International Journal of Plant & Soil Science
6(6): 310-318, 2015; Article no.IJPSS.2015.121
ISSN: 2320-7035

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^{14}C-Glyphosate Mineralization in Soils Enriched with Glucose and Phosphate

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Authors' contributions

This work was carried out in collaboration between both authors. Author HS designed the study, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Author CLS carried out analyses of the study. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2015/16646
Editor(s):
(1) Dionisios Gasparatos, Soil Science Laboratory, Faculty of Agriculture, Aristotle University of Thessaloniki, Greece.
(2) Marco Trevisan, Institute of Agricultural Chemistry and Environmental Research Centre BIOMASS, Faculty of Agriculture, Catholic University of the Sacred Heart, Italy.
Reviewers:
(1) Anonymous, USA.
(2) Anonymous, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=958&id=24&aid=8652

Original Research Article

Received 9th February 2015
Accepted 11th March 2015
Published 1st April 2015

ABSTRACT

^{14}C-Glyphosate (N-(phosphonomethyl) glycine) mineralization was studied in three upper soils, two Entic Haplustoll and one Typic Ustifluvent, with different physical and chemical characteristics but with similar climatic conditions, soil use and management. The evolved ^{14}C-CO_2 and CO_2 were tracked over 28 d, sampled to 2 d, 7 d, 15 d, and 28d, while soil was incubated under laboratory conditions in three different treatments: glyphosate (GLY), glyphosate + glucose (GLY+C) and glyphosate + glucose + phosphate (GLY+C+P). The incubations were conducted in closed jars at 28±1ºC. At the end of the incubation period, the percentage of ^{14}C-CO_2 released ranged from 46.9% to 61.2% (GLY), 44.6% to 60.3% (GLY+C) and 45.1% to 62% (GLY + C+ P). In the S1 soil, ^{14}C-glyphosate mineralization in the GLY+C+P treatment was significantly decreased ($p<0.05$) with respect to the GLY and GLY+C treatments. The observed changes in glyphosate mineralization may be associated with the presence of exogenous inorganic P which may have been utilized by microorganisms as a preferred alternative P source. S2 soil had the highest ^{14}C-glyphosate mineralization but did not show any significant differences among the three treatments. S3 soil, with the highest concentration of organic C in the >50 µm, also revealed the lowest ^{14}C-glyphosate mineralization, perhaps because the organic C was acting as an easily available carbon source.

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Addition of glucose (GLY+C) also depressed glyphosate mineralization in S3 soil. Soil organic matter (SOM) accumulation in the coarser soil size fraction (>50 µm) was easily oxidizable by the soil microorganisms, although it is not clear if this process negatively or positively impacted glyphosate mineralization.

Keywords: ^{14}C-glyphosate; mineralization; organic carbon; phosphorus; total microbial activity.

1. INTRODUCTION

Glyphosate (N-(phosphonomethyl) glycine) is a broad-spectrum organophosphate herbicide used to control annual and perennial weeds; it is used mainly in transgenic soybean cultivation and to chemically fallow cropland. Glyphosate is currently the most widely sold herbicide in Argentina, accounting for 76% of total market herbicides [1].

The environmental risk of glyphosate is linked to its release to the atmosphere or its discharge to the hydrosphere through runoff or deep transport. Mineralization of glyphosate is considered a significant dissipation pathway. The half-life of glyphosate is reported to be quite variable, ranging from a few days (< 5 d) to 60 d although a half-life of >100 d has been reported [2,3].

Land use strategies impact the soil characteristics which, in turn, affect glyphosate degradation. The impact of various strategies has been the focus of recent research, with particular attention on the important role on soil microbial activity [4,5,6].

It has not been clearly determined if the slightly decomposed organic matter that accumulates in the upper soil layers of no-till agricultural fields accelerates, depresses or has no effect on glyphosate mineralization. It has been determined that incorporation of corn residues in soil caused a decrease in glyphosate mineralization [7]. It was proposed that microorganisms prefer glyphosate over soil organic matter (SOM) as a C source due to the low C to N ratio (3:1) of glyphosate [8]. Studies of mineralization of glyphosate in crop residues, found that crop residues were the primary C source [9].

However, the authors proposed that glyphosate could act as a readily available C source, permitting an increase in microbial activity. Some studies have shown that addition of a small amount of readily available substrate can lead to an increase in microbial activity and a subsequent rise in mineralization of living and/or non-living SOM [10]. It has also been proposed that soil microflora could use glyphosate as a P source [11]. It was observed that microbial utilization of glyphosate in the soils also occurs at a high rate in the presence of P, N and C [12].

Previously, was proposed that the degree to which glyphosate stimulates soil microbial activity appears to be unique to each soil [13]. If the interactions among soil characteristics and sources of labile C and P are unique and system-specific, the resulting impacts to glyphosate mineralization may also be particular to the system, but little is known about how such modifications may occur. Our hypothesis is that a small amount of readily available C and P substrate would accelerate glyphosate mineralization, although soil characteristics could further modify the behavior pattern. The goal of this study was to assess the effect of inorganic P and a labile source of organic C on short term degradation of glyphosate in soils.

2. MATERIALS AND METHODS

2.1 Soils

Soil was collected near the town of Ambul, Córdoba, Argentina (30º30′0″S 64º30′0″W). The annual mean temperature at the collection location is 17.8ºC, the annual mean precipitation is 620 mm and the annual mean potential evapotranspiration is 1050 mm [14]. Three active crop sites were selected (S1, S2, S3). Soils were classified according to [15] as Entic Haplustoll (S1 and S2) and Typic Ustifluvent (S3). The soil textural class was sandy loam (S1), loam (S2) and loamy sand (S3). Crop sequence is soybean [*Glycine max* (L.) Merr.] followed by maize (*Zea mays* L.), a rotation that has been practiced for 10 years. Glyphosate is regularly applied to the field two or three times each year.

Soil samples were collected from a 0–15 cm depth. The soil samples were air-dried, ground with a porcelain mortar and pestle and sieved through a 2 mm screen. All samples were characterized by measuring various parameters.

Particle-size was determined by sedimentation by pipette of Robinson technique [16]; soil pH and electrical conductivity (EC) were measured in a water extract (soil:water of 1:2.5). Total organic C content (TOC) was determined using wet combustion and converted to soil organic matter (SOM) by multiplying by a factor of 1.72. Total N (N_t) was determined by Kjeldahl, extractable phosphorus (P_{ext}) was measured using Bray 1 and cation-exchange capacity (CEC) determined by ammonium acetate saturation. All techniques are described in [17]. Summary of soil characteristics is shown in Table 1.

Soil size fractionation analysis was based on an adaptation of the technique described by [18] where distilled water was added to the soil samples (soil:water of 1:2) with 20 glass beads (0.5 cm in diameter) by triplicate. The 50-2000 µm and <50 µm size fractions were recovered from the dispersed suspension by sieving and drying at 50ºC. Soil weight and organic carbon concentration in each fraction (OC_f) were quantified.

2.2 Incubations and Analysis

A radiolabelled solution was prepared with ^{14}C-glyphosate (phosphonomethyl-^{14}C-glyphosate, radiopurity > 98%, specific activity: 6.66 10^7 Bq mmol^{-1}, purchased from Sigma) and unlabelled glyphosate in water to reach a final concentration of 10 mg L^{-1} glyphosate and 3.36 10^6 Bq L^{-1}. The laboratory incubations were conducted in closed jars for 28 d at 28±1ºC. Triplicate incubations were done in hermetically closed glass jars. Three treatments were included in the analysis. Treatment 1: Soil + glyphosate (GLY) – 10 g of each soil were weighed into 250 mL closed glass jars and 1 mL of labelled glyphosate solution (1 mg of glyphosate kg^{-1} soil; 3.36 10^5 Bq kg^{-1} soil) was added to each jar. Treatment 2: Soil + glyphosate + glucose (GLY+C) – 10 g of each soil were weighed into 250 mL closed glass jars and 1 mL of labelled glyphosate solution (as treatment 1) and 500 µL of 14 mM glucose solution (154 mg glucose kg^{-1} soil) were added to each jar. Treatment 3: Soil + glyphosate +glucose + phosphate (GLY+C+P) – 10 g of each soil were weighed into 250 mL closed glass jars, 1 mL of labelled glyphosate solution and 500 µL of glucose solution (as treatment 2) and 1 mL of K_2HPO_4 0.78 mM were added to each jar. 1.5 mL was the final total volume (treatment solution and distilled water) added at all treatments. Small vials with 3 mL of 1 M NaOH were placed in the jars to capture CO_2 produced during incubation. The vials were sampled and changed at 2, 7 and 15 d after incubation initiation, with a final sampling on day 28. Additional triplicate blank jars containing only the vials with NaOH served as controls to account for the CO_2 trapped from the air inside the jars.

Total CO_2 absorbed by the NaOH solution was measured by titration of an aliquot (1 mL) with 0.1 M HCl against phenolphthalein after addition of a 0.5 M $BaCl_2$ solution [19]. The ^{14}C activity in CO_2 collected in the NaOH solution was measured according description from [20]. 7.5 ml of Ecolite ICN scintillation cocktail were added to 1 ml of CO_2-NaOH solution and using a liquid scintillation counter (Beckman LS 6000IC, Fullerton, California, USA).

2.3 Kinetic Parameters and Statistical Analysis

Cumulative ^{14}C-CO_2 glyphosate and C-CO_2 evolved were adjusted to a first order model:

$$C_t = C_0 - C_0e^{-kt} \tag{1}$$

Where: C_t is the percentage of ^{14}C-CO_2 or C-CO_2 mineralized at time t, C_0 is an estimation of the percentage of ^{14}C or C, potentially mineralizable, *k* is the daily mineralization rate (days^{-1}) and t is time in days.

Half-life time

From the equation $C_t = C_0e^{-kt}$ and this expression linearized which takes the form:

$$lnC_t = -kt \ln C_0$$

Reordering:

$$kt = ln\frac{C_0}{C_t}$$

When C_t is the half of C_0 then $ln\frac{C_0}{C_t}$ = 2 and the half-life time, $t_{1/2}$, (days) can be calculated as:

$$t_{1/2} = \frac{\ln 2}{k} \tag{2}$$

The degradation of glyphosate was estimated by the efflux of $^{14}CO_2$ from ^{14}C-glyphosate and the results were presented as percentages of initial ^{14}C applied. Total microbial activity (TMA) was estimated by the efflux of total respired CO_2 from soils. Glyphosate mineralization between the three soils was evaluated by one-way ANOVA. For each soil, the effect of C (glucose) and P (K_2HPO_4) on decomposition of ^{14}C-glyphosate (glyphosate mineralization) and TMA were evaluated using one-way ANOVA for significant ($p<0.05$) differences among the treatments in each soil, followed by a LSD Fisher test of mean comparisons. InfoStat statistical program was used for the statistical analysis [21].

3. RESULTS AND DISCUSSION

3.1 Soil Properties and ^{14}C-glyphosate Mineralization

Cumulative ^{14}C-glyphosate mineralization, together with soil C-CO_2 evolution, is shown in Fig. 1 (a, b, respectively). At the end of the incubation period, the percentage of ^{14}C-CO_2 released ranged from 46.9% to 61.2% of the ^{14}C applied. Significant differences ($p<0.01$) among soils were detected during all incubation periods S2>S1≈S3. The first order equation parameters (Eq. 1) to characterize ^{14}C-glyphosate mineralization and TMA are shown in Table 2, together with the $t_{1/2}$ (Eq. 2). The estimated values of potentially mineralizable ^{14}C-CO_2 were slightly lower than experimental values on day 28, suggesting two stages during the process, and initial “fast” stage followed by a second “slow” stage.

S1 and S2 soils presented similar TMA behavior as well as similar physical and chemical characteristics, as shown in Table 1 and Table 2. The P_{ext} content of the S2 soil was three times of the S1 soil. The relationship between glyphosate and the phosphate anion is not yet clearly understood, although mutual interaction is generally accepted. [19] found a negative correlation between glyphosate adsorption and phosphorus soil content. The greatest glyphosate mineralization in S2 soil, relative to S1 soil, could be associated with lower glyphosate retention in S2 soil due to considerable glyphosate adsorption at adsorption sites not occupied by a phosphate anion. Previous studies have pointed out that glyphosate adsorption decreases in P-enriched soils because phosphate anion has higher affinity for sorption sites than glyphosate [22]. On the other hand, [23] have reported competitiveness between glyphosate and phosphate by the same adsorption sites.

Several studies have demonstrated that herbicide molecules accumulated on the interface of a soil/water solution are protected against microbial attack [24,25]. It has been reported that the highest sorption and lowest desorption of glyphosate coincided with the absence of or minor mineralization [26].

S3 soil was sandier than the other soils, with a greater proportion (70%) of sand fraction. The physical fractionation results coincided with granulometric analysis (Fig. 2a). The >50 µm soil size fraction for S3 soil was, on average, twice that of S1 and S2 soils. Capitalizing on the soil fractionation analysis without SOM elimination, it is possible to analyze differences in SOM quality. Soil organic matter physical fractionation allows for separation of fresh SOM or lightly transformed and humified SOM [17], as represented by the >50 µm soil size fraction or <50 µm soil size fraction, respectively. S3 soil, in addition to having the lowest SOM content, also had the lowest SOM concentration in the >50 µm soil size fraction (Fig 2b). The highest TMA obtained for S3 may be related to the abundance of organic C accumulated in the coarse soil fractions (>50 µm) which is easily oxidized due to great exposition, small chemical interactions between the organic and mineral fractions [27,28] and a higher C:N ratio than the organic matter of the soil as a whole [29]. In S3 soil, mineralization of TOC ranged from 9.4 to 12.7% after two days of incubation; normal values of organic carbon mineralization in soil are around 5% [30,31].

However, the highest TMA of the S3 soil did not have a positive impact on ^{14}C-glyphosate mineralization as the S3 soil had the lowest ^{14}C-glyphosate mineralization. Rampoldi et al. [20] reported that, even though glyphosate mineralization has been described as a cometabolic process, sometimes the highest TMA was not the highest glyphosate mineralization. Previous studies determined that in a sandy soil, incorporation of corn residues caused a decrease in glyphosate mineralization [7]. Earlier, [8] proposed that microorganisms prefer glyphosate over soil organic carbon. It is possible that SOM composition should be considered when preferences of soil microorganisms between glyphosate and other substrates are evaluated.

On the other hand, the lowest glyphosate mineralization in S3 soil, could be associated to the high P content of S3 soil, given that an external phosphorus source could inhibit glyphosate mineralization [32].

3.2 Effect of the Addition of Glucose and P on ^{14}C-glyphosate Mineralization and TMA

Cumulative $^{14}CO_2$ and CO_2 evolution in the treatments GLY, GLY+C, GLY+C+P for each soil are shown in Fig. 3. The addition of glucose and glucose+phosphate did not significantly affect TMA or ^{14}C-glyphosate mineralization. It is possible that the pattern of behavior of glyphosate mineralization in soils under different situations can be modified by the physical and chemical properties of each soil, leading to unexpected patterns related to the resulting interactions.

3.3 Interactions of Soil and Treatment on ^{14}C-glyphosate Mineralization

Differences in glyphosate mineralization, as measured using $^{14}C-CO_2$ evolution, were apparent among the tested soils. Soil S2 showed the highest $^{14}C-CO_2$ released at 28th day, although there were no significant differences among the three treatments. These data indicate that the addition of C and P little improved the glyphosate mineralization capacity of S2 soil.

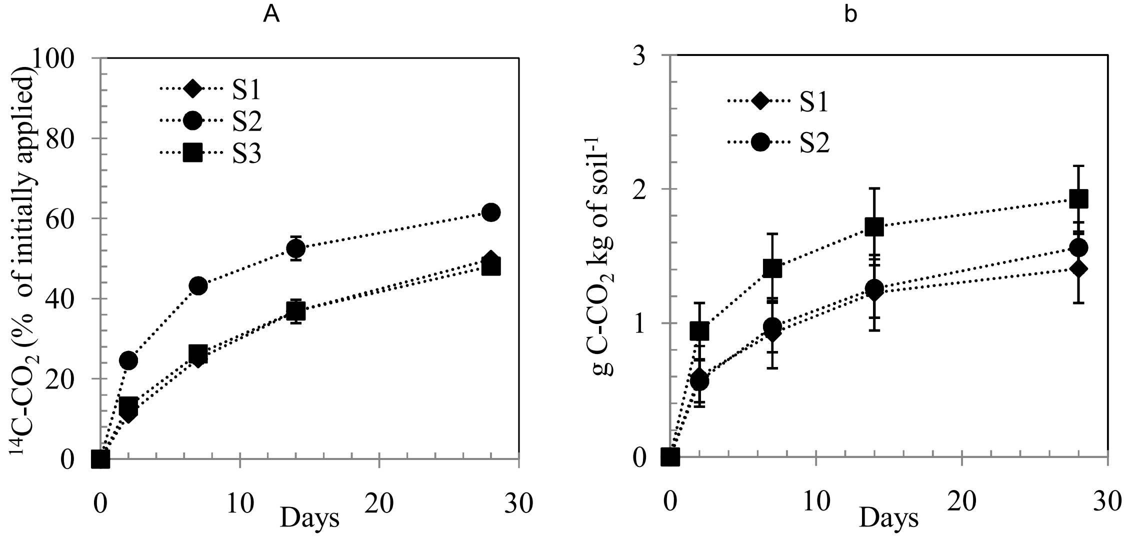

Fig. 1. Cumulative ^{14}C-glyphosate mineralization (a) and soil $C-CO_2$ (b) during laboratory incubations in three agricultural soils. The standard deviations (error bars) are shown when larger than the symbol size

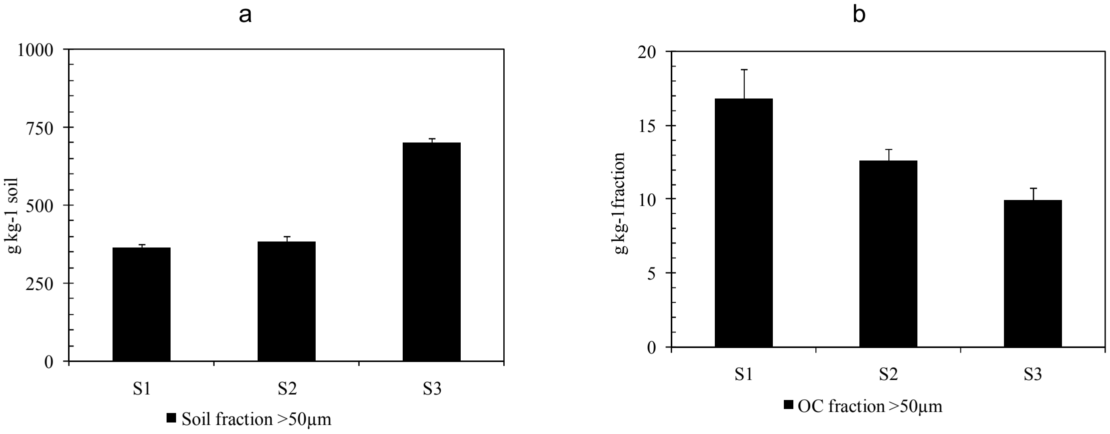

Fig. 2. Soil mass (a) and organic C content (b) in the coarsest soil size fraction (> 50µm) in three agricultural soils. Error bars represent one standard deviation

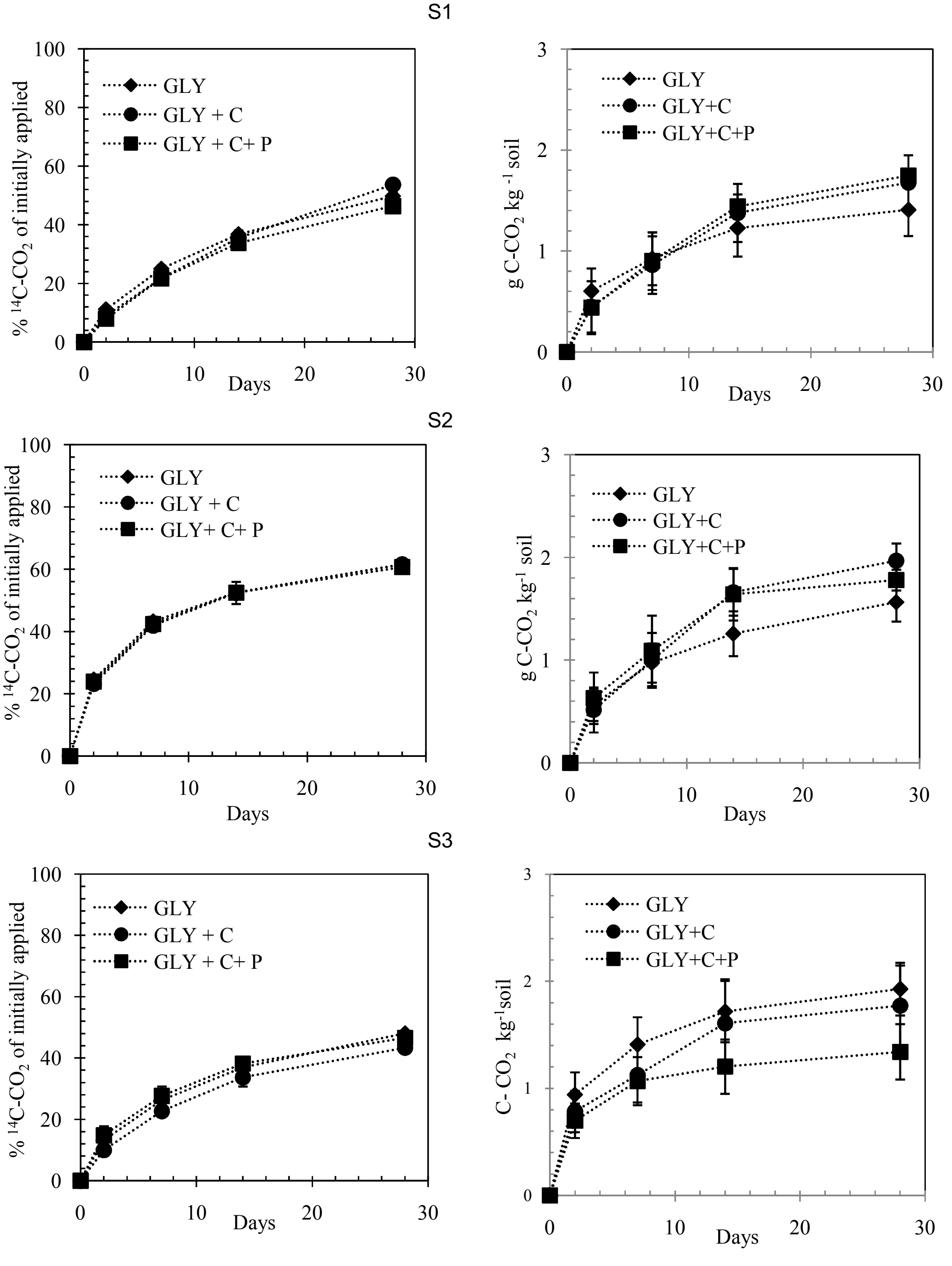

Fig. 3. Cumulative ^{14}C-glyphosate mineralization and soil C-CO$_2$ evolution, during laboratory incubations in three agricultural soils and three treatments: GLY (glyphosate), GLY+C (glyphosate+glucose), and GLY+C+P (glyphosate+glucose+K_2HPO_4). The standard deviations (error bars) are shown when larger than the symbol size

Table 1. Physical and chemical characteristics of the three tested soils

Parameter	Units	Soil 1		Soil 2		Soil 3	
TOC	g kg^{-1}	10.7	±0.5	11.5	±0.6	7.4	±0.4
Nt		1.09	±0.05	1.16	±0.06	0.8	±0.04
C:N		9.8		9.9		9.2	
P_{ext}	mg kg^{-1}	7.1	±0.4	21.2	±1	24.1	±1.2
pH $_{soil:water1:2.5}$		7.4	±0.4	6.8	±0.03	7.1	±0.4
CEC	c_{mol} kg^{-1}	18.1	±0.9	19.8	±1	9.8	±0.5
EC	dS m^{-1}	0.5	±0.02	0.5	±0.02	0.9	±0.02
Sand	g kg^{-1}	472	±24	426	±21	718	±20
Silt		432	±22	440	±20	208	±10
Clay		96	±5	134	±7	74	±4

TOC: Total organic carbon, N_t: Total nitrogen; CEC: Cation exchange capacity, EC: Electrical conductivity

Table 2. First order model parameters, potentially mineralizable carbon (C_0) and daily rate of mineralization (*k*), for ^{14}C-glyphosate mineralization and total microbial activity (TMA) in three agricultural soils

		Treatment								
		GLY (T1)			**GLY+C (T2)**			**GLY+C+P (T3)**		
		S1	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
Glyphosate mineralization	C_0 † (^{14}C-CO$_2$, %)	53.6±1.9	58.4±1.7	47.6±1.6	68.5±3.6	57.9±1.7	47.7±1.5	50.2±1.4	58.9±1.8	48.8±1.6
	k, days^{-1}	0.090±0.007	0.218±0.022	0.118±0.011	0.054±0.005	0.203±0.020	0.093±0.007	0.081±0.005	0.201±0.021	0.132±0.012
	Half-life time ($t_{1/2}$), days	8	3	6	13	3	7	9	3	5
Total Microbial Activity (TMA)	C_0† C-CO$_2$, mg kg soil^{-1}	1809±157	1437±88	1694±106	2045±177	2098±228	1258±88	1908±178	1704±119	1148±68
	k, days^{-1}	0.162±0.034	0.19±0.03	0.336±0.071	0.123±0.023	0.105±0.024	0.461±0.124	0.134±0.029	0.152±0.026	0.455±0.105

†^{14}C-CO$_2$ and C-CO$_2$ potentially mineralizabl9339e

In contrast to S2, S1 and S3 soils showed significant interactions ($p<0.05$). In S1 soil, ^{14}C-glyphosate mineralization changed over the incubation period for the GLY+C+P treatment, and was significantly lower ($p<0.05$) than GLY and GLY+C treatments. These differences may be linked to the exogenous inorganic P which may have replaced the glyphosate as a source of P. It has been proposed that soil microflora could use glyphosate as P source, and addition of P to a test matrix would then depress the degradation of glyphosate by inhibition of the C-P lyase enzyme [11,32,33]. The S3 soil displayed a different response, with significantly decreased ^{14}C-CO_2 evolution over the incubation period in the GLY+C treatment, suggesting substitution of glyphosate for glucose as an easily oxidized carbon source.

4. CONCLUSION

The results of this study showed differences in glyphosate mineralization capacity among soils, despite similarities in climate type, use and management. Phosphorus availability can modify the glyphosate mineralization capacity of the soil depending on phosphate content, although substantive questions remain that should be addressed through additional research into microbial composition. SOM accumulation in the coarser soil size fraction (>50 µm) was easily oxidizable by TMA, however, the impact of this process on glyphosate mineralization is not known.

ACKNOWLEDGEMENTS

This work was granted by the National Agency for Scientific and Technological Promotion PICT 0250/10 project.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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