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Microbiological attributes in a latosol in glyphosate application under water deficit conditions

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The objective of this study was to evaluate the effects of glyphosate on soil microbial activity under appropriate and water deficit conditions. The factors consisted of four treatments with different Roundup Ready® soybean cultivars, two herbicidal treatments [glyphosate (1.080 and 1.800 g a.e. ha^{-1}) + control] and two soil moisture treatments (appropriate and water deficit conditions), plus two soybean conventional varieties subject only to the soil moisture treatments. It was evaluated total organic carbon (TOC), basal respiration rate (CO_2), microbial carbon biomass (MCB), metabolic quotient (*q*CO_2), microbial quotient (*q*mic), nodule number (NN), and dry weight of nodules per plant (DWNP). Except for TOC, all other microbiological parameters were significantly influenced by the application of glyphosate, and the effects are more evident at a 1.800 g a.e. ha^{-1} dose in Roundup Ready® soybean cultivars associated to water deficit condition. The soil microbiota stability was favored by the absence of glyphosate application and water deficit condition on soil. MCB and *q*CO_2 showed to be quite sensitive to changes resulting from the use of glyphosate. In general, in conventional cultivars there was lesser effect of water deficit conditions on the microbiological attributes, when compared to treatments with glyphosate application under the same conditions.

Key words: *Glycine max* (L.) Merrill, inhibitors of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs), microbiota, Roundup Ready® soybean seed.

INTRODUCTION

Due to the release and expansion of the cultivated area with genetically modified crops, especially those with Roundup Ready® soybean technology, the intensity of glyphosate use, which was already high, because of its use in desiccation management, has become even higher with the possibility of post-emergence use of crops (Petter et al., 2007).In sensitive plants, glyphosate acts by inhibiting the key enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs), activity in the synthetic process of the aromatic amino acids tryptophan, phenylalanine, and tyrosine. In turn, in crops resistant to this herbicide, plants present a EPSPs enzyme

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insensitive to this molecule, which is not metabolized (Santos et al., 2004), and, therefore, it is released on soil under its original form or under the form of aminomethylphosphonic acid (AMPA) (Monquero et al., 2004).

These two forms under which glyphosate is excreted through the root system can interfere with the microbial activity on soil, affecting the N_2-fixing bacteria associated to the root system of the *Bradyrhizobium* genus soybean, since these microorganisms present enzymatic sensitivity, and thus there can be interference with symbiotic interaction (Santos et al., 2007). Another effect of glyphosate may be associated to a possible effect on the activity of other edaphic microorganisms, such as, for instance, those involved in redox reactions on soil, or even in the enzymatic activity (phosphatases, ureases, and β-glucosidase). Such hypothesis is supported by studies showing that the use of glyphosate changes the soil microbial activity (Andréa et al., 2003; Arantes et al., 2007; Zobiole et al., 2010a).

The possible effect of glyphosate on soil microbial activity may influence the plants growth, and this effect can indirectly manifest itself through lower nutrient absorption by plants. The reduction of nutrient absorption by plants may be associated to decreased activity of N-fixing bacteria, formation of complexes with metal ions (Fe, Cu, Mn and Zn) by excretion of glyphosate through the root system, or also through decrease activity of the microbiota involved in redox reactions, thus affecting the availability of N, Fe, Cu, Zn and Mn.

However, some studies (Santos et al., 2007; Serra et al., 2011) demonstrate the deleterious effect of glyphosate on the microbial activity, especially in N_2-fixing bacteria, there are other ones (Figueiredo et al., 2011) which present controversial data, thus demonstrating the need for further studies aimed at explaining this herbicide behavior in the microbial activity. These effects can manifest themselves in a more intense manner under water deficit conditions, since the availability of ions and the microbial activity in soil are highly influenced by soil moisture, because they involve biological processes (Bekku et al., 2003; Pavinato and Ceretta, 2004). What technicians and producers often observe at field sites is a lower agronomic performance of Roundup Ready® soybean cultivars when compared to conventional soybean cultivars under water deficit conditions (periods without rain), and such an effect may be associated to the glyphosate effect on soil microbial activity.

This way, in addition to the clearly highlighted divergent results with regard to the glyphosate effect on microorganisms, in the Cerrado region (e.g. Cerrado region of Brazilian State of Piaui), there is also the aggravating usual occurrence of periods without rain throughout the crop cycle, something which may favor the deleterious effect of glyphosate.

Therefore, this study aimed to evaluate the possible effects of this herbicide on soil microbial activity and on soybean nodulation under appropriate and water deficit conditions in the Cerrado region of Brazilian State of Piaui.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse at the experimental area in the campus of Federal University of Piaui (UFPI) in the city of Bom Jesus, Brazilian State of Piaui, within the crop season 2011/2012 at 09º04'28" South latitude, 44º21'31" West longitude, and 277 m average altitude.

The climate in the region is Aw, according to the Köppen global climate classification, with two well-defined seasons, that is, a dry season from May to September and a rainy season from October to April. Climatic data were collected at the weather bureau of the National Institute of Meteorology (INMET), about 200 m far from the experimental site, and they are displayed in Figure 1.

The soil used in the experiment was classified as dystrophic yellow latosol. The soil had the following granulometric constitution: 640, 80, and 280 g kg^{-1} of sand, silt, and clay, respectively. The chemical composition of soil in the experimental area is displayed in Table 1.

The experimental design adopted consisted of randomized blocks under a factorial scheme 4 × (3 × 2) + (2 × 2), with the factors consisting of four treatments with different Roundup Ready® soybean varieties (P98Y12, P98Y51, M9144, and M9056), two herbicidal treatments [glyphosate (1.080 and 1.800 g e.a. ha^{-1}) and control] and two soil moisture conditions (adequate moisture and water deficit) forming twenty-four treatments, in addition to two further conventional soybean varieties (M-soy 9350 and UFUS-Milionaria), which were subjected only to soil moisture treatments, totaling twenty-eight treatments with 4 replications. Glyphosate was not applied to the soil in the treatments with conventional cultivars due to the absence of high sorption desorption of glyphosate.

The experiment did not aim to compare the cultivars with each other, since each one has intrinsic characteristics which may interfere with the results. This way, the experiment was analyzed as a double factorial (glyphosate × moisture condition), aiming to verify the glyphosate effect and the soil moisture conditions, as well as the behavior of various cultivars with regard to the treatments.

The seeds were treated with pyraclostrobin + thiophanate methyl + fipronil at a 2 ml p.c. kg^{-1} dose of seed, and inoculated with *Bradyrhizobium japonicum*, at the dose of 3 ml pc kg^{-1} of seed. The fertilization conducted was 2 g of formulaic N-P_2O_5-K_2O 04-24-12 per pot, what is equivalent to 500 kg ha^{-1} of the fertilization.

Sowing was conducted on 20 December, 2011, in pots with an 8 dm^{-3} capacity and at a 2 to 3 cm sowing depth, placing five seeds per pot, with subsequent thinning, leaving only the most vigorous plant.

The application of glyphosate was conducted at 25 days after emergence (DAE) of crop, using a pressurized backpack sprayer with CO_2, connected to the bar with four XR 110.02 spraying nozzles at a constant 2 kgf cm^{-2} pressure, applying a spray volume equivalent to 125 L ha^{-1}. The environmental conditions at the time of treatment application were: 28°C average temperature, 78% relative humidity and wind speed ranging from 3 to 8 km h^{-1}.

Soil moisture control was determined as follows: a) the weight of pots in field capacity (FC) was measured using a methodology adapted from Bonfim-Silva et al. (2011), where the pots were saturated with water, left at rest for 12 h, in order to drain the excess water and weight determined; b) then, with the FC value, the appropriate moisture condition (80% of FC), and the soil water deficit (30% of FC) was calculated. In all treatments the soil was kept with an 80% moisture of FC up to 25 DAE. After this treatment period, the water deficit treatments were applied which lasted until 55 DAE. During this period, the plants were monitored to keep moisture around 30% of FC and avoid they entered a permanent wilting point at the onset of pod formation (R3), soil samples were

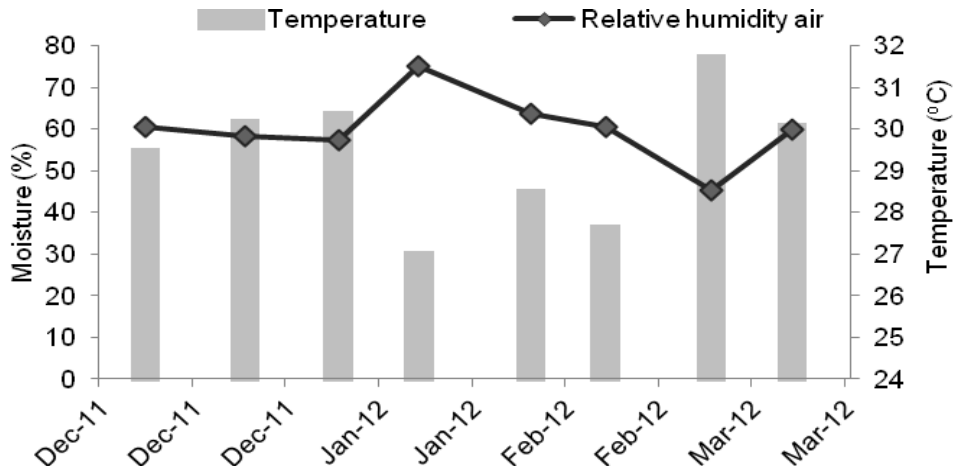

Figure 1. Relative moisture and average air temperature in the city of Bom Jesus. Brazilian State of Piaui (crop season 2011/2012). during the conduction of experiment (data from INMET - Bom Jesus weather bureau).

Table 1. Chemical composition of the soil layer (0-0.20 m) collected from the experimental area prior to the installation of the experiment in the city of Bom Jesus, Brazilian State of Piaui (crop season 2011/2012).

pH $CaCl_2$	P (Mehlich)	K	Ca	Mg	Al	H + Al	O.M.[1] (g dm^{-3})	V[2] (%)	CEC[3]	Fe	B	Mn	Zn	Cu	S
	(mg dm^{-3})		(cmol$_c$ dm^{-3})						(cmol$_c$$dm^{-3}$)	(mg dm^{-3})					
4.6	47.0	74.0	2.1	1.0	1.1	3.1	15	51.49	6.39	88.7	0.24	7.3	2.5	0.2	2.8

[1]O.M.: organic matter; [2]V%: base saturation; [3]CEC: cation-exchange capacity at pH 7.

collected from each pot for microbial evaluations. Subsequently, the soil samples were transported to the laboratory, sieved in a 2 mm sieve, and stored in a refrigerator at 4°C until the evaluation time at the Soils Laboratory of the UFPI.

The basal respiration (CO_2) of soil was evaluated (Alef and Nannipieri, 1995), the microbial carbon biomass (MCB), estimated through the extraction-irradiation method (Mendonça and Matos, 2005) and the soil total organic carbon (TOC) determined through colorimetry according to Quaggio et al. (1987). The microbial quotient was calculated through the soil MCB/TOC ratio (Sparling, 1992), and the metabolic quotient (qCO_2) was determined through the ratio between the soil CO_2 rate per MCB unit (Anderson and Domsch, 1993). For determining the soil CO_2, the samples moisture were adjusted to 70% of FC, then, the evolved CO_2 was determined from 20 g of soil incubated for 72 h with a NaOH 0.05 mol L^{-1} solution and titered with HCl 0.05 mol L^{-1} (Alef and Nannipieri, 1995). The number of nodules per plant (NN) and dry weight of nodules per plant (DWNP) were also determined in the root system

After collecting and tabulating data, the variance analysis was performed, and the averages of significant variables were grouped according to the Tukey test at a 5% significance level ($p > 0.05$), using the statistical software Sisvar 4.1.

RESULTS AND DISCUSSION

Except for TOC and regardless of the Roundup Ready® cultivar evaluated, it was found out that the glyphosate doses significantly influenced the soil microbiological parameters: basal respiration (CO_2), MCB, microbial quotient (*q*mic), metabolic quotient (qCO_2), and total DWNP (Table 2). These results corroborate those obtained by Ratcliff et al. (2006) for CO_2, Haney et al. (2002) for MCB, Bohm et al. (2007) for *q*mic, Santos et al. (2005) for qCO_2, Oliveira Júnior et al. (2008) for NN, and Dvoranen et al. (2008) for DWNP. In turn, for the soil moisture conditions, it was found out a differentiated behavior of cultivars with regard to the evaluated parameters. There was a significant interaction between the factors glyphosate doses and moisture conditions for the parameters CO_2 and *q*mic in the cultivar P98Y12RR, CO_2, qCO_2 in the cultivars P98Y51RR and M9144RR, and MCB and *q*mic in the cultivar M9056RR. In turn, for NN and DWNP there was a significant interaction for all Roundup Ready® cultivars.

The differentiated behavior of the metabolic activity under appropriate moisture and soil water deficit conditions and in the presence of glyphosate in cultivars may be associated to intrinsic characteristics (genetic) of each material, among them tolerance to water deficit and, hence, the maintenance of cellular metabolism at the root system level, something which directly influences on the microbial activity, as this is influenced by root exudates of

Table 2. Variance analysis (F-values) for total organic carbon (TOC), basal respiration (CO_2), microbial biomass carbon, microbial quotient (*q*mic), metabolic quotient (qCO_2), number of nodules per plant (NN), dry weight of nodules per plant (DWNP) in treatments with Roundup Ready® soybean cultivars and conventional soybean cultivars subject to glyphosate application under different soil moisture conditions in the city of Bom Jesus, Brazilian State of Piaui (crop season 2011/2012).

Source variation	**Cultivar P98Y12RR**						
	TOC	**CO_2**	**MBC**	***q*mic**	**qCO_2**	**NN**	**DWNP**
Dose (D)	0.61^{ns}	56.50**	6.67**	5.83*	34.68**	168.33**	51.54**
Condition (C)	0.33^{ns}	2.52*	0.69^{ns}	2.42^{ns}	0.06^{ns}	217.18**	261.35**
D × C	1.23^{ns}	11.47**	2.19^{ns}	5.86*	2.95^{ns}	79.38**	11.86**
CV	15.00	16.84	21.07	17.86	29.38	19.22	23.18
Source variation	Cultivar P98Y51RR						
Dose (D)	0.44^{ns}	69.87**	17.51**	13.13**	0.48**	37.45**	225.04**
Condition (C)	0.14^{ns}	2.68*	21.38**	31.11**	9.16**	112.62**	373.04**
D × C	0.41^{ns}	1.86**	0.61^{ns}	0.03^{ns}	25.68**	6.06*	65.64**
CV	17.79	16.37	19.31	17.14	20.87	22.51	14.34
Source variation	Cultivar M9144RR						
Dose (D)	0.32^{ns}	69.69**	27.16**	8.06**	19.58**	43.78**	74.01**
Condition (C)	0.37^{ns}	11.89**	4.38^{ns}	2.76^{ns}	1.83*	163.92**	399.00**
D × C	2.97^{ns}	4.13**	0.10^{ns}	0.59^{ns}	31.50**	10.03**	10.06**
CV	16.69	14.23	16.68	33.18	22.25	20.39	14.67
Source variation	Cultivar M9056RR						
Dose (D)	0.47^{ns}	12.21**	32.57**	24.25**	4.74**	35.74**	87.29**
Condition (C)	3.74^{ns}	0.96^{ns}	60.94**	36.18**	17.74**	171.77**	403.05**
D × C	0.30^{ns}	0.42^{ns}	6.84**	5.62*	0.10^{ns}	7.21**	22.32**
CV	13.39	15.52	18.55	13.84	29.24	23.63	15.26
Source variation	Cultivar M-Soy 9350***						
Condition (C)	1.44^{ns}	13.37*	0.64^{ns}	1.41^{ns}	16.48*	153.50**	352.33**
CV	14.85	20.12	13.65	24.60	29.80	18.85	4.91
Source variation	Cultivar UFUS-Milionária***						
Condition (C)	0.92^{ns}	19.60*	14.07*	5.00^{ns}	3.98^{ns}	39.24**	244.44**
CV	22.22	10.47	18.17	21.89	10.95	22.57	11.03

and*: significant at 1 and 5%. respectively; ns: non-significant; * conventional soybean not subject to glyphosate application; CV - coefficient of variation.

the crop´s root system. According to Peña et al. (2005), the plant exudates stimulate the microbial activity associated to the rhizosphere, besides being more readily available than plant remains.

Regardless of the Roundup Ready® cultivar, the basal respiration rate (CO_2) was higher in treatments which received the highest glyphosate dose (1.800 g e.a. ha^{-1}) (Figure 2). However, analyzing the application of glyphosate doses under the various soil moisture conditions, the effect of moisture only in the M9144RR cultivar was observed, although there is a tendency to decrease CO_2 in all cultivars. The lowest respiration rates were found in the absence of glyphosate and under the low water content condition on soil (30% of FC). These data are similar to those obtained by Gimsing et al. (2004), who also observed a CO_2 increase due to the application of glyphosate.

These results show that the microorganisms are able to use glyphosate as a carbon source and that soil moisture determines the magnitude of this response. According to Haney et al. (2002), a higher CO_2 release is usual in the presence of glyphosate, a fact which, according to the authors, may be associated to ease of the glyphosate molecule metabolism by soil microorganisms. According to Gimsing et al. (2004), glyphosate mineralization is directly related to the heterotrophic bacteria population.

In turn, differences in CO_2 rates between cultivars are probably due to the metabolism ability of the molecule in the plant, which interfered with the amount of glyphosate excreted through the root system and the way how this

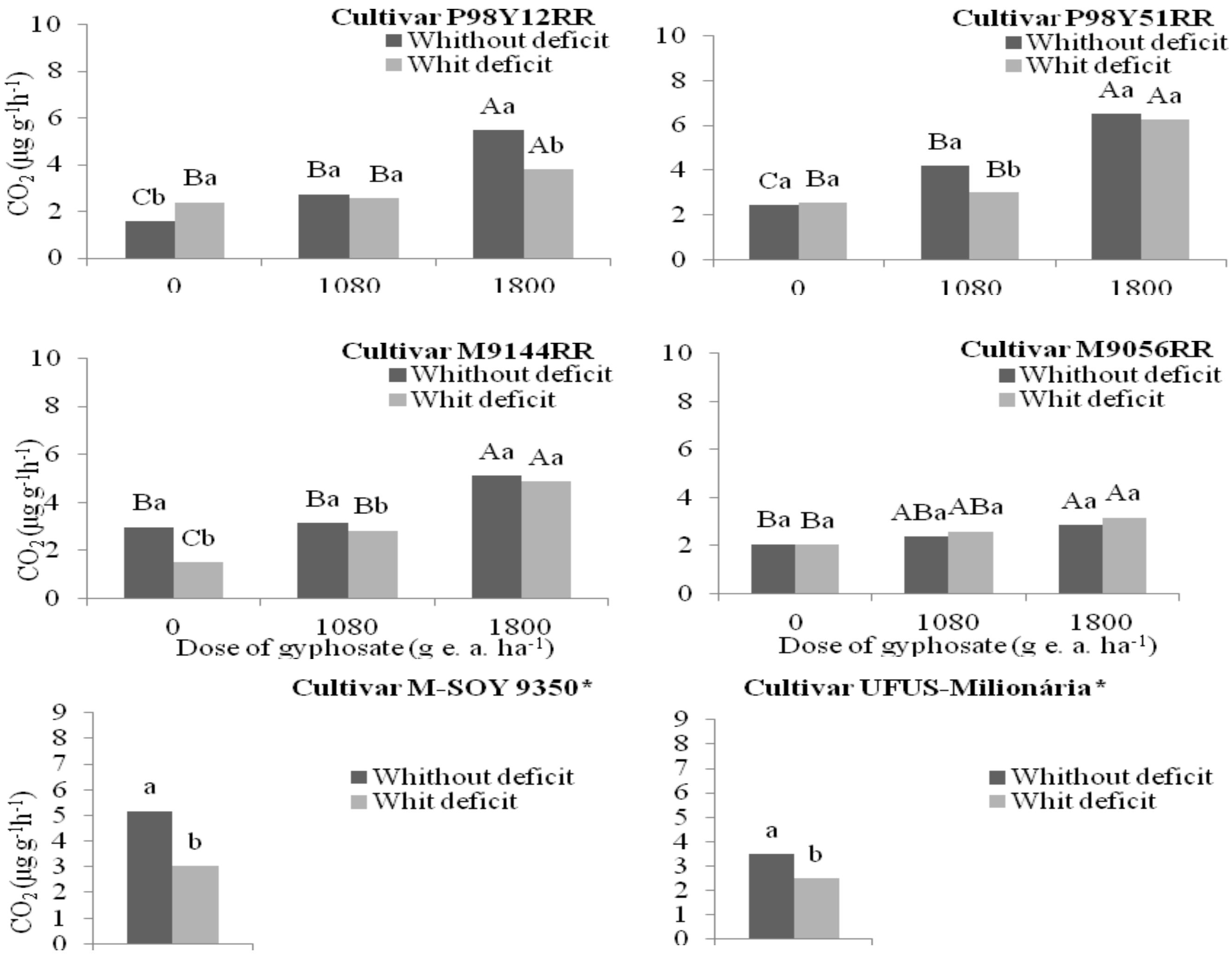

Figure 2. Basal respiration (CO_2 - µg g^{-1} h^{-1}) on soils with Roundup Ready® soybean cultivars subject to glyphosate application under appropriate and water deficit conditions. and conventional soybean cultivars subject only to water conditions the city of Bom Jesus. Brazilian State of Piaui (crop season 2011/2012). Average values followed by the same letters capitalized under the same water condition and lowercase letter at the same dosage do not significantly differ from each other using the Tukey test at 5%. *Conventional soybean cultivars did not receive glyphosate applications.

compound is exudated (AMPA or intact molecule). As reported by Reddy et al. (2004), this feature is highly dependent on the genotype and the edaphic and climatic conditions during cultivation.

The MCB values were significantly decreased due to glyphosate application, indeed, more evident at the 1.800 g e.a. ha^{-1} dose (Figure 3). Under water deficit conditions, the lowest MCB values were observed for the P98Y51RR, M9056RR, and UFUS-Milionaria cultivars. These results corroborate those obtained by Santos et al. (2007) and Bohm et al. (2007), who also found a decrease in MCB with glyphosate application in Roundup Ready® soybean.

The lower MCB values indicate that, despite an increase in microbial activity (CO_2) (Figure 2), there is no MCB increase, that is, the microorganisms incorporate minor carbon amounts to their structures in the presence of glyphosate, especially under water deficit conditions. For Bekku et al. (2003), the soil microbial activity is strongly regulated by soil water content, since it is an indispensable cell protoplasm component, helping to regulate soil gas exchanges, and also to determine the solubility and availability of nutrients.

Active microorganisms, such as bacteria and fungi, are the main organic matter decomposers present on soil and water, and incorporation of chemical compounds in these environments, acts as a nutrient source, especially carbon, nitrogen, and phosphorus (Dallman et al., 2010). However, according to Kremer and Means (2009), and as observed in this study, glyphosate exudation by Roundup

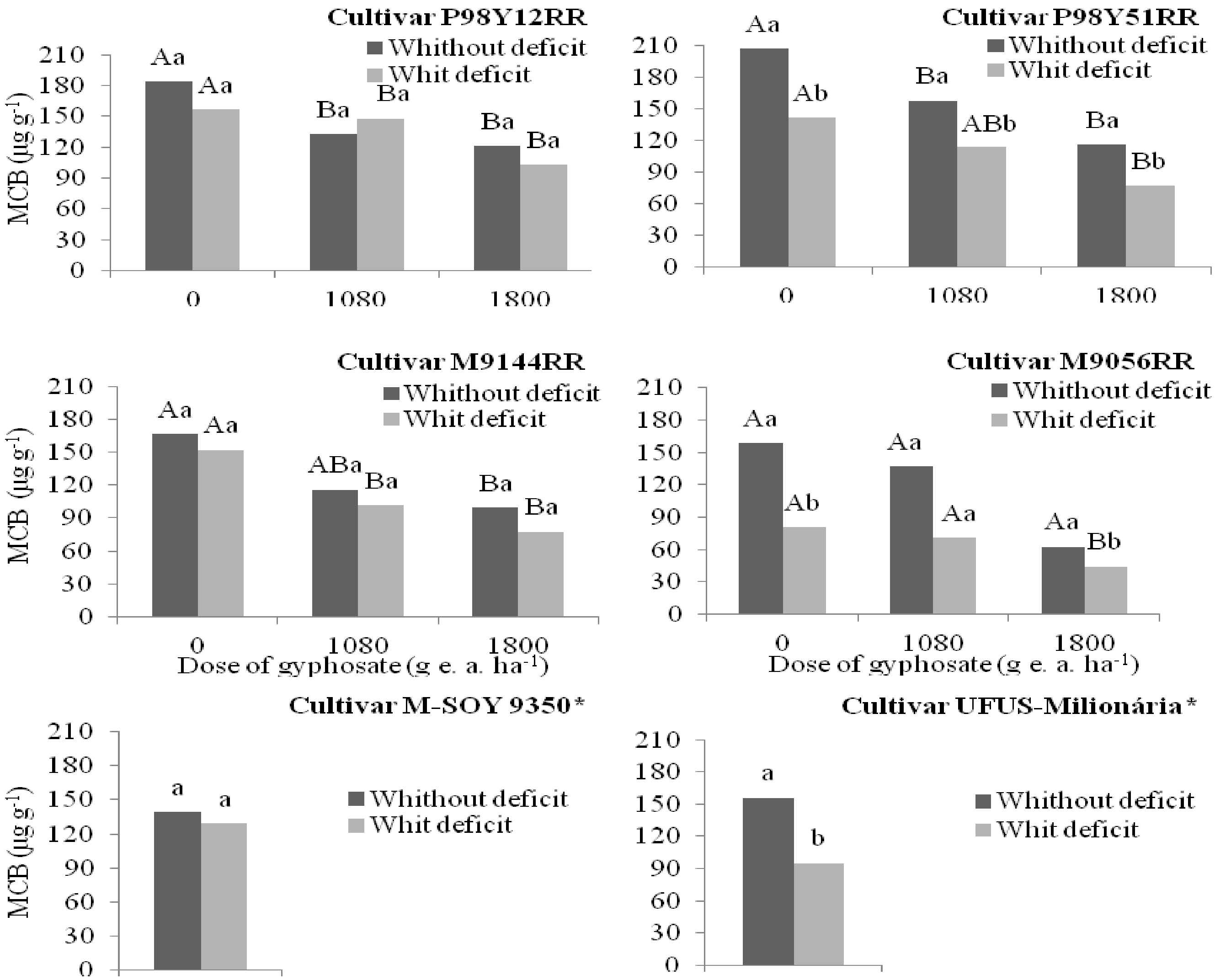

Figure 3. Microbial carbon biomass (MCB) (µg g^{-1}) on soils with Roundup Ready® soybean cultivars subject to glyphosate application under appropriate and water deficit conditions; and conventional soybean cultivars subject only to water conditions the city of Bom Jesus, Brazilian State of Piaui (crop season 2011/2012). Average values followed by the same letters capitalized under the same water condition and lowercase letter at the same dosage do not significantly differ from each other using the Tukey test at 5%. *Conventional soybean cultivars didn't receive glyphosate applications.

Ready® soybean cultivar roots can promote a negative effect on microorganisms associated to the rhizosphere, interfering with the metabolic activity.

It is clearly evident that the microorganisms, in the presence of glyphosate, present higher respiration rates (CO_2), possibly due to the degradation activity of this molecule. However, by degrading glyphosate, the microorganisms decrease the activity in organic matter on soil, which is the organic fraction that would result in incorporation of carbon to the microbial biomass, since the glyphosate molecule does not provide carbon in large amounts. This situation is further enhanced by decrease in the microbial quotient (*q*mic) (Table 3).

The *q*mic is the ratio expressing how much TOC is immobilized on soil in the microbial biomass. In face of this, one observed a lesser effectiveness of microorganisms in TOC immobilization in treatments with glyphosate application (Table 3), especially when associated to lower soil water content (30% of FC). This is possible to verify that the glyphosate effect predominates in this parameter, since by analyzing data in the conventional cultivars one does not observe an effect of water deficit on *q*mic. Therefore, this is possible to highlight that glyphosate potentiates the deleterious effect of water deficit on cultivars which received the application. These results are similar to those found by Liphadzi et al. (2005), who also report decrease in *q*mic due to glyphosate application in the Roundup Ready®

Table 3. Microbial quotient (*q*mic) of soil in treatments with Roundup Ready® soybean cultivars subject to different glyphosate doses under appropriate and water deficit conditions and conventional soybean cultivars subject only to water conditions the city of Bom Jesus. Brazilian State of Piaui (crop season 2011/2012).

Glyphosate	*q*mic (%)					
	P98Y12RR			P98Y51RR		
	Without deficit	With deficit	Average	Without deficit	With deficit	Average
0 g e.a. ha^{-1}	1.05Aa	0.71Ab	0.88A	1.03Aa	0.75Ab	0.89A
1080 g e.a. ha^{-1}	0.71Ba	0.83Aa	0.77AB	0.88ABa	0.61ABb	0.75A
1800 g e.a. ha^{-1}	0.66Ba	0.63Aa	0.65B	0.72Ba	0.41Bb	0.56B
Media	0.81a	0.72a	0.76	0.88a	0.59b	0.73

Glyphosate	M9144RR			M9056RR		
	Without deficit	With deficit	Average	Without deficit	With deficit	Average
0 g e.a. ha^{-1}	1.05Aa	0.79Aa	0.92A	0.82Aa	0.43Ab	0.63A
1080 g e.a. ha^{-1}	0.71Aa	0.53Aa	0.62B	0.67Aa	0.39Ab	0.53A
1800 g e.a. ha^{-1}	0.49Aa	0.47Aa	0.48B	0.32Ba	0.26Aa	0.29B
Media	0.75a	0.59a	0.67	0.61a	0.36b	0.48

Glyphosate	M-Soy 9350*			UFUS-Milionária*		
	Without deficit	With deficit	Average	Without deficit	With deficit	Average
0 g e.a. ha^{-1}	0.80a	0.65a	0.72	0.72a	0.50a	0.61

Average values followed by the same letters capitalized in columns and lowercase in the line do not significantly differ from each other using the Tukey test at 5%. *Conventional soybean cultivars did not receive glyphosate applications.

soybean crop.

The lower *q*mic values are directly related to the decrease in the MCB values, that is, it may also be regarded as indicative of microbial stress, since the incorporation of carbon derived from the glyphosate molecule in microbial biomass is low. However, respiratory activity is kept or increased. This effect leads to decrease in the carbon present in microbial biomass, thus indicating that the herbicide and water deficit condition present an inhibiting effect on soil micro-organism populations, as reported by Bohm et al. (2007).

At worth stressing that, in general, despite the increase in microbial respiration (CO_2), there was a decrease in MCB. The microbial activity (CO_2) measures the intensity with which the biochemical processes take place in the ecosystem, and the interpretation of these results should be performed with discretion, since high rates of C-CO_2 release on soil not always indicate favorable conditions (Islam and Weil, 2000), as found out in this study. Thus, the evaluation of the CO_2/MCB ratio which constitutes qCO_2 has shown to be more appropriate, since this component infers on the metabolic effectiveness and not only the metabolic activity of microorganisms.

Overall, regardless of the Roundup Ready® cultivars, there was an increase tendency in the metabolic quotient (qCO_2) (Table 4) with glyphosate application. These results corroborate those found by Bohm and Rombaldi (2010), which also report increased qCO_2 due to the application of different glyphosate doses. In turn, the lowest qCO_2 values and the highest *q*mic values (Table 3) were obtained in treatments without glyphosate application, showing that this treatment provides the microbiota stability maintenance.

The highest qCO_2 values indicate increased microorganism activity on soil, since it is a consequence of increased CO_2 release per MCB unit, which, as previously reported, reflects a microbiota stress condition. Such a fact confirms the presence of an easy degrading substrate (glyphosate), however, low assimilation, because the effect of higher basal respiration came along with the decreased MCB and it consequently resulted in a lower metabolic effectiveness of microorganisms.

Therefore, the lowest qCO_2 values observed in treatments without glyphosate application, when compared to treatments receiving the application of this herbicide, reflect an economic condition in energy use, indicating that this system may be adjusting to a new balance state as reported by Tótola and Chaer (2002). These results allow one to state that qCO_2 significantly detected changes due to the various soil moisture conditions and the presence or absence of glyphosate, thus highlighting, as a good indicator of soil quality and very sensitive to human interventions, according to what is documented in the literature (Islam and Weil, 2000; Baretta et al., 2005).

Regardless of the Roundup Ready® cultivar, there were decreased in NN and DWNP with glyphosate application, and considering that this is the most noticeable effect at a 1.800 g e.a. ha^{-1} dose (Figures 4 and 5) and, especially when associated to lower water content on soil. These

Table 4. Metabolic quotient (qCO_2) of soil in treatments with Roundup Ready® soybean cultivars subject to different glyphosate doses under appropriate and water deficit conditions. and conventional soybean cultivars subject only to water conditions the city of Bom Jesus. Brazilian State of Piaui (crop season 2011/2012).

Glyphosate	qCO_2 (g C-CO^2 g^{-1} MBC dia^{-1})					
	P98Y12RR			**P98Y51RR**		
	Without deficit	**With deficit**	**Average**	**Without deficit**	**With deficit**	**Average**
0 g e.a. ha^{-1}	0.20^{Ba}	0.42^{Ba}	0.31^{B}	0.49^{Ba}	0.53^{Ba}	0.51^{A}
1080 g e.a. ha^{-1}	0.51^{Ba}	0.41^{Ba}	0.46^{B}	0.50^{Bb}	0.94^{Aa}	0.72^{A}
1800 g e.a. ha^{-1}	1.09^{Aa}	0.91^{Aa}	1.00^{A}	0.99^{Aa}	1.11^{Aa}	0.96^{A}
Média	0.60^{a}	0.58^{a}	0.59	0.74^{b}	0.86^{a}	0.80
Glyphosate	**M9144RR**			**M9056RR**		
	Without deficit	**With deficit**	**Average**	**Without deficit**	**With deficit**	**Average**
0 g e.a. ha^{-1}	0.43^{Ba}	0.45^{Ba}	0.44^{B}	0.72^{Ba}	0.39^{Aa}	0.56^{B}
1080 g e.a. ha^{-1}	0.67^{Ba}	0.46^{Bb}	0.56^{A}	0.98^{ABa}	0.55^{Ab}	0.76^{AB}
1800 g e.a. ha^{-1}	1.25^{Aa}	1.17^{Aa}	1.21^{A}	1.20^{Aa}	0.79^{Ab}	1.00^{A}
Média	0.78^{a}	0.69^{a}	0.73	0.97^{a}	0.58^{b}	0.77
Glyphosate	**M-Soy 9350***			**UFUS-Milionária***		
	Without deficit	**With deficit**	**Average**	**Without deficit**	**With deficit**	**Average**
0 g e.a. ha^{-1}	0.40^{b}	1.00^{a}	0.70	0.53^{a}	0.62^{a}	0.57

Average values followed by the same letters capitalized in columns and lowercase in the line do not significantly differ from each other using the Tukey test at 5%. *Conventional soybean cultivars did not receive glyphosate applications.

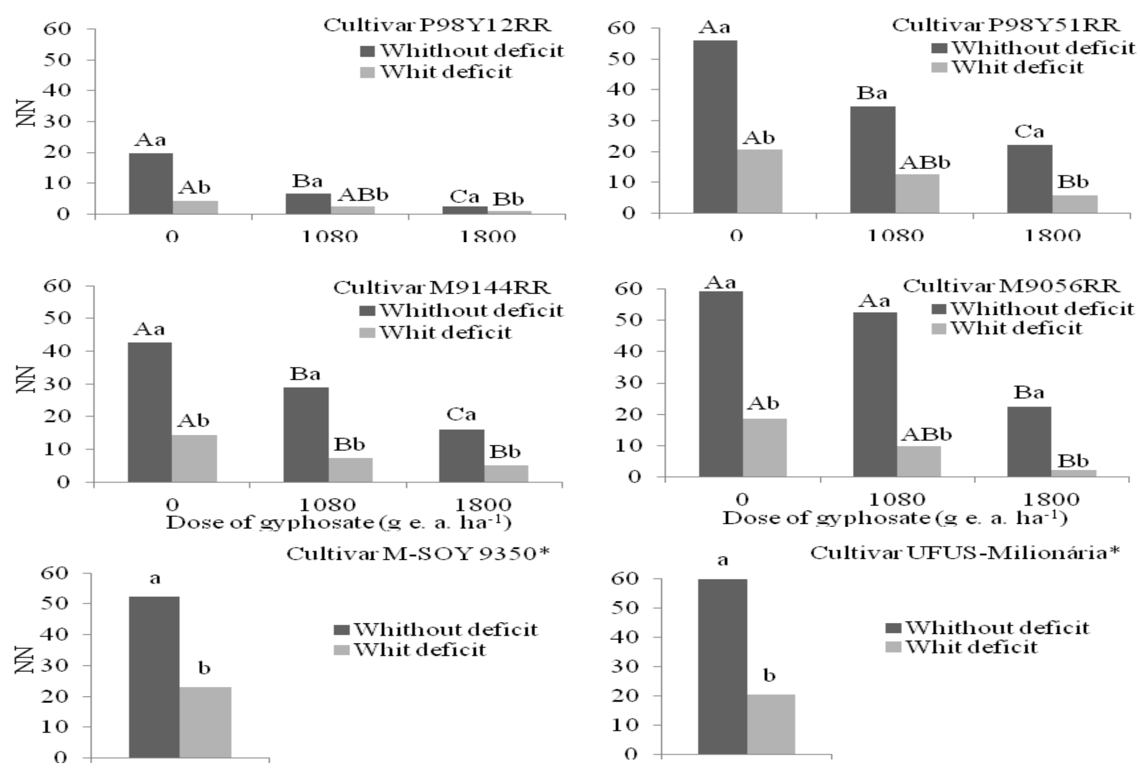

Figure 4. Number of nodules per plant (NN) in Roundup Ready® soybean cultivars subject to glyphosate application under appropriate and water deficit conditions, and conventional soybean cultivars subject only to water conditions the city of Bom Jesus. Brazilian State of Piaui (crop season 2011/2012). Average values followed by the same letters capitalized under the same water condition and lowercase letter at the same dosage do not significantly differ from each other using the Tukey test at 5%. *Conventional soybean cultivars did not receive glyphosate applications.

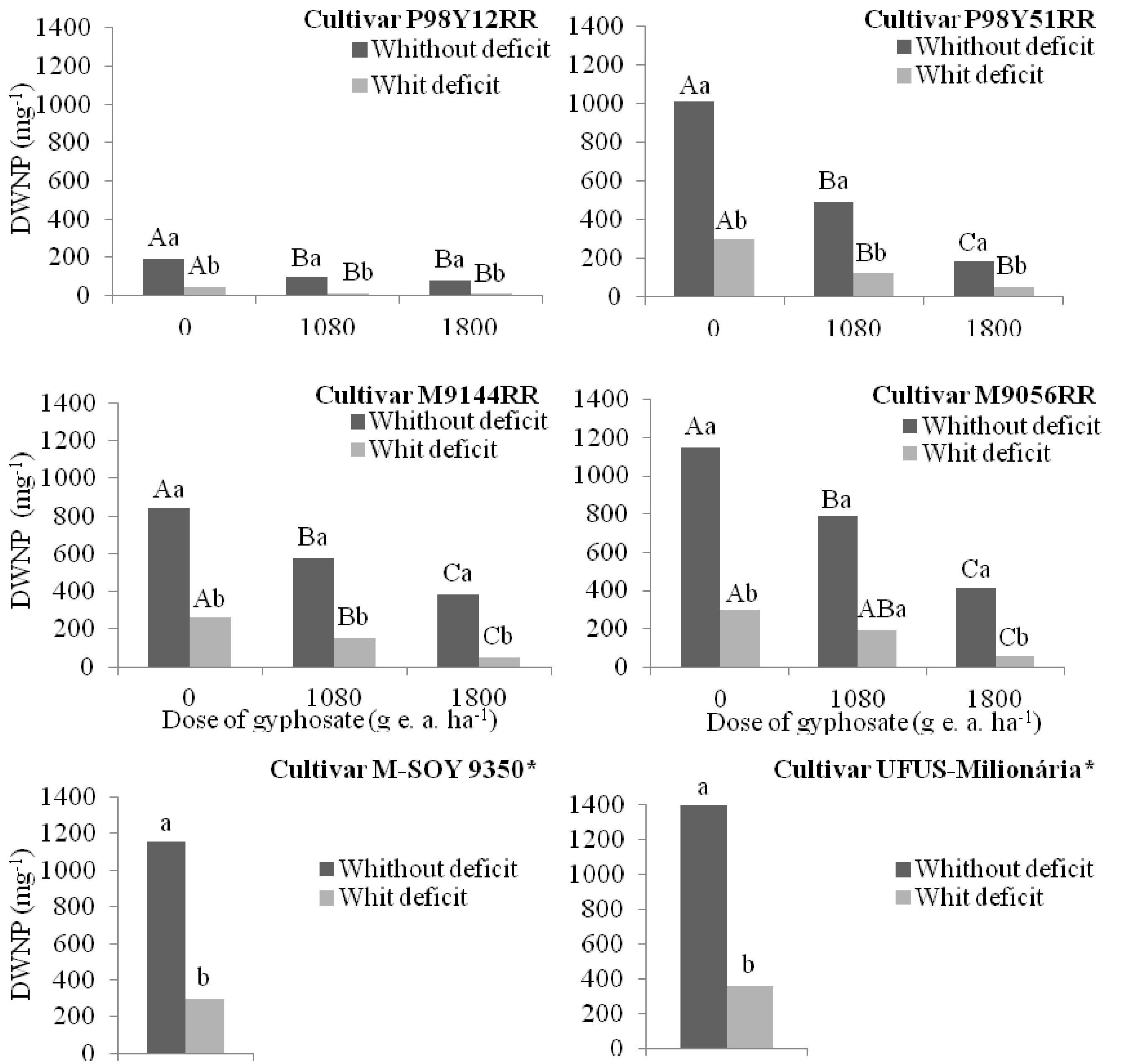

Figure 5. Dry weight of nodules per plant (DWNP) in Roundup Ready® soybean cultivars subject to glyphosate application under appropriate and water deficit conditions; and conventional soybean cultivars subject only to water conditions the city of Bom Jesus, Brazilian State of Piaui (crop season 2011/2012). Average values followed by the same letters capitalized under the same water condition and lowercase letter at the same dosage do not significantly differ from each other using the Tukey test at 5%. *Conventional soybean cultivars didn't receive glyphosate applications.

data corroborate those obtained by Santos et al. (2004), Oliveira Júnior et al. (2008), and Zobiole et al. (2010b), who also found out decrease in NN and DWNP caused by glyphosate application. The results clearly showed the phytotoxic effect of glyphosate on nodulation. This fact is related to N_2-fixing bacteria associated to the soybean root system of the *B. japonicum* genus, which has an EPSPs enzyme sensitive to glyphosate, which may occur this way, the accumulation of shikimic, hydroxybenzoic, and protocatechuic acids, and, hence, inhibit the synthesis of aromatic amino acids, interfering with symbiotic interaction or directly acting on microorganisms (Bellalloui et al., 2006; Santos et al., 2007).

For the condition of soil moisture, the results showed decrease in NN and DWNP when subject to water deficit. This fact is related to the sensitivity of biological nitrogen fixation (BNF) to water deficit (Serraj et al., 2001). The basis of this sensitivity is associated to the transportation of ureides (allantoic and allantoin acid) through xylem to the leaves, from the nodules, whose metabolism is affected by water deficit. With this, a new transportation through phloem and accumulation in nodules, something which inhibits BNF as a result of a retro-inhibiting effect, which can cause a decrease in vigor, respiration, and the water flow reaching the nodules (Kron et al., 2008). The interruption of ureides transportation through xylem is a result of stomatal closure under of water deficit conditions (30% of FC).

The results clearly show the effect of glyphosate on the soil microbiota, affecting the soil bioindicators in a negative way, and this effect is most noticeable under water deficit conditions. Comparing the microbial activity in conventional cultivars to Roundup Ready® cultivars, this activity is much less affected under water deficit conditions, thus reinforcing the hypothesis of higher effect of this herbicide on the soil microbiota stability under water deficit conditions.

The MCB and the metabolic quotient showed to be quite sensitive to changes resulting from the application of glyphosate, presenting a great potential for soil quality studies under these conditions.

Conflict of Interest

The authors have not declared any conflict of interest.

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