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Full Length Article

Impact of Herbicide 2, 4-Dichlorophenoxyacetic Acid Butyl Ester on Soil Nitrogen-transforming Bacterial Populations in Two Soils

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Abstract

Herbicides applied to the field affect the soil microbial community. Here, we evaluated the impact of the herbicide 2,4-dichlorophenoxyacetic acid butyl ester (2, 4-Dbe) on the microbial community and nitrogen-transforming bacterial populations at three doses of 0, 5 (field application rate) and 50 mg (ten-fold field rate) active ingredient (a.i.) kg^{-1} dry soil applied to Oxisol and Fluvo-aquic soils in the laboratory, respectively. After addition of 2, 4-Dbe, the populations of aerobic bacteria and actinomycete significantly depressed in the Oxisol soil (pH 5.9), while promoted in the Fluvo-aquic soil (pH 8.3). An increasing dose of 2, 4-Dbe combined with much less fungal populations in both soils ($p < 0.05$). Application of 5 mg a.i. 2, 4-Dbe significantly stimulated ammonifiers, and had no influence on nitroso-bacteria and denitrifying bacteria; 50 mg a.i. 2, 4-Dbe inhibited all of them ($p < 0.05$). Both doses of 2, 4-Dbe inhibited autotrophic nitrifying bacteria, but stimulated heterotrophic nitrifying bacteria in both soils ($p < 0.05$). These results revealed that the sensitivity of different microbial species to 2, 4-Dbe differed significantly, and the impacts were also associated with herbicide application rates and soil types. Fungi and autotrophic nitrifying bacteria are more sensitive, and heterotrophic nitrifying bacteria are insensitive to 2, 4-Dbe toxicity.

Keywords: Herbicide; Bacterium; Fungus; Actinomycetes; Nitrogen-transforming bacteria

Introduction

Herbicides with differential toxicity applied in crop production not only control targeted weeds, but may also have residual impact on microbial community structure and function of soils (Pampulha and Oliveira, 2006; Zabaloy *et al*., 2008). In fact, researches on the influence of herbicides on soil microorganisms had been initiated in the 1940s and 1950s (Smith *et al*., 1945; Gamble *et al*., 1952; Aldrich, 1953), and there have been a great quantity of literature reported until now. However, these researches had drawn different conclusions that the impacts depended on herbicide types (Martens and Bremner, 1993; Zabaloy *et al*., 2008), applied concentrations (Haney *et al*., 2000; Sofo *et al*., 2012), soil types (Banks *et al*., 2014; Paul *et al*., 2015), and microbial species (Moorman, 1989; Cycoń *et al*., 2013).

Soil fertility often is restricted by various microbial metabolic cycles. The addition of any potentially toxic herbicide will impact on balances of soil-inhabiting microorganisms, and the balances could be changed by direct toxic action or by selective toxicity for certain groups of microbes or by promoting the growth of one or more types of soil organism (Edwards, 1989; Moorman, 1989). These effects were involved in the types and concentrations of the chemicals as well as environmental conditions exposed (Moorman, 1989).

The herbicide 2, 4-dichlorophenoxyacetic acid (2, 4-D) was one of the first herbicides developed in the early 1940s (Peterson, 1967), since then a serial of 2, 4-D products were developed, such as 2.4-D butyl ester, 2, 4-D isobutyl ester, 2, 4-D isooctyl ester, 2, 4-D dimethylamine salt, 2, 4-D sodium salt etc. Up to now 2, 4-D herbicides are still widely used throughout the world (Germaine *et al*., 2006). Although the herbicide was considered as a short half-life in soils, it may influence soil microbial communities (Zabaloy *et al*., 2010), and be potential threat to the environment and human health, especially its extensive use (Chinalia *et al*., 2007).

It had been reported that the herbicide 2, 4-dichlorophenoxyacetic acid butyl ester (2, 4-Dbe) had appreciable impact on soil microorganisms (Rai, 1992; Zhang *et al*., 2010). However, based on the limited information in several studies regarding the toxicity of 2, 4-D to soil bacteria and fungi is insufficient for drawing definitive conclusions about the toxicity of 2, 4-D to soil microorganisms (USDA, 2006), besides, there is a serious lack of information on the effect of the herbicide on soil nitrogen-transforming bacteria so far. Therefore, this work was carried out to explorer the effects of 2, 4-Dbe on ammonifiers, nitrosomonas, nitrifying bacteria (autotrophic

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and heterotrophic) and denitrifying bacteria which involved in the soil nitrogen cycle.

Materials and Methods

Experimental Soils

Two original soils at the different ecological sites were chosen in the study. The Oxisol soil, which is representative in southeast China, was sampled from 0 to 20 cm depth in a long-time vegetable rotation field at Institute of Ecological Agriculture, Fujian Academy of Agricultural Sciences, Fuzhou, Fujian province (26°07'N, 119°20'E); and the Fluvo-aquic soil, representative in north China plain, sampled in a long-time maize-wheat rotation field at Fengqiu agricultural ecology station of Chinese academy of sciences, Fengqiu county, Henan province (35°00'N, 114°24'E). The general characteristics of the tested soils are shown in Table 1.

Experimental Herbicide

The herbicide 2, 4-Dbe is commonly applied in crop systems for weed control in the above two ecological regions. The 2, 4-Dbe used in this experiment is 57% active ingredient emulsifiable concentrate (EC), brand name Greenland® which was produced by Shangdong Vicome Greenland Chem Co. Ltd. Shangdong, China.

Experimental Design

Our investigation on the influence of 2, 4-Dbe on soil microorganisms was conducted in the laboratory with a 45 day incubation period. Three doses of 2, 4-Dbe i.e. 0, 5 (field application rate) and 50 mg (ten-fold field application rate) active ingredient (a.i) kg^{-1} (Zabaloy *et al*., 2008) were applied to an Oxisol soil and a Fluvo-aquic soil with four replicates, respectively. The fresh soils sampled from two experimental sites, respectively were sieved through 2 mm, weighted 150 g (equal to dry soil weight) of each soil and placed into a 300 mL wide-mouth bottle. Subsequently, soil samples were adjusted to 60% of water holding capacity with double deionized water in which 2, 4-Dbe could be dissolved. Control soils were also prepared only with double deionized water. All soil samples were incubated at 25ºC, and 10 g soil was sampled each time by destructive sampling from each bottle at 2, 8, 16, 28 and 45 days after incubation.

Methods for Soil Microbial Populations

Microbial numbers were determined using conventional methods and calculated. Ten grams of each soil sample incubated was suspended in 90 mL sterile water and shaken in a shaker for 30 min. Then, these soil suspensions were diluted in 10-fold series.

The Colony Counting Method

0.05 mL soil suspensions of 10^{-3}–10^{-7} dilutions were used to inoculate in PDA medium, gauserime synthetic agar medium, beef extract peptone medium (Smith and Mayfield, 1977) and acetamide agar medium (Kidd *et al*., 2008), respectively, which determined the numbers of fungi, actinomycete, aerobic bacteria and heterotrophic nitrifying bacteria. Each microorganism was cultured with four dilutions and every concentration had four parallel culture dishes. All the dishes were incubated in dark at 25℃. Total number of microorganisms on the agar mediums just need to be counted.

The Most Probable Number (MPN) Method

One mL soil suspensions of 10^{-3}–10^{-7} dilutions were used to inoculate tubes containing selective liquid media. Ammonifier were cultured in peptone ammonification medium (Fargo and Fleming, 1977), nitroso-bacteria medium reported by Kihn *et al*. (2000), autotrophic nitrifying bacteria medium described by Lin *et al*. (2006), and denitrifying bacteria medium followed by Johns *et al*. (2004). Each microorganism was cultured with four dilutions and every concentration has four parallel tubes. All the tubes were incubated in dark at 25℃. After incubation, the pattern of positive and negative tubes is noted, and a standardized MPN table was consulted to determine the most probable number of organisms (causing the positive results) per unit volume of the original sample.

Statistical Analysis

All statistical analyses were performed using SPSS19.0 (SPSS for Windows, Version 13.0). The differences of microbial populations among three treatments were analyzed using ANOVA, and the differences were considered significant at $p < 0.05$.

Results

Our study revealed that the populations of fungi, aerobic bacteria and actinomycetes in Oxisol soil were significantly higher than in Fluvo-aquic soil (Fig. 1), that could be associated with soil organic matter content (Table 1). Application of herbicide 2, 4-Dbe had different effects on microbial populations in two soils, aerobic bacteria and actinomycetes were significantly inhibited in the Oxisol soil, in contrast, they were stimulated in the Fluvo-aquic soil under two dose treatments, but fungi were appreciably depressed in both soils ($p < 0.05$). These impacts were more obvious with increasing 2, 4-Dbe dose. The results revealed that fungi were more sensitive to the herbicide than aerobic bacteria and actinomycetes. The impacts of 2, 4-Dbe depended on the soil types, and even the low dose of 2, 4-Dbe could affect populations of aerobic bacteria, actinomycetes and fungi in the Oxisol soil. However, high

Table 1: General characteristics of the tested soil

Characteristics	Oxisol soil	Fluvo-aquic soil
Organic matter (%)	1.8	1.2
pH (1:2.5)	5.9	8.3
Total nitrogen (g/kg)	1.8	0.8
NH_4^+-N (mg/kg)	6.1	4.6
NO_3^--N (mg/kg)	9.8	30.2
Cation exchange capacity [cmol(+)kg^{-1}]	9.7	6.1
Mechanical analysis of soil particles		
>0.01 mm (%)	74.2	88.0
0.01-0.001 mm (%)	16.0	6.6
<0.001 mm (%)	9.8	5.4

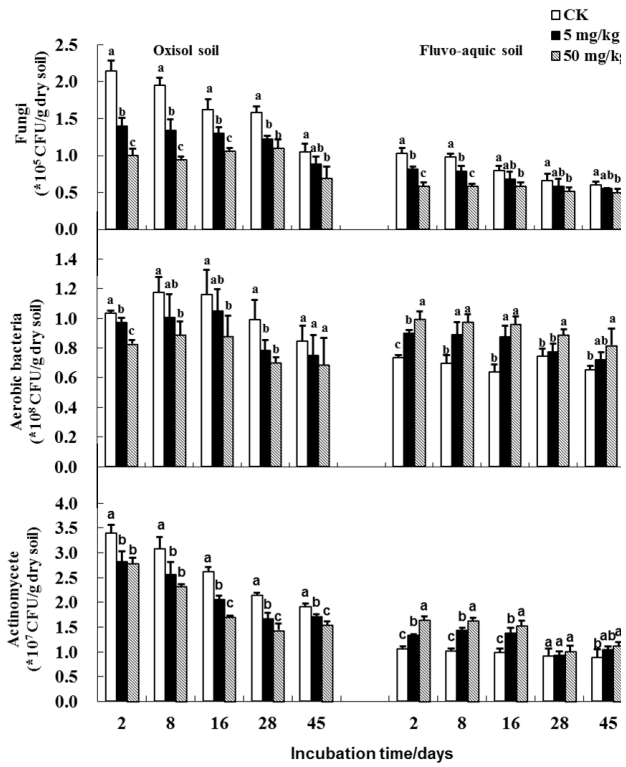

Fig. 1: Impact of 2, 4-D on microbial community in two soils

dose of 2, 4-Dbe did not affect aerobic bacteria and actinomycetes in the Fluvo-aquic soil.

On the influences of 2, 4-Dbe on soil nitrogen-transforming bacteria, the tested results implied that the field dose of 2, 4-Dbe promoted ammonifiers ($p < 0.05$), but had no influence on nitrosomonas and denitrifying bacteria, while a ten-fold field dose of 2, 4-Dbe could depress populations of ammonifiers, nitrosomonas-bactria and denitrifying bacteria in the two types of soil (Fig. 2). Both dose of 2, 4-Dbe inhibited autotrophic nitrifying bacteria ($p< 0.05$), while slightly or significantly stimulated heterotrophic nitrifying bacteria in the two soils. These revealed that autotrophic nitrifying bacteria were more sensitive to 2, 4-Dbe than other nitrogen-transforming bacteria. High dose of 2, 4-Dbe would result in negative

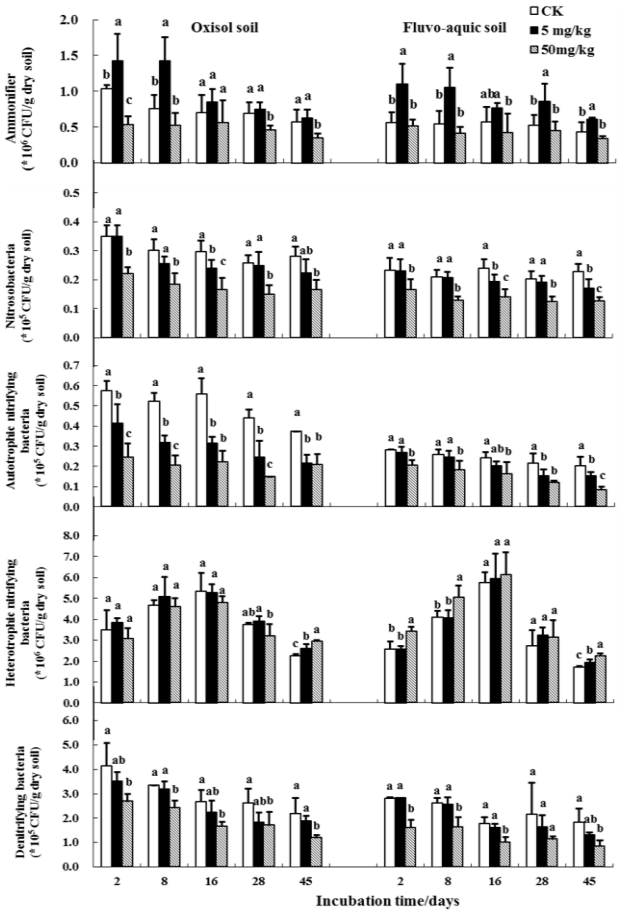

Fig. 2: Impact of 2, 4-D on soil nitrogen-transforming bacterial populations in two soils

effects on nitrogen-transforming bacteria except for heterotrophic nitrifying bacteria.

Discussion

Certainly, the effects of herbicide doses and types on microorganisms have great differences. It has been shown that fungal, bacterial and actinomycete populations were reduced by the 2, 4-D treatment (Rai, 1992; Mohiuddin, 2013), and the reduction being more marked where the ester formulation was used relative to amine (Rai, 1992). Han *et al*. (2014) considered that fungus number could be a sensitive indicator which assessed the ecological effect of environment herbicide 2, 4-D polluted. However, Durga Devi *et al*. (2008) reported that application of 2, 4-D benefited soil fungi while the bacterial populations were depressed initially in paddy soils, but the populations in the treated and untreated plots were similar by 30 days after spraying. In addition, Zhang *et al.* (2010) considered that the addition of 2, 4-Dbe significantly altered the microbial community in two experimental soils with significantly different organic matter and fertility levels, bacteria and

actinomycetes were significantly higher with 100 μg g^{-1} dose, compared with other dose treatments.

The results described in above literatures indicated that impacts of the herbicide were greatly different even opposite under different conditions. It was thought that the physical-chemical properties of soils would affect the effect of the herbicide, including soil pH, organic matter and texture. It was reported that soil properties with high clay and organic matter would enhance the negative effect by prolonging persistence of herbicide as compared to ones with light-textured and low organic matter (Boivin *et al*., 2005; Abbas *et al*., 2015), the Oxisol soil in this experiment was in line with such characteristics; the Fluvo-aquic soil was comparatively in line with the soil characteristics and experimental results reported by Zhang *et al*. (2010).

Nitrogen is an important nutrient element with different existing forms, its transformation processes in soil classically involve (1) ammonification of organic-N substrate nitrogen by ammonifiers, (2) oxidation of NH_3 or NH_4^+ by nitroso-bacteria, (3) nitrification of NO_2^- by autotrophic or/and heterotrophic nitrifying bacteria, and (4) denitrification of NO_3^- by denitrifying bacteria. However, the sensitivities of these nitrogen-transforming bacteria to herbicides are still not clearly understood.

Due to lack of literatures, we cited some indirect research literatures on impact of 2, 4-D or other herbicides on nitrogen-transforming bacteria. Martens and Bremner (1993) reported that addition of 5 mg a.i. kg^{-1} soil 2, 4-D did not inhibit urea hydrolysis in four soils tested or nitrification of urea nitrogen in two fine-textured soils, but inhibited nitrification of the nitrogen in two coarse-textured soils. The effects of the herbicide on nitrification activities in soils were significantly affected by incubation time, soil texture and soil organic-carbon content. Autotrophic nitrifiers were more sensitive to both sulfonylureas than other microorganisms (Gigliotti and Allievi, 2001), that was agreement with the experimental results of 2, 4-Dbe. But when paraquat was added to four different soils, it did not obviously affected nitrification, while slightly retarded ammonification of organic-matter nitrogen (Tu and Bollen, 1968).

Yuan *et al*. (2005) reported that there was no relationship between nitrification potential and numbers of nitrifiers in soil. Generally, autotrophic nitrifying bacteria were dominant in converting NH_4^+ to NO_2^-/NO_3^- compared to heterotrophic nitrifying bacteria. Therefore, nitrification is mainly carried out by autotrophic nitrifying bacteria but not total nitrifying bacteria. Then, would inhibiting autotrophic bacteria reduce soil nitrification potential? It was reported that the hetrotrophic bacterial population significantly increased in a soil with a long historical use of glyphosate (Partoazar *et al*., 2011), and addition of 500 ug g^{-1} 4-(2, 4-dichlorophenoxy) butyric acid significantly increased total heterotrophic bacteria of soils in microcosms (Cuadrado *et al*., 2008). Zabaloy *et al*. (2010) thought that low rates of 2, 4-D did not affect heterotrophic bacteria counts, our experimental results supported these conclusions, addition of 2, 4-Dbe increased numbers of heterotrophic nitrifying bacteria. Because González *et al*. (2012) and Han *et al*. (2015) have isolated an indigenous bacterial strain which utilize 2, 4-D as the sole carbon and energy source from a polluted river and from a soil of growing wheat with a long-term history of herbicide use, respectively. However, nitrifying bacteria appeared to be the most sensitive microorganisms to napropamide (Cycoń *et al*., 2013), the conclusion also was supported by our experimental results with herbicide 2, 4-D. Sometimes, changes of the microbial community were minimal when herbicides were applied, but this could be indicators of potential long-term effects. Therefore, 2, 4-Dbe might has substantial long-term effects on soil microorganisms in agricultural soils, especially the effects were greater in soils with low organic matter and fertility level than in ones with high organic matter and fertility level (Zhang *et al*., 2010). To determine the changes of microbial community structure after several year applications of herbicide under different soil types and management practices, it is considered be necessary to carry out long-term studies (Banks *et al*., 2014).

Conclusion

Impact of herbicide 2,4-Dbe on bacteria and actinomycetes populations were positive and negative, respectively in alkaline Fluvo-aquic soil and acidic Oxisol soil, but depressed fungal populations in both soils. Effect on microbial species of nitrogen-transformation bacteria also was significantly different, and autotrophic nitrifying bacteria were more sensitive to toxicity of 2, 4-Dbe.

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