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Glyphosate and AMPA, “pseudo-persistent” pollutants under real-world agricultural management practices in the Mesopotamic Pampas agroecosystem, Argentina[☆]

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ABSTRACT

In the Pampas, public concern has strongly risen because of the intensive use of glyphosate for weed control and fallow associated with biotech crops. The present study was aimed to evaluate the occurrence and concentration of the herbicide and its main metabolite (AMPA) in soil and other environmental compartments of the mentioned agroecosystem, including groundwater, in relation to real-world agricultural management practices in the region. Occurrence was almost ubiquitous in solid matrices (83–100%) with maximum concentrations among the higher reported in the world (soil: 8105 and 38939; sediment: 3294 and 7219; suspended particulate matter (SPM): 584 and 475 µg/kg of glyphosate and AMPA). Lower detection frequency was observed in surface water (27–55%) with maximum concentrations in whole water of 1.80 and 1.90 µg/L of glyphosate and AMPA, indicating that SPM analysis would be more sensitive for detection in the aquatic ecosystem. No detectable concentrations of glyphosate or AMPA were observed in groundwater. Glyphosate soil concentrations were better correlated with the total cumulative dose and total number of applications than the last spraying event dose, and an increment of 1 mg glyphosate/kg soil every 5 spraying events was estimated. Findings allow to infer that, under current practices, application rates are higher than dissipation rates. Hence, glyphosate and AMPA should be considered “pseudo-persistent” pollutants and a revisions of management procedures, monitoring programs, and ecological risk for soil and sediments should be also recommended.

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1. Introduction

Glyphosate (*N*-[phosphonomethyl]-glycine) is a post emergent systemic herbicide used for weed control from domestic gardening to extensive crops (Franz et al., 1997). In the last years, public concern has risen about potential environmental and health problems linked with the agriculture use of glyphosate, mainly because of the vast areas and volumes involved in the production of genetically modified crops, like soybeans, corn and cotton (Bonny, 2008). In Argentina, the use of glyphosate has increased

drastically since its introduction in the 80's, reaching approximately 200000 tons in 2012, and representing 80% of total commercialized herbicides (CASAFE, 2012). Such increase was mainly driven by the expansion of the agriculture frontier boosted by the biotech soybean adoption, reaching more than 19.8 million ha in 2013–2014 produced mainly under no-till practice (78% of the total seeded area) (SAGyP, 2016). Under that practice glyphosate is not only used for weed control, but also for chemical fallow. In addition, doses have been increased four-fold between 1996 and 2012, and several cases of weed resistance reported. An equivalent process has been occurred in other South American countries like Brazil, Bolivia, Paraguay and Uruguay (Fischer et al., 2014).

The environmental fate of glyphosate and its major metabolite (aminomethylphosphonic acid, AMPA) have been well studied in North America since the 80's (Battaglin et al., 2014; Newton et al.,

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1984; Scribner et al., 2007), but the issue has been more recently addressed in South America. In Argentina, environmental concentrations were assessed for the first time in the Rolling Pampa, a core agricultural area, using UV-HPLC less than ten years ago (Peruzzo et al., 2008). More recently, a couple of studies were also conducted in a marginal agricultural area, the Southern Pampas (Aparicio et al., 2013; Lupi et al., 2015). Although common distribution patterns can be observed among studies, clear differences in the concentrations were found between core and marginal areas, indicating that important variations exist among regions. Given such differences, a new question arose regarding how the occurrence and concentrations of glyphosate and its metabolite are related to the use of the herbicide under current agricultural practices.

In addition, higher occurrence and concentrations of glyphosate and AMPA in solid matrices were found in mentioned studies. Those results were in accordance with the high sorption affinity of soil for glyphosate demonstrated under experimental conditions and used to explain the retention of the herbicide in the upper soil (Okada et al., 2016). However, some other studies have also showed that preferential flow of the herbicide through macropores is feasible and it would allow the herbicide mobility across the soil, explaining its occurrence in groundwater (Kjær et al., 2011; Vereecken, 2005). In addition, relatively high occurrence frequencies (up to 58%) were reported in groundwater samples collected from intensive agricultural areas of Spain (Sanchís et al., 2012). The heavy use of the glyphosate in the Pampas has risen concern regarding the potential leaching of the herbicide and its metabolite to groundwater, since it is the major drinking water source for cattle and people. However, no data is yet available about glyphosate/AMPA occurrence in groundwater of areas under the above mentioned production system.

On the other hand, a common belief has been installed among farmers and agronomists of the region, considering that glyphosate is innocuous for the environment. This idea was based on earlier field experiments showing that glyphosate was rapidly inactivated in soil after spraying (Sprankle et al., 1975) and quickly dissipated in soil (Newton et al., 1984), and sometimes it has driven to the overuse of the herbicide. However, differences among single spraying experiments and real-world agriculture management practices in the Pampas could change the environmental distribution and persistence of the herbicide and its metabolite, but it has not been previously assessed.

In the present study, the environmental fate of the glyphosate and AMPA was assessed in the Mesopotamic Pampas agroecosystem, including for the first time the analysis of groundwater samples. In addition, the relationships between concentrations in soil and the use of the herbicide under the current agricultural practices of the region, was assessed on the bases of the information provided by management records of 14 local farms between 2007 and 2012. The Mesopotamia region has been chosen because it is an important agricultural area of the Argentine Pampas where information on glyphosate concentrations still is not available.

2. Materials and methods

2.1. Area of study

The area of study (Fig. 1) was located in the southeast of the Entre Ríos Province, “Gualeduaychú” district. The sampling sites were distributed within a circle of 14 km radius, centered in the Urdinarrain city (W58°53'0", S32°41'0"), an important agricultural district. According with the updated Köppen–Geiger climate classification system (Kottek et al., 2006), the study area is placed in a warm temperate, fully humid, hot summer region (Cfa), with

maximum and minimum mean temperatures of 23.0 and 12.5 °C, and average annual precipitation of 1155 mm. Landscape is characterized by rolling prairies 40–60 m above sea level. Soils are classified as vertisol type (uderte suborder, peluderte group, argico subgroup) and mormorillonite class, because of the presence of esmectite clay minerals and 5.6% of organic matter in horizon A (INTA, 2014).

2.2. Sampling

Samples were collected from 17 agricultural farms between January and March 2012. Farms were selected on the bases of the accessibility, the cooperation of agronomists and the availability of the farm managing records.

Composite soil samples were collected from plots of the selected farms, most of them (14) having available full historical managing records. At each plot, composite samples were formed by pooling 50 subsamples randomly collected from the top-soil layer (0–5 cm depth). Groundwater samples were collected in those plots where windmills were available, usually pumping from 40 to 60 m-deep aquifer. In addition, surface water and sediment samples were collected from 1st and 2nd order waterways, at catchments draining either to the “Gualeduay” or “Gualeduaychú” rivers. Water samples were collected in pre-washed 1L polypropylene bottles. All the samples were stored at –20 °C until analysis.

2.3. Sample analysis

Analytical procedures for glyphosate determination in collected samples was performed according a previous study (Aparicio et al., 2013). Soil samples were conditioned using a hot-air heater set at 30 °C, and then homogenized, crushed, and sieved through 2 mm mesh. Subsamples were dried to constant weight at 105 °C for analyzing moisture content. Sediment samples were dried for 12 h at 35 °C, then dried milled and sieved as above. Suspended particulate matter (SPM) was determined gravimetrically from surface water samples (60 mL) filtered in the field immediately after sample collection through 0.45 µm nitrocellulose-acetate filters. Subsamples of soil (5 g), sediments (2 g) and SPM (≈0.4 g) were extracted with dihydrogen phosphate buffer according with Peruzzo et al. (2008). All subsamples were spiked with an appropriate amount of isotope-labeled glyphosate (1, 2-¹³C, ¹⁵N) previously to the extraction step. After sonication and centrifugation, the supernatant was decanted. The pH of soil, sediment and SPM extracts, as well as, whole surface water and groundwater samples, were adjusted to 9 with borate buffer. Groundwater samples were previously treated with HCl (pH = 1) to release the analyte from a possible complex with other substance or ion, and then neutralized according with Ibáñez et al. (2006). The obtained buffered aliquots were then derivatized overnight with 9-fluorenylmethylchloroformate (FMOC-Cl) in acetonitrile in the dark. The excess of FMOC-Cl was removed by liquid/liquid extraction with dichloromethane. Aqueous phases were passed through a 0.22 µm nylon filter into chromatographic vials.

Glyphosate and AMPA analysis was performed by UPLC-MS/MS on an Acquity UPLC system (Waters) using an Acquity UPLC BEH C18 column (1.7 µm, 50 × 2.1 mm) column (Waters), with methanol – 5 mM NH₄Ac_(aq) gradient. Detection was accomplished using a Quattro Premier XE Tandem Quadrupole Mass Spectrometer (Waters) equipped with and ESI source operated in positive mode. Precursor ions were *m/z* 392.1, 394 and 334.1 for glyphosate, glyphosate 1, 2-¹³C, ¹⁵N, and AMPA, respectively. Quantification and confirmation product ions were *m/z* 88.1 and 179.1 for glyphosate, *m/z* 90.1 and 216.1 for glyphosate 1, 2-¹³C, ¹⁵N, and *m/z* 179.1 and 112.1 for AMPA. Positive findings were considered when the

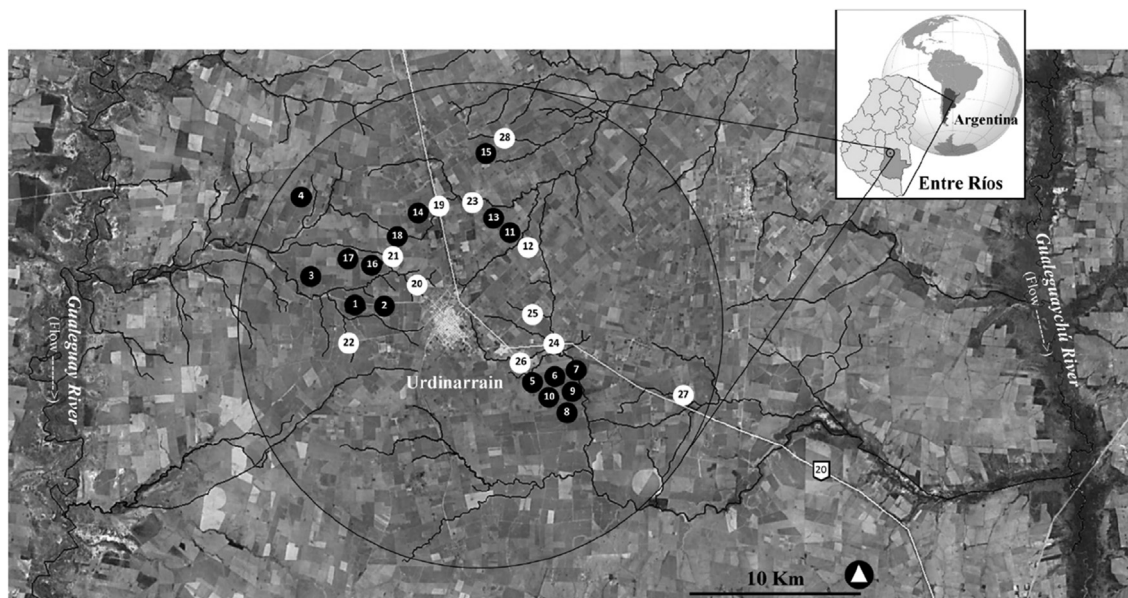


Fig. 1. Geographical location of the studied area and the sampling sites in the Mesopotamic Pampas. Sampling was conducted between January and March 2012. Black dots: soil samples, white dots: water and sediment samples.

concentration ratio between the quantification (Q) and confirmation transitions (q) was in the range 0.8–1.2 of the reference standard (SANCO/10684/2009). Retention times for glyphosate and AMPA were 2.0 and 2.5 min, respectively. Retention times for peaks in samples were compared with standards and accepted when a deviation lower than 2.5%. Quantification was performed by the standard external method and checked by the isotopic dilution methods. Recoveries were calculated from the isotope-labeled glyphosate spiking. The limits of detection (LOD) and limits of quantification (LOQ) were calculated in the different matrices analyzed as the lowest concentration of the standard with the signal-to-noise ratio of 5 and 10, respectively.

2.4. Agricultural management practices

In all the studied farms, no-till farming with chemical fallow and terrestrial spraying were used as base management practice. The information provided by the agronomists about the agricultural practices in each farm plot during the last five years is summarized in Table 1. No records were available for farms 5, 6 and 7. Glyphosate spraying information is presented as: i) total number of applications between 2007 and 2012 (TNA), ii) mean number of applications per year between 2007 and 2012 (MNA), iii) time (days) from the last application (TLA), iv) last application dose (LAD), v) last-year cumulative dose (YCD), and vi) total cumulative dose between 2007 and 2012 (TCD), vii) mean application dose between 2007 and 2012 (MAD). Doses were expressed as kg of active ingredient as acid equivalent (a.e.) per ha.

Estimated glyphosate soil concentrations (EGSC) were calculated from the LAD, assuming a soil layer of 5 cm with a density of 1200 kg/m^3 . Meteorological data were obtained from the Urdinarrain Municipal Station.

2.5. Data analysis

Results are expressed as the mean \pm standard error (number of samples), considering values between LOD and LOQ as the mean of both limits. The median was used with the AMPA-to-glyphosate ratio, and the median test was used for comparison of ratios

among compartments. Linear correlation was used to assess the relationship among i) the concentrations of glyphosate and AMPA in the different studied compartments, ii) the soil glyphosate concentration and the spraying events, and iii) the ratio of glyphosate/AMPA and the agricultural management practices.

3. Results and discussion

3.1. Environmental occurrence and concentrations

In soil, the LOD and LOQ were 0.3 and 1.1 $\mu\text{g/kg}$ for glyphosate and 0.6 and 2.5 $\mu\text{g/kg}$ for AMPA. The average recovery was $83.7 \pm 0.56\%$ (17) and the ion suppression 23%. The soil moisture was between 3.5 and 6.5%. The concentrations ranges and detection frequencies for the herbicide and its metabolite are presented in Fig. 2. The occurrence of both compounds was ubiquitous in soil (100%). Observed frequencies were higher than those found in the Southern Pampas, a marginal agricultural area of Argentina (62.5%) (Aparicio et al., 2013). In addition, values were also higher than those reported for other regions of the world (50–66%) (Ibáñez et al., 2005; Scribner et al., 2007), but in this case, differences could be explained because sampling in the present study was targeted directly to agricultural plots. The average concentrations of glyphosate and AMPA in soil were 2299 ± 476 (17) and 4204 ± 2258 (17) $\mu\text{g/kg}$, respectively. Although measured concentrations of the herbicide were in the range of those reported for other regions of the world (Heinonen-Tanski et al., 1985; Ibáñez et al., 2005; Scribner et al., 2007; Veiga et al., 2001), the maximum concentration obtained in the present study was 5 fold the highest concentration reported in the literature for soils of barley fields in Finland (Heinonen-Tanski et al., 1985). AMPA concentrations were also within previously published values, but again, maximum concentration was 7 fold greater than the highest value reported in the literature (Ibáñez et al., 2005). In comparison with previous studies conducted in Argentina, concentrations were similar to those found in the core soybean and corn agricultural area (Peruzzo et al., 2008), but 10 fold higher than those found in a marginal area (Aparicio et al., 2013). Together, these results indicate that the presence of glyphosate and AMPA in soils of biotech-crop

Table 1
Information about the studied farms and agricultural management practices.

Farm Location ^a	Plot surface (ha)	Crop rotation (2007–2012)	TNA	MNA	TLA	LAD	YCD	TCD	MAD	EGSC
1	68	C/S/W/S/C/S	21	4.2	15	0.9	5.3	17.7	0.84	1482.0
2	54	S/Ra/W/S/W/S/C	29	5.8	23	0.6	3.4	22.1	0.76	960.0
3	95	W/S/Su/C/S/W/S	24	4.8	15	0.8	5.4	20.3	0.90	1375.3
4	104	S/W/S/C/S	26	5.2	8	1.0	9.1	24.2	0.93	1606.5
8	27	S/W/S/W/C/S	14	2.3	7	0.5	2.6	12.0	0.86	912.3
9	18	S/C/S/W/C/S	14	3.3	7	0.5	2.6	12.0	0.86	912.3
10	18	S/C/S/W/C/S	14	3.3	7	0.5	2.6	12.0	0.86	912.3
11	71	C/S/So/S/W/S	13	2.6	30	0.7	4.5	12.0	0.92	1131.7
13	30	S/W/S/C/S/W/S	10	2.0	6	0.8	4.1	9.0	0.90	1371.7
14	24	W/S/Su/W/S/C/S	12	2.0	56	0.9	2.1	10.0	0.83	1464.6
15	25	W/S/Su/W/S/C/S	14	2.3	30	0.7	1.9	10.8	0.77	1176.9
16	40	W/S/S/W/S/S	13	2.6	12	0.7	3.9	9.4	0.72	1131.7
17	46	S/W/S/C	13	2.6	13	1.3	3.5	11.9	0.92	2107.7
18	58	W/S/W/S/C/S	20	3.0	5	0.7	3.6	15.4	0.77	1131.7

C: Corn; Su: Sunflower; W: Wheat; S: Soybean; Ra: Rapeseed; So: Sorghum.

TNA: total number of applications between 2007 and 2012.

MNA: mean number of applications per year between 2007 and 2012.

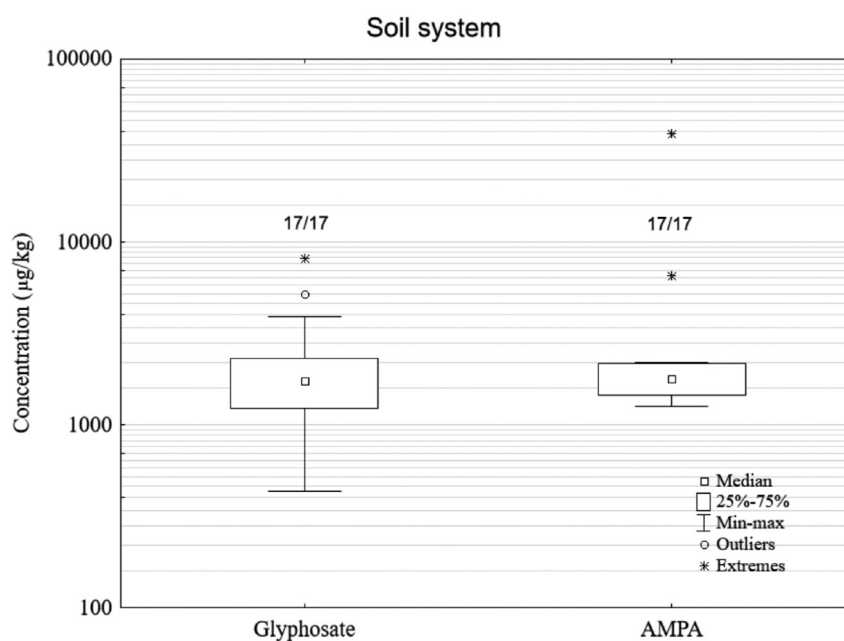
TLA: time from the last application (days).

LAD: last application dose (kg a.e./ha).

YCD: last-year cumulative dose (kg a.e./Ha).

TCD: total cumulative dose between 2007 and 2012 (Kg a.e./ha).

MAD: mean application dose between 2007 and 2012 (kg a.e./ha).

EGSC: estimated glyphosate soil concentration from the LAD ($\mu\text{g}/\text{kg}$).^a It correspond with the number of sampling place in Fig. 1.**Fig. 2.** Concentrations of glyphosate and AMPA in agricultural soils. The number on top of each box plot are number of detections/total number of analyzed samples.

core areas is ubiquitous and with concentrations usually above the mg/kg, values higher than those found in areas with less agricultural intensity. It should be not only be matter of environmental concern, but also a productive issue. In the last years, an increasing number of cases of weed resistance to glyphosate have been reported by farmers and documented in several studies (Cerdeira et al., 2011; Vila-Aiub et al., 2008).

In groundwater, the LOD and LOQ of the method were 0.07 and 0.22 $\mu\text{g}/\text{L}$ for glyphosate, and 0.15 and 0.44 $\mu\text{g}/\text{L}$ for AMPA respectively. The recovery for both compounds was $53.5 \pm 4.94\%$ (5). Concentrations of the herbicide and its metabolite were below the LOD in all analyzed samples. These result were different of those reported for other countries where occurrence and detected

concentrations ranged from 5 to 44% and 0.0005–0.98 $\mu\text{g}/\text{L}$ for glyphosate, and from 5.8 to 72% and 0.0001–2.62 $\mu\text{g}/\text{L}$ for AMPA (Battaglin et al., 2014; Hanke et al., 2008; Mörtl et al., 2013; Sanchís et al., 2012; Scribner et al., 2007). The availability of pollutants to reach groundwater depends on several factors, including to the proprieties of the source of pollution, the weather, the soil and the contaminant itself. Recent studies simulating mobility of glyphosate through the soil under conventional agricultural practices in Argentina, have demonstrated that the high adsorption coefficient of the herbicide was a dominant factor influencing the mobility through the soil profile, with most of the herbicide retained at the first 5 cm-soil layer (Okada et al., 2016). However, mobility of the herbicide by peripheral flow through macropores was also

demonstrated experimentally (Kjær et al., 2011; Sione et al., 2017; Vereecken, 2005). In addition, it was detected in groundwater of several places of U.S. since 2001 (Battaglin et al., 2014) and in intensive agricultural areas of Spain with increasing frequencies from 2007 to 2010 (Sanchís et al., 2012). The sorption properties of glyphosate, together with the soil characteristics and depth of the assessed aquifer would explain the undetected concentrations in groundwater, indicating no current contamination of the drinking water source used in the region for cattle and people. However, due to the heavy use and potential mobility through preferential flow, a periodic monitoring of the resource would be recommended.

The occurrence and concentrations of glyphosate and AMPA in surface waters (whole water and particulate suspended matter) and sediment samples obtained from 1st and 2nd order agricultural watercourses are shown in Fig. 3. The LOD and LOQ in whole water samples were 0.06 and 0.18 µg/L for glyphosate and 0.10 and 0.30 µg/L for AMPA, respectively, and recovery was $82.7 \pm 1.81\%$ (11). Only half or less of the samples showed detectable levels of the AMPA or glyphosate. Those frequencies were relatively low in comparison with the one obtained in solid matrices. The average concentration of glyphosate and AMPA in samples above LOD were 0.73 ± 0.65 (3) and 0.53 ± 0.48 (6) µg/L, respectively. In general, reported values for other regions of the world, were within the range obtained in the present study (Battaglin et al., 2009, 2014; Glozier et al., 2012; Hanke et al., 2008; Mörtl et al., 2013; Newton et al., 1984; Popp et al., 2008; Scribner et al., 2007; Struger et al., 2008; Wan et al., 2006). However, observed concentrations were several orders of magnitude lower than that found in the Rolling Pampas (Peruzzo et al., 2008), but in that study, sampling was conducted particularly soon after a significant rainfall event. On the other hand, results were similar to those obtained in several low

order streams of the Southern Pampas (Aparicio et al., 2013), under less extreme conditions. In high-order watercourses, tributaries of the Paraná River, occurrence and concentrations of glyphosate in whole water were comparable, but AMPA was not detected in any sample (Ronco et al., 2016). Hence, surface water concentrations would be less indicative of the agricultural intensity and more variable among watercourses, depending on the region and the order of the watercourse.

The LOD and LOQ obtained for the SPM were, respectively, 0.15 and 0.45 µg/kg for glyphosate, and 0.30 and 0.90 µg/kg for AMPA. For both compounds the occurrence was ubiquitous in this matrix and mean concentrations were 340.2 ± 57.6 (9) µg/kg (0.049 ± 0.009 µg/L) and 223.2 ± 43.3 (9) µg/kg (0.032 ± 0.007 µg/L) for glyphosate and AMPA respectively. Maximum concentrations were similar of those obtained in the Southern Pampas (Aparicio et al., 2013), but occurrence and mean values were higher than in the Mesopotamic Pampas. Reported results for Paraná River tributaries (Ronco et al., 2016), presented similar concentrations of glyphosate, but lower of AMPA, as well as lower occurrences of both compounds. The reported glyphosate and AMPA concentrations in SPM in other regions of the world are scarce. In a Canadian forest watershed, the maximum measured concentrations of glyphosate in SPM was 0.06 µg/L, and the occurrence 14% (Feng et al., 1990). Although, the concentration was similar to those obtained here, the occurrence was markedly lower, as observed in higher order watercourses, such as Paraná River tributaries. That could be explained by the scale analysis and intensiveness of the agriculture in the studied area.

In sediments, the LOD and LOQ for glyphosate were 0.35 and 1.10 µg/kg, respectively. For AMPA, LOD and LOQ were 0.64 y 2.59 µg/kg, respectively. Recoveries were the same for both

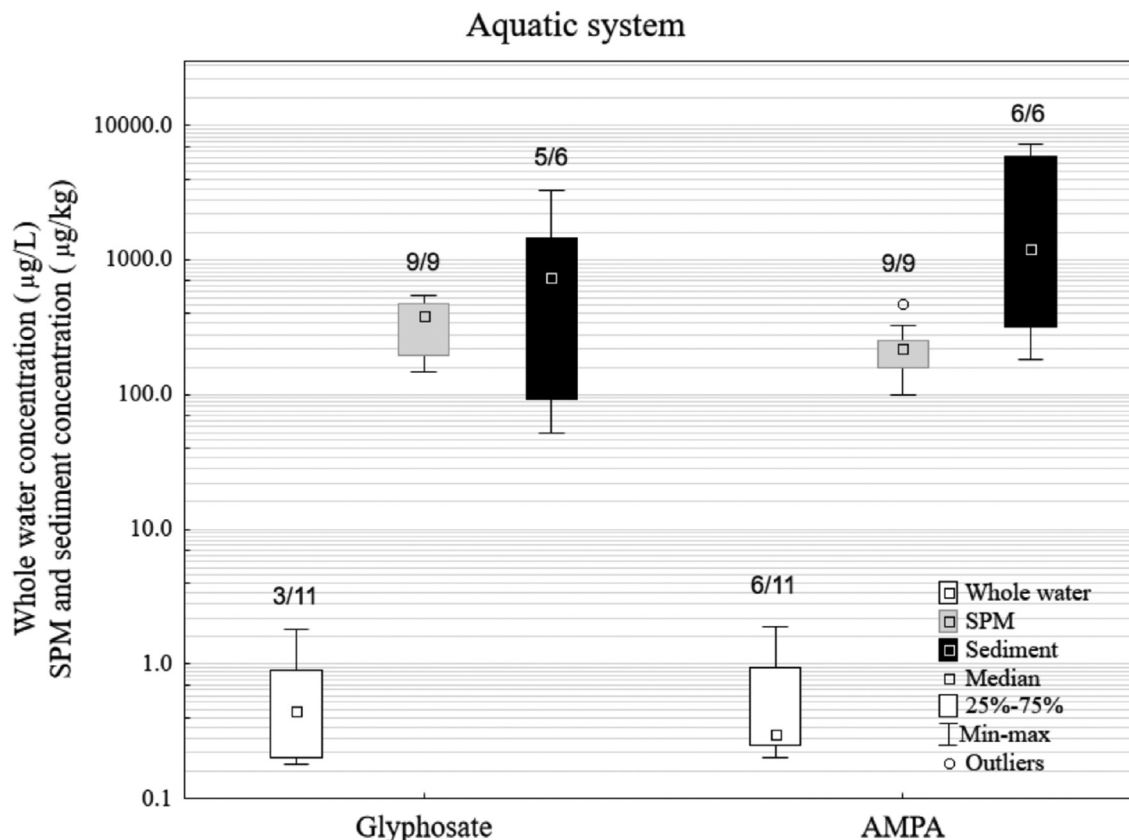


Fig. 3. Concentrations of glyphosate and AMPA in low order waterways. The number on top of each box plot are number of detections/total number of analyzed samples.

compounds with values of $43.4 \pm 0.77\%$ (6). As observed for the SPM, a high level of occurrence was found for glyphosate, and AMPA was ubiquitous in sediment samples. The average measured concentrations were 1126 ± 423 (6) and 2660 ± 1368 (6) $\mu\text{g}/\text{kg}$, respectively. Available information from other regions of the world showed that concentrations of the herbicide and its metabolite in farm ditches (Wan et al., 2006) and forest watershed of Canada (Feng et al., 1990) were similar of that reported here, with maximum values above the mg/kg . In the Southern Pampas (Aparicio et al., 2013), occurrence values were similar, but the maximum concentrations were less than 10-fold lower. On the other hand, in the Rolling Pampas (Peruzzo et al., 2008), values were of the same order of magnitude. In Paraná tributaries (Ronco et al., 2016), concentrations were low or undetected for those located in the upper sector of the basing, but concentrations were similar of that obtained in the present study for those in the lower sector, across the agricultural district. According to all these results, it is clear that sediment compartment is an important sink of the herbicide and its metabolite. In addition, the occurrence and concentration in sediments would reflect the intensiveness of the agricultural practices. Moreover, results obtained in the present study showed that average values in sediments were approximately the half of the average concentrations observed in soil.

3.2. Glyphosate and AMPA relationship

The percentage of samples with higher concentration of the herbicide over the metabolite, or *vice versa*, obtained for solid matrices is shown in Fig. 4 (insert). Significant differences (Median test, $p = 0.0032$) were observed in such proportion among the assessed environmental compartments. All samples presented higher levels of AMPA than the glyphosate in sediments, with AMPA/glyphosate ratio (AGR) ranging 1.5 to 15.5, and a median value of 4.0. On the other hand, a clear prevalence of samples with higher concentration of glyphosate was observed in SPM, with a AGR ranging 0.4 to 3.0, with a median ratio of 0.5. In soil, a slight predominance of samples with higher concentration of AMPA was found, but the pattern was not so conclusive. The AGR in soil ranged from 0.4 to 4.8, with a median of 1.3.

Although degradation rates among compartments would have some variations, the metabolite-to-pesticide ratio is usually used to estimate environmental fate and transport processes, with smaller or larger ratio values indicating fresher or more aged materials, respectively (Battaglin et al., 2014). According with obtained results, sediments come up as the oldest material, in agreement with the hypothesis it is the final sinks of pollutants. Almost exactly the same pattern can be deduced from data reported for low-order streams of the Southern Pampas (Aparicio et al., 2013). Differently, the ratio deduced from the Paraná tributaries (Ronco et al., 2016) was no so clear, probably reflecting the higher diversity and complexity of sources and processes driving the fate of the herbicide and its metabolite in rivers draining larger areas, including urban-industrial land uses. On the other hand, SPM appeared as the freshest material, indicating a more recent input, probably from runoff or atmospheric depositions. Experimentally, significant loss of glyphosate by runoff (11–28% of applied glyphosate) were reported after a rainfall simulation (1 h with 60 mm/h) on Entre Ríos soil previously sprayed with the herbicide (Sasal et al., 2015). In addition, wet depositions of glyphosate and AMPA were reported in USA ranging $3.9\text{--}16.0 \mu\text{g}/\text{m}^2$ and $1.7\text{--}5.2 \mu\text{g}/\text{m}^2$, respectively, showing higher values of the herbicide than the metabolite (Chang et al., 2011). In soil, a slight predominance of AMPA was observed, but the ratio was not so clear. However, the same pattern, even more clear, can be also deduced from the soil data published for the Southern Pampas (Aparicio et al., 2013).

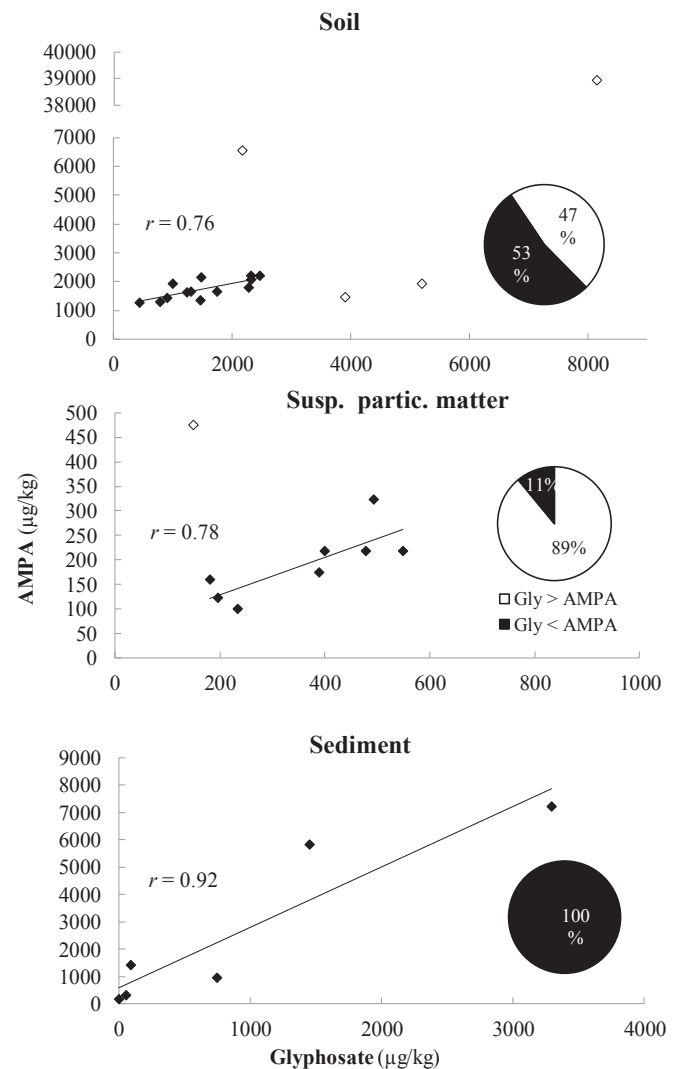


Fig. 4. Relationship between glyphosate and AMPA concentrations in the studied solid matrices. Insert: Percentage of samples with higher concentrations of glyphosate over AMPA, or *vice versa*.

Therefore, results suggest that expected soil concentrations of AMPA would be usually higher than those of glyphosate, but it could vary among samples.

Relationships between glyphosate and AMPA concentrations in the different studied solid matrices are shown in Fig. 4. A statistically significant positive correlation was found between the herbicide and its metabolite for all studied compartments (Pearson correlation, $p < 0.05$). In soil, the relationship was clear only for concentrations of both compounds below $3000 \mu\text{g}/\text{kg}$. Above those values the relationship became more erratic. Even better was the correlation found in the SPM, with the exception of one outlier value, presenting unusually high concentrations of AMPA. Finally, the strongest relationship was observed in the sediments. The obtained results differ from those obtained by Battaglin et al. (2005) in which no significant correlation was found, probably because of the broader scale of the study. The clear relationship between the concentrations of the herbicide and its metabolite obtained here could be explained by the homogeneity of the studied agroecosystem and bounded spatiotemporal relationship between the spraying events (source), the transport and the fate of the herbicide at the assessed scale. In addition, the higher strength in the relationship observed in sediments could be related with a higher

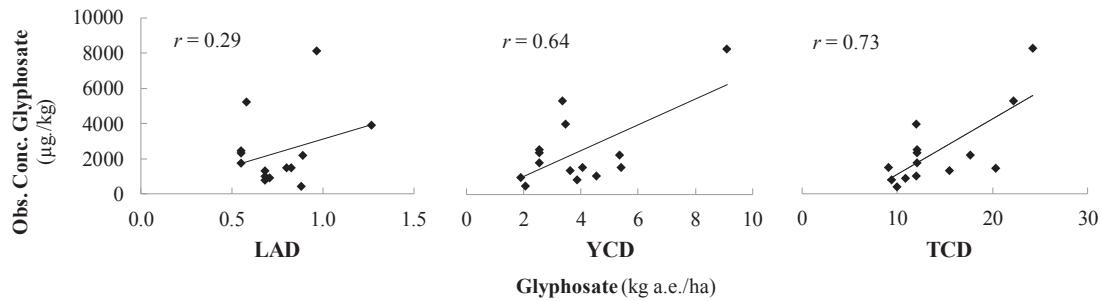


Fig. 5. Relationship between LAD, YCD and TCD with the measured soil glyphosate concentration (OBS).

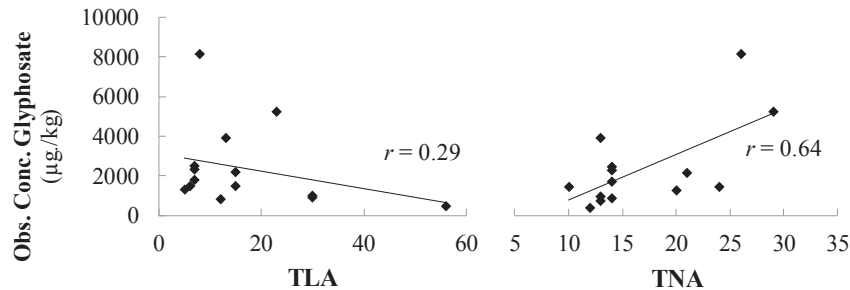


Fig. 6. Relationship between TLA and TNA with the measured soil glyphosate concentration (OBS).

stability of that compartment favoring biotic degradation over other processes (i.e. new sprayings, erosion, leaching, etc.). That also agree with the higher proportion of the metabolite over the herbicide observed in all sediment samples.

3.3. Agricultural management practice and glyphosate in soil

Seeding of wheat during cold season and corn or soybean in the warm season, usually sandwiched with other crops like sorghum, sunflower and rapeseed was the basic crop rotation system used in the studied farms, and representative of the agricultural practice in the region (Table 1). Under this production system, glyphosate is used not only for weed control but also for chemical fallow. Thus, several applications are performed during the annual cycle. The annual mean number of herbicide applications in the studied farms was 3.3. In addition, the average glyphosate application per spraying event was 0.8 kg a.e./ha and the mean accumulate annual dose was 3.9 kg a.e./ha, but maximum values of 1.3 and 9.1 kg a.e./ha, respectively, were observed in some cases.

Regarding the relationship between the measured glyphosate concentration (OBS) and the used doses, no correlation was observed with LAD ($p = 0.308$). However, it was positively correlated with YCD ($p = 0.013$) and TCD ($p = 0.002$), increasing the strength of the relationship as higher the number of included spraying events (Fig. 5). In addition, no significant relationship was observed with TLA ($p = 0.298$), but OBS was positively correlated with TNA ($p = 0.013$), estimating an increment of 1 mg/kg of glyphosate in soil every 5 spraying events (Fig. 6). When OBS was compared with the concentrations estimated from LAD (EXP), higher OBS than EXP were observed in 71% of the samples, with a mean ratio OBS/EXP of 1.92 ± 0.39 (17), and no relationship ($p = 0.317$) was observed between the two variables (Fig. 7A). In addition, a positive correlation ($p = 0.009$) was observed between the TNA and the difference between OBS and EXP (OBS-EXP) (Fig. 7B), but not with TLA (data not shown in the graph, $r = 0.330$, $p = 0.248$). All together these results indicate that measured

concentrations of glyphosate were better explained by the long-term records than the last application, with a higher prediction error (respect LAD) as higher the TNA in the plot. In addition, an accumulation process of the herbicide with the number of applications was detected.

Although, it was expected that the AGR increases with the time from the last application (TLA), no significant correlation was observed between these two variables ($r = 0.22$ $p = 0.44$). In addition, no relationship was observed between AGR and TNA ($r = 0.19$ $p = 0.51$), LAD ($r = 0.32$ $p = 0.27$) or TCD ($r = 0.311$ $p = 0.27$). On the other hand, it was significantly correlated with

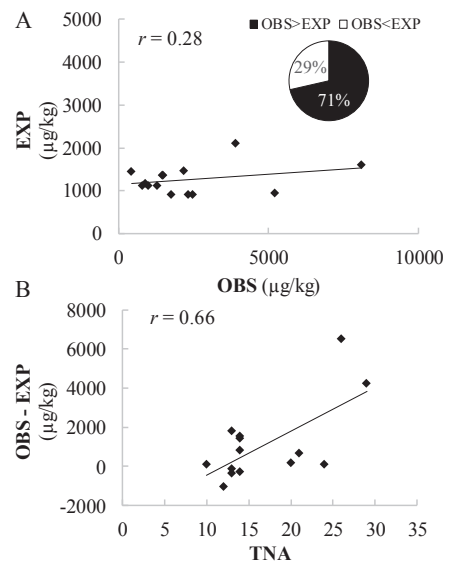


Fig. 7. Panel A: Relationship between the OBS and the expected glyphosate concentrations (EXP) in soil (insert percentage of cases where OBS > EXP or vice versa). Panel B: Relationship between TNA and de difference of OBS-EXP.

YCD ($r = 0.66$, $p = 0.009$), but no clear explanation was found for such observation.

Dissipation time 50% (DT50) and 90% (DT90) for glyphosate in the Mesopotamic Pampas soils (Paraná district) was estimated between 20–23 d and 290–390 d, respectively (Okada et al., unpublished). In other regions of the world the estimated values of DT50 were between 30 and 40 d (Newton et al., 1984) or 45–60 d (Feng and Thompson, 1990) and 12 weeks for DT90 (Rueppel et al., 1977). Considering those studies and the average number of applications used under studied agricultural practices (around one every four months), it would be expected that not all the glyphosate would have dissipated between spraying events. This would help to understand why measured glyphosate concentrations in soil were higher than the explained by the last spraying event and why they were correlated with the TNA. Therefore, in contrast with regional common beliefs, these results would indicate that, under agricultural practices, soil characteristics and climatic conditions of the Mesopotamic Pampas, the application rate of the herbicide would be higher than the dissipation rate, and consequently the glyphosate would accumulate from one application to the other. These results agree with the definition of “pseudo-persistent” pollutants coined by Daughton (2003) to those compounds that are continuously introduced to the environment, and for which new molecules are permanently replenishing those that are being removed.

4. Conclusions

In the present study the environmental fate of glyphosate and AMPA in the Mesopotamic Pampas agroecosystem was assessed for the first time in relations with current agricultural practices in local farms. The major finding of the study was that according with the spraying dosages and frequencies of glyphosate obtained from the agricultural management records, and the measured concentrations of the herbicide and its metabolite in soil, it is possible to state these compounds are behaving, in the studied agroecosystem, as “pseudo-persistent” pollutants. In addition, from the above mentioned information, an increment rate of 1 mg of glyphosate per kg of soil every five spraying events was estimated. Regarding the environmental fate of the glyphosate and AMPA, the affinity of these compounds for the solid matrices was confirmed, with higher concentrations in soil (usually above the mg/kg), sediment and SPM. On the other hand, the concentration in the dissolved fraction was low, and therefore the analysis of the SPM shows up as a more sensitive strategy for detection of the herbicide and the metabolite in surface waters. Finally, the concentrations of glyphosate and AMPA in groundwater was assessed for the first time in the region, showing that the concentrations of these compounds are still undetectable in the deep aquifer used as water source for cattle and people in the region.

According to the main conclusions reached in the study, a quick revision of the current agricultural management practices together with strict monitoring program (including groundwater) are recommended, in order to stop the observed accumulation of these compounds in studied environmental compartments. A reevaluation of the ecotoxicological risk would be also required for soil and sediment under the light of the high concentrations found in these compartments.

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