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Impact of fomesafen on the soil microbial communities in soybean fields in Northeastern China

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ABSTRACT

Fomesafen, a widely adopted residual herbicide, is used throughout the soybean region of northern China for the spring planting. However, the ecological risks of using fomesafen in soil remain unknown. The aim of this work was to evaluate the impact of fomesafen on the microbial community structure of soil using laboratory and field experiments. Under laboratory conditions, the application of fomesafen at concentrations of 3.75 and 37.5 mg/kg decreased the basal respiration (R_B) and microbial biomass carbon (MBC). In contrast, treatment with 375 mg/kg of fomesafen resulted in a significant decrease in the R_B, MBC, abundance of both Gram+ and Gram− bacteria, and fungal biomass. Analysis of variance showed that the treatment accounted for most of the variance (38.3%) observed in the soil microbial communities. Furthermore, the field experiment showed that long-term fomesafen application in continuously cropped soybean fields affected the soil bacterial community composition by increasing the relative average abundance of *Proteobacteria* and *Actinobacteria* species and decreasing the abundance of *Verrucomicrobia* species. In addition, *Acidobacteria* and *Chloroflexi* species showed a pattern of activation-inhibition. Taken together, our results suggest that the application of fomesafen can affect the community structure of soil bacteria in the spring planting soybean region of northern China.

1. Introduction

Pesticides have greatly contributed to improved agricultural production. However, the use of pesticides may alter biological processes in the soil by either direct or indirect actions (Lo, 2010). Soil microbes play a central role in the ecosystem by driving the Earth's biogeochemical cycles (Fierer et al., 2012; Philippot et al., 2013; Van Der Heijden et al., 2008) and have been used as an early indicator of alterations in soil processes (Hernández-Allica et al., 2006). Whereas certain pesticides stimulate the growth of microorganisms, others have a depressive effect or have no effect (Lo, 2010). Therefore, changes in the soil microbial community could be of help in evaluating the impact of pesticides on soil ecological risk. Laboratory microcosms, which represent a small but stable ecosystem, have allowed the short-term analysis of soil spiked with pesticides at concentrations often much higher than the recommended usage (Zhang et al., 2010; Muñoz-Leoz et al., 2013; Cycoń et al., 2013; Cai et al., 2015). However, Kampichler et al. (2001) suggest that laboratory microcosm research alone is not sufficient for determining the role of species interactions in the field. Karpouzas et al. (2014) suggest that a tiered microcosm-to-field analysis could provide a comprehensive assessment of the toxicity of pesticides toward soil microbes. In addition, the soil microbial community structure is influenced by vegetation and environmental factors such as temperature, light, water, and nutrients (Hartmann et al., 2009; Bronick and Lal, 2005). Field experiments, which represent a large and complex ecosystem, have the advantage of investigating the microbial toxicity of pesticides applied at more realistic exposures. Hence, a combination of laboratory and field study is an appropriate procedure for developing a thorough understanding of the relationship between pesticide application and soil microbial populations.

Fomesafen, 5-[2-chloro-4-(trifluoromethyl)phenoxy]-N-(methylsulfonyl)-2-nitrobenzamide, a diphenyl ether herbicide, has been used for post-emergence weed control in peanut and soybean crops. However, fomesafen can persist for long periods in the soil, and its residue is implicated in phytotoxicity and damage caused during crop rotation (Cobucci et al., 1998; Guo et al., 2003; Rauch et al., 2007). In our

Abbreviations: R_B, basal respiration; MBC, microbial biomass carbon; DW, dry weight; qCO_2, metabolic quotient; PLFA, phospholipids fatty acid; Gram+, Gram-positive bacteria; Gram−, Gram-negative bacteria; OTUs, Operational Units; ANOVA, analysis of variance; ANOSIM, analysis of similarities; SIMPER, similarity percentage; PCoA, principal coordinate analysis; RDA, regularized discriminant analysis; PSA, primary secondary amine; $MgSO_4$, anhydrous magnesium sulfate

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previous study, we examined the effect of fomesafen on soil microbes in peanut fields and found that higher fomesafen levels induce long-term changes (that last for > 90 days) in the microbial community (Wu et al., 2014). Zhang et al. (2014a) found a transient change in the soil microbial community in corn fields after 60 days of incubation with a low dosage of fomesafen (0–500 μg/kg). However, Santos et al. (2006) observed a decrease in MBC and the microbial quotient of soil cultivated with common bean (*Phaseolus vulgaris*) in conventional-till systems after applying 25% fomesafen (aqueous solution, AS) at a dosage of 250 g a.i./ha. Thus study findings can be affected by pesticide concentration, and environmental conditions. Northeastern China contains the largest area dedicated to the production of soybeans in China (Xue, 2013). In the Heilongjiang Province alone, 1021.9 t of fomesafen was used on > 1.62×10^6 ha of soybean fields in 2012 (Hu et al., 2015). With the low degradation rate of fomesafen, repeated application can ultimately lead to its progressive accumulation in soil. The heavy use of fomesafen constitutes a potential risk to the soil microbial community in soybean fields in northeastern China. Yet only one study has analyzed its application. When 25% fomesafen (AS) was applied to soil in a bean-wheat field at a dosage of 150 L/hm^2, a decrease in soil urease, protease, peroxidase activities, and microbes was observed (Zheng et al., 2013). Hence, it is necessary to study the effect of fomesafen on soil ecology by carrying out in-depth studies on the soil microbial community and microbial activity.

The objective of this study was (1) to assess fomesafen impact on soil microbial activity and the microbial community at three different doses (3.75, 37.5, and 375 mg/kg dry weight soil) relative to an untreated control under laboratory conditions, and (2) to assess the impact of continuous application of fomesafen at the recommended dose on soil microorganisms in soybean fields in northeastern China.

2. Materials and methods

2.1. Experimental set-up—laboratory incubation

A 3-month laboratory incubation was carried out as described by Wu et al. (2014). Soil samples that had not been previously treated with fomesafen or other pesticides were collected from the top layer (0–15 cm) of an experimental soybean field at the Northeast Agriculture University in the Heilongjiang Province of China. In the laboratory, stones and roots were removed and the soil was sieved through a 2 mm mesh; thereafter, the sample was pre-incubated in a dark room at 25 ± 1 °C for 2 weeks. Fomesafen was applied at four dosages, 0 (CK), 3.75 (T1), 37.5 (T10), and 375 (T100) mg/kg dry weight soil (DW). The lower fomesafen concentration (3.75 mg/kg) is the recommended dosage for soybean crops. The dose of 375 mg/kg (100 times that of the field rate) has been used in routine ecotoxicological studies to evaluate the potential hazardous effect on soils under an accidental discharge of uncontrolled amounts of pesticides to the environment (Cycoń et al., 2010). Each treatment (4000 g) was carried out in separate polyvinyl chloride tanks by spraying a fomesafen solution in methanol (30 mL) and then mixing using a rotary mixer (Hana Mixer, AHM-P125B). Control (CK) soil samples received the same volume of methanol only. After the evaporation of the methanol, each freshly treated soil sample was equally distributed (200 g) into brown wide-mouth bottles (15 cm height, 8 cm diameter). Each treatment was carried out in triplicate for each sampling time, which resulted in a total of 60 bottles in the experiment. The water content was adjusted to 60% of the maximum water holding capacity of the soil. All bottles were covered with a porous plastic film and incubated in growth cabinets for a period of 90 days at 50% relative humidity in the dark and at a temperature of 25 ± 1 °C (night/day). Throughout the incubation period, moisture loss was compensated for by adding sterile deionized water every 2 days to maintain a constant soil moisture status. Soil samples were collected from each bottle at different time intervals, i.e., 7, 15, 30, 60, and 90 days after fomesafen application.

The soil is of a silty loam character (USDA soil classification system) with the following properties: 3.5% clay, 60.2% silt, 36.3% sand, 32.4 g/kg organic matter, 114.4 mg/kg available P, 358.6 mg/kg available K, 13.7 mg/kg NH_4^+-N, 60.8 mg/kg NO_3^--N, and pH 8.14.

2.1.1. Fomesafen extraction and determination

Residual fomesafen was extracted from the soil and its levels were determined as described by Zhang et al. (2012). In brief, a portion of each soil sample (5 g) was placed in a 50-mL centrifuge tube and mixed with 20 mL of 0.5% (v/v) formic acid in acetonitrile. The tubes were shaken for 2 h and then centrifuged at 2077*g* for 5 min. Then, 1.5 mL of the upper layer was transferred to a 2-mL centrifuge tube and cleaned using dispersive solid-phase extraction with 50 mg primary secondary amine (PSA) and 150 mg anhydrous magnesium sulfate ($MgSO_4$). The samples were then vortexed for 1 min and centrifuged as described above. Next, the supernatant was filtered through a 0.22-μm syringe filter (15-mm diameter; Agela Technologies, China) and transferred into a 2-mL glass vial and diluted before injection.

All analyses were performed using the Waters Acquity UPLC system coupled with a triple-quadrupole TQD mass spectrometer (Waters Corp., Milford, MA, USA). Chromatographic separation was performed on a UPLC BEH C_{18} column (1.7 μm, 2.1 × 50 mm) maintained at 45 °C. The mobile phases, which consisted of acetonitrile (A) and a 0.2% aqueous solution of formic acid (B), were pumped at a flow rate of 0.3 mL/min. The gradient elution was as follows: 0–2.0 min with 10–90% A, 2.0–2.1 min with 90–10% A, and then holding at 10% A for 3.0 min. Quantification was performed using ESI^-; 437 (*m/z*) was selected as the precursor ion, and its quantitative and qualitative product ions were 286 (*m/z*) and 316 (*m/z*) when the cone voltages and collision energies were 45 and 23 V, respectively.

2.1.2. Microbiological analysis

The R_B was measured as described by Wu et al. (2014). Briefly, 20 g of fresh soil was placed in a flask with a glass vial containing 10 mL 0.1 M NaOH to trap the released CO_2 and incubated at 25 °C for 24 h. Excess NaOH was then back titrated with 0.05 M HCl using Brand Titrette (Germany).

The MBC in the soil was determined using a fumigation-extraction method described by Lin et al. (1999). MBC is given by MBC = 2.64Ec, where Ec = (C extracted from fumigated soil) – (C extracted from non-fumigated soil). The microbial metabolic quotient (qCO_2) = R_B/MBC.

A phospholipid fatty acid (PLFA) analysis was performed according to the protocol described by Bossio et al. (1998). Briefly, lipids were extracted in a one-phase mixture of $CHCl_3$/CH_3OH/phosphate buffer (1:2:0.8, v/v/v). Polar lipids were separated from glycol lipids and neutral lipids on silica gel columns (500 mg; Supelco, Inc. USA). After the methylation of the polar lipids, PLFA methyl esters were separated and analyzed using Polaris Q ion-trap GC-MS (Thermo Fisher Scientific, Inc. USA) with a HP-5MS column (60 m × 0.25 mm × 0.25 m). The Supelco 37-Component Fatty FAME Mix and Bacterial Acid Methyl Esters (Sigma-Aldrich) were used for peak identification and quantification. Seventeen fatty acids with chain lengths of 14–20 carbon atoms, i.e., 14:0, i15:0, a15:0, 15:0, 16:0, i16:0, i17:0, 16:1ω7c, cy17:0, 17:0, 10Me18:0, 18:2ω6,9, 18:1ω9c, 18:1ω9t, 18:0, cy19:0, and 20:0, were identified as biomarkers of the different microbial groups. Thirteen fatty acids were used together to assess the bacterial biomass (14:0, i15:0, a15:0, 15:0, 16:0, i16:0, i17:0, 16:1ω7c, cy17:0, 17:0, 18:0, cy19:0, and 20:0), of which i15:0, a15:0, i16:0, and i17:0 were used to represent gram-positive (Gram +) bacteria and 16:1ω7c, cy17:0, cy19:0 were used to represent Gram-negative (Gram −) bacteria (Kong et al., 2008). The unsaturated PLFAs 18:1ω9c, 18:1ω9t, and 18:2ω6,9 were used as an indicator of fungal biomass (Papadopoulou et al., 2011; Zhang et al., 2010). The sum of all of the PLFAs was used to represent the total microbial lipid biomass.