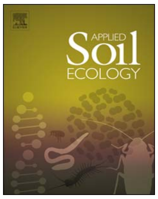
ARTICLE IN PRESS

Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

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



Simultaneous application of two herbicides and green compost in a field experiment: Implications on soil microbial community

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ARTICLE INFO

Keywords:
Triasulfuron
Prosulfocarb
Organic amendment
Soil microbiology
Field

ABSTRACT

The simultaneous use of herbicides and organic amendments is a common agricultural practice that may modify the behavior of the herbicides themselves and affect the microbial community in soils. There is little information about the changes in the soil microbial community by this agricultural practice under real field conditions. The aim of this work was to assess the effects on the soil microbial community (abundance, activity, and structure) of the following two herbicides triasulfuron (TSF) and prosulfocarb (PSC) applied as individual or combined formulations in an unamended soil (Soil) and in a soil amended with green compost (Soil + GC) at field scale. Herbicide dissipation, soil biomass, respiration, dehydrogenase activity (DHA), and the profile of phospholipid fatty acids (PLFA) were monitored for 100 days. Triasulfuron recorded a slower dissipation rate than PSC. The dissipation rate of TSF decreased in the GC-amended soils compared to the unamended ones. Furthermore, the Soil + GC recorded a higher soil biomass and respiration than the unamended ones. In the GC-amended soil, biomass values decreased with individual or combined TSF application compared to the Soil + GC control, while biomass values in the unamended soil increased with the application of combined herbicides after 100 days. In general, soil respiration values decreased with the application of herbicides in both the unamended and GC-amended soils. This negative effect was higher for the combined TSF + PSC application. DHA values decreased over time in the unamended soils treated with herbicides, but this decrease was not observed in the GC-amended soil. The microbial structure clearly changed throughout the experiment under the different conditions assayed. After 100 days of simultaneous TSF + PSC application, there was a significant increase in Gram-positive bacteria and a significant decrease in Gram-negative bacteria and *Actinobacteria* in the unamended soil. The GC-amended soil minimized the effects of TSF + PSC, and only the relative abundance of *Actinobacteria* increased at 100 days. The microbial community in the unamended and GC-amended soils behaved differently with herbicide application; however, the combined application of TSF and PSC altered soil microbial activity and structure compared to their individual application or non-application. The application of GC to soil buffered the impact of TSF and PSC on microbial biomass and activity, and reduced the shift in the soil microbial structure.

1. Introduction

The application of pesticides in modern agriculture is a widespread practice around the world designed to increase crop yields (Imfeld and Vuilleumier, 2012). However, the extensive use of these chemicals also releases pollutants into the environment (Herrero-Hernández et al., 2016; Pose-Juan et al., 2015b; Sánchez-González et al., 2013). Given the toxic nature of pesticides, considerable effort has been made to monitor, understand and minimize their environmental impact (Herrero-Hernández et al., 2015, 2016; Odukkathil and Vasudevan, 2013).

In this respect, the application of organic amendments to the soil could act as a barrier to avoid pesticide leaching, minimizing the spread of pollutants (Álvarez-Martín et al., 2016b; Marín-Benito et al., 2013, 2017). On the other hand, the use of organic amendments is a common practice in agriculture and in soil remediation processes for increasing the soil content of nutrients and organic carbon (OC) (Clemente et al., 2015; Medina et al., 2012). This agricultural practice improves soil properties and crop yield, and enhances soil microbial activity (López-Rayo et al., 2016; Medina et al., 2015; Zornoza et al., 2016). Organic amendments can inoculate new microorganisms in the soil or promote the growth of specific microorganisms that modify the soil's microbial

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https://doi.org/10.1016/j.apsoil.2018.03.004
Received 20 December 2017; Received in revised form 2 March 2018; Accepted 3 March 2018

Please cite this article as: García-Delgado, C., Applied Soil Ecology (2018), https://doi.org/10.1016/j.apsoil.2018.03.004

activity and structure (Álvarez-Martín et al., 2016a; García-Delgado et al., 2015; Sun et al., 2017).

However, organic amendments may also modify the persistence and dissipation of pesticides in soils by increasing soil OC (Marín-Benito et al., 2012, 2013, 2014). In some cases, organic amendments have led to a decrease in the half-life of a pesticide, while in others there was an increase or even no effect at all (Álvarez-Martín et al., 2016a; Hussain et al., 2015; Marín-Benito et al., 2014). As the application of organic amendments affects the behavior of pesticides in the soil, they will be able to regulate the bioavailability and concentration of pesticides in the soil solution, conditioning their possible impact on soil microbial communities. Therefore, the soil microbial community's function and activity could be affected by the simultaneous application of pesticides and organic amendments (Hussain et al., 2009; Pose-Juan et al., 2015a).

Information about the effects pesticides have on soil microorganisms and assessing the toxicity of these compounds for soil microbial communities is increasing nowadays due to is a pre-requisite for improving pesticide regulation in the short term (Karpouzas et al., 2014). According to the reviews by Hussain et al. (2009) and Imfeld and Vuilleumier (2012), the presence of pesticides and their degradation products in the soil may inhibit, promote, or have no effects on microbial diversity and its functions. Therefore, considering the inconsistent results and the significance of microbial biomass, diversity and activity in many soil cycles and soil health, there is considerable scientific interest in determining the impact pesticides has on the soil microbial community (Martin-Laurent et al., 2010).

Moreover, there is little information about the soil microbial community's response and function when pesticides and organic residues are simultaneously applied (Pose-Juan et al., 2017, 2015a). Many of the published studies on soil microbial community's response to pesticides have been conducted at laboratory or greenhouse scale (Cycoń et al., 2012, 2013; Karpouzas et al., 2014; Pose-Juan et al., 2017, 2015a), while field-scale assays that replicate real conditions are scarce (Petric et al., 2016; Spyrou et al., 2009).

The largest share of pesticides involves herbicides, which play key roles in promoting crop yields. These compounds have also posed serious issues of environmental pollution (Huang et al., 2016), and soil bacteria are sensitive to some of them, such as sulfonylureas, affecting the universal biological processes in living systems (Patyka et al., 2016).

Two groups of herbicides widely used today are sulfonylureas and thiocarbamates. They have good selectivity, and are characterized by broad-spectrum weed control for many cereal crops, such as rice, wheat or maize, soybean and sugar beet or vegetables (e.g., carrots, peas, and potatoes) (Sofo et al., 2012). Triasulfuron is a sulfonylurea that inhibits the behavior of acetolactate synthase, and it is responsible for the biosynthesis in plants and bacteria of three branched-chain amino acids (leucine, isoleucine, and valine). Since the enzyme is absent in humans and animals, it is a safe choice to apply sulfonylureas in the field (Wang et al., 2010). Triasulfuron is a weak acid which presents a high solubility in water and low hydrophobicity. In field dissipation studies, TSF exhibited an elevated mobility and moderate persistence in soils (PPDB, 2017). The time required for the concentration to decline to half of the initial value (DT_{50}) ranged between 15.9 and 65.4 days (EFSA, 2015). The adsorption of TSF by soils influences its biodegradation and bioavailability (Said-Pullicino et al., 2004). Its transformation to metabolites is due to microbial degradation and chemical hydrolysis (Pose-Juan et al., 2017; Singh and Kulshrestha, 2006). Soil bacterial communities or activities could be affected by this herbicide (Pose-Juan et al., 2017; Wang et al., 2010). Nevertheless, little is currently known about the impact TSF and other sulfonylurea herbicides have on soil microbes (Karpouzas et al., 2014).

Thiocarbamate herbicides inhibit the elongase enzyme, hence the main effect of these herbicides is the inhibition of the synthesis of very-long-chain fatty acids, while also affecting meristematic tissues. Among these herbicides, and as a secondary effect, PSC has previously been reported to inhibit shikimate synthesis, leading to a decrease in flavonoid content and a variation in amino acid composition and content. The changes can be interpreted as secondary effects, probably related to the stress caused by the primary effects of PSC (Hjorth et al., 2006). Prosulfocarb has low solubility in water and high hydrophobic character. This herbicide presents high adsorption, is slightly mobile and non-persistent in soils (PPDB, 2017). Under field conditions, prosulfocarb DT_{50} values ranged between 6.5 and 13.0 days (EFSA, 2007). The dissipation of PSC is due mainly to microbial degradation (Gennari et al., 2002). The high adsorption of PSC by soil organic matter fractions could lead to a decrease in leaching (Nègre et al., 2006).

The physicochemical behavior of TSF and PSC herbicides, including their dissipation, mobility and persistence in a field experiment in an unamended soil and one amended with green compost (GC), has been evaluated in a previous study (Marín-Benito et al., 2018). Herbicide concentrations were determined at various times in the surface soil and at different depths up to 50 cm to evaluate the effect of the increased OC in the amended soil and the influence on the dissipation and mobility of individual Logran® and Auros®, or the combined commercial formulation Auros Plus® of both compounds.

The present work supports a simultaneous study designed to evaluate the effect of herbicides applied on microbial communities and their evolution over the dissipation process. To our knowledge, there are no studies on the impact of PSC on soil microbial communities, and little is known about the impact of TSF on soil microbes under real field conditions (Karpouzas et al., 2014).

Accordingly, the aim of this work was to determine the possible modifications of soil microbial communities due to the application of the herbicides TSF and PSC on an unamended and a GC-amended soil. A field experiment was set up, and the effects of the individual or combined commercial formulations of the herbicides were studied on the following: (i) the soil microbial biomass, respiration, and dehydrogenase activity, as parameters indicating the abundance, overall activity and function of microbial communities, and (ii) the profile of phospholipid fatty acids (PLFAs) extracted from the soil, as indicator of the soil microbial structure. Changes were evaluated at various times during the dissipation of herbicides in the soil surface.

2. Materials and methods

2.1. Herbicides

This study used the commercial formulations of triasulfuron (TSF) (Logran® 20% p/p), prosulfocarb (PSC) (Auros® 80% p/v), and triasulfuron + prosulfocarb (TSF + PSC) (Auros Plus®) (Syngenta Agro S.A., Madrid, Spain). Analytical standards of both compounds were purchased from PESTANAL® (purity > 98.9%) (Sigma Aldrich Química S.A., Madrid, Spain). The chemical name, structure and characteristics of these compounds are included in Table S1 (in Supplementary material) (PPDB, 2017).

2.2. Green compost

A composted organic residue of vegetal origin from the pruning of plants and trees in parks and gardens in the city of Salamanca (Spain) has been used. It was supplied by the city council. The physicochemical characteristics of this green compost (GC) on a dry weight basis are as follows: pH 7.33, determined in a GC/water suspension (1:2); OC content 9.80%, determined by the modified Walkley-Black method; dissolved organic carbon (DOC) 0.353%, determined in a suspension of GC in deionized water (1:2) after shaking (24 h), centrifuging (20 min at 10,000 rpm) and filtering, using a Shimadzu 5050 (Shimadzu, Columbia, MD, USA) organic carbon analyzer; total N, 1.04% determined by the Kjeldahl method. The C/N rate was 9.42, and the ash percentage determined by weight difference after ignition at 540 °C for