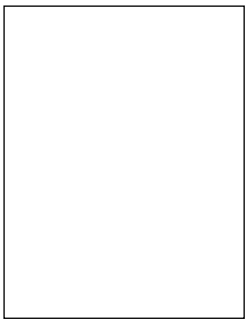

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Glyphosate Dissipation in Different Soils under No-Till and Conventional Tillage

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ABSTRACT

Glyphosate is the most used herbicide in Argentina, accounting for 62% of the commercialized pesticides in the market. It is used as a weed controller in no-till systems, and it is also applied in various genetically modified crops (e.g. soybean, corn, cotton). Though it has a high solubility in water, it tends to adsorb and accumulate in agricultural soils. The main objectives of this work were to compare the dissipation of glyphosate and the accumulation of its metabolite aminomethylphosphonic acid (AMPA) over time in three soils from agricultural areas of Argentina, under long-term management of no-till (NT) and conventional tillage (CT) practices. Forty percent of the applied glyphosate was degraded within the first three days in all soils, indicating a fast initial dissipation rate.

However, the dissipation rate considerably decreased over time and the degradation kinetics followed a two-compartment kinetic model. No differences were found between tillage practices. Dissipation was not related to the microbial activity measured as soil respiration. The fast decrease in the concentration of glyphosate at the beginning of the dissipation study was not reflected in an increase on the concentration of AMPA. The estimated half-lives for glyphosate ranged between 9 and 38 days. However, glyphosate bioavailability decreases over time as it is strongly adsorbed to the soil matrix. This increases its residence time which may lead to its accumulation in agricultural soils.

Key Words: dissipation, pesticide, two-compartment kinetic model

INTRODUCTION

Glyphosate (N-(phosphonomethyl) glycine) is a post-emergence, non-selective, foliar herbicide. It is the most used herbicide in Argentina, accounting for 62% of the commercialized pesticides in the market (CASAFE, 2012). It is used as a weed controller in no-till (NT) systems,

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and it is also applied on various genetically modified crops (e.g. soybean, corn, cotton). Glyphosate has an amine, carboxylate, and phosphonate group. It is a highly water-soluble compound (11.6 g L^{-1} at 25º C), but it also has a high affinity to soil particles, such as clays and aluminum and iron oxides (Vereecken, 2005).

The biological degradation of a pesticide in the soil is dependent on various factors such as: microbiological activity, organic matter (OM) content, nutrient availability, pH, salinity, temperature, oxygen content, humidity and bio-availability (Aislabie and Lloydjones, 1995; Walker *et al*., 2001; Borggaard and Gimsing, 2008). Glyphosate is mainly degraded by biological activity, although evidence of an abiotic pathway via metal interaction has been recently reported (Ascolani Yael *et al*., 2014). In general, microorganisms use glyphosate aerobically or anaerobically, as a source of organic phosphorous (Zboinska *et al*., 1992; Dick and Quinn 1995; Obojska *et al*., 2002; Ermakova *et al*., 2008). However, some species have been described to be capable of using glyphosate as a carbon or nitrogen source (Kryosko and Lupicka, 1997; Obojska et al., 1999). There are two pathways known for the degradation of glyphosate (Fig. 1). In one pathway, the herbicide is hydrolyzed to inorganic phosphorous and sarcosine, by the activity of a C-P lyase enzyme. Sarcosine is then metabolized into glycine and formaldehyde by a sarcosine oxidase, until its complete mineralization to CO_2 and NH_3. Some examples of bacteria that use this pathway are *Arthrobacter* spp., *Rhizobium* spp. and *Pseudomonas* spp. (Dick and Quinn, 1995; Kishore and Jacob, 1987; Liu *et al*., 1991). Other organisms degrade glyphosate by an oxidoreductase, producing glyoxylate and aminomethylphosphonic acid (AMPA). Glyoxylate enters the glyoxylate bypass of the Krebs cycle, while AMPA is exported into the extracellular space (Jacob *et al*., 1988). Some bacteria are capable of metabolizing AMPA by a C-P lyase, yielding inorganic phosphorous and methylamine (Pipke *et al*., 1987; Kertesz *et al*., 1994), which further fully metabolizes to CO_2 and NH_3. Examples of microorganisms that use this pathway are *Ochromobactrum anthropi* (Sviridov *et al*., 2012) and *Geobacillus caldoxylosilyticus* (Obojska *et al*., 2002).

Fig. 1

Fig. 1 Glyphosate degradation pathways into the metabolites AMPA and sarcosine, until complete mineralization.

The AMPA pathway is most commonly described in the literature because this compound is persistent in the environment and it accumulates in the soil *(*Simonsen *et al*., 2008). Sarcosine, on the other hand, is easily degradable and does not accumulate in soil (Borggaard and Gimsing, 2008). However, this does not imply that the preferential degradation path is via AMPA formation since energetically it is more convenient for the cell to produce sarcosine.

Generally, values of glyphosate DT_{50} (time for the dissipation of 50% of the initial concentration) in soils are in between 3 to 40 days (e.g. Rueppel *et al*., 1977; Smith and Aubin, 1993; Grunewald *et al*., 2001; Simonsen *et al*., 2008; Zablotowicz *et al*., 2009; Bergström *et al*., 2011). The mineralization of glyphosate varies according to the soil type (Smith and Aubin, 1993) because of differences in the biological activity of the soil or the degree of bio-availability. Regarding soil biological activity, some studies have found a positive correlation between the degradation rate and the soil respiration rate (Franz *et al*., 1997; Torstensson, 1985), whereas others have reported that the degradation of glyphosate is positively correlated to the microbial biomass (Von Wirén-Lehr *et al*., 1997).

The degree of glyphosate bio-availability is determined by the physico-chemical characteristics of the soil and it is strongly related to the degree of adsorption to the soil matrix (Gerritse *et al*., 1996; Cheah *et al*., 1997; Vereecken, 2005; Sorensen *et al*., 2006). In this sense results are variable; degradation rate has been positively correlated to the soil pH and negatively to the soil organic carbon (OC) and adsorption (*Kf*) (Mamy *et al*., 2005; Ghafoor *et al*., 2011). Since glyphosate has a zwitterion structure, with four dissociation constants (pKa = 2.0, 2.6, 5.8 and 10.8;

Sprankle *et al*., 1975), the soil pH modifies the electric charge of glyphosate. As pH increases, glyphosate becomes more anionic, increasing the repulsion by the negatively charged particles of the soil (Zhao *et al*., 2009) and therefore becomes more bio-available. In another study, mineralization was positively correlated to OC, total nitrogen content, electrical conductivity, and total microbial activity, but no relationship was found with pH or texture (Zablotowicz *et a*l., 2009). This reflects that glyphosate degradation is controlled by several factors or by an interaction of factors that can vary from soil to soil and even have opposite effects. For example, soil organic matter, and in particular humic substances, may reduce its bio-availability since glyphosate can be adsorbed via hydrogen bonds to the phenolic groups (Albers et al., 2009). However, organic matter content increases biological activity, offering favorable conditions for pesticide degradation (Thorstensen and Lode, 2001).

There is a growing concern about glyphosate's behavior in the environment regarding its accumulation in soils and water bodies, especially since there is evidence of its risks for living organisms, including humans (Guyton *et al*., 2015). The description of the dissipation of glyphosate in soils with a long term history under agricultural practices is of interest since it is one of the most sensitive factors that determine possible losses to surface water and groundwater (Ghafoor *et al*., 2011). The objectives of this study were to compare glyphosate dissipation in different soils under long-term NT and CT management and to evaluate AMPA accumulation over time.

MATERIAL AND METHODS

Site description and sampling

Soil samples were obtained from three different Experimental Stations with long-term field trials under different tillage systems of the Instituto Nacional de Tecnología Agropecuaria (INTA). The studied soils are situated in areas of high agronomic land use and of different edaphoclimatic conditions. Manfredi (MAN) experimental site is located in Córdoba Province, in the central region of Argentina. The soil is a coarse-silty, mixed, thermic, Entic Haplustoll of the Oncativo series (INTA, 1987). The field trial was established 30 years ago. Samples were taken from treatments under NT and CT with a maize-soybean rotation. Paraná (PAR) experimental site is located in Entre Ríos Province, belonging to the Mesopotamia region. The soil is classified as fine, mixed, thermic, Aquic Argiudoll from the Tezanos Pinto series (INTA, 1998). Soil samples were taken from a long-term field trial of 16 years under NT and CT, with a wheat/soybean-maize rotation. Pergamino (PER) site is located in Buenos Aires Province, part of the Pampas geographical region. The soil is classified as a fine, thermic, illitic, Typic Argiduoll (Pergamino series) (INTA, 1972). The field trial was established 34 years ago and includes NT and CT under a maize-wheat/soybean rotation.

All of the field trials sampled have been under NT and CT for more than 16 years and have received glyphosate applications with doses ranging from 3--6 L ha^{-1} $year^{-1}$ (1.0--2.1 Kg a.i. ha^{-1} $year^{-1}$), depending on the crop rotation. Disturbed soil samples of the top 15 cm of the soil profile were collected in a completely randomized blocks design with 2 treatments, NT and CT, and 4 blocks per treatment. The main characteristics of the sampled soils are shown in Table I. The physico-chemical characteristics of the soils have been analyzed before by Okada et al. (2016). PAR soil has the highest clay content, and CEC and Ca^{2+} values, while MAN soil has lower OC content and a higher pH than PAR and PER ($P < 0.05$). Glyphosate and AMPA concentration were measured before the experiments to quantify the initial load of the pesticide in the soils. Traces of glyphosate were detected at concentrations below the limit of quantification (LQ), while AMPA concentration ranged from 0.1--0.3 mg Kg^{-1}.

Chemicals

The standard curves were prepared using a stock solution of analytical glyphosate (PESTANAL®, 99.9%) and AMPA (PESTANAL®, 99%). Isotope-labeled glyphosate (1,2-^{13}C, ^{15}N, Sigma-Aldrich) was used as an internal standard. For the degradation experiment, the stock solution that was added to the soil was prepared using commercial glyphosate (ATANOR II®, 35.6% acid equivalent) in ultra-pure water. HPLC-grade methanol and HPLC-grade acetonitrile for analytical procedures were purchased from Seasinglab. Ultra-pure water (ELGA PURELAB® Ultra, Illinois, USA) was used in all the analytical procedures and solution preparations.

TABLE I

General characteristics of the studied soils (Okada et al., 2016).

Soil	PAR	PER	MAN
Location	Paraná, Entre Rios Province	Pergamino, Buenos Aires Province	Manfredi, Córdoba Province
Coordenates	31°51’ 15’’ S, 60°32’ 10’’ W	33º 57’ S, 60º 33’ W	31º 56´ 55´´ S 63º 46 ` 30´´ W
Years under NT and CT	16	34	30
Soil type	Aquic Argiduoll	Typic Argiuduoll	Entic Haplustoll
Series	Tezanos Pinto	Pergamino	Oncativo
Texture	Silty clay loam	Silty loam	Silty loam
Sand (%)	9.2 b[a)]	12.5 ab	16.9 a
Silt (%)	54 b	64.8 a	66.8 a
Clay (%)	36.8 a	22.7 b	16.3 c
CEC (meq 100 g^{-1})	28.9 a	20.7 b	17.4 b
pH	6.0 b	5.8 b	6.4 a*
OC[b)] (%)	1.6 a	1.7 a	1.1 b
P-Bray[c)] (mg Kg^{-1})	34.4 b	29.4 b	64.0 a
Fe [d)] (mg Kg^{-1})	1677.7 b	3184.3 a	1191.1 c
Al[d)] (mg Kg^{-1})	221.6 b	185.2 b	323.8 a

[a)]Different letters indicate significant differences between soils ($P < 0.001$, $*P < 0.05$).
[b)]OC: organic carbon measured with the oxidation chromic acid method (Walkley and Black, 1934)
[c)]P-Bray: available phosphorous according to Bray and Kurtz (1945).
[d)]Amorphous Al and Fe oxides extracted with 0.2 M acidified ammonium oxalate (pH 3) (Blackemore *et al*., 1987). Al was determined with a UV spectrophotometer with the Aluminon method (Barnhisel and Bertsch, 1982) and Fe using a specific atomic adsorption lamp.

Dissipation studies

The term dissipation refers to the group of processes that reduces the concentration of a pesticide following its application (Gustafson and Holden, 1990). To study glyphosate dissipation in soil, 400 g of dry soil sample were placed in 1.5 L flasks. A dose corresponding to 6 L ha^{-1} of commercial glyphosate ATANOR II® (35.6 % a.i.) was applied on day 0. The dose applied was equivalent to a final concentration in soil of 4000 μg Kg^{-1} of active ingredient. The moisture of the soil samples was kept at 60 % of the field capacity. Samples were incubated in the dark at a constant temperature of 22°C ± 1°C. A sub-sample of 5 g was taken from each flask at day 0 (after application), 1, 3, 7, 15, 20, 28, 44 and 62, and analyzed for glyphosate and AMPA.

AMPA concentration was transformed into a glyphosate equivalent mass and expressed as a percentage of the initially applied glyphosate by the following equation (Coupe *et al*., 2011):

% AMPA = ($AMPA_t$ (µg L^{-1}) x MW_{gly}/MW_{AMPA}) x 100 / GLY_i (µg L^{-1}) (Eq. 1)
$AMPA_t$: concentration of AMPA at different times of sampling.
GLY_i: initial concentration of glyphosate at day 0.
MW_{gly}= 169 g mol^{-1} (molecular weight of glyphosate)
MW_{AMPA}= 111 g mol^{-1} (molecular weight of AMPA)

Dissipation kinetics

The dissipation of glyphosate was described using a first order (Eq. 2) and a bi-exponential kinetic model (Eq. 4).

First order kinetic model:

$$C_t = C_o e^{-kt} \quad \text{(Eq. 2)}$$

Where C_t (mg kg^{-1}) is the concentration at time t (days), C_o is the initial concentration, k is the first-order dissipation constant ($days^{-1}$). This model assumes that the degradation rate is proportional to the remaining herbicide concentration in the soil. If the dissipation kinetics follows a first order equation, the DT_{50} can be calculated as:

$$DT_{50} = \frac{Ln\,2}{k} \quad \text{(Eq. 3)}$$

Bi-exponential or two-compartments kinetic model:

$$C_t = C_1\, e^{-k_1 t} + C_2\, e^{-k_2 t} \quad \text{(Eq. 4)}$$

with: $C_1 + C_2 = C_0$

where C_1 and C_2 represent the initial concentration in each compartment (mg kg^{-1}), adding up to the initial concentration at time zero (C_0). k_1 and k_2 are the degradation constants for compartment 1 and 2, respectively ($k_1 > k_2$). This model implies that the herbicide is distributed in fractions of different degradability in the soil (two-site model) (Laabs *et al*., 2000). In the first compartment, the compound is found in a soluble or biologically available form. In the second compartment, the herbicide bio-availability is radically reduced due to the strong adsorption to the soil particles and is less available for microbial degradation (Hamaker and Goring, 1976). In this model, the DT_{50} values cannot be found analytically, but they can be estimated from readings of the curve or by iterative methods (Fomsgaard, 2004).

Desorption studies

A batch experiment was used to measure glyphosate adsorption and desorption at an equivalent concentration to that of the initial dose used in the dissipation study. For this, 2 g of soil from each sample were placed in a 50 ml flask and re-suspended with 40 mL of a 0.01 M $CaCl_2$ solution for 24 h at a constant temperature of 20 ºC (OECD, 2000). The soil slurry was spiked with 400 µL of a 20 mg L^{-1} glyphosate solution. Samples were shaken for 24 h at a constant temperature (20 ºC) and then centrifuged for ten minutes at 664 *g*. An aliquot of 2 ml of the aqueous phase was used to quantify the remaining glyphosate in the solution, and indirectly estimate the amount of glyphosate in the soil. The rest of the aqueous phase was discarded carefully to avoid any soil loss

during manipulation. The volume of the solution that was removed was replaced with an equal volume of 0.01 M $CaCl_2$ and the soil was re-suspended and shaken at a constant temperature for another 24 h. After this, samples were centrifuged and glyphosate was measured again in the aqueous solution in order to quantify the glyphosate that desorbed from the soil matrix. The procedure was repeated four times and each desorption step was considered as Desorption 1, 2, 3 and 4.

Glyphosate and AMPA analysis

The extraction and quantification of glyphosate and AMPA in the soil samples was performed as follows. Five g of soil were spiked with 50 µL of a 10 $\mu g L^{-1}$ isotope-labeled glyphosate (1,2-^{13}C, ^{15}N), and then shaken vigorously to ensure homogeneous distribution and left 30 min to stabilize. Afterward, 25 mL of extracting solution (100 mM $Na_2B_4O_7 \cdot 10H_2O$/ 100 mM K_3PO_4, pH=9) was added to the samples and placed in an ultrasonic bath for 30 min. This is a strong basic extraction method that has 90% of recovery in soil samples; therefore it allows the quantification of most of the adsorbed glyphosate. Tubes were then centrifuged for 10 min to separate the phases. An aliquot of 2 mL of the liquid phase was derivatized overnight with 2 mL of FMOC-CL (1 mg mL^{-1} in acetonitrile). Afterward, 4.5 ml of CH_2Cl_2 were added to the samples and shaken vigorously, to remove organic impurities and minimize matrix effects (clean-up step). The aqueous fraction was separated from the organic solvent after centrifuging for 10 min and the supernatant was collected and filtered through a 0.22 µm nylon filter. For the soil samples, the background solution used for the standard curve was the extractant solution. Each point of the standard curve was spiked with 4 µL of 10 $\mu g L^{-1}$ isotope labeled glyphosate (1,2-^{13}C, ^{15}N) in order to evaluate the analytical recovery of the method.

The extraction and quantification in water samples from the adsorption/desorption studies was performed by adding 1 ml of extracting solution to the 2 ml water sample. After shaking, the samples were derivatized by adding 1 ml of 1 mg mL^{-1} FMOC-CL solution and incubated over-night at room temperature. A clean-up step was performed according to the soil samples procedure. The standard curve for the water samples was prepared with ultra-pure water. The analytical recovery was evaluated by adding 10 µL of isotope-labeled glyphosate solution (10 $\mu g L^{-1}$) to each sample and point of the standard curve prior to the extraction step.

The chromatographic analysis was performed using a Waters® ACQUITY® UPLC (C18, 1.7 um 2.1 x 50 mm). The injection volume was 20 µL. The mobile phase flow was set at 0.4 mL min^{-1} at 60 ºC. The solvents used were 5 mM $NH_4(CH_3COO)$ in water (A) and 5 mM $NH_4(CH_3COO)$ in methanol (B), with a gradient set as follows: 100% A (0 -- 0.2 min); 30% A:70%B (0.2 --2.5 min); 100% B (2.5 --4.5 min); and 100% A (4.5--6 min). The target molecules were detected with a Waters® Micromass® Quattro Premier XE Mass Spectrometer (MS/MS). The source of ionization was set in positive mode using a capillary voltage of 3 kV. Collision gas was Argon 99.99% at a pressure of 44×10^{-3} mbar. The limit of detection (LD) in the soil samples was 0.5 $\mu g Kg^{-1}$ and the LQ was 10 $\mu g Kg^{-1}$, both for glyphosate and AMPA. In water samples, LD was 0.1 $\mu g L^{-1}$ and LQ was 0.5 $\mu g L^{-1}$.

Soil biological activity

The soil biological activity of each soil under NT and CT was measured indirectly in four replicates by quantifying the CO_2 produced by microbial respiration according to the methodology by Anderson (1982). Briefly, 25 g of soil sample were placed in a sealed 1.5 L jar containing a flask with 25 mL of NaOH (0.05 M). The jar was sealed airtight and samples were incubated for 3 days at 22 ºC ± 1°C. After incubation, the NaOH concentration remaining in the flask was measured by titration. For this, 2.5 mL of $BaCl_2.2H_2O$ (0.5 M) and two drops of phenolphthalein were added to

the flask containing the NaOH (0.05 M) solution. Then titration was done by adding drops of HCl (0.05 M). Four jars containing the 0.05 N NaOH solution without any soil sample were used as blanks.

Statistical analysis

Analysis of variance (ANOVA) was performed using a mixed linear model with the PROC MIXED procedure (SAS Institute version 9.0, 2002). Soil and tillage type were considered as fixed effects, and time as a repeated measure.

RESULTS AND DISCUSSION

Dissipation

In all the studied soils under NT and CT, there was an immediate degradation response to the glyphosate applied, without any lag phase (Fig. 2). Other authors have also noted that glyphosate mineralization starts rapidly without a lag phase (Mamy *et al*., 2005; Zablotowicz *et al*., 2009; Gimsing *et al*., 2004), even if the soil has never received any glyphosate treatment (e.g. de Andréa et al., 2003). This is an indication that the degrading enzyme system is active before the herbicide is applied (Torstensson, 1985; Franz *et al*., 1997) and it can be considered a general property although the rate of degradation can be very different from soil to soil (Borggaard and Gimsing, 2008). In our soils the initial dissipation was fast, and 40% of the applied glyphosate was degraded within the first three days. Afterward, the concentration remained between 50 and 40 percent until the end of the experiment in PAR and PER soils. In MAN soil, the concentration decreased by up to 80 % on day 62. The PROC MIXED analysis results show there was an interaction of soil type and time, meaning that there were significant differences in glyphosate concentration between locations per day ($P < 0.001$). On the other hand, there were no significant differences between the mean concentration of glyphosate for each day between tillage practices of the same soil ($P > 0.01$).

Fig. 2

Fig. 2 Percentage of degraded glyphosate with respect to the initial concentration. Error bars represent standard deviation (n=4).

Dissipation kinetics

Our results indicate that the glyphosate dissipation process in the studied soils follows a bi-exponential kinetic model (R^2= 0.955--0.987, Table II), whereas the first order kinetic model did not provide satisfactory results (not shown). Some studies have described glyphosate degradation using first order kinetics (Mamy *et al*., 2005; Ghafoor *et al*., 2011; Bergström *et al*., 2011), although others have used a bi-exponential approach (Zablotowicz *et al*., 2009; Simonsen *et al*., 2008). In the bi-exponential model, the dissipation rate k_1 of the first phase is dependent on the microbial ability of the soil to metabolize the glyphosate present in the soil solution. The degradation constant of the slow pool, k_2, is smaller than k_1 because microbial degradation is limited by a decrease in the compound's bioavailability due to its strong retention to the soil (Zaranyika and Nyandoro, 1993). In this way, degradation is limited by processes of rapid adsorption to soil particles and slow desorption from the soil matrix into the solution (Eberbach, 1998; Scow and Hutson, 1992).

TABLE II

Estimated kinetic dissipation parameters for glyphosate.

Soil	Tillage system	Bi-exponential kinetic model						
		A[a)] mg Kg^{-1}	B[a)] mg Kg^{-1}	k_1[a)] $days^{-1}$	k_2[a)] $days^{-1}$	R^2	DT_{50}[b)]	DT_{90}[b)]
PAR	NT	1.64	1.83	1.27	0.002	0.969	23	390
	CT	1.57	2.08	1.84	0.006	0.955	20	290
PER	NT	1.14	1.63	2.82	0.005	0.987	31	355
	CT	0.71	1.74	1.89	0.009	0.987	38	220
MAN	NT	0.99	1.61	1.52	0.020	0.977	11	90
	CT	0.89	1.89	2.95	0.003	0.980	9	58

[a)]Estimated parameter from Eq.4.
[b)]Estimated parameters from Eq. 3.

One of the most important properties that regulate retention of the herbicide in soil is adsorption. The results from the adsorption-desorption batch experiment show that at the initial concentration of 4000 μg Kg^{-1}, most of the glyphosate was completely adsorbed in all soils (Table III). At this initial concentration, no difference was found in adsorption between tillage practices or soils ($P < 0.05$). Glyphosate desorption was measured on four consecutive days (Table III). In general, the percentage of desorbed glyphosate was very low. No glyphosate was detected in neither of the desorption steps in PAR soil. There were no significant differences in desorption between tillage practices, therefore the results are shown for the average of each soil (Table III). For MAN and PER soil, desorption was < 0.5%, being significantly higher in desorption step 1 for MAN ($P < 0.05$). In the PAR soil, no glyphosate was detected in the aqueous solution. According to the Freundlich adsorption coefficient (*Kf*) reported by Okada *et al.* (2016), glyphosate adsorption capacity follows the order: PAR > PER > MAN. In this case, the higher adsorption capacity of PAR soil is associated to its high clay content and CEC (Okada *et al*., 2016) since glyphosate can be complexed with cations released from the clays via a cation exchange reaction (Glass, 1987). Another factor that influences the degree of adsorption is the amount of pre-adsorbed phosphate, which decreases adsorption since both molecules compete for the same sorption sites (Gimsing *et al*., 2004). MAN soil has a lower adsorption capacity, as this soil contains less clay and more phosphate (Okada *et al*., 2016). This also explains why desorption was higher, increasing glyphosate bio-availability. In summary, these processes resulted in a greater dissipation at the end of the experiment. Although some studies suggest that glyphosate adsorption is strongly related to the Fe and Al amorphous oxides content (Morillo *et al*., 2000), such relationship could not be detected for the soils used in the present study (Okada *et al*., 2016).

TABLE III

Adsorption and desorption of an initial glyphosate concentration of 4000 μg Kg^{-1}. Each desorption step corresponds to the glyphosate measured in the aqueous solution after 24 hr of equilibrium with a 0.01 M $CaCl_2$ solution. The procedure was repeated 4 times (Desorption 1, 2, 3 and 4).

Soil	Adsorbed glyphosate (%)	Desorbed glyphosate (%)			
		Desorption 1	Desorption 2	Desorption 3	Desorption 4
PAR	99.93 (0.10)	ND	ND	ND	ND
PER	99.11 (0.50)	0.13 (0.09) aA[a)]	0.12 (0.13) bA	0.16 (0.10) bA	0.07 (0.07) bA

MAN	98.10 (0.85)	0.33 (0.24) aB	0.13 (0.09) aA	0.13 (0.05) aA	0.12 (0.07) aA

ND: non-detectable. Standard deviation in parenthesis.
[a)]Lower case letters indicate significant differences between extraction steps in the same soil, upper case letters indicate significant differences between soils in the same extraction step ($P < 0.05$).

Dissipation time and biological activity

The DT_{50} and the time necessary to dissipate 90 % of the initial concentration (DT_{90}) were estimated from the bi-exponential model (Table III). For the studied soils, the values of DT_{50} ranged from 9 to 38 days, indicating a fast initial dissipation of glyphosate. However, the persistence in the soils is high as evidenced by the DT_{90} values. Even though glyphosate half-life time in these soils was not greater than 40 days, the time needed to dissipate 90% of the initially applied doses is over 7 months for PAR and PER soil, indicating the long persistence of the herbicide after the application.

Soil respiration has been related to the degradation of glyphosate mainly because it is an unspecific biological process. However, results reported in the literature are not conclusive. Some authors found a positive correlation between both parameters (Torstensson, 1985; von Wirén-Lehr *et al*., 1997), while others found no relationship (Gimsing *et al*., 2004; Rampoldi *et al*., 2014). In this study, the biological activity, measured as the soil respiration, was higher in PAR and PER compared to MAN soil and there were no differences between tillage practices ($P<0.01$) (Fig. 3). Therefore, there was no relationship between glyphosate total dissipation and microbial activity since MAN was the soil with the highest degradation but with the lowest soil respiration. Soil respiration is an unspecific soil microbial activity marker, thus the differences in degradation may be due not only to poor soil activity but also to differences in the microbial composition of the soils. For example, glyphosate mineralization was reported to be strongly correlated to the specific abundance of *Pseudomonas spp.* bacteria but not to the overall respiration activity (Gimsing *et al*., 2004).

Fig. 3

Fig. 3 Biological activity of each soil and tillage measured as soil respiration.

AMPA

Before the dissipation experiment, glyphosate and AMPA were measured in the soil samples. Though only traces of glyphosate residues were detectable, AMPA was present in all the samples (Fig. 4). On day 0 after glyphosate application, PAR and MAN contained 0.2 mg Kg^{-1} of AMPA and PER contained 0.3 mg Kg^{-1}. In all the soils and tillage practices, AMPA concentration remained constant for the first days, indicating a steady state between its formation and degradation. AMPA slowly accumulated over time, increasing its concentration above the initial soil's concentration in less than 10% in PAR and PER, and 30 % in MAN (Fig. 4). No significant difference was found between tillage practices within the same soil ($P > 0.01$). The fast decrease of glyphosate concentration at the beginning of the dissipation study was not reflected by an increase in AMPA concentration. From these results we are not able to estimate the actual amount of AMPA generated from the initially applied glyphosate since degradation of AMPA may also be occurring at the same time. However, since AMPA is a more persistent compound in soil than glyphosate (Simonsen *et al*., 2008), the fact that there was no substantial increase in AMPA concentration during the first three days suggests that the preferred degradation pathway in the studied soils is via the sarcosine metabolite. The sarcosine pathway is usually underestimated because it is harder to link to glyphosate degradation, since it is an unspecific compound also produced from other sources

and because it rapidly degrades to glycine, so it does not accumulate in soils (Karpouzas and Singh, 2006). However, the sarcosine pathway is metabolically more convenient since it is a quicker source of phosphorus and it is energetically more efficient than the AMPA pathway (Wang *et al*., 2016).

Fig. 4

Fig. 4 Accumulated AMPA expressed as the percentage of the initially applied glyphosate.

CONCLUSIONS

In all the studied soils the initial dissipation of glyphosate was fast, followed by a decrease in the dissipation rate. Over time, glyphosate becomes less bioavailable, making the remaining fraction more persistent especially in those soils with high adsorption capacity (PAR and PER). In this sense, the partitioning of the herbicide between the aqueous and the solid phase will influence the degradation, as it becomes less available to microorganisms, while it adsorbs to the soil matrix. There were no differences in dissipation between NT and CT, indicating that glyphosate degrading microflora was not modified with the different tillage managements. Also, tillage practices did not alter the general soil properties therefore; glyphosate bio-availability was not affected by NT or CT management.

Glyphosate initial dissipation was fast, whereas the accumulation of its metabolite, AMPA, was scarce. This suggests a fast AMPA degradation or that the preferred degradation pathway in these soils is via sarcosine.

The estimated half-lives for glyphosate in the studied soils under optimal temperature and moisture conditions ranged between 9 and 38 days. However, the less available residues can remain in the soil for almost a year after application. In the field, glyphosate might persist for even longer periods if conditions are temporarily less favorable for degradation (e.g. cold or dry seasons). The implications of this study are that glyphosate residues may accumulate in agricultural soils, especially if it is applied 2 to 3 times per year which is frequently the case. This may lead to negative impacts on the soil biota and furthermore, it increases the risk of polluting surface waters, by soil runoff, and groundwater resources, by vertical transport. It is important that glyphosate applications are kept to the needed minimum, in order to avoid its environmental accumulation and distribution.

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Figure captions

Fig. 1. Glyphosate degradation pathways into the metabolites AMPA and sarcosine, until complete mineralization.
Fig. 2. Percentage of degraded glyphosate with respect to the initial concentration. Error bars represent standard deviation (n=4).
Fig. 3**.** Biological activity measured as soil respiration.
Fig. 4. Accumulated AMPA expressed as the percentage of the initially applied glyphosate.

Table I. General characteristics of the studied soils (Okada et al., 2016).

Soil	PAR	PER	MAN
Location	Paraná, Entre Rios Province	Pergamino, Buenos Aires Province	Manfredi, Córdoba Province
Coordenates	31°51’ 15’’ S, 60°32’ 10’’ W	33º 57’ S, 60º 33’ W	31º 56´ 55´´ S 63º 46 ` 30´´ W
Years under NT and CT	16	34	30
Soil type	Aquic Argiduoll	Typic Argiuduoll	Entic Haplustoll
Series	Tezanos Pinto	Pergamino	Oncativo
Texture	Silty clay loam	Silty loam	Silty loam
Sand (%)	9.2 b[a)]	12.5 ab	16.9 a
Silt (%)	54 b	64.8 a	66.8 a
Clay (%)	36.8 a	22.7 b	16.3 c
CEC (meq 100 g^{-1})	28.9 a	20.7 b	17.4 b
pH	6.0 b	5.8 b	6.4 a*
OC[b)] (%)	1.6 a	1.7 a	1.1 b
P-Bray[c)] (mg Kg^{-1})	34.4 b	29.4 b	64.0 a
Fe [d)] (mg Kg^{-1})	1677.7 b	3184.3 a	1191.1 c
Al[d)] (mg Kg^{-1})	221.6 b	185.2 b	323.8 a

[a)]Different letters indicate significant differences between soils ($P < 0.001$, $*P < 0.05$).
[b)]OC: organic carbon measured with the oxidation chromic acid method (Walkley and Black, 1934)
[c)]P-Bray: available phosphorous according to Bray and Kurtz (1945).
[d)]Amorphous Al and Fe oxides extracted with 0.2 M acidified ammonium oxalate (pH 3) (Blackemore *et al*., 1987). Al was determined with a UV spectrophotometer with the Aluminon method (Barnhisel and Bertsch, 1982) and Fe using a specific atomic adsorption lamp.

Table II. Estimated kinetic dissipation parameters for glyphosate.

Soil	Tillage system	Bi-exponential kinetic model						
		A[a)] mg Kg^{-1}	B[a)] mg Kg^{-1}	k_1[a)] $days^{-1}$	k_2[a)] $days^{-1}$	R^2	DT_{50}[b)]	DT_{90}[b)]
PAR	NT	1.64	1.83	1.27	0.002	0.969	23	390
	CT	1.57	2.08	1.84	0.006	0.955	20	290
PER	NT	1.14	1.63	2.82	0.005	0.987	31	355
	CT	0.71	1.74	1.89	0.009	0.987	38	220
MAN	NT	0.99	1.61	1.52	0.020	0.977	11	90
	CT	0.89	1.89	2.95	0.003	0.980	9	58

[a)]Estimated parameter from Eq.4.
[b)]Estimated parameters from Eq. 3.

Table III. Adsorption and desorption of an initial glyphosate concentration of 4000 μg Kg^{-1}. Each desorption step corresponds to the glyphosate measured in the aqueous solution after 24 hr of equilibrium with a 0.01 M $CaCl_2$ solution. The procedure was repeated 4 times (Desorption 1, 2, 3 and 4).

Soil	Adsorbed glyphosate (%)	Desorbed glyphosate (%)			
		Desorption 1	Desorption 2	Desorption 3	Desorption 4
PAR	99.93 (0.10)	ND	ND	ND	ND
PER	99.11 (0.50)	0.13 (0.09) aA[a)]	0.12 (0.13) bA	0.16 (0.10) bA	0.07 (0.07) bA
MAN	98.10 (0.85)	0.33 (0.24) aB	0.13 (0.09) aA	0.13 (0.05) aA	0.12 (0.07) aA

ND: non-detectable. Standard deviation in parenthesis.
[a)]Lower case letters indicate significant differences between extraction steps in the same soil, upper case letters indicate significant differences between soils in the same extraction step ($P < 0.05$).

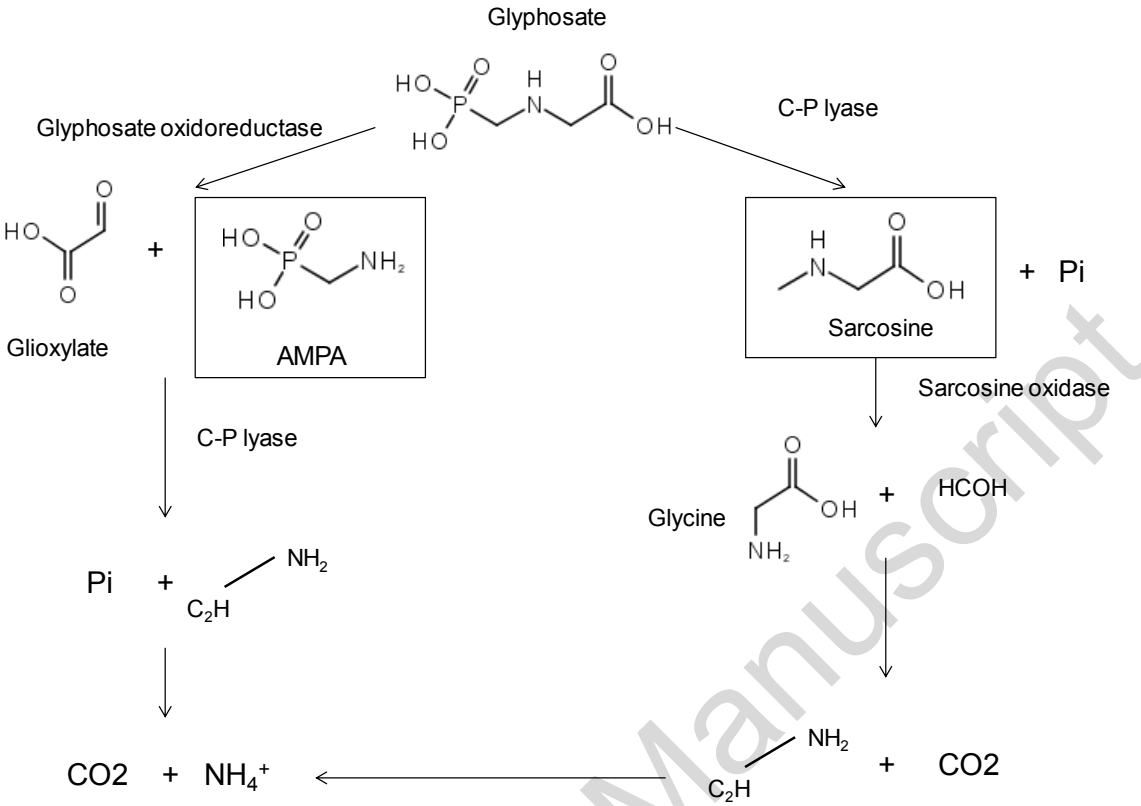


Fig. 1. Glyphosate degradation pathways into the metabolites AMPA and sarcosine, until complete mineralization.

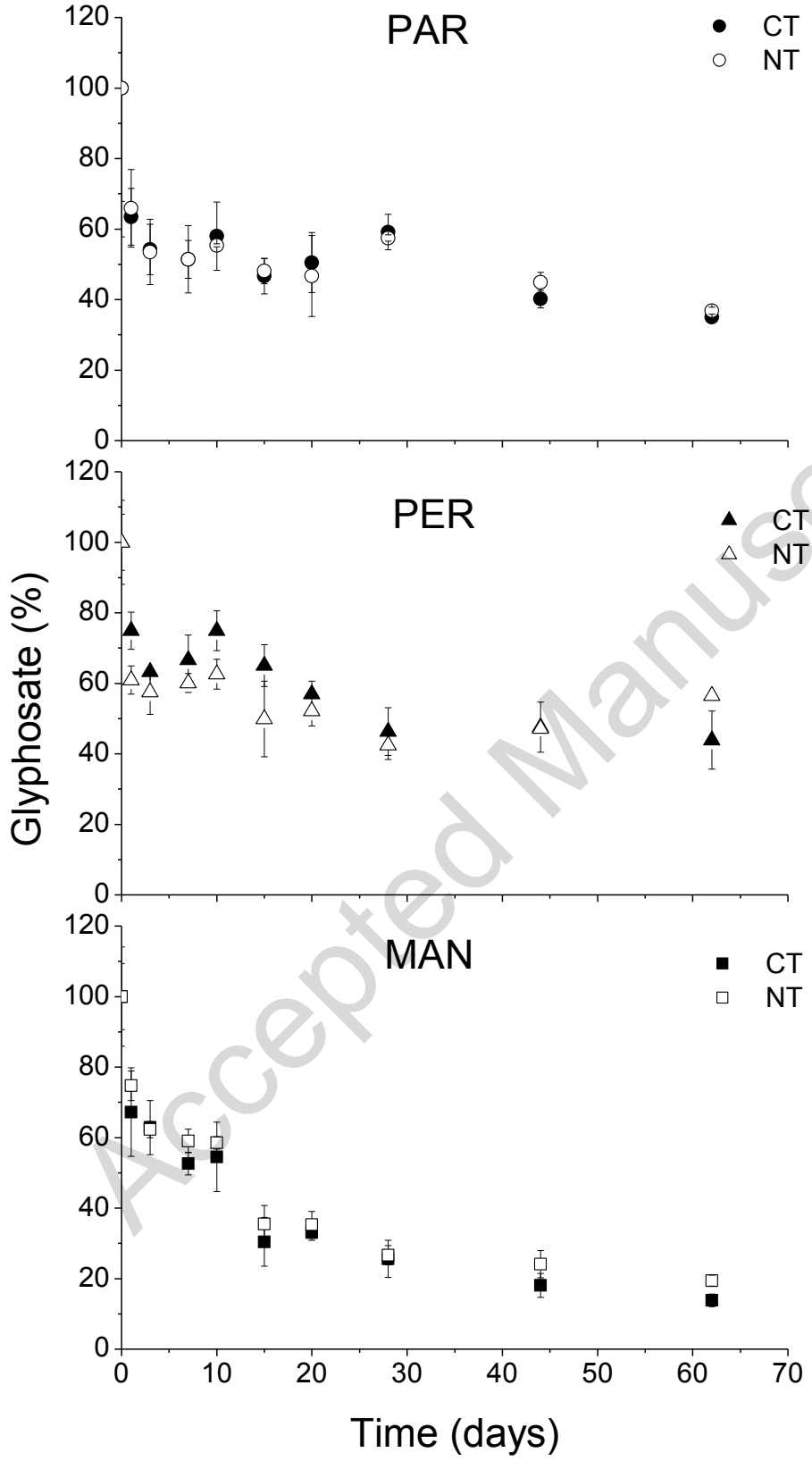


Fig. 2. Percentage of degraded glyphosate with respect to the initial concentration. Error bars represent standard deviation (n=4).

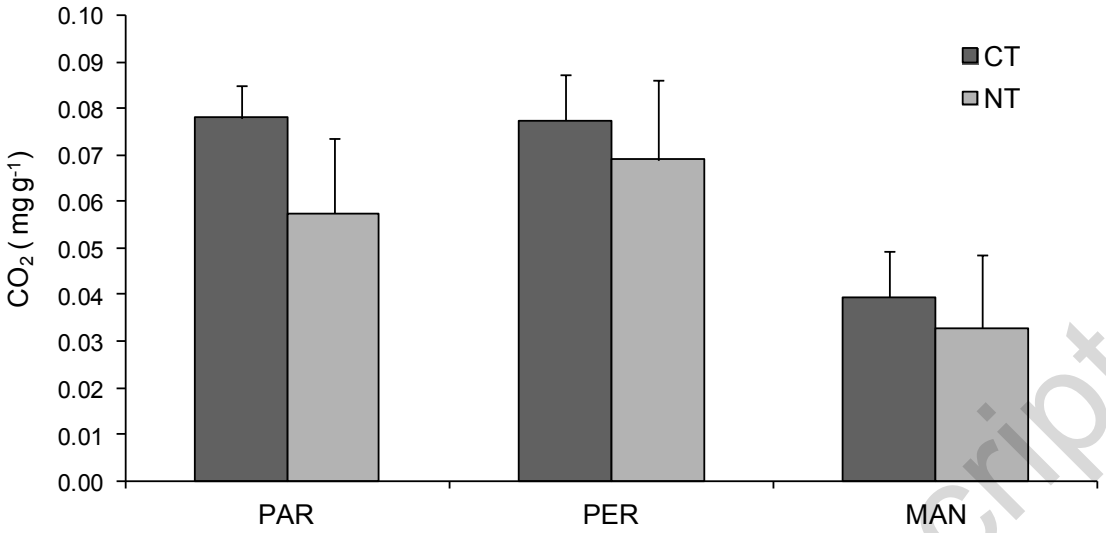
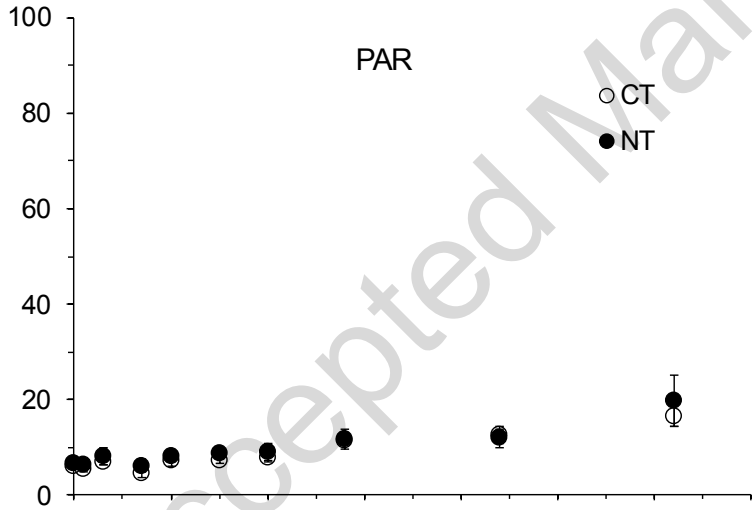


Fig. 3. Biological activity of each soil and tillage measured as soil respiration.



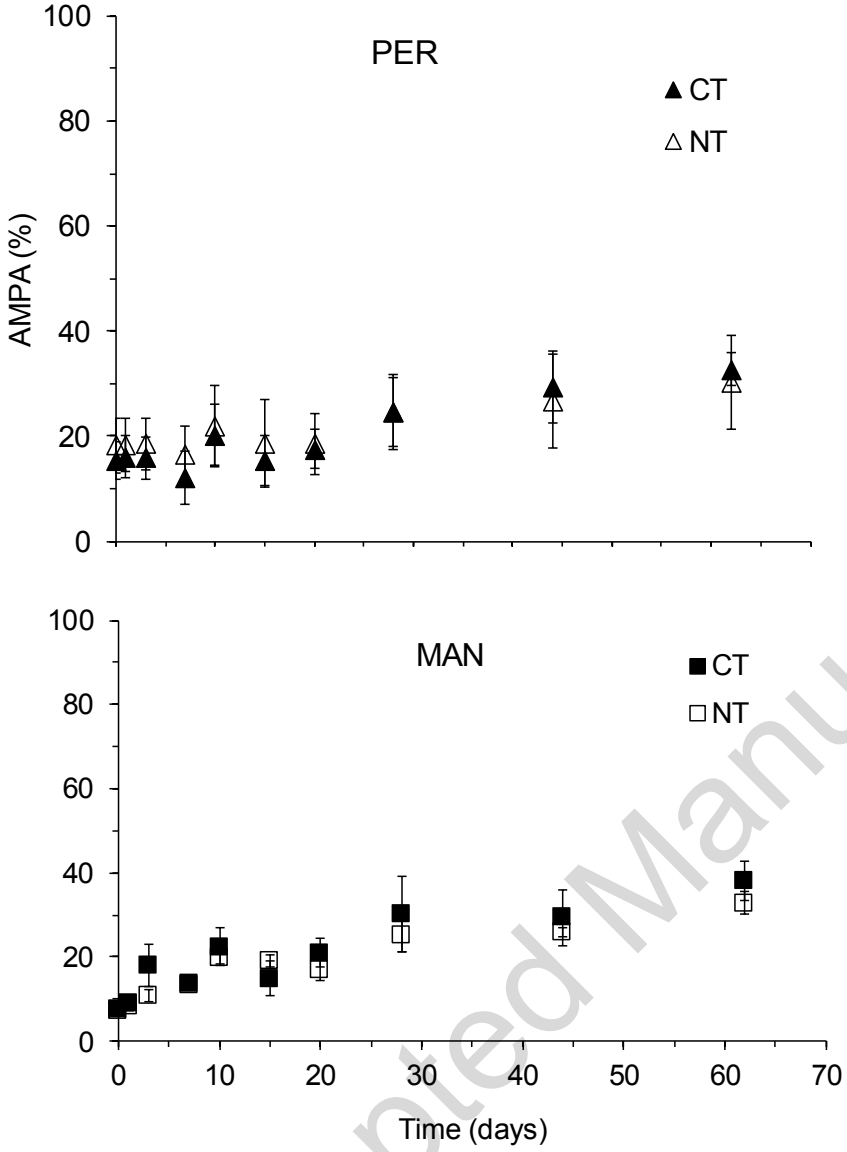


Fig. 4. Accumulated AMPA expressed as the percentage of the initially applied glyphosate.