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Response of Soil Microbial Communities to Different Doses of Glyphosate and Sulfosulfuron in a Calcareous Soil

M. Mollaee[1], H. Ghadiri[2], M. Zarei[3], B. Heidari[2], M. Cheshmi[4], and B. Singh Chauhan[5*]

ABSTRACT

To investigate the response of soil microbial populations to different doses of glyphosate and sulfosulfuron, a factorial experiment based on a complete block design was conducted at Shiraz University, Iran. The factors included different herbicides and dose rates (glyphosate at 0, 540, 1,080, and 4,320 g ae ha^{-1} and sulfosulfuron at 0, 12.5, 25, and 50 g ai ha^{-1}), and time of measurements (4, 15, 45, and 65 days after herbicides spray). Microbial respiration, microbial biomass carbon, metabolic quotient, dehydrogenase activity, and aerobic heterotrophic bacteria were measured in soil samples. The results showed that microbial respiration, microbial biomass carbon and metabolic quotient were highest for glyphosate 1,080 g ae ha^{-1} at 4 days after herbicide application. Dehydrogenase activity had a decreasing trend in all herbicide treatments in comparison with the control treatment in all measuring times, except 4 days after spraying. There was no significant difference in dehydrogenase activity between herbicide treatments. The effect of sulfosulfuron on microbial respiration and metabolic quotient was not significant, whereas time and its interaction with herbicide dose rate affected these two variables significantly. Generally, all the measured indices for sulfosulfuron and glyphosate treatments decreased with time after herbicide application. Sulfosulfuron at 50 g ha^{-1} and glyphosate at 4,320 g ha^{-1} had the lowest amounts of aerobic heterotrophic bacteria after 65 days, decreased by 23.7 and 50%, respectively compared with the control. Our results demonstrate that the effects of herbicides on soil microbial communities are strongly related to the herbicide dose and the time after herbicide spray. In conclusions, the herbicides at doses more than the recommended doses showed inhibitory effects on soil microbial communities in the alkaline soil, where the inhibitory effect was more at 4,320 g ae ha^{-1} glyphosate.

Keywords: Aerobic heterotrophic bacteria, Metabolic quotient, Microbial biomass carbon, Microbial respiration.

INTRODUCTION

The growing use of herbicides has now become an important environmental concern (Myers *et al*., 2016), because of the adverse effect of these 0 or their secondary metabolites on the environment, groundwater, soil microorganisms and soil stability (Araujo *et al*., 2003; Gomez *et al*., 2009; Mierzejewska *et al*., 2019; Rojas *et al*., 2016). The soil serves as the repository for all agricultural contaminants; however, soil is also an important habitat for bacteria, fungi and actinomycetes whose activities influence soil fertility through degradation of organic material and nutrient cycling (Nguyen, *et al*., 2018; Zain *et al*., 2013). Soil microorganisms have many crucial

[1] Department of Agrotechnology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Islamic Republic of Iran.
[2] Department of Crop Production and Plant Breeding, College of Agriculture, Shiraz University, Shiraz, Islamic Republic of Iran.
[3]Department of Soil Science, College of Agriculture, Shiraz University, Shiraz, Islamic Republic of Iran.
[4]Department of Crop Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Islamic Republic of Iran.
[5] Queensland Alliance for Agriculture and Food Innovation and the School of Agriculture and Food Sciences, The University of Queensland, Gatton 4343, Queensland, Australia.
*Corresponding author; e-mail: b.chauhan@uq.edu.au

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roles such as enhanced water availability, protection against pathogens, and the transformation of nutrients, especially carbon (Dobbelaere *et al*., 2003; Gopalakrishnan *et al*., 2017; Kuzyakov and Xu, 2013; Nguyen *et al*., 2016). Therefore, any change in the soil could have implications for the microorganism population and their activity, indirectly influencing plant growth and other soil functions (Nannipieri *et al*., 2017; Wang *et al*., 2008). For determining the effect of different chemicals on soil, there are several biological, physical, and chemical parameters will respond rapidly to environmental changes (Avidano *et al*., 2005). Carbon dioxide is one of the main metabolic products in heterotrophic microorganisms; therefore, it can be used as a microbial activity indicator (Accinelli *et al*., 2002; Blagodatskaya *et al*., 2014). The term microbial activity encompasses all the metabolic reactions and interactions by and between soil microorganisms (Nannipieri *et al*., 1990), which are the key factors mitigating the effect of pollutants on soil quality (Kuperman and Carreiro, 1997; Schloter *et al*., 2018; Tang *et al*., 2019).

The biomass and quantity of microorganisms are also widely used in ecological studies to assess microbial activity and soil health (Bolter *et al*., 2002; Tang *et al*., 2019). Measurements of enzymatic activity and respiration of the whole microbial community are also sensitive parameters used to evaluate the effects of pollutants on soil (Anderson, 2003; Thiele-Bruhn and Beck, 2005; Yang *et al*., 2016). Among soil enzymes, dehydrogenase is a reliable common enzyme for estimating the impacts of different pesticides on soil microbial communities (Accinelli *et al*., 2002; Shaw and Burns, 2006) and is also a good indicator of soil biogeochemical processes (Kremer and Li, 2003; Song *et al*., 2017).

The use of herbicides can cause metabolic changes in microbial populations and soil enzyme activities and previous studies have mentioned different effects of herbicides on soil microbial communities (Kepler *et al*., 2020; van Hoesel *et al*., 2017; Zabaloy *et al*., 2008; Zhang *et al*., 2010).

Effects of some of the herbicide treatments were felt shortly after their application, whilst other herbicide treatments had a long-lasting effect on most microorganisms, which could be as a result of differences in herbicide persistence, type of herbicide decomposition, concentration of the active ingredient in the formulation, and environmental factors (Adomako and Akyeampong, 2016; Lupwayi *et al*., 2010; Nguyen *et al*., 2016). There are also different tolerance levels in microbial populations to herbicides (Druille *et al*., 2016; Shahid *et al*., 2018).

Glyphosate and sulfosulfuron are post-emergent, systematic broad-spectrum herbicides used for weed control in crops and pastures. Glyphosate is the most commonly used herbicide in the world due to its non-selective nature, low mammalian toxicity, and extensive application in glyphosate-tolerant crops (Busse *et al*., 2001; Gill *et al*., 2017). It is also used in forest production, often at higher rates than recommended in cropping situations (Tanney and Hutchison, 2010), and in natural grasslands for the control of invasive weed species (Rodriguez and Jacobo, 2010). Glyphosate inhibits 5-Enolpyruvylshikimate-3-Phosphate Synthase (EPSPS), a key enzyme in the shikimate pathway for producing aromatic amino acids in plants. This pathway is also present in microorganisms (Kepler *et al*., 2020). Therefore, there is a possibility that glyphosate can affect microbial growth and activity by inhibiting the shikimate pathway, which produces the amino acids that are important for growth, survival and decomposition of microorganisms (Nguyen *et al*., 2016). Glyphosate is known as a non-mobile herbicide in the soil because of high sorption to soil particles, especially in clay soils. It has moderate persistence in the soil and is degraded primarily by co-metabolic microbial processes (Accinelli *et al*., 2005; Mandal *et al*., 2020). Sulfonylurea

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herbicides are categorised by high biochemical activity at low usage rates and they are highly persistent in the environment (Brown, 1990; Mandal *et al*., 2020). Sulfosulfuron is in this class of herbicides and is relatively persistent and mobile (Mandal *et al*., 2020). This herbicide acts by inhibiting the enzyme Acetolactase Synthase (ALS), which produces amino acids leucine, isoleucine, and valine. This enzyme is present in both microorganisms and plants, and exposure to sulfonylureas would inhibit microbial growth (Rose *et al*., 2016).

Several studies have investigated the effect of herbicide applications on soil microorganisms (Andrea *et al*., 2003; Bottrill *et al*., 2020; Lancaster *et al*., 2010; Rosenbaum *et al*., 2014; Zabaloy *et al*., 2012). Some of these studies have described a transitory increase in the amount of microbial respiration and microbial carbon biomass (Nguyen *et al*., 2016; Wardle and Parkinson, 1990a, b), or negative effects on microbial communities (Adhikary *et al*., 2014; Andrea *et al*., 2003; Du *et al*., 2018; Lancaster *et al*., 2010), while others have not found significant effects (Rosenbaum *et al*., 2014; Zabaloy *et al*., 2012). Such studies for sulfosulfuron are limited. Also, these effects could be altered with different herbicide doses, soil characteristics and diversity of soil microbial communities (Kepler *et al*., 2020; Lupwayi *et al*., 2010; Nguyen *et al*., 2016). Nguyen *et al*. (2016) mentioned that the effect of glyphosate on soil microbial biomass and respiration can be altered by soil pH and organic carbon content. They noticed in their meta-analysis survey that microbial respiration was stimulated by glyphosate in soils with pH< 5.5; however, soils with pH between 5.5-7.5 showed a negative effect of glyphosate on respiration. Limited data is available for alkaline soils (pH> 7.5). They also mentioned that soil organic carbon content can moderate the effect of glyphosate on soil but its role is not noticeable as much as pH.

The aim of this study was to assess the response of microbial communities to different doses of glyphosate and sulfosulfuron at different time periods after herbicide application in a calcareous soil with no herbicide application history. For this purpose, factors such as microbial respiration, microbial carbon biomass, microbial metabolic quotient, dehydrogenase activity and the number of cultivable bacteria, which are important indicators of microbial communities, were planned to be measured. We hypothesised that differences in herbicide rates and time of herbicide exposure in soils with calcareous (alkaline) characteristics may lead to different responses in soil microbial activities. We selected the two herbicides (glyphosate and sulfosulfuron) due to their different modes of action and persistence in the soil which allows to make a good comparison. Lower than the recommended doses of herbicides were selected to represent the probability of receiving low doses of the herbicides to the soil via foliar application, drift, and incorrect application. The application of doses higher than the recommended dose represents situations of herbicide-resistant weeds, garden and pasture applications, pre-harvest and desiccant use patterns (Benbrook, 2016; Myers *et al*., 2016). Variability in factors such as temperature, soil moisture content, and C to N ratio could overshadow the effect of herbicides on microorganisms (Bottrill *et al*., 2020; Domsch *et al*., 1983; Xue *et al*., 2020). For this reason, we attempted to remove the effects of other parameters using controlled conditions. This is the first published study on the response of soil microbial communities to herbicides in this region in alkaline (calcareous) soils.

MATERIALS AND METHODS

Soil Sampling

This experiment was carried out at the Agricultural Research Station of Shiraz University, Iran, in 2012. No herbicide

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was applied in the experimental area before soil sampling. Soil samples were taken from depths of 5 to 10 cm and mixed to make a uniform composite sample. The soil was sandy clay loam (sand 47%, silt 28%, clay 25%) with an organic matter of 0.93%, pH of 7.96 and EC of 0.33 dS m^{-1}. The soil classifications were fine, mixed (calcareous), mesic, Typic Calcixerepts according to the Keys to Soil Taxonomy (Soil Survey Staff, 2014) from the Daneshkadeh series, Shiraz, Iran. The soil samples were immediately transferred to the laboratory, sieved (< 2 mm), placed in trays, and incubated at 25°C in an incubator. Herbicides were applied at different doses to the soil surface. During the study, the soil was kept at 75% water holding capacity by weighing the trays every day and adding distilled water (Gomez *et al*., 2009). This study was conducted as a factorial experiment in a completely randomized design and the treatments were dose rates of herbicides and time after herbicide application. Herbicide treatments included 0, 12.5, 25, and 50 g ai ha^{-1} of sulfosulfuron and 540, 1,080, and 4,320 g ae ha^{-1} of glyphosate. The time treatment consisted of 4, 15, 45 and 65 Days After Spray (DAS). The field recommended doses of sulfosulfuron and glyphosate were 25 and 1,080 g ae ha^{-1}, respectively. Factors such as Microbial Respiration (MR), Microbial Biomass Carbon (MBC), metabolic quotient (qCO_2), Dehydrogenase Activity (DHA), and Aerobic Heterotrophic Bacteria (AHB) were measured. All factors were measured for all the time-treatments, except MBC which was measured (three times) at 4, 45, and 65 DAS.

Laboratory Measurements

Microbial Respiration (MR) was estimated using the closed jar method and titration (Isermeyer, 1952). In this method, carbon dioxide from soil respiration is trapped by the sodium hydroxide solution and then determined using titration as g CO_2 per g soil. Microbial Biomass Carbon (MBC) was determined by the fumigation incubation method (Jenkinson and Powlson, 1976). In this method, the soil microbial community is killed by fumigation with chloroform steam, and thus appropriate for mineralization. The microbial metabolic quotient (qCO_2) was measured by dividing basal respiration (g CO_2-C g^{-1} dry soil h^{-1}) to microbial C (g C_{mic} g^{-1} dry soil) (Dilly and Munch, 1998). The common range for qCO_2 in a neutral soil is between 0.5 and 2.0 mg CO_2-C g^{-1} C_{mic} h^{-1} (Anderson, 2003).

Dehydrogenase Activity (DHA) was measured using the triphenyltetrazoliumchloride (TTC) method (Thalman, 1966), which is based on the assessment of the decrease rate of TTC to Triphenylformazan (TPF) in soils after incubation at 30°C for 24 hours. The soil samples were prepared by incubating with TTC under moist conditions at 37°C for 24 hours. Determination of TPF, which is resultant from TTC as a product of enzyme activity, was done spectrophotometrically. Measurements were done at 485 nm wavelength and enzyme activity was given as µg TPF g^{-1} soil (Tabatabai, 1982). The number of Aerobic Heterotrophic Bacteria (AHB) was estimated through the plate count method (Zabaloy *et al*., 2008). Soil suspension and dilutions were prepared and appropriate dilutions were transferred to Petri dishes containing agar, and the number of cultivable AHB was expressed as $\log_{10}$ CFU g^{-1} soil (Colony Forming Unit: CFU).

Statistical Analyses

This factorial experiment was based on a randomized complete block design with six replications. The hypothesis of normality and homogeneity of variance was tested with the Levene test. The data were homogenous (P> 0.05) and the normality test revealed that no data transformation was needed. The data of each herbicide were compared with LSD (Least Significant

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Difference P= 0.05) values using Analysis Of Variance (ANOVA). All statistical procedures were performed using SAS (ver. 9.1).

RESULTS AND DISCUSSION

Microbial Respiration

Four days after glyphosate application, the rate of Microbial Respiration (MR) in all the glyphosate treatments was significantly higher than 0 g ai ha^{-1} (control). The 1,080 g ae ha^{-1} glyphosate treatment (the recommended dose of glyphosate) recorded the highest amount, increased by 71% compared with the control treatment (Figure 1-a). At the end of the incubation stage, all the glyphosate treatments showed a decreasing trend. In the final measurement, there was no difference between glyphosate doses in MR, which was less than the control. Microbial respiration analysis of sulfosulfuron treatments showed initial stimulation for 12.5, 25 and 50 g ai ha^{-1} treatments. The highest MR rate of sulfosulfuron treatments was observed at 50 g ai ha^{-1} during the first incubation period (4 DAS) by an increase of 36% relative to the control (herbicide×time; P= 0.01) (Figure 1-b). The MR trend in both herbicide treatments decreased with time after incubation, but no significant difference was observed between different doses of sulfosulfuron and the control at 65 DAS.

The initial increase in released CO_2 in the presence of glyphosate indicated that the soil microorganisms may use herbicides or dead microorganisms as a source of carbon. Previous studies have reported similar effects of glyphosate on microorganisms and soil microorganisms appeared to utilize herbicides as a substrate (Duke *et al*., 2012; Pertile *et al*., 2020). Furthermore, this initial increase in MR may be related to a stress response in herbicide sensitive species. These findings are consistent with Pertile *et al*. (2020) who reported that the parameter related to soil microbial activity (DHA and MR) increased initially as a reaction to the possible stress caused by the herbicides and then decreased to the level of the control treatment. All herbicide treatments showed a decreasing trend toward the end of the incubation period, probably due to depletion of the nutrient resource. In the control treatment, MR was slightly higher at 15 DAS than at 4 DAS. A possible explanation could be better conditions in the experimental trays and incubation environments compared with field

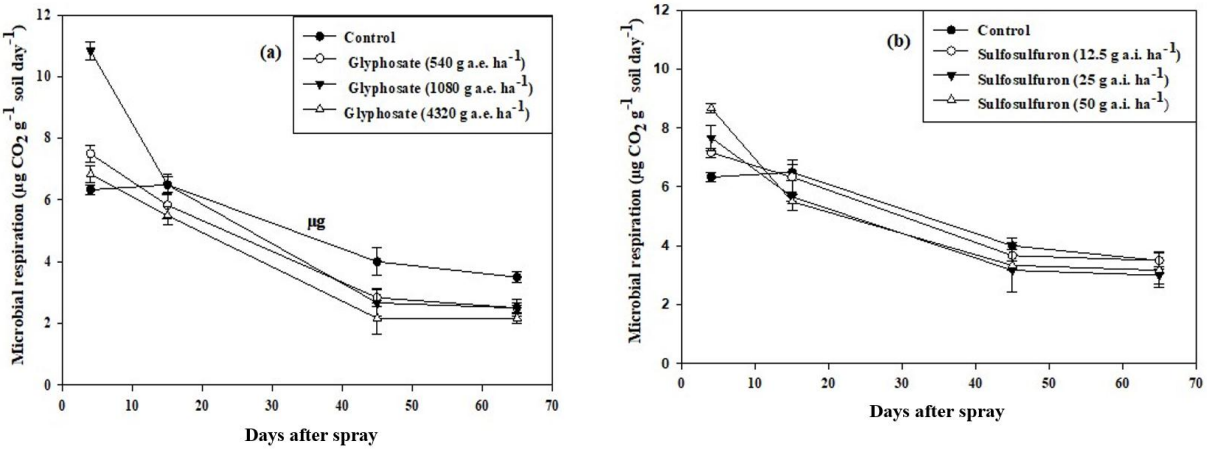

Figure 1. Microbial respiration changes (P≤ 0.05) after application of different doses of glyphosate (a) and sulfosulfuron (b), over time. The vertical bars represent standard errors of the mean.

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conditions. Optimum conditions led to increasing MR until 15 DAS in the control treatment compared with the start of the study in which samples were brought from the field (Figures 1-a and -b). However, beyond 15 DAS, MR declined, perhaps due to insufficient nutrients for microorganism populations and an increase in competition between microorganisms (Figures 1-a and -b).

Microbial Biomass Carbon and Metabolic Quotient

At 4 DAS, Microbial Biomass Carbon (MBC) (reflecting the size of the microbial community) decreased by 10% in the 4,320 g ae ha^{-1} glyphosate treatment, but increased by 0.6 and 15% at 540 and 1,080 g ae ha^{-1} glyphosate, respectively, compared with the control (Figure 2-a). The lowest amount of MBC in all measurements was observed at 4,320 g ae ha^{-1} glyphosate, which could be due to the suppression effect of glyphosate at high doses on susceptible microorganisms. For sulfosulfuron, there were no significant differences between treatments from the start to the end of the study, except for the 50 g ai ha^{-1} treatment, which had the lowest amount of MBC. The MBC was 24% lower than the control treatment at 65 DAS (Figure 2-b). For both herbicides, MBC displayed a generally decreasing trend until the end of the incubation stage (Figures 2-a and -b).

The results showed an increase in the amount of MBC at 540 and 1,080 g ae ha^{-1} glyphosate and 12.5 and 25 g ae ha^{-1} sulfosulfuron (Figures 2-a and -b) at the beginning of incubation compared with the control treatment. It could be attributed to boosting the carbon source via compositing the herbicides in the soil (Kremer *et al*., 2005). The reduction of MBC at 4,320 g ae ha^{-1} glyphosate treatment and 50 g ai ha^{-1} sulfosulfuron may be due to the preventive effect of higher herbicide doses, which led to the quick extinction of microorganisms and the reduction of the amount of MBC (Gomez *et al*., 2009; Nguyen *et al*., 2016). Our results differ from some previous studies, in which glyphosate application at the recommended dose did not alter microbial biomass (Bottrill *et al*., 2020; Wardle and Parkinson, 1991). Using the recommended and lower doses of glyphosate as a nutrient in our study can be the reason for increasing MBC in this treatment. However, at 4,320 g ae ha^{-1} treatment (higher than the recommended dose) the toxic effect of the herbicide was stronger.

At the first incubation period, the amount of microbial metabolic quotient (qCO_2), which can explain the level of stress in the soil, was higher for both herbicide treatments compared with the control (Figures 2-c and -d), but over time, a decreasing trend was observed. The decreasing trend could be due to the tolerance ability of the microbial community. Most bacteria are able to tolerate minor variations in an environmental factor and can acclimatize over time (Grover *et al*., 2011; Hill *et al*., 1995). Also, microorganisms make suitable provisions for survival by yielding to the stress conditions (Herbert, 1989).

Dehydrogenase Activity

In the beginning of the incubation time (4 DAS), DHA was affected by herbicides. An increase in DHA in all the herbicide treatments was observed. However, this increase was transitory (Figures 3-a and -b). The results showed 82±2 and 77±1% inhibition in DHA at 65 DAS in glyphosate and sulfosulfuron treatments, respectively, compared with the control. In contrast, the control treatment showed an increase in DHA immediately after incubation and its DHA at 65 DAS increased by 60% relative to 4 DAS. The initial increase of DHA in herbicide treatments and then a decrease to the end of the incubation period was similar to the MR pattern, and it could be due to stress response to herbicide (Pertile *et al*., 2020) or increasing microbial activity

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through utilizing substrates (Duke *et al*., 2012).

Aerobic Heterotrophic Bacteria

Overall, the number of Aerobic Heterotrophic Bacteria (AHB) in all treatments displayed a reduction from the beginning to the end of incubation, although this reduction was more noticeable at the highest dose of glyphosate (50% reduction compared with the control). The lowest doses of glyphosate (540 and 1080 g ae ha^{-1}) caused a short temporal increase (8.8 and 5.6%, respectively) in bacterial density 4 DAS (Figure 4-a). This initial growth of bacteria compared with the control treatment (Figure 4-b) could be attributed to higher nutrient availability in the lowest herbicide treatments, because at low doses the herbicides are themselves used by microorganisms as a food source. Similar observations were reported by Araujo *et al*. (2003) and Ratcliff *et al*. (2006). However, with time, we observed a decrease in the number of AHB at 540 and 1080 g ae ha^{-1}glyphosate, which could be due to the effect of competition, i.e., the nutrition source was not sufficient for additive AHB. Glyphosate at 4,320 g ae ha^{-1} exerted high deterring

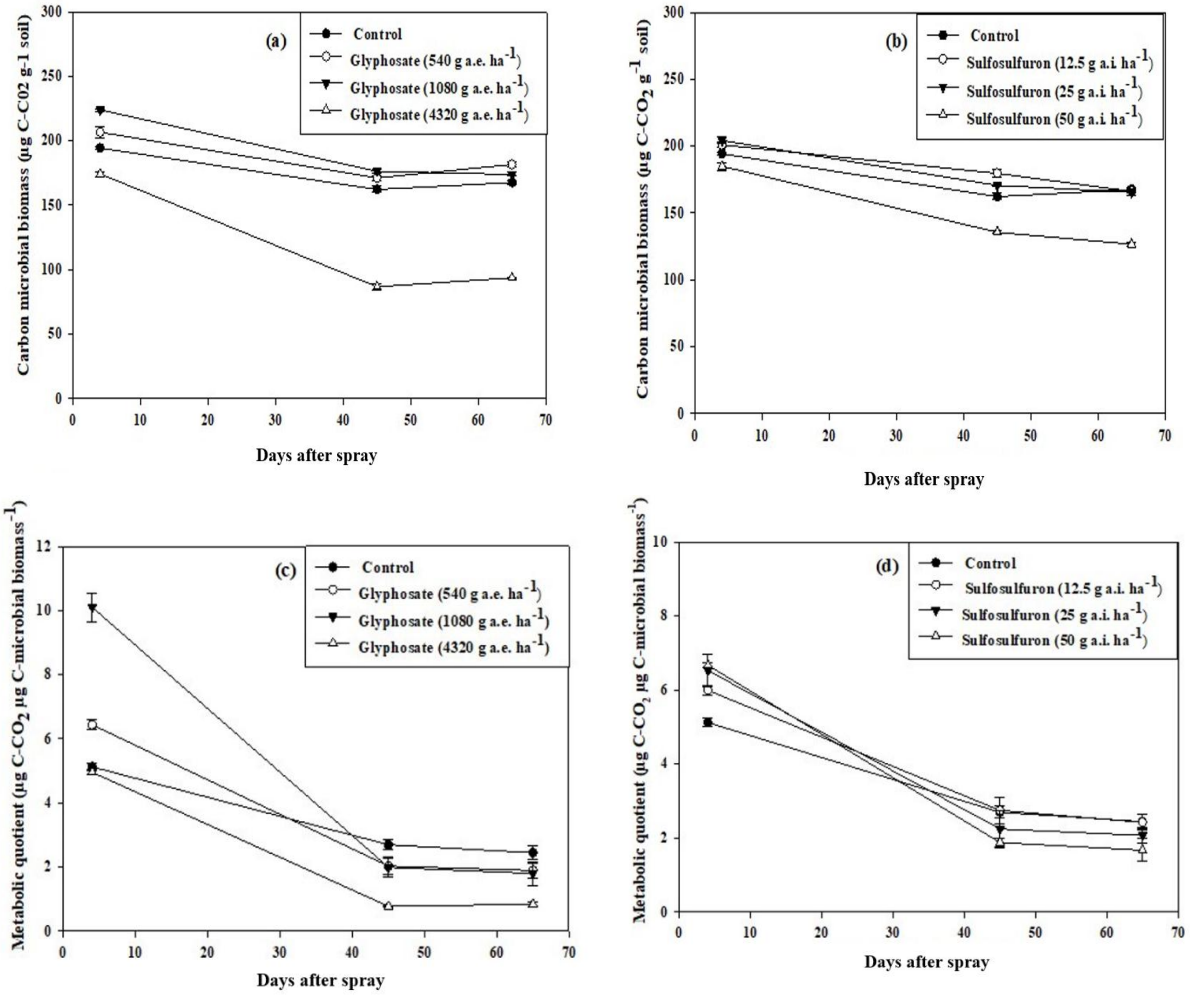

Figure 2. Microbial biomass carbon (a and b) and metabolic quotient (c and d) affected (P≤ 0.05) by different doses of glyphosate (a and c) and sulfosulfuron (b and d) at different times after herbicide application. The vertical bars represent standard errors of the mean.

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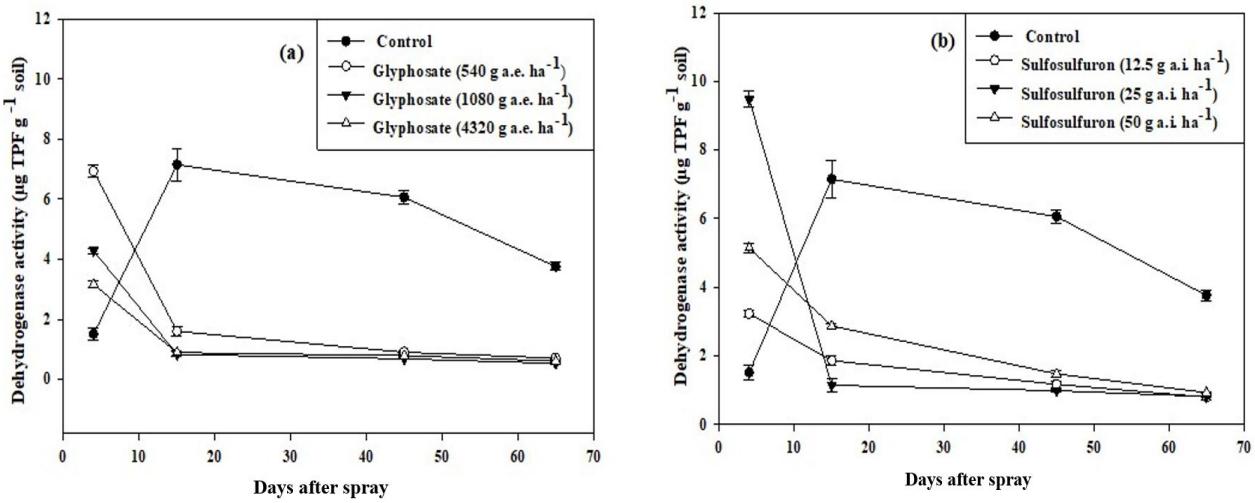

Figure 3. Dehydrogenase activity changes ($P \leq 0.05$) after application of different doses of glyphosate (a) and sulfosulfuron (b) over time. The vertical bars represent standard errors of the mean.

effects during the period of the study (Figure 4-a). The number of bacteria in the sulfosulfuron treatments had a similar decreasing trend (Figure 4-b).

The decreasing trend of different parameters in all treatments during incubation can be explained in terms of limited nutrient availability for microbial communities; however, there may be other reasons. We observed this reduction more in the herbicide treatments than in the control, which could be due to the fatal effect of herbicides on sensitive microorganisms (Gomes *et al*., 2009; Nguyen *et al*., 2016; Radivojevic *et al*., 2011). This response to different doses could mean that a range of sensitivity exists amongst the microbial population, such that fewer hardy species are suppressed by increasing glyphosate doses over 10 mg kg^{-1}, while a resistant degrader population rewards at higher doses (Nguyen *et al*., 2016).

Kryuchkova *et al*. (2014) observed that the existence of glyphosate in the soil could lead to a short-term rise in the number of bacteria and their microbial activity. In our study, the temporary rise in bacterial numbers and activities could be due to the regaining of the original population because of a better supply of nutrients coming from dead bacteria (Ismail *et al*., 1998; Zabaloy *et al*., 2008). It could also be due to degradation of herbicides by microorganisms, which then use the degraded chemicals as a source of C (Araujo *et al*., 2003; Bottrill *et al*., 2020; Duke *et al*., 2012; Ratcliff *et al*., 2006).

Although herbicides are not designed to deter microorganisms, their negative effects on microbial activities and communities, particularly sensitive microbial communities, have been observed (Nguyen *et al*., 2016). Microbial response to herbicides depends on the properties and exposure duration of herbicides, soil characteristics, environmental conditions, and the type of soil microbial communities (Dennis *et al*., 2018; Lupwayi *et al*., 2010; Nguyen *et al*., 2016; Zabaloy and Gomez, 2008). Many studies showed a non-significant effect of herbicide applications at recommended field rates on microbial communities (Bottrill *et al*., 2020; Nguyen *et al*., 2016; Radivojevic *et al*., 2011; Rosenbaum *et al*., 2014; Zabaloy *et al*., 2012). The recommended dose (1,080 g ae ha^{-1} of glyphosate and 25 g ai ha^{-1} of sulfosulfuron) used in our study had negligible inhibitory effects on microbiological factors. Negative effects of herbicides on soil microbial populations have been often observed at doses higher than the recommended doses (Busse *et al*.,

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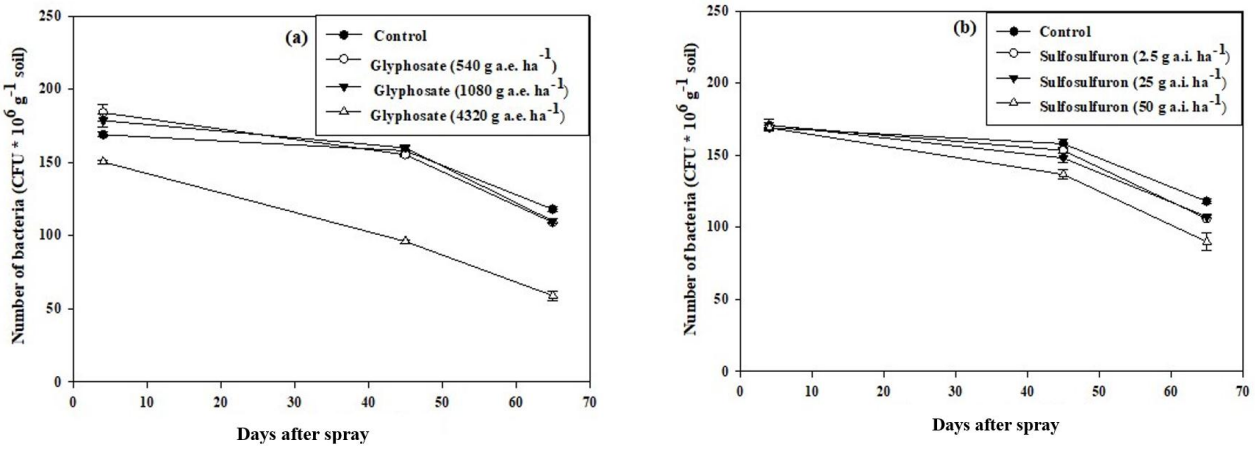

Figure 4. Aerobic heterotrophic bacteria variation ($P \leq 0.05$) after application of different doses of glyphosate (a) and sulfosulfuron (b) over time. The vertical bars represent standard errors of the mean.

2001; Gomes *et al*., 2009; Nguyen *et al*., 2016; Radivojevic *et al*., 2011).

Herbicides include carbon-containing compounds that microbes can use as a nutrient source. In addition to the stress response to herbicides by microbial communities, there is an assumption that the dead microbes (killed by lethal doses of herbicide) provide carbon for microbial metabolism and this can be a reason for the temporary increase in the rate of microbial respiration and dehydrogenase enzyme activity at the first of incubation period (Figures 1 and 3).

Differences between our results and some of the previous studies (Rosenbaum *et al*., 2014; Zabaloy *et al*., 2012) can be due to different soil characteristics, such as different pH and amount of organic component (Bottrill *et al*., 2020; Lupwayi *et al*., 2010; Nguyen *et al*., 2016). There is a relationship between soil pH and phosphorus availability, and alkaline soils have more phosphate availability (da Silva Cerozi and Fitzsimmons, 2016; Lindsay, 1979). Glyphosate and phosphate may both bond to the surface of soil particles, potentially leading to antagonism between the two herbicides. Consequently, in soil with high pH, via the availability of P, sorption of glyphosate would decrease (Borggaard and Gimsing, 2008). Furthermore, glyphosate mobility and availability for degradation via microorganisms is higher in alkaline soil.

CONCLUSIONS

Our results suggest that the lower doses of the herbicides do not have a toxic effect on microbial communities. The results confirm that the effects of herbicides on soil microbial communities are closely related to the dose of herbicides and the time after using herbicides. Although glyphosate and sulfosulfuron can stimulate the growth and reproduction of microorganisms by boosting the nutrient availability, they can also inhibit this growth and cause a reduction in MBC and metabolic activity at their higher doses in a calcareous soil.

Low and recommended doses of the herbicides may be used as a source of energy by microorganisms immediately after herbicide application because they have high levels of C, N and P compounds. In addition, the microorganisms killed by herbicides are another source of carbon. Hence, herbicides can have a short time benefit and stimulate metabolic activities of microorganisms capable of degrading them. In general, herbicides lethal effects reduced the number of bacteria during the incubation period. Thus, the use of herbicides can cause changes in microorganisms' functions and

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soil enzyme activities. It is evident that the impact of herbicides is also affected by soil pH. The present study may contribute to understanding of the effect of the herbicides on calcareous soils.

The impact of herbicides on microbial biomass and respiration depends on the dose and duration of exposure and repetition of herbicide application; therefore, their augmentative effects must be considered in future studies. The effect of herbicides can be altered with different formulations, additive and surfactant compounds; therefore, for future studies, we suggest estimation of different commercial herbicide types by considering the effect of their additive chemicals on soil microbial communities in different soil types with different sorption conditions. Also, more molecular and field-based studies are needed for improving knowledge on microorganism community structure and diversity of herbicides tolerance.

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پاسخ اجتماعات میکروبی خاک به دوزهای مختلف گلیفوسیت و سولفوسولفورون در خاک آهکی

م. مولایی، ح. غدیری، م. زارعی، ب. حیدری، م. چشمی، و ب. سینگ چاوهان

چکیده

جهت بررسی پاسخ جمعیت‌های میکروبی خاک به علف‌کش‌های گلیفوسیت و سولفوسولفورون، آزمایشی در قالب طرح فاکتوریل در دانشگاه شیراز - ایران انجام شد. تیمارها شامل علف‌کش‌ها و دوزهای مختلف آن‌ها (گلیفوسیت ۰، ۵۴۰، ۱۰۸۰ و ۴۳۲۰ گرم ماده موثر/ هکتار و سولفوسولفورون ۰ و ۱۲.۵، ۲۵ و ۵۰ گرم ماده موثر/ هکتار) و زمان آزمایشات (۴، ۱۵، ۴۵ و ۶۵ روز بعد از کاربرد علف‌کش). نتایج نشان داد که بیشترین میزان تنفس میکروبی، کربن بیوماس میکروبی و ضریب متابولیک در تیمار ۱۰۸۰ گرم ماده موثر/ هکتار گلیفوسیت در ۴ روز بعد از کاربرد علف‌کش حاصل شد. فعالیت آنزیم دهیدروژناز در تمامی تیمارهای علف‌کشی در مقایسه با تیمار شاهد در زمان‌های اندازه‌گیری به غیر از ۴ روز بعد از کاربرد علف‌کش، روند کاهشی نشان داد. تفاوت معنی داری در فعالیت آنزیم دهیدروژناز بین تیمارهای علف‌کشی وجود نداشت. اثر علف‌کش سولفوسولفورون بر میزان تنفس میکروبی و ضریب متابولیک معنی‌دار نبود، درحالیکه زمان و اثر متقابل آن با دوز علف‌کش، این دو فاکتور (میزان تنفس میکروبی و ضریب متابولیک) را به طور معنی‌داری تحت تاثیر قرار داد. به طورکلی تمامی شاخص‌های اندازه‌گیری شده با گذشت زمان، برای تیمارهای سولفوسولفورون و گلیفوسیت کاهش پیدا کرد. سولفوسولفورون ۵۰ گرم ماده موثر / هکتار و گلیفوسیت ۴۳۲۰ گرم ماده موثر / هکتار، کمترین میزان باکتری‌های هتروتروفیک هوازی را ۶۵ روز بعد از کاربرد علف‌کش نشان دادند، که در مقایسه با تیمار شاهد، به ترتیب ۲۳.۷٪ و ۵۰٪ کاهش یافتند. نتایج نشان داد که اثرات علف‌کش‌ها بر اجتماعات میکروبی خاک بسیار وابسته به دوز علف‌کش و زمان بعد از کاربرد علف‌کش است. علف‌کش‌ها در دوزهای بیشتر از دوز توصیه شده، اثرات بازدارنده‌ای بر اجتماعات میکروبی خاک در خاک‌های قلیایی نشان دادند.

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