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ORIGINAL ARTICLE

# Transient effects of glyphosate on microbial parameters in soil microcosms

## *Efeitos transitórios do glifosato sobre parâmetros microbianos em microcosmos de solo*

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**ABSTRACT:** Microbial parameters were determined during 28 days in soil microcosms spiked with glyphosate at the recommended dose (RD) and 10-fold RD (10X), as well in non-contaminated controls (CM). Soil respiration rates were stimulated by glyphosate after 1-3 days. Cumulative soil respiration in 10X was 52% and 32% higher than CM and RD, respectively. Total hydrolytic activity was similar between treatments during 0-14 days; lower activities were measured in CM/RD after 21 days and in RD after 28 days. Lower soil dehydrogenase activity was detected in contaminated soils after 7 and 21 days, but activities were comparable to CM at day 28. Total heterotrophic bacteria were higher in CM after 14 days; after 28 days, higher counts were observed in CM/10X microcosms. Microbial biomass carbon (MBC) at day 1 was negatively affected by 10X; however, it returned to CM/RD values at day 28. The metabolic quotient ($qCO_2$) indicated a higher microbial stress in 10X at day 1; after 28 days, values were similar between treatments. In summary, the microbial indicators were changed in a transitory way by glyphosate. Therefore, no short-term detrimental effects are expected in terms of microbial numbers and activities. Studies to assess the diversity and structure of the soil bacterial community in response to glyphosate application are indicated.

**RESUMO:** *Parâmetros microbianos foram determinados durante 28 dias em microcosmos de solo adicionados de glifosato na dose recomendada (RD) e 10 vezes a DR (10X), bem como em controles não contaminados (CM). As taxas de respiração do solo foram estimuladas pelo glifosato após 1-3 dias. A respiração cumulativa do solo no tratamento 10X foi 52% e 32% superior àquela em CM e RD, respectivamente. A atividade hidrolítica total foi semelhante entre os tratamentos durante 0-14 dias; atividades mais baixas foram mensuradas em CM/RD após 21 dias, e em RD após 28 dias. A menor atividade da desidrogenase do solo foi detectada em solos contaminados após 7 e 21 dias, mas as atividades foram comparáveis aos CM no dia 28. A população de bactérias heterotróficas totais foi superior em CM após 14 dias; após 28 dias, contagens mais altas foram observadas nos microcosmos CM/10X. O carbono da biomassa microbiana (MBC) no dia 1 foi afetado negativamente em 10X; no entanto, retornou aos valores de CM/RD no dia 28. O quociente metabólico ($qCO_2$) indicou maior estresse microbiano em 10X no dia 1; após 28 dias, os valores foram semelhantes entre os tratamentos. Em suma, os indicadores microbianos foram alterados de forma transitória pelo glifosato. Portanto, efeitos prejudiciais de curto prazo não são esperados em termos de número e atividades microbianas. Estudos para avaliar a diversidade e estrutura da comunidade bacteriana nos solos em resposta à aplicação de glifosato são indicados.*

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# 1 Introduction

The use of synthetic organic pesticides is intimately associated to agricultural practices, usually benefiting aspects related to productivity (Popp *et al.*, 2013). Herbicides prevail among agricultural pesticides, considered to be essential due to the interference of undesirable plants on crop productivity, as well as the relative inefficacy of alternative control strategies (Fernandez *et al.*, 2009).

Glyphosate (N-(phosphonomethyl) glycine) is a systemic, post-emergence, broad spectrum non-selective herbicide (Singh & Singh, 2016). It is among the most widely used herbicides worldwide, and major users are United States, Argentina, and Brazil, in large part due to the introduction of glyphosate-resistant (especially soybean) crops. The increased use of glyphosate is also justified in the context of a more conservationist soil management, particularly the facilitation of adopting no-tillage strategies (Nguyen *et al.*, 2016).

However, beyond the desired effects due to its application, pesticides might also affect non-target organisms. Considering the widespread use of pesticides, several concerns arise in the context of soil and water quality, the potential impacts towards organisms associated with these environmental matrices, as well as the effects related to human health (Marin-Morales *et al.*, 2013).

The soil microbiota, mainly represented by bacteria and fungi, performs several functions directly related to the maintenance of soil quality and fertility, thus supporting major ecosystem services (Thiour-Mauprivez *et al.*, 2019). Energy and nutrient fluxes in soils are dominated by the microbiota, regulating the complex and dynamic soil processes. Soil microorganisms are mainly heterotrophic, acquiring carbon, nitrogen and energy through the decomposition of organic materials. Such activity is essential for humus formation and nutrient cycling, which involves the mineralization of organic compounds and the transient immobilization of nutrients within microbial biomass (Jacoby *et al.*, 2017; Thiour-Mauprivez *et al.*, 2019).

Pesticides might negatively affect microbial activities and populations due to their potential toxicity; on the other hand, from the large microbial diversity, microorganisms could be stimulated if they are able to use pesticide molecules as nutrient and energy sources (Wołejko *et al.*, 2020). From the wide use of glyphosate, and the relevant activities performed by the edaphic microbiota, soil respiration, microbial biomass, microbial metabolic quotient ($qCO_2$), and enzyme activities, are increasingly employed to assess the potential impacts of herbicides towards the soil microbial community (Bonfleur *et al.*, 2015). Microcosms (or miniaturized ecosystems) are widely used to investigate the effects of xenobiotics on natural soil microorganisms. Such approach permits experimental manipulations to evaluate microbial activities and/or composition in response to environmental perturbations. From these premises, we aimed to assess the effects of glyphosate, applied to soil at two rates in microcosms, on indicators of microbial activity and biomass, as compared to non-contaminated soil.

# 2 Material and methods

## 2.1 Soil microcosms

The soil, an Oxisol (Rhodic Hapludox), was collected (0-10 cm) from an area without recent history of agrochemical applications, in the Cerro Largo municipality, Rio Grande do Sul state, Brazil. After drying (22 ± 5 °C for 2 days) and sieving (< 2 mm), soil was homogenized and used to assemble the microcosms. Soil physicochemical properties are presented in Table 1.

**Table 1.** Physicochemical properties of the Oxisol (0-10 cm) employed in microcosm experiments.
**Tabela 1.** Propriedades físico-químicas do Latossolo Vermelho (0-10 cm) usado nos experimentos de microcosmo.

| Property (unit) | Determined value |
|---|---|
| Clay (%) | 64.0 |
| pH | 5.0 |
| Organic matter (%) | 2.8 |
| Carbon (%) | 1.768 |
| Nitrogen (%) | 0.167 |
| P-Mehlich (mg $dm^{-3}$) | 10.7 |
| Ca ($cmol_c$ $dm^{-3}$) | 3.7 |
| Mg ($cmol_c$ $dm^{-3}$) | 1.3 |
| K ($cmol_c$ $dm^{-3}$) | 0.675 |
| Al ($cmol_c$ $dm^{-3}$) | 0.5 |
| H + Al ($cmol_c$ $dm^{-3}$) | 6.2 |
| CTC efective ($cmol_c$ $dm^{-3}$) | 6.2 |

Isopropylamine salt of glyphosate was acquired as a commercial formulation (Atanor 48®), which presents 480 g of active ingredient (a.i.) $L^{-1}$. Two doses of the herbicide were applied to soil microcosms, one representing the recommended dose (RD; 3.19 mg a.i. $kg^{-1}$ dry soil) and another representing 10-fold the RD (10X; 31.90 mg a.i. $kg^{-1}$ dry soil), calculated from the maximal dose of the commercial product recommended for soy cropping (6 L $ha^{-1}$). Control microcosms (CM) received equal volumes of distilled water.

Microcosms were assembled using sterile airtight glass flasks (3.5 L). Treated (RD, 10X) or untreated (CM) soils (1.25 kg) were added to flasks in quadruplicates. Sterile distilled water was added to adjust initial water content to 60% of field capacity, and soils were then homogenized. The three microcosm groups (RD, 10X and CM) were incubated for 28 days at room temperature, in the dark, with periodic shaking under aseptic conditions, with the period of the experiment being from May 31 to June 27, 2018. Soil humidity was determined gravimetrically, and sterile distilled water was added if needed. Minimum and maximum temperatures were

registered daily throughout the incubations.

2.2 Basal soil respiration

Soil respiration was evaluated through the capture of $CO_2$ by a 0.5 mol $L^{-1}$ NaOH solution. Microcosms were equipped with plastic vials containing the NaOH solution and hermetically closed. Periodically, microcosm flasks were open to remove NaOH vials containing the captured $CO_2$, and immediately replaced by new vials with freshly prepared NaOH solution. Withdrawn vials were added with 1 mL of 300 g $L^{-1}$ $BaCl_2$ and two drops of phenolphthalein (1%, m $v^{-1}$). Residual NaOH was titrated with 0.5 mol $L^{-1}$ HCl, and converted to released C-$CO_2$ (mg C-$CO_2$ $kg^{-1}$ dry soil). Two flasks (3.5 L) without soil served as blanks. Obtained results were presented as cumulative soil respiration and respiration rates.

2.3 Hydrolysis of fluorescein diacetate (FDA)

FDA is a colorless compound used to evaluate hydrolytic activity in soils. This substrate is hydrolyzed by a broad variety of non-specific extracellular and membrane-bound enzymes, releasing fluorescein which is measured spectrophotometrically at 490 nm. Soil evaluations, in triplicates, were performed as described elsewhere, and the released fluorescein (µg fluorescein $g^{-1}$ dry soil) was determined using a fluorescein standard curve.

2.4 Dehydrogenase activity

Soil dehydrogenase activity was estimated in triplicates by the reduction of 2,3,5-triphenyltetrazolium chloride (TTC). Five mL of Tris-HCl buffer (100 mol $L^{-1}$; pH 8.0) containing TTC (10 g $L^{-1}$) were added to 5 g of soil. After incubation (37 °C, 16 h), the released formazan was extracted with 20 mL acetone. This mixture was maintained in the dark for 2 h, with intermittent agitation. Then, the absorbance at 546 nm was measured in supernatants. Dehydrogenase activity results were expressed as percentage (%) related to the incubation time resulting in maximal absorbance (100% relative activity).

2.5 Total culturable heterotrophic bacteria

Soil samples were collected from the microcosms, and serially diluted (from $10^{-1}$ to $10^{-8}$) using sterile saline (8.5 g $L^{-1}$ NaCl). Then, 20 µL of each dilution were used to inoculate, in triplicates, 180 µL of sterile Tryptone Soya Broth (TSB) contained in 96-well polystyrene plates. After incubation (30 °C, 5 days), evaluations were performed verifying medium turbidity as indicative of microbial growth. The most probable number (MPN) of heterotrophic microorganisms (Log MPN $g^{-1}$ dry soil) was obtained from reference tables, considering sample volume and dilution factor.

2.6 Microbial biomass carbon (MBC) and microbial metabolic quotient ($qCO_2$)

MBC was determined through the fumigation-extraction method, at the beginning (day 1) and at the end (day 28) of the experiment. Fumigation was performed using by adding ethanol-free chloroform directly to soil samples. After extraction with $K_2SO_4$ and wet oxidation with $K_2Cr_2O_7$, excess $K_2Cr_2O_7$ was titrated with ferrous ammonium sulfate. MBC was estimated by the difference between the carbon extracted in fumigated and non-fumigated samples, using a correction factor of 0.33.

Metabolic quotient was calculated from the ratio between soil respiration (mg C-$CO_2$ $kg^{-1}$ dry soil) and MBC (mg microbial carbon $kg^{-1}$ dry soil) determined at day 1 and day 28, and expressed as mg C-$CO_2$ $mg^{-1}$ microbial carbon.

2.7 Data analysis

Soil respiration was determined in quadruplicates. All other assays were performed in triplicates, from compound soil samples. Results were used to calculate means and standard deviations. Comparisons among means were performed through the Tukey test at 95% significance ($p < 0.05$).

# 3 Results and Discussion

Cumulative soil respiration, after 28 days, was higher in 10X microcosms when compared to CM and RD ($p < 0.05$), whereas RD and CM were similar ($p > 0.05$; Figure 1a). Previously, glyphosate (5 and 50 mg $kg^{-1}$) have not affected cumulative soil respiration (Busse *et al.*, 2001), as similarly reported elsewhere (Fernandez *et al.*, 2009; Bonfleur *et al.*, 2015). However, enhanced cumulative soil respiration after glyphosate application was demonstrated to occur, usually in a dose-dependent manner (Araújo *et al.*, 2003; Lane *et al.*, 2012).

The higher cumulative respiration in glyphosate treatments resulted, mainly, from the increased basal respiration rates observed during the initial incubation period (Figure 1b). In contaminated microcosms, higher respiration rates were noticed, particularly, at day 3 in RD and days 1-3 in 10X; subsequently, respiration rates displayed no clear trend in comparison to CM (Figure 1b). This behavior was also reported previously following glyphosate application (Haney *et al.*, 2000). At 10 mg $kg^{-1}$, glyphosate had no effect on soil respiration rate; whereas at 20 mg $kg^{-1}$, respiration rates were increased from 12 to 20 days of soil incubation (Accinelli *et al.*, 2002). Specifically, Yang *et al.* (2018) indicated that glyphosate (11.5 and 23.0 mg $kg^{-1}$) increased respiration rates during the first 3 days of incubation as compared to non-contaminated soils.

Our results are in accordance with a meta-analysis performed by Nguyen *et al.* (2016), which demonstrates that glyphosate application at <10 mg $kg^{-1}$ had no significant effect on soil respiration, and that higher glyphosate rates resulted in transient increases on this parameter. The reason for enhanced respiration at the higher application rate (10X) might be due to microbial decomposition of glyphosate, contributing directly to increased $CO_2$ release. Andrighetti *et al.* (2014) indicated that higher soil respiration resulted from the ability of soil microbiota to metabolize glyphosate.

Nevertheless, Lane *et al.* (2012) suggest that this is not

always the case, since soil respiration is a nonspecific marker of microbial activity. In this sense, secondary effects, due to the availability of nitrogen and phosphorus from the glyphosate molecules, could also support microbial growth and enhance respiration, stimulating the mineralization of native soil organic matter (Haney *et al.*, 2002a). An enhanced soil respiration might even indicate toxic effects of glyphosate towards the soil microbiota, which respond by increasing energy production (Zabaloy *et al.*, 2012). In addition, a higher respiration might result, indirectly, from mineralization of the sensitive microbial biomass killed by herbicides (Lane *et al.*, 2012; Bonfleur *et al.*, 2015).

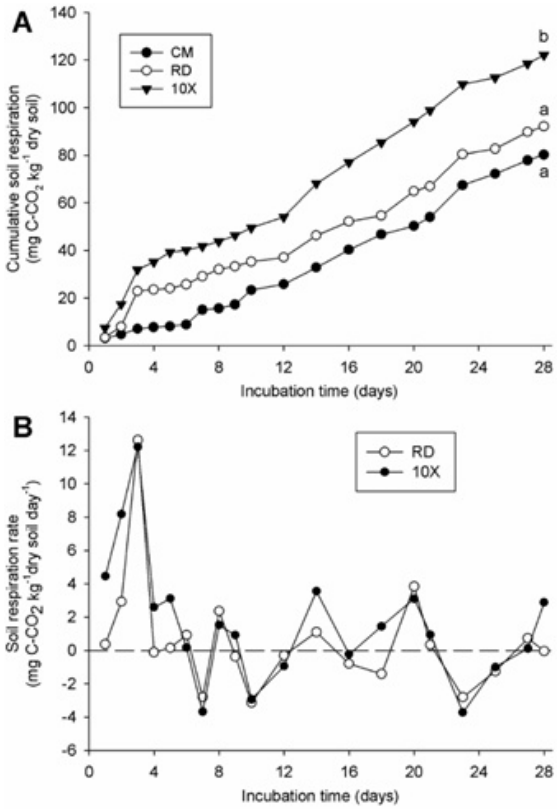


**Figure 1.** (A) Cumulative respiration and (B) respiration rate, in soil microcosms contaminated with glyphosate at recommended dose [RD] and 10-fold the RD [10X], and in non-contaminated controls [CM], as a function of incubation time. Different letters indicate significant differences ($p < 0.05$) between treatments at the same incubation time.

**Figura 1.** (A) Respiração cumulativa e (B) taxas de respiração, em microcosmos de solo contaminados com glifosato na dose recomendada [RD] e 10 vezes a RD [10X], e em controles não contaminados [CM], em função do tempo de incubação. Letras distintas indicam diferenças significativas ($p < 0.05$) entre os tratamentos no mesmo tempo de incubação.

Hydrolysis of FDA is generally used as a measure of the total microbial activity in soils. FDA hydrolysis was similar ($p > 0.05$) between CM and contaminated soils up to 14 days of incubation (Figure 2a). Higher FDA hydrolysis ($p < 0.05$) was detected in 10X microcosms after 21 days and, after 28 days, RD displayed lower values as compared to CM and 10X (Figure 2a). Previously, a consistent increase in FDA hydrolysis was reported 32 days after glyphosate application (2.16 mg kg$^{-1}$ soil) (Araújo *et al.*, 2003). Glyphosate (33 mg kg$^{-1}$ soil) displayed no effect on FDA hydrolysis during 30 days of incubation (Dennis *et al.*, 2018), as also reported by Fernandez *et al.* (2009) in an experiment carried out for 28 days.

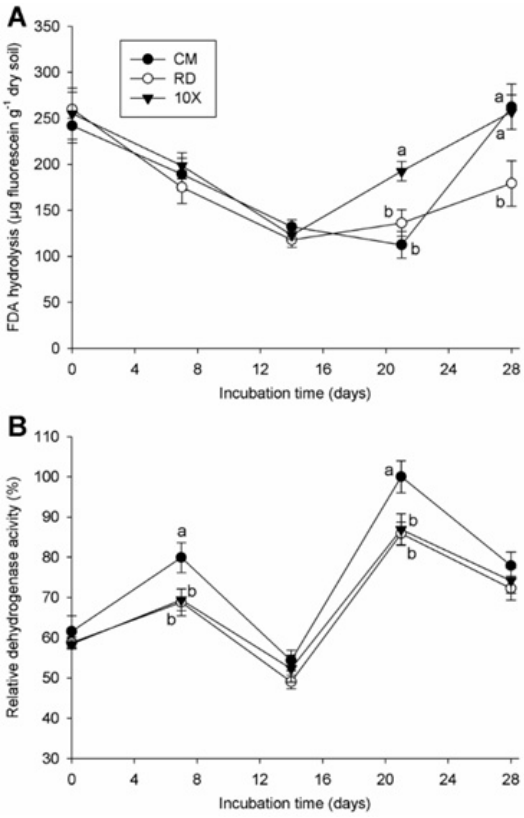


**Figure 2.** (A) Total hydrolytic activity and (B) relative dehydrogenase activity, in soil microcosms contaminated with glyphosate at recommended dose [RD] and 10-fold the RD [10X], and in non-contaminated controls [CM], as a function of incubation time. Different letters indicate significant differences ($p < 0.05$) between treatments at the same incubation time.

**Figura 2.** (A) Atividade hidrolítica total e (B) atividade relativa de desidrogenase, em microcosmos de solo contaminados com glifosato na dose recomendada [RD] e 10 vezes a RD [10X], e em controles não contaminados [CM], em função do tempo de incubação. Letras distintas indicam diferenças significativas ($p < 0.05$) entre os tratamentos no mesmo tempo de incubação.

In soil without previous glyphosate application, hydrolytic activities were higher than non-contaminated soil at days 1 and 32, but similar to control soil at day 17 (Andrighetti *et al.*, 2014). A trend of decreased FDA hydrolysis was observed during 14 days in soils receiving glyphosate at 150 mg kg$^{-1}$; however, such effects disappeared at day 21 (Zabaloy *et al.*, 2012). In another investigation, glyphosate was also demonstrated to exerted small (although significant) and transient negative effects on FDA hydrolysis (Weaver *et al.*, 2007). From our results (Figure 2a), glyphosate displayed no consistent effects on soil hydrolytic activity.

Soil dehydrogenase activity was also assessed, as an indicator of the oxidative capacity of soil microbiota. Although CM displayed a trend of superior average percentages of dehydrogenase activity throughout incubations, significant differences ($p < 0.05$) were only

detected between CM and RD/10X at days 7 and 21 (Figure 2b). At the end of incubations, dehydrogenase activity was similar ($p > 0.05$) between CM and glyphosate treatments.

The effects of glyphosate on soil dehydrogenase are diverse. Glyphosate was reported to decrease dehydrogenase activity (Fernandez *et al.*, 2009). Contrarily, Partoazar *et al.* (2011) showed that glyphosate increased dehydrogenase activity, whereas De Andréa *et al.* (2004) observed that single glyphosate applications had no effect on soil dehydrogenase.

Glyphosate is also reported to affect dehydrogenase activity in a transient manner. For instance, the application of glyphosate at doses usually employed in the field, increased dehydrogenase activity after 4 days, but such effects disappeared after 45 days (Gomez *et al.*, 2009). In incubation experiments, glyphosate only temporarily increased dehydrogenase activity (Panettieri *et al.*, 2013). Similarly, our results (Figure 2b) indicate that glyphosate had no prominent effects on the oxidative activities of the soil microbiota.

Total heterotrophic bacteria were estimated using the MPN technique. From Figure 3a, bacterial populations were similar between treatments at days 0 and 7. Subsequently, higher numbers ($p < 0.05$) were detected in CM after 14 days, in 10X after 21 days, and in CM/10X at the end of incubations (Figure 3a).

Reduced bacterial counts were reported following glyphosate application at 1 mg $kg^{-1}$ (Mekwatanakarn & Sivasithamparam, 1987). Samal *et al.* (2019) indicated that, after an initial increase in bacterial counts, soil treated with high glyphosate doses (100-200 mg $kg^{-1}$) exhibited lower counts than control soil after 28 days. Although glyphosate may exert potential negative impacts towards soil bacteria (Andrighetti *et al.*, 2014), the MPN results (Figure 3a) indicate transitory effects. Previously, glyphosate displayed no effect on culturable soil bacteria (Araújo *et al.*, 2003; Lane *et al.*, 2012). When applied at 2 mg $kg^{-1}$, the density of soil bacteria was not significantly changed, whereas positive but temporary effects were observed when glyphosate was applied at 20 mg $kg^{-1}$ (Wardle & Parkinson, 1990).

It should be noted that MPN is a culture-dependent approach, that is, results refer only to bacteria that were able to grow at the assay conditions. Thus, soil microbial biomass carbon (MBC) was also determined (Figure 3b). MBC can indicate the trends of microbial population dynamics and activities in soils, and is regarded as a sensitive variable to evaluate environmental impacts. One day after treatment, glyphosate at 10X exerted a negative effect on MBC ($p < 0.05$). After 28 days, the MBC in CM was similar to initial values, whereas increases were detected in RD and 10X microcosms ($p < 0.05$). At the end of incubations, MBC values were similar between treatments (Figure 3b).

Haney *et al.* (2000) reported that MBC was not affected by glyphosate, as evaluated three days after application. Glyphosate also tended to display no effects towards soil MBC as evaluated after 1 and 28 days (Castilho *et al.*, 2016). Similarly, the MBC after 21 and

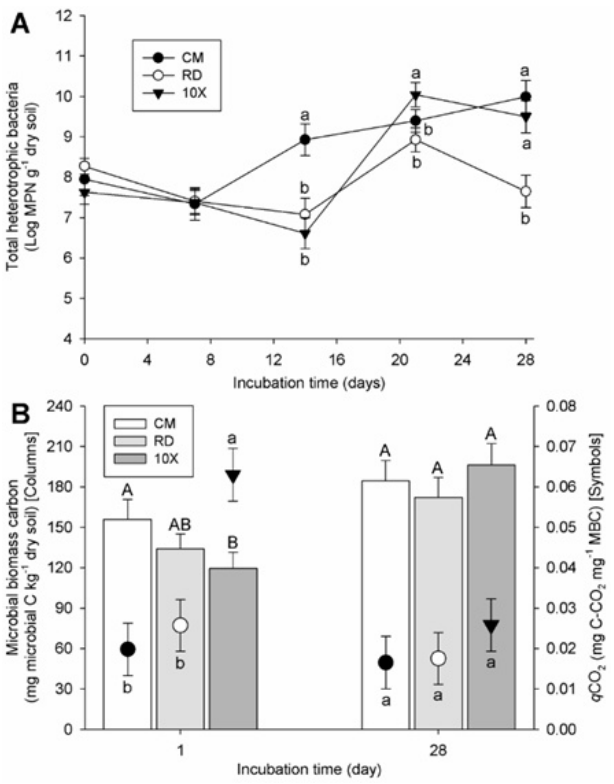


**Figure 3.** (A) Most probable number [MPN] of total heterotrophic bacteria in soil microcosms contaminated with glyphosate at recommended dose [RD] and 10-fold the RD [10X], and in non-contaminated controls [CM], as a function of incubation time. (B) Microbial biomass carbon [MBC; Columns] and microbial metabolic quotient [$qCO_2$; Symbols: ●, CM; ○, RD; ▼, 10X] in soil microcosms, determined at days 1 and 28. Different lowercase letters indicate significant differences ($p < 0.05$) between treatments at the same incubation time for MPN and $qCO_2$ determinations. Distinct uppercase letters indicate significant differences ($p < 0.05$) between treatments at the same incubation time for MBC determinations.

**Figura 3.** (A) Número mais provável [MPN] de bactérias heterotróficas totais em microcosmos de solo contaminados com glifosato na dose recomendada [RD] e 10 vezes a DR [10X], e em controles não contaminados [CM], em função do tempo de incubação. (B) Carbono da biomassa microbiana [MBC; Colunas] e quociente metabólico microbiano [$qCO_2$; Símbolos: ●, CM; ○, RD; ▼, 10X] em microcosmos de solo, determinados nos dias 1 e 28. Letras minúsculas distintas indicam diferenças significativas ($p < 0.05$) entre os tratamentos no mesmo tempo de incubação para determinações de MPN e $qCO_2$. Letras maiúsculas distintas indicam diferenças significativas ($p < 0.05$) entre os tratamentos no mesmo tempo de incubação para determinações de MBC.

63 days of glyphosate application were comparable to control soils (Bonfleur *et al.*, 2015). Contrarily, Haney *et al.* (2002b) observed higher MBC, measured 14 days after glyphosate application, in five out of nine agricultural soils.

Nguyen *et al.* (2016) indicated that glyphosate at <10 mg $kg^{-1}$ soil had no significant effect on soil microbial biomass, but this indicator was diminished at doses between 10 and 100 mg $kg^{-1}$. Analogously to our investigation, Gomez *et al.* (2009) observed that increasing doses of glyphosate exerted negative effects on soil MBC after 4 days as compared to control soil; after 45 days, MBC was higher or similar to controls. Thus,

glyphosate temporarily affected soil MBC (Figure 3b), suggesting that, after an initial negative impact, the soil microbiota was recovered, which might indicate its ability to use the herbicide as a source of nutrients (Gomez *et al.*, 2009).

The microbial metabolic quotient ($qCO_2$) was then calculated from soil respiration rates and MBC determined at days 1 and 28. The $qCO_2$ is used as a measure of microbial stress in soils after disturbances, such as herbicide application. The higher $qCO_2$ values in were observed after 1 day in the 10X treatment ($p < 0.05$), as compared to CM and RD (Figure 3b). Hence, an increased energy production rate (respiration) per unit of MBC was noticed right after contamination in 10X microcosms. In this situation, the energy resources of the remaining microbiota were mainly allocated for survival and cell maintenance (Bonfleur *et al.*, 2015). Such lower metabolic efficiency might reflect a stressful condition for the microbial community due to the deleterious effect of glyphosate. The reduced MBC, coupled with higher respiration and $qCO_2$ values, even though indicating a decreased microbial population, might also suggest that selected microorganisms were increased in numbers and activities (Santos *et al.*, 2012).

On the other hand, $qCO_2$ results obtained after 28 days were similar between treatments (Figure 3b), emphasizing the temporary effects of glyphosate, and suggesting the recovery of metabolic efficiency of soil microorganisms over time (Gomez *et al.*, 2009; Castilho *et al.*, 2016). Nevertheless, MBC and $qCO_2$ indicate a higher stability of microbial numbers and activities in non-contaminated microcosms. Even considering the absence of significant effects of glyphosate at RD on MBC and $qCO_2$ (Figure 3), the herbicide could modify the composition of microbial populations (Bonfleur *et al.*, 2015).

## 4 Conclusion

The results suggest that the herbicide affected transiently the evaluated indicators of microbial activity and biomass. Specifically, soil respiration rates (days 1-3) and cumulative soil respiration tended to increase with the addition of glyphosate. Total hydrolytic activity was similar between treatments from day 0 to day 14. The lowest soil dehydrogenase activity was detected in contaminated soils after 7 and 21 days. Total heterotrophic bacteria were higher in CM after 14 days and, higher counts were observed in CM/10X microcosms after 28 days. The initial decrease of microbial biomass carbon, and higher metabolic quotient ($qCO_2$) indicated higher microbial stress in 10X on day 1; after 28 days, the values were similar between treatments. No deleterious effects should be expected in the short-term, after application at RD and even at 10X, in soils without history of glyphosate use. The parameters evaluated do not elucidate aspects of microbial diversity and community structure and, therefore, further studies are needed to clarify the effects of glyphosate, including successive applications and potential impacts on the microbial diversity of the studied soil.

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