Journal of Hazardous Materials 273 (2014) 155–164

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

# Responses of soil microbial community to different concentration of fomesafen

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## HIGHLIGHTS

- 100× recommended application rate reduced soil microbial activity.
- 100× recommended application rate changed soil microbial community composition.
- 100× recommended application rate changed microbial functional diversity.
- Nitrogen-fixing gene abundance was reduced by high fomesafen application.

## ARTICLE INFO

*Article history:*
Received 13 September 2013
Received in revised form 10 March 2014
Accepted 21 March 2014
Available online 30 March 2014

*Keywords:*
Fomesafen
Microbial activity
Community structure
Functional diversity
*nifH*

## ABSTRACT

Fomesafen degrades slowly in soils and has been linked to crop damage. However, the effect of its residues on soil microbial communities is unknown. The goal of this work was to assess the effect of applying three different doses of fomesafen on microbial community structure and functional diversity as measured by phospholipid fatty acid (PLFA) levels, community-level physiological profiles (CLPPs) and real-time PCR. Our results indicate that applying 100 times the recommended dose of fomesafen (T100) adversely affects soil microbial activity and stresses soil microbial communities as reflected by the reduced respiratory quotient ($qCO_2$, $Q_R$). The PLFA analysis showed that high levels of fomesafen treatment (T100) decreased the total amount of PLFAs and both bacterial (both Gram-positive (GP) bacteria and Gram-negative (GN) bacteria) and fungal biomass but increased the microbial stress level. However, the BIOLOG results are not consistent with our other results. The addition of fomesafen significantly increased the average well color development, substrate utilization, and the functional diversity index ($H'$). Additionally, the abundance of the *nifH* ($N_2$-fixing bacteria) gene was reduced in the presence of high concentrations of fomesafen (T100). Taken together, these results suggest that the addition of fomesafen can alter the microbial community structure and functional diversity of the soil, and these parameters do not recover even after a 90-day incubation period.



## 1. Introduction

Fomesafen (5-[2-chloro-4-(trifluoromethyl) phenoxy]-*N*-[methylsulfonyl]-2- nitrobenzamide) is a diphenyl ether that is specifically used for early post-emergent control of broad-leaved weeds among bean and soybean crops. This herbicide acts by inhibiting protoporphyrinogen oxidase, which is a key enzyme in the porphyrin biosynthesis pathway in plants [1]. After being imported to China, fomesafen soon became one of the most frequently used herbicides in soybean and peanut fields because of its high herbicidal activity at low concentrations [2]. However, U.S. Department of Agriculture Soil Conservation Service (USDASCS) [3] reported that fomesafen has a large relative leachability and a medium relative runoff potential. Jumel et al. [4] found that fomesafen has high reproductive toxicity in the freshwater snail *Lymnaea stagnalis*. Moreover, fomesafen degrades slowly in the soil with a half-life of 100–240 days, and its residue is implicated in phytotoxicity and damage during crop rotation [5]. A significant

*Abbreviations:* dw, dry weight; $R_B$, basal respiration; SIR, substrate-induced respiration; MBC, microbial biomass carbon; $qCO_2$ and $Q_R$, respiratory quotient; PLFA, phospholipids fatty acid; GP, Gram-positive bacteria; GN, Gram-negative bacteria; CLPPs, community level physiological profiles; AWCD, average well-color development; *H*, functional diversity index; PCA, principal component analysis; PVC, polyvinyl chloride.

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http://dx.doi.org/10.1016/j.jhazmat.2014.03.041
0304-3894/

proportion of these pesticides frequently end up in the soil where they undergo a biological and physicochemical transformation [6]. Thus, the intensive use of fomesafen constitutes a potential risk to environmental safety, and may have particularly adverse consequences for soil quality.

The diversity of soil organisms is tremendous; one gram of soil contains as many as $10^{10}$–$10^{11}$ bacteria [7], 6000–50,000 bacterial species [8], up to 200 m fungal hyphae [9] and 3000–11,000 different genomes [10]. Soil microbes are a crucial component of the biosphere because they are responsible for many ecosystem services, such as the promotion of plant growth, the cycling of carbon, nitrogen, and other nutrients and the degradation of pollutants and pesticide [7,11,12]. All of these functions are of great importance to productivity of agricultural soils [13]. Moreover, soil microbes represent the unseen majority in soil and comprise a large portion of the genetic diversity on Earth [14]. Soil microorganisms are very sensitive to any ecosystem perturbation, which rapidly alters their diversity and activity [15]. Properties that reflect the biomass, activity and diversity of microbial community populations in the soil serve as useful indicators of the impact of outside disturbances (including pesticide application) on soil health [16]. However, high inputs of pesticides can cause changes in bacterial diversity [17], and may also influence biogeochemical cycles. While researchers have previously investigated the effect of fomesafen on microbial activity (including microbial biomass C and metabolic quotient ($qCO_2$), they have not focused on the structure and function of the microbial community [18].

The aim of this study was to assess the response of soil microbial populations to fomesafen-induced disturbances and to monitor the recovery of soil health after fomesafen treatment. Several soil microbial parameters that have the potential to act as bioindicators of soil quality were determined, including basal respiration, substrate-induced respiration, ecophysiological indices, microbial biomass C, community composition, functional community profiling and the abundance of nitrogen-fixing bacteria. To our knowledge, no previous studies have examined the effect of fomesafen on the composition and functional diversity of microbes in agricultural soils in China.

## 2. Materials and methods

### 2.1. Soil characterization and experimental design

Soil (top 0–15 cm) was collected from an experimental plot near Lang fang (N39°82′, E116°70′) in Hebei Province. The field had not been tilled or planted with crops in the last 2 years. The soil was classified as silty loam, and its properties were as follows: 10.0% clay, 78.2% silt, 11.8% sand, 16.9 g organic matter $kg^{-1}$ soil, 27.8 mg available P $kg^{-1}$ soil, 308.8 mg available K $kg^{-1}$ soil, 14.1 mg $NH_4^+$—N $kg^{-1}$ soil, 58.9 mg $NO_3^-$—N $kg^{-1}$ soil and pH 7.36. After collection, the soil samples were mixed, sieved with 2 mm mesh to remove any plant tissue and preincubated under the experimental conditions described below for two weeks in large polyvinyl chloride (PVC) tanks. The effects of fomesafen on soil microbial communities were studied using three different doses: 3.75 mg $kg^{-1}$ soil dry weight (a.i./dw; T1), 37.5 mg $kg^{-1}$ soil (a.i./dw; T10) and 375 mg $kg^{-1}$ soil (a.i./dw; T100). T1 corresponds to the conventional recommended field application dose for spring soybean crops (375 g active ingredient $ha^{-1}$), assuming a soil bulk density of 1 g $cm^{-3}$ and an effective soil depth of 1 cm. T10 and T100 correspond to10 or 100 times the recommended dosage. For each fomesafen concentration, a set of three replicated mesocosms was prepared by transferring subsamples of 4 kg dry weight soil to separate PVC tanks. Each treatment was artificially contaminated by adding 30 mL of fomesafen (purity, 96.3%, Shandong Binnong Technology Co., Ltd, China) solution (in methanol). An equal volume of pure methanol (30 mL) was added to fomesafen-free controls (CK). Sub-samples were thoroughly mixed with a rotary mixer (Hana Mixer, AHM-P125B) to assure uniform pesticide distribution and kept for 24 h in a dark room at 22 ± 1 °C to allow the methanol to evaporate. Next, each freshly-treated soil sub-sample was distributed equally (200 g) among brown wide mouth bottles that were 15 cm high and 8 cm in diameter. The water content of the soil was adjusted to 60% capacity and was held constant by the daily addition of deionized water. The pots were covered with porous plastic film and placed in an environmental chamber at 25 °C and 50% humidity. Each experiment was conducted in triplicate. The pots were removed from the environmental chamber after various intervals (7, 15, 30, 60 and 90 d), and the soils were then analyzed for concentration of fomesafen, basal respiration, substrate-induced respiration, microbial biomass carbon, community structure, and functional diversity as described below.

### 2.2. Fomesafen concentration

The fomesafen was extracted from the soil and its levels were determined as previously described by Zhang et al. [19] with some modifications. Briefly, 5.0 g sample of soil was extracted for 2 h in 20 mL acetonitrile (containing 0.5% (v/v) formic acid). After centrifugation, 1.5 mL of the upper layer was transferred into a 2.0 mL dispersive-SPE tubes containing PSA 50 mg and 150 mg $MgSO_4$. Then the tubes were vortexed for 1 min and centrifuged for 5 min at RCF 2077 g. The resulting supernatants were filtered through 0.22 μm nylon syringe filters for UPLC–MS/MS analysis. Analysis of fomesafen was conducted on a triple-quadrupole mass spectrometer (TQD, Waters Crop.) using the multiple reaction monitoring (MRM) mode and negative ESI mode. 437 ($m/z$) was selected as the precursor ion, and its quantitative and qualitative product ions were 286 ($m/z$) and 316 ($m/z$), respectively, when the collision energies were both 23 V.

### 2.3. Microbial respiration and biomass carbon

Soil basal respiration ($R_B$; an indicator of overall microbial activity) and substrate-induced respiration (SIR; an indicator of potentially active microbial biomass) were measured as previously described by Zeng et al. [20] with some modifications. To measure the basal respiration, 20 g fresh soil was transferred to a flask along with a vial containing 0.1 N NaOH (10 mL) to trap the released $CO_2$. As a blank, one flask did not contain soil. The soil samples and the blank were all incubated at 25 °C for 24 h. Then NaOH solution was precipitated with 3 N $BaCl_2$ and back-titrated with 0.05 N HCl using Brand Titrette® (Germany). The substrate-induced respiration (SIR) was measured by adding 10,000 mg glucose $kg^{-1}$ dry weight soil to the soil samples and then measuring $CO_2$ emission 6 h later.

Microbial biomass carbon (MBC), an indicator of the overall size of the soil microbial community, was measured by the chloroform-fumigation–extraction method [21]. The MBC was calculated using the following equation:

$$\mathrm{MBC} = 2.64E_C \quad (1)$$

where $E_C$ = (C extracted from fumigated) − (C extracted from non-fumigated soil) with a conversion factor of 2.64.

### 2.4. Phospholipid fatty acid (PLFA) profiles

The microbial community structure of the soil was determined by analyzing its phospholipid fatty acid (PLFA) composition as previously described by Bossio et al. [22]. The microbial biomass was assessed by analyzing the presence of 17 fatty acids, 14:0, i15:0, a15:0, 15:0, 16:0, i16:0, i17:0, 16:1ω7c, cy17:0, 17:0, 10Me18:0,