DOI: http://dx.doi.org/10.1590/1678-4499.203

Microbial activity of soil with sulfentrazone associated with phytoremediator species and inoculation with a bacterial consortium

Christiane Augusta Diniz Melo*, Wendel Magno de Souza, Felipe Paolinelli de Carvalho, André Marcos Massenssini, Antonio Alberto da Silva, Lino Roberto Ferreira, Maurício Dutra Costa

Universidade Federal de Viçosa - Fitotecnia - Viçosa (MG), Brazil.

ABSTRACT: Phytostimulation plays a key role in the process of rhizodegradation of herbicides in soil. Additionally, bio-enhancement associated with phytoremediation may increase the efficiency of the decontamination process of soils with herbicides. Therefore, the objective of this study was to evaluate the biomass and microbial activity of soil contaminated with sulfentrazone and cultivated with phytoremediator species plus a bacterial consortium. The experiment was conducted in a greenhouse, carried out with a 2 × 4 × 4 completely randomized factorial design with 4 replications. The first factor consisted of the presence or absence of bio-enhancement with a bacterial consortium composed of *Pseudomonas* bacteria; the second factor consisted of a monoculture or mixed cultivation of 2 phytoremediator species *Canavalia ensiformis* and *Helianthus annuus*, besides the absence of cultivation; the third factor was made up by the bio-remediation time (25, 45, 65, and 85 days after thinning). Uncultivated soils displayed low values of microbial biomass carbon and microbial quotient as well as high values of metabolic quotient throughout the bio-remediation time, indicating the importance of cultivating phytoremediator species for the stimulation of soil microbiota. Bio-enhancement with the bacterial consortium, in general, promoted an increase in the microbial biomass and activity of soil contaminated with sulfentrazone. In the presence of the bacterial consortium, *Canavalia ensiformis* stimulated a greater activity of associated microbiota and supported a higher microbial biomass. Phytoremediation associated with microbial bio-enhancement are thus promising techniques for the bio-remediation for soils contaminated with sulfentrazone. This technique enhances the biomass and activity of soil microorganisms.

Key words: bio-enhancement, phytostimulation, herbicide, metabolic quotient, microbial quotient.

*Corresponding author: chrisadinizmelo@yahoo.com.br

Received: May 22, 2016 – Accepted: Oct. 3, 2016

INTRODUCTION

Soil is a complex and dynamic ecosystem, where biological activity is determined mainly by microorganisms (Zhou et al. 2011). Its microbes play an important role in nutrient cycling, pest control, maintenance of the soil structure and quality as well as in crop productivity (Gil et al. 2011; Lupwayi et al. 2012). Moreover, microorganisms influence the behaviour of herbicides in the soil since they have the capacity to obtain energy and nutrients for their survival from the transformation of these compounds into less toxic substances for the environment.

Many studies have shown the impact of herbicides on the soil microbial community. The results are varied, with direct or indirect effects, null (Zilli et al. 2008; Nakatani et al. 2014) or negative effects (Santos et al. 2006; Lane et al. 2012), depending on the products and their physical and chemical properties, dosage, type of application, and time after application. However, there are few reports on the microbial activity of soils contaminated with herbicides, and in particular with sulfentrazone, submitted to bio-remediation.

The application of sulfentrazone adversely affects soil microbial activity and biomass (Vivian et al. 2006; Silva et al. 2014). On the other hand, following the application of herbicides, a natural process of adaptation of existing native microbiota in the soil tends to occur, selecting for microorganisms capable of tolerating and or degrading the molecule, therefore, reducing the concentrations of those substances over time.

Sulfentrazone has high residual activity and can persist for years in the soil. Due to its persistence, it can cause injury of sensitive crops grown in succession (Artuzi and Contiero 2006) as well as increase the risk of leaching and environmental contamination. To decontaminate soils with a history of application of this herbicide, bio-remediation techniques involving phytoremediator plants and microorganisms with remedial potential can be used.

Plants contribute significantly to the process of bio-remediation of soil contaminated with herbicides. Through the phytoremediation process, *Helianthus annuus* and *Canavalia ensiformis* are able to reduce toxic levels of sulfentrazone in soils (Belo et al. 2011; Madalão et al. 2012). Phytoremediation may occur by extraction, stabilization, volatilization, accumulation or degradation of the herbicide in a plant tissue or by stimulating associated indigenous microbiota, known as phytostimulation. Plants are able to support large microbial populations in the rhizosphere by exuding substances through the roots, such as carbohydrates and amino acids (Turpault et al. 2007), which supply important sources of nutrients for the microorganisms in the soil-root interface with a consequent stimulus to rhizodegradation.

Phytoremediation associated with bio-enhancement, which is the introduction of microorganisms or consortia to accelerate and to enhance the removal efficiency of in situ toxic compounds (Martin-Hernandez et al. 2012), promotes faster soil decontamination, allowing for a faster planting of sensitive crops in previously contaminated fields. Studies confirm that the increase in initial biomass level improves the biodegration rate of pollutants and increases the treatment efficiency (Pimmata et al. 2013; Szulc et al. 2014).

Soil respiration and enzyme activity are soil biological and biochemical processes regarded as useful indicators of quality and health of this ecosystem (Xiong et al. 2013). In addition, the metabolic and microbial quotients are widely studied as they reflect the microbiota efficiency in the use of carbon and in the quality of soil organic matter (Thirukkumaran and Parkinson 2000; Santos et al. 2006). The study of such indicators in sulfentrazone contaminated soil can help in the understanding of the process of bio-remediation of this herbicide by soil microorganisms and remediator plants.

Thus, the objective of this study was to evaluate the microbial biomass and the microbial activity of sulfentrazone contaminated soil and cultivated with phytoremediator plants as well as a bacterial consortium.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the Federal University of Viçosa, in Viçosa (lat 20°45′S; long 42°52′W), Minas Gerais State. During the experiment, the average maximum and minimum temperatures were 27.6 and 15.5 °C, with 78% of average relative humidity.

The experiment was carried out in a $2 \times 4 \times 4$ completely randomized factorial design with 4 replications. The first factor consisted on the presence or absence of inoculation with a previously selected bacterial consortium; the second factor consisted of a monoculture or a mixed cultivation of 2 phytoremediator species and the absence of cultivation; the third factor was composed of bio-remediation time: 25; 45; 65 and 85 days after thinning (DAT).

Pots with 12.0 dm^3 capacity were coated with a plastic bag and filled with 10.0 dm^3 of medium texture soil, without herbicide application history, with the following chemical characteristics: pH (water) of 6.1; organic matter content = 4.12 $dag \cdot kg^{-1}$; P = 6.9 and K = 200 $mg \cdot dm^{-3}$; Ca = 3.7; Mg = 1.0; Al = 0.0; H + Al = 1.15; and $CTC_{effective}$ = 5.21 $cmol_c \cdot dm^{-3}$. The soil was fertilized with ammonium sulphate (0.20 $g \cdot dm^{-3}$ of N) and simple superphosphate (1.80 $g \cdot dm^{-3}$ of P_2O_5). After that, sulfentrazone was applied in all pots using a backpack sprayer with constant pressure maintained by CO_2, bar coupled with 2 fan type tips TT110 02, spaced by 0.5 m, and working pressure of 250 kPa with spray volume of approximately 140 $L \cdot ha^{-1}$ at a dose of 1,000 $g \cdot ha^{-1}$ a.i. The environmental conditions at the time of application were T = 27 °C, RH = 72%, and wind speed of 1.9 $km \cdot h^{-1}$.

One day following herbicide application, the remedial species *Canavalia ensiformis* (Madalão et al. 2012) and *Helianthus annuus* (cultivar Tera 860 HO) (Belo et al. 2011) were sown. Sowing was performed at 5 cm depth using 6 seeds per pot. After 15 days of sowing, thinning was conducted, leaving 2 plants of the same species or 1 of each (mixed cultivation) in each experimental unit.

The bacterial consortium used was selected based on the degradation potential presented by the isolates in a previous study (Melo et al. 2016). The consortium consisted of 6 isolates identified as *Pseudomonas putida*, *P. lutea*, and *P. plecoglossicida*, as well as 3 isolates of *Pseudomonas* sp. They were grown separately on nutrient broth medium (1.0 L water; 2.0 g Na_2HPO_4; 3.0 g NaCl; 3.0 g of meat extract; 5.0 g peptone; 6.8 pH) for about 10 h at 30 °C and 150 rpm until reaching an optical density of 0.6. The medium was centrifuged (5,000 rpm; 5.0 min; 4 °C) and bacterial cells, suspended in saline solution (NaCl 0.85%). The consortium was established by pipetting volumes of solution of each of the 6 isolates to assure equal amount of colony forming units ($CFU \cdot mL^{-1}$) of each species, totalling 12.0 mL of solution, and inoculating 4.5×10^4 $CFU \cdot g^{-1}$ of soil in the corresponding treatments, right after the thinning.

The phytoremediator species were grown for 25; 45; 65 and 85 DAT, at which they were cut close to the ground. At the harvest dates, *Canavalia ensiformis* plants were at the vegetative stages V2 to V3; V4 to V5; V6 to V7 and V8 to V9 and *Helianthus annuus* plants were at the stages V16 to V18; R1 to R3; R5.1 to R5.8 and 5.10 to R6, respectively. The root system of the plants was removed, and all the soil was homogenized to take samples from each pot. The samples were placed in plastic bags and kept under refrigeration for 24 h until moisture determination. Respiratory rate, microbial biomass carbon, soil metabolic quotient, and microbial quotient were all estimated.

To assess respiratory rate, the respirometric method was used to determine C-CO_2 evolved from the soil in which samples of 100.0 g of sieved soil (2 mm mesh) and humidity equal to 60% of field capacity, in duplicate, were incubated for 15 days in airtight bottles at room temperature. The C-CO_2 released from soil was carried by continuous flow of free CO_2 air into another bottle containing 100.0 mL of NaOH 0.5 $mol \cdot L^{-1}$. Fifteen days after incubation, the C-CO_2 evolved was estimated from the titration of 10.0 mL of NaOH (0.5 $mol \cdot L^{-1}$) solution, added with 5.0 mL of $BaCl_2$ (0.5 $mol \cdot L^{-1}$) with solution of HCl 0.5 $mol \cdot L^{-1}$ and 3 drops of phenolphthalein (1%).

Following the incubation period, 18.0 g of soil from each jar were weighed for determination of microbial biomass carbon (MBC) according to a methodology modified from Islam and Weil (1998). From the obtained values of C-CO_2 evolution and MBC, the metabolic quotient (qCO_2, in $\mu g \cdot \mu g^{-1} \cdot d^{-1}$) was calculated by dividing the daily average of soil evolved C-CO_2 by MBC determined in the soil (Anderson and Domsch 1993). The microbial quotient (qMIC, in %) was calculated as the ratio between the MBC and organic carbon (OC) (Anderson and Domsch 1989) of each experimental unit obtained through the content of soil organic matter (OM = OC × 1.724) estimated by the Walkley-Black method.

Data were submitted to analysis of variance by the F-test at 5% probability. Significant interactions were evaluated and the means, compared by Tukey's multiple comparisons test ($p < 0.05$). For bio-remediation times, simple linear models, $Y = \alpha + \beta_1 \cdot X + \varepsilon$, and also the quadratic ones, $Y = \alpha + \beta_1 \cdot X + \beta_2 \cdot X^2 + \varepsilon$, were adjusted. The models were adjusted and compared for equality of the parameters α, β1, and β2 (Littell et al. 2006).

RESULTS AND DISCUSSION

The triple interaction among cultivation, inoculation, and bio-remediation time was significant for all assessed variables. The respiratory rate found in soils cultivated simultaneously with the 2 phytoremediator species was 42% higher than that of soils cultivated with →

C. ensiformis in the absence of inoculation, on the 25th DAT. On the 65th DAT, in treatments with inoculation of the bacterial consortium, the soil cultivated with *H. annuus* had a higher respiratory rate when compared to the other soils (Table 1).

Soil inoculated with the bacterial consortium promoted specific effects depending on the species cultivated and on the bio-remediation time. On the 25th DAT, inoculation provided an increase of 44% in the respiratory rate of soil cultivated with *C. ensiformis*. The same behavior was observed for soils with *H. annuus* and mixed cultivation on the 85th DAT. On the other hand, the lowest respiration rate was found in soil cultivated with *C. ensiformis*, in the presence of inoculation on the 65th DAT (Table 1).

The respiratory rate of the soil cultivated with *H. annuus* and inoculation linearly increased over bio-remediation time, as well as uncultivated soil, regardless of the presence of bacterial consortium. Those treatments presented higher evolution of C-CO_2 (Figure 1a).

Soil cultivated with *H. annuus* and mixed cultivation, both without inoculation, also presented a linear behavior over time; however, lower microbial activity was observed (Figure 1a). Treatments with *C. ensiformis* and mixed cultures, both in the presence of inoculation, did not differ from each other and were adjusted into a common curve with quadratic effect and increase in microbial respiration from the 40th DAT.

Uncultivated soil, regardless of inoculation and bio-remediation time, had lower microbial biomass carbon than cultivated soils (Table 2). Regarding the presence of cultivation, *C. ensiformis*, on the 65th and 85th DAT in the presence and absence of microbial consortium, respectively, supported higher microbial biomass than *H. annuus* and mixed cultivation. On the 45th and 85th DAT, soils inoculated and solely cultivated with *C. ensiformis* and *H. annuus* exhibited greater MBC (Table 2).

Inoculation provided an increase in MBC in soils cultivated with the 2 species together on the 25th DAT, by *C. ensiformis* on the 65th and the 85th DAT and by *H. annuus* on the 85th DAT (Table 2).

Uncultivated soils, inoculated and non-inoculated showed a decreased MBC from the 63th DAT and, in general, lower microbial biomass in relation to the cultivated ones. For the other treatments, an increase in the MBC was found over bio-remediation time (Figure 1b). In the presence of the bacterial consortium, *C. ensiformis* favoured the establishment of high microbial biomass in the soil over the 85 days of cultivation (Figure 1b).

Soil metabolic quotient with no cultivation was the highest, regardless of inoculation on the 65th and 85th DAT (Table 3). On the 85th DAT, the average difference between cultivated and uncultivated soils reached 66% in the presence of inoculation and 71% in the absence of the bacterial consortium (Table 3).

Table 1. Respiratory rate of soil with sulfentrazone cultivated with the phytoremediator species *Canavalia ensiformis* and *Helianthus annuus* in monoculture or mixed cultivation, in the absence or presence of bacterial consortium.

Respiratory rate ($\mu g \cdot g^{-1} \cdot d^{-1}$)								
Crops	Inoculation				Inoculation			
	Presence		Absence		Presence		Absence	
	25 DAT				45 DAT			
Canavalia ensiformis	88.98	Aa[1]	49.87	Bb	90.44	Aa	87.39	Aa
Helianthus annuus	71.12	Aa	68.93	Aab	92.89	Aa	100.22	Aa
Mixed cultivation	75.53	Aa	86.53	Aa	100.83	Aa	97.78	Aa
No crop	63.03	Aa	54.27	Aab	122.83	Aa	78.83	Aa
Crops	**65 DAT**				**85 DAT**			
Canavalia ensiformis	95.33	Bb	121.00	Aa	150.33	Aa	129.56	Aa
Helianthus annuus	134.44	Aa	115.50	Aa	163.78	Aa	129.56	Ba
Mixed cultivation	95.33	Ab	107.56	Aa	151.56	Aa	124.06	Ba
No crop	114.89	Aab	125.89	Aa	140.56	Aa	138.72	Aa
CV (%)	16.85							

[1]Means followed by the same lowercase letters in the column and uppercase letters on the row for each season do not differ by the Tukey's test ($p > 0.05$). DAT = Days after thinning; CV = Coefficient of variation.

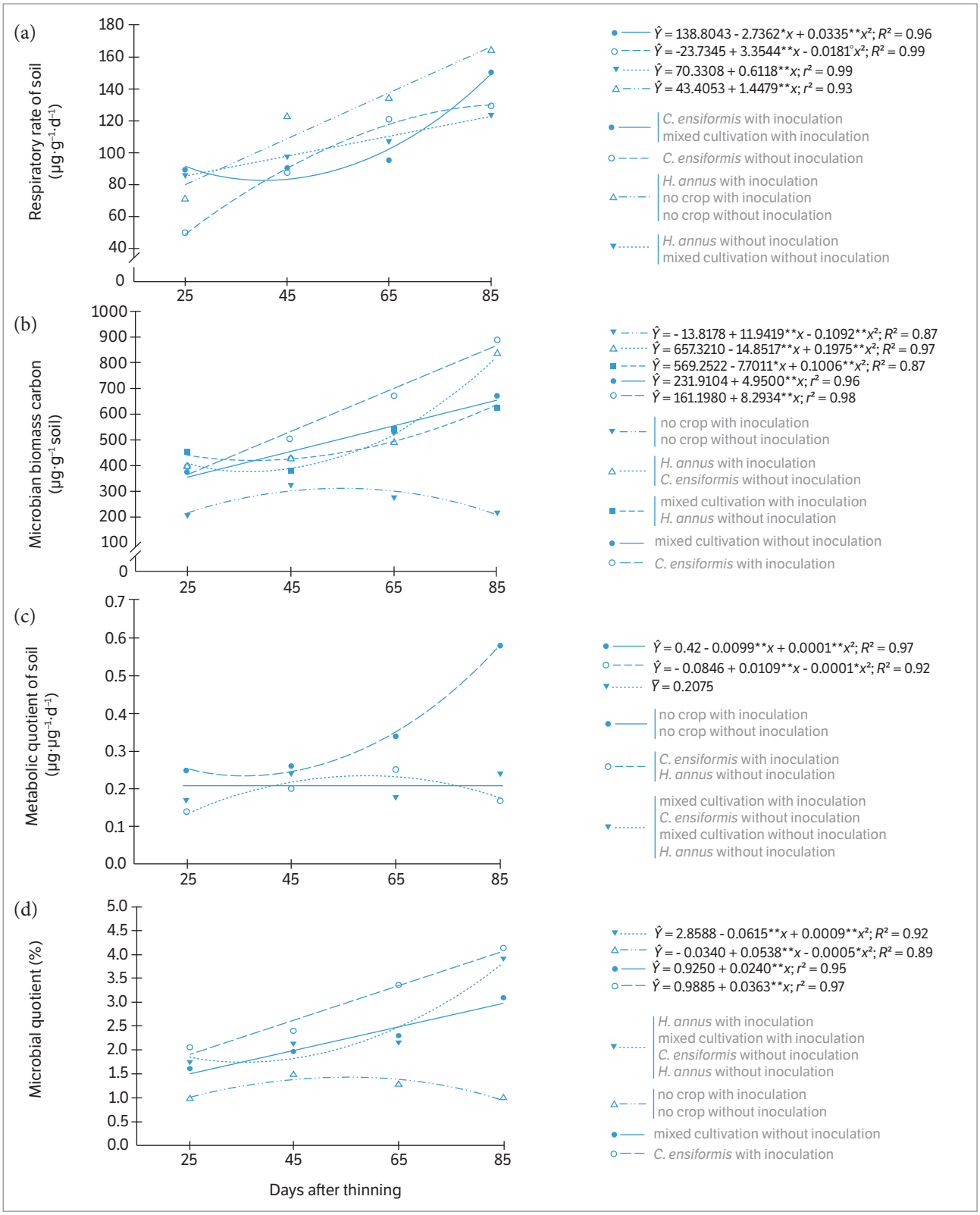

Figure 1. Respiratory rate (a), microbial biomass carbon (b), metabolic quotient (c), and microbial quotient (d) of soil contaminated with sulfentrazone and cultivated with phytoremediator species *Canavalia ensiformis* and *Helianthus annuus*, in monoculture or mixed cultivation, for different times, in the absence or presence of bacteria consortium. Models that did not differ from each other were presented in a common curve by the Model Identity Test ($p \leq 0.05$). **,* and $^{\circ}$Significant at 1, 5 and 10%, respectively, by the t-test.

Table 2. Microbial biomass carbon of soil with sulfentrazone cultivated with the phytoremediator species *Canavalia ensiformis* and *Helianthus annuus* in monoculture or mixed cultivation, in the absence or presence of a bacterial consortium.

Crops	Microbial biomass carbon (μg·g^{-1} soil)							
	Inoculation				Inoculation			
	Presence		Absence		Presence		Absence	
	25 DAT				45 DAT			
Canavalia ensiformis	396.00	Aa[1]	369.33	Aa	506.00	Aa	434.50	Aa
Helianthus annuus	396.00	Aa	440.00	Aa	429.33	Aab	432.67	Aa
Mixed cultivation	454.00	Aa	379.50	Ba	381.33	Ab	429.00	Aa
No crop	262.50	Ab	209.00	Ab	385.33	Ab	325.00	Ab
Crops	**65 DAT**				**85 DAT**			
Canavalia ensiformis	674.67	Aa	495.00	Ba	892.67	Aa	795.00	Ba
Helianthus annuus	486.00	Ab	473.50	Aa	835.33	Aa	645.33	Bb
Mixed cultivation	539.00	Ab	533.00	Aa	626.67	Ab	674.67	Ab
No crop	341.50	Ac	278.67	Ab	245.00	Ac	220.00	Ac
CV (%)	10.99							

[1]Means followed by the same lowercase letters in the column and uppercase letters on the row for each season do not differ by the Tukey's test ($p > 0.05$). DAT = Days after thinning; CV = Coefficient of variation.

Table 3. Metabolic quotient of soil with sulfentrazone cultivated with the phytoremediator species *Canavalia ensiformis* and *Helianthus annuus*, in monoculture or mixed cultivation, and in the absence or presence of a bacterial consortium.

Crops	Metabolic quotient (qCO_2; μg·μg^{-1}·d^{-1})							
	Inoculation				Inoculation			
	Presence		Absence		Presence		Absence	
	25 DAT				45 DAT			
Canavalia ensiformis	0.25	Aa[1]	0.14	Bb	0.18	Ab	0.20	Aa
Helianthus annuus	0.18	Aa	0.16	Ab	0.30	Aab	0.24	Aa
Mixed cultivation	0.17	Aa	0.23	Aab	0.25	Aab	0.23	Aa
No crop	0.25	Aa	0.28	Aa	0.26	Aa	0.24	Aa
65 DAT					**85 DAT**			
Canavalia ensiformis	0.14	Bc	0.25	Ab	0.17	Ab	0.17	Ab
Helianthus annuus	0.28	Aab	0.25	Ab	0.20	Ab	0.19	Ab
Mixed cultivation	0.18	Abc	0.20	Ab	0.24	Ab	0.20	Ab
No crop	0.34	Ba	0.45	Aa	0.58	Aa	0.65	Aa
CV (%)	22.70							

[1]Means followed by the same lowercase letters in the column and uppercase letters on the row for each season do not differ by the Tukey's test ($p > 0.05$). DAT = Days after thinning; CV = Coefficient of variation.

On the 25th DAT, soil without inoculation cultivated with *C. ensiformis* exhibited lower qCO_2 (Table 3) in an inverse manner and at the same proportion as on the 65th DAT. A significant difference caused by the presence of the bacterial inoculum was also observed in uncultivated soil on the 65th DAT (Table 3).

Over bio-remediation time, uncultivated soils displayed increasing values of metabolic quotient from the 50th DAT (Figure 1c). Soil cultivated with *C. ensiformis* without inoculation and cultivated with *H. annuus* with inoculation presented an average reduction in qCO_2 from the 55th DAT (Figure 1c). The mixed cultivation, regardless of inoculation, as well as *C. ensiformis* with inoculation and *H. annuus* without inoculation, did not have metabolic quotient changes over the cultivation period, especially with low values close to 0.20 μg·μg^{-1}·d^{-1} (Figure 1c).

The microbial quotient was influenced by all studied factors, being lower in uncultivated soils, despite the season and inoculation (Table 4). Monocultures of *C. ensiformis* and *H. annuus*, in the presence of inoculation,

Table 4. Microbial quotient of soil with sulfentrazone cultivated with phytoremediator species, in monoculture or mixed cultivation, and in the presence or absence of a bacterial consortium.

Microbial quotient (*q*MIC; %)								
Crops	Inoculation				Inoculation			
	Presence		Absence		Presence		Absence	
	25 DAT				45 DAT			
Canavalia ensiformis	2.04	Aa[1]	1.76	Aa	2.40	Aa	2.04	Aa
Helianthus annuus	1.75	Aa	1.89	Aa	2.14	Aab	2.05	Aa
Mixed cultivation	2.08	Aa	1.62	Ba	1.79	Abc	1.97	Aa
No crop	1.23	Ab	0.97	Ab	1.61	Ac	1.48	Ab
Crops	**65 DAT**				**85 DAT**			
Canavalia ensiformis	3.36	Aa	2.22	Ba	4.14	Aa	3.69	Ba
Helianthus annuus	2.16	Ab	2.13	Aa	3.92	Aa	3.03	Bb
Mixed cultivation	2.45	Ab	2.30	Aa	2.84	Ab	3.10	Ab
No crop	1.66	Ac	1.28	Bb	1.08	Ac	1.00	Ac
CV (%)	12.20							

[1]Means followed by the same lowercase letters in the column and uppercase letters on the row for each season do not differ by the Tukey's test ($p > 0.05$). DAT = Days after thinning; CV = Coefficient of variation.

had higher *q*MIC values on the 45th and 85th DAT. The *q*MIC associated with *C. ensiformis* was higher on the 65th and 85th DAT, with the presence and absence of inoculum, respectively (Table 4).

Inoculation promoted beneficial effects as *q*MIC increased in soils cultivated with *H. annuus* and *C. ensiformis* simultaneously on the 25th DAT, with *C. ensiformis* and uncultivated soil on the 65th DAT and with *C. ensiformis* and *H. annuus* separately on the 85th DAT (Table 4).

Cultivation of *C. ensiformis* added with microbial consortium inoculation provided an increase in *q*MIC over bio-remediation time, compared to the other treatments (Figure 1d). The *q*MIC of soil cultivated with both phytoremediator species simultaneously without inoculation increased linearly over time as well. In uncultivated soils, regardless of inoculation, the *q*MIC presented decreasing values from the 54th DAT (Figure 1d).

Microbial degradation of herbicides in the soil depends on the presence and activity of microorganisms able of (co)metabolizing them. The presence of herbicide in the soil, and particularly the cultivation of phytoremediator species, may support microbial growth and induce the release of increasing C-CO_2 over bio-remediation time, as observed in Figure 1a. The respiratory rate of the soil has been widely used in studies on waste decomposition and bio-remediation, associated with the quantification of the pollutant levels in the soil (Thirukkumaran and Parkinson 2000; Lamy et al. 2013). The evolution of CO_2 in the soil, alone, does not allow consistent interpretations since a high respiratory rate may indicate an active and degrading population in an ecosystem with a high level of productivity as well as an ecological disorder or disturbance. The latter may explain the high respiratory rates observed over time in uncultivated soils (Figure 1a).

MBC was very sensitive to the presence of phytoremediator species, reducing dramatically in the absence of cultivation (Table 2, Figure 1b). In uncultivated soils, it is very common to find fewer microorganisms and lower metabolic activity compared to cultivated soils because there is no supply of carbon and energy via plant root exudation (Sandmann and Loos 1984). Cultivated soils support higher microbial biomass by means of the root exudates, which influence the growth of bacteria and fungi that colonize the rhizosphere with environmental changes in the surrounding soil serving as a substrate for selective growth of microorganisms (Cardoso and Nogueira 2007). Greater activity and microbial biomass in the rhizosphere soils treated with herbicides, in relation to non-vegetated soils, were also found in other studies (Pires et al. 2005; Santos et al. 2007; Santos et al. 2010), and this effect is attributed to phytostimulation of the associated microbiota.

This study failed to draw conclusions on the reduction of sulfentrazone concentration in the soil upon the action of →

microorganisms. However, there is a relationship between the size of soil microbial biomass and herbicide degradation capacity and contaminants in this ecosystem (Voos and Groffman 1997; Lamy et al. 2013). In the context of bio-remediation, high and active microbial biomass associated with phytoremediator species *C. ensiformis* and *H. annuus* is of great interest because it may enhance the soil decontamination process with sulfentrazone.

Given the complexity of the soil ecosystem, the variety of compounds exuded by the roots, and the countless interactions established between plants and microorganisms, there are many possibilities for the transformation of xenobiotic compounds by rhizodegradation, a mechanism that contributes, in an integrated manner, to phytostimulation (Santos et al. 2007). Moreover, the variety of exudate compounds as well as the phenological growth stage of plants may influence their effectiveness in the bio-remediation process (Santos et al. 2010). The species *Helianthus annuus* and *Canavalia ensiformis*, previously identified as sulfentrazone phytoremediators (Belo et al. 2011; Madalão et al. 2012), display quite different morphophysiological and growth characteristics, which may result in distinct capacities for phytostimulation, as found in this study, and, therefore, for herbicide rhizodegradation in the soil.

Overall, the bio-enhancement provided positive results, improving the activity and microbial biomass associated with certain phytoremediator species at certain times. The effects observed over time suggest the existence of tolerance and possible degradation of sulfentrazone by the most adapted microbiota and selected after application of the herbicide. According to Wang et al. (2014), one of the limitations of bio-enhancement is that, in many occasions, contaminated sites may be deficient in nutrients that do not support the rapid growth of the added bacteria. However, in this study, although the survival of the bacterial consortium was not evaluated, it is believed that the presence of cultivation was crucial to ensure favorable conditions for growth and viability of these inoculated microorganisms. There is not much information in the literature regarding bio-enhancement in the bio-remediation of soils contaminated with herbicides.

The treatment consisting of *C. ensiformis* added with the bacterial consortium stood out among the others in relation to MBC and *q*MIC over bio-remediation time. Such evidence reinforces that *C. ensiformis* is effective in stimulating microbiota and very promising for the remediation of soils contaminated with sulfentrazone. Pires et al. (2005), evaluating the rhizospheric activity of species with potential for phytoremediation of the herbicide tebuthiuron, found that the microbial community associated with roots of *C. ensiformis* was the largest and the most active among the treatments studied.

The low qCO_2 values observed during bio-remediation in soils cultivated with *C. ensiformis* added with bacterial consortium, *H. annuus* without inoculation, and mixed cultivation indicate savings in the use of energy by microorganisms and presumably reflect a more stable environment or closer to its equilibrium state. This result indicates a lower C-CO_2 loss and higher incorporation of carbon in the microbial cells, which may evidence an active and more efficient microbiota in nutrient acquisition. On the other hand, the high qCO_2 values found in uncultivated soils are indicative of ecosystems that underwent stress or disturbance (Tótola and Chaer 2002), which may be related to the presence of herbicide and exhaustion of the nutrients in these soils. Such conditions stimulate the oxidation of organic matter by microorganisms resulting in lower soil conservation. These achievements thus indicate the long-term carbon losses that may occur if soils contaminated with sulfentrazone are not submitted to any bio-remediation technique as well as the importance of cultivation to encourage microorganisms to promote rhizodegradation and to improve the efficiency of soil herbicide decontamination.

A similar behaviour of the curves adjusted to MBC and *q*MIC evidences the great influence of the MBC on the results of *q*MIC and a small influence of the soil organic carbon that has shown low variation among treatments. Carbon utilization capacity was increased (> *q*MIC) over time in inoculated soil, cultivated with *C. ensiformis*; however, in uncultivated soils, regardless of the inoculation, the microorganisms showed less economical metabolism (< *q*MIC). Overall, less organic carbon (C) was channelled into energy metabolism and more C was fixed in the microbial cells in cultivated soils. These results indicate a microbiota efficient in the use and incorporation of carbon into biomass and an active population in the soil, as well as desirable features that may reflect on the degradation of the herbicide.

CONCLUSION

By means of microbiological indicators, it was observed that uncultivated soils displayed lower microbial biomass and activity than the cultivated ones. The phytoremediator species, in monoculture or mixed cultivation, have different capacities of stimulating the rhizosphere microorganisms. *Canavalia ensiformis* in the presence of a bacterial consortium is able to provide greater activity of associated microbiota and to support a higher microbial biomass. Phytoremediation associated with microbial bio-increase is a promising technique for the bio-remediation of soils contaminated with sulfentrazone.

ACKNOWLEDGEMENTS

We thank the National Council of Scientific and Technological Development (CNPq) and the Minas Gerais Research Foundation (FAPEMIG) for the scholarship and support provided.

REFERENCES

Anderson, T. H. and Domsch, K. H. (1989). Ratios of microbial biomass carbon to total organic carbon in arable soils. Soil Biology and Biochemistry, 21, 471-479. http://dx.doi.org/10.1016/0038-0717(89)90117-X.

Anderson, T. H. and Domsch, K. H. (1993). The metabolic quotient of CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental condition, such as pH, on the microbial of forest soil. Soil Biology and Biochemistry, 25, 393-395. http://dx.doi.org/10.1016/0038-0717(93)90140-7.

Artuzi, J. P. and Contiero, R. L. (2006). Herbicidas aplicados na soja e produtividade do milho em sucessão. Pesquisa Agropecuária Brasileira, 41, 1119-1123. http://dx.doi.org/10.1590/S0100-204X2006000700007.

Belo, F. A., Coelho, A. T. C. P., Ferreira, L. R., Silva, A. A. and Santos, J. B. (2011). Potencial de espécies vegetais na remediação de solo contaminado com sulfentrazone. Planta Daninha, 29, 821-828. http://dx.doi.org/10.1590/S0100-83582011000400012.

Cardoso, E. J. B. N. and Nogueira, M. A. (2007). A rizosfera e seus efeitos na comunidade microbiana e na nutrição de plantas. In A. P. D. Silveira and S. S. Freitas (Eds.), Microbiota do solo e qualidade ambiental (p. 79-96). Campinas: Instituto Agronômico.

Gil, S. V., Meriles, J., Conforto, C., Basanta, M., Radl, V., Hagn, A., Schloter, M. and March, G. J. (2011). Response of soil microbial communities to different management practices in surface soils of a soybean agroecosystem in Argentina. European Journal of Soil Biology, 47, 55-60. http://dx.doi.org/10.1016/j.ejsobi.2010.11.006.

Islam, K. R. and Weil, R. R. (1998). Microwave irradiation of soil for routine measurement of microbial biomass carbon. Biology and Fertility of Soils, 27, 408-416. http://dx.doi.org/10.1007/s003740050451.

Lamy, E., Tran, T. C., Mottelet, S., Pauss, A. and Schoefs, O. (2013). Relationships of respiratory quotient to microbial biomass and hydrocarbon contaminant degradation during soil bioremediation. International Biodeterioration and Biodegradation, 83, 85-91. http://dx.doi.org/10.1016/j.ibiod.2013.04.015.

Lane, M., Lorenz, N., Saxena, J., Ramsier, C. and Dick, R. P. (2012). Microbial activity, community structure and potassium dynamics in rhizosphere soil of soybean plants treated with glyphosate. International Journal of Soil Biology, 55, 153-159. http://dx.doi.org/10.1016/j.pedobi.2011.12.005.

Littell, R. C., Milliken, G. A., Stroup, W. W., Wolfinger, R. D. and Schabenberger, O. (2006). SAS® for mixed models. 2. ed. Cary: SAS Institute.

Lupwayi, N. E., Lafond, G. P., Ziadi, N. and Grant, C. A. (2012). Soil microbial response to nitrogen fertilizer and tillage in barley and corn. Soil Tillage Research, 118, 139-146. http://dx.doi.org/10.1016/j.still.2011.11.006.

Madalão, J. C., Pires, F. R., Cargnelutti Filho, A., Chagas, K., Nascimento, A. F. and Garcia, G. O. (2012). Fitorremediação de solos contaminados com o herbicida sulfentrazone por espécies de adubos verdes. Revista Ciências Agrárias, 55, 288-296. http://dx.doi.org/10.4322/rca.2012.068.

Martin-Hernandez, M., Suarez-Ojeda, M. E. and Carrera, J. (2012). Bioaugmentation for treating transient or continuous p-nitrophenol shock loads in an aerobic sequencing batch reactor. Bioresource Technology, 123, 150-156. http://dx.doi.org/10.1016/j.biortech.2012.07.014.

Melo, C. A. D., Massensini, A. M., Passos, A. B. R. J., Carvalho, F.P., Ferreira, L. R., Silva, A. A. and Costa, M. D. (2016). Isolation and characteristics of sulfentrazone-degrading bacteria. Journal of Environmental Science and Health: Part B, 52,115-121. http://dx.doi.org/10.1080/03601234.2016.1248136.

Nakatani, A. S., Fernandes, M. F., Souza, R. A., Silva, A. P., Reis Júnior, F. B., Mendes, I. C. and Hungria, M. (2014). Effects of the glyphosate-resistance gene and of herbicides applied to the soybean crop on soil microbial biomass and enzymes. Field Crops Research, 162, 20-29. http://dx.doi.org/10.1016/j.fcr.2014.03.010.

Pimmata, P., Reungsang, A. and Plangklang, P. (2013). Comparative bioremediation of carbofuran contaminated soil by natural attenuation, bioaugmentation and biostimulation. International Biodeterioration and Biodegradation, 85, 196-204. http://dx.doi.org/10.1016/j.ibiod.2013.07.009.

Pires, F. R., Souza, C. M., Cecon, P. R., Santos, J. B., Tótola, M. R., Procópio, S. O., Silva, A. A. and Silva, C. S. W. (2005). Inferências sobre atividade rizosférica de espécies com potencial para fitorremediação do herbicida tebuthiuron. Revista Brasileira de Ciência do Solo, 29, 627-634. http://dx.doi.org/10.1590/S0100-06832005000400015.

Sandmann, E. R. and Loos, M. A. (1984). Enumeration of 2,4-D-degrading microorganisms in soils and crop plant rhizospheres using indicator media: high populations associated with sugarcane (*Saccharum officinarum*). Chemosphere, 13, 1073-1084. http://dx.doi.org/10.1016/0045-6535(84)90066-3.

Santos, E. A., Costa, M. D., Ferreira, L. R., Reis, M. R., França, A. C. and Santos, J. B. (2010). Atividade rizosférica de solo tratado com herbicida durante processo de remediação por *Stizolobium aterrimum*. Pesquisa Agropecuária Tropical, 40, 1-7. http://dx.doi.org/10.5216/pat.v40i1.4670.

Santos, E. A., Santos, J. B., Ferreira, L. R., Costa, M. D. and Silva, A. A. (2007). Fitoestimulação por *Stizolobium aterrimum* como processo de remediação de solo contaminado com trifloxysulfuron-sodium. Planta Daninha, 25, 259-265. http://dx.doi.org/10.1590/S0100-83582007000200004.

Santos, J. B., Jakelaitis, A., Silva, A. A., Costa, M. D., Manabe, A. and Silva, M. C. S. (2006). Action of two herbicides on the microbial activity of soil cultivated with common bean (*Phaseolus vulgaris*) in conventional-till and no-till systems. Weed Research, 46, 284-289. http://dx.doi.org/10.1111/j.1365-3180.2006.00510.x.

Silva, G. S., Melo, C. A. D., Fialho, C. M. T., Tuffi Santos, L. D., Costa, M. D. and Silva, A. A. (2014). Impact of sulfentrazone, isoxaflutole and oxyfluorfen on the microorganisms of two forest soils. Bragantia, 73, 292-299. http://dx.doi.org/10.1590/1678-4499.0061.

Szulc, A., Ambrozewicz, D., Sydow, M., Lawniczak, Q., Piotrowska-Cyplik, A., Marecik, R. and Chrzanowski, L. (2014). The influence of bioaugmentation and biosurfactant addition on bioremediation efficiency of diesel-oil contaminated soil: Feasibility during field studies. Journal of Environmental Management, 132, 121-128. http://dx.doi.org/10.1016/j.jenvman.2013.11.006.

Thirukkumaran, C. M. and Parkinson, D. (2000). Microbial respiration, biomass, metabolic quotient and litter decomposition in a lodgepole pine forest floor amended with nitrogen and phosphorous fertilizers. Soil Biology and Biochemistry, 32, 59-66. http://dx.doi.org/10.1016/S0038-0717(99)00129-7.

Tótola, M. R. and Chaer, M. G. (2002). Microrganismos e processos microbiológicos como indicadores da qualidade dos solos. In V. H. Alvarez, V. C. E. G. R. Schaefer, N. F. Barros, J. W. V. Mello and L. M. Costa (Eds.), Tópicos em ciência do solo (p. 195-276). Viçosa: Sociedade Brasileira de Ciência do Solo.

Turpault, M. P., Gobran, G. R. and Bonnaud, P. (2007). Temporal variations of rhizosphere and bulk soil chemistry in a Douglas fir stand. Geoderma, 137, 490-496. http://dx.doi.org/ 10.1016/j.geoderma.2006.10.005.

Vivian, R., Reis, M. R., Jakelaitis, A., Silva, A. F., Guimarães, A. A., Santos, J. B. and Silva, A. A. (2006). Persistência de sulfentrazone em Argissolo Vermelho-Amarelo cultivado com cana-de-açúcar. Planta Daninha, 24, 741-750. http://dx.doi.org/10.1590/S0100-83582006000400015.

Voos, G. and Groffman, P. M. (1997). Relationships between microbial biomass and dissipation of 2,4-D and dicamba in soil. Biology and Fertility of Soils, 24, 106-110. http://dx.doi.org/10.1007/BF01420229.

Wang, T., Sun, H., Jiang, C., Mao, H. and Zhang, Y. (2014). Immobilization of Cd in soil and changes of soil microbial

community by bioaugmentation of UV-mutated *Bacillus subtilis* 38 assisted by biostimulation. European Journal of Soil Biology, 65, 62-69. http://dx.doi.org/10.1016/j.jhazmat.2014.06.028.

Xiong, D., Gao, Z., Fu, B., Sun, H., Tian, S., Xiao, Y. and Quin, Z. (2013). Effect of pyrimorph on soil enzymatic activities and respiration. European Journal of Soil Biology, 56, 44-48. http://dx.doi.org/10.1016/j.ejsobi.2013.02.005.

Zhou, X. G., Yu, G. B. and Wu, F. Z. (2011). Effects of intercropping cucumber with onion or garlic on soil enzyme activities, microbial communities and cucumber yield. European Journal of Soil Biology, 47, 279-287. http://dx.doi.org/10.1016/j.ejsobi.2011.07.001.

Zilli, J. E., Botelho, G. R., Neves, M. C. P. and Rumjanek, N. G. (2008). Efeito de glyphosate e imazaquin na comunidade bacteriana do rizoplano de soja (*Glycine max* (L.) Merrill) e em características microbiológicas do solo. Revista Brasileira de Ciência do Solo, 32, 633-642. http://dx.doi.org/10.1590/S0100-06832008000200018.