Impact of sulfosate on functional groups of microorganisms of the C and N cycles in the soybean rhizosphere

Marcos Antonio Avanzi[1], Leopoldo Sussumu Matsumoto[1], Ulisses Brigatto Albino[1], Janaina Emiliano[1], Gabriel Liuti[1], Matheus Felipe de Lima Andreata[1], Mickely Liuti Dealis[1], Erika Tyemi Goya Niekawa[1], Miguel Octavio Pérez Navarro[1] and Galdino Andrade[1,*]

[1] State University of Londrina, CCB, Department of Microbiology, Laboratory of Microbial Ecology, PO Box 6001, Londrina, PR, 86051-990, Brazil. *Corresponding author, E-mail: andradeg@uel.br.

ABSTRACT

The use of herbicides in Brazil has increased in recent years, along with the concern about the effect these products may have on the environment. The objective of this work was to evaluate the effect of sulfosate herbicide on functional groups of microorganisms of the N and C cycles in soybean rhizosphere. The experiment was carried out in a greenhouse using a completely randomized experimental design with five replicates. The treatments included a control without herbicide application and application of N-phosphonomethylglycine trimethyl sulfonic salt (sulfosate) at doses of 0.96 and 1.92 kg ha^{-1} . The populations of heterotrophic bacteria, saprophytic fungi, actinomycetes, *Pseudomonas fluorescens*, cellulolytic, amylolytic, proteolytic and free-living nitrogen-fixing bacteria were evaluated. The dry mass of root and shoot, nodule number and dry mass were evaluated in the plants. At the 1.92 kg ha^{-1} dose of sulfosate, an inhibitory effect appeared on the populations of fungi and actinomycetes. The sulfosate herbicide did not affect plant growth and nodulation in either doses. Analysis of correlations between microorganism populations and between microorganism populations and plant attributes showed differences between herbicide treatments and control plants.

Key words: herbicide, *Glycine max*, microbial population, nodulation.

INTRODUCTION

The rhizosphere is the region of soil influenced by roots, a microcosm where complex interactions between microorganisms present in both soil and plant occur. The microorganisms that live in this region are important for natural and agricultural systems, actively participating in the biogeochemical cycles of nutrients and organic matter transformations (Andrade 2004).

The rhizosphere microbiome influences plant growth, as well as its resistance to biotic and abiotic stresses, through changes in nutrient absorption and chemical signals exchanged during metabolic processes. The rhizosphere microbial community composition is affected by environmental conditions, soil physical and chemical properties, stage of plant development and plant genotype (Qiao et al., 2017).

All microorganisms that participate directly in some stages of one of the biogeochemical cycles are considered functional groups with nitrogen, carbon, sulfur and phosphorus being considered the most important for plant nutrition (Andrade 2004).

Modern agricultural practices, which include the use of herbicides, pesticides and insecticides, are necessary to meet the growing demand for food in the world, having a high impact on the environment due to its unspecificity or unspecifity in target organisms and their accumulation on ecosystem. Agrochemical accumulation in soil not only decreases microbial diversity, but also affects the production of indole acetic acid, plant growth promoting hormone, phosphate solubilization, processes of nitrogen transformation and inhibits soil enzymes (dehydrogenases, ureas and phosphatases) (Walvekar et al., 2017).

Sulfosate is an herbicide that contain N (phosphonomethyl) glycine acid (glyphosate) as the active ingredient, inhibitor enol-piruvil shikimate phosphate synthase involved in the synthesis of shikimic acid. Glyphosate participates in the biosynthesis of three essential amino acids: phenylalanine, tyrosine and tryptophan, thus interrupting photosynthesis and plant cell metabolism. It is a non-selective broad-spectrum systemic herbicide that can be used to control most of the weeds, annuals and perennials. Due to its adsorption on clay particles, glyphosate remains unaltered for different periods of time (Singh and Singh 2016).

It is now known that more than 32 species of weeds have developed resistance to glyphosate, and the strategies used to overcome these problems is the development of agricultural cultivars resistant to higher doses of this herbicide. Consequently, these measures are increasingly affecting the local biota and microbiota. Initially, glyphosate was considered benign to the environment. However, it should not be assumed that specific chemicals have only one mechanism or mode of action in the ecosystem, since the products and by-products of these chemicals can interact with numerous biochemical processes in cells, tissues and organs of various organisms (Cuhra et al., 2016).

Zobiole et al. (2011) observed that the herbicide glyphosate negatively impacted interactions between rhizosphere microbial groups, in which there was a reduction of fluorescent pseudomonas, Mn-reducing bacteria and plant growth promoting rhizobacteria, and an increase of Fusarium spp. Newman et al. (2016), work in the analyzes of glyphosate treated corn and soybean rhizosphere microbiota showed increased abundance of proteobacteria (fluorescent pseudomonas), decreased acid-bacteria (involved in biogeochemical processes), and actinobacteria.

Some herbicides also influence biological nitrogen fixation. Silva et al. (1998) observed a decrease in cowpea nodulation (*Vigna unguiculata (L.) Walp*) when trifluralin, linuron and imazaquim herbicides were applied at the doses recommended in the initial stages of plant development. Arruda et al. (2001) found that the number and dry mass of soybean nodules decreased with increasing sulfentrazone herbicide dose.

Glyphosate herbicides can be biodegraded or mineralized in the environment, these processes being important for the elimination of pollutants from the soil and the environment (Skipper 1998). Levanon (1993) has shown that fungi and bacteria actively participate in the degradation of malate, alachlor, atrazine and carbofuran pesticides. Soil depth influences herbicide degradation. According to Savin and Amador (1998), norflurazon was more degraded by bacteria in the B horizon than in the A horizon.

To better interpret the positive or negative effects of herbicides on the soil microbial population, this study aimed to evaluate the impact of the sulfosate herbicide on functional groups of microorganisms associated with the C and N cycles in the soybean rhizosphere.

MATERIALS AND METHODS

Experimental draw

The experimental design was completely randomized with 5 replicates. The treatments were composed of salt of N-phosphonomethylglycine trimethyl sulfonic (sulfosate) at the usual dose, 0.96 kg ha^{-1} (D1) and twice the dose, 1.92 kg ha^{-1} (D2) (doses referring to salts in commercial product), maintaining a control without herbicide.

The evaluations were accomplished before herbicide application and 1, 7, 28, 45 and 75 days after applications (T0, T1, T2, T3, T4 and T5, respectively), totalizing 90 pots. The logarithmic data of the number of colony-forming units (CFU) per soil gram of the six evaluations in the three treatments (factorial 3 x 6) were analyzed statistically through analysis of variance (one-way ANOVA) and mean test (Tukey honest significant difference - HSD). The effect of sulfosate on correlations between populations of functional microorganism groups was evaluated using Pearson´s correlation coefficient (r) for the significance level of $p < 0.05$ for each treatment (Statistic for Windows Version 5.1).

Soil and sulfosate application

A red eutroferric latosol (> 70% clay) was used, as well as organic matter 32.12 g dm^{-3}; pH in $CaCl_2$ 6.00, V (%) 72; Al^{+3} 0.00 $cmol_c$ dm-3 (KCl 1 N); total N 1.61 gdm^{-3}; Ca^{+2} 5.00 $cmol_c$ dm^{-3} (KCl 1N); Mg^{+2} 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); $SO4^{-2}$ 9.56 mg dm^{-3} (CH_3COONH_4); Mn 17.20 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 transferred into 1 liter pots (10 cm in diameter).

The pots were left in a resting place for seven days, being irrigated with distilled water when necessary. After this period, the first soil sampling was performed to evaluate the microorganisms (T0). The herbicide was applied to the soil surface using a coastal spray equipment with a cone tip, following the manufacturer's recommendations.

Functional groups of microorganisms

The sulfosate effects on the population sizes of heterotrophic bacteria, saprophytic fungi, actinomycetes and *Pseudomonas fluorescens* were evaluated. In addition, populations of functional microorganisms that participate in the cycles of C (cellulolytic, amylolytic and proteolytic) and of the N [free-living nitrogen fixing bacteria that use malate (MFC) or glucose (GFC) as carbon source] were evaluated. Soybean roots were collected, and the soil adhered intimately to the roots was considered rhizosphereric. This soil was collected, homogenized and used to evaluate the number of colony forming units (CFU) (Zuberer 1994).

Each sample (1 g of fresh soil) was suspended in 9 mL of sterile saline (0.85% NaCl) and maintained at 5 °C. Aliquots (100 µL) of the decimal serial dilutions (10^{-6} for heterotrophic bacteria, *P. fluorescens* and free-living N fixing bacteria MFC, free-living N fixing bacteria GFC and actinomycetes, and 10^{-3} for amylolytic, cellulolytic, proteolytic and saprophytic fungi) were inoculated and scattered over the surface of petri dishes.

The culture media used, described in Frame 1, were: TSA for heterotrophic bacteria; ABD medium for saprophytic fungi; amide casein for actinomycetes; King´s B medium for *P. fluorescens;* medium with cellulose for cellulolytic; minimal medium for amylolytic; medium with casein for proteolytics; Nfb medium for MFC free-living N fixing bacteria, and Burk medium for GFC free-living N fixing bacteria (Matsumoto et al., 2005).

The inoculated plates were incubated at 28 °C and the colonies were counted after the third day, and recounted

on the fifth day of incubation. The log of UFC g^{-1} of dry soil was determined. The colonies that presented degradation halos in their respective culture media were considered cellulolytic, amylolytic and proteolytic.

Frame 1: Culture media for functional groups of microorganism of cycle C (cellulolytic, amylolytic and proteolytic) and of cycle N (Nfb, Burk and proteolytic).

TSA medium for heterotrophic bacteria	Casein peptone 17 g, soybean peptone 3 g, glycose 2,5 g, NaCl 5 g, K_2HPO_4 2,5 g, agar 15 g and distilled water 1000 mL, pH= 6,8.
ABD medium for saprophytic fungi	Potato infusion (from 200 g of potato) 4 g, glycose 20 g, agar 15 g and distilled water 1000 mL, pH= 5,6.
Casein amide medium for actinomycetes (Kuster & Willians, 1960)	Soluble amide 10 g, caseine 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. medium for *P. fluorescens* (Scher & Baker, 1982)	Peptone 20 g, glycerine 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.
Cellulose medium for cellulolytics (Wood, 1980)	Carboxymethyl cellulose 5 g, NO_3NH_4 1 g, solution of NaCl (0,85%) 50 mL, soil extract (v/v) 950 mL, agar 15 g, pH = 7,0. identification: Scatter a solution of NaCl 1M over the agar surface for 5 minutes; eliminate; add Congo red solution 0,1% for 30 minutes, wash with distilled water until halos around the colonies are observed, count halo-forming colonies
Minimum medium for amylolytics (Pontecorvo et al., 1953)	Soluble amide 10 g, caseine 10 g, glyose 1 g, Na_2HPO_4 3 g, $MgSO_4.7H_2O$ 0,1 g, agar 15 g. Identification: Cover medium surface with lugol´s solution, eliminate excess, count halo-forming colonies.
Casein medium for protelytics (Wood, 1980 modified by Andrade in our lab)	Caseine 10 g, yeast extract 0,1 g,KH_2PO_4 1,5 g, $MgSO_4.7H_2O$ 0,5 g, solution of NaCl (0,85%) 50 ml, agar 15 g, soil extract (v/v) 950 mL, pH= 6,8. Cell lysis halo preparation: Add a solution of 0,1N HCl to medium surface for 2 min, eliminate, count halo-forming colonies
Nfb medium for N free-living fíxer bacteria MCS (Döbereiner & 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, bromothymol blue 0,5% 5 mL, agar 15 g, distilled wter 1000 mL, pH = 6,8
Burk medium for N free-living fixer bacteria GCS (Wilson & 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; Medium Composition: Solution A 100 mL, Solution B 100 mL, Solution C 100 mL, glycose 5 g, agar 15 g and distilled water 700 ml, pH = 7,0.

Plants

The experiment was conducted in a greenhouse (65% ± 2 relative humidity, 27 ºC ± 2). *Glycine max* soybean seeds (var. CD-202) were surface disinfested with 1% sodium hypochlorite solution for one minute, washed three times with sterile distilled water and seeded at 0.03 m depth one day after herbicide application (T1). Four seeds were sown per pot. Irrigation was done on the surface with distilled water, whenever necessary, aiming to maintain the soil in the field capacity.

The emergence of the seedling started at time T2, when thinning was performed, staying one plant per pot. The evaluations made at the T0, T1 and T2 times occurred in the absence of plants and in the T3, T4 and T5 evaluations, the plants were in stages V2 (trifolium fully developed in the second node above the unifoliated node), R1 (complete flowering), and R6 (complete seed formation).

RESULTS AND DISCUSSION

The populations of saprophytic fungi and actinomycetes were significantly reduced when the sulfosate dose (D2) was applied twice. However, the populations of heterotrophic bacteria, *P. fluorescens*, cellulolytic and proteolytic did not have a significant influence of the herbicide when compared to those of the control (Table 1).

Table 1. Log UFC number of heterotophic bacteria (BH) (BH), saprophytic fungi (FS), Actinomycetes (Act), *P. fluorescens* (Pf), celulolytics (Cel) and proteolytics (Pro) in soybean rhizosphere treated with two doses (D1 and D2) of sulfosate. Means followed by the same letter in the column do not differ from each other according to Tukey´s test HSD ($p<0.05$). Analysis of variance (one-way ANOVA) (n=30).

	BH	FS	Act	Pf	Cel	Pro
	Log ufc g^{-1} dry soil					
Control	6,76 a	4,97 a	6,26 a	4,91 a	6,26 a	5,50 a
Herbicide						
D 1	6,89 a	4,92 ab	6,20 a	4,67 a	5,71 a	5,38 a
D 2	6,91a	4,70 b	5,99 b	4,73 a	5,85 a	5,40 a
ANOVA (*p*)	0,24	0,03	0,02	0,11	0,08	0,75

Microbial metabolism is a process of energy conversion, and is governed by enzymatic mechanisms, in which reaction intermediates play a vital role in herbicide bioremediation. The microorganisms well known for their ability to degrade glyphosate, include Pseudomonas sp., *Arthrobacter atrocyaneus* and Flavobacterium sp., among others (Singh and Singh 2016). The herbicide produced inhibitory and stimulatory effects on populations of microorganisms, as reported for other herbicides. Johnsen et al. (2001) found that some groups of microorganisms can be suppressed and others stimulated.

The growth of soybean plants was not affected by the application of sulfosate, even at the D2 dose, in the which dry masses of the aerial part and root did not present significant differences, possibly due to the fact that the product is strongly adsorbed to soil colloids, with no residual effect that could influence the plant development (Singh and Singh 2016). Likewise, nodulation was not affected either (Table 2). Among recent research works, Aynalem and Assefa (2017) showed that the herbicide glyphosate does not affect the growth and nodulation of Faba bean (*Vicia faba L.*) inoculated with Rhizobium. Furthermore, the results under *in vitro* tests showed reduction of cell population in different concentrations of glyphosate used.

In the soil without application of sulfosate (control), the population of saprophytic fungi presented a significant positive correlation with those of actinomycetes (r = 0.67) and with those of N GFC fixers (r = 0.63). The population of actinomycetes presented a significantly negative correlation with that of amylolytic microorganisms (r = -0.71) and positive with *P. fluorescens* (r = 0.57) and fixers of N GFC (r = 0.88). *P. fluorescens* was negatively correlated with the cellulolytic population (r = -0.64) and positive with N GFC fixers (r = 0.64). Significant positive correlation was also observed in populations of cellulolytic and amylolytic microorganisms (r = 0.52) and MFC free-living fixers (r = 0.55). On the other hand, the amylolytic population showed a significant negative correlation with free-living fixers GFC (r = -0.80) (Table 3).

Table 2. Root dry mass (MSR), dry mass of the aerial part, (MSPA), number of nodules (NN), nodule dry mass (MSN) of soybean treated with 2 doses of sulfosate. Means followed by the same letter in the column do not differ from each other according to Tukey´s test HSD (p<0,05). Analysis of variance (one-way ANOVA) (n=15).

	MSR	MSPA	NN	MSN
	G	G	--	g
Control	0,82 a	1,96 a	7,73 a	0,65 a
Herbicide				
D 1	0,79 a	2,39 a	10,47 a	0,67 a
D 2	0,71 a	2,13 a	10,10 a	0,53 a
ANOVA (*p*)	0,80	0,80	0,69	0,78

Table 3. Pearson´s correlation coefficient (r) in plant rhizosphere without sulfosate application, among heterophic bacteria (BH), saprophitic fungi (FS), actinomycetes (ACT), *P. fluorescens* (Pf), celulolytics (PC), amilolytics (PA), proteolytics (PP), N fixers using malathion as carbon source (MFC), N fixers using glycose as carbon source(GFC) (n= 30).

	BH	FS	ACT	Pf	PC	PA	PP	MFC
FS	0,28							
ACT	0,40	0,67*						
Pf	0,29	0,45	0,57*					
PC	0,01	-0,18	-0,38	-0,64*				
PA	-0,43	-0,37	-0,71*	-0,43	0,52*			
PP	-0,39	0,48	0,12	0,35	0,01	0,09		
MFC	-0,20	-0,14	-0,45	-0,13	0,55*	0,30	0,50	
GFC	0,41	0,63*	0,88*	0,64*	0,47	-0,80*	0,19	-0,27

Correlations among the functional groups observed in the D1 treatment were different from those observed in the control, except among the populations of actinomycetes and fungi, which also showed a significant positive correlation but with a lower coefficient (r = 0.52). The heterotrophic bacteria had a significant positive correlation with MFC free-living fixers (r = 0.55). *P. fluorescens* showed significant positive correlations with the actinomycetes (r = 0.65), with proteolytics (r = 0.58) and with the free-living fixers GFC (r = 0.81) and negative with the free-living fixers MFC (r = -0.68). In the presence of sulfosate (D1), the significant correlation between amylolytic and cellulolytic was negative (r = -0.67) when compared to the control. On the other hand, the

significant correlation between the amylolytic population was positive with that of proteolytics (r = 0.57) and negative with the free-living fixers MFC (r = -0.66). Within the N cycle, proteolytics had a significant negative correlation with MFC free-living fixers (r = -0.58) and positive with GFC free-living fixers (r = 0.76). The correlation between the two populations of free-living fixers was significant and negative (r = -0.83) (Table 4).

Table 4. Pearson´s correlation coefficient (r) in plant rhizosphere with sulfosate D1, among heterophic bacteria (BH), saprophitic fungi (FS), actinomycetes (ACT), *P. fluorescens* (Pf), celulolytics (PC), amilolytics (PA), proteolytics (PP), N fixers using malathion as carbon source (MFC), N fixers using glycose as carbon source(GFC) (n= 30).

	BH	FS	ACT	Pf	PC	PA	PP	MFC
FS	0,40	1						
ACT	0,09	0,52*	1					
Pf	-0,38	0,17	0,65*	1				
PC	0,14	0,30	-0,14	-0,32	1			
PA	-0,32	-0,18	0,14	0,49	-0,67*	1		
PP	-0,01	-0,08	0,43	0,58*	-0,50	0,57*	1	
MFC	0,55*	0,31	-0,32	-0,68*	0,51	-0,66*	-0,58*	1
GFC	-0,43	-0,17	0,48	0,81*	-0,41	0,45	0,76*	-0,83*

After the second dose of sulfosate was applied (D2), correlations between functional groups were different from those obtained in the control. Comparing the correlations between the D1 with the D2 dose populations, it was observed that only the correlations between the GFC free-living fixers with *P. fluorescens* (r = 0.84) and proteolytics (r = 0.58) maintained the same correlations significant found in the D1 dose. The heterotrophic bacteria correlated significantly, but negatively with saprophytic fungi (r = -0.61); however, it was positively correlated with cellulolytics (r = 0.73). Actinomycetes showed a significant negative correlation with the proteolytic population (r = -0.93). The population of I had a significant positive correlation with amylolytics (r = 0.77) and free-living fixers MFC (r = 0.75) and GFC (r = 0.84). MFC free-living fixers also showed a significant positive correlation with amylolytics (r = 0.58). The proteolytics had a significant positive correlation with free-living fixers GFC (r = 0.58). The correlation between the two populations of free-living fixers was different from that obtained at dose D1; in D2 the correlation was significant and positive (r = 0.61) (Table 5). When comparing the results of significant correlations at dose D1 and D2, it was verified that the MFC free-living fixer group was the only to change from negative to positive with *P. fluorescens*, amylolytic and with GFC free-living fixers.

Most of the significant correlations observed among functional groups of microorganisms did not occur simultaneously in all treatments except for *P. fluorescens*, which showed a positive correlation with N GFC fixers at doses D1 (r = 0.81), D2 (r = 0, 84) and control (r = 0.64). The results observed in the correlations show that, although the herbicide did not cause a significant change in the population size of *P. fluorescens*, it altered the interactions of this group with other functional groups.

The GFC free-living fixators showed positive correlation with proteolytics in both D1 and D2. On the other hand, the interactions between MFC free-living fixers with *P. fluorescens* and amylolytic and between the two free-living fixer populations changed from negative in D1 to positive in D2. These correlations were influenced by the presence of sulfosate, whereas in the control this was not observed.

The actinomycetes population had a greater number of significant correlations in the control than in D1. Only fungi and *P. fluorescens* showed correlation with actinomycetes in both treatments. The correlations between the two populations in the C cycle, cellulolytic and amylolytic, changed from positive, in the control, to negative in D1.

Table 5. Pearson´s correlation coefficient (r) in plant rhizosphere with sulfosate D2, of heterophic bacteria (BH), saprophitic fungi (FS), actinomycetes (ACT), P. fluorescens (Pf), celulolytics (PC), amilolytics (PA), proteolytics (PP), N fixers using malathion as carbon source (MFC), N fixers using glycose as carbon source(GFC) (n= 30).

	BH	FS	ACT	Pf	PC	PA	PP	MFC
FS	-0,61*							
ACT	-0,20	0,35						
Pf	-0,37	0,06	-0,27					
PC	0,73*	-0,24	-0,23	0,06				
PA	-0,47	0,29	0,14	0,77*	-0,02			
PP	0,06	-0,17	-0,93*	0,32	0,18	-0,10		
MFC	-0,20	0,06	0,07	0,75*	0,25	0,58*	0,07	
GFC	-0,26	0,05	-0,49	0,84*	0,17	0,48	0,58*	0,61*

Microbial communities are important components of the soil ecosystem. A functioning agroecosystem is dependent on these microbial communities, having their abundance, activities and biodiversity as good indicators. In the work of Oladele and Ayodele (2017), it was observed that the herbicides glyphosate, paraquat and atrazine caused a decrease in fungal communities and an increase in some genera of bacteria. Johnsen et al. (2001) found that interactions among different populations of soil microorganisms are influenced by herbicide applications and other agrochemicals. These authors have reported that actinomycetes play an important role in the degradation of more complex molecules of C, and the bacteria in N transformation in the composition of these products.

In the absence of the herbicide (control), the heterotrophic bacterial population showed a significant negative correlation with the gorwth of the aerial part of the plant (r = -0.53), number of nodules (r = -0.69) and nodule dry mass (R = -0.64). The saprophytic fungi had a significant negative correlation with root dry mass (r = -0.70). Actinomycetes showed a significant negative correlation with root dry mass (r = -0.78) and aerial part (r = -0.68), number of nodules (r = -0.71) and nodule dry mass (r = -0.63). The amylolytic population showed a significant positive correlation with the dry masses of the root (r = 0.79), aerial part (r = 0.67), number of nodules (r = 0.68) and dry mass of nodules (r = 0.65). The GFC free-living fixers presented a significant negative correlation with the dry root mass (r = -0.88) and aerial part (r = -0.71) and number of nodules (r = -0.69) and dry mass of nodules (r = -0.66) (Table 6).

In the dose of sulfosate (D1), the heterotrophic bacterial population had a significant positive correlation with root dry masses (r = 0.52), aerial part (r = 0.53) and nodules (r = 0.52). These results differ from the control plants, where significant correlations with aerial part dry mass and nodule were negative. The saprophytic fungi showed a significant positive correlation with dry mass aerial part (r = 0.55). *P. fluorescens* showed a significant negative correlation with root dry masses (r = -0.76) and aerial part (r = -0.59), number of nodules (r = -0.73) and nodule dry mass (r = -0.64). The proteolytic had a significant negative correlation with root dry masses (r = -0.69), aerial part (r = -0.59), number of nodules (r = -0.65) and nodule dry mass (R = -0.59). The MFC free-living fixers showed a significant positive correlation with root dry masses (r = 0.87) and aerial part (r = 0.87), number of nodules (r = 0.77) and nodule dry mass (R = 0.85). GFC free-living fixers presented a significant negative correlation with root dry masses (r = -0.93) and aerial part (r = -0.83), number of nodules (r = -0.82) and nodule dry mass (r = -0.84) (Table 7).

At twice the dose of sulfosate (D2), significant correlations were observed for actinomycetes, being positive with root dry masses (r = 0.92) and aerial part (r = 0.91) and number (r = 0, 85) and nodule dry mass (r = 0.93). The proteolytics had a negative correlation with the root dry masses (r = -0.94) and aerial part (r = -0.91), number of nodules (r = -0.89) and nodule dry mass (r = -0.95). (Table 8). Comparing the significant correlations between control and dose D2, it was observed that all correlations between actinomycetes and plant growth moved from negative to positive.

In both treatments and control, no similar correlations were observed between functional groups of microorganisms with plant growth and nodulation. Between the control and D1, some common correlations were observed, such as those between the GFC free-living fixers that had negative correlation with dry mass of the aerial part, root, nodule and number of nodules, not being observed in dose D2. Yet, correlations between N fixing bacteria MFC and plant growth were significant and positive only in D1.

Some negative correlations between heterotrophic bacteria and mass dry of the aerial part and nodule dry mass were observed in the control, but they were positive in D1 treatment. At this dose, a very large effect on the interaction between amylolytic and plant growth was present. In the control, the correlations were significant and positive, whereas in the dose D1, despite being not significant, they were negative. Among proteolytics and plant growth, the correlations changed from not significant in the control to significant and negative in the D1 dose. These effects should be related to the presence of sulfosate and its C: N ratio, which has a direct influence on the proteolytics that are mostly actinomycetes, organisms involved in herbicide degradation in the soil (Johnsen et al., 2001).

No equal correlations between control and D2 were observed. Correlations between actinomycetes and plant growth and nodulation were negative in the control and positive in D2 treatment. The interactions between proteolytics and plant growth were also influenced by dose D2, which changed from non-significant in control to significant and negative in D2. The same was observed at dose D1.

Table 6. Pearson´s correlation coefficient (r) in plant rhizosphere without sulfosate application among heterotrophic bacteria (BH), saprophitic fungi (FS), actinomycetes (ACT), *P. fluorescens* (Pf), celulolytics (PC), amilolytics (PA), proteolytics (PP), N fixers using malathion as carbon source, (MFC), N fixers using glycose as carbon source (GFC), root dry mass (MSR), dry mass of the aerial part (MSPA), number of nodules (NN) and nodule dry mass (MSN) (n= 15).

	BH	FS	ACT	Pf	PC	PA	PP	MFC	GFC
MSR	-0,48	-0,70*	-0,78*	-0,30	0,24	0,79*	-0,08	0,24	-0,88*
MSPA	-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*
MSN	-0,64*	-0,46	-0,63*	-0,01	0,00	0,65*	0,31	0,37	-0,66*

Table 7. Pearson´s correlation coefficient (r) in pant rhizosphere with D1, among heterotrophic bacteria (BH), saprophytic fungi (FS), actinomycetes (ACT), *P. fluorescens* (Pf), celulolytics (PC), amilolytics (PA), proteolytics (PP), N fixers using malathion as carbon source, (MFC), N fixers using glycose as carbon source (GFC), root dry mass (MSR), dry mass of the aerial part (MSPA), number of nodules (NN) and nodule dry mass (MSN) (n= 15).

	BH	FS	ACT	Pf	PC	PA	PP	MFC	GFC
MSR	0,52*	0,33	-0,45	-0,76*	0,48	-0,50	-0,69*	0,87*	-0,93*
MSPA	0,53*	0,55*	-0,21	-0,59*	0,47	-0,50	-0,59*	0,87*	-0,83*
NN	0,42	0,23	-0,50	-0,73*	0,45	-0,49	-0,65*	0,77*	-0,82*
MSN	0,52*	0,45	-0,32	-0,64*	0,49	-0,49	-0,59*	0,85*	-0,84*

Table 8. Pearson´s correlation coefficient (r) in plant rhizosphere (r) with sulfosate D2, among heterotrophic bacteria (BH), saprophytic fungi (FS), actinomycetes (ACT), *P. fluorescens* (Pf), celulolytics (PC), amilolyitics (PA), proteolytics (PP), N fixers using malathion as carbon source (MFC), N fixers using glycose as carbon source (GFC), root dry mass (MSR), dry mass of the aerial part (MSPA), number of nodules (NN) and nodule dry mass (MSN) (n= 30).

	BH	FS	ACT	Pf	PC	PA	PP	MFC	GFC
MSR	-0,23	0,22	0,92*	-0,23	-0,30	0,15	-0,94*	0,03	-0,42
MSPA	-0,17	0,25	0,91*	-0,11	-0,20	0,32	-0,91*	0,13	-0,33
NN	-0,38	0,22	0,85*	-0,05	-0,36	0,26	-0,89*	0,14	-0,35
MSN	-0,19	0,22	0,93*	-0,10	-0,18	0,33	-0,95*	0,19	-0,38

CONCLUSIONS

The effect of the sulfosate herbicide on groups of microorganisms, plant growth and nodulation was small, but evident at dose D2. However, correlations between functional groups of microorganisms and between those and plant growth were altered by the herbicide. Reduction of saprophytic fungi populations and actinomycetes did not influence plant growth.

This work demonstrates that the sulfosate herbicide affects interactions between microbial populations in the soybean rhizosphere, presenting possible toxicity for some populations. However, it is believed that this herbicide biodegradation occurs by some groups of microorganisms or even the adsorption of these molecules on clay soil, influencing the results obtained.

ACKNOWLEDGEMENTS

The authors are grateful to CNPq, PIBIQ and CAPES for their financial support.

REFERENCES

Andrade G (2004) Role of functional groups of microorganisms on the rhizosphere microcosm dynamics. Varma A, Abbott L, Werner D and Hampp R (eds.) Plant surface microbiology. Springer-Verlag, Berlim, p. 51-69.

Arruda JS, Lopes NF and Bacarin MA (2001) Nodulação e fixação do dinitrogênio em soja tratada com sulfentrazone. Pesquisa Agropecuária Brasileira 36: 325-330.

Aynalem B and Assefa F (2017) Effect of glyphosate and mancozeb on the rhizobia isolated from nodules of Vicia faba L. and on their N_2 fixation, north Showa, Amhara Regional State, Ethiopia. Advances in Biology 2017:7

Cuhra M, Bohn T and Cuhra P (2016) Glyphosate: too much of a good thing?. Frontiers in Environmental Science 4: 28.

Johnsen K, Jacobsen CS and Torsvik V (2001) Pesticide effects on bacterial diversity in agricultural soils – a review. Biology and Fertility of Soils 33: 443-453.

Levanon D (1993) Roles of fungi and bacteria in the mineralization of the pesticides atrazine, alachlor, malathion and carbofuran in soil. Soil Biology and Biochemistry 25: 1097-1105.

Matsumoto LS, Martines AM, Avanzi MA, Albino UB, Brasil CB, Saridakis DP, Rampazo LGL, Zangaro W and Andrade G (2005) Interactions among functional groups in the cycling of, carbon, nitrogen and phosphorus in the rhizosphere of three successional species of tropical woody trees. Applied Soil Ecology 28: 57-65.

Newman MM, Hoilett N, Lorenz N, Dick RP, Liles MR, Ramsier C, Kloepper JW (2016) Gyphosate effects on soil rhizosphere-associated bacterial communities. Science of The Total Environment 543:155-160.

Oladele S and Ayodele O (2017) Glyphosate, 1,1'- dimethyl-4,4'-bipyridinium dichloride and Atrazine induces changes in Soil organic carbon, bacterial and fungal communities in a tropical alfisol. Eurasian Journal of Soil Science 6: 238-248.

Qiao Q, Wang F, Zhang J, Chen Y, Zhang C, Liu G, Zhang H, Ma C and Zhang J (2017) The variation in the rhizosphere microbiome of cotton with soil type, genotype and developmental stage. Scientific Reports 7: 3940.

Savin MC and Amador JA (1998) Biodegradation of norflurazon in a bog soil. Soil Biology and Biochemistry 30: 275-284.

Silva JCC, Colaço W, Burity HÁ, Ferreira NCM, Figueiredo MVB and Martinez CR (1998) Herbicidas na nodulação e na fixação de N_2 em caupi. Pesquisa Agropecuária Brasileira 33: 77-86.

Singh B and Singh K (2016) Microbial degradation of herbicides. Critical Reviews in Microbiology 42: 245-261.

Skipper HD (1998) Bioremediation of Contaminated Soils. Sylvia DM, Fuhrmann JJ, Hartel PG and Zuberer DA (eds.) Principles and applications of soil microbiology. Prentice Hall, New Jersey, p. 469-481.

Walvekar VA, Bajaj S, Singh DK and Sharma S (2017) Ecotoxicological assessment of pesticides and their combination on rhizospheric microbial community structure and function of Vigna radiate. Environmental Science and Pollution Research International 24:17175-17186.

Zobiole L, Kremer R, Oliveira R and Constantin J (2011) Glyphosate affects micro-organisms in rhizospheres of glyphosate-resistant soybeans. Journal of applied microbiology 110:118-127.

Zuberer DA (1994) Recovery and enumeration of viable bacteria. *In:* Weaver RW, Angle JS and Bottomley PS (eds.) Methods of Soil Analysis. Part 2. Microbiological and biochemical properties. American Society of Agronomy, Madison, p. 119-144.

Received: July 14, 2017.
Accepted: October 24, 2017.
Published: February 12, 2018.