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IMPACT OF GLYPHOSATE ON MICROBIAL ATTRIBUTES OF SOIL PLANTED WITH TWO SPECIES OF PASSION FRUIT[1]

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ABSTRACT - Glyphosate is one of best known agrochemicals and is used to prevent the spread of weeds. However, little is known about the impact of this chemical on non-target organisms such as the soil microbial community. Therefore, the objective of this study was to evaluate the effect of glyphosate on the microorganism population and the microbial attributes of soils cultivated with yellow and sweet passion fruits. The experimental design used was complete randomized blocks in a 3 x 2 factorial scheme with the times of soil sample collection (0, 5 and 47 days after herbicide application- DAH) and the two species of passion fruit yellow (*Passiflorae dulis* f. flavicarpa O. Deg.) and sweet (*Passiflora alata* Dryand) as the factors,with three replications. No impact of the glyphosate herbicide was foundon the bacterial communities of soil. However, a mild and transitory impact was observedon the fungal populations, encouraging these populationsat 47 DHA. Glyphosate changed the carbon microbial biomass and soil microbial attributes, except for total organic carbon. Multivariate, principal component analysis revealed that the total bacteria, endospore-forming bacteria, total fungi, carbon microbial biomass and metabolic quotient attributes of soil are the most sensitive factors for predicting the impact of glyphosate on biological indicators of soil planted with two species of passion fruit yellow (*P. edulis* f. *Flavicarpa*) and sweet (*P. alata*).

Key-words: *Passiflora alata*. *Passiflora edulis*.Carbon microbial biomass.Soil respiration.Endospore-forming bacteria.

IMPACTO DO GLIFOSATO SOB OS ATRIBUTOS MICROBIANOS DE SOLOS PLANTADOS COM DUAS ESPÉCIES DE MARACUJÁ

RESUMO - Glifosato é um dos agroquímicos mais conhecidos e é utilizado para prevenir a propagação de ervas daninhas. No entanto, pouco se sabe sobre o impacto desse químico sobre organismos não-alvos, tais como a comunidade microbiana do solo. Portanto, o objetivo deste estudo foi avaliar o efeito de glifosato sobre a população de micro-organismos e atributos microbianos de solos cultivados com maracujazeiro amarelo e doce. O delineamento experimental foi em blocos casualizados em esquema fatorial de 3 x 2, sendo o primeiro fator os tempos de coleta de amostras de solo (0, 5 e 47 dias após a aplicação do herbicida-DAH) e o segundo, as duas espécies de maracujá amarelo ("*Passiflora edulis* f. Flavicarpa O. Deg.") e doce (*Passiflora alata* Dryand) com três repetições. Nenhum impacto do herbicida foi encontrado sobre as comunidades bacterianas dos solos. No entanto, um leve e transitório impacto foi observado nas populações de fungos, incentivando esta população aos 47 DAH. O glifosato mudou o carbono da biomassa microbiana e atributos microbianos do solo, exceto para o carbono orgânico total. A análise multivariada de componentes principais revelou que as bactérias totais, bactérias formadoras de endósporos, fungos totais, carbono da biomassa microbiana e quociente metabólico do solo são os fatores mais sensíveis para prever o impacto do glifosato sobre a população e atividade microbiana de solos plantados com maracujá amarelo(*P.edulis* f. *Flavicarpa*) e doce (*P. alata*).

Key-words: *Passiflora alata*. *Passiflora edulis*.Carbono da biomassa microbiana. Respiração do solo. Bactérias formadoras de endósporos.

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INTRODUCTION

The use of herbicides is an essential practice becauseno more efficient form of management has been found to yieldshort-term results. The broad-spectrum control of several weed species at any stage of development and the lack of waste makes the herbicide glyphosate (Roundup®) the main herbicide used in agriculture.

Glyphosate is an organophosphorated, non-selective post-emergent that inhibits the action of the enzyme 5-enol-pyruvylshiquimate-3-phosphate synthase (XU et al., 2011), resulted in a reduction inthe quantity of the aromatic amino acids essential for the growth and survival of weeds.

However, there is growing interest about the impact of herbicides on non-target organisms (SEBIOMO; OGUNDERO; BANKOLE, 2011) such as the soil microbial community. Because these communities are highly sensitive to environmental changes, the introduction of pesticides may reduce the functions that these communities play in soil-organic matter dynamics, nutrient cycling, decomposition processes (ACOSTA-MARTINÉZ et al., 2008).

The above effects are very specific to the groups of microorganisms and herbicides used, which may cause either beneficial or harmful effects to specific community groups. In Mississippi soils Weaver et al. (2007) found that this chemical has transient effects on and low toxicity to the microbial community, even when applied at a concentration greater than indicated. In Brazil Araújo; Monteiro and Abarkeli (2003) found an increase in microbial activity through basal respiration after glyphosate. The effect of herbicides on the microbiological quality of soil has been studied in the management of various crops, especially in soybeans (MALTY; SIQUEIRA; MOREIRA, 2006). Despite the importance of glyphosate for weed management, few studies show the impact of pesticides on the biological quality and the microorganism populations of soil from fruit.

In passion fruit cultivation, despite the lack of records in Brazil, the active ingredients most commonly used for weed control are diuron, oxyfluorfen and alachlor and post-emergence glyphosate and paraquat. However, no studies have been conducted on the impacts of these herbicides on the population and microbial activity in soils cultivated with passion fruit. The objective of this work was to evaluate the effect of glyphosate on the microorganism populations, microbial biomass and microbial attributes in soils cultivated with two passion fruit species.

MATERIALS AND METHODS

The experiment was conducted in an experimental area in the Garanhuns, Pernambuco State, located at8°33'25" south latitude and 36°29'34" westlongitude and at approximately 842 m of altitude. The region's climate, according to Köppen classification, is the CSA type "Temperate Mediterranean" mesothermal with a hot dry summer. The average temperature is 20°C, dropping as low as 15.4°C, and the average accumulated rainfall is 1333 mm annually. The experimental area has approximately 242 m^2 of land usable for agricultural activities.

In the experimental area one composite soil-sample, classified as Argissolo Vermelho-Amarelo (EMBRAPA, 2006) was collected at 0-30 cm depth, representative of the area covered and set aside to dry. After drying, this sample was passed through a sieve of 2 mm mesh for chemical characterization in accordance with the methodology recommended by EMBRAPA (2009). The pH was determined in water at the ratio 1:2.5 soil: solution, the available P was determined by colorimetry after extraction with $Mehlich^{-1}$, the exchangeable K was determined by flame photometry after extraction with $Mehlich^{-1}$, and the exchangeable Ca, Mg and Al were extracted with 1 mol L^{-1}KCl and determined by titrimetry (Table 1).

Table 1. Chemical analysis of soil collected before the experimental for the evaluation of the effect of glyphosate on the microbial attributes of soils cultivated with two species of passion fruit.

	pH[1]	P[2]	K[2]	Ca[3]	Mg[3]	Al[3]	OM
	(H_2O 1:2,5)		----------cmolcdm^{-3}----------				(%)
Solo	5,95	0,28	0,10	2,19	1,54	0,05	1,41

[1]water extractor (1:2.5) (EMBRAPA, 2009), [2]Mehlich^{-1}extractor (EMBRAPA, 2009); [3]Extrator KCL molL^{-1} (EMBRAPA, 2009); OM – organic matter (muffle method).

The types of passion fruit used in the experiment were known as yellow (*Passiflora edulis* f. *flavicarpa* O. Deg.) and sweet (*Passiflora alata* Dry) and passion fruit. The seedlings were obtained from seeds of the hybrid IAC- Instituto Agronômico de Campinas 277 provided by the InstitutoAgronômico of Pernambuco (IPA). The seedlings were transported to the field in October after the emergence of the first tendrils. The seedlings were planted in pits with the dimensions 40 x 40 x 40 cm. After planting the side shoots were pruned weekly, leaving only a single stem until the stem reached 1.7 m in height to start driving the lateral cords. The system used was a driving system with vertical cordon wire strands of smooth wire, secured with 2 m high stakes, and with plant spacing of 2 m and 3 m between rows.

Fertilization was performed manually with the application of the fertilizer near the local irrigation because this soil frequently remains moist. The fertilization for training and production, using nitrogen and potassium, followed the recommendation for an expected yield of more than 35 t $ha^{-1}$and was applied by means of topdressing.

The experimental design used three replicates of randomized blocks in a 3 x 2 factorial scheme, with the three sampling times (0, 5 and 47 days after herbicide application- DAH) as the first factor and the two species of passion fruit (sweet and yellow) as the second factor. The plots consisted of two plants each.

Drip irrigation was used inserted on the line. The irrigation time was defined by the voltage of the soil water, measured by a tensiometer placed 0.15 m distant from the plant and at 0.20 m depth. The plants were irrigated whenever the voltage measured at a 0.20 depth reached the closest possible value to 40 kPa. The herbicide was applied using a CO_2 knapsack sprayer coupled to a bar. The recommended dose was applied.

We collected soil samples at 0-10 cm depth at different locations in the rhizosphere of the plants in the usable area of the plots. Eight individual samples were collected that constituted a composite sample of each plot. Immediately after collection, the samples were refrigerated and transported to the laboratory. These samples were subjected to partial drying in air and sieved using a 2 mm shaded mesh.

The populations of total bacteria (TB), endospore-forming bacteria (EFB), total fungi (TF) *Trichoderma* spp. (TRI), carbon microbial biomass (CMB),basal respiration (BRS); total organic carbon (TOC); microbial quotient (qMIC) and metabolic quotient (qCO_2) of the soil were evaluated.

To detect the microorganism populations in the soil, the samples from each treatment were weighed in triplicate,10g of dry soil was placed in 250 mL flasks with 90 mL of sterile distilled water (SDW) and homogenized in a shaker at 250 rpm for 30 minutes. Next, one mL of this suspension and nine mL of SDW were transferred to a test tube, generating a serial dilutions (JONHSON; CURL, 1972). The aliquots of 0.1 mL were plated in culture medium for each particular microorganism group: nutrient agar to TB, potato dextrose agar to TF, King to EFB and Martin to TRI.

The plates were incubated at 25 °C and with a photoperiod of 12 h. The bacterial populations (TB and EFB) were evaluated after 24 h of incubation, whereas the TF was incubated for 48 h. The populations of TRI were evaluated after 120 h of incubation. In each plate, the colonies were quantified using a colony counter. The numbers of microorganisms were used in the following formula: Population = number of colonies x dilution factor x ten with the latter representing the adjustment factor for plating 1 mL of suspension per plate (JONHSON; CURL, 1972).

The population values given are the average result of the colony numbers measured in all the tested samples and are expressed as colony forming units per gram of soil (CFU g^{-1} soil)

To determine the carbon microbial biomass (CMB), the samples were submitted to an irradiation process according to Mendonça and Matos(2005). For this measurement, we used 0.5 mol K_2SO_4as the extract and placed 80 mL of 0.5 mol K_2SO_4 in 20 g of soil moisture content. The carbon in the K_2SO_4 extracts was determined by colorimetry (TATE; ROSS; FELTHAM, 1988).

The basal respiration of soil (BRS) was determined by quantifying the carbon dioxide (CO_2) released in the process of microbial respiration (CO_2 evolution) using the alkali adsorption method with adjustments for the humidity of the soil samples to 60% of its field capacity (ANDERSON; DOMSCH, 1985). Aliquots of 30 g each were drawn from the soil samples and placed in individual airtight containers, where the CO_2 produced was captured by 0.5 mol L^{-1} NaOH. After 72 hours of incubation, the amount of CO_2 was quantified by titration with 0.25 mol L^{-1}HCl, and the addition of barium chloride solution (0.05 mol L^{-1} $BaCl_2$) to the NaOH solution, using phenolphthalein diluted in 100 mL of ethyl alcohol (95% v / v) as an indicator.

The total organic carbon (TOC) was determined according to Yeomans and Bremner (1988), whose procedure is hot oxidation with potassium dichromate and titration of the remaining dichromate ammonium ferrous sulfate.

The metabolic quotient (qCO_2) was calculated as the ratio between the BRS / CMB (ANDERSON; DOMSCH, 1985) and the microbial quotient (qMIC) was calculated by the CMB / TOC ratio, according to Sparling (1992).

The results of the variables were submitted to ANOVA, and the means were compared by Scott-Knott test at 5% probability. To meet the requirements for homogeneity of variance and normality, root (x+1) transformations were performed for the microorganisms populations, and the averages of the

observed data were used.

Therefore, the task of working with multivariate statistics to interpret the impact of the timing of the application of glyphosate on two species of passion fruit was examined by principal component analysis, yielding a group reduced to two principal components identified in a two-dimensional graph representing the original information.

RESULTS AND DISUSSION

An analysis of variance showed no effect in the interactions with the time of collection and passion fruit species factors or to the isolated factors of the variables BT and EFB, with averages of 4.7 x 10^6 CFU g^{-1} of soil and 1.02 x 10^6 CFU g^{-1} of soil, respectively (Table 2).

Although no difference was found, the mean TB in the soils cultivated with sweet passion fruit presented one order of magnitude higher of CFU g^{-1} of soil than the soils cultivated with yellow passion fruit, indicating that the rhizosphere of the different species can influence the microorganism population density due to the difference in the signals made by the roots as exudates (BAREA et al., 2005).

The densities of TB and EFB in the soils cultivated with two species of passion fruit showed high resilience capacity and power of adaptation of the bacteria in the soils subjected to the action of glyphosate. According to Peixoto (2005), microorganisms are extremely sensitive to the pressures faced by ecosystems, and determining their population levels is advantageous to determining the response time of the microorganisms acting on these pressures (thus interfering in the ecological functions) the resilience of the impacted soil the quality of the plant and especially the sustainability of the ecosystem.

Table 2. Population density of total bacteria, endospore-forming bacteria, total fungi and *Trichoderma* spp. found in the rhizosphere of two species of passion fruit at three times of collection after glyphosate application.

	Times ofcollection (DAH)		
Species	0	5	47
	Total bacteria (x 10^6CFU g^{-1} of soil)		
Sweet	7.0 aA	1.9 aA	6.7 aA
Yellow	3.1 aA	0.5 aA	4.1 aA
	Endospore-forming bacteria (x 10^6CFU g^{-1} of soil)		
Sweet	0.5 aA	0.7 aA	1.3 aA
Yellow	0.2 aA	2.4 aA	0.8 aA
	Total fungi (x10^4CFU g^{-1} of soil)		
Sweet	32.5 aA	3.7 aA	99.3 bA
Yellow	24.0 abA	8.1 bA	65.8 aA
	Trichoderma spp.(x10^4CFU g^{-1} of soil)		
Sweet	6.3 bA	2.0 bA	78.8 aA
Yellow	1.1 aA	0.6 aA	32.0 aB

*Values followed by the same capital letter in the column and minuscule on the line did not differ (Skott-Knott test, $p<0.05$).

The TF variable showed interactions with the time of collection and passion fruit species factors (Table 2) and was the smallest population in the sweet passion fruit soil in the period five DAH application. Other studies have shown that the use of herbicides has little or no effect on fungal populations and may contribute to the growth of these populations (ARAÚJO; MONTEIRO; ABARKELI, 2003) and that the inhibitory or stimulatory effect will depend on the dosage used (CARRILO; HONRUBIA, 2003).

The TF and TRI populations decreased immediately after herbicide application (5 DHA) and later (47 DHA) increased dramatically over the soil levels observed prior to the application (0 DHA) which is due to the degradation of the herbicide molecules. Other factors may have caused this increase, including the use of the herbicide molecules as substrates for the microorganisms; the availability of nutrients such as carbon, nitrogen and phosphorus (MONTEIRO, 2001), and the use of tissues from the weeds killed by the herbicide as a nutrient source for the fungi. In addition, microorganisms killed by the action of the herbicide become available substrates for the survivors or invaders, causing these survivors or invaders to proliferate abundantly without competition.

The population density of TRI showed statistical significance only for the time of collection factor, with the third time point showing a higher population density. This result demonstrates that a toxic effect occurred immediately after the herbicide appli-

cation, but this negative effect was shortlived, agreeing with Weaver et al. (2007) who obtained similar results when assessing the effect of this herbicide in soils from the rhizosphere of soybeans resistant to glyphosate in Mississippi.

The analysis of variance for the microbial variables and biological indicators found in the rhizosphere of the two species of passion fruit at the three times examined after the application of the glyphosate herbicide revealed that the soils of the rhizosphere of the two species of passion fruits how different responses in the CMB of the soil; the CMB of the soil surrounding the sweet passion fruit increased immediately after application (5 DHA), while in the yellow passion fruit, the increase occurred at 47 DHA. This differential response of each species can be attributed to the different degrees of sensitivity of the species to the herbicide, as plants show different responses after intoxication due to the changing pattern of root exudation (KREMER; MEANS; KIM, 2005), which influences the different levels and compositions of the microbial community. These findings are corroborated in this study by the similarity between the behavior of the populations of TF, TRI and CMB.

Increased levels of CMB in the soil after the application of glyphosate were also observed in other studies (HANEY et al., 2000). According to Panettieri et al. (2013) glyphosate application acts as a source of organic carbon to the first 37 days of incubation, providing greater CMB and enzyme activity. After this period, mainly starting from 57 days of incubation, there was a decrease of CMB and enzyme activity. In soils from the rhizosphere of herbicide-resistant soybeans in no tillage straw, the CMB in creased to three times the value at the time of soybean planting (ZILLI et al., 2007).Studies in soils cultivated with vine conventionally management, show that the application of pesticides also reduce microbial density comparing with agro ecological-management (RECH et al., 2013).

The microbial activity demonstrated by the BRS had an effect only in the soils from the sweet passion fruit, which showed a variation between 1.0 to 1.10 mg C-CO_2 Kg^{-1}soil $hour^{-1}$ with higher sensitivity and greater ability of the microorganisms to metabolize the herbicide on the carbon sources in this niche.

Table 3. Microbial biomass and microbial attributes of soil founded in the rhizosphere of two species of passion fruit at three times after application of the herbicide glyphosate.

	Times ofcollection (DAH)		
Species	0	5	47
	Carbon microbial biomass (mg de C-CMB kg^{-1} dry soil)		
Sweet	348,33 aB	394.00 aA	98.33 bB
Yellow	307.00 aA	234.00 bB	481.00 bA
	Total organic carbon (dag Kg^{-1})		
Sweet	1.14 aA	1.45 aA	1.33 aA
Yellow	0.92 aA	1.15 aA	1.26 aA
	Basal respiration of soil (mg C-CO_2 Kg^{-1}soil $hour^{-1}$)		
Sweet	1.10 aA	1.05 aA	1.00 aA
Yellow	0.8 aB	0.8 aB	0.8 aB
	Metabolic quotient		
Sweet	0.0032aA	0.0023aA	0.0105aA
Yellow	0.00099aB	0.0034aB	0.0029aB
	Microbial quotient (%)		
Sweet	2.93 aB	3.6 aA	0.75 bB
Yellow	9.09 Aa	2.04 bA	8.31 aA

*Values followed by the same capital letter in the column and minuscule on the line did not differ (Skott-Knott test, $p<0.05$).

Different concentration of glyphosate (PARTOAZAR; HOODAJI; TAHMOURESPOUR, 2013), different textural classes of soil and organic matter content, are factors that may influence the basal soil respiration. Souza et al. (1996) observed that up to 30 days after the application of glyphosate, little increase could be observed in the microbial activity through the evolution of CO_2 due to adsorption of the herbicide by clay soil, ranging from 0 to 1.5 meq CO_2 $100g^{-1}$ soil, and the two Brazilian soils (Hapludult and Hapludox) ranged up to 0.5 µg C-CO_2 g^{-1}soil (ARAÚJO; MONTEIRO; ABARKELI, 2003), similar to the values found in this experiment.

In Hapludult and Hapludox soils, both from Brazil, an increase in CO_2 on the order of 10-15% was observed when glyphosate was applied (ARAÚJO; MONTEIRO; ABARKELI, 2003). However, in this experiment, the microbial activity in the soils from the rhizosphere of the yellow passion fruit was not altered by the herbicide application, but in the sweet passion fruit, a reduction in activity of 4.54% was observed immediately after the application of glyphosate.

The TOC in the soils of both species increased with the herbicide application, ranging from 0.92 to 1.45 dag kg^{-1}. These low amounts of TOC in the soil may be due to the high rate of decomposition.

We generated principal components (Figure 1) as an auxiliary tool in the distinction of treatments using glyphosate on the population of microorganisms and microbial activity in two species of passion fruit, using the TB, EFB, TF and TRI populations, together with the CMB, BRS, TOC, qCO2 and qMIC. This analysis produced a correlation matrix that showed a correlation between the different variables.

The TB population increased along with increases in the populations of the other microorganisms analyzed as EFB, TF, TRI and the qCO_2, with a correlation coefficient of (0.77; 0.8; 0.85 and 0.74) showing a co-evolution of specific groups of microorganisms in the soil, even after the introduction of the glyphosate herbicide. High and positive correlations can explain the relationship between the microorganisms to maintain the stability of the soil microbial environment (SANTOS et al., 2011).

The high values of the correlations between qCO_2 with all the populations of microorganisms, TOC and MBC with a correlation coefficient of (-0.77 and 0.40) reflect the interaction between the population and the soil microbial activity. The introduction of a xenobiotic in the soil produced a positive correlation between the BRS and TOC; that is, the concentration of carbon in the surface increased, followed by an increased concentration of microorganisms (also verified by the high correlation between TOC and EFB 0.53) and, consequently, an increase in carbon dioxide resulted from application of glyphosate.

The increase in BRS followed by a decrease of CMB (-0.43) and *q*MIC (-0.58) was also found in other studies (SANTOS et al., 2011), showing that all the variables are involved with CMB and revealing the importance of this analysis to determine the activity of the soil. BRS can be used as a sensitive indicator in the evaluation of soil quality, as demonstrated in other studies (MARTINS et al., 2010).

The analysis of all the variables with only the information of the first two principal components does not cause significant loss of information because factor 1 and factor 2 explained 83.29% of the variation in the data obtained. Other studies that evaluate the microbial quality of the soils have been performed using the first two factors (GARCÍA-RUIZ et al., 2008, GARDNER et al., 2011).

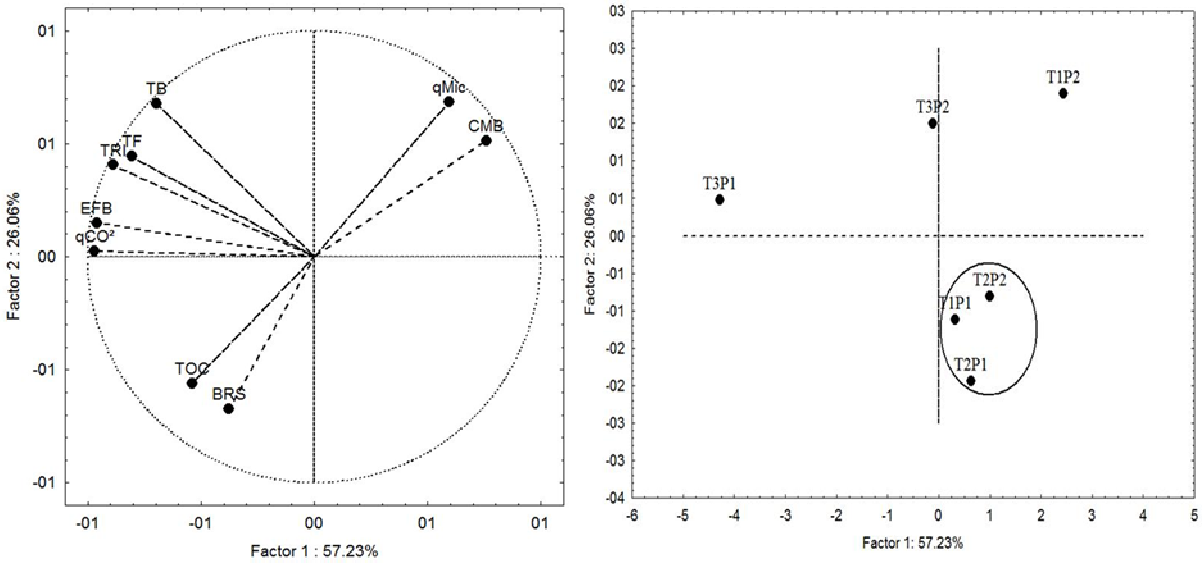
The importance of each variable in each principal component is shown by the value of the modular weight identifying which variables are correlated with each principal component.

The first factor explained 57.23% of the total variation of the attributes studied on the effect of herbicide glyphosate in two species of passion fruit. The variables TB, EFB, TF, TRI, CMB and qCO_2 correlated in factor1, showing that their mean values increased when going from left to right on the graph (Figure 1a). These attributes were the most sensitive in detecting the differences between the treatments. The variables TOC, BRS and *q*MIC correlated with factor 2, indicating that their mean values increase from the bottom to the top of the graph.

Figure 1 is a graph showing factor 1 versus factor 2. This graph easily distinguishes the group of microorganisms (EFB, TF and TRI) and the microbial indicators group (TOC, BRS and *q*MIC) found in the soil of two species of passion fruit. The reason for this can be observed in the weights of the factors. The first factor weights are the largest in EFB (-0.95) and TRI (-0.88), and the higher weights in the second factor are *q*MIC (0.68) and BRS (-0.67).

Through the relationship between these chosen attributes, diagrams were formed to visualize the two-dimensional ordering vector. The first factor explained 57.23% of the total variation of the chosen attributes with the highest correlation coefficients mentioned above, which are the most sensitive factors in distinguishing the different treatments (Figure 1b).

The variance explained by factor 2 was 26.06%, and the TOC, BRS, and qMIC attributes were identified as sensitive, showing a greater distance from the vector with respect to factor 2.



TB = total bacteria, EFB = endospore-forming bacteria, TF= total fungi and TRI = *Trichoderma* spp. CMB=carbon microbial biomass soil, BRS= basal respiration of soil, TOC= total organic carbon, qMIC= microbial quotient and qCO_2 =metabolic quotient.

Figure 1. a) Diagram of the projection vectors of the microbial populations and biological attributes and b) ordination diagram of the principal components of the soils in the rhizosphere of two species of passion fruit at three times after the application of the herbicide glyphosate.

CONCLUSIONS

No impact of the glyphosate herbicide was found on the bacterial communities of soil. However, a mild and transitory impact was observed on the fungal populations.

Glyphosate can change the carbon microbial biomass and soil microbial attributes, except for total organic carbon.

Multivariate, principal component analysis revealed that the total bacteria, endospore-forming bacteria, total fungi, carbon microbial biomass and metabolic quotient attributes of soil are the most sensitive factors for predicting the impact of glyphosate on biological indicators of soil planted with two species of passion fruit.

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