



Occurrence of glyphosate and AMPA in an agricultural watershed from the southeastern region of Argentina

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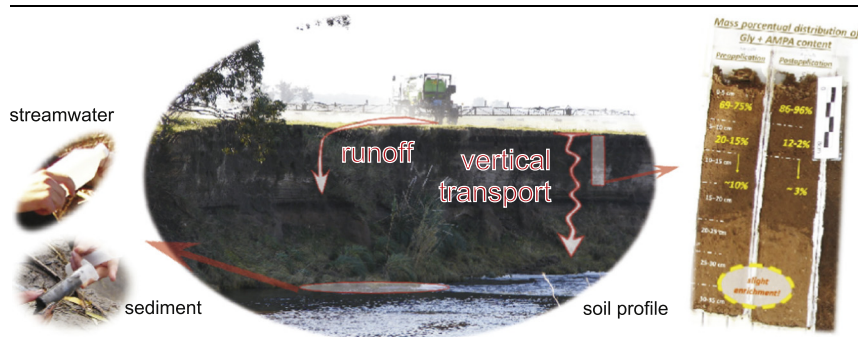
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HIGHLIGHTS

- The fate of GLY + AMPA was studied in agricultural soil profiles from soybean fields.
- GLY + AMPA in soil profile were well correlated with organic carbon content and pH.
- GLY was concentrated in the upper soil layer after application.
- GLY and AMPA were detected in streamwater and sediment at lower levels than soils.

GRAPHICAL ABSTRACT



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ABSTRACT

Glyphosate (GLY) and AMPA concentrations were determined in sandy soil profiles, during pre- and post-application events in two agricultural soybean fields (S1 and S2). Streamwater and sediment samples were also analyzed. Post-application sampling was carried out one month later from the event. Concentrations of GLY + AMPA in surface soils (0–5 cm depth) during pre-application period showed values 20-fold higher (0.093–0.163 µg/g d.w.) than control area (0.005 µg/g d.w.). After application event soils showed markedly higher pesticide concentrations. A predominance of AMPA (80%) was observed in S1 (early application), while 34% in S2 for surface soils. GLY + AMPA concentrations decreased with depth and correlated strongly with organic carbon (r between 0.74 and 0.88, $p < 0.05$) and pH (r between -0.81 and -0.76 , $p < 0.001$). The slight enrichment of pesticides observed from 25 cm depth to deeper layer, in addition to the alkaline pH along the profile, is of high concern about groundwater contamination. Sediments from pre-application period showed relatively lower pesticide levels (0.0053–0.0263 µg/g d.w.) than surface soil with a predominance of glyphosate, indicating a limited degradation. Levels of contaminants (mainly AMPA) in streamwater (ND-0.5 ng/mL) denote the low persistence of these compounds. However, a direct relationship in AMPA concentration was observed between sediment and streamwater. Despite the known relatively short half-life of glyphosate in soils, GLY + AMPA occurrence is registered in almost all matrices at different sampling times (pre- and post-application events). The physicochemical characteristics (organic carbon, texture, pH) and structure of soils and sediment in addition to

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the time elapsed from application determined the behavior of these contaminants in the environment. As a whole, the results warn us about vertical transport through soil profile with the possibility of reaching groundwater.

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1. Introduction

Glyphosate (N-[phosphonomethyl] glycine) is a broad spectrum systemic herbicide. It was introduced in the market in 1974 as an active ingredient by Monsanto Co, in the Roundup product. Glyphosate base salts are very soluble in water, although it is strongly retained in soil matrix. Particularly, the glyphosate-phosphonic moiety would be responsible of its adsorption on oxides and specially on the poor crystallized fractions (Sheals et al., 2002). In this way, Ulén et al. (2012) reported a correlation between glyphosate and phosphorus (both bounded to particles) in drainage water from clay soils in a leaching experiment. Otherwise, organic matter is also an important soil property that influences the degree of glyphosate adsorption (Albers et al., 2009). Besides, soil pH plays an important role in adsorption, considering that it governs the electrostatic interaction between adsorbents and glyphosate (Pessagno et al., 2008). Once in the topsoil, glyphosate horizontal mobility is related to runoff process and sediment transport. Moreover, if rain-fall occurs shortly after the herbicide application on bare soil, there is a strong risk for pesticide offsite movement (Yang et al., 2015). In addition, even soils with high glyphosate adsorbing capacity might be subjected to severe loss of herbicide with unfavorable soil structure (Todorovic et al., 2014).

On the other hand, glyphosate vertical mobility is related mainly to preferential flow and particle-facilitated transport in well-structured soil (Kjær et al., 2011). Although glyphosate is directly applied on surface soil, leaching column experiments indicated that up to 50% of the particles in the leachate came from the top 0.5 cm of the soil, and particles would be generated both inside the column and as a result of the splash process during intensive rain (Styczen et al., 2011). Furthermore, studies in a wide geographic region scale in the United States, concluded that both, the glyphosate and its main metabolite (aminomethyl)phosphonic acid (AMPA) are mobile and occurred widely in the environment, being detected in rivers, rain water and groundwater (Battaglin et al., 2014). The glyphosate-based herbicide consumption increased widely in Argentina with introduction of genetic modified organism in 1995, and 197 million kg of glyphosate-based products was applied in 2012. Argentina is the top exporter of soybean oil and soybean meals in the world's export market, and the third-largest exporter of soybeans. Despite the growing importance of soybean production in Argentina and the widely use of glyphosate, few studies have been reported at local and regional levels about glyphosate and AMPA behavior in agricultural watersheds. Particularly, Aparicio et al. (2013), found pesticide levels in surface soil from Buenos Aires province, one of the most important soybean production areas, which ranged between 0.035–1.502 µg/g and 0.299–2.256 µg/g for glyphosate and AMPA, respectively. The relationship of a glyphosate-based commercial product and phosphorus fertilizer application in a runoff experiment was recently evaluated in Aquic Argiudoll soil (Sasal et al., 2015). However, there is no evidence of field studies about glyphosate behavior through soil profile and its potential transport to groundwater. In the agricultural production, based on no-till system, genetic modified organism and intensive use of agrochemicals have been object of demonstration and struggle in rural villages in Argentina (Arancibia, 2013). Moreover, information about risk for human health considering glyphosate as a carcinogenic compound was reported (De Roos et al., 2005; Paganelli et al., 2010). The recent classification of glyphosate toxicology as *probably carcinogenic to human* (Group 2A) by the International Agency for Research on Cancer (IARC, 2015) evidence the need of performing studies about glyphosate behavior mainly focused on the protection of human health. The knowledge about the chance of

glyphosate to reach groundwater is essential and urgent in order to generate prevention policies. The levels of glyphosate and AMPA found in the region encourage us to study the occurrence and fate of these contaminants in aquatic and terrestrial environments. Several studies have revealed that glyphosate-based formulations affect the aquatic communities modifying the structure and quality of freshwater ecosystems (Sandrini et al., 2013; Vera et al., 2010). Therefore, the knowledge about persistence, degradation and transport of glyphosate and AMPA in soil profiles is necessary to evaluate the behavior of these compounds in order to find possible strategies of prevention or mitigation of watershed contamination. The aim of this work was to determine glyphosate and AMPA levels in different matrices (streamwater, sediment and soil) with spatial and temporal samplings considering pre- and post-application periods. Also, raining events were considered in order to evaluate the behavior of these contaminants in a typical soybean agricultural watershed.

2. Materials and methods

2.1. Study area

Pampean region of Argentina, is responsible of 80% of soybean production with intensive use of agrochemicals, being glyphosate the most applied herbicide. The Quequén Grande River (QGR) basin has a total area of 9.990 km², which is drained by several streams with a north–south orientation and is located in the south of de Pampa region of Buenos Aires Province. The QGR drains in the Atlantic Ocean and is the most important stream of the basin with an approximately mean flow rate of 12 m³/s rising to 758 m³/s during flooding episodes, as was observed in 1985. The mean annual temperature is 14 °C with a minimum of 7.3 °C and a maximum of 21 °C. The climate is sub-humid with mean rainfall of 891 mm for the 1960–2009 period and with heavy rains during summer months. The occurrence of natural vegetation between farms and stream is scarce, permitting the contaminants to reach the aquatic environment.

2.2. Sampling area

The matrices were sampled at two different moments considering the pesticide (glyphosate-based herbicide) application events (November–December). Therefore the pre- and post-application periods were June (2012) and January (2013), respectively. The samples were collected at three distinct areas. The first one was a buffer zone (natural area) adjacent to a river gully which was separated from the fields by a truck and a row of trees (soil control area — CA_S: 38°14'35.0"S, 59°06'44.5"W, sediment and water control area — CA_W: 38°11'53.93"S, 59°7'0.12"W). Also, soil samples from CA were located on a gentle hill and have never been directly sprayed. The agricultural sampling stations were settled at Site 1 (S1: 38°14'38.5"S, 59°06'52.5"W) 150 m far from the river shore and Site 2 (S2: 38°14'51"S, 59°05'52.2"W) adjacent to the river (Fig. 1). Both plots located 250 m (S1) and 2500 m (S2) far from CAS, were used for extensive agriculture (system barley–soybean) since the year 2010. The applied rate of glyphosate was considered according to INTA (National Institute of Agriculture Technology) recommendation and on the basis of information provided by the plots owner. The formulated product applied on bared soil was Roundup Full II (at 1.1 kg/ha active ingredient) using a bloom sprayer. Moreover agricultural areas were in fallow period and during the first sampling date scarce litter in surface soil (0–5 cm) was found. Topographic differences laid between both

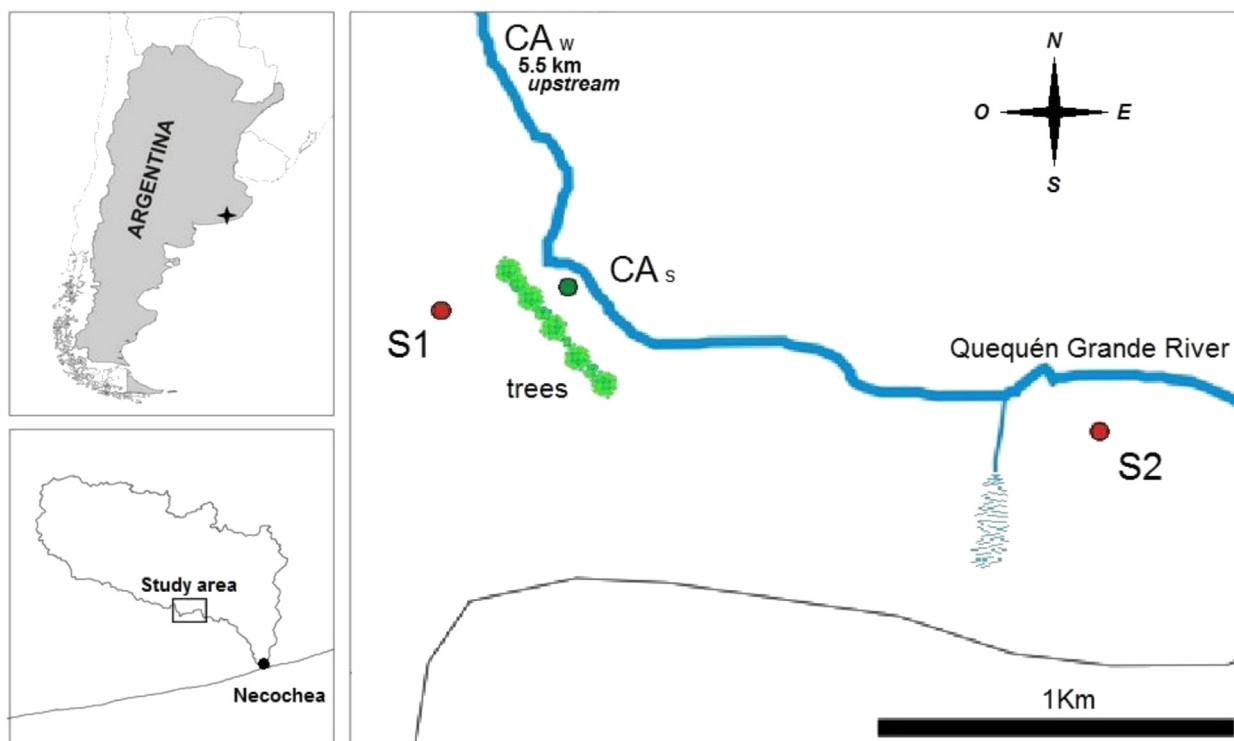


Fig. 1. Study area and sampling points. Soil Control Area: CA_s , sediment and streamwater Control Area: CA_w and agricultural plots: S1 and S2.

plots, S2 is located in lowland region adjacent to a creek which was linked to a natural wetland settled in the middle of other agricultural plots.

Three intact soil columns, separated by at least 50 m, were obtained from each site. Soil samples were collected with a 40 cm long aluminum corer and 10 cm of diameter, which was introduced into the soil by hitting with a hammer and removed with the help of a spade. The corer was opened along in the laboratory; the different layers were identified according to changes in color or texture and then subsampled. In the case of homogeneity, the core was divided in segments of 5 cm long. In the case of the presence of litter in surface soil (0–5 cm), it was manually removed before sample grinding.

Streamwater samples from QGR, were collected by triplicate using 500 mL polypropylene bottles at the three different sites (CA_w , S1 and S2). Temperature, conductivity and pH were measured in the field.

River bottom sediment samples (0–10 cm depth) were collected with plastic tubes of 12 cm of length and 4 cm of diameter at about 50 cm from the shore line to avoid resuspension of sediment during sampling and to ensure that sediment has not been subjected to air-oxidation processes. Three samples were collected at each sampling site (CA_w , S1 and S2). The CA_w , located 5.5 km upstream from CA_s , was settled as control point of upstream contamination source, considering that QGR was also surrounded in large extension by agricultural areas with agrochemical use. All samples were stored at $-20\text{ }^{\circ}\text{C}$ until analysis. Soil texture was determined according to Gee and Bauder (1986), soil pH was determined with an electrode with 1:2.5 soil-water relation (Nelson and Sommers, 1982). Walkley and Black (1965) method was used for organic carbon determination.

2.3. Glyphosate and AMPA determination

Prior to analysis, the subsamples of soil and sediments were dried at $30\text{ }^{\circ}\text{C}$ in a force-convection oven (24 h) to constant weight, grounded and sieved (2 mm). For glyphosate and AMPA determination, 2 mL of streamwater and 5 g of river sediment or soil were used. The water,

sediment and soil samples were fortified with 20 μL and 250 μL of 1 $\mu\text{g/mL}$ stock solution of isotope-labeled glyphosate ($1,2\text{-}^{13}\text{C}$, ^{15}N), respectively to determine matrix effects and recovery. After 30 min, the liquid and solid samples were extracted with 0.5 mL and 12 mL of extracting solution (0.1 M of KH_2PO_4) respectively. After supernatant collection, the same volume of buffer solution (0.1 M $\text{K}_3\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, pH 9) was added to the samples prior derivatization. The samples were sonicated for 30 min and centrifuged. An aliquot of 2 mL was taken from the supernatant and derivatized with 2 mL of a solution composed of 1 mg/mL of 9-fluorenylmethylchloroformate (FMOC-CL) in acetonitrile. A standard curve in the range 0–0.2 $\mu\text{g/g}$ of glyphosate and AMPA was prepared with each set of samples to compensate degradation of FMOC-CL between sets of samples. An equivalent amount of isotope-labeled glyphosate was added to each point of the curve. The samples and the standard curve were let to rest overnight in darkness and then they were manually shaken for 3 min with 6 mL of dichloromethane to extract and possible derivatizing by-products (e. g. FMOC-OH) and matrix interferences (Hanke et al., 2008) were removed. Then, the aqueous phase was filtrated through a $0.22\text{ }\mu\text{m}$ nylon filter and disposed into a 1 mL vial for LC-MS/MS determination (Aparicio et al., 2013). The limit of detection (LOD) and quantification (LOQ) of the method was 0.05 ng/mL and: 0.2 ng/mL, respectively. The LOQ for soil and sediment samples was set at 0.001 $\mu\text{g/g}$, while for streamwater samples at 0.5 ng/mL. When replicates were below the LOQ, quantifiable replicates were averaged with LOD. Recoveries for spiked matrices were higher than 70%. Soil and sediment concentrations are expressed as μg of glyphosate or AMPA per gram of soil (dry weight: d.w.) and streamwater samples in ng/mL. The percentage AMPA was calculated according to Battaglin et al. (2014), and was defined as:

$$\% \text{AMPA} = \frac{C_{\text{AMPA}}}{C_{\text{Gly}} + C_{\text{AMPA}}} \times 100$$

where C_{AMPA} and C_{Gly} are the AMPA and glyphosate concentration expressed in $\mu\text{g/g}$.

2.4. Instrumental analysis

Ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS) analysis was performed using an ACQUITY UPLC™ system coupled to a Quattro Premier™ XE tandem quadrupole mass spectrometer (Waters). For the chromatographic separation, an Acquity UPLC BEH C18 column (1.7 μm , 50×2.1 mm) (Waters) fitted with an Acquity VanGuard BEH C18 pre-column (1.7 μm , 5×2.1 mm) (Waters) was used. The flow rate for the mobile phase was 0.4 mL min^{-1} . Mobile phase was a time-programmed gradient using organic-free water modified with ammonium acetate 5 mM (phase A) and methanol modified with ammonium acetate 5 mM (phase B). The injection volume was 20 μL . Drying as well as nebulizing gas was nitrogen, obtained from a nitrogen generator. The cone gas and desolvation gas flows were optimized at 2 L/h flow and 600 L/h, respectively. For operation in MS/MS mode, collision gas was Argon 99.99% with a pressure of 4.04×10^{-3} mbar in the T-Wave cell. Masslynx NT v 4.1 (Waters) software was used to process quantitative data obtained from calibration standards and from samples.

2.5. Statistical analysis

The Kruskal–Wallis non-parametric test (KW test) was used to compare the glyphosate + AMPA concentrations (in $\mu\text{g/g}$ units) in different sites, considering that parametric test requires larger data set (USEPA, 2002) and normal distribution was not assumed. Mann–Whitney U test (MW test) was applied for pairwise comparisons. Wilcoxon test for paired measurements was used for comparisons between sampling periods. Spearman correlation coefficient was employed to evaluate correlation between glyphosate + AMPA (expressed as $\mu\text{g/g}$) and soil physicochemical properties (particle size distribution, organic carbon content and pH). All tests were set at 95% confidence level and α : 0.05 and only single measurements above LOQ were considered.

3. Results and discussion

3.1. Soil samples

3.1.1. Physicochemical properties

Soil texture was sandy loam with a slight reduction of sand content concomitantly with depth increase. Organic carbon (OC) content and pH values of soil profiles were similar for CA and agricultural plots (Table 1). Thus, OC decreased with depth and soil-pH remained almost constant. Alkalization of the first 10 cm of the cultivated areas compared with the CA could be explained by a reduction in the OC content.

By comparing CA_s with both S1 and S2, a reduction in OC content of 45% and 50%, for the upper most layer (0–5 cm) in the pre-application period was observed. Moreover a similar behavior was observed regarding to clay content being 29% and 13% for S1 and S2, respectively, as an evidence of fine particles losses by soil erosion due to management practices in the cultivated plots. The highest OC content is normally observed on the surface soil, mainly in non-tillage plots. The decrease of OC observed in post-application period in relation to pre-application, could be explained by the fact that there was a flooding episode between the two sampling dates (Fig. 2), which could have removed the OC from the upper soil layer.

3.1.2. Glyphosate and AMPA content in Soil Control Area (CA_s)

Although CA_s has never been sprayed, both compounds (parental and metabolite) were detected (Fig. 3). Run-off process was discarded as possible mechanism for glyphosate and AMPA delivering, considering that CA_s samples were located on gentle hill. Nevertheless, glyphosate could reach the air and be delivered in CA_s with the spray drift during application or through wind erosion of soil particles. On the other hand, AMPA would be also related with wind erosion from

Table 1

Physicochemical soil properties. Particle size distribution, % organic carbon content (OC%) and pH along the soil profile. The soil control area (CA_s) samples were taken only in pre-application period (Pre, Post). Agricultural fields: S1 and S2.

Site	Soil layer (cm)	Particle size distribution %			OC% ¹		pH	
		Sand	Silt	Clay	Pre	Post	Pre	Post
CA _s	0–5	76.5	11.0	12.5	3.1	–	7.5	–
	5–10				1.4	–	7.5	–
	10–15				1.0	–	8.3	–
	15–20				0.9	–	8.5	–
	20–25	68.7	16.5	14.8	1.0	–	8.4	–
	25–30	69.9	15.8	14.3	1.0	–	8.4	–
S1	30–35	68.0	16.8	15.1	0.9	–	8.5	–
	0–5	81.40	9.75	8.90	1.7	0.8	8.2	8.6
	5–10	75.9	13.4	10.8	1.2	1.1	8.3	8.4
	10–15				0.9	1.1	8.4	8.5
	15–20				0.7	0.9	8.5	8.5
	20–25				0.6	0.7	8.6	8.5
S2	25–30	75.3	15.43	9.20	0.5	0.7	8.6	8.6
	30–35	73.0	17.3	9.75	0.6	0.7	8.6	8.5
	0–5	70.43	18.73	10.83	1.5	0.9	8.1	8.6
	5–10	72.0	16.1	11.9	1.1	1.1	8.2	8.4
	10–15	72.37	17.73	9.93	1.2	1.0	8.4	8.5
	15–20	73.70	16.53	9.77	1.0	0.9	8.4	8.5
	20–25	73.57	17.33	9.10	1.0	0.7	8.6	8.6
	25–30	66.17	23.50	10.40	1.0	0.8	8.6	8.6
	30–35	72.70	19.10	8.30	1.0	1.1	8.6	8.5

agricultural close areas, although one of the main reasons of AMPA occurrence in CA_s would be related to glyphosate degradation (Gimsing et al., 2004). The magnitude of spray drift depends on meteorological (i.e. wind speed, humidity, air temperature) and technical factors (i.e. droplet size, spray chemical composition, working pressure, boom high) (Arvidsson et al., 2011). In application events with bloom sprayers, less than 1% of the applied dose is associated with the spray drift, although in other results reach until 24% (Jensen and Olesen, 2014). Even though the spray drift originated with bloom sprayer is supposed to be low, a droplet with size < 100 μm will begin to move practically horizontal to the ground at a close distance from the point of release (i.e. the nozzle) and will contribute significantly to drift losses due to a slow sedimentation velocity (Holterman, 2003). Chang et al. (2011) observed maximum glyphosate concentration of 2.5 ng/mL in rain water when rainfall follows the application period, while AMPA reached 2 ng/mL in rain water. Farenhorst et al. (2015) detected

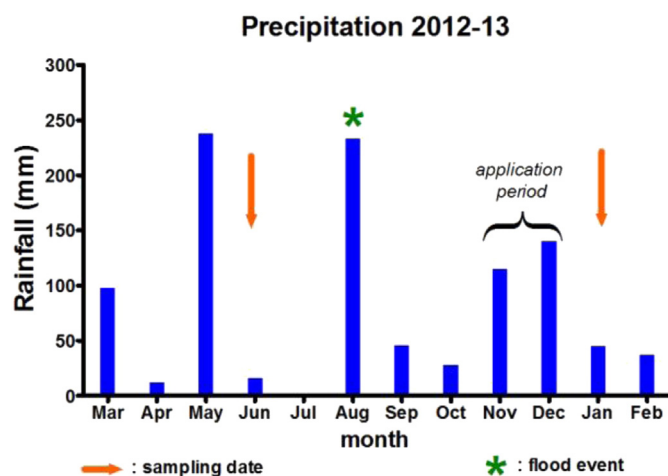


Fig. 2. Monthly distribution of precipitation during the year 2012–13. Application period, samplings is indicated in the figure.

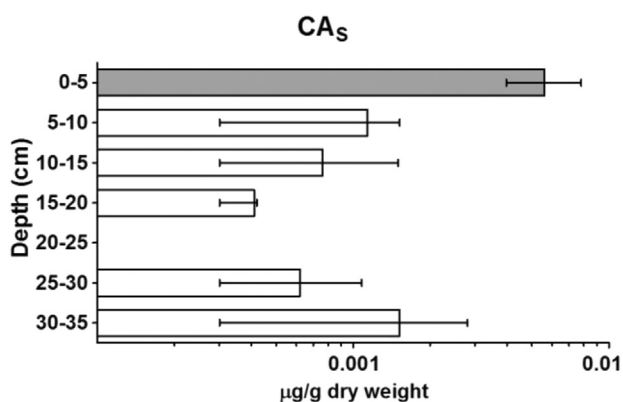


Fig. 3. Distribution (in µg/g) of glyphosate (white bars) and AMPA (gray bar) in the soil profile (semilog scale) for the soil Control Area (CA_S). Significant difference was observed (KW: $p < 0.05$) between 0–5 cm and 10–15 cm soil layer. Error bars show the range for the glyphosate and AMPA. The absent bar indicates all samples below LD.

eighteen pesticides in tow city sampling stations located 2.5 and 1.2 km north from agricultural land and glyphosate was positively associated with agricultural applications. As a whole, glyphosate and AMPA can travel long distances and can be washed out of the atmosphere by rain. Consequently, glyphosate and AMPA were present in CA_S. In addition, the total pesticide concentrations (glyphosate + AMPA: GLY + AMPA) observed for CA_S and the two plots was similar from 10 cm to deeper layer, independently of the sampling date (KW test: $p > 0.05$). For the first 10 cm of soil, the GLY + AMPA content was higher (an order of magnitude) in the agricultural plots than in CA_S (KW test: $p < 0.05$). Particularly, the highest concentration of glyphosate in soil was found in the first 5 cm of depth for S1 and S2, but it was not detectable in CA_S at the same depth. In addition, the presence of AMPA in the upper layer in CA_S evidences a possible strong and irreversible sorption of AMPA and complete degradation of the parental product. Landry et al. (2005) reported studies in undisturbed soil columns with glyphosate application concluding that grass cover improved the glyphosate degradation and AMPA sorption in relation to bare columns. On the other hand, glyphosate shows more capacity to lixiviate deeper in the soil, based on the fact that it was found at 30–35 cm in CA_S with a concentration close to 0.002 µg/g d.w. Moreover, it could remain unaltered as a result of a reduction of the microbial activity with depth, as was observed by Grondona et al. (2014) for soils from the same study region. Shushkova et al. (2010) have demonstrated, under experimental conditions, that glyphosate can lixiviate up to 30 cm of depth. The authors also found that, after glyphosate application and simulated rainfall, about 14–18% moved from the top soil to a lower 10–20 cm layer and 6–8% was washed down to the 20–30 cm layer. This behavior resulted in an enrichment of glyphosate in the lower horizons characterized by the absence (or low level) of biodegradative processes. Also, Bending and Rodriguez-Cruz (2007) found a significant reduction of dehydrogenase activity with depth, as an indicator of microbial degradation capacity for the herbicide isoproturon. Particularly, considering that glyphosate degradation in soil is primarily related to microbial activity, Stenrød et al. (2006) also found a reduction of microbial biomass and activity with depth. Although glyphosate and AMPA have never been applied in CA_S, the degradation product shows higher persistence under natural conditions. Other reports determined that both compounds were found several years after the herbicide application (Kjær et al., 2005; Simonsen et al., 2008). In addition, both pesticides were also found at 30 cm of depth as was observed in other studied soil profiles (Landry et al., 2005; Veiga et al., 2001). Candela et al. (2010) found glyphosate and AMPA concentrations at 1.9 m depth in coarse sandy soils from Barcelona, Spain with the risk of groundwater contamination.

3.1.3. Glyphosate and AMPA content in agricultural plots

3.1.3.1. Pre-application period. Total concentrations of GLY + AMPA ranged between 0.0008–0.163 µg/g and the highest levels were found in surface layer (0–5 cm). There were no significant differences between plots (MW test: $p > 0.05$). A predominance of AMPA was usually observed in all profiles, with a clear exception of S1 at 15–20 cm layer where only glyphosate was found (Fig. 4). This behavior was also observed in CA_S for similar layer, indicating the absence or very low degradation processes at that depth. Moreover, in the lowest layers of S1 and similar depths in S2, lower proportion of AMPA in relation with glyphosate was observed, supporting the assumption that lower microbial activity was carried out. The percentage AMPA is showed in Table 2.

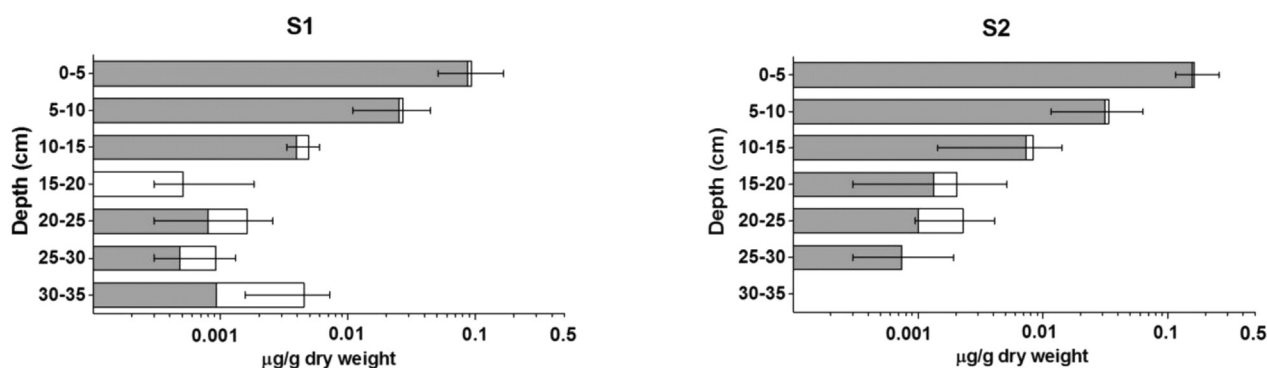
3.1.3.2. Post-application period. For the first soil layer (0–5 cm), both plots showed in post-application period a marked rise in GLY + AMPA contents (increment: 0.271 µg/g d.w. for S1 and 0.209 µg/g d.w. for S2), although no significant differences were observed (Wilcoxon test, $p > 0.05$) between sampling periods (Fig. 4). The S1 increase was mainly due to the enrichment in AMPA (75% of GLY + AMPA increment), justified by mineralization of recently applied glyphosate. On the other hand, the glyphosate was responsible of the enhanced pesticide levels in S2, as a consequence of a fresh application. Considering that more time elapsed in S1 since pesticide application, it can be expected that this plot would have a high AMPA/GLY ratio, as a consequence of degradation processes, while S2 does not, given that all pesticide increment was due to glyphosate enrichment. Therefore, these results denote a later application in S2 plot. The AMPA decrease observed in surface layers from S2 plot during post-application period could be mainly a consequence of the erosion due to flooding episode occurred before the application event, in addition to AMPA mineralization, where residual AMPA concentrations could have been markedly depleted (Fig. 4).

The soil layer (5–10 cm) from S1, evidenced an AMPA increment in post-application period, indicating probably lixiviation from the upper layer. Rampazzo et al. (2013) reported migration of glyphosate and AMPA up to 10 cm of depth after 28 days (loamy soil) and 12 days (sandy soil) of the application event. The concentration of AMPA in the soil profile decreased one order of magnitude for each 5 cm of depth in all plots and sampling times, with exception of S2 in post-application period where a decrease in two orders of magnitude was observed. A reduction in the AMPA/GLY ratio was evidenced along the soil profile, indicating glyphosate vertical transport and scarce degradation (Table 2).

3.1.4. Contaminants levels and physicochemical characteristics

The GLY + AMPA concentration was correlated with the OC content for CA (Spearman, $n = 10$, $r = 0.74$, $p < 0.05$) and S1 ($n = 17$, $r = 0.88$, $p < 0.001$). No correlation was observed between particle size distribution (sand, lime and clay) and GLY + AMPA content (Spearman: r between 0.2 and -0.17 , $p > 0.05$ in all cases). Piccolo and Celano (1994) have described the interaction of glyphosate with humic substances, suggesting sorption associated with a hydrogen bonding formation. Albers et al. (2009) observed that 40% of the sorbed glyphosate added at the beginning of the experiment was associated to humic and fulvic acids of the organic matter in a sandy soil. Regarding pH, a strong negative correlation was observed for S1 ($n = 21$, $r = -0.81$, $p < 0.0001$) and S2 ($n = 21$, $r = -0.76$, $p < 0.001$) in the pre-application period indicating a reduction in the GLY + AMPA content with the increment of pH. As the pH in the soil raise, the carboxylate and phosphonic acid moieties in glyphosate deprotonate and impart negative net charge to the molecule. Also the soil increments its negative net charge, by neutralization of the non-bridging hydroxyls in silanols groups ($>Si-OH$). Thus, glyphosate and the surface soil repeat each other diffculting the sorption of the molecule. Gimsing et al. (2004) have also found a diminishing of glyphosate adsorption on a sandy soil with increment of pH in a laboratory study. Therefore, in this work, samples from pre-

Pre-application period



Post-application period

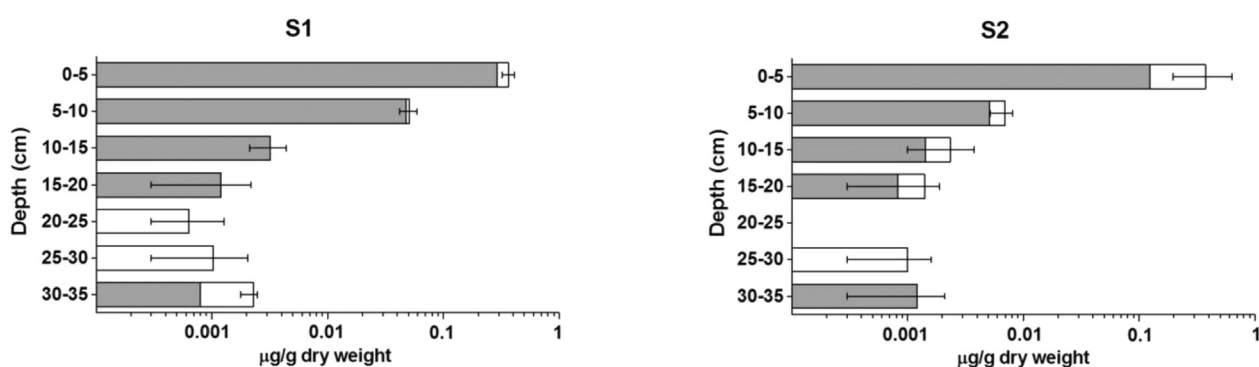


Fig. 4. Distribution (in µg/g) of glyphosate (white bars) and AMPA (gray bars) in the soil profile for agricultural plots (S1, S2) in the pre- and post-application periods (semilog scale). At the right side of the bars, the total Gly + AMPA content is indicated. No significant differences were observed (Wilcoxon test, $p > 0.05$) between application periods for S1 and S2 in the 0–5 cm soil layer. Error bars show the maximum and minimum concentration for the Gly + AMPA content. The absent bars indicate all samples below LD.

application period showed a strong negative correlation between pesticide concentration and pH. However, in the post-application period this behavior was not observed. It could be explained by the fact that a short time elapsed from pesticide application and sampling date, and therefore glyphosate and AMPA did not have much time to penetrate the soil and to be partitioned in the matrix.

All sites showed minimum concentration in the soil layers (15–25 cm) with a slightly enrichment at deeper layer, which is not completely consistent with the OC content or pH change, with the exception of S2 in the pre-application period.

Sorption of glyphosate is a complex phenomenon influenced by many factors and the presence of clays, iron oxides and especially

poor crystallized amorphous fraction seem to play an important role (Dion et al., 2001; Morillo et al., 2000). Piccolo et al. (1996) and Albers et al. (2009) also have proposed organic carbon content as a main factor in glyphosate adsorption. In addition, Gimsing and Borggaard (2002) observed competition between phosphate and glyphosate for active sites in Danish surface soils and pH influenced strongly the phosphate and glyphosate adsorption and Ulén et al. (2012), showed a correlation between glyphosate and phosphorus (both bounded to particles) in drainage water from clay soils in a leaching experiment. In our study, in the upper soil layer, glyphosate and AMPA would be mainly retained associated with OC content, while clay fraction would not play an important role. As the depth increase, the OC content was reduced as

Table 2
Percentage AMPA. Total glyphosate and AMPA content (G + A) in µg/g and percentage AMPA (%AMPA) along the soil profile (soil control area: CA_s, agricultural fields: S1 and S2) in pre- and post-application periods. No detectable levels refer as nd.

Depth (cm)	Pre-application						Post-application			
	CA _s		S1		S2		S1		S2	
	G + A	%AMPA	G + A	%AMPA	G + A	%AMPA	G + A	%AMPA	G + A	%AMPA
0–5	0.0056	100	0.0932	93	0.1633	96	0.3641	80	0.3726	34
5–10	0.0010	0	0.0271	92	0.0334	94	0.0508	94	0.0070	73
10–15	0.0007	0	0.0049	81	0.0083	88	0.0032	100	0.0023	61
15–20	0.0003	0	0.0005	0	0.0020	66	0.0012	100	0.0014	59
20–25	nd	–	0.0016	49	0.0023	44	0.0006	0	nd	–
25–30	0.0005	0	0.0009	52	0.0007	100	0.0010	0	0.0010	0
30–35	0.0014	0	0.0045	21	nd	–	0.0023	36	0.0012	100

well as the contaminant content. Probably, other soil properties would be also involved in glyphosate and AMPA retention between 20 and 30 cm.

In unstructured sandy soil with the absence of macropores (e.g. root channels, biopores) or fractures, glyphosate vertical transport could be associated to colloidal particles and piston flow movement (Borggaard and Gimsing, 2008) being preferential flow impeded. In this way, glyphosate can be retained in the uppermost soil layer justifying the increment on GLY + AMPA concentrations observed in the 0–5 cm soil layer after the application. Although most of the applied dose of herbicide was retained in the uppermost layer of soil (with more than 90% of GLY + AMPA in the first 10 cm), glyphosate and metabolite were detected in the deepest layer (30–35 cm) in both plots and sampling periods, at concentrations between 0.0012–0.0045 µg/g d.w. These findings warn us about the subsequent vertical transport of pesticides through the soil profile, particularly in soils with high pH where glyphosate sorption is decreased and therefore the probability of reaching groundwater could be enhanced.

3.2. Streamwater and Sediments samples

Water samples showed detectable levels of glyphosate and AMPA, although most of them were below the LOQ (Table 3). Since glyphosate has a half-life of 4.2 days in streamwater and ready degradation is associated with the presence of microorganism in aqueous phase (Vera et al., 2010), this could be the main reason for the similar herbicide levels found in different sampling periods. Glyphosate and AMPA were detected in 33% and 20% of the total water samples, respectively. Similar results were found by Aparicio et al. (2013) in streamwater samples collected from the same watershed although sampling period was different. Coupe et al. (2012) observed higher levels of glyphosate in streamwater associated with new application event. They concluded that highest glyphosate concentration in water will occur during the first run-off event following the application and posteriorly glyphosate concentration will decrease with time. In contrast, AMPA concentration in soil will increase due to degradation of parental and also the concentration in streamwater will rise because of delivering processes (e.g. run-off). Although streamwater concentrations were below LOQ, glyphosate was detected in CA_W and S2 in post-application period suggesting a recent application. On the other hand, AMPA was only detected in pre-application period particularly in CA_W, denoting the transport of this compound from the source (agricultural field). A pesticide sediment–runoff partition coefficient (K_p) expressed as L/kg was calculated in order to understand the partition of pesticide between sediment and streamwater. In the case of S2 the K_p was 26, denoting a high adsorption on sediments. Silburn et al. (2013) reported similar results for pyriithiobac sodium and metalochlor after 34 days of application. Ibáñez et al. (2005) also found glyphosate concentration of 0.48 ng/mL and 0.18 ng/mL for AMPA in streamwater from a Valencian Mediterranean region. On the other hand, the concentrations of glyphosate and AMPA in surface sediment were one order of magnitude higher than those found in streamwaters (Table 4). In this way, river bottom sediments represent a sink for water soluble contaminants. AMPA

Table 4

Sediment samples. Physicochemical properties and contaminant content (pre-application period) in µg/g d.w. Sediment control area: CA_W. Agricultural fields: S1 and S2.

Site	Physicochemical properties				Contaminant content		
	% Particle size distribution			OC% ¹	pH	Pre-application period	
	Sand	Silt	Clay			Glyphosate	AMPA
CA _W	83.2	8.8	8	0.8	8.8	0.014	0.013
S1	72.4	15.1	12.5	1.0	9.1	0.003	0.002
S2	81.6	10.4	8	1.0	8.7	0.007	0.005

¹ OC%: organic carbon content.

represented between 37–47% of the total contaminant levels (GLY + AMPA) in water samples suggesting a limited degradation compared to surface soil. There were no marked differences in streamwater between pre- and post-application periods. Detectable concentrations of glyphosate were only found in S2 for both sampling times. This situation could be attributed to the input of higher amounts of herbicide from the creek next to S2. Strongly sorbing pesticides runoff is enhanced when the rainfall events occur a short time after pesticide application (Shipitalo et al., 2006). However, before the post-application sampling time scare rainfall was registered (Fig. 2) diminishing the probability of detecting high pesticide levels in streamwater.

4. Conclusions

- Although glyphosate-based herbicide was applied in agricultural plots, this compound and its metabolite, AMPA, were found at low concentrations in a buffer zone (CA_S).
- In spite of the known relatively short half-life of glyphosate in soils, the occurrence of glyphosate and AMPA was registered in almost all matrices at different sampling times (pre- and post-application events).
- Sediment samples from pre-application period showed relatively lower pesticide levels (0.005–0.026 µg/g d.w.) than surface soil with a predominance of glyphosate, indicating a limited degradation in sediments in relation to soils, where the AMPA/GLY ratio > > 10. The low contaminant levels found in streamwater (ND–0.5 ng/mL) denote the low persistence of these compounds. However, a direct relationship in AMPA concentration was observed in the aquatic environment between sediment and streamwater.
- The physicochemical characteristics (mainly OC and pH) and structure of soils, in addition to the time elapsed from the application period determined the behavior of these pesticides in the environment.
- A strong and positive correlation was observed between organic carbon fraction and GLY + AMPA contents in pre-application period while a strong and negative correlation was observed with soil pH, denoting the increase of pesticide dispersion risk under alkaline soil pH values. Thus, in these sandy soils, the statement about the importance of organic carbon content in glyphosate retention is evidenced.
- After a month of application event, most of the herbicide was retained in the upper 10 cm of soil profile. However, although glyphosate and

Table 3

Streamwater samples. Physicochemical properties (temperature in °C, conductivity in mS/cm) and contaminant content (ng/mL). Water control area: CA_W. Agricultural fields: S1 and S2.

Site	Physicochemical properties						Contaminant content			
	Pre-application period			Post-application period			Pre-application period		Post-application period	
	Temp.	Cond.	pH	Temp.	Cond.	pH	Glyphosate	AMPA	Glyphosate	AMPA
CA _W	13.5	1.1	8.8	23.3	1.6	8.7	nd ¹	0.5	<LQ	nd
S1	9.0	1.2	8.9	–	–	–	nd	nd	–	–
S2	10.5	1.2	8.9	21.1	1.1	8.4	<LQ	nd	<LQ	nd

¹ nd: no detectable.

AMPA concentrations diminished drastically along the soil profile, both compounds were found at 35 cm of depth. As a whole, the results warn us about vertical transport through soil profile being of high concern about groundwater contamination.

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