Glyphosate impact on C and N microbial functional groups in soybean rhizosphere

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ABSTRACT

The no-tillage system requires a higher use of herbicides to control weeds before sowing, increasing the concern over the effect that such products might have on the environment. However, few studies reporting the influence of glyphosate on soil microorganisms are available in the literature. The aim of this work was to evaluate the effect of two formulations of glyphosate on functional groups of microorganisms in soybean rhizosphere. The experiment was performed in a greenhouse, and the treatments were the isopropylamine salt and the ammonium salt of glyphosate at the maximum and twice the maximum allowed dose. The control treatment had no application. The populations of soil bacteria, fungi, actinomycetes, fluorescent pseudomonas, cellulolytics, amylolytics, proteolytics and free-living N-fixing were determined by plate counts on selective media. Plant root dry weight, shoot dry weight and the number and dry weight of nodules were determined. The application of herbicides had little effect on the populations of microorganisms, as well as on plant growth. Nevertheless, the interactions among some populations of microorganisms and between these and the plant were influenced by the formulation of the glyphosate applied.

Key words: Risk analysis, soil microorganisms, soybean, glyphosate, rhizosphere.

INTRODUCTION

For almost a century, rhizosphere had been defined as the narrow zone of soil subject to the influence of living roots (Hiltner 1904). It is characterised by intense bacterial activity as a result of a leakage or exudation of substances from the root (Cely et al., 2016). The microorganisms that live in this region are important for natural and crop systems, as they play an active role in biogeochemical cycles of nutrients and organic matter transformations (Andrade 2004). Microorganisms can be divided into functional groups, which include all microorganisms participating in a biogeochemical cycle. The most important biogeochemical cycles for plant nutrition are N, C, K and P (Smith et al., 2015).

Although specific for plants, herbicides not only affect the target organisms, but may also alter the ecological balance directly or indirectly (Nye et al., 2014). However, they can be biodegraded or mineralised in the environment, being of great importance for bioremediation of both soil and environment (Singh et al., 2004; Ramos and Yoshioka 2012).

Glyphosate (N-(phosphonomethyl) glycine acid) inhibits the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) enzyme, as well as aromatic amino acid biosynthesis (Powles and Yu 2010; Maeda and Dudareva 2012; Varanasi et al., 2017). Although glyphosate is not applied directly to soil, a significant amount of the product may reach the soil surface during post-emergence applications. The amount of glyphosate available for soil microorganisms also depends on several abiotic factors, such as pH, nutrient, temperature and soil composition and the importance of these factors varies according to the agrochemical utilized (Zabaloy et al., 2011).

Glyphosate is a polar compound, being intensively adsorbed by Fe and Al oxides and clay in soil (Nye et al., 2014). It is predominantly degraded in soil by the microbial flora (Sviridov et al., 2015), but may still influence some non-target soil microbial groups such as *Bradyrhizobium japonicum* (Mertens et al., 2017).

In the groundnut rhizosphere, an increase in doses of herbicides reduced the total population and the phosphate solubilizing bacteria (Abbas et al., 2014). In *in-vitro* experiments, glyphosate reduced the bacterial population and the fungal spore production. However, such effects were not observed when applied to soil in the recommended field rate (Ratcliff et al., 2006).

Some studies evaluated the effect of other herbicides on free-living N fixing bacteria. The population of *Azospirillum brasilense* was inhibited by benzoic acid, but not by phenoxy acid, yet, nitrogen fixation was only inhibited by benzoic acid (Martinez Toledo et al., 1990). In addition, Mekonnen and Dessie (2016) observed that the use of pendimethalin may negatively affect nodulation, reducing the photosynthate allocation to the nodules, or restricting root growth and the number of root sites availabes for infection.

The objective of this work was to evaluate the effect of two formulations of the herbicide glyphosate on microbial functional groups in a soybean rhizosphere at two doses, and measure their influence on plant growth.

MATERIALS AND METHODS

Experimental design

The experiment was conducted under greenhouse conditions (approx. 65 % relative humidity, 27 °C) in 1 L pots containing one soybean plant. The experimental units were completely randomised with five replicates. The treatments were two formulations of glyphosate; G1, (tradename Roundup, conc. a.e. 360 g l^{-1} glyphosate, present as 480 g l^{-1} isopropylamine salt of glyphosate, Monsanto do Brazil Co) 0.96 Kg a.e. ha^{-1} and 1.92 Kg a.e. ha^{-1} (D1 and D2, respectively) and G2 (tradename Roundup w.g., conc. a.e. 760 g kg^{-1} present as 792.5 g kg^{-1} as ammonium salt of glyphosate, Monsanto do Brazil Co) 0.72 Kg ha^{-1} and 1.44 Kg ha^{-1} (D1 and D2, respectively), D1 refers to the maximum allowed field dose and D2 to twice the maximum allowed field dose. Control pots received no herbicide. Six evaluations were made, one before herbicide application (T0) and 1, 7, 28, 45 and 75 days after application (T1 to T5, respectively). All the functional group evaluations of microorganism populations and plant growth were made after herbicide application, except for nodules, which was evaluated when small nodules were present (T3 to T5).

The analysis of variance (ANOVA) and Tukey's significant difference test (HSD) at the 5% significance level were used to compare the mean of each microbial population and plant growth parameter between the control, G1 and G2 at each dose (D1 and D2). The Pearson correlation coefficient (r) at the 5% significance level was performed separately from the control G1 and G2, using the overall mean from the six evaluations (Statistic for Windows Version 5.1).

Substrate and herbicide application

The non-sterile soil used was a Typic Haplorthox, (>70% clay); pH $CaCl_2$ 6.00, containing organic matter 32.12 g dm^{-3}; Al 0.00 $cmol_c$ dm^{-3} (KCl 1 N); N 1.61 g dm^{-3}; Ca 5.00 $cmol_c$ dm^{-3} (KCl 1N); Mg 1.17 $cmol_c$ dm^{-3} (KCl 1N); K 1.43 $cmol_c$ dm^{-3} (Mehlich-I); P 103.06 mg dm^{-3} (Mehlich-I); SO_4 9.56 mg dm^{-3} (CH_3COONH_4); Mn 170.20 mg dm^{-3} (Mehlich-I); Fe 71.30 mg dm^{-3} (Mehlich-I); Cu 33.10 mg dm^{-3} (Mehlich-I); Zn 25.00 mg dm^{-3} (Mehlich-I); B 0.53 mg dm^{-3} (HCl 0.05 N). The soil was mixed with sand in the proportion 1:1 and put in pots. To stabilize the soil, pots were kept for seven days, watered with distilled water so as to maintain moisture close to 90% field capacity. After this period, the first evaluation was made (T0) and the herbicides were applied at the respective doses, by spraying according the manufacturer's recommendation.

Plants

Soybean seeds (var. COODETEC CD-202) were surface sterilised with 1% sodium hypocloride solution for one minute, washed three times with sterile distilled water and four seeds were sown, 3 mm deep per pot at 0.03 m deep, 1 day after herbicide application (T1). Pots were watered from above with distilled water whenever necessary, to maintain the soil close to 90% of field capacity (water holding capacity= 80%). Seedling emergence started at time T2, when they were thinned to one plant per pot. The evaluations made at times T0, T1 and T2 occurred in the absence of plants. In the evaluations T3, T4 and T5, the plants were at growth stage V2, R1 and R6, respectively (Fehr and Caviness 1977). The shoots were cut and roots were washed in tap water and dried. Nodules were removed and counted. Dry weight of roots, shoots and nodules were determined after drying at 70°C for 3 days.

Microbial Functional Groups

The populations of heterotrophic bacteria, saprophytic fungi, actinomycetes and fluorescent pseudomonads were evaluated. We also evaluated the populations of some microbial functional groups that participated in biogeochemical cycles of carbon (cellulolytic, amylolytic and proteolytic) and nitrogen [free-living N fixing bacteria that used malate (MCS) and others that used glucose (GCS) as C source]. All soil samples were collected by core. Bulk soil was collected before herbicide application from the pots (T0). Roots were removed from the pots; soil that remained attached to the roots was considered rhizospheric. The rhizospheric soil was collected, homogenized and a one-gram sample was used to evaluate the colony forming units (CFU) of the populations as described previously (Zuberer 1994). One gram of each soil sample was suspended in 9 mL of sterile saline solution with 0.85% of NaCl and kept at 5 °C. Aliquots (100 μL) of the tenfold serial dilutions (10^{-5}, 10^{-6} and 10^{-7} for heterotrophic bacteria, fluorescent pseudomonad and free-living N-fixing bacteria MCS and GCS and actinomycetes, and 10^{-2}, 10^{-3} and 10^{-4} for amylolytics, cellulolytics, proteolytics and saprophytic fungi) were inoculated and spread on the surface of two Petri dishes with the appropriate medium for each population. The culture media used were: TSA for heterotrophic bacteria, PDA for saprophytic fungi, starch casein for actinomycetes, King's B for fluorescent pseudomonad, media with cellulose for cellulolytics, minimum medium for amylolytics, casein medium for proteolytics, Nfb medium for free-living N-fixing MCS and Burk´s medium for free-living N-fixing GCS. The formulations of culture media used are detailed in Table 1. The Petri dishes were incubated at 28 °C and colonies counted after 3 and 5 days. The colonies considered cellulolytic, amylolytic and proteolytic were those that showed degradation halos on their respective medium.

Table 1. The culture media for enumerating functional group populations of microorganisms from C cycling (cellulolytic, amylolytic and proteolytic media) and N cycling (Nfb, Burk and proteolytic media).

TSA media for heterotrophic bacteria	Casein peptone 17 g, soybean peptone 3 g, glucose 2.5 g, NaCl 5 g, K_2HPO_4 2.5 g, agar 15 g and distilled water 1000 mL, pH= 6.8.
PDA media for saprophytic fungi	Potato Infusion (obtained from 200 g of potato) 4 g, glucose 20 g, agar 15 g and distilled water 1000 mL, pH= 5.6.
Starch casein media for actinomycetes (Kuster and Willians 1964)	Soluble starch 10 g, casein 0.3 g, KNO_3 2 g, NaCl 2 g, K_2HPO_4 2 g, $MgSO_4.7H_2O$ 0.05 g, $CaCO_3$ 0.02 g, $FeSO_4$ 0.01 g, agar 15 g and distilled water 1000 mL, pH = 6.5 to 7.0.
King's B media for *P. fluorescens* (Scher and Baker 1982)	Peptone 20 g, glycerin 10 mL, K_2HPO_4 1.5 g, $MgSO_4.7H_2O$ 1.5 g, agar 15 g and distilled water 1000 mL, pH = 6.5 to 7.0.
Media with cellulose for cellulolytics (Wood 1980)	Carboxymethyl cellulose 5 g, NO_3NH_4 1.0 g, NaCl solution (0.85%) 50 mL, soil extract (v/v) 950mL, agar 15 g, pH = 7.0. Halo develop: Cover the media surface with NaCl 1M solution during 5 minutes; remove it; add red Congo 0.1% solution during 30 minutes, wash with distilled water until degradation halos around colonies are formed and count them.
Minimum media for amylolytics (Pontecorvo et al., 1953)	Soluble starch 10 g, casein 10 g, glucose 1 g, Na_2HPO_4 3 g, $MgSO_4.7H_2O$ 0.1 g, agar 15 g. Halo develop: Cover the media surface with lugol solution, remove excess, count colonies with degradation halos.
Casein media for proteolytic (Wood, 1980 modified by Andrade in our Laboratory),	Casein 10 g, yeast extract 0.1 g,KH_2PO_4 1.5 g, $MgSO_4.7H_2O$ 0.5 g, NaCl solution (0.85%) 50 mL, agar 15 g, soil extract 950 mL (v/v), pH= 6.8. Halo develop: Add 0.1N HCl solution over media surface during 2 minutes, remove, count colonies with degradation halos around colonies.
Nfb media for free-living N-fixing MCS (Döbereiner and Day 1976)	KH_2PO_4 0.4 g, K_2HPO_4 0.1 g, $MgSO_4.7H_2O$ 0.2 g, NaCl 0.1 g, $CaCl_2$ 0.02 g, $FeCl_3$ 0.01 g, $MoO_4Na.2H_2O$ 0.002 g, sodium malate 5 g, bromotimol blue 0.5% 5 mL, agar 15 g, distilled water 1000 mL, pH = 6.8.
Burk´s media for free-living N-fixing GCS (Wilson and Knight 1952)	Solution A: K_2HPO_4 6.4 g, KH_2PO_4 1.6 g, distilled water 1000 mL; Solution B: NaCl 2 g, $MgSO_4.7H_2O$ 2 g, $CaSO_4.2H_2O$ 0.5 g, distilled water 1000mL; Solution C: $NaMoO_4.2H_2O$ 0.01 g, $FeSO_4$ 0.03 g, distilled water 1000 mL; Media composition: Solution A 100 mL, Solution B 100 mL, Solution C 100 mL, glucose 5 g, agar 15 g and distilled water 700 mL, pH = 7.0.

RESULTS AND DISCUSSION

Significant differences were observed after 75 days. The treatments with glyphosate in the two formulations and at the two doses tested did not affect significantly the population of heterotrophic bacteria, actinomycetes, amylolytic, cellulolytic, proteolytic or free-living N fixing MCS and GCS. The two glyphosate formulations tested had little or no effect on the microbial groups quantified. In this experiment, the general absence of an effect, even at twice the maximum allowed dose, may be associated to the strong adsorption of glyphosate by Fe and Al oxides and clay (Nye et al., 2014), all of which were major components of the soil used in this experiment (clay >70%; AlO_3 24%; Fe_2O_3 25%).

Similar results for microorganisms were described by Duke et al. (2012), who observed that the metabolic activity, respiration and the biomass of soil microorganisms could increase when glyphosate was applied in higher doses. The population of saprophytic fungi was reduced by dose D1 of glyphosate G2 but not for G1, whereas the herbicide G1 at both doses used decreased only the population of fluorescent pseudomonads when compared to the control (Table 2). The fact that the differences were small and that the same effect was not observed with the high dose (D2) could indicate that, although significant at the 5% level, this difference may not be real. As fungi are heterotrophic microorganisms, their activity may have increased by glyphosate (Kremer and Means 2009). On the other hand, the population of fluorescent pseudomonad was significantly reduced in relation to the control by the herbicide G1, but not G2 at both doses applied. This reduction may be associated with its formulation, since evidence shows that the availability in soil is the same for both products (Trezzi et al., 2001; Zabaloy et al., 2011).

Table 2. Log CFU of heterotrophic bacteria (HB), saprophytic fungi (SF), Fluorescent Pseudomonads (FP) and cellulolytic community (CL) in the soybean rhizosphere treated with two formulations of glyphosate in two doses after 75 days.

	HB	SF	FP	CL
	Log cfu g dry soil^{-1}			
Control	6.76 a[1]	4.97 a	4.91 a	5.78 a
Glyphosate 1				
D 1	6.99 a	4.98 a	4.55 b	5.84 a
D 2	6.69 a	4.74 ab	4.53 b	5.79 a
Glyphosate 2				
D 1	6.99 a	4.65 b	4.74 ab	5.89 a
D 2	6.66 a	4.79 ab	4.74 ab	5.74 a
ANOVA (p)	0.12	0.03	<0.01	0.68

[1]Values in the column followed by the same letter do not differ significantly at level $p< 0.05$ by Tukey HSD test.

Twice the maximum allowed rate was used as this is the highest concentration of glyphosate likely to

come into contact with soil under normal agricultural operations, when small areas of a field headland are sprayed over twice in the same spray operation.

Plant growth and nodulation were not significantly affected by glyphosate in either formulations or dose when compared to the control (Table 3). As expected, plant growth did not suffer any effect from the two glyphosate formulations applied pre-planting, even at twice the maximum allowed dose. The nodule number and dry weight did not differ between the treatments and the control, irrespective of the dose. Formulations of glyphosate have shown to have different effects, usually affecting negatively or not affecting nodulation (Duke et al., 2012). In contrast, Chagas Junior et al. (2013) observed that the herbicide in sequential application increased nodulation and dry weight of nodules for soybean.

Table 3. Root dry weight (RDW), shoot dry weight (SDW), number of nodules (NN), nodule dry weight (NDW) of soybean treated with two formulations of glyphosate at two doses after 75 days.

	DWR	DWS	NN	DWN
	g	g	--	g
Control	0.83 a	1.96 a	7.73 a	0.14 a
Glyphosate 1				
D 1	0.84 a	2.43 a	10.20 a	0.15 a
D 2	0.86 a	2.17 a	9.93 a	0.15 a
Glyphosate 2				
D 1	0.81 a	2.07 a	10.40 a	0.12 a
D 2	0.76 a	2.03 a	8.67 a	0.12 a
ANOVA (*p*)	0.89	0.79	0.61	0.71

[1]Values in the column followed by the same letter do not differ significantly at level $p < 0.05$ by Tukey HSD test.

Correlations among the microbial populations were determined separately for the control and formulations G1 and G2. The results showed that, each treatment showed different significant correlations between different microbial groups (Tables 4, 5 and 6). The positive correlation among N-fixing GCS with actinomycetes and fluorescent pseudomonad were not influenced by the two herbicides, since they also occurred in the control. Among the correlations common to the control group and G1, we can conclude that no difference was seen between the free-living fixing GCS with saprophytic fungi, actinomycetes and amylolytics, and neither between actinomycetes with saprophyte fungi and fluorescent pseudomonads.

Herbicide application caused distortion in some correlations, which changed from significant in the control groups to non-significant in the treatments with herbicides. The correlations between actinomycetes and amylolytics, cellulolytics and amylolytics and fluorescent pseudomonads and N fixing MCS were observed only in the roots of the control plants. Other correlations that were not significant in the control were established in the presence of the herbicide. This was the case of the correlations

between saprophytic fungi and fluorescent pseudomonad, amylolytics with proteolytics and with MCS N-fixing and between proteolytics and MCS N-fixing.

In the presence of the herbicide G2, the smallest number of significant correlations was observed between the microbial groups evaluated, when compared with those of G1 and the control (Table 6). This would suggest that G2 caused more disturbances in the correlations between the functional populations evaluated.

Table 4. Pearson correlation coefficient (r, *p< 0.05*, n= 25) between microbial groups in the soybean rhizosphere without glyphosate for the times (T1, T2, T3, T4 and T5).

	HB[1]	SF	ACT	FP	Cl	Am	Pr	MCS
SF	0.28							
ACT	0.40	0.67*						
Pf	0.29	0.45	0.57*					
Cl	0.01	-0.18	-0.38	-0.64*				
Am	-0.43	-0.37	-0.71*	-0.43	0.52*			
Pr	-0.39	0.48	0.12	0.35	0.01	0.09		
MCS	-0.20	-0.14	-0.45	-0.13	0.55*	0.30	0.50	
GCS	0.41	0.63*	0.88*	0.64*	0.47	-0.80*	0.19	-0.27

[1]Heterotrophic bacteria (HB), saprophytic fungi (SF), actinomycetes (ACT), Fluorescent pseudomonad (FP), cellulolytics (Cl), amylolytics (Am), proteolytics (Pr), free-living N fixing MCS (MCS), and free-living N fixing GCS (GCS).

Table 5. Pearson correlation coefficient (r, *p< 0.05*, n= 25) between microbial groups in soybean rhizosphere treated with glyphosate 1 D1 for the times (T1, T2, T3, T4 and T5).

	HB[1]	SF	ACT	FP	Cl	Am	Pr	MCS
SF	-0.25							
ACT	-0.07	0.50*						
Pf	-0.23	0.41*	0.56*					
Cl	-0.09	-0.14	0.17	0.24				
Am	0.52*	-0.07	-0.03	0.00	0.07			
Pr	0.44*	-0.11	0.16	0.29	0.11	0.63*		
MCS	0.32	-0.44*	-0.12	0.00	0.27	0.51*	0.62*	
GCS	-0.58*	0.52*	0.65*	0.53*	0.07	-0.39*	-0.22	-0.31

[1]Heterotrophic bacteria (HB), saprophytic fungi (SF), actinomycetes (ACT), Fluorescent pseudomonad (FP), cellulolytics (CP), amylolytics (AP), proteolytics (PP), free-living N-fixing MCS (MCS), and free-living N-fixing GCS (GCS).

The number of significant correlations between plant growth, nodulation parameters and the microbial functional groups also varied between the different treatments (Tables 7, 8 and 9). The correlations between free-living N fixing GCS and amylolytics and plant growth and nodulations did not suffer any herbicide effect, since they were observed in the two treatments and in the control.

The same was observed between saprophytic fungi and root dry weight, and between actinomycetes and nodule number. Herbicide G1 produced significant positive correlations between heterotrophic bacteria and shoot dry weight and nodule number and dry weight. These correlations were the opposite of what was observed in the control. They were also significant, but negative. The correlation found in the control and in the G2 treatment was only between actinomycetes and root dry weight, which remained negative at both doses.

The results of this work are related to the effects of these products in soil, as the soil characteristics are fundamental in the process of herbicide biodegradation, as well as the behaviour of such products in soils (Gimsing et al., 2004).

Table 6. Pearson correlation coefficient (r, *p< 0.05*, n= 25) between microbial groups in the rhizosphere treated with glyphosate 2 D1 for the times (T1, T2, T3, T4 and T5).

	HB[1]	SF	ACT	FP	Cl	Am	Pr	MCS
SF	-0.16							
ACT	-0.46*	0.32						
Pf	-0.23	0.54*	0.35					
Cl	0.03	-0.23	0.19	0.01				
Am	0.05	-0.30	-0.04	-0.46*	0.02			
Pr	0.27	-0.03	0.04	-0.20	0.31	0.49*		
MCS	-0.10	-0.14	0.27	-0.22	0.07	0.63*	0.41*	
GCS	-0.35	0.33	0.74*	0.67*	0.28	-0.30	-0.21	-0.15

[1]Heterotrophic bacteria (HB), saprophytic fungi (SF), actinomycetes (ACT), Fluorescent pseudomonad (FP), cellulolytics (Cl), amylolytics (Am), proteolytics (Pr), free-living N fixing MCS (MCS), and free-living N fixing GCS (GCS).

Table 7. Pearson correlation coefficient (r, *p< 0.05*, n= 15) between plant parameters and microbial functional groups evaluated in the soybean rhizosphere without glyphosate for the times (T3, T4 and T5).

	HB[1]	SF	ACT	FP	Cl	Am	Pr	MCS	GCS
RDW	-0.48	-0.70*	-0.78*	-0.30	0.24	0.79*	-0.08	0.24	-0.88*
SDW	-0.53*	-0.48	-0.68*	-0.01	0.05	0.67*	0.21	0.36	-0.71*
NN	-0.69*	-0.34	-0.71*	-0.19	0.14	0.68*	0.47	0.50	-0.69*
NDW	-0.64*	-0.46	-0.63*	-0.01	0.00	0.65*	0.31	0.37	-0.66*

[1]Heterotrophic bacteria (HB), saprophytic fungi (SF), actinomycetes (ACT), Fluorescent pseudomonad (FP), cellulolytics (Cl), amylolytics (Am), proteolytics (Pr), free-living N fixing MCS (MCS), free-living N fixing GCS (GCS), root dry weight (RDW), shoot dry weight (SDW), number of nodules (NN), and nodule dry weight (NDW).

Table 8. Pearson correlation coefficient (r, *p< 0.05*, n= 15) between plant parameters and microbial functional groups evaluated in the soybean rhizosphere with glyphosate 1 D1 for the times (T3, T4 and T5).

	HB[1]	SF	ACT	FP	Cl	Am	Pr	MCS	GCS
RDW	0.50*	-0.55*	-0.35	-0.23	0.12	0.54*	0.44*	0.76*	-0.56*
SDW	0.49*	-0.44*	-0.18	-0.06	0.20	0.53*	0.46*	0.79*	-0.41*
NN	0.51*	-0.51*	-0.43*	-0.31	0.00	0.46*	0.33	0.60*	-0.58*
NDW	0.50*	-0.52*	-0.30	-0.17	0.14	0.47*	0.44*	0.79*	-0.44*

[1]Heterotrophic bacteria (HB), saprophytic fungi (SF), actinomycetes (ACT), Fluorescent pseudomonad (FP), cellulolytics (CP), amylolytics (AP), proteolytics (PP), free-living N-fixing MCS (MCS), free-living N-fixing GCS (GCS), root dry weight (RDW), shoot dry weight (SDW), number of nodules (NN), and nodule dry weight (NDW).

CONCLUSIONS

The direct effect of the two herbicide formulations, even at twice the maximum allowed dose, was slight on the population size of microbial groups, plant growth and nodulation. However, the effects were more evident in the correlations between microbial populations, and even more so in the correlations between microbial populations and plant parameters. Although, adsorption and bio-mineralisation would have played a fundamental role in these processes. For this reason, further studies should be conducted to identify the effect of glyphosate on microbial populations and activities in soil.

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