Adsorption and mobility of glyphosate in different soils under no-till and conventional tillage

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ABSTRACT

Glyphosate (N-(phosphonomethyl) glycine) is a post-emergence, non-selective, foliar herbicide. Around 200 million liters of this herbicide are applied every year in Argentina, where the main agricultural practice is no-till (NT), accounting for 78.5% of the cultivated land. In this work, we studied the adsorption of glyphosate in different soils under long-term management (more than 16 years) of NT and conventional tillage (CT). Samples were taken from different regions of Argentina corresponding to: Paraná soil (PAR), a silty clay loam soil (<37% clay), Manfredi (MAN) and Pergamino (PER), both silty loam soils (<26% clay). We found that the adsorption was very high in all the soils, and it was particularly influenced by the soil clay content and CEC and negatively related to pH and phosphorus. In general, the adsorption coefficient (Kf) was higher in the CT samples. We also studied the vertical transport of glyphosate in undisturbed columns (15 cm long) and compared the effect of NT and CT. Less than 0.24% of the applied pesticide leached in all soils. No significant difference was found between the total amount of leached glyphosate between soils or tillage practice. The highest glyphosate concentration (67.53% of the initially applied doses) was found in the top 5 cm of the columns. The strong retention of glyphosate to the soil matrix, as confirmed by the high Kf values obtained in the isotherm studies, was the dominant factor influencing glyphosate mobility through the soil profile.

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1. Introduction

When a pesticide is applied in the field, a great proportion of it reaches the soil where several factors can influence its final destiny. Some factors depend on the intrinsic properties of the pesticide (e.g. adsorption, solubility, and persistence). Other factors depend on the physico-chemical and biological properties of the soil (e.g. organic content, humidity, biomass, pore connectivity, and pH) (Holland, 2004). The soil properties are also influenced by climate factors such as rainfall and temperature, as well as cropping and managing practices.

The tillage system can modify the chemical and biological properties of the soil. It also alters the porous space, modifying the hydraulic properties and the solute and water transport through the soil profile (Larsbo et al., 2009). In soils under conventional tillage (CT), macropores are generally destroyed, and only the intraaggregate space is preserved (Mapa et al., 1986). In no-till (NT) systems the formation of continuous macropores is promoted (Locke and Bryson, 1997), allowing the preferential flow of water and chemical substances to groundwater levels (Harris et al., 1993; Kamau et al., 1996; Ogden et al., 1999). In some cases, NT can increase the organic matter (OM)

content, which in return promotes the retention of certain pesticides (Levanon et al., 1994; Novak et al., 1996).

Glyphosate (N-(phosphonomethyl) glycine) is a post-emergence, non-selective, foliar herbicide. Around 200 million liters of this herbicide are applied every year in Argentina, where the main agricultural practice is NT, accounting for 78.5% of the cultivated land (Aapresid, 2012). Glyphosate has an amine, carboxylate, and phosphonate group, and it behaves as an amphoteric molecule with four ionization constants: pKa = 2, 2.6, 5.8 and 10.8 (Sprankle et al., 1975). It has a high water solubility (11.6 g L^{-1} at 25 °C) (Montgomery, 1993), that may increase the risk of being transported in the aqueous phase. On the other hand, it has a tendency to highly adsorb to soil particles, which can lower the potential to contaminate surface waters or groundwater (Vereecken, 2005). The ability to adsorb to soil particles contributes to the accumulation of glyphosate in soil. Nevertheless, the degree of adsorption can be affected by several factors. Adsorption has been related to the soil clay content and the cation exchange capacity (CEC) (Hiera da Cruz et al., 2007), suggesting that glyphosate can be complexed by cations released from clays via cation-exchange reaction with solution protons (Glass, 1987). Other important factors that influence adsorption are crystalline and amorphous aluminum and iron oxides (Morillo et al., 2000). Within the soils pH range (between 4 and 8), glyphosate is found in its anionic form and has a high affinity

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for Al^{3+} and Fe^{3+} trivalent cations (Sheals et al., 2002; Gimsing and Borggaard, 2007; Barja and Dos Santos Afonso, 2005). As the soil pH increases, glyphosate's adsorption decreases (Zhao et al., 2009). Additionally, inorganic phosphate competes strongly for the same adsorption sites, thus increasing glyphosate's mobility in soil (Prata et al., 2005).

Soil structure is an important factor influencing glyphosate transport (Aronsson et al., 2011; Gjettermann et al., 2009). Several laboratory and lysimeter studies have indicated that glyphosate may be transported by preferential flow in structured soils (Vereecken, 2005). Kjaer et al. (2011) have demonstrated rapid macropore mediated transport of glyphosate in a field study that was monitored for eight months after pesticide application. The study's results demonstrated that even though glyphosate is a strong sorbing pesticide, leaching can occur via preferential transport pathways. The average concentrations found in the drainage runoff exceeded the EU limit value for groundwater of $0.1 \mu g\,L^{-1}$. In another field study, glyphosate percolated to the drainage water in a clayey soil, whereas no leaching was detectable under the same conditions in a sandy soil (Aronsson et al., 2011). Vertical transport has also been studied in laboratory conditions using undisturbed or repacked soil columns (e.g. de Jonge et al., 2000; Dousset et al., 2004; Strange-Hansen et al., 2004; Barrett and McBride, 2006; Gjettermann et al., 2009, 2011). The results from the studies are variable and depend on the selected experimental conditions, such as input flow, herbicide concentration, time duration and the type of soil studied.

Once glyphosate reaches the soil, it can be mineralized by microbial activity (Rampoldi et al., 2014; Gimsing et al., 2009; Dick and Quinn, 1995). The main pathway described for glyphosate mineralization in the environment is via the glyphosate oxidoreductase enzyme, which yields aminomethylphosphonic acid (AMPA) and glyoxylate. The AMPA molecule accumulates in the soil since its generation is faster than its degradation (Simonsen et al., 2008). Glyphosate can also be hydrolyzed to sarcosine, by the activity of the C–P lyase enzyme. Contrary to AMPA, sarcosine is easily degradable does not accumulate in soil (Borggaard and Gimsing, 2008) and is not an exclusive metabolite of glyphosate's degradation.

Very few studies have addressed the effect of tillage practices on glyphosate adsorption and mobility using samples from long-term field trials. Therefore, the focus of this work was to compare the effect of NT and CT management practices in different soils from Argentina on the adsorption and leaching of glyphosate. For this purpose, first we performed batch isotherm studies to compare the adsorption between soils and tillage systems. To study the vertical movement, we used undisturbed soil cores from long-term field trials under NT and CT. The transport of an inert molecule (bromide) in these columns was previously described in Okada et al., 2014. In the latter work, the authors described bromide transport using the convection–dispersion equation and compared the effect of tillage and soil on the velocity and the hydrodynamic dispersion coefficient. Thus, the second objective of the present work was to study the effect of different soils under NT and CT on glyphosate transport. The formulated hypotheses are: (i) glyphosate's adsorption is influenced by the physico-chemical properties of the soils; (ii) the vertical transport of glyphosate is higher in soils under NT than in CT.

2. Material and methods

2.1. Soils and sampling

Soil samples were obtained from long-term field trials from three different Experimental Stations of the National Institute of Agronomical Technology (INTA). The Manfredi (MAN) experimental site is located in Córdoba Province and was established 30 years ago. The soil corresponds to a coarse-silty, mixed, thermic Entic Haplustoll of the Oncativo series (INTA, 1987). Samples were taken from treatments under NT and CT with a maize-soybean rotation. Parana (PAR)

experimental site is located in Entre Ríos Province. The soil belongs to the Tezanos Pinto series, which is characterized as a fine, mixed, thermic Acuic Argiudoll (INTA, 1998). It is a deep soil and moderately well drained. Soil samples were taken from a long-term field trial (16 years) under NT and CT, with a wheat/soybean–maize rotation. Pergamino (PER) site is located in Buenos Aires Province. The soil is classified as fine, thermic, illitic, Typic Argidioll (Pergamino series) (INTA, 1972). They are well-drained soils with medium permeability. The field trial was established 34 years ago under NT and CT, and it has a maize–wheat/soybean rotation.

Undisturbed soil columns were sampled in a completely randomized blocks design, resulting in 4 columns from each tillage practice of the studied soils (total number of columns = 24). Core samples were obtained introducing stainless steel cylinders of 8 cm wide inner diameter and 15 cm length into the top soil. Samples were then sealed with plastic lids and stored at 4 °C until the transport studies were conducted.

Disturbed soil samples from 0 to 15 cm depth of top soil were also collected from each block of the sampled columns for the adsorption isotherm experiments (n = 24). Subsamples from each replicate were also used for physical and chemical analysis.

Particle size distribution was obtained by the pipette method (Soil Conservation Service, 1972), and organic carbon content (OC) was measured through oxidation using the chromic acid method (Walkley and Black, 1934).CEC was determined by displacement with 1 M ammonium acetate at pH 7 (Chapman, 1965), and pH was measured by an electrode in a soil:water ratio of 1:2.5. Available phosphorous (P-Bray) was determined according to Bray and Kurtz (1945). Specific surface area (SSA) was measured by the ethylene glycol monoethyl ether method (Heilman et al., 1965; Carter et al., 1986). Al and Fe amorphous oxides were extracted with 0.2 M acidified ammonium oxalate (pH 3) (Blackemore et al., 1987). Al was determined using the Aluminon method (Barnhisel and Bertsch, 1982) and Fe using a specific atomic adsorption lamp.

2.2. Chemicals

Stock solutions for the standard curves of all the glyphosate measurements and the isotherm studies were prepared using pure analytical glyphosate (PESTANAL[R], 99.9%) purchased from Sigma-Aldrich, and AMPA (PESTANAL[R], 99%). HPLC-grade methanol and HPLC-grade acetonitrile (ACN) for analytical procedures were purchased from Seasinglab. Nanopure water was obtained by purifying demineralized water in ELGA Purelab ultra (Illinois, USA). For the column experiment, the stock solution of glyphosate was prepared using commercial glyphosate (ATANOR II®, 35.6% acid equivalent).

2.3. Glyphosate and AMPA analysis

To quantify glyphosate and AMPA from water samples of the column experiments and isotherm studies, an aliquot of 3 ml of each sample was transferred to a 15 mL polyethylene flask. Then, 0.5 ml of borate buffer (0.04 mM $Na_2B_4O_7 \cdot 10\,H_2O$, pH = 9) and 0.5 ml of ACN were added. After shaking, the samples were derivatized with 0.5 ml of 9-fluorenylmethylchloroformate (FMOC-CL) dissolved in ACN ($6\,g\,L^{-1}$), and incubated overnight at room temperature. As a clean-up step, to remove any organic impurities and minimize matrix effects, 4.5 ml of CH_3Cl_2 were added to the samples and shaken vigorously. Samples were centrifuged for 10 min to separate the aqueous fraction from the organic solvent. The supernatant was collected and filtered through a 0.22 μm nylon filter, and then analyzed by liquid chromatography (LC) coupled to a tandem mass spectrometer (MS/MS).

To extract and quantify glyphosate and AMPA from the soil samples of the column experiments, 5 g of soil from each depth were subsampled and placed into 50 mL tubes for analysis in the lab. Previous to the extraction method, samples were spiked with 50 μl of an

isotope-labeled glyphosate (1,2-^{13}C, ^{15}N, Sigma-Aldrich) stock solution (10 mg L^{-1}). Samples were vigorously shaken for homogenization and then left 30 min to stabilize. Afterward, 25 mL of extracting solution (100 mM Na$_2$B$_4$O$_7\cdot$10H$_2$O/100 mM$\cdot$K$_3$PO$_4$, pH = 9) was added to the subsamples and then agitated using an ultrasonic bath for 15 min. Tubes were then centrifuged for 10 min. An aliquot (2 mL) of the liquid phase was then derivatized with 2 mL of FMOC-CL (1 mg mL^{-1} in ACN). Clean up step and UPLC MS/MS analysis was performed according to the water samples procedure. For the soil samples, the background solution used for the standard curve was the extracting solution. An equivalent amount of isotope-labeled glyphosate was added to each point of the standard curve to evaluate the analytical recovery of the method. The chromatographic analysis was carried out as previously described in Aparicio et al. (2013). The limit of detection (LD) and the limit of quantification (LQ) both for glyphosate and AMPA in water samples were 0.1 μg L^{-1} and 0.5 μg L^{-1}, respectively. For soil samples, the LD of both compounds was 0.5 μg Kg^{-1} and the LQ was 10 μg Kg^{-1}.

Before the experiments, glyphosate and AMPA concentration was measured in the soil samples to quantify the initial charge of pesticide. Glyphosate was detected below the LQ while AMPA concentration ranged from 103.5 to 320.5 μg L^{-1} (Appendix A).

2.4. Adsorption isotherms

Adsorption isotherms were conducted according to the Batch Equilibrium Method (OECD, 2000). 40 mL of a 0.01 M CaCl$_2$ solution was added to 2 g of soil and shaken for pre-equilibration for 24 h at a constant temperature of 20 °C. After reaching equilibrium with the solution, different concentrations of glyphosate solutions were added. The initial concentrations (C_o) used were 0, 0.5, 1, 1.5, 2, 5, 10 and 20 mg L^{-1}. Samples were shaken for 24 h at a constant temperature (20 °C) and then centrifuged. An aliquot of the supernatant was then analyzed for pesticide concentration. The experiment was conducted with four replicates.

Isotherms were fitted using the non-linear Freundlich equation:

$$C_s = K_f \cdot C_{aq}^{1/n}$$

where C_s (mg Kg^{-1}) is the concentration of glyphosate adsorbed to the solid phase at equilibrium, C_{aq} (mg L^{-1}) is the mass concentration of the herbicide in the aqueous phase at adsorption equilibrium and K_f (mg$^{1-1/n}\cdot$L$^{1/n}\cdot$Kg^{-1}) is the Freundlich adsorption coefficient and n is an empirical constant.

The curves were modeled using the NLIN procedure with SAS version 9.0 software (SAS Institute, 2002). The estimated parameters were compared among soils and tillage (as fixed effects) using a linear mixed model (PROC MIXED). Mean comparisons were evaluated with a significance level of 0.05 using LSMEANS.

2.5. Column experiments

Solute transport studies were carried out under controlled laboratory conditions at a constant temperature of 20 °C. Previous to the leaching experiment, columns were slowly pre-saturated by capillary action with a 0.01 M CaCl$_2$ solution. Afterward, they were sealed with a cap containing a stainless steel plate with holes on both ends of the column, which allowed a uniform distribution of the inlet flow. Columns were irrigated with a 0.01 M CaCl$_2$ solution at a constant flow of 4.16 mm h^{-1} using a syringe pump during the whole duration of the experiment. At the lower boundary condition, the columns were connected to a vacuum chamber keeping a constant tension of −11 KPa. Inside the chamber, a fraction collector was used to collect the effluent at different time intervals. Previous to the glyphosate injection, bromide (Br$^-$) was used as a tracer to characterize non-reactive solute movement in the studied columns (Okada et al., 2014). After all the Br$^-$ was leached (approximately 3 PV), a pulse of a commercial solution of glyphosate (ATANOR II®) equivalent to 6 L ha was applied for 15 min (volume of injection: 6 ml; pulse concentration: 199 mg L^{-1} of active ingredient dissolved in a 0.01 M CaCl$_2$ solution). The applied rate in this study corresponds to the agricultural doses used in the fields, assuming three applications of 2 L ha^{-1} per year. Immediately after, columns were leached at a constant rate (4.16 mm h^{-1}) with a 0.01 M CaCl$_2$ solution for 8 PV (7 days). In some cases (PER CT and MAN CT) columns ponded and no effluent could be collected at approximately 5 PV. Samples were collected in the fraction collector and weighted to calculate effluent volume. The water samples were frozen until laboratory analysis to determine glyphosate and AMPA concentration.

After the leaching experiment, all columns were weighted and then cut into three segments corresponding to the upper part (0–5 cm from the top of the column), middle section (5–10 cm) and lower section (10–15 cm). The soil was then air dried at 30 °C for three days to remove excess water. Soil samples from each segment were sieved using a 0.5 mm mesh, and then kept frozen until analysis in the laboratory to determine glyphosate and AMPA concentration.

Selected physical properties, such as pore volume (PV), bulk density (δb), total porosity ($\emptyset$) and dispersion coefficient (D) are shown in Appendix B.

3. Results

3.1. Soils

Table 1 shows the physico-chemical characteristics of the soils. No significant difference was found between tillage systems regarding texture, exchangeable cations, pH, OC, P-Bray, SSA and Al and Fe amorphous oxides content ($P < 0.001$) (not shown). Overall, PAR was the soil with the highest clay content, while there were no significant differences between MAN and PER ($P < 0.001$). The CEC and Ca^{2+} content were also higher in PAR soil. The lowest organic carbon content (OC) was found in MAN soil ($P < 0.001$). In the case of pH, MAN values were higher than PAR and PER ($P < 0.05$). There was no significant difference in the OC between tillage practices. NT generally increases organic matter (OM) content in the superficial layer of 0–5 cm depth (Montoya et al., 2006). However this can invert as depth increases yielding no net change between systems since the OC contents become higher at 15 cm depth in CT management while they decrease in NT (VandenBygaart and Angers, 2006; Montoya et al., 2006). Since the soil samples in this study were taken from the first 15 cm of the top soil, the homogenization of the samples might have masked any differences between tillage systems.

3.2. Adsorption isotherms

Overall, glyphosate's adsorption was very high in all the tested initial concentrations (Table 2). In PAR and PER, more than 89% of the herbicide was sorbed in the range of initial concentrations applied (from 0.5 to 20 mg L^{-1}). In MAN soil, the percentage of adsorbed glyphosate decreased to 73% when the initial concentration was 20 μg L^{-1} in both CT and NT. This behavior indicates that sorption becomes less efficient as the adsorption sites become fully saturated with glyphosate molecules (Barrett and McBride, 2006).

Glyphosate isotherms are shown in Fig. 1. Adsorption isotherms were modeled using the non-linear Freundlich model to estimate the empirical adsorption coefficients Kf and nf (Table 3). Isotherms exhibited a L-type ($1/nf < 1$) curve according to the classification of Giles et al. (1960), indicating that the adsorption firstly occurred on the higher energy sites of adsorption, followed by the low energy sites (Alok and Xu, 2001). The values of Kf ranged from 100 to 457. In general they are higher than others reported in the literature (Mamy and Barriuso, 2005; Yu and Zhou, 2005; de Jonge and de Jonge, 1999; Candela et al., 2007). Adsorption had the following order: PAR > PER > MAN ($P <$

Table 1
General physico-chemical characteristics of the studied soils.

	Texture (%)			Exchangable cations (meq 100 g^{-1})					pH	OC %	P-Bray (mg L^{-1})	SSA (m^2 g^{-1})	Fe^2 (mg·Kg^{-1})	Al^2 (mg·Kg^{-1})
	Sand	Silt	Clay	Ca^{2+}	Mg^{2+}	Na$^+$	K$^+$	CEC						
PAR	9.23 b[1]	54 b	36.77 a	19.59 a	3.48 a	0.13 c	1.44 b	28.79 a	6.01 b	1.6 a	34.4 b	140.1 a	1677.78 b	221.6 b
PER	12.5 ab	64.8 a	22.7 b	12.54 b	2.95 b	0.57 a	1.5 b	20.74 b	5.76 b	1.77 a	29.4 b	66.0 b	3184.34 a	185.2 b
MAN	16.87 a	66.8 a	16.34 c	9.22 b	1.16 c	0.24 bc	2.84 a	17.43 b	6.42 a*	1.12 b	64.0 a	61.8 b	1191.09 c	323.8 a

OC: organic content, CEC: cation exchange capacity, SSA: specific surface area.
[1] Different letters indicate differences between soils ($P < 0.001$, $*P < 0.05$).
[2] Amorphous Al and Fe oxides.

0.05). There was a tendency of a higher Kf in the CT samples, but this difference was only significant in the PER and MAN soils ($P < 0.05$) (Table 3).

The clay content was strongly correlated to the Kf (r = 0.85, $P < 0.001$), CEC(r = 0.85, $P < 0.001$) and by a less extent to OC (r = 0.44, $P < 0.05$) (Table 4). Bergström, et al. (2011) obtained similar results, with a correlation between glyphosate adsorption and clay of r = 0.987. The amount and type of clays in the soil is one of the main factors controlling glyphosate adsorption (Glass, 1987; Dion et al., 2001; de Santana et al., 2006). In this case, PAR soil not only had the highest amount of clay content, but also presented a very high SSA, due to the fact that the main clay minerals are montmorillonite type (INTA, 1998). A greater SSA has more available adsorption sites for the herbicide to bond (Petersen et al., 1996). Although adsorption has been strongly related to Fe and Al amorphous oxides (Morillo et al., 2000), in our study we found no relationship between those parameters and the adsorption coefficients.

The reason the clay fraction may increase glyphosate retention is explained by CEC. The CEC, which is a variable dependent on the clay content (Cremers and Laudelout, 1966) and OM, has been associated to glyphosate retention mainly because the herbicide can be complexed with cations released from the clays via a cation exchange reaction with solution protons (Glass, 1987). Hiera da Cruz et al. (2007) also found an increase in glyphosate sorption with increasing CEC. De Jonge and de Jonge (1999) concluded that glyphosate's adsorption was influenced by the ionic strength and major cations (Ca^{2+}, K$^+$ or NH^{4+}) in solution. Complexation of glyphosate with surface-exchanged multivalent cations has been suggested as one of the possible sorption mechanisms (McConnell and Hossner, 1989; Sprankle et al., 1975). This could also explain stronger glyphosate sorption at higher ionic strength levels, because the diffuse double layer is compressed and Ca cations become more strongly attached to the clay surfaces (de Jonge and de Jonge, 1999).

Other factors that influence adsorption are the soils pH and pre-adsorbed phosphate. In this case, we found a negative relationship between the Kf and pH (r = −0.44, $P < 0.05$) and the P-Bray (−0.57, $P < 0.05$) (Table 4). Glyphosate adsorption can be related to pH because the iron oxides become more protonated as the pH decreases, favoring the adsorption of the negatively charged glyphosate molecule (Morillo et al., 2000; Mamy and Barriuso, 2005; Wang et al., 2006). Phosphate also influences the degree of adsorption in a negative way, since both molecules compete for the same sorption sites (Gimsing et al., 2004). Additionally, the presence of pre-adsorbed phosphate can inhibit glyphosate's adsorption since it increases the negative charges of the soil, which increases the repulsion with the negatively charged molecules of glyphosate (Gimsing and Borggaard, 2007).

When we compared the Kf of each tillage practice within the same soil, we found an interaction between soil and tillage ($P < 0.05$). In this case, the Kf CT values were higher than in NT in the PER and MAN soils. PAR soil exhibits the same tendency though the differences were not statistically significant. This behavior cannot be attributed to the physico-chemical properties of the soils since no significant difference in the studied parameters was found between NT and CT. A possible explanation of the higher adsorption found in the CT samples is that the amount of particulate organic matter (POM) between macroaggregates is increased with the plowing of the soil (Six et al., 2000). This increases the exposed adsorption sites for glyphosate, since it bonds to the OM via hydrogen bond (Piccolo et al., 1996). So even though the net OM content is the same in NT and CT, there could be more exposed adsorption sites in the tilled soil.

3.3. Transport

The transport of Br$^-$ in these columns was previously described in Okada et al. (2014). The authors found that the Br$^-$ breakthrough curves (BTC) exhibited an asymmetric behavior and tailing, indicating the occurrence of preferential flow. No difference between tillage practices was found regarding velocity or dispersion, except in the PAR columns. NT PAR had significantly higher hydrodynamic dispersion coefficient (D) than CT PAR suggesting that structure in this particular soil is influencing the water movement via preferential flow.

Regarding glyphosate transport, no systematic effect of tillage or soil could be observed because the results show a high variability between samples (Fig. 2). In general, glyphosate's mobility was very low. The results in Fig. 2 indicate that glyphosate leached in small amounts without showing a particular pattern or BTC. In some columns, there was an early peak of glyphosate, suggesting that a fraction of the molecule was transported with the water flow without being retained by the soil matrix after the pulse application. At the end of the experiment, the total eluted mass of glyphosate from the columns was less than 0.13% of the initially injected glyphosate mass (Table 5). The strong retention of glyphosate to the soil matrix, as confirmed by the high Kf values obtained in the isotherm studies, was the dominant factor influencing glyphosate mobility in the soil profile. Dousset et al. (2004) found that only 0.011% of the applied glyphosate leached in 20 cm long columns irrigated for 12 days. Zhao et al. (2009) reported no leaching of glyphosate in columns of a clay loam soil. On the other hand, glyphosate was found in the leachate in columns from a sandy loam and sandy soil. Barret et al. (2007) applied a commercial solution of glyphosate 10 times higher than the one used in the current study, and found that 80% of the pesticide was retained in the disturbed coarse soil columns. In this case, the elevated sorption of glyphosate could be

Table 2
Percentage of adsorbed glyphosate to the soil, with the different initial concentrations tested in the aqueous phase.

	Adsorbed glyphosate (%)					
	PAR		PER		MAN	
C_o (mg L^{-1})	NT	CT	NT	CT	NT	CT
0.5	100	100	100	100	95.2	100
1	100	100	99.5	100	93.0	99.5
1.5	100	100	99.5	100	96.8	98.0
2	99.9	100	97.9	100	96.3	99.0
5	99.4	99.8	90.4	99.8	89.3	97.5
10	98.7	98.6	89.1	98.6	72.4	91.9
20	96.1	96.6	93.7	96.6	73.9	73.2

C_o: initial concentration of glyphosate in the aqueous phase. NT: no-till management; CT: conventional tillage.
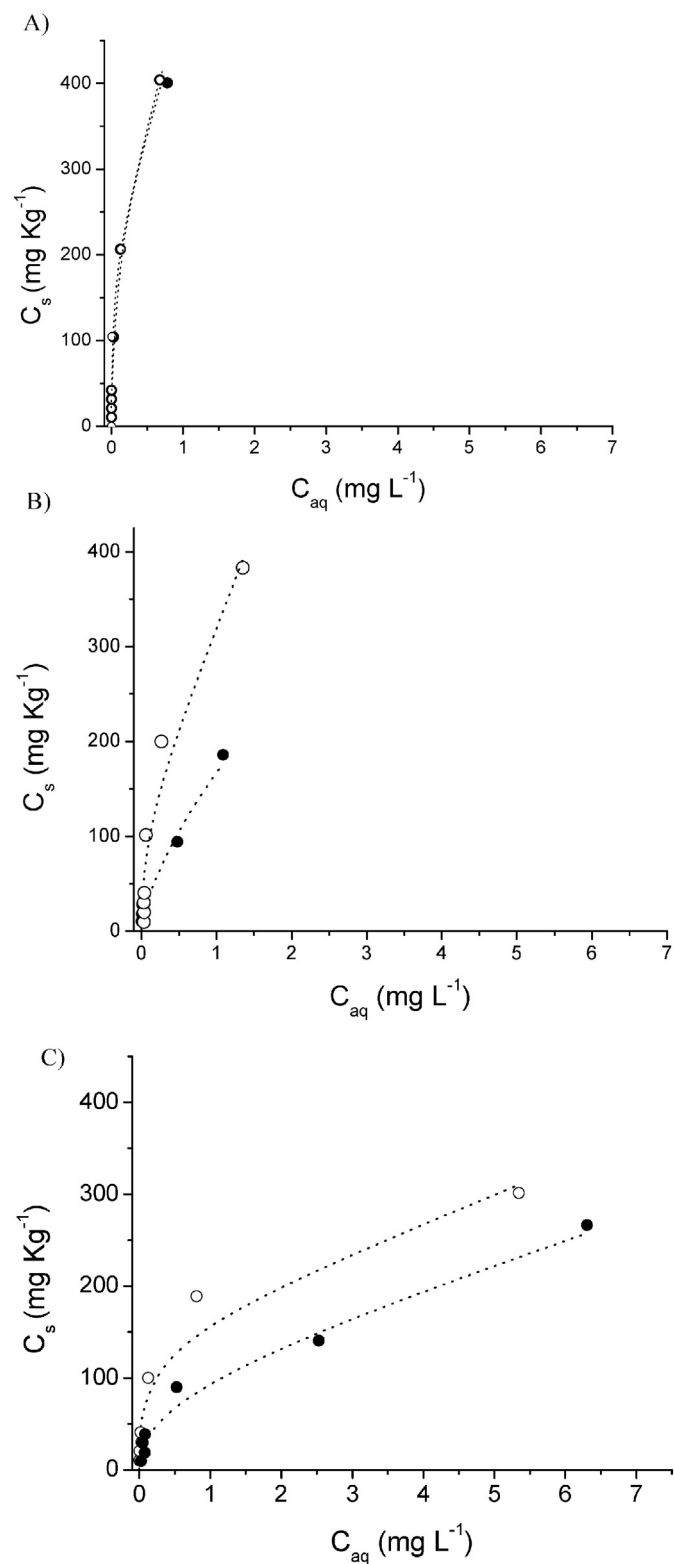
A)

B)

C)

Fig. 1. Adsorption isotherms of glyphosate for A) PAR, B) PER and C) MAN soils under NT (●) and CT (○). The dotted line shows the Freundlich model fit.

explained by the high organic matter content of the soils, suggesting that the pesticide binds by a metal bridge to organic functional groups. Gjettermann et al. (2009) had a maximum recovery in the leached samples of 0.32% from the initially applied glyphosate. They found that 68% of the leached glyphosate was bound to the soil particles

Table 3
Freundlich adsorption isotherm coefficients (Kf and $1/nf$) of glyphosate for the soils under NT and CT.

Soil	Kf		$1/nf$		R^2
	CT	NT	CT	NT	
PAR	457.4 aA[1]	414.2 aA	0.33	0.33	0.96–0.99
PER	345.7 aB	173.4 bB	0.33	0.43	0.94–0.99
MAN	182.6 aC	101.7 bC	0.52	0.47	0.96–0.99

NT: no-till, CT: conventional tillage.
[1] Different capital letters indicate significant differences between different soils under the same tillage treatment. Different lower case letters indicate significant differences between tillage practices in the same soil ($P < 0.05$).

(>20 nm), suggesting that particle-facilitated transport is an important process involved in glyphosate transport.

Other studies have found opposite results. For example, de Jonge et al. (2000) found a rapid breakthrough of glyphosate in sandy loam topsoil columns. Since the Kf for this soil was rather high ($Kf = 78.4$) a higher retention would have been expected. Nevertheless, glyphosate was detected from the first effluent samples (corresponding to less than 10 mm of the applied water), indicating that the pesticide solution bypassed the soil matrix. On the contrary, in the same study with columns from an unstructured sandy soil the leached glyphosate was 50 to 100 times lower than in the structured soil.

Leaching in field conditions have been reported before (Kjaer et al., 2011; Gjettermann et al., 2009). After glyphosate application in the field, the presence of the herbicide is limited to the surface layer of the soil, favoring the association between soil particles. Thus, facilitated particle transport via surface runoff (of splash eroded particles) and through preferential flow (macropores), could be the main transport modes of glyphosate (Kjaer et al., 2011; Gjettermann et al., 2009).

In this study, we used a 0.01 M CaCl$_2$ solution in order to prevent soil structure collapse. Thus, there were no particles leached in the effluent. If glyphosate's leaching mechanism is mainly via particle facilitated transport, we could be underestimating the leaching concentrations that could occur in the field under rain water condition. It is also worth noting that the applied concentration of the pulse equals to 199 mg L^{-1}. Considering that on average 1 PV of the studied columns corresponds to a volume of approximately 430 mL (Appendix B), when 1 PV has passed the column the glyphosate solution is diluted to 2.8 mg L^{-1}. As it can be seen in from the adsorption studies, 90% of the glyphosate is absorbed when the initial concentration in the solution is ≤5 mg L^{-1} in all the studied soils (Table 2). These results suggest that glyphosate was retained inside the column and this was further confirmed in the glyphosate soil quantification analysis.

3.4. Glyphosate retention in soil columns

After the leaching experiment, all columns were cut into three segments corresponding to the upper part (0–5 cm from the top of the column), middle section (5–10 cm) and lower section (10–15 cm). After being air dried, glyphosate and AMPA were quantified from each section. The glyphosate concentration in the upper part of the columns ranged from 0.35 to 1.56 mg·Kg^{-1} and 0.25 to 1.03 mg·Kg^{-1} for AMPA. These values are similar to those found in a field survey from samples across 16 agricultural farm soils of the southeast of Buenos Aires Province (Argentina), in which the detected concentrations of glyphosate in the top soil ranged from 0.035 to 1.50 mg·Kg^{-1} (Aparicio et al., 2013). In another environmental field study in the Province of Entre Ríos (Argentina), the values of glyphosate and AMPA ranged from 0.43 to 8.10 and 0.0013 to 38.9 mg·Kg^{-1}, respectively (Primost, 2013).

Glyphosate and AMPA distribution showed a similar pattern, being the highest concentration of both molecules in the top part of the column (Table 5), indicating that a portion of the applied glyphosate degraded into AMPA. AMPA was expressed on a glyphosate mass

Table 4
Pearson correlation coefficients (r) between the estimated Freundlich adsorption coeficiente (Kf) and selected soil properties.

	Kf	Sand	Silt	Clay	CEC	pH	OC	P-Bray	Fe	Al
Kf	1									
Sand	−0.64**	1								
Silt	−0.63**	0.27	1							
Clay	0.85**	−0.56*	−0.81**	1						
CEC	0.85**	−0.50	−0.82**	0.95**	1					
pH	−0.44*	0.27	0.05	−0.39	−0.30	1				
OC	0.44*	−0.42	−0.22	0.51	0.35	−0.60*	1			
P-Bray	−0.57*	0.09	0.29	−0.17	−0.16	0.11	0.08	1		
Fe[a]	0.35	−0.08	0.07	0.04	0.04	−0.14	−0.005	0.20	1	
Al[a]	−0.50	0.43	−0.39	−0.20	−0.20	0.06	−0.58	0.53	−0.79	1

CEC: cation exchange capacity, OC: organic content.
* $P < 0.05$.
** $P < 0.001$.
[a] Amorphous oxides.

equivalent basis and added to the glyphosate concentration to obtain the total extracted glyphosate (TEG) as follows (Coupe et al., 2011):

$$\text{Total extracted glyphosate (TEG)} = \text{glyphosate} \left(mg \cdot Kg^{-1} \right)$$
$$+ \left[AMPA \left(mg \cdot Kg^{-1} \right) \times MW_{gly}/MW_{AMPA} \right]$$

where MW_{gly}: glyphosate molecular weight (169 g mol^{-1}) and MW_{AMPA}: AMPA molecular weight (111 g mol^{-1}).

Concentrations of TEG decreased in the middle section and reached its minimum in the lower part of the columns (Table 5). No significant difference was found between tillage practices and soils regarding the distribution of TEG ($P = 0.8798$). The statistical analysis showed that there was a highly significant difference between depths (Fig. 3). The
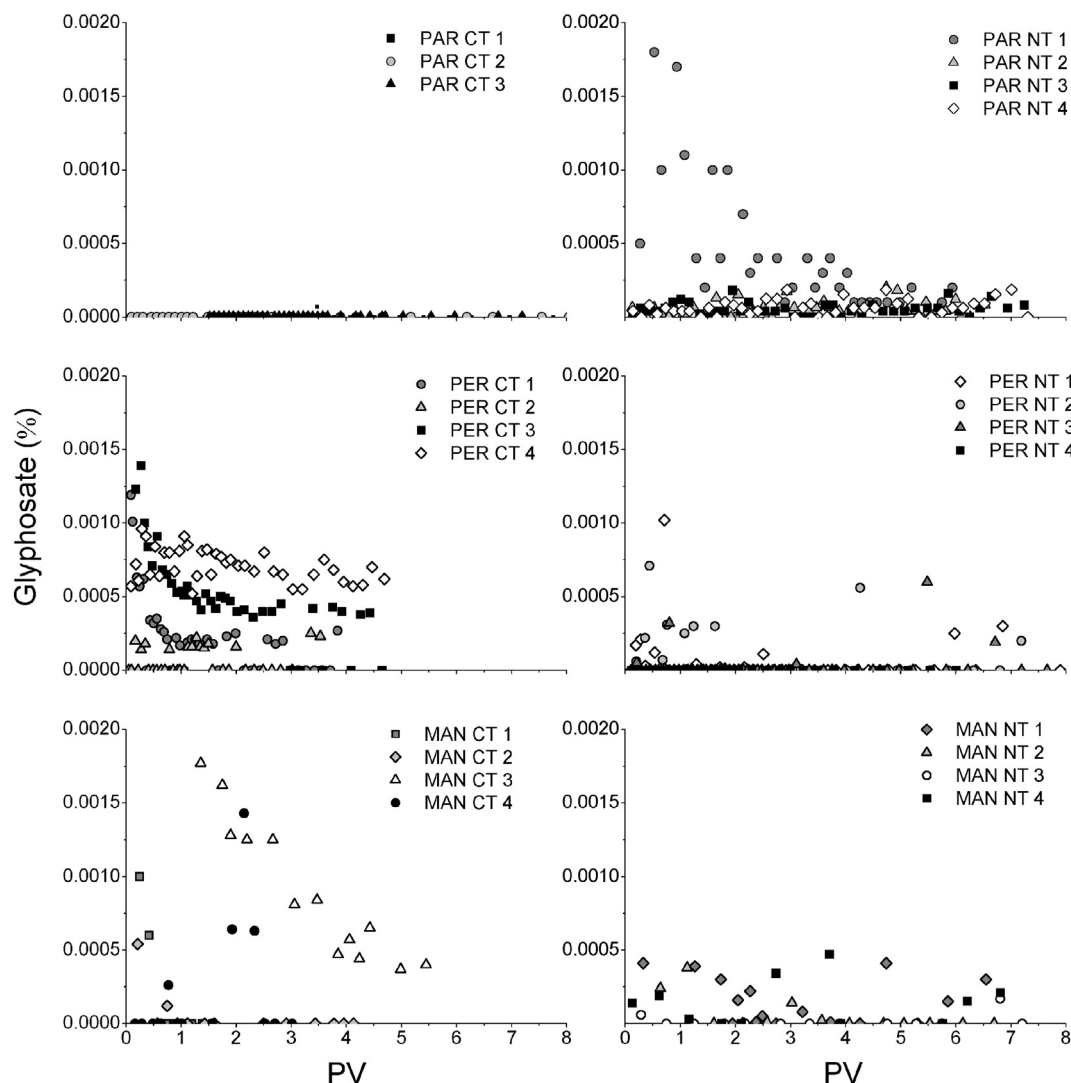
Fig. 2. Percentage of glyphosate found in the effluent samples with respect to the initially applied dose in the columns. Different symbols represent a different column. NT: no-till management; CT: conventional tillage, PV: pore volume.

Table 5
Percentage of the total leached glyphosate found in the water samples and distribution of glyphosate, AMPA and TEG in the soil columns at the end of the leaching experiment.

Column	Leachate	Soil			
	Total leached glyphosate	Depth	Glyphosate	AMPA	TEG
	(%[a])	(cm)	(%[a])		
MAN NT	0.01	0–5	51.1	8.5	59.6
		5–10	6.9	0.05	7.0
		10–15	2.7	1.98	4.7
MAN CT	0.07	0–5	52.9	12.5	65.5
		5–10	2.0	7.0	9.0
		10–15	0.3	3.1	3.4
PAR NT	0.05	0–5	47.2	10.2	57.4
		5–10	20.6	4.4	25.0
		10–15	4.33	1.83	6.1
PAR CT	0.00	0–5	58.6	16.5	75.2
		5–10	5.8	2.5	8.4
		10–15	0.0	0.7	0.7
PER NT	0.01	0–5	40.6	41.8	81.8
		5–10	3.97	9.14	13.1
		10–15	0.4	2.0	2.5
PER CT	0.13	0–5	40.9	24.5	65.5
		5–10	4.4	7.7	12.1
		10–15	2.9	9.71	12.6

[a] Calculated from the initially applied mass (μg) of glyphosate in the columns. NT: no-till management; CT: conventional tillage.

highest glyphosate retention was on the first top 5 cm of the columns (67.53%) ($P < 0.001$). Between the middle and lower section the difference was not significant. These results are similar to the ones reported by Zhao et al. (2009), in which almost all of the initially applied glyphosate was retained by the soil matrix in a clay loam soil (61% in the upper 5 cm of the column, 37% in the middle 5–9 cm and 0.7% in the last 11 to 15 cm). Feng and Thompson (1990) also reported that after glyphosate application, more than 90% of the applied herbicide was found in the first 5 cm of the soil profile due to the high adsorption of the herbicide to clay particles and OM.

The percentage of TEG remaining in the columns ranged from 71.4 to 97.5% of the initially applied glyphosate. Glyphosate's degradation can occur rapidly in the soil, with values of DT50 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). Since the recovery of TEG was not 100%, some of the glyphosate could have degraded into sarcosine or completely dissipated by microbial activity during the transport process.
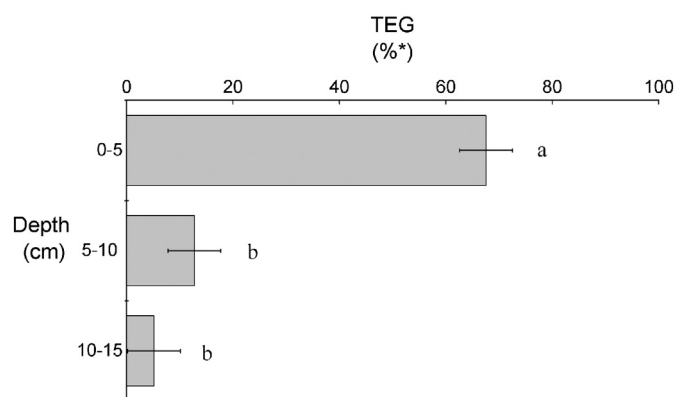
Fig. 3. TEG [mg·Kg^{-1} glyphosate + (mg·Kg^{-1} AMPA × 169/111)] distribution in the undisturbed soil columns after completion of the leaching experiment. Different letters indicate significant differences ($P < 0.001$). *Percentage of the initially applied glyphosate mass.

4. Conclusions

Glyphosate was strongly adsorbed in all soils. The Kf was influenced positively by the clay content and CEC and was negatively related to pH and phosphorus. There was a tendency of higher adsorption of glyphosate in the CT soils, which could not be explained by differences in the measured physico-chemical properties of the soils under each management practice.

Glyphosate was found to move rapidly in small amounts, although most of the applied herbicide was retained in the upper part of the soil columns. Tillage practice did not have an influence on the leaching of glyphosate at the applied doses rate.

Appendix A. Initial load of glyphosate and AMPA of the studied soils

	Glyphosate		AMPA	
	(μg·Kg^{-1})		(μg·Kg^{-1})	
	NT	CT	NT	CT
PAR	<LQ	>LQ	103.48 (24.5)	127.5 (28.1)
PER	<LQ	<LQ	264.1 (58.8)	320.5 (172.3)
MAN	<LQ	<LQ	160.9 (53.9)	145.1 (30.1)

<LQ: below limit of quantification.

Appendix B. Physical properties of the soils obtained from the undisturbed columns (Okada et al., 2014)

Parameter	Soil	Tillage	
		NT	CT
PV (ml)	PAR	401.4	348.1
	PER	454.8	453.2
	MAN	484.1	473.6
δ_b (g ml^{-1})	PAR	1.37	1.34
	PER	1.21	1.22
	MAN	1.15	1.17
Ø	PAR	0.48	0.49
	PER	0.55	0.54
	MAN	0.56	0.55
D (cm^2 h^{-1})	PAR	4.40	1.68
	PER	1.36	1.00
	MAN	0.51	1.33

PV: pore volume; δ_b: bulk density; Ø: total porosity; D: hydrodynamic dispersion coefficient.

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