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Soil microbial communities and glyphosate decay in soils with different herbicide application history

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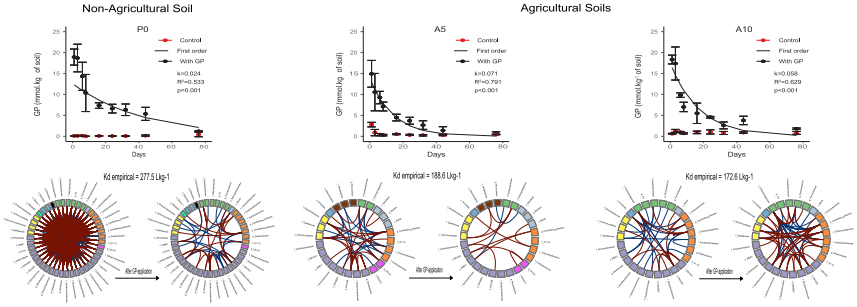
HIGHLIGHTS

- Glyphosate dissipation in soil is evaluated under field conditions.
- Redundant bacterial populations of potential degraders
- Application of glyphosate disrupt bacterial association network.
- Bioavailability is a key factor for the persistence of GP and AMPA.

GRAPHICAL ABSTRACT



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ABSTRACT

This study evaluates the glyphosate dissipation under field conditions in three types of soil, and aims to determine the importance of the following factors in the environmental persistence of herbicide: i) soil bacterial communities, ii) soil physicochemical properties, iii) previous exposure to the herbicide. A soil without previous record of GP application (P0) and two agricultural soils, with 5 and >10 years of GP exposure (A5 and A10) were subjected to the application of glyphosate at doses of 3 mg·kg^{-1}. The concentration of GP and AMPA was determined over time and the dynamics of soil bacterial communities was evaluated using 16S ARN ribosomal gene amplicon-sequencing. The GP exposure history affected the rate but not the extent of GP biodegradation. The herbicide was degraded rapidly, but P0 soil showed a dissipation rate significantly lower than soils with agricultural history. In P0 soil, a significant increase in the relative abundance of *Bacteroidetes* was observed in response to herbicide application. More generally, all soils displayed shifts in bacterial community structure, which nevertheless could not be clearly associated to glyphosate dissipation, suggesting the presence of redundant bacteria populations of potential degraders. Yet the application of the herbicide prompted a partial disruption of the bacterial association network of unexposed soil. On the other hand, higher values of linear (Kd) and nonlinear (Kf) sorption coefficient in P0 point to the relevance of cation exchange capacity (CEC), clay and organic matter to the capacity of soil to adsorb the herbicide, suggesting that bioavailability was a key factor for the persistence of GP and AMPA. These results contribute to understand the relationship between bacterial taxa exposed to the herbicide, and the importance of soil properties as predictors of the possible rate of degradation and persistence of glyphosate in soil.

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1. Introduction

The herbicide glyphosate [N-(phosphonomethyl)-glycine] is a synthetic phosphonate used extensively in the entire world. The introduction of glyphosate-resistant crops, pre-emergence applications and weed control between crops has broaden its application (Székács and Darvas, 2012), with a concomitant increase in the volume applied per hectare. Environmental concern related to the widespread use of glyphosate has derived in a large number of experimental studies (reviewed by Cerdeira and Duke, 2006; Borggaard and Gimsing, 2008; Mamy et al., 2016) and modelling studies (la Cecilia and Maggi, 2018; Wang et al., 2016) focusing on its fate. The persistence of glyphosate in the environment increases the possibility of freshwater and groundwater contamination, as well as the interception and absorption by weeds and crops (Bento et al., 2017; Doublet et al., 2009).

Degradation of glyphosate (GP) in soils is mainly microbiological (Sprankle et al., 1975), and the role of abiotic factors on its dissipation is negligible (Bento et al., 2016). Therefore, the role of soil microorganisms is critical in minimizing the environmental concentration of the herbicide. GP biodegradation occurs by two alternative pathways (Singh and Walker, 2006). One of the pathways, carried out by microorganisms that utilize the herbicide as a source of phosphorous, involves the conversion to sarcosine, which is subsequently mineralized to carbon dioxide and water. This pathway rarely occurs in natural environment because the enzymes involved are induced when the intracellular P_i is deficient, a situation not typically encountered in agricultural soils (Sviridov et al., 2015). In the other pathway, GP is metabolized to glyoxylate and aminomethylphosphonic acid (AMPA) by microorganisms that use GP as a source of N. The capacity of many soil bacteria to degrade GP, yielding both sarcosine and AMPA, has been demonstrated in the laboratory (Sviridov et al., 2015). The biodegradation of GP via AMPA has been well documented, and this metabolite has been detected at higher concentrations than GP in agricultural fields (Aparicio et al., 2013; Battaglin et al., 2014; Primost et al., 2017; Silva et al., 2018). An important body of literature, mostly performed in microcosms, has revealed that GP exposure affects the structure of soil microbial communities. A wide variety of responses have been described, ranging from transient to permanent changes, affecting members of phylum *Acidobacteria* (Newman et al., 2016a), ammonia-oxidizing bacteria (Allegrini et al., 2017), mycorrhiza (Druille et al., 2013), and others. Either the reduction or the enhancing of the microbial activity and biomass in soil has also been reported (Gómez et al., 2009; Haney et al., 2002).

It has been shown that repeated use of the same pesticide for several years brings about the ability of soil biota to degrade it rapidly (James et al., 2010). However, this process depends on the intervals between successive pesticide applications, and on the stability of the active microbiota (Kaufman et al., 1985). Previous studies on the effect of repeated applications of GP focused on the activity of microbial communities rather than on the kinetics of biodegradation. Araújo et al. (2003) found an increase in respiration and FDA activity in agricultural soils after GP application, compared to soils with no history of GP exposure. On the other hand, Allegrini et al. (2015) did not find differences in the microbial community tolerance to GP from contrasting soils with and without history of exposure to the herbicide.

Because soil is a very complex and dynamic environmental matrix, herbicide degradation in soil is not only determined by the microorganisms and the environmental factors, such as land use, soil moisture, temperature and sources of nitrogen and carbon (Girvan et al., 2003; Lauber et al., 2008; Bento et al., 2016; Zabaloy et al., 2016), but it is also critical that the molecule is available for enzymatic attack (Throckmorton et al., 2015). Once GP is sprayed, a part of the herbicide attaches to soil particles due to their high adsorption capacity, and will be more or less bioavailable, depending on the reversibility of adsorption equilibrium. Some mechanisms have been proposed to describe the interaction between GP and soil particles (Cruz et al., 2007; Gimsing and Borggaard, 2002; Ololade et al., 2014). Yet it is not entirely clear what are the main factors that control the adsorption of GP to soil. Weber et al. (2004) proposed a pedotransfer function for predicting the linear sorption coefficient (Kd) of different pesticides, and more recently Dollinger et al. (2015) put forward a specific function for GP, in which Kd is mainly driven by cation exchange capacity (CEC) and clay content.

Although it is known that sorption influences both the immobilization and the microbial degradation of the herbicide, less attention was devoted to studying the interplay between soil properties, microbial community composition and GP biodegradation. Our working hypothesis is that soil characteristics, rather than the dependency on specific microorganisms, determine the glyphosate dissipation in soil. To that aim, the specific objectives of this work are: i) to evaluate the degradation rates in soils with and without previous exposure to GP, ii) to establish the relationship between the dynamics of biodegradation and the changes in soil bacterial communities iii) to elucidate the influence of soil properties on microbial community structure and GP bioavailability. We based our study in three soils located in the southeast of Buenos Aires Province, Argentina, with similar edapho-climatic conditions, but different history of land use and herbicide exposure. These fields have contrasting characteristics in clay, CEC and soil organic matter, which make them appropriate for evaluating, on a field experiment, the relationship between soil parameters and herbicide dissipation, as well as the role of native bacterial communities in response to glyphosate application.

2. Materials and methods

2.1. Experimental design

The field experiments took place between November 2013 and February 2014 at INTA Balcarce Agronomic Experimental Station, Province of Buenos Aires. Soils studied are classified as Luvic Phaeozem (IUSS Working Group WRB, 2007). Three locations were selected: a soil without previous exposure to herbicides (P0: S37°45′47.9″ WO58°18′28.4″) belonging to a football stadium surrounded by a row of trees, and two agricultural soils, with 5 and almost 10 years of GP application history (A5: S37°45′49.7″ WO58°17′33.1″ and A10: S37°45′17.4″ WO58°17′51.8″, respectively). Agricultural soils were managed under conventional tillage with maize-wheat/soybean rotation. The history of GP use and spraying dosage during the last year and the 5 years before this experiment are shown in Table 1 and Supplementary Table S1. A randomized block design with six plots of 10 m^2 was made at each location. Commercial glyphosate (DuPont® Premium HL 48% w/v) was sprayed onto three of the six plots, whereas the other three plots remained as controls (no herbicide added). Considering a depth of 5 cm and a soil bulk density of 1.2 t m^{-3}, the sprayed soils received uniform manual application of approximately 3 mg of active ingredient kg^{-1} of soil.

Soil samples were collected the day before application and on days 1, 3, 5, 8, 16, 24, 32, 44 and 72 after herbicide application. Each sample was a composite of ten sub-samples per plot, collected from the top 0 to 5 cm, using a soil core device, which was cleaned by flashover to avoid cross-contamination between samples. Samples were homogenized, dried at 30 °C and sieved through 2-mm mesh, and stored at −20 °C until DNA extraction.

Soil texture was determined using the pipette method (Gee and Bauder, 1986). Cation-exchange capacity (Chapman, 1965), pH (1:2.5 soil: water ratio), total organic carbon (Nelson and Sommers, 1982) and available phosphorus (Bray and Kurtz, 1945) were determined by standard procedures.

Temperature and rain data during the experiment were collected in the Meteorological Station of INTA Balcarce (http://anterior.inta.gov.ar/balcarce/info/meteorologia/meteoro2.htm) and the information is summarized in Fig. S1.