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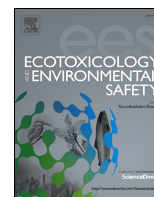

Effects of the herbicide mesotrione on soil enzyme activity and microbial communities

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A B S T R A C T

Mesotrione (2-[4-(methylsulfonyl)-2-nithobenzoyl]-1, 3-cyclohexanedione) is a selective triketone herbicide that has been widely used in corn production for the past 15 years. However, its potential for risk to soil ecosystems is poorly documented. The present study investigated the soil enzyme activity and soil microbial community responses to a 20 days' mesotrione exposure at doses of 0.1, 1.0 and 5.0 mg/kg. On days 2, 5, 10 and 20, activities of soil β-glucosidase, urease and acid phosphatase, soil microbe abundances, soil microbial community structure and abundance of the *AOA-amoA* and *AOB-amoA* genes were measured. Results showed that activities of urease and acid phosphatase were relatively stable, with no difference found between the mesotrione-treated group and control at the end of exposure. But β-glucosidase activity was reduced in the 5.0 mg/kg mesotrione treatment. In the 1.0 and 5.0 mg/kg mesotrione-treated soil, abundance of bacteria, fungi and actinomycetes all were reduced. In the 0.1 mg/kg mesotrione-treated soil, only fungi abundance was reduced by the end of the exposure. The analysis of terminal restriction fragment length polymorphism (T-RFLP) revealed soil microbial community structure could be affected by mesotrione at all experimental doses, and microbial diversity declined slightly after mesotrione exposure. Abundance of *AOA-amoA* and *AOB-amoA* genes were reduced markedly in 1.0 and 5.0 mg/kg mesotrione-treated soil. The present study suggests that mesotrione at higher doses might induce negative impacts on soil microbes, a finding which merits additional research and which should be accounted for when application of this herbicide is considered.

1. Introduction

Modern agriculture requires not only ensuring and improving productivity, but also minimizing the environmental impacts of agricultural production activities. Pesticides have played a huge role in ensuring and improving food production, but they have also brought many negative impacts to the environment and ecosystems (Ackerman, 2007; Pereira et al., 2009).

Mesotrione (2-[4-(methylsulfonyl)-2-nithobenzoyl]-1, 3-cyclohexanedione) is a selective triketone herbicide which is developed as a replacement for atrazine from a natural phytotoxin in the bottlebrush plant (*Callistemon citrinus*) by Syngenta Participations AG in Switzerland (Mitchell et al., 2001). mesotrione can effectively clear corn fields from weeds, such as abutilon, cockle and amaranth (Nakka et al., 2017). mesotrione has been found to be easily degraded in the environment, with a half-life range from 5 to 15 days in soil, which can prevent long-term environmental impacts and post-crop damage (Sun et al., 2013). Due to these favorable properties, mesotrione has been extensively used in maize production (Nigel, 2011). Even with so many advantages, its impacts on the environment are inevitable. Studies have shown that mesotrione also induced negative effects on non-target organisms. For example, Kreutz et al. (2008) reported that the 96 h LC_{50} of this herbicide to the neotropical silver catfish *Rhamdia quelen* was 532.0 mg/L, significantly higher than that of atrazine (10.2 mg/L). Wang et al. (2018) reported that the environmental relevant concentration in water (1.8 μg/L) of mesotrione could not induce any negative effects but higher concentration (180 μg/L) markedly induced oxidative stress and DNA damage on the common carp *Cyprinus carpio*. Piancini et al. (2015) also reported that mesotrione could induce oxidative stress and DNA damage on two Brazilian fish, *Oreochorimis*

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niloticus and *Geophagus brasiliensis*, at sub-lethal concentrations. Xie et al. (2010) made a 90-day toxicity test and found that the maximal no-effective dose of mesotrione on male and female rats was 25.0 mg/kg·day.

A few studies have estimated the risk of mesotrione to soil organisms. Crouzet et al. (2010) studied the response of the soil microbial community to mesotrione applied at doses of 1 × FR (recommended field rates, 0.45 mg/kg in the first 5 cm of the soil), 10 × FR (4.5 mg/kg) and 100 × FR (45 mg/kg) in a chernozem soil, and reported that mesotrione applied at the recommended field rates was quickly dissipated from this chernozem soil with no consistent impact on soil microbial communities. However, at doses far exceeding the recommended rates, mesotrione had impacts on non-target soil microorganisms. Crouzet et al. (2016) also studied functional and structural responses of soil N-cycling microbial communities to the mesotrione, and found that mesotrione at a dose of 1 × FR (0.45 mg/kg) did not strongly affect soil N-nutrient dynamics while 100 × FR (45 mg/kg) mesotrione induced short-term inhibition of nitrification and a lasting stimulation of denitrification. Pose-Juan et al. (2015) investigated effects of mesotrione on the soil microbial biomass and activity in a sandy loam soil, and reported mesotrione at a dose of 50 mg/kg increased soil biomass but reduced soil dehydrogenase activity. However, more soil studies are needed in order to draw a more accurate picture of the environmental impact of mesotrione, considering that the soil microbial responses to chemical anthropic disturbances often vary depending on the chemical properties and application rates of the pesticide, soil characteristics, microbial communities investigated and the methodological approaches employed (Busse et al., 2001; Pal et al., 2006).

Soil enzymes, microbial communities and functional genes are important indicators to reflect the soil status. Soil enzymes are directly involved in the biochemical reactions in the soil. For example, soil β-glucosidase, urease and acid phosphatase are hydrolases responsible for hydrolysis of cellobiose, urea and organophosphorus respectively, and provide glucose, inorganic nitrogen and phosphorus elements to plants and other soil organisms (Adewole et al., 2017). Soil microbial communities perform various functions such as material cycle, energy conversion and contaminant degradation through their metabolism in soil and thereby contribute to the sustainable use and environmental quality of agrosystems (Millennium Ecosystem Assessment (MEA), 2005). Soil functional genes encode enzymes responsible for many biochemical processes in soil. Measurements of functional genes using quantitative PCR (qPCR) provide a powerful tool for evaluating microbial contributions to different steps of mass cycles. For example, the *amoA* genes which mainly exist in *ammonia-oxidizing bacteria* (*AOB*) and *ammonia-oxidizing archaea* (*AOA*), are responsible for the ammonia oxidation process, a limiting step of nitrification. Soil enzyme activity, abundance, diversity and structure of soil microbial communities, as well as abundance of soil functional genes respond rapidly to environmental change (Wallenstein and Vilgalys, 2005). Thus, they are widely employed to study the soil response to environmental contaminants.

In the present study, responses of soil enzyme activities (β-glucosidase, urease and acid phosphatase), soil microbe abundances, soil microbial community structure and functional gene abundance (*AOA-amoA* and *AOB-amoA*) to mesotrione exposure were discussed. The aim of the present study is to understand the effects of mesotrione on soil microbial communities and on soil enzymatic activity in a typical brunisolic soil, thereby providing useful data to clarify the potential environmental risks of such compounds.

2. Materials and methods

2.1. Chemical and reagents

Mesotrione (CAS NO. 104206-82-8) with purity ≥ 99% was obtained from Tanmo Quality Inspection Co., Ltd, Beijing, China. All other chemicals and reagents used in the following experiments were analytical grade and purchased from Sangon Biological Engineering Technology and Service Co. Ltd (Shanghai, China), and Takara Biotechnology Co. Ltd (Dalian, China).

2.2. Soil and mesotrione exposure

The soil (brunisolic soil) used in the present study was gathered from the surface horizon (0–20 cm) of the experimental field of Shandong Agricultural University (36°09′50.47″N 117°09′20.40″E, Taian, China) that never utilized mesotrione, with debris removed. Basic characteristics of the soil are listed in Table S1 in the Supplementary material.

Before the experiments, the soil was incubated for 2 weeks at 25 °C and 60% of the maximum water-holding capacity (WHC), to activate the microbial communities and reach a stable status.

For the exposure, mesotrione was firstly dissolved in acetone with different concentrations, then 500 μL of a certain mesotrione solution was added into 50.00 g soil (wet weight, wt) for each treatment, with the final concentrations of mesotrione in soil being 0, 0.1, 1.0 and 5.0 mg/kg. Then the mixtures were manually stirred on enamelled plates for 2 h to promote the mesotrione distribution and acetone volatilization, after which the treated soil samples were adjusted to 60% WHC with ultrapure water and transferred into brown bottles. For each concentration, 12 parallel bottles were prepared. The control group was also prepared with the same method as the mesotrione-treated groups, with just the mesotrione solution replaced with pure acetone. All soil samples were incubated at 25 °C in dark, with soil water content adjusted every other day. Soils were sampled on days 2, 5, 10 and 20. When sampling, three bottles of each treatment group were taken as 3 replications in the following tests.

2.3. Soil enzyme activities determination

In the present study, activities of three soil enzymes (urease, acid phosphatase, and β-glucosidase) were determined in order to evaluate the ecotoxicology of mesotrione, according to the methods described in a previous study (Sun et al., 2017). The specific methods are described below.

For urease assay, 10 g soil and 2.0 mL methylbenzene were mixed in a 100-mL volumetric flask first. After a 15 min standing, 10 mL urea (10%) and 20 mL citrate buffer (pH = 6.7) were added to the reaction system, followed by a gentle shaking. Then the system was incubated at 37 °C for 3 h, diluted with ultrapure water (38 °C) to 100 mL, cleared of methylbenzene and then filtrated. After that, 1.0 mL filtrates, 19.0 mL ultrapure water, 4.0 mL sodium phenate and 3.0 mL hypochlorite were mixed in another 50 mL volumetric flask with a gentle shaking, followed by a 20 min standing for the chromogenic reaction. Finally, the mixture was diluted to 50 mL with ultrapure water and its absorbance at 578 nm was measured using an ultraviolet-visible spectrophotometer (U-V spectrophotometer, UV-2600, Shimadzu, Japan) in 1 h. The urease activity was expressed as mg NH^{4+}-N produced by per kilogram of dry soil per 24 h.

Activity of the acid phosphatase was tested as Dick et al. (1996) described. In detail, 1.0 g soil was mixed with 0.2 mL methanol, 4.0 mL modified universal buffer (MUB, pH 6.5) and 1.0 mL 4-nitrophenyl phosphate disodium salt solution (0.05 M) in a 50 mL conical flask and incubated at 37 °C for 1 h. Then the action was ended by adding 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH. After a filtration, the filtrate was diluted 10 times by MUB (pH 6.5). Finally, the content of p-nitrophenol phosphate (pNP) in the filtrate was measured at 410 nm. The results were expressed as micrograms of pNP g^{-1} of dry soil h^{-1}.

The method of β-glucosidase activity test was similar to the acid phosphatase assay, just replacing the 4-nitrophenyl phosphate disodium salt solution (0.05 M) with p-nitrophenyl-β-D-glucopyranoside (pNG) solution (0.025 M). The content of the hydrolysis reaction

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