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Assessment of the ecotoxicological impact of natural and synthetic β-triketone herbicides on the diversity and activity of the soil bacterial community using omic approaches

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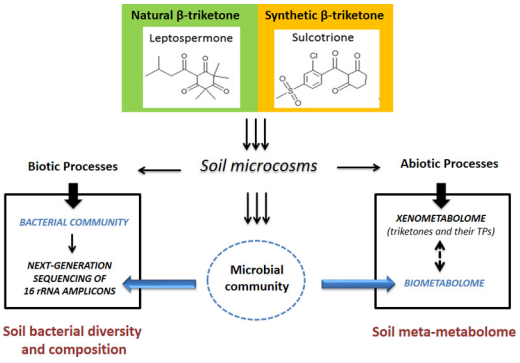
HIGHLIGHTS

- Leptospermone strongly affected the soil bacterial diversity and structure.
- Less effects were observed for sulcotrione.
- Both leptospermone and sulcotrione modified the soil meta-metabolome.
- Ecotoxicological effects of both triketones were resilient at recommended field dose.

GRAPHICAL ABSTRACT



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ABSTRACT

The emergence of pesticides of natural origin appears as an environmental-friendly alternative to synthetic pesticides for managing weeds. To verify this assumption, leptospermone, a natural β-triketone herbicide, and sulcotrione, a synthetic one, were applied to soil microcosms at 0× (control), 1× or 10× recommended field dose. The fate of these two herbicides (*i.e.* dissipation and formation of transformation products) was monitored to assess the scenario of exposure of soil microorganisms to natural and synthetic herbicides. Ecotoxicological impact of both herbicides was explored by monitoring soil bacterial diversity and activity using next-generation sequencing of 16S rRNA gene amplicons and soil metabolomics. Both leptospermone and sulcotrione fully dissipated over the incubation period. During their dissipation, transformation products of natural and synthetic β-triketone were detected. Hydroxy-leptospermone was almost completely dissipated by the end of the experiment, while CMBA, the major metabolite of sulcotrione, remained in soil microcosms. After 8 days of exposure, the diversity and structure of the soil bacterial community treated with leptospermone was significantly modified, while less significant changes were observed for sulcotrione. For both herbicides, the diversity of the soil bacterial community was still not completely recovered by the end of the experiment (45 days). The combined

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use of next-generation sequencing and metabolomic approaches allowed us to assess the ecotoxicological impact of natural and synthetic pesticides on non-target soil microorganisms and to detect potential biomarkers of soil exposure to β-triketones.

1. Introduction

Microorganisms play a major role in the regulation of soil functions such as nutrient cycling (Nannipieri et al., 2003; Whitman et al., 1998). However, side effects of plant protection products (PPPs) on soil microbial communities have been neglected by the European regulation which only considers impacts of PPPs on carbon mineralization in soil (Martin-Laurent et al., 2013). Although numerous reports show that soil microbial communities are sensitive to PPPs, there are no requirement to assess their effects on the abundance, diversity and activity of microbial communities and further consequences on ecosystems functions (Ingram et al., 2005; Wang et al., 2006). Hence, monitoring the dissipation of pesticides in soils is only one of the components to evaluate their ecotoxicological risk. Indeed, PPPs can stimulate the growth of microbial populations capable of degrading and using them as a nitrogen and carbon source for their growth, but can also be toxic for sensitive microbial populations harboring the enzyme targeted by the pesticides. Therefore, new approaches need to be developed to evaluate the ecotoxicological impact of PPPs on soil microorganisms supporting ecosystem functions as recently recommended by an EFSA scientific opinion (EFSA, 2017).

To assess the ecotoxicological impact of pesticides on soil microbial diversity and activity, we used β-triketone herbicides (*e.g.* sulcotrione, mesotrione and tembotrione) as a model. This new generation of herbicides was developed by mimicking the structure of leptospermone (2,2,4,4-tetramethyl-6-(3-methyl-1-oxobutyl)-1,3,5-cyclohexanetrione), an allelopathic compound first isolated from the bottlebrush plant (*Callistemon citrinus*) (Beaudegnies et al., 2009; Gray et al., 1980; Mitchell et al., 2001). Since their introduction into the market, synthetic β-triketones are increasingly used in several European countries as selective pre- and post-emergence herbicides to control the development of a wide range of broadleaves weeds in corn crop to replace banned *s*-triazines. Recently, leptospermone, isolated from essential oil distilled from manuka tree (*Leptospermum scoparium*), was used as a natural herbicide to control several broadleaf and grass weeds in different crops (Dayan et al., 2007; Dayan et al., 2011). Being not chemically optimized, leptospermone is known to be less efficient than synthetic triketones. Then, to get identical weed control efficacy, leptospermone should be applied at a dose three-times higher than that of sulcotrione (Dayan et al., 2011). Given the increasing environmental concerns over synthetic herbicides, natural ones are generally considered as an environmental-friendly alternative for crop protection. However, most of these claims have not been evaluated scientifically (Dewhurst, 2001). In the particular case of triketones, one can hypothesize that since natural and synthetic ones are structural analogs that have the same mode of action (*i.e.* inhibition 4-hydroxyphenylpyruvate dioxygenase (HPPD), a key enzyme in plant carotenoid biosynthesis (Dayan et al., 2007; Meazza et al., 2002; Rocaboy-Faquet et al., 2014; Schulz et al., 1993)), they might behave similarly in the soil and have similar impact on non-target soil organisms, among which microorganisms. This hypothesis is further reinforced by the fact that a large proportion of soil microorganisms harbor HPPD enzymes and can consequently be directly affected by triketones.

In an earlier study, we showed that the soil bacterial diversity was significantly modified by leptospermone treatment, but these changes were found to be resilient when leptospermone was fully dissipated in soil (Romdhane et al., 2016a). This study reports for the first time the comparison of the fate in soil and the ecotoxicological effect of natural and synthetic triketones (leptospermone *vs.* sulcotrione) on bacterial community diversity and activity of an agricultural soil. Therefore, the fate of both triketones was monitored in the soil using classical analytical chemistry analysis to determine exposure scenarios of soil microorganisms. An innovative strategy that combined next-generation sequencing of 16S rRNA amplicons generated from soil DNA extracts and analysis of the soil biometabolome using a metabolomic approach was then applied to study the ecotoxicological impact of triketones on soil microbial diversity and activity, respectively.

2. Methods

2.1. Chemicals

Sulcotrione (2-[2-chloro-4-(methylsulfonyl)benzoyl]-1,3-cyclohexanedione, 98.8% purity) was purchased from Sigma-Aldrich (France). Pure leptospermone (2,2,4,4-tetramethyl-6-(3-methylbutanoyl)cyclohexane-1,3,5-trione) was synthesized as described by Owens et al. (2013). Stock solutions of leptospermone and sulcotrione were prepared in methanol at a concentration of 4 g L^{-1} and 1.2 g L^{-1}, respectively.

2.2. Soil sampling and microcosm set up

Soil was collected from the surface layer (0–20 cm) of an experimental field located at the University of Perpignan, France. Soil was sieved to 2 mm and stored at 4 °C until use. The physicochemical characteristics of the soil was 13.9% clay, 60.5% silt, 25.6% sand, 1.7% organic matter, 0.98% organic carbon, 15.5 meq 100 g^{-1} cation exchange capacity (CEC), 214% Ca^{2+}/CEC, a pH in water of 8.1 and a soil humidity of 20%. Soil samples were treated or not (control) with natural and synthetic β-triketones applied at the agronomical dose or at ten-times the agronomical dose to test for realistic- and worst- scenario of exposure, respectively. Soil samples (20 g equivalent dry weight) were spiked with 250 μL of a ten time dilution of the leptospermone or sulcotrione stock solution corresponding to 1× recommended agronomical dose (D1, 5 μg g^{-1} (Dayan et al., 2011) and 1.5 μg g^{-1} (ACTA, 2015), respectively), and 250 μL of the stock solution to reach 10× recommended agronomical dose (D10, 50 μg g^{-1} and 15 μg g^{-1}, respectively). Soil samples treated with methanol was used as a control (D0, control). After soil treatment, methanol was evaporated and soil were moistened to reach about 35% of the soil water-holding capacity. Soil microcosms were incubated at 22 ± 2 °C in the dark. Soil samples were collected after 8 and 45 d of incubation for further analyses. Five replicates were prepared for each treatment (D0, D1 and D10) and time points.

2.3. Dissipation of herbicides in soil microcosms

The same procedure was used to extract leptospermone and sulcotrione from soil as described by Patil et al. (2016). Briefly, 10 g of soil were suspended in 6 mL aqueous solution of 0.1 M HCl and vortexed for 30 s. They were then added with 30 mL of ethyl acetate and mixed by agitation at 300 rpm for 30 min. They were centrifuged for 10 min at 3000*g*. The supernatant was recovered. Soil was retrieved and submitted to a second extraction. The soil extracts were dried and re-suspended in 5 mL of methanol. Recoveries were estimated to 91% (RSD 6%) and 40% (RSD 5%) for leptospermone and sulcotrione, respectively. They were stored at −20 °C until their analysis by LC-MS.