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Effect of glyphosate and a commercial formulation on soil functionality assessed by substrate induced respiration and enzyme activity

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ABSTRACT

Glyphosate is the most widely used herbicide globally. While concerns have been raised that glyphosate may modify soil ecosystems, the specific effects of glyphosate and commercial formulations of glyphosate on soil microbial community function are still not fully understood. This study investigated the effect of increasing doses (0–79 mg kg^{-1}) of glyphosate and the formulation RoundupCT® on substrate induced respiration (SIR) and enzyme activities representing C-, N-, S- and P- cycling in three contrasting agricultural soils, over a 27 d period. Soil characteristics and the form of herbicide were dominant factors controlling potential effects on soil functionality. The light-textured Tenosol was more responsive to herbicide treatments than either the clay Vertosol or loamy Chromosol. In the Tenosol, there was a significant interaction between dose and herbicide form at 3 d after treatment: application of RoundupCT® at the two highest doses (26 and 79 mg glyphosate kg^{-1} soil) enhanced SIR of a number of C-substrates, while the highest dose of glyphosate inhibited SIR. Roundup CT® also triggered significantly greater consumption of arabinose, glucose, N-acetylglucosamine and proline in the Tenosol 27 d after application compared to glyphosate alone, but application dose was no longer significant. Effects in both the Chromosol and Vertosol were less clear, with glyphosate increasing SIR of glucose and malic acid in the Chromosol at day 3 only cf. Roundup CT, while SIR of arabinose, glucose and malic acid was stimulated by RoundupCT® in the Vertosol cf. glyphosate. In the Vertosol, glyphosate and RoundupCT® application at 79 mg kg^{-1} significantly increased respiration of arabinose 3 d after application, and oxalic acid at both time points, compared with the untreated control. Although some minor effects on enzyme activities were observed, they were generally less sensitive than measures of SIR. The exception was a significant reduction of cellulase activity in Vertosol 27 d after treatment with glyphosate (but not RoundupCT®) at rates equal or greater than 2.9 mg kg^{-1}. Overall, effects of glyphosate or RoundupCT® at label rates were minor or periodic. This study demonstrated that the soil type and formulation of the herbicide are important factors when assessing potential impacts of herbicides on soil functions.

1. Introduction

Over the last two decades, global herbicide use has increased rapidly as farmers have adopted conservation tillage practices which rely on herbicides as the primary means of weed control [1]. A key herbicide in conservation tillage systems is the broad-spectrum active ingredient (a.i.) glyphosate [N-(phosphonomethyl)-glycine], which inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) - an enzyme in the shikimate pathway involved in the production of aromatic amino acids and other secondary metabolites [2]. Globally, more than 746 million kg of glyphosate was used in 2014, roughly 17 times the amount used in 1994 [3]. The increase in glyphosate application is also partly due to the increasing development and use of glyphosate-tolerant crops [4].

Because of its widespread usage, the impact of glyphosate on soils and the environment has come under scrutiny. Issues surrounding the use of glyphosate include the risk of surface and groundwater pollution via runoff and leaching [5]; the development of glyphosate-resistant weeds [6]; and non-target effects on soil microbiology [7,8]. A wide range of soil microorganisms use the shikimate synthesis pathway, leading to concerns about the potential impact of glyphosate on soil microbial community structure and function [7,9].

Soil microorganisms play a critical role in the degradation of soil organic matter, nutrient turnover and pathogen suppression, and are therefore an essential component of sustainable farming systems. However, the results of published studies on the impact of glyphosate on soil microorganisms are highly variable. For example, glyphosate

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has been reported to have no significant impact [10] or significant impacts [11] on microbial community structure. Contradictory results are also observed in published studies on the impact of glyphosate on soil microbial biomass (SMB) and soil respiration (SR) [1]. However, in a meta-analysis of over 30 peer-reviewed studies [12], showed that addition of glyphosate to soils at typical field application rates had no significant effect on soil microbial biomass (SMB) and soil respiration (SR), while higher rates induced transitory increases in SMB and SR up to 60 d after glyphosate application, followed by a reduction in SMB and SR beyond 60 d.

Standard measures of microbial functional diversity in soil include substrate induced respiration (SIR) and soil enzyme activities, both of which are considered appropriate ecotoxicological indicators because of their sensitivity and relatively rapid response to changes caused by natural or anthropogenic disturbance [13]. SIR involves monitoring CO_2 (e.g. Microresp™; [14]) or proton (e.g. Biolog; [15]) evolution from soil or soil slurries after the addition of a range of C substrates, indicating the capacity of the soil to metabolize specific organic inputs [16]. The relatively limited number of studies examining the effects of glyphosate on SIR suggest that glyphosate application at typical commercial application rates (equivalent to $< 10\ mg\ kg^{-1}$ in topsoil [12]; does not affect functional diversity [17,18], but higher glyphosate concentrations cause significant disturbance [18,19].

Although a number of studies have investigated the effects of glyphosate on soil enzyme activity, the diversity of enzymes investigated to date has been limited, thus lowering the ability to directly compare results across studies. For example, glyphosate (applied as Roundup®) at 360 g a.i. ha^{-1} to soil had no significant effects on the activities of phosphatases, catalase, protease and fluorescein diacetate hydrolase at 2 months after application, whereas the same formulation at 3.6 kg a.i. ha^{-1} stimulated catalase activity [20]. In another study, glyphosate at 21.6 kg a.i. ha^{-1} inhibited the activity of dehydrogenase, phosphatases and urease enzymes [21]. In addition to the variation in enzyme activities measured, another potential cause of differences in results across studies is the use of the active ingredient glyphosate versus the application as a commercial herbicide formulation, since these formulations contain additional surfactants, salts and other additives [22,23].

We hypothesized that the observed variability in effects of glyphosate on the activity and function of soil microbial communities across published studies may be due to differences in glyphosate dose, soil properties, glyphosate formulation, or time elapsed between glyphosate application and measurement. To test this hypothesis, we conducted a dose-response incubation using a range of enzyme activities and C substrates in three contrasting agricultural soils. Specifically, we hypothesized that dose thresholds for significant effects would be lower for RoundupCT®, than equivalent doses of glyphosate, due to synergistic effects of various constituents in the formulation.

2. Materials and methods

2.1. Soils and chemicals

Three soils with different physical and chemical characteristics and variation in levels of previous exposure to glyphosate were chosen for this study (Table 1). Soil from the top 200 mm layer was collected from three different agricultural regions of Australia: Wongan Hills, Western Australia; Temora, New South Wales; and Warwick, Queensland, with the soil type from each region classified as Tenosol, Chromosol and Vertosol, respectively [24]. Soils were air-dried and passed through a sieve (< 2.0 mm) prior to use.

Enzyme substrates and standards were purchase from Biosynth AG (Basel, Switzerland). Carbon substrates for MicroRespTM assays were purchase from Sigma Aldrich (Australia) and were of technical grade or higher. Glyphosate (N-(phosphonomethyl)glycine, technical grade, > 98%) was kindly provided by Adama Australia. RoundupCT® (Sinochem, Melbourne) was purchased locally. Chemicals were dissolved in deionized water to obtain appropriate dilutions for soil spiking. Each stock solution of glyphosate/RoundupCT® was made up separately for each soil type. The dose of glyphosate applied as a maximum label application rate (excluding sugarcane ratoon regrowth control) was 2.93 mg glyphosate kg^{-1} soil, based on the application of 2.2 kg glyphosate ha^{-1} to a dry soil of bulk density 1.5 g cm^{-3} with an average depth of herbicide penetration into the soil assumed to be 50 mm for surface herbicide applications [25]. The herbicide dose was standardized between glyphosate and RoundupCT® based on a glyphosate concentration of 450 g dm^{-3} in RoundupCT®. Doses were 0 (control), 1.0, 2.9, 8.8, 26.4 and 79.1 mg glyphosate kg^{-1} soil, equivalent to 0, 0.33, 1, 3, 9 and 27 times the maximum label rate of 2.2 kg glyphosate ha^{-1}.

Table 1
Physicochemical properties residues of the soils used in this study.

Property	Unit	Tenosol	Chromosol	Vertosol
EC	dS/m	0.036	0.34	0.12
pH ($CaCl_2$)	pH units	5	4.7	5.7
pH (Water)	pH units	5.8	5.2	6.7
Sulfur (KCl40)	mg/kg	5.2	30	8.4
Colwell Phosphorus	mg/kg	5.8	150	83
Phosphorus Buffer Index $_{+Col\ P}$	L/kg	15	60	110
Organic Carbon	%	0.24	2.3	1.5
Total Nitrogen	%	0.03	0.25	0.15
Total Carbon	%	0.3	2.8	2
Chloride	mg/kg	8.9	49	7.1
Boron	mg/kg	0.71	1.1	1.4
KCI Extractable Ammonium	mg/kg	< 0.3	31	0.84
KCI Extractable Nitrate	mg/kg	5.9	140	35
Exchangeable Cations				
Aluminium	cmol(+)/kg	0.15	0.15	< 0.1
Calcium	cmol(+)/kg	0.98	6.8	23
Potassium	cmol(+)/kg	0.15	2.1	0.99
Magnesium	cmol(+)/kg	0.33	1.5	17
Sodium	cmol(+)/kg	0.071	0.14	1.5
CEC	cmol(+)/kg	1.7	11	42
Calcium/Magnesium Ratio		3	4.5	1.3
Aluminium Saturation	%	9.2	1.4	< 0.1
Exchangeable Calcium	%	58	64	54
Exchangeable Potassium	%	8.8	20	2.3
Exchangeable Magnesium	%	19	14	40
Exchangeable Sodium	%	4.2	1.4	3.7
DTPA-extractable micronutrients				
Copper	mg/kg	0.11	2.1	1.9
Iron	mg/kg	79	140	33
Manganese	mg/kg	0.76	61	53
Zinc	mg/kg	0.3	4.2	2.5

2.2. Experimental design

Three soil types (described above) were treated with one of two amendments (glyphosate or RoundupCT®) at six doses, replicated four times per treatment combination. Incubations were set up for destructive sampling at 3 and 27 d after the treatment was applied. Treatments were established in two different formats, depending on the analysis to be undertaken.

For SIR assays, approximately 0.30 g of soil was added to each well of large volume (2 cm^3) 96-well plates as per manufacturer's instructions (MicrorespTM, James Hutton Institute, Aberdeen, Scotland). The average mass per well varied between soils due to differences in soil bulk densities and was calculated by weighing the total mass of soil added per plate. For enzyme activity assays, 5.0 g of soil was accurately weighed into individual 50 cm^3 sterile polypropylene centrifuge tubes with screw caps.

After filling both deep-well plates and 50 cm^3 tubes, soils were brought to 40% of maximum water holding capacity (WHC) and incubated for 7 d at 25 °C prior to the establishment of herbicide