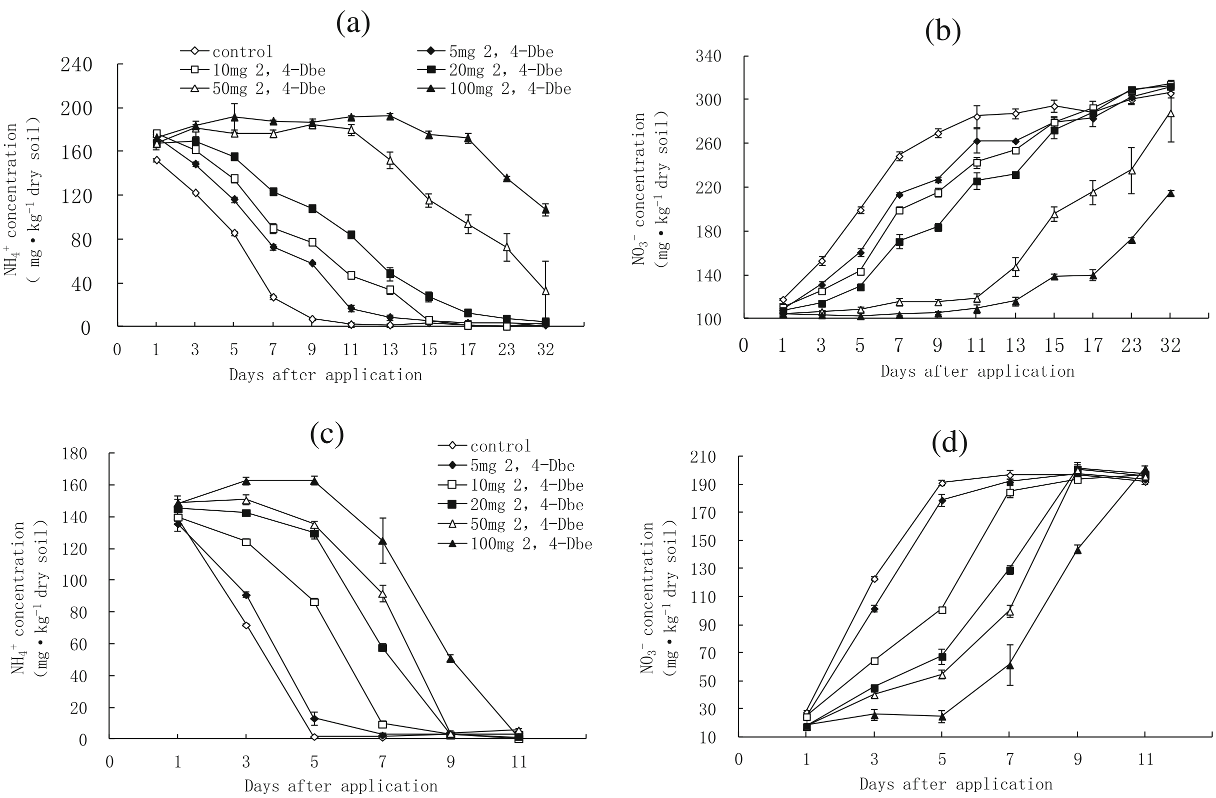

Fig. 2 Changes in the contents of ammonium and nitrate in vegetable-planting red soil (**a**, **b**) and in Fluvo-aquic soil (**c**, **d**) under different doses of 2,4-Dbe

4 Discussion

Currently, many types of herbicides are broadly being used in agriculture, and previous studies have indicated that most of these herbicides have an effect on soil nitrification or nitrogen transformation especially at higher application rates (Martens 1987; Martens and Bremner 1993; Csitari et al. 1996; Sheoran et al. 2016). To understand the toxicity on the level and the residual of different types of chemical herbicides, more intensive work is still needed to be done.

Although the impact on soil nitrification is one of the properties of herbicides, the level of inhibition and mechanism is still not clearly understood. Whether the inhibitory effects of herbicides are comparable with the commonly known nitrification inhibitors is to be examined. In fact, the toxicity of herbicides is related to many factors such as type of chemicals and their derivatives (Debona and Audus 1978; Martens 1987; Narain Rai 1992; Martens and Bremner 1993; Kucharski et al. 2009a), doses of application (Martens 1987; Nada and Mitar 2002), and degradation (Corke and Thompson 1970), as well as related to the properties of soil (Martens and Bremner 1994) and microbial species (Nada and Mitar 2002). Therefore, evaluating the effectiveness of herbicide needs the support of more evidences.

2,4-D is a frequently used herbicide and has a long history, which is not only employed as a plant growth regulator but also as a herbicide. However, it is still unknown whether it could be used as a nitrification inhibitor. Different derivatives of 2,4-D had different toxicity (acid/amine and ester groups) (Narain Rai 1992; Qurratu and Reehan 2016). There exists two viewpoints on the effect of 2,4-D, where Biederbeck et al. (1987) considered that the effects of long-term applications of 2,4-D were neither ecologically significant nor interfere with nutrient cycling to adversely affect the fertility of soil. Narain Rai (1992) believed that the application of 2,4-Dbe reduced the populations of fungal, bacterial, and actinomycete, C and N of microbial biomass, as well as mineralization of N, nitrification, and potentially mineralizable N. This indicates that 2,4-Dbe probably interfered with nutrient cycling.

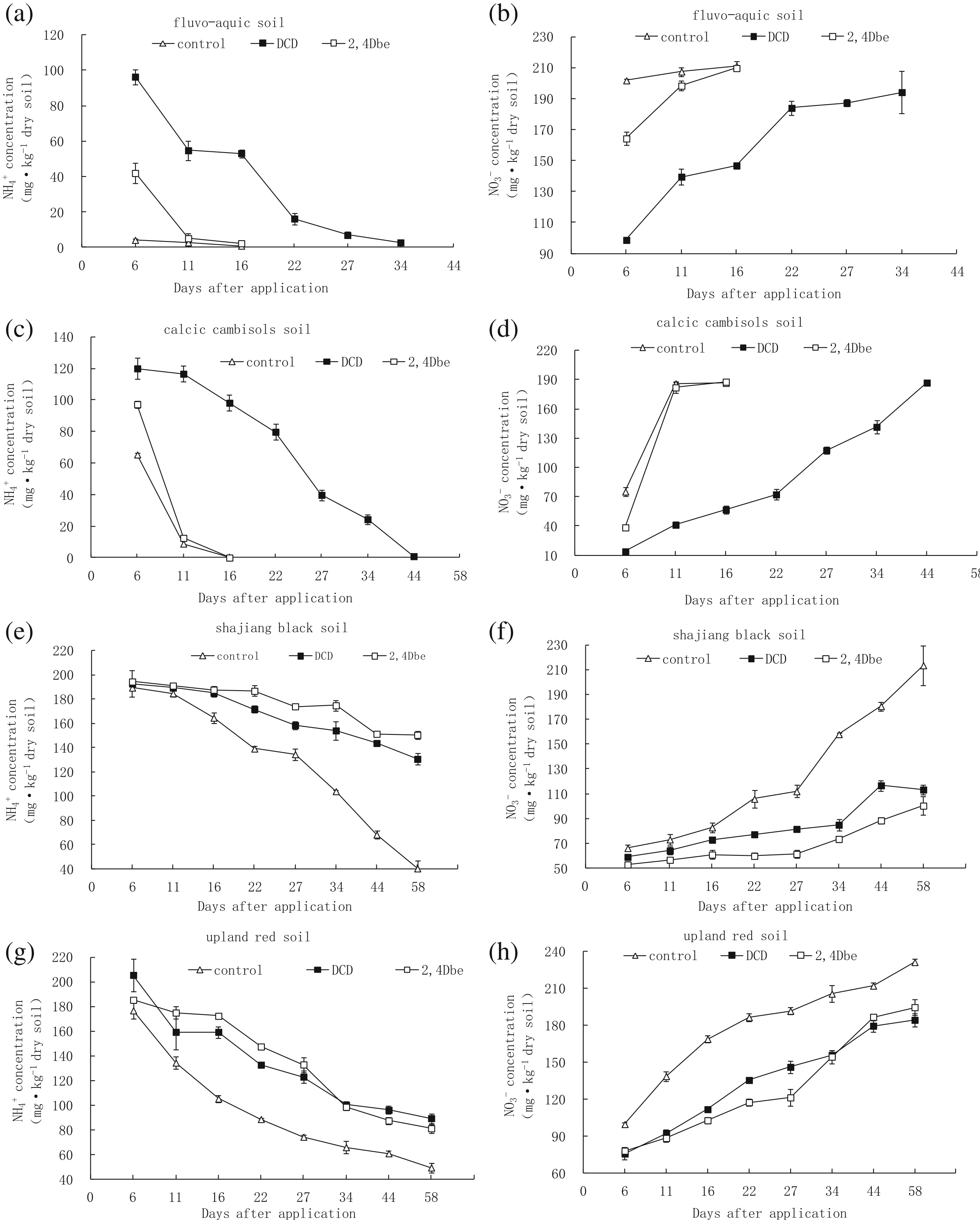

Fig. 3 Changes in the contents of ammonium and nitrate in different types of soils using herbicides 2,4-D and DCD

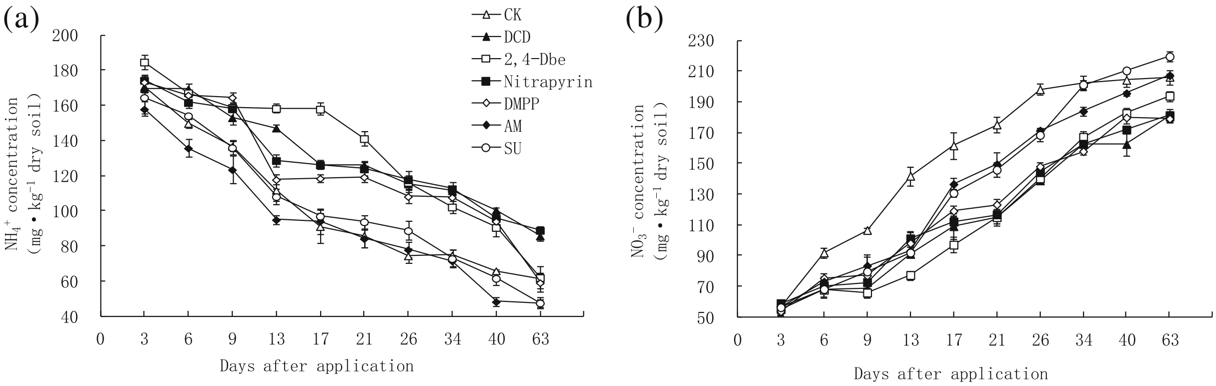

Fig. 4 Changes in the contents of ammonium (**a**) and nitrate (**b**) in dryland red soil using 2,4-Dbe herbicide and five nitrification inhibitors

Our previous studies also indicated that the application of 2,4-Dbe to Oxisol and Fluvo-aquic soils affected microbial community and nitrogen-transforming bacterial populations at the doses of 5 mg (field application rate) and 50 mg (Ding et al. 2017).

Although, 2,4-Dbe displayed a better effect in inhibiting nitrification compared to other types of herbicides in this trial, it still needs the support of more experimental data to prove it as an inhibitor. The performance of herbicide in different types of soils may be related with the properties of soil (pH, etc.) and microbial species (Nada and Mitar 2002). Previous publications reported that autotrophic nitrification bacteria did not grow below pH 4.5 and grow faster in near-neutral condition (De Boer and Kowalchuk 2001). Therefore, wide range in soil pH likely brought marginal effect to the soil nitrification processes (Yu et al. 2016a, b). However, these results indicate that 2,4-Dbe had at least influences on the nitrification in all different types of tested soils, and its inhibiting effectiveness are certainly related not only with the properties of soil but also on the applied doses. In this study, the doses of 2,4-Dbe were applied only from the range of field application rate to 20 times (Martens and Bremner 1993; Zabaloy et al. 2008), especially when the applied doses were more than four times the field application rates, the inhibition became more effective. In the case of incubation experiments, usually, a larger range of herbicide doses was used to study their effects (Debona and Audus 1970; Olson and Lindwall 1991; Zabaloy et al. 2008; Kucharski et al. 2009b). Therefore, if the ranges in the application doses of 2,4-Dbe are acceptable in agriculture or adding into nitrogen fertilizer, 2,4-Dbe may play a role of nitrification inhibitor. In conclusion, to boost crop yielding, 2,4-Dbe might be more beneficial as an agricultural inhibitor of nitrification to conserve soil N by improving soil N fertility compared with nitrification inhibitors.

5 Conclusions

1. Different types of herbicides have both significant and little inhibitory effects on soil nitrification. 2,4-Dbe displayed the most significant effect among the eight selected herbicides which represent different chemical taxonomy, but the effects of 2,4-Dbe showed differences in different types of soils and the inhibitory effects were enhanced with increments in its dose.
2. The inhibitory effect of 10 mg of 2,4-Dbe was better than AM and SU and equivalent to that of DCD, nitrapyrin, and DMPP at their recommended application rates in dryland red soil, where it may be playing a role of nitrification inhibitor.

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