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Interaction Effects of Glyphosate and Cypermethrin on Soil Basal Respiration and Carbon Mineralization Quotient

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ABSTRACT

A factorial experiment laid out in completely randomized design (CRD) to examine the combined effects of glyphosate and cypermethrin on basal respiration (BR) and mineralization quotient (qM) of soil samples collected from an agro ecosystem was conducted under controlled laboratory conditions. The insecticide was applied to the soil at two field recommended rates (75 and 100 g a.i/ha) and the herbicide at 1.44 kg a.i./ha. The incubation study was carried out using soil samples maintained at 60 % water holding capacity at room temperature for 12 weeks. The main effects of cypermethrin appeared as transient inhibition, which was followed by mild stimulation of basal respiration and mineralization quotient of soil samples. However, whether in sole application, or judging from its contributions from treatment combinations, glyphosate engendered stimulatory effects on carbon mineralization. Results further showed that the interaction between the herbicide and insecticide produced stimulation of soil microbial activity from the beginning to the end of incubation. It is therefore presumed that the degradation of glyphosate in soil may confer a co-metabolic influence on the cypermethrin insecticide in soils.

Key words: Cypermethrin, Glyphosate, Basal respiration (BR), Mineralization quotient (qM), Co-metabolism, Field recommended rate (FR).

INTRODUCTION

Soil quality has been properly captured by Karlen *et al*. (1997) as "the capacity of a specific type of soil to function, within natural or managed ecosystem boundaries to sustain plant and animal health and productivity, maintain or enhance water and air quality, and support human health and habitation" Soil quality is very critical to crop production. Soil quality depends on a large number of physical, chemical, biological, microbiological and biochemical properties, the last two being the most sensitive due to their rapid respond to changes in soil or environmental conditions (Dick and Tabatabai, 1993; Trasar-Cepeda *et al.*, 2000; Bastida *et al.*, 2008). Processes that adversely impact these properties always lead to soil productivity problems. Certain farm inputs, in particular, pesticides have been implicated as potential soil contaminants, hence they have great tendencies to alter soil quality. Prominent among these are herbicides and insecticides. Pesticides had no doubt been very useful to fighting pests and diseases and as such made great contributions to global food security. However, their widespread and long term use often results in pesticide resistance and bio magnification, toxicity, residual effects and contamination across the food web. Pesticides in the soil affect non-target and beneficial microorganisms and their activities which are essential for maintaining soil fertility (Schuster and Schroder, 1990). Investigation into the way in which these potential soil contaminants influence soil functioning is important and very critical to selecting a set of farm inputs when sustainable crop production and soil management are key priorities. Traditionally, this is done by measuring a pre-established set of physical, chemical and biological soil parameters. Among these, the characteristics of soil microbial communities, i.e. community size, activity and community structure and functions are key parameters, since they respond quicker than any other soil component to changes in soil status (Bloem *et al*., 2006). Ecophysiological indices such as qM(mineralization quotients) are generated by determining the ratio of physiological performances (such as oxygen consumption or carbon dioxide evolution) to the total microbial biomass per unit time, or total organic carbon. The qM (carbon mineralization quotient) expresses the fraction of total organic carbon mineralized throughout the incubation time (Pinzari *et al*., 1999).

Glyphosate (N-(phosphonomethyl) glycine) though non selective, hence not registered for specific crops is desirable

in arable crops. This is because when applied preplant, it controls a broad spectrum of annual and perennial grasses, broadleaves and sedges (Franz *et al*., 1997). It is widely known to prevent arable crops from early weed competition when applied preplant. Although glyphosate is not recommended for direct application in soils, a significant amount may reach the soil during early-season or preplant applications (Haney *et al*., 2000). Cypermethrin, a pyrethroid insecticide is widely used to control certain insect pests. It is becoming increasingly popular as agricultural and commercial pesticide following the gradual phase-out of organochlorine and some organophosphate (Wenjun *et al*., 2007) in response to their environmental toxicity. Reports that cypermethrin has been found in soils and sediments (Tyler *et al*., 2000) indicate the potential of the insecticide to impact on certain components of soil quality. The effects of each of these two pesticides on soil microbial activities have been documented.

Glyphosate at the field recommended rate has been reported to stimulate soil basal respiration, increases microbial biomass (Wardle *et al*., 1994; Haney *et al*., 2000), and reduce the growth of earthworms after repeated applications (Springett and Gray, 1992). Souza *et al*. (1999a, 1999b) verified that microorganisms used the herbicide as C source, but the process was extremely dependent on soil moisture. However, Krzysko-Lupicka & Orlik (1997) verified that glyphosate used as a unique source of P or C inhibited the soil fungi population and changed the strain composition, thus acting as environmental selecting agent. On the other hand, Bromilow *et al*. (1996) and Busse *et al*. (2001) did not detect differences in the microbial processes of soil treated with glyphosate among other pesticides. Cypermethrin at field recommended dose resulted in transient toxic effect on soil microbial biomass – C, Basal and substrate induced respiration rate and fluorescein diacetate hydrolyzing activity (Goswami *et al*., 2013). The ecophysiological status of the soil microbial communities as expressed by microbial metabolic quotient (qCO2) and microbial respiration quotient (QR) were also altered for a short period, indicating cypermethrin induced disturbance at field recommended dose of application (Goswami *et al*., 2013).

Since glyphosate and cypermethrin are often used together on same crop field to control weeds and insect pests respectively, making them to be simultaneously present in same soil, it is presumed that their joint action on certain soil properties may differ from their individual effects on the soil microbial community. Studies have revealed that herbicide-insecticide interaction usually results in synergistic action and injury to crop plants. Few studies have also been done on the influences of pesticide-pesticide interactions on soil persistence of certain pesticides. Carbofuran persistence was shown to increase by the presence of 2,4-D, while terbufos and glyphosate decreased alachlor persistence, and butylate increased atrazine persistence in laboratory incubation studies (Peterson, 1990). However, while the influence of pesticide interaction on pesticide persistence may give an insight into their interaction with the soil microbial community, which has been identified as the major pesticide degraders, direct measurement of soil microbial characteristics will provide more direct information on how these xenobiotics jointly affects soil life. The present study therefore seeks to examine the interaction effects of glyphosate and cypermethrin on soil microbial properties in laboratory incubation study.

MATERIALS AND METHODS

Soil Sample

The soil sample used in this study was collected from the Agricultural Experimental Farm, Department of Crop, Soil and Pest management, Federal University of Technology, Akure, Nigeria ($7^0$16´N,$5^0$12´E). The soil sample was collected from an area in the field having no pesticide treatment history during the last four years. The physico-chemical properties of the soil show 31.2% clay 52.8% silt 16% sand with 1.82% organic carbon, 1.5g/kg of soil total nitrogen, 10.8 mg/kg available phosphorus and 0.42 Cmol/kg available potassium. The pH of the soil was found to be 5.5 (1: 2 H_2O).

Soil sample preparation

Soil samples from the field were collected from a depth of 0-15 cm in the field and brought to the laboratory in sealed polyethylene bags. At laboratory soil sample were sieved (<2mm) to remove plant materials, soil macro fauna and stones. After sieving, the soil samples were homogenized and brought to a maximum water holding capacity (WHC) of 60% by adding water. This was achieved by first determining the initial moisture content of the soil samples, thereafter distilled water was added to make up the samples to 60% WHC. The samples were finally kept in a plastic box. They were then stabilized at 30°C in the dark for one week. 600 g of the stabilized soil samples was transferred into glass jars to be simultaneously treated with glyphosate and cypermethrin. The control and the insecticide treated soils were incubated in moist condition at room temperature throughout the incubation period. This condition adopted following reports that maximum growth and activity of microorganisms occur in such condition (Alexander, 1977).

Pesticide treatment and experimental plan

The insecticide cypermethrin and herbicide glyphosate were obtained from the local agro dealers. Cypermethrin (10% E.C) was applied at two field recommended rates (75

and 100g.a. i/ha or 0.75 and 1.0 mg^{-1} kg soil respectively) while glyphosate application was made at 2.0 L/ha. Treatments also included a control where neither herbicide nor insecticide was applied. The application rates of these pesticides were chosen to minimize the effect of adsorption of pesticide on the studied properties of soil and emphasize the side effects of pesticides on soil microorganisms (Goswami *et al*., 2013). In addition, the recommended dose in laboratory tests was to assess the side effects of pesticides on soil microorganisms. The conversion of the field application rates of cypermethrin and glyphosate into mg/kg of soil was done assuming even distribution in 0-15cm of soil with a soil density of 1.5 g cm^{-3}. Prior to its application in soil, calculated amount of cypermethrin and glyphosate doses were dissolved in the water that was used to bring the soil to the 60% water holding capacity, and the mixture was poured into the prepared soil. The control soil samples received equal volumes of sterile distill water. The plastic jars were then covered tightly using paper tapes to seal the jars' cover and incubated at room temperature for a period of 12 weeks and titrated at one week interval. The moisture condition of the soils was maintained at 60% of maximum water holding capacity by the addition of sterile distill water at periodic intervals throughout the incubation period. To achieve this, the initial weights of the jars were determined and recorded at the beginning of incubation. These weights were confirmed from time to time, and any deviation, which indicated moisture loss, was corrected by adding water to arrive at the original weight.

Determination of physico-chemical properties of soil

Soil texture, pH, Organic matter and soil nutrient status of the air dried soil sample were determined following standard methods (AOAC, 1990). The soil samples were analyzed for total N using Kjeldahl digestion and distillation method. Available phosphorus was by the Bray 1 method, exchangeable K, Ca and Mg were determined by extraction with 1M ammonium acetate at pH 7.0. K, Ca and Mg contents were determined with flame photometer. Soil pH (1:2 soil-water) was determined by pH meter, while organic matter (OM) was determined by dichromate oxidation method.

Measurement of soil basal respiration (SBR)

Basal respiration (µg CO_2-C g^{-1} soil) was determined by the alkali sorption-titration method described by Anderson and Domsch (1990). A 10 mL solution of 0.5M NaOH was dispensed into a 50ml beaker and placed inside the plastic jars containing the treated soil to trap CO_2 evolved from the soil. The trapping solutions were replaced after titrating at every 7days and covered back with lids (air tight seal). At the end of each week of incubation, 5mL of 1.0M $BaCl_2$ was added to the solutions from the jars to precipitate carbonate (as $BaCO_3$), thus facilitating the determination of CO_2 evolution (as µg CO_2-C g^{-1} soil) from the treated soil. The evolved CO_2-C was then determined by titration. NaOH in solution was titrated against 0.5 M HCl using phenolphthalein indicator. Two blanks without soil were prepared to assess the amount of CO_2 trapped without respiratory activity. qM (mineralization quotient) was measured at the end of incubation. This was taken as the ratio of CO_2-C (µg CO_2-C g^{-1} soil) to organic carbon (mg g^{-1} soil).

Data analysis

Data collected from the experiment were subjected to an analysis of variance while treatment means were compared using the Tukey test.

RESULTS

Results of the single effects of glyphosate and cypermethrin on soil respiration are presented in Tables 1a and b respectively. Microbial respiration followed similar trends when both the herbicide and insecticide were considered singly. Soil respiration increased from 1 to 5 weeks after incubation at all levels of glyphosate and cypermethrin application. The peak of soil respiration was attained at 5 WAT, and thereafter there was a decline and values of soil respiration obtained no longer follow any consistent trend. Neither glyphosate nor cypermethrin significantly ($P<0.05$) affected microbial respiration in virtually all the weeks from start of incubation to the end of the experiment. Results however indicated that glyphosate slightly stimulated carbon mineralization from the first to the last week of incubation, but this stimulation was not observed with the two rates of cypermethrin until the fifth week after incubation. Cypermethrin was initially observed to inhibit soil respiration.

Table 2 shows the effects of glyphosate-cypermethrin interaction on carbon mineralization. All cypermethrin treatments combined with the zero level of glyphosate to inhibit carbon mineralization from week 1 to 4, and thereafter engendered a stimulation, which however was not consistent. Contrary to what obtained in the single effects of cypermethrin, the two rates of the insecticide combined with glyphosate to cause a stimulatory effect on soil microbial respiration from the beginning to the end of incubation.

Table 1a: Main effects of glyphosate on carbon mineralization (mg-C g^{-1} Soil)

Treatments	Weeks after treatment application											
	1	2	3	4	5	6	7	8	9	10	11	12
Gly -0	4.2a	7.2a	9.1a	13.3b	15.4a	14.2a	14.2a	15.4a	16.2a	14.3a	13.2a	14.9a
Gly -2L	5.4a	8.3a	9.2a	15.0a	17.3a	14.2a	14.6a	15.6a	16.8a	14.6a	14.3a	15.3a
se	0.43	0.37	0.54	0.45	0.49	0.33	0.46	0.55	0.38	0.32	0.51	0.45

Means in a column followed by the same letter(s) are not significantly (P<0.05) from one another, se = standard error

Table 1b: Main effects of cypermethrin on carbon mineralization (mg-C g^{-1} Soil)

Treatments	Weeks after treatment application											
	1	2	3	4	5	6	7	8	9	10	11	12
Cyp-0	5.2a	8.6a	10.1a	15.5a	16.2a	13.8a	13.9a	14.9a	16.1a	14.3a	13.5a	14.7a
Cyp-0.75	4.8a	7.8a	8.8a	15.2a	16.2a	14.8a	14.5a	15.7a	16.7a	14.2a	13.7a	15.0a
Cyp-1.0	4.6a	6.9a	8.6a	13.1b	16.8a	14.8a	14.8a	15.8a	16.8a	14.9a	14.1a	15.7a
se	0.61	0.52	0.77	0.64	0.69	0.46	0.65	0.78	0.53	0.45	0.72	0.63

Means in a column followed by the same letter(s) are not significantly (P<0.05) from one another, se = standard error

Table 2. Effects of herbicide – insecticide interactions on carbon mineralization (mg-C g^{-1} Soil)

Treatment combinations		Weeks after treatment application											
		1	2	3	4	5	6	7	8	9	10	11	12
Gly -0	Cyp-0	4.4a	7.5ab	9.0a	15.0a	14.9a	13.3a	13.8a	15.0a	15.3a	13.8a	13.0a	13.5a
	Cyp-0.75	4.4a	7.4ab	8.5a	14.4a	15.5a	13.8a	13.8a	14.8a	16.7a	14.6a	13.2a	14.6a
	Cyp-1.0	3.2a	6.3b	8.0a	10.5b	15.7a	14.0a	14.5a	15.5a	16.4a	14.7a	13.4a	14.8a
Gly – 2L	Cyp-0	5.1a	7.7ab	9.2a	15.8a	16.6a	14.2a	15.2a	15.6a	16.9a	14.0a	14.0a	14.8a
	Cyp-0.75	5.9a	7.9ab	9.2a	15.9a	17.4a	14.6a	14.1a	15.9a	16.9a	14.6a	14.0a	16.5a
	Cyp-1.0	5.9a	9.6a	11.0a	16.0a	18.0a	15.6a	15.0a	16.0a	17.0a	15.1a	15.0a	16.6a
se		0.61	0.52	0.77	0.64	0.69	0.46	0.65	0.78	0.53	0.45	0.72	0.63

Means in a column followed by the same letter(s) are not significantly (P<0.05) from one another, se = standard error

Table 3a: Main effects of glyphosate on soil organic carbon (mg g^{-1} Soil)

Treatments	Weeks after treatment application											
	1	2	3	4	5	6	7	8	9	10	11	12
Gly -0	7.0a	11.3a	13.3a	21.3a	23.7a	20.6a	20.9a	22.4a	23.9a	20.9a	19.9a	7.4a
Gly -2L	7.0a	11.2a	13.3a	21.2a	23.8a	20.7a	21.0a	22.5a	24.1a	21.1a	20.0a	7.5a
se	0.43	0.37	0.54	0.45	0.49	0.33	0.46	0.55	0.38	0.32	0.51	0.45

Means in a column followed by the same letter(s) are not significantly (P<0.05) from one another, se = standard error

Table 3b: Main effects of cypermethrin on soil organic carbon (mg g^{-1} Soil)

Treatments	Weeks after treatment application											
	1	2	3	4	5	6	7	8	9	10	11	12
Cyp-0	6.1a	9.9a	11.7a	18.6b	25.4a	22.2a	22.4a	24.0a	25.7a	22.5a	21.4a	8.0a
Cyp-0.75	7.3a	11.8a	14.0a	22.3a	24.9a	21.7a	22.0a	23.6a	25.2a	22.1a	21.0a	7.8a
Cyp-1.0	7.5a	12.0a	14.2a	22.7a	20.8b	18.2b	18.4b	19.7b	21.1b	18.4b	17.6b	6.5a
se	0.61	0.52	0.77	0.64	0.69	0.46	0.65	0.78	0.53	0.45	0.72	0.63

Means in a column followed by the same letter(s) are not significantly (P<0.05) from one another, se = standard error

Data on the single effects of glyphosate on soil organic carbon show that the herbicide slightly reduced soil organic carbon, whereas this parameter was increased by the two levels of cypermethrin few weeks into the incubation period when compared to the control (Tables 3a and b). Table 4 also indicates that cypermethrin with no glyphosate combination initially increased soil organic carbon, but later reduced it. Combination of the insecticide with glyphosate at the rate of 2l/ha however lowered soil organic carbon all through the incubation period.

Results of the effects of the herbicide-insecticide interaction on qM (carbon mineralization quotients) are presented in Figure 1. The two rates of cypermethrin when in combination with the field recommended rate of glyphosate raised carbon mineralization quotient above the control treatment at 1WAT. However, only cypermethrin application at 100g a.i./ha sustained this trend in subsequent weeks after treatment application. All the other treatment combinations lowered qM during the first 4 weeks of incubation when compared with the control treatment. The lowest values for qM at this period were recorded in the jars treated with cypermethrin at 100g a.i./ha with no glyphosate application. There was a sharp drop in qM in the control treatment at 5WAT, causing qM to become higher in all the treatment combinations than in the control treatment. This trend was sustained till the end of the experiment, and the highest value of qM was recorded against sole application of 100g a.i./ha rate of cypermethrin without glyphosate combination.

Table 4. Effects of herbicide – insecticide interactions on soil organic carbon (mg g^{-1} Soil)

Treatment combinations		Weeks after treatment application											
		1	2	3	4	5	6	7	8	9	10	11	12
Gly -0	Cyp-0	5.9a	9.4a	11.2a	17.8b	29.0a	25.3a	25.5a	27.4a	29.3a	25.6a	24.9a	19.1a
	Cyp-0.75	6.6a	10.6a	12.6a	20.1b	22.4b	19.6b	19.8bc	21.2abc	22.7b	19.9b	18.9abc	17.0a
	Cyp-1.0	8.5a	13.7a	16.2a	25.9a	20.0b	17.4b	17.6c	18.9c	20.2b	17.7b	16.8c	16.3a
Gly – 2L	Cyp-0	8.4a	13.4a	15.9a	25.4a	28.4a	24.7a	25.0ab	26.9ab	28.7a	18.5b	17.6c	16.6a
	Cyp-0.75	6.4a	10.3a	12.2a	19.4b	21.7b	18.9b	19.2c	20.6bc	22.0b	19.2b	18.3bc	16.8a
	Cyp-1.0	6.2a	9.9a	11.7a	18.7b	20.9b	18.2b	18.4c	19.8c	21.1b	25.1a	23.9ab	18.9a
se		0.61	0.52	0.77	0.64	0.69	0.46	0.65	0.78	0.53	0.45	0.72	0.63

Means in a column followed by the same letter(s) are not significantly (P<0.05) from one another, se = standard error

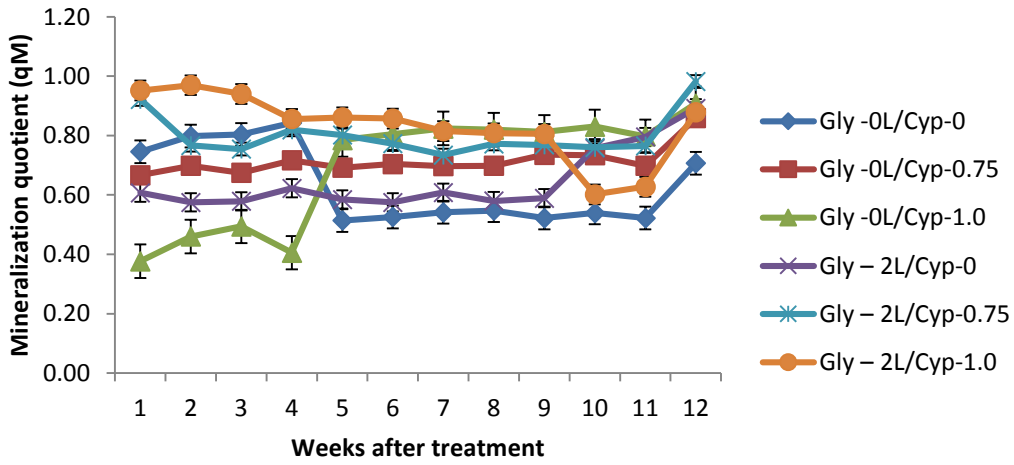

Figure 1: Effects of glyphosate-cypermethrin interaction on carbon mineralization quotient (qM)

DISCUSSIONS

The decrease in soil respiration engendered by the two rates of cypermethrin can be associated with toxic effects of cypermethrin on certain soil microorganisms, which probably did not adapt to the insecticide. Similar results were obtained by Goswami *et a*l., (2013) and Handa *et al*., (1999), who treated soil samples with cypermethrin and deltamethrin respectively. The rise in soil respiration above the control treatment observed with cypermethrin after the fourth week of incubation may be due to the fact that the insecticide or its metabolites and the dead microbial cells killed by the insecticide served as a source of carbon or energy source for the surviving microorganisms for cell proliferation (Tu and Miles, 1976). Application of insecticide to soils has been reported to inhibit some sensitive species while others rapidly appear to replace the sensitive species subsequently maintaining the metabolic integrity of the soil (Goswami *et al*., 2013). When pesticides are applied to the soil at sufficient and optimum concentrations, microbes are killed or reduced in number. However, the bacterial number increases quickly to reach a level far in excess of the untreated soil due to reduction in microbial competition (Adams, 2001) and use of cell debris of killed cells by the survivor population (Goswami *et al*., 2013).

The stimulatory effects of glyphosate on carbon mineralization compared to the control indicates glyphosate has no toxic effects on soil microorganisms present in the incubated soil samples. Adverse effects of glyphosate on soil microorganisms in laboratory studies are reported (Quinn et al., 1988; Santos and Flores, 1995). Findings of this study however contradict most of these reports. Microbial growth in artificial media containing glyphosate as the sole source of C or N is rare, and only a limited number of bacterial species and fungi are able to grow when glyphosate is provided as the sole source of P (Liu *et al*., 1991). However, unlike the results of laboratory, studies on field have shown either no effect or a slight stimulation of soil microbial activities by glyphosate (Benslama and Boulahrouf, 2013). The differences between laboratory and field studies can be explained in part by the high concentrations of the herbicide used in numerous laboratory studies and by the herbicide chemistry (Wardle, 1995). The present study, though carried out in the laboratory, strictly employed the field recommended rate of the herbicide, hence the differences in the experimental outcome. Similar results (using field rates on the field) were observed by Haney et al., (2000) and Weaver *et al*. (2007). Relative to the control, higher amount of carbon dioxide released in soil treated with glyphosate suggest that some microbiota of the incubated soil are able to use glyphosate as a nutrient source.

These results are consistent with the results of Benslama and Boulahrouf, (2013); and Wardle and Parkinson (1990) who suggest that the production of carbon dioxide is related to the decomposition of glyphosate in soil. This may also be related to a stress response in glyphosate sensitive species, due to the energy drain resulting from the ATP used in the accumulation of shikimate and hydroxibenzoic acids (Zablotowicz and Reddy, 2004). The mineralization of organic substances (such as the carbon mineralization) is mainly mediated by chemoorganotrophic microorganisms, which should not be damaged by the application of glyphosate, because this herbicide acts on a metabolic route that is typical of lithotrophic organisms (Damin and Trivelin, 2011). In fact, Acinelli *et al*. (2002) observed that the application of certain doses of glyphosate did not interfere with soil microbial activity, while some other rates resulted in greater microbial activity. This can be connected to the death of lithotrophic micro-organisms, ensuring a competitive advantage to heterotrophs. Similar results were found for glyphosate by Haney *et al*. (2000), Ratcliffe et al. (2006) and Zabaloy & Gómez (2008). Some studies have shown that the N mineralization in the soil is increased by the application of glyphosate. Grossbard (1985) assessed the effect of glyphosate on soil N mineralization and observed an increase in mineralization when the herbicide was used.

Despite the fact that cypermethrin in sole application initially reduced CO_2 evolution, the insecticide's combination with glyphosate was synergistic with respect to soil microbial activity as measured by soil carbon mineralization all through the period of incubation. The apparent contradiction between the single and combined effects of cypermethrin on soil respiration can be explained by the presence of a microbial community, which presumably was capable of using the substrates produced from the cometabolic decay of cypermethrin for respiration rather than for assimilation alongside with glyphosate degradation and assimilation. Haney *et al*. (2002) evaluated under laboratory conditions the effect of applying atrazine, and a mix of atrazine and glyphosate on the mineralization of total-C and total-N present in the soil. These authors observed a greater C and N mineralization in the soil treated with the mixture. The results showed that glyphosate favored the chemoorganotrophic microbiota, causing an increase in the soil edaphic organic matter mineralization.
The synergy formed by glyphosate-cypermethrin interaction also reflected in their effects on qM. The low values of qM recorded against sole cypermethrin treatments during the first four weeks of incubation confirmed cypermenthrin toxicity to soil microbiota. Overcoming this low level of qM after the fourth week suggests there are certain strains of soil microorganism, which were able to degrade the insecticide.

CONCLUSION

Glyphosate in this study did not only increase soil microbial activities, but the herbicide also exhibited a synergy with cypermethrin, which in sole application did not initially favour soil microbial activity as measured by soil carbon mineralization and qM (carbon mineralization quotient). Combination of glyphosate and cypermethrin was found to raise carbon mineralization above the control soil samples all through the incubation period. Glyphosate has earlier been reported to engender such a synergy with atrazine, and the herbicide has also been observed to decrease alachlor persistence. Further studies are however suggested to confirm the herbicide – glyphosate as a body-guide to other pesticides and xenobiotics to cushion their toxic effects on the soil microbial community.

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