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Glucose mineralization in soils of contrasting textures under application of S-metolachlor, terbuthylazine, and mesotrione, alone and in a mixture

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ABSTRACT: Microbial adaptation may occur in surface soils under agricultural uses exposed to herbicides. However, little is known about herbicide mixture effects in the soil, especially in tropical regions like Brazil. The aim of this study was to evaluate glucose mineralization in soils of contrasting textures (sandy clay and sandy loam) from areas cultivated with maize under application of S-metolachlor, terbuthylazine and mesotrione, alone and in a mixture. The methodology was established according to the soil microorganisms: carbon transformation test with ^{14}C-glucose solution (D-[U-^{14}C] glucose) in biometric flasks. After the addition of ^{14}C-glucose, the amount of ^{14}C in cumulative CO_2 of microbial respiration was measured several times during the 28-day incubation. For unamended soil control (without herbicide), microbial activity followed a similar behavior to amended soil with herbicides in total $^{14}CO_2$ released and accumulated, ranging from 23 to 27%. Overall, mineralization constant rate (k) values for all treatments were also similar, with an average value of 0.0038% $CO_2.d^{-1}$. Consequently, mineralization half-life times (MT50) were from 173 to 198 d. Microbial respiration for all treatments was slightly higher in the sandy clay compared with sandy loam soil; although soil samples with application of herbicides (alone and in a mixture) did not show decreased basal microbial respiration or mineralization rates of glucose. To corroborate these findings, additional research with different organic substrates and in cultures with different applications of herbicides are needed to prove the non interference of these herbicides on the microbial respiration in the soil.

Key words: microbial respiration, tropical condition, herbicides.

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INTRODUTION

Microbial activity influences the dynamics of nutrients in the soil, promoting the decomposition of soil organic matter (SOM), what means mineralization and solubilization of nutrients in the soil solid phase. These organisms also have other important functions such as the suppression of pathogens, the production of phytohormones and decomposition of pesticides, including herbicides; and multitrophic level microbial interactions drive eco and agro-biotechnological processes such as bioremediation, wastewater treatment, plant growth promotion, and ecosystem functioning (Bottomley 2005; Saleem and Moe 2014).

However, microbial adaptation may occur in surface soil under agricultural uses exposed to herbicides, interfering positively, providing an increase in the metabolism of these products by the microorganisms (Reis et al. 2008; Hussain et al. 2009a), but also interfering negatively by intoxicating soil biota (non-adapted organisms) or having no effect (Pereira et al. 2008; Mahía et al. 2008; Blume and Reichert 2015). Soils rich in SOM and with residual concentration of herbicides may increase the herbicide degradation rate, as shown for atrazine by Mirgain et al. (1993) in laboratory microcosm conditions, possibly because of microbial adaptation to repeated herbicide exposure.

S-metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(1S)2methoxy1- methylethyl]acetamide), terbuthylazine (6-chloroN-(1,1-dimethylethyl)-N'ethyl-1,3,5 triazine-2,4-diamine), and mesotrione (2-[4-(methylsulfonyl) 2-nitrobenzoyl]-1,3- cyclohexanedione) are widely used in pre- or early post-emergence to control weeds in maize. S-metolachlor is a member of the chloroacetanilide family and inhibits the very long chain fatty acid (VLCFA) formation, which interferes with normal cell development and curbs both cell division and growth (Trenkamp et al. 2004). Terbuthylazine is a member of the triazine family and inhibits photosynthesis by supressing electron transfer at the reducing site of photosystem II in the chloroplasts (Good 1961). Mesotrione is a weak acid, member of the triketone family of herbicides. Susceptible plants are controlled through inhibition of the 4-hydroxyphenyl-pyruvate dioxygenase (4-HPPD) enzyme, which affects carotenoid biosynthesis (Mitchell et al. 2001). Given the high efficiency of these herbicides in weed control, a commercial mixture is recommended, e.g. Lumax® used in Italy (Pinna et al. 2014; Otto et al. 2016), with a label dose of 37.5, 212.5, and 187.5 $g \cdot L^{-1}$ of mesotrione, S-metolachlor, and terbuthylazine, respectively.

Little is known about the impact of herbicide mixtures on soils (Joly et al. 2015), especially in tropical regions like Brazil. Studies regarding this subject are usually carried out considering single molecules. However, Mendes et al. (2016) found that mesotrione, applied both alone and in a mixture with S-metolachlor + terbuthylazine, had no influence on its sorption or desorption, and Mendes et al. (2017) reported that mesotrione degradation rate was influenced by soil texture regardless if applied alone or in mixture. Concurrently, mesotrione sorption and biotransformation in the soil are relatively low and quick, respectively, indicating leaching potential, which can enter the groundwater in maize production fields. But there is little knowledge about the interference of these herbicides on the microbial respiration of soils, which can be measured by ^{14}C-labeled glucose mineralization in the amount of ^{14}C in CO_2 (Tian et al. 2015).

The aim of this study was to evaluate ^{14}C-labeled glucose mineralization in tropical soils of contrasting textures from areas cultivated with maize under application of S-metolachlor, terbuthylazine, and mesotrione, alone and in a mixture. Information on microbial respiration of these herbicides in the soils is crucial in assessing environmental impact and risk from the chemical applications.

MATERIAL AND METHODS

Soil sampling and preparation

The carbon transformation experiments with the herbicides S-metolachlor, terbuthylazine and mesotrione, alone and in mixture, were performed at the Ecotoxicology Laboratory of the Center of Nuclear Energy in Agriculture - CENA, University of São Paulo - USP, Piracicaba, SP, Brazil. The methodology was established according to the guidelines of the OECD – 217, Soil Microorganisms: Carbon Transformation Test (OECD 2000).

Soil samples with contrasting textures were collected from the surface layer (0 -10 cm depth), after pre-cleaning the residue or vegetation layer in two different locations from soil under maize cultivation in Piracicaba, SP, Brazil (Alfisol – Paleudult, sandy clay, lat 22°42'34"S, →

long 47°37'18"W, and Ultisol - Typic Hapludalf, sandy loam, lat 22°42'52"S, long 47°37'10"W). After drying, samples were sieved through a 2.0 mm mesh and stored at room temperature. The physical and chemical properties of the samples and classification are shown in Table 1.

Incubation and microbial respiration

Soil samples (300 g, dry weight) were placed into 3,000 mL jars. The moisture content was adjusted to 50% of the water holding capacity (WHC), and the soil was then pre-incubated at 20 + 2 ºC for one week. The soil then reached a final soil moisture content of 75% WHC by mixing deionized water. In total, a 2 × 5 × 5 factorial experiment was established corresponding to two soils (sandy clay and sandy loam), five herbicide treatments (control – without herbicide, S-metolachlor + terbuthylazine + mesotrione, S-metolachlor, terbuthylazine, and mesotrione), and five incubation times (0, 7, 14, 21 and 28 days) prior to glucose addition.

After application of herbicide doses in the soil samples, they were mixed carefully with a spatula assuring complete homogenisation of the samples. After application and homogenization of the soil sample a 10 g aliquot was taken from each initial sample (300 g) and transferred to a biometric flask (250 mL). This procedure was performed in triplicate.

An aliquot of 1 mL of standard analytical ^{14}C-glucose solution (D-[U-^{14}C] glucose) with specific activity of 11 GBq mmol·L^{-1} and total activity of 37 MBq·mC^{-1} was added to soil samples of each biometric flask at 0, 7, 14, 21 and 28 days after herbicide application.

After application of ^{14}C-glucose solution, each biometric flask was sealed with a rubber stopper attached to a filter "lime soda", containing the stopper between the filter and the flask. The entry of atmospheric CO_2 into the flask was blocked by the filter "lime soda" ensuring that $^{14}CO_2$ was collected only from the microbial respiration. An aliquot of 10 mL of a sodium hydroxide (NaOH) solution 0.2 mol·L^{-1} was added in all lateral tubes of each biometric flask.

Aliquots (1 mL) of radiolabeled NaOH solutions were collected 6 hours after application of ^{14}C-glucose solution and transferred in duplicate to separate vials containing 10 mL of the scintillation solution insta-gel plus, and the initial concentration of ^{14}C-glucose after 15 minutes was determined by liquid scintillation counting with a Tri-Carb 2910 TR LSA counter (PerkinElmer). The remaining old NaOH solution was removed from the lateral tube and then it was again filled with 10 mL of a new NaOH solution (non-radiolabeled).

Chemical products

The stock solutions (1,200 µL) were prepared using non-radiolabeled analytical standards of mesotrione (150 g·ha^{-1}), S-metolachlor (1250 g·ha^{-1}) and terbuthylazine (750 g·ha^{-1}), alone and mixed with purities of 99.9, 98.2 and 98.8%, respectively (Sigma Aldrich, Saint Louis, MO, USA). Herbicide doses were calculated according to the collection depth of 0.1 m, soil density 1,200 kg·m^{-3} and 300 g soil mass on a dry basis. Non-radiolabeled standards were carefully mixed in acetone to reach the final volume of stock solution.

Mineralization rates

A kinetic assessment of mineralization rates was conducted to compare different treatments. First order reaction models were fitted to observe cumulative evolved CO_2 for each herbicide (control, S-metolachlor + terbuthylazine + mesotrione, S-metolachlor, terbuthylazine, and mesotrione), using linear and nonlinear regression analyses (Blume and Reichert 2015). The selection of model

Table 1. Physicochemical properties of contrasting soil textures (0-10 cm of depth) studied in this experiment.

Soil[a]	pH	K	Ca^{2+}	Mg^{2+}	H + Al	BS	P	V	OC	Sand	Clay	Silt	Texture class	CEC
	(H_2O)	(mmol$_c$ · kg^{-1})					(mg·kg^{-1})			(%)				
NVef	6.4	11	51	26	41	88	18	68	1.80	46.6	37.6	15.8	Sandy clay	129
PVAd	6.9	1	18	7	29	26	15	47	0.52	81.6	15.1	3.3	Sandy loan	55

[a]According to the Soil Taxonomy and Brazilian Soil Science Society (Embrapa, 2013). *Nitossolo Vermelho eutroférrico* – NVef (Alfisol – Paleudult) and *Argissolo Vermelho-Amarelo distrófico* – PVAd (Ultisol - Typic Hapludalf). pH = Potential of Hydrogen; K = potassium; Ca = Calcium; Mg = Magnesium; H + Al = Potential acidity; BS = Base Saturation; CEC = Cation Exchange Capacity; P = Phosphorus; V = Base Saturation Levels; OC = Organic Carbon. Source: Soil Science Department - ESALQ/USP, Piracicaba, SP, Brazil.

order was based on goodness-of-fit of the model to observed data, measured by the coefficient of determination (R^2).

Statistical data processing

An analysis of variance (ANOVA) was used to detect differences in herbicide treatments within each soil and incubation time. When significant, means were compared by Tukey's test ($p< 0.05$), whereas mineralization kinetic and decomposition parameters were estimated by the Sigma Plot® (Version 10.0 for Windows, Systat Software Inc., Point Richmond, CA).

RESULTS AND DISCUSSION

Microbial respiration

The triple interaction between soil type, herbicide type, and incubation time was statistically significant. Cumulative $^{14}CO_2$ (% of added ^{14}C-glucose) of microbial respiration for all 5 herbicide treatments (control – without herbicide; S-metolachlor + terbuthylazine + mesotrione; S-metolachlor, terbuthylazine, and mesotrione) in the different incubations was higher statistically ($p< 0.05$) in the sandy clay compared with sandy loam soil; however it exhibited a similar behavior with steady growth exponential over time (Figure 1). This fact can be attributed to higher OC (1.80%) and clay (37.6%) content in the sandy clay in relation to the sandy loam soil (0.52% and 15.1%, respectively) (Table 1). In the same conditions of this experiment, Mendes et al. (2017) also found that mineralized ^{14}C-mesotrione accumulation (alone and in a mixture with S-metolachlor + terbuthylazine), represented by $^{14}CO_2$ accumulation, was lower in the sandy loam (65 – 70%) than in the sandy clay soil (85 – 83%). According to the same authors the differences in mesotrione levels observed between the two soil types may be attributed to the varied OC and pH of the soils, wich are in turn directly affected by their respective microbial population density and diversity. Thus, measurements of the microbial activity in the soil would be required to validate these assumptions.

Soil microorganisms degrade natural and synthetic organic compounds whereas their degradation products may either accelerate or decrease microbial activities (Schmidt et al. 2011; Hussain et al. 2009b). As a result, an

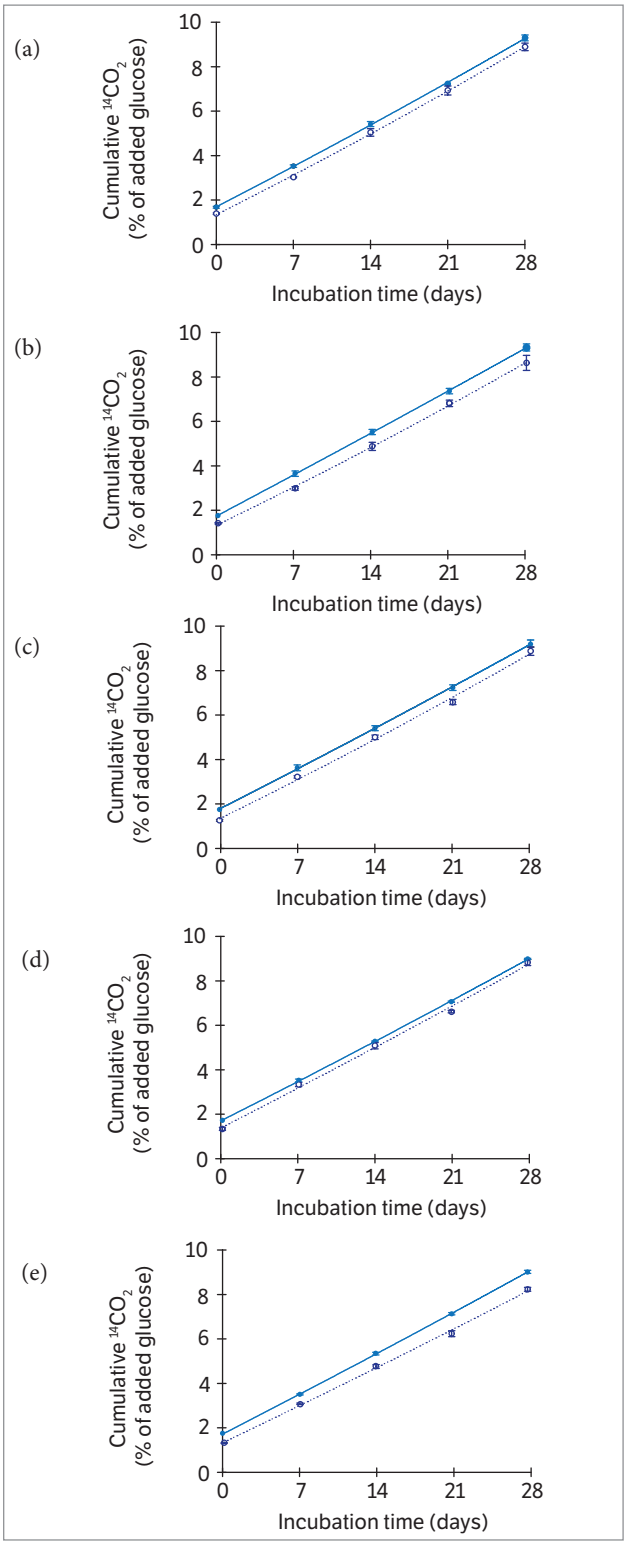

Figure 1. Cumulative $^{14}CO_2$ (% of added ^{14}C-glucose) of microbial respiration for 5 herbicide treatments: (a) control – without herbicide, (b) S-metolachlor + terbuthylazine + mesotrione, (c) S-metolachlor, (d) terbuthylazine and (e) mesotrione — and different incubation periods in sand clay and sandy loam soil. Error bars represent standard error of the means (n = 3).

environmental change happens: for exemple, herbicides used for weed control can influence soil carbon cycling through modifications in both metabolic activity and community structure. Tejada (2009) reported that the application of a glyphosate + diflufenican mixture to soil increased the inhibition of the soil microbial biomass-C and soil enzymatic activities compared to the action of both herbicides applied individually. However, the persistence period of the herbicides depended on the soil texture, and influenced its toxic effect, corroborating with data found in the present study.

Initially, at 0 day of incubation of both soils, 1.2 – 1.7% of the added glucose was mineralized, and up to 8 – 9% of the glucose was mineralized within 28 days (Figure 1). Tian et al. (2015) reported that high glucose addition (204 $\mu \cdot Cg^{-1}$ soil) increased the percentage of glucose-C mineralized to CO_2, but it decreased the proportion of the added glucose incorporated into microbial biomass, compared to the low glucose level (20.4 $\mu g \cdot Cg^{-1}$ soil). Thus, future studies are required to integrate the soil microbial community structure with the different levels of glucose additions.

Blume and Reichert (2015) reported that adding substrates (glucose and ground banana leaves) to soil increased microbial respiration for all sites [5 banana plantations with diverse pesticide management (herbicide, nematicide, and fungicide), plantation age (5 yr and 20 yr)], and microhabitats (bare area, litter pile, and nematicide ring). With the addition of glucose, a readily degradable substrate, no differences were observed for plantation age or pesticide use rate. Independent of plantation history, soil microorganisms were capable of responding to a simple organic carbon source. In addition, the same authors described that by adding a greater amount of carbon from banana leaves (0.25 g compared with 0.05 g glucose), nitrogen immobilization may reduce its availability to microorganisms and plants.

Overall, cumulative $^{14}CO_2$ of microbial respiration for treatments in mixture (S-metolachlor + terbuthylazine + mesotrione) and alone (S-metolachlor, terbuthylazine, and mesotrione) was very similar in the sandy clay and sandy loam soil, showing that addition of three herbicides in mixture did not affect the microbial respiration compared to treatment with each herbicide alone or to control, without herbicides (Figure 1). These results are comparable with previous studies, which reported that soil samples from high pesticide input (application of herbicides, nematicides, and fungicides) did not have decreased basal microbial respiration or mineralization rates, because some pesticide residues could serve as carbon or energy source to microorganisms and are degraded and assimilated by these microorganisms (Hussain et al. 2009b; Blume and Reichert 2015).

Joly et al. (2015) also reported that although the pollution pressure was maintained throughout the experiment (by herbicides: S-metolachlor, mesotrione, and nicosulfuron; adjuvants, and/or degradation products), the Limagne soil microbial communities appeared to be quite resistant to the different treatments; and the bacterial and fungal diversity estimated by fingerprinting analyses remained unchanged, such as microbial biomass evaluated by the microbial carbon measurement in the presence of metolachlor (White et al. 2010). This absence of effects, already shown by other authors concerning herbicide mixtures (Joly et al. 2015), could be explained by a real absence of effect or by the hypothesis of functional redundancy proposed by Wardle and Parkinson (1990), whereby microbial communities under the effect of herbicides were presumably in a considered state of flux, with susceptible microbes being killed and others, thereby, having a readily available source of carbon, explaining a balance in the microbial parameters.

Mineralization rates

The response of microbial communities to applications of S-metolachlor, terbuthylazine, and mesotrione, alone and in a mixture, and the mineralization rates of glucose are describable by reaction kinetics. We calculated decay constants (k) for site only (Table 2) without correction for microbial biosynthesis; thus, the constants reflect the net mineralization rates as described by Blume and Reichert (2015).

For unamended soil – control (without herbicide), microbial activity followed a similar behavior to amended soil with herbicides in total $^{14}CO_2$ released and accumulated, ranging from 23 to 27% (Table 2). In the sandy clay, a lower amount of CO_2 was observed in the soil-applied terbuthylazine (~26%); in the sandy loam, a higher amount of CO_2 was observed in the soil-applied herbicides mixture (S-metolachlor + terbuthylazine + mesotrione) with ~27%; and a lower amount was observed in the soil-applied mesotrione (~23%).

Table 2. $^{14}CO_2$ released and accumulated at the 28th day of incubation (%) and parameters of the first order kinetics (mineralization constant rate - k, mineralization half-life - MT50, and coefficient of determination - R^2) of the applied ^{14}C-glucose in sand clay and sandy loam soil with S-metolachlor + terbuthylazine + mesotrione (in mixture), S-metolachlor, terbuthylazine, mesotrione (alone), and without herbicide (control).

Herbicide	Texture soil	Parameter			
		$^{14}CO_2$ (%)	k (d^{-1})	MT50 (d)	R^2
Control	Sandy clay	27.00 Aa	0.0039	177.73	0.99
	Sandy loam	24.63 Bb	0.0037	187.33	0.99
S-metolachlor + terbuthylazine + mesotrione	Sandy clay	27.42 Aa	0.0040	173.28	0.99
	Sandy loam	25.30 Ba	0.0038	182.40	0.99
S-metolachlor	Sandy clay	27.07 Aa	0.0039	177.73	0.99
	Sandy loam	24.26 Bb	0.0037	187.33	0.99
Terbuthylazine	Sandy clay	26.15 Ab	0.0038	182.40	0.99
	Sandy loam	24.29 Bb	0.0037	187.33	0.99
Mesotrione	Sandy clay	26.91 Aa	0.0038	182.40	0.99
	Sandy loam	23.34 Bc	0.0035	198.04	0.99

Means followed by the same capital letter for each herbicide and small caps with respect to soil type do not differ by Tukey's test ($p< 0.05$). DMS (soil) = 0.0861; DMS (herbicide) = 0.1206; and and CV (%) = 2.32.

Therefore, neither microbial toxic effects were observed from the chemical mixtures with respect to reductions in the microbial community, nor in reduced glucose mineralization. There was little difference between the types of soil, as previously indicated, and this difference probably did not have an environmental impact on the microbial community. S-metolachlor, terbuthylazine, and mesotrione applied alone at the recommended field rates exert only few or in consistent minor effects on soil microbial communities and its influence depended on the rate of application and duration of activity (Salminen et al. 1996; Joly et al. 2012; Radivojevic et al. 2013). Overall, k values for all treatments were also similar, with an average value of 0.0038% $CO_2 \cdot d^{-1}$, and consequently mineralization half-life times (MT50) were from 173 to 198 d.

Blume and Reichert (2015) reported that decreased k for long-term cultivation independently of pesticide input clearly shows an age effect on mineralization rate for readily decomposable organic material. It is possible that high SOM maintains an active microbial population in the field, which is stimulated by readily degradable carbon sources. Thus, continuous input of fresh organic materials is required to maintain adequate microbial activity for sustainable crop production in the long run. Therefore, other factors like soil properties environmental conditions, concentration of herbicide used, and activity and production of secondary metabolites also contribute to determine the effect of herbicides on soil biological activities (Hussain et al. 2009b).

Agricultural soils receiving herbicide mixtures differ in their mineral and organic composition: essential factors in the herbicide sorption, bioavailability and degradation processes, which prevent us to conclude at a larger scale on the safety of the use of this three herbicide mixture (Joly et al. 2015). Knowing that the increased use of herbicide mixtures is the current trend in agricultural practices, it is essential that we investigate more precisely their impact with more sensitive methodologies and approaches, and focus on specific microbial communities which ensure key functional steps in biogeochemical cycles (Joly et al. 2012). Generally, understanding the mechanisms underlying molecular responses in microorganisms in answer to herbicide application could be helpful to elucidate the risk assessment of herbicide contamination and its consequent adverse impacts on soil microbial diversity, enzymatic activity, and biochemical reactions (Hussain et al. 2009b).

CONCLUSION

According to the above results, we conclude that microbial respiration for all treatments was slightly higher in the sandy clay compared with the sandy loam soil. Soils of contrasting textures from areas cultivated with maize under application of S-metolachlor, terbuthylazine, and mesotrione alone and in a mixture did not have decreased basal microbial respiration or mineralization →

rates of glucose. To corroborate these findings, additional research with different organic substrates and in cultures with different applications of herbicides are needed to prove the non interference of these herbicides on soil microbial respiration.

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