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RESEARCH ARTICLE

Impact of fluxapyroxad on the microbial community structure and functional diversity in the silty-loam soil

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Abstract

The aim of this work was to assess the effect of applying three different doses of fluxapyroxad on microbial activity, community structure and functional diversity as measured by respiration, microbial biomass C, phospholipid fatty acid (PLFA) and community-level physiological profiles (CLPPs). Our results demonstrated that substrate-induced respiration (on day 15) and microbial biomass C (on days 7 and 15) were inhibited by fluxapyroxad, but stimulation was observed thereafter. In contrast, fluxapyroxad addition increased the basal respiration and metabolic quotients (qCO_2) and respiratory quotients (Q_R). Analysis of the PLFA profiles revealed that the total and bacterial biomass (both Gram-positive bacteria (GP) and Gram-negative bacteria (GN)) were decreased within the initial 15 days, whereas those as well as the GN/GP ratio were increased at days 30 and 60. Fluxapyroxad input decreased the fungi biomass but increased the bacteria/fungi ratio at all incubation time. Moreover, high fluxapyroxad input (75 mg fluxapyroxad kg^{-1} soil dry weight) increased the microbial stress level. A principal component analysis (PCA) of the PLFAs revealed that fluxapyroxad treatment significantly shifted the microbial community structure, but all of the observed effects were transient. Biolog results showed that average well color development (AWCD) and functional diversity index (H´) were increased only on day 60. In addition, the dissipation of fluxapyroxad was slow in soil, and the degradation half-lives varied from 158 to 385 days depending on the concentration tested.

Keywords: fluxapyroxad, microbial activity, community structure, functional diversity

1. Introduction

Fluxapyroxad, 3-(difluoromethyl)-1-methyl-*N*-(3´,4´,5´-trifluoro[1,1´-biphenyl]-2-yl)-1*H*-pyrazole-4-carboxamide, a new active ingredient developed by BASF Corporation (Germany) to control a broad spectrum of fungal disease in cucumbers and tomatoes, is in the process of being registered in China. It belongs to the carboxamide class of chemicals, and its mode of action is to inhibit succinate dehydrogenase in complex II of the mitochondrial respiratory chain, which results in inhibition of spore germination, germ tubes and mycelia growth within the fungus target species (EPA 2012). In addition, fluxapyroxad has been demonstrated to persist for a long period in soils (half-lives ranging from 213 to 1 827 days) (EPA 2012). Pimentel (1995) and Prado *et al.* (2009) have estimated that as much as 99.7% of the applied load of pesticides were dispersed in the environment, not reaching their target. In this way, a large portion of pesticides accumulates in the soil system where it undergoes biological and physicochemical

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transformations (Pimentel 1995; Prado *et al.* 2009). This suggests that input of fluxapyroxad may pollute the soil environment and affect the health of soil ecological systems.

Soil microbes are a basic component of the soil ecosystem and are vital for soil fertility and the degradation of organic pollutants in soil. Soil microbes are highly sensitive to environmental changes (Schloter *et al.* 2003). Soil microbial properties, particularly those reflecting the biomass, activity, and diversity of the soil microbial communities, have great potential as bioindicators of the effects of pesticide on soil health (Epelde *et al.* 2010). Several studies on widely-used pesticides have already shown that pesticide application leads to changes in soil nutrient levels and alterations to soil microbial community structure and functional diversity (Monkiedje *et al.* 2002; Zhang *et al.* 2010b; Muñoz-Leoz *et al.* 2011). To prevent potential fluxapyroxad-induced adverse effects on the soil ecosystem, further research is needed to measure degradation of fluxapyroxad in soil and to accurately assess the effect of fluxapyroxad on soil microbial communities and functional diversity.

The aim of this study was (1) to understand the fluxapyroxad degradation dynamics in soil under laboratory conditions when added at three different rates (0.75, 7.5 and 75 mg kg^{-1}); (2) to assess the impact of fluxapyroxad on microbial community composition; (3) to examine the impact of fluxapyroxad on soil microbial functional diversity. To our knowledge, this work provides the first report about the effects of fluxapyroxad on soil microbial community structure and function in agricultural soils in China.

2. Results

2.1. Fluxapyroxad degradation

The degradation of fluxapyroxad in soil under laboratory conditions from three different rates (T1, field rate, 0.75 mg fluxapyroxad kg^{-1} soil dry weight; T10, 10-fold of field rate, 7.5 mg fluxapyroxad kg^{-1} soil dry weight; T100, 100-fold of field rate, 75 mg fluxapyroxad kg^{-1} soil dry weight) was showed in Table 1. There was a slow decrease in fluxapyroxad content for T1 and T10 treated soils during the experiment. The initial deposits of chlorimuron-ethyl in soil after application were 0.71, 6.45 and 70.97 mg kg^{-1} on the 1st day for T1, T10 and T100, which declined to 0.54, 5.78 and 69.13 mg kg^{-1} after 60 days respectively. In soils treated with 0.75 mg kg^{-1} dry weight (DW; T1) and 7.5 mg kg^{-1} DW (T10), the degradation process of fluxapyroxad appeared to follow the first-order kinetic equation, $C_t=C_0e^{-kt}$

Where, C_t represents the concentration of the pesticide residue at time t; C_0 represents the initial concentration and k is the rate constant per day. By the end of the experimental period, fluxapyroxad concentration was degraded by 23.51 and 10.33% with half-life values of 158 and 385 days, respectively (Table 1). Little degradation was observed in the test period of 60 days when fluxapyroxad was supplied at 75 mg kg^{-1} (T100) (Table 1).

2.2. Soil microbial biomass and respiration

In our study, the impact of fluxapyroxad on microbial parameters was dependent upon fluxapyroxad concentration and incubation time (Table 2). Significant interactions between fluxapyroxad concentration and incubation time occurred for soil microbial biomass C (MBC), basal respiration (R_B), substrate-induced respiration (SIR), metabolic quotients (qCO_2) and respiratory quotients (Q_R).

As shown in Fig. 1-A, fluxapyroxad applied at recommended doses (T1) had no clear effect on microbial biomass C at all incubation times, whereas the higher doses (T10, T100) induced significant decreases within the first 15 days (P<0.05). However, the inhibitory effect of fluxapyroxad on microbial biomass C disappeared at days 30 and 60 (P>0.05).

Table 1 The degradation of fluxapyroxad in soil at three different application rates (T1, T10 and T100)[1)]

Incubation time (d)[2)]	T1		T10		T100	
	Residue (mg kg^{-1})	Degradation (%)	Residue (mg kg^{-1})	Degradation (%)	Residue (mg kg^{-1})	Degradation (%)
1	0.71	–	6.45	–	70.97	–
7	0.66	5.95	6.35	1.48	69.71	–
15	0.62	12.04	6.09	5.45	69.50	–
30	0.57	19.16	5.92	8.08	70.43	–
60	0.54	23.51	5.78	10.33	69.13	–
Regression eq.	C=0.6803$e^{-0.004t}$ r^2=0.8869		C=6.3734$e^{-0.0018t}$ r^2=0.8777		–	
$t_{1/2}$ (days)	158		385		–	

[1)] T1, 0.75 mg fluxapyroxad kg^{-1} soil dry weight; T10, 7.5 mg fluxapyroxad kg^{-1} soil dry weight; T100, 75 mg fluxapyroxad kg^{-1} soil dry weight.
[2)] $t_{1/2}$, half-life or time required for a 50% dissipation of the initial fluxapyroxad concentration.
– indicates no significant degradation was observed.