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Spatial-temporal and flow dynamics of pesticides in a basin.

SPATIAL AND TEMPORAL TRENDS AND FLOW DYNAMICS OF GLYPHOSATE AND OTHER PESTICIDES WITHIN AN AGRICULTURAL WATERSHED IN ARGENTINA

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Abstract: In the present study, we evaluated the spatial and temporal trends of current use pesticides in surface water and sediments and to determine its relation with hydrological stream dynamics within the agricultural watershed of El Crespo stream. We sampled two contrasting sites: Site 1 (upstream) surrounded by agricultural lands, and Site 2 (downstream) surrounded by natural grasslands. Most of the applied pesticides (i.e. glyphosate, 2,4-D, atrazine, tebuconazole and imidacloprid) were detected in high frequencies in surface water samples at both sites. However, only glyphosate and AMPA (aminomethylphosphonic acid) were present at high concentrations and had a significant spatial-temporal trend. The highest concentrations were found during spring 2014 in Site 1, in association with the intense rains that occurred in that season. The fact that glyphosate and AMPA concentrations were higher than the rest of the studied compounds is closely related to the land use within the watershed, since glyphosate was the most applied herbicide during the fallow period in glyphosate-resistant crops (soybean, maize). The pesticide mixture had a significant spatial-temporal trend, reaching the highest levels during storm flow events in spring 2014. The intensive rains in spring 2014 could be the main factor that influenced the stream hydrology and pesticide behavior at El Crespo watershed. The estimated annual pesticide losses were 3.11 g/ha in Site 1 and 0.72 g/ha in Site 2. This result indicates that possible attenuation process could be decreasing the pesticides loads during the downstream transport from Site 1 to Site 2. This article is protected by copyright. All rights reserved

Keywords: Surface water, Glyphosate, Pesticide mixture, Stream discharge, Pesticide loss

INTRODUCTION

The nature of pesticide usage often requires broad distribution over large areas of lands, having a direct impact on natural systems. For this reason, they are probably the most studied environmental contaminants [1]. Frequently, agricultural lands and wetlands are associated, increasing the probabilities that pesticides reach the aquatic ecosystems by runoff after rainfalls and by drift during application [2].

Recent studies have focused on the presence of glyphosate in wetlands because this herbicide is one of the most commonly used in agricultural production [3]. The reported levels of glyphosate and its main metabolite, aminomethylphosphonic acid (AMPA), in USA surface waters ranged between 0.08 and 450 µg/L [4-5], while the concentrations in sediments reached 470 µg/Kg [5]. In Switzerland, the reported glyphosate concentrations in surface water ranged from 0.024 to 3.3 µg/L [6]; while in Argentina, levels in surface water are within 0.5 - 7.6 µg/L and from 5 to 200 µg/Kg in sediments [7]. These environmental levels can induce adverse effects in no-target aquatic biota [8]. Moreover, in 2015 the International Agency for Research on Cancer classified glyphosate as a probable human carcinogen [9], creating public concern about its presence in the environment.

The above-mentioned studies focus on the detection of one single molecule and its metabolite. However, in environmental samples, it is common to find a mixture of various pesticides in water and sediments. The composition of the mixture of pesticides present in the environment has a major toxicological relevance since mixtures of different chemicals may lead to a lower or higher toxic effect than would be expected from exposure to a single compound [10]. Thus, the associated risks of pesticides in surface water are better described when most of the current-use pesticides are analyzed together because it provides a realistic situation of the stream pollution and the possible toxicological effects to aquatic biota [5].

Monitoring studies often focus on the simultaneous detection of several current-use pesticides from each region. For example, Allison et al. [11] and Wightwick et al. [12] reported the presence of various herbicides and fungicides in Australian streams associated with horticultural production. Gilliom [13], Belden et al. [14] and Smiley et al. [15], reported the presence of pesticide mixtures in USA streams under agricultural and urban impacts. Ccanccapa et al. [16] studied the spatio-temporal variations of more than 40 pesticides in two Spanish rivers associated with different impacts. In a recent study, Schreiner et al. [17] monitored the presence of pesticide mixtures in several European and USA streams, demonstrating that herbicides and their metabolites were the most frequently detected (e.g. diuron, isoproturon, atrazine and glyphosate).

In Argentina, one study included the simultaneous detection of herbicides and insecticides, such as atrazine, acetochlor, cypermethrin, chlorpyrifos and endosulfan [18]. Another study analyzed the presence of 29 pesticides in several streams and reported that atrazine was the most detected compound [19]. These studies demonstrated the presence of pesticide mixtures in surface water but did not include glyphosate, which is the main herbicide used in the Argentinian agricultural production [20].

Pesticide concentrations in surface water can fluctuate depending on seasonal variations, environmental conditions, and the stream hydrology of the watershed. High levels of pesticide mixtures in surface water have been related to surface runoff from croplands caused by late spring and early summer rainfalls [21-22]. In this sense, it is important to understand how the hydrological conditions may influence the behavior of pesticide mixtures within a watershed [23] in order to obtain relevant data such as the estimated loads and losses of pesticides from agricultural lands to aquatic environments [6, 24-25].

In Argentina, agricultural production requires the application of several pesticides throughout the year (e.g. insecticides for summer pests control, pre-emergent herbicides in winter-spring for the fallow period, post-emergent herbicides over summer and winter crops, and fungicides in spring for fungi control). The southeast of the Pampas plains or Austral Pampas [26] is one of the major agricultural zones in Argentina and it is highly irrigated by wetlands, rivers, and streams. The river and stream flow dynamics are strongly related to the environmental humidity and results from the runoff water and the level of the phreatic table [27]. In addition, the hydrology regime of the Buenos Aires streams can change dramatically during storm events, reaching a discharge more than 100 times greater than the average discharge [28]. Despite the importance of hydrological characteristics in the behavior of pesticides in the environment, to our knowledge, there are no studies linking the variations in levels of pesticides with the hydrological dynamics of streams in Argentina. The main objectives of the present study were a) to study the spatial and temporal trends of pesticides (herbicides, insecticides and fungicides) as individual compounds and as a pesticide mixture in surface water and sediments, and to link the hydrological stream dynamics with the pesticide behavior, in order to estimate the loads and losses of pesticides from croplands to the aquatic ecosystem.

MATERIAL AND METHODS

Description of study area, sampling sites, and climate conditions

El Crespo is a third-order stream following Strahler [29] classification. It is located in the southeast of the Buenos Aires Province- Argentina with a catchment area of 489.42 km^2 and flows from south to north through 65 km (Figure 1). This watershed is only influenced by farming activities without urban or industrial impact, being an optimal site to study processes like pollution, transport and dynamic of pesticides. The headwaters of El Crespo are located in the Tandilia Hills system at the southern area with Typic Argiudols soils. The drainage system has a continuous flow because it captures groundwater baseflow. The mouth of El Crespo, at the northern end, is located on floodplains characterized by Typic Natraquoll soils [30]. The historic (from 1970 to 2015) and sampling period (from October 2014 to October 2015) climate conditions, as daily temperatures and rainfall of El Crespo watershed are presented in Supplemental Data, Table S1. The information was obtained from the data base of EEA INTA Balcarce Weather Station.

Sampling Site 1 was located upstream, 20 km away from the headwater, and sampling Site 2 was located downstream, close to the stream mouth (Figure 1). Both sites are 45 km away from each other (Figure 1). Site 1 is surrounded by cultivated fields and Site 2 is located in an area covered with native grassland used for extensive livestock production, without historical pesticide applications. The study was carried out from October 2014 to October 2015, covering all the pesticide applications along the year in this area of Argentina. The main cultivated crops and used pesticides in the study area during the 2014-2015 campaign are listed in Table 1. These data were obtained from surveys made to the farmers of the study area and from estimates of the cultivated area of each crop in the Balcarce district obtained from the Argentinian Agroindustry Ministry database [31].

Discharge measurements

At each site, there was a bridge two meters above the stream water surface, from which the water depth and water velocity were measured (Supplemental Data, Figure S1). To calculate the total area of the channel cross-section profile, the bridges were divided into equidistant sections of 0.50 m across a transect of 5.50 m in Site 1 and 8.00 m in Site 2. Then, the water depth was measured in each section using a 10 Kg submersible buoy coupled to a ten-meter tape and the area of the cross-section profile of the stream was calculated (Supplemental Data, Figure S1). To obtain the mean water velocity of the stream, water velocity was measured at each section at different depths (0.30, 0.60, 0.90 and 1.20 m) using a mechanical current meter (SIAP N°870) coupled to a five-meter stick. The mean water velocity was measured at 5 different water depths that included the storm flow and low-base flow periods during the sampling time. The stream discharge was calculated by the relation between the mean water velocity (m/s) and the cross-section area (m^2) at that particular depth, expressed in m^3/s. The relationship between water depth and stream discharged was modeled using an exponential equation ($R^2 = 0.95$). The stream water depth was measured from the middle of the bridge at each sampling time and the discharge was estimated using the model.

Surface water and sediments sampling

The sampling periods were divided according to contemplated a temporal sequence from in spring 2014 (October – November 2014), summer (December 2014 – March 2015), fall (April – June 2015), winter (July –August 2015) and spring 2015 (September – October 2015), according of the Southern Hemisphere (Table 2). Surface water and sediments were sampled monthly and after heavy rainfall or a storm event, except on February and May 2015 (Table 2). In total, sites were sampled 15 times. On August 10, the stream was overflowed and only surface water for Site 1 could be sampled, since access to Site 2 was not possible. The total number of water samples for Site 1 were n= 16 and for Site 2 was n=15, in triplicate. The total number of sediment samples in Site 1 were n= 22 and in Site 2 n= 35. In Site 1, during fall there were no sediments in the stream edge, in winter and spring 2015 only two and three samples were collected, respectively.

Water samples were collected from the middle of the bridge in 1 L polypropylene bottles and immediately the pH and electrical conductivity was measured. Later, the samples were stored at -20°C until pesticide analysis. Sediment samples were collected at the stream edges using a PVC cylinder of 5 cm diameter and 20 cm of length coupled to a two-meters stick. The upper 5 cm of sediments were used for pesticide and physicochemical analysis. They were dried at a constant temperature in an oven at 30°C for 3 days and then were milled. A subsample was sieved using a 0.5 mm mesh to measure the total organic carbon (TOC) by the loss-on-ignition method [32]. Another two subsamples were sieved using a 2 mm mesh, one for particle size distribution (PSD) and one pesticide analysis (described in Pesticide analysis section). The PSD was determined according to the pipette method for estimating three sizes: clay (< 2 µm), silt (2 – 50 µm) and sand (50 µm – 2000 µm) [33]. The pH and electrical conductivity were measured in sediment:water ratio of 1:2.5.

Pesticide analysis

Glyphosate and AMPA determination. A subsample of 2 mL of surface water and 5 gr of sediments were used for analysis. The surface water and sediment samples were fortified with 10µL and 50µL of 10 µg/mL stock solution of [1,2-^{13}C,^{15}N] glyphosate, respectively, to determine matrix effects and recovery. After 30 min, the liquid and solid samples were extracted with 1 mL and 25 mL of borate buffer solution (100 mM $Na_2B_4O_7 \cdot 10H_2O$/100 mM K_3PO_4, pH=9), respectively. After the sediment samples were sonicated three times for 15 min and centrifuged at 3000 rpm, an aliquot of 2 mL was taken from the supernatant. A standard curve with six points (0.5, 1, 10, 20, 50 and 100 μg/L) of glyphosate and AMPA was prepared with each set of surface water and sediment samples, which had an equivalent amount of [1,2-^{13}C,^{15}N] glyphosate to that expected in the analyzed samples at each point of the curve. Later, surface water samples, sediment samples and the standards were derivatized with 2 mL of a solution of 1 mg/mL of 9-fluorenylmethylchloroformate (FMOC-CL) in acetonitrile during 24 h in darkness. Then, samples and the standards were shaken for 3 min with 5 mL of dichloromethane to end the clean-up step. The samples were centrifuged at 3000 rpm and the aqueous phase was filtrated through a 0.22 μm nylon filter and dispensed into a 1.5 mL vial for UHPLC–MS/MS determination [7]. Analyses were performed by injecting 20 μL of the final extract in the UHPLC–MS/MS system (Water Acquity) equipment calibrated for positive detection, using a column Acquity UHPLC BEH C18 (1.7 μm, 50 x 2.1 mm) (Waters). To perform the chromatographic separation, the mobile phases were water (phase A) and methanol (phase B), both modified with ammonium acetate 5 mM. The flow rate for the mobile phase was 0.4 mL min^{-1}. The percentage of organic modifier (B) was changed linearly as follows: 0 min, 0%; 0.2 min, 0%; 2.5 min, 70%; 3.5 min, 100%; 4.5 min, 100%; 5.0 min, 0%; and 6 min, 0%. The column was kept at 60 °C. The limit of detection (LOD) and quantification (LOQ) for glyphosate and AMPA in surface water and sediment are listed in Table 3. Recoveries for spiked matrices were higher than 70%.

Multiresidues pesticide determination. A subsample of 100 mL of surface water and 5 gr of sediments were used for the multiresidue analysis of pesticides (Table 3). The surface water samples were filtered through a 0.45 µm nylon membrane and 1 mL of concentrated formic acid was added. Then the samples were fortified with 10 µL of 1µg/mL stock solution of [^{5}D] atrazine to determine matrix effects and recovery. Oasis HLB (60 mg) cartridges were conditioned with 5 mL of acetonitrile, 5 mL of methanol and 5 mL of acidified water (with 1% of formic acid). Then, the sample was passed through the cartridge. After drying under vacuum, pesticide residues were eluted with 3 mL of methanol. The extract was evaporated to dryness under a gentle nitrogen stream (40 °C) and finally reconstituted with 1mL of methanol:water (50:50, v/v). The samples were filtrated through a 0.22 µm nylon filter and dispensed into a 1.5 mL vial for UHPLC–MS/MS determination [19].

Sediment samples were fortified with 50 µL of 1µg/mL stock solution of [^{5}D]- Atrazine and 5 mL of nanopure water and 20 mL of acetonitrile was added. After 30 min, the samples were sonicated three times for 15 min and centrifuged at 3000 rpm for 15 min. An aliquot of 1 mL was taken from the supernatant and mixed with 1 mL of ultrapure water and was later filtrated through a 0.22 µm nylon filter and disposed into a 1 mL vial for UHPLC–MS/MS determination [34].

Multiresidues analysis for surface water and sediments was performed by injecting 20 µL of the final extract in the UHPLC–MS/MS system (Water Acquity), using a column Acquity UHPLC BEH C18 (1.7 µm, 100 x 2.1 mm) (Waters) fitted with an Acquity VanGuard BEN C18 pre-column (1.7 µm, 5 x 2.1 mm). The matrix effect for water and sediments was evaluated for the selected pesticides by spiking a blank sample with 10 µg/L (equivalent to 0.1 µg/L in the water sample and 100 µg/Kg in sediment sample) of each individual pesticide. A standard curve with six points, 0.5, 1, 10, 20, 50 and 100 µg/L of pesticide mixture was prepared with each set of surface water and sediment. The LOD and LOQ in water and sediment samples of the multiresidue analysis are shown in Table 3. The identification of the target compound in the samples was based on liquid chromatography retention time compared to that of a standard and the selection of two ion products from the corresponding precursor ion. The most intensive ion product from each precursor ion was selected for quantification (Q), whereas a less sensitive secondary transition (q) was used as the second criterion for confirmation purposes. Positive findings were confirmed calculating the peak area ratios between the Q/q and comparing them with ion-ratios obtained from a reference standard. A finding was considered positive when the concentration ratio Q/q was in the range of 0.8–1.2 [7, 19, 34]. For UHPLC analysis, Masslynx NT v 4.1 (Waters) software was used to process quantitative data obtained from calibration standards and from the samples.

Data and statistical analyses

Pesticide levels and spatial-temporal trends. The mean pesticide residues concentrations were calculated using all data. In samples where the pesticide concentration was below the LOD, values were set to zero. When the concentration of the compound was below the LOQ, the concentration was set to the LOD value (censored data) [23]. The pesticide mixture concentrations in surface water were calculated as the sum of all the residues in a single sample.

The concentration data was rank-transformed in order to meet the normality and homogeneity of variance assumptions. Spatial and temporal trends of individual pesticides and pesticide mixtures were analyzed using the linear mixed model PROC MIXED of SAS version 9.0 software [35], with sampling sites and seasons as fixed effects. Mean comparisons were evaluated with a significance level of 0.05 or 0.01using LSMEANS.

Pesticide mixture and stream discharge relation. To analyze if the concentration of the pesticide mixture was related to the hydrological stream dynamics in each sampling site, a Spearman correlation analyzes was done with a significance level of 0.05. We estimated the daily pesticide loads of individual pesticides and of the pesticide mixture in surface water at each sampling site using the follow equation

$$\text{Pesticide load (g/d)} = \text{Stream discharge } (\text{m}^3/\text{s}) \text{ x Pesticide concentration } (\mu\text{g/L}) * \text{CF}$$

CF (Conversion Factor): 86.95 s L g / d m^3 µg.

To estimate the annual pesticide losses from agricultural lands to surface water, we used the average annual loads and the drainage area of each sampling site

$$\text{Pesticide loss (g/ha)} = \frac{\text{Average loads (g/d) x 365 (d)}}{\text{Drainage area (ha)}}$$

Being the drainage area at Site 1= 214.50 km^2 (21,450 ha) and at Site 2= 367.25 km^2 (36,725 ha).

RESULTS

Water and sediment physical-chemical properties

Surface water at both sampling sites was alkaline (pH 8 - 9) and had electrical conductivities near 1 mS/cm (Supplemental Data, Table S2), similar to other streams of the southeast Pampas [36]. Sediments at both sites were slightly alkaline, with a pH range of from 7.60 to 8.60 and with conductivities from 0.20 to 0.60 mS/cm. Sediments were classified as sandy loam and sandy silt loam and had a TOC content between 1.55 - 3.20 % (Supplemental Data, Table S2).

Spatial and temporal trends of individual pesticides in surface water

To analyze the spatial and temporal trends of individual pesticides, we focused on the compounds that had a detection frequency above 40 % (Table 4). The pesticides that were detected in less than 10 % of the samples were not included in the analysis (Supplemental Data, Table S3). The most detected pesticide residues were glyphosate, AMPA, atrazine, hydroxy-atrazine (At-OH), 2,4 D, metolachlor, imidacloprid, and tebuconazole (Table 4). The mean concentrations of these compounds were very variable. Glyphosate had the highest mean concentration and maximum value compared to the other pesticide residues (Table 4). At-OH was the second compound with the highest mean concentration, reaching a maximum value of 2.27 µg/L, followed by AMPA with a maximum concentration of 2.00 µg/L. The herbicide 2,4-D had a maximum level of 0.99 µg/L. The rest of the compounds had mean concentration values less than 0.10 µg/L (Table 4).

The pesticide residues that had a spatial and/or temporal significant variation were glyphosate, AMPA, atrazine and At-OH (Figure 2). The herbicides 2,4D, metolachlor, acetochlor, metsulfuron methyl, flurocloridone, the insecticides imidacloprid and chlorpyrifos, and the fungicides, tebuconazole, epoxiconazole and metalaxyl did not show significant spatio-temporal trends during the period of this study ($p > 0.05$).

Glyphosate and AMPA had a significant site-season interaction ($p < 0.01$). During spring 2014, glyphosate levels were significantly higher at Site 1 (2.09 µg/L) than at Site 2 (0.68 µg/L) (Figure 2A). In summer, the opposite occurred since the levels of glyphosate at site 2 were four times higher than at site 1 (Figure 2A). In the following seasons, glyphosate concentrations remained low (< 0.4 µg/L) and did not differ significantly between sites. Regarding the temporal differences within sites, during spring 2014 Site 1 had the highest glyphosate concentration compared to the other seasons (Figure 2A). On the other hand, at Site 2 glyphosate levels during the summer were significantly higher than in the rest of the seasons.

In the case of AMPA, the concentrations during spring 2014 were significantly higher at Site 1 (1.13 µg/L) than the concentrations found at Site 2 (0.15 µg/L). The AMPA levels at Site 1 then decreased (<0.25 µg/L) and did not differ significantly with those of Site 2 in the rest of the seasons (Figure 2B). Regarding variations within sites, AMPA concentration was only significantly different during spring 2014 at Site 1 compared to the rest of the seasons, whereas at Site 2 AMPA concentrations did not differ between months.

The concentrations of atrazine and its metabolite, At-OH, varied significantly between seasons but not between sites ($p < 0.01$). Atrazine concentrations were significantly higher during spring 2014 than in the rest of the seasons (Figure 2C). On the other hand, At-OH concentrations during spring 2014, summer and fall were lower than 0.15 µg/L and then increased significantly during winter reaching a concentration of 1.75 µg/L. Concentrations then decreased to 1.0 µg/L in spring 2015, not differing significantly from the rest of the months (Figure 2D).

Spatial and temporal trends of pesticide mixtures in surface water and its relation to stream discharge

Pesticide mixture concentrations had spatial-temporal trends (Figure 3A), with a significant site-season interaction ($p < 0.05$). The concentrations of the pesticide mixture only differed significantly between sites during spring 2014 (Figure 3A). Site 1 had a greater concentration of pesticide mixtures in spring 2014. In the following seasons, concentrations in

Site 1 were lower than in Site 2, although not significantly different. Variations along seasons in the same site were only significantly different during spring 2014 for Site 1. Concentrations in Site 2 did not differ between seasons (Figure 3A).

In spring 2014, El Crespo stream was going through a storm flow period as a consequence of intense rains (approximately 110 mm) that occurred from October 7 to November 19 (Table 2). As a consequence of these heavy rainfalls the stream discharge reach a maximum of approximately 3 m^3/s and 2 m^3/s at Site 1 and Site 2, respectively (Figure 3B). During summer and fall, the stream had a low-base flow period (Table 2). The discharges did not exceed 1 m^3/s at both sites (Figure 3B). Until early winter, the low base-flow period continued (Table 2). However, on August 10, the stream overflowed due to the intensive rains that hit this region during August 5-10, of approximately 158.2 mm (Table 2). The discharge in this sampling date at Site 1 was impossible to measure manually. The El Crespo stream overflowing is presented in Supplemental Data, Figure 2S. During spring 2015, the stream returned to low base-flow regime with a discharge of less than 1 m^3/s at both sites (Figure 3B).

The pesticide mixture concentration was correlated with the stream discharge at Site 1 (Spearman coefficient $r = 0.72$, $p = 0.0024$; Supplemental Data, Figure S2 A). At this site, the highest pesticide mixture concentrations occurred in spring 2014 during the storm flow period. Then, during the low-base flow period, pesticide concentrations decreased. At Site 2, no correlation was observed between pesticide mixture concentration and stream discharge (Spearman coefficient $r = 0.22$, $p = 0.4244$; Supplemental Data, Figure S2 B).

Based on the stream discharge and pesticide mixture concentrations, the annual pesticide losses were estimated as 3.11 g/ha at Site 1 and 0.72 g/ha at Site 2 (Table 5). Glyphosate annual losses were the highest of all the pesticides residues, reaching 1.38 g/ha at Site 1 and 0.32 g/ha at

Site 2 (Table 5). The stream regime and total loads and losses at each sampling site are detailed at Supplemental Data, Table S4.

Occurrence of pesticides in sediments

Glyphosate, AMPA, atrazine, At-OH, metolachlor, acetochlor and chlorpyrifos were the only pesticide residues present in sediments. AMPA was the most detected compound, followed by glyphosate, acetochlor and metalochlor (Table 6). The rest of the residues had detection frequencies less than 12 %. Although At-OH was detected in few samples, this residue had the highest concentration in sediment samples, reaching a peak of 157 μg/Kg (Table 6). Sediments were absent in the stream edges at Site 1 during fall. Also, we were only able to collect a small number of samples during winter and spring 2015. Therefore, we did not analyze the spatial and temporal trends of pesticide residues in this environmental compartment.

DISCUSSION

Spatial and temporal trends of individual pesticides in surface water

Glyphosate and its metabolite, AMPA, were detected at a high frequency and high concentrations in surface water samples of El Crespo stream (Table 4). This is probably due to the repeated and high doses of glyphosate that were applied over a wide area of the watershed. Glyphosate was the main herbicide used in the study zone based on surveys of producers (Table 1). The commercial formulations with 54% of active ingredient were the most commonly applied by the farmers. In general, there are three application periods: one during the fallow period in winter-spring, the second previous to sowing, and the third during the growth stage of transgenic crops. During the 2014-2015 campaign, the area of El Crespo watershed under no-till management covered about 96% of the cultivated area and the doses used of glyphosate in the first two applications were 2.5 L/ha. The transgenic crops (soybean and maize) covered about

60% of the cultivated fields (Table 1) and the third application of glyphosate reached 2.5 L/ha (total annual application dose: 5 L/ha on transgenic crops).

Glyphosate and AMPA levels in surface water had a spatial and temporal trend, reaching the maximum levels at Site 1 during spring 2014 (Figure 2A-B). This can be attributed to the intense precipitations that occurred during that season, which increased the glyphosate flush from agricultural lands to the stream. The significant increase of glyphosate during summer in Site 2 could be explained by the downstream transport of glyphosate residues from Site 1, since at Site 2 pesticides were not applied.

Glyphosate has a high polarity and water solubility (Log Kow = -3.57 at 20°C) [37]. However, it also has a high affinity for soil particles [38], which may lead to its accumulation in agricultural soils. Glyphosate can be transported to aquatic environments by runoff dissolved in water or bond to colloids [39]. The main concern about the presence of glyphosate in freshwater ecosystems comes from the fact that this herbicide can induce several adverse effects in aquatic organisms [8]. For example, glyphosate induces deleterious reproduction effects on the native South American fish *Rhamdia quelen* at chronic exposure levels [40], and it induces algae mortality in the periphyton communities [41]. The Canadian Water Quality Guidelines for the Protection of Aquatic Life indicates that a concentration of 65 μg/L of glyphosate is the maximum level allowed in surface water to protect the aquatic life [42]. The levels of glyphosate and AMPA in surface water detected in the present study were similar to others previously reported in streams of the Pampas [7] and in European streams [6] and these levels did not surpassed those established by the Canadian Water Quality Guidelines for the Protection of Aquatic Life. However, the levels detected in USA streams [4-5] sometimes can surpass this limit.

The systemic herbicide 2,4-D is one of the most used in the world for weed control and it is the second most used in Argentina, after glyphosate [20]. Based on surveys to the farmers, there were two applications of 2,4-D. The first application was done during the chemical fallow at the same time as the application of glyphosate and before the sowing of sunflowers and transgenic soybeans. The second application occurred during the growing of wheat and maize (Table 1). In El Crespo watershed the herbicide 2,4-D was detected in more than 83.87 % of the surface water samples (Table 4). The maximum levels were detected during spring 2014 in Site 1, but concentrations were not significantly different between seasons and sampling sites. The short half-life of 2,4-D in soils (4-7 days) and its rapid biodegradation and photolysis in water [43] may explain the lower levels detected in Site 2. There is a lack of information about the occurrence of 2,4-D in aquatic ecosystems, but the present study indicates that 2,4-D is a frequent pollutant in surface water of agricultural areas, and it should be included in monitoring programs. Also, it is relevant to study the presence of 2,4-D in surface water because it can induce adverse effects on aquatic biota, as reported by Vigário and Simoe [44] in the South American fish *Poecilia vivipara*.

Atrazine is a chloro-s-triazine belonging to the triazine group of synthetic organic herbicides and it is frequently applied for the selective control of broadleaf and grassy weeds [45]. Atrazine was the third most used herbicide in El Crespo watershed (Table 3). This herbicide was used during the fallow and growing periods, on maize and sorghum crops, which cover an 11.85% of the cultivated area (Table 1). Atrazine and its metabolite, At-OH, had a high detection frequency, but at lower concentrations than glyphosate and 2,4-D (Table 4). Atrazine and At-OH residues had a significant temporal variation. The parental compound increased during spring 2014 in Site 1 (Figure 2C), and its metabolite during winter 2015 (Figure 2D). The moderate half-life of atrazine, approximately 78 days in soils of the study zone [46] and 86 days in surface water [45], could explain why atrazine was frequently detected during spring 2014 and At-OH was detected more frequently after that. Similar levels of atrazine have been reported in other Argentinian basins [18-19] and Australian [11] and Spanish rivers [16]. Atrazine is a ubiquitous pollutant of surface water and it was banned in the European Union in 2003 [47] because it has a high risk of reaching groundwater [45] and it is considered as a possible carcinogen [48]. However, in other countries including Argentina it is widely used.

The other studied herbicides, such as metolachlor, acetochlor, metsulfuron methyl and flurochloridone were less applied in the cultivated area (Table 1). The occurrence of these compounds ranged from 45 to 83 % with concentrations below 0.50 µg/L (Table 4) and did not show spatial-temporal trends. Data of acetanilide herbicides (metolachlor, acetochlor and alachlor) in Argentina streams are scarce. For example, De Gerónimo et al. [19] detected the presence of acetochlor in several streams of the Buenos Aires province, but at levels below the LOQ. Nevertheless, the concentrations of acetanilide herbicides in surface water of different countries may reach up to 5 µg/L [16, 49-50].

In El Crespo basin, the most used insecticide to control summer pests was the neonicotinoid imidacloprid and the second most used was the organophosphate chlorpyrifos. The occurrence frequency was 93.3% and 45.16% for imidacloprid and chlorpyrifos, respectively (Table 4). Although these insecticides are only applied in the summer season, we detected no significant spatial-temporal trends. Imidacloprid has a high solubility in water (Log Kow = 0.57 at pH: 7, 20°C) [51], therefore it can be expected to be found in aquatic environments. Surface water contamination by imidacloprid, particularly after storm events that cause runoff pulses from cultivated fields [52] deserves attention. Generally, the levels do not exceed 1 µg/L in

European [53] and Australian surface waters [54], as it is the case of El Crespo stream. However, levels exceeding 8 μg/L have been reported in Spanish rivers [16]. This neurotoxic insecticide has a great impact on the structure of aquatic invertebrate communities [55] and could induce high mortality on wild pollinator species [56]. As a consequence, it was banned from the EU (from 2013-2015) [57]. Also other countries like USA, Canada, Netherlands and Sweden have established water quality guidelines with limits for imidacloprid of 0.13-1.05 μg/L [54]. In Argentina, there is a lack of mitigation measures, although there is a recent report of its genotoxicity effects in a native fish species (*Australoheros facetus*) [58].

From the analyzed fungicides, tebuconazole was the most detected, followed by metalaxyl and epoxiconazole (Table 4). However, other fungicides that were applied at El Crespo watershed, such as cyproconazole, pyroclasrobin, azoxystrobin and carbendazim (Table 1), were not included in this study. Nevertheless, their presence in surface water cannot be discarded [59]. The occurrence of fungicides in Argentinian streams has seldom been studied; however, the concentrations found in this study could induce adverse effects on aquatic biota. For example, Toni et al. [60] demonstrated oxidative stress of tebocunazole on *Cyprinus carpio*.

Spatial and temporal trends of pesticide mixtures in surface water and its relation to stream discharge

The understanding of spatial-temporal trends of pesticide mixtures is a critical first step in evaluating the risks of pesticides within agricultural streams [15]. Some studies have demonstrated that the highest levels of pesticides in surface water occur as pulses in response to late spring and early summer rainfall events [21-22], as it was observed in the present study. The maximum levels of concentration of pesticide mixtures in El Crespo stream was detected at Site 1 during spring 2014 (Figure 3) after a heavy rainfall event (Table 2). Several studies have shown that herbicides are the predominant compounds in the mixture of pesticides that are present in surface water [15,17] as also observed in this study. In the present study, glyphosate was the main pesticide residue present in surface water samples (Table 4). It is possible to hypothesize that the rain could wash adsorbed glyphosate residues from the stubbles, because glyphosate adsorption to stubbles is a reversible process [61], so glyphosate molecules remain in the aqueous solution until they reach the stream.

The study of the combinations of pesticides that are present in surface water can be used to predict the possible hazards in aquatic environments [8], because the pesticide mixture can strongly exceed the toxic effects of individual compounds [10]. Polard et al. [22] demonstrated the potential genotoxicity of surface water containing a complex pesticide mixture and how the temporal trends in the pesticide mixtures levels associated with different hydrological contexts can influence the degree of the genotoxic damage in fish.

On the other hand, it is important to know the relation of pesticides mixture with the stream regime. Some authors have defined the low base-flow regime as a period in which, under average climatic conditions, there are few precipitation events and groundwater is the main contributor to the stream [23]. The high base-flow (storm flow) is defined as a period in which, under average climatic conditions, there are frequent precipitation events and the stream discharge is derived to a greater degree from recent rainfall [23]. The hydrologic dynamics of the Pampas streams is influenced by different factors such as rainfall events, groundwater contribution (depending of the water table depth) and soil moisture [27]. Within a period of high soil moisture and intense rains, the stream discharges may increase more than 100 times [27-28]. Generally, the high base-flow period in the Pampas streams occurs during winter-spring seasons and the low base-flow period occurs during the summer-fall seasons [27]. In the case of El

Crespo stream, we detected a high flow period during spring 2014, when the discharge was increased more than five times compared to the rest of the seasons (Figure 3B), as consequence of the heavy rainfalls that occurred in this season (Table 2). However, during winter at low base-flow, the pesticide levels increased at Site 1. This result could be explained by the common observation that during the low base-flow period pesticides are concentrate in the surface water [16]. At Site 1, which is surrounded by crops, is affected directly by pesticides and there was an intrinsic relation with the stream regime (Supplemental Data, Figure S2 A). However Site 2, which is surrounded by extensive livestock with no historical pesticide applications, the concentrations of pesticide residues in surface water would only depend on those that were transported downstream. In this sense, pesticides can suffer different attenuation processes when they are transported downstream, such as degradation, accumulation in sediments as it is demonstrated in the present study (Table 6) and bioaccumulation, as have demonstrated at macrophytes that inhabit the El Crespo stream [62]. Also, at Site 2 pesticide dilution could be an important process to take into account, because the drainage area at Site 2 comprises almost the whole watershed.

The annual pesticide losses at El Crespo watershed mainly corresponded to glyphosate and AMPA residues (Table 5), which were the major pesticide residues detected in surface water (Table 4). The major proportion of glyphosate losses were registered during the fallow period of spring 2014. Depending on the season, agricultural losses may account for the highest share of pesticide pollution [25]. Some studies have estimated pesticide losses such as atrazine [24-25] or glyphosate [4,6], from urban and agricultural inputs. Different strategies to reduce diffuse agricultural losses and spills due to improper management are still needed [25]. In Argentina, and in particularly at El Crespo watershed, there was not mitigation measurements to reduce the pesticide contamination to freshwater ecosystems. One possibility to decrease agricultural impact is the implementation of riparian vegetation barriers at the stream edge, because these barriers can reduce runoff of pesticide from agricultural lands to surface water [63].

Occurrence of pesticides in sediments

Glyphosate and AMPA were the main detected pesticides in sediments (Table 6). Other pesticides such as atrazine, At-OH, acetochlor, metolachlor and chlorpyrifos were detected in lower frequencies (Table 6). Glyphosate and AMPA levels detected in the present study were lower than other report at the Pampas streams [7]. This can be related to the sandy texture of the sediments at El Crespo stream (Supplemental Data Table S2), since glyphosate is generally adsorbed to the clay fraction [38]. Our results are in agreement with others studies that reported low levels of pesticide residues in sandy sediments [16]. The presence of glyphosate and AMPA in sediments is in concordance that this herbicide is the main used at El Crespo watershed.

CONCLUSION

From the whole current-use pesticide residues analyzed, glyphosate and its metabolite AMPA predominated in surface water and sediment samples. Other pesticide residues, such as atrazine, At-OH, 2,4-D, acetochlor, metolachlor, imidacloprid and tebuconazole were frequently detected in surface water but at much lower concentrations than glyphosate and AMPA. Pesticide residues, specially glyphosate and AMPA concentrations in surface water had a significant spatial and temporal trend, increased at Site 1 during spring 2014. It was mainly associated with consecutive storm events that occurred during this season in the study area.

The correlation found between pesticide mixture levels and stream discharge at the Site 1 (a site surrounded by agricultural lands) shows the relevance of analyzing the hydrologic stream dynamics to understand pesticides behavior. Major estimated annual losses corresponded to glyphosate and its metabolite AMPA.

Currently, in Argentina there are few regulations about the maximum levels of pesticides in surface waters to protect aquatic life. Therefore, it is necessary to work on guidelines to mitigate the diffuse losses of pesticide from agricultural field.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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Data availability—Data can be accessed by contacting the authors directly (perez.debora@inta.gob.ar).

REFERENCES

1. Connell DW. 2005. Pesticides, in Connell DW, ed, *Basic Concepts of Environmental Chemistry* 2nd ed, Taylor and Francis CRC Press, Boca Raton, FL, USA, pp 167-189.
2. Tang X, Zhu B, Katou H. 2012. A review of rapid transport of pesticides from sloping farmland to surface waters: Processes and mitigation strategies. *J Environ Sci* 24:351–361.
3. Baylis AD. 2000. Why glyphosate is a global herbicide: strengths, weaknesses and prospects. *Pest Manag Sci* 56:299-308.
4. Coupé RH, Kalkhoff SJ, Capl P, Gregoire C. 2012. Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins. *Pest Manage Sci* 68:16–30.
5. Battaglin WA, Meyer MT. 2014. Glyphosate and its degradation product AMPA occur frequently and widely in U.S. soils, surface water, groundwater, and precipitation. *J Am Water Resour Assoc* 50:275-290.
6. Hanke I, Wittmer I, Bischofberger S, Stamm C, Singer H. 2010. Relevance of urban glyphosate use for surface water quality. *Chemosphere* 81:422-429.
7. Aparicio VC, De Gerónimo E, Marino D, Primost J, Carriquiriborde P, Costa JL. 2013. Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and soil of agricultural basins. *Chemosphere* 93:1866-73.
8. Myers JP, Antoniou MN, Blumberg B, Carroll L, Colborn T, Everett LG, Hansen M, Landrigan PJ, Lanphear BP, Mesnage R, Vandenberg LN, vom Saal JS, Welshons WV, Benbrook CM. 2016. Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. *Environ Health* 17:15-19.

9. Guyton KZ, Loomis D, Grosse Y, El Ghissassi F, Benbrahim-Tallaa L, Guha N, Scoccianti C, Mattock H, Straif K. 2015. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *Lancet Oncol* 16:490-491.
10. Reffstrup TK, Larsen JC, Meyer O. 2010. Risk assessment of mixtures of pesticides. Current approaches and future strategies. *Regul Toxicol Pharm* 56:174–192.
11. Allison G, Bui A, Zhang P, Rose G, Wightwich AM, Allinson M, Pettigrove V. 2014. Investigation of 10 herbicides in surface waters of a horticultural production catchment in southeastern Australia. *Arch Environ Contam Toxicol* 67:358-73.
12. Wightwick AM, Bui AD, Zhang P, Rose G, Allinson M, Myers JH, Reichman SM, Menzies NW, Pettigrove V, Allinson G. Environmental fate of fungicides in surface waters of a horticultural-production catchment in southeastern Australia. *Arch Environ Contam Toxicol* 62:380–390.
13. Gilliom RJ. 2007. Pesticides in U.S. streams and Groundwater. *Environ Sci Technol* 41: 3409-3414.
14. Belden JB, Gilliom RJ, Martin JD, Lydy MJ. 2007. Relative toxicity and occurrence patterns of pesticide mixtures in streams draining agricultural watersheds dominated by corn and soybean production. *Integrated Environ Assess Manag* 3:90-100.
15. Smiley PC, King KW, Fausey NR. 2014. Annual and seasonal differences in pesticide mixtures within channelized agricultural headwater streams in central Ohio. *Agr Ecosyst Environ* 193:83-95.
16. Ccanccapa A, Masia A, Andreu V, Picó Y. 2016. Spatio-temporal patterns of pesticide residues in the Turia and Júcar Rivers (Spain). *Sci Tot Env* 540:200-210.

17. Schreiner VC, Szöcs E, Bhowmik AK, Vijver Martina G, Schäfer RB. 2016. Pesticide mixtures in streams of several European countries and the USA. *Sci Tot Env* 573:680–689.
18. Bonansea RI, Amé MV, Wunderlin DA. 2013. Determination of priority pesticides in water samples combining SPE and SPME coupled to GC–MS. A case study: Suquía River basin (Argentina) *Chemosphere* 90:1860-1869.
19. De Gerónimo E, Aparicio VC, Barbaro S, Portocarrero R, Jaime S, Costa JL. 2014. Presence of pesticides in surface water from four sub-basins in Argentina. *Chemosphere* 107:423-31.
20. Cámara Argentina de Sanidad Agropecuaria y Fertilizantes (CASAFE). 2016. Buenos Aires, Argentina. [cited 2017, June 1]. Available from: www.casafe.org/publicaciones/estadisticas/
21. Thurman EM, Goolsby DA, Meyer MT, Kolpin DW. 1991. Herbicides in surface waters of the Midwestern United States: The effect of spring flush. *Environ Sci Technol* 25:1794-1796.
22. Polard T, Jean S, Gauthier L, Laplanche C, Merlina G, Sánchez-Pérez JM, Pinelli E. 2011. Mutagenic impact on fish of runoff events in agricultural areas in south-west France. *Aquat Toxicol* 101:126–134.
23. Sprague LA, Nowell LH. 2008. Comparison of pesticide concentrations in streams at low flow in six metropolitan areas of the United States. *Environ Toxicol Chem* 27: 288-298.
24. Wittmer IK, Bader HP, Scheidegger R, Singer H, Luck A, Hanke I. 2010. Significance of urban and agricultural land use for biocide and pesticide dynamics in surface waters. *Water Res* 44: 2850-2862.
25. Wittmer IK, Scheidegger R, Bader HP, Singer H, Stamm C. 2011. Loss rates of urban biocides can exceed those of agricultural pesticides. *Sci Tot Env* 409:920-932.
26. Viglizzo E, Pordomingo A, Castro M, Lértora F. 2002. La sustentabilidad del agro pampeano. Ediciones INTA. 84pp.

27. Kruse E, Ainchil J. 2011. El agua en la llanura bonaerense. *Revista de la Universidad* 36: 109-119.
28. Jergentz S, Mugni H, Bonetto C, Schulz R. 2005. Assessment of insecticide contamination in runoff and stream water of small agricultural streams in the main soybean area of Argentina. *Chemosphere* 61:817–826.
29. Strahler AN. 1957. Quantitative Analysis of Watershed Geomorphology. *Trans Amer Geophys Union* 38:913-920.
30. Cartas de suelos de la República Argentina - Provincia de Buenos Aires. 2017. Argentina. [cited 2017, June 1]. Available from: http://anterior.inta.gov.ar/suelos/cartas/.
31. Siembra, Cosecha, Producción y Rendimiento. Ministerio de Agroindustria. Presidencia de la Nación. Argentina. [cited 2016, October 2]. Available from: www.siia.gob.ar/_apps/siia/estimaciones/estima2.php
32. Schulte EE, Hopkins BG. 1996. Estimation of organic matter by weight loss-on-ignition. In Magdoff FR, Tabatsai MA, Hanlon EA, eds, Soil organic matter: Analysis and interpretation. SSSA Inc, Madison, USA. pp 21-31.
33. Gee GW, Bauder JW. 1986. Particle size analysis. In Klute A, eds, Methods of Soil Analysis. Part 1. Physical and Mineralogical methods, 2nd ed Agronomy Madison USA, 383-411.
34. De Gerónimo E, Botero-Coy AM, Marín JM, Aparicio VC, Costa JL, Sancho JV, Hernández F. 2015. A simple and rapid analytical methodology based on liquid chromatography-tandem mass spectrometry for monitoring pesticide residues in soils from Argentina. *Anal Methods* 7:9504-9512.
35. SAS Institute. 2002. *The SAS System, Ver 9.* Cary, NC, USA.

36. Romanelli A, Massone HE, Quatrozl OM. 2011. Integrated hydrogeological study of surface and groundwater resources in the southeastern Buenos Aires Province, argentina. *Int J Environ Res* 5: 1053-1064.

37. Schuette J. 1998. Environmental fate of glyphosate. [cited 2017, June 2]. Available from: www.cdpr.ca.gov/docs/emon/pubs/fatememo/glyphos.pdf.

38. Okada E, Costa JL, Bedmar F. 2016. Adsorption and mobility of glyphosate in different soils under no-till and conventional tillage. *Geoderma* 263:78–85.

39. Daouk S, De Alencastro LF, Pfeifer HR. 2013. The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: proof of widespread export to surface waters. Part II: The role of infiltration and surface runoff. *J Environ Sci Heal B* 48:725-736.

40. Soso AB, Barcellos LJG, Ranzani-Paiva MJ, Kreutz LC, Quevedo RM, Anziliero D, Lima M, Bolognesi da Silva L, Ritter F, Calliari Bedin A, Finco JA. 2007. Chronic exposure to sub-lethal concentration of a glyphosate-based herbicide alters hormone profiles and affects reproduction of female Jundiá (*Rhamdia quelen*). *Environ Toxicol Phar* 23:308-313.

41. Vera MS, Lagomarsino L, Sylvester M, Pérez GL, Rodríguez P, Mugni H, Sinistro R, Ferraro M, Bonetto C, Zagarese H, Pizarro, H. 2010. New evidences of Roundup®(glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems. *Ecotoxicology* 19:710-721.

42. Presence and levels of priority pesticides in selected Canadian aquatic ecosystems. Canada. [cited 2017, June 2]. Available from: www.ec.gc.ca/Publications/FAFE8474-C360-46CC-81AB-30565982E897/PresenceAndLevelsOfPriorityPesticidesInSelectedCanadianAquaticEcosystems.pdf.

43. World Health Organization. 2003. 2,4-D in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality. [cited 2017, June 2] Available from: www.who.int/water_sanitation_health/dwq/chemicals/2,4-D.pdf
44. Vigário AF, Sabóia-Morais SMT. 2014. Effects of the 2,4-D herbicide on gills epithelia and liver of the fish *Poecilia vivipara*. *Pesq Vet Bras* 34:523-528.
45. Lazorko-Connon S, Achari G. 2009. Atrazine: its occurrence and treatment in water *Environ Rev* 17: 199–214.
46. Fuscando F, Bedmar F, Monterubbianesi MG. 1999. Persistence of atrazine, metribuzin and simazine herbicides in two soils. *Pesq Agropec Bras* 34:2037-2044.
47. Bethsass J, Colangelo A. 2006. European Union Bans Atrazine, While the United States Negotiates Continued Use. *Int J Occup Environ Health* 12:260–267
48. MacLennan PA, Delzell E, Sathiakumar N, Myers SL, Cheng H, Grizzle W, Chen VW, Wu XC. 2002. Cancer incidence among triazine herbicide manufacturing workers. *J Occup Environ Med* 44:1048-1058.
49. Kalhoff JW, Kolpin D, Ferreri TE, Barceló D. 1998. Degradation of chloroacetanilide herbicides: The Prevalence of sulfonic and oxanilic acid metabolites in Iowa groundwaters and surface waters. *Environ Sci Tech* 32:1738-1740.
50. Byer JD, Struger J. Sverko E, Klawunn P, Todd A. 2011. Spatial and seasonal variations in atrazine and metolachlor surface water concentrations in Ontario (Canada) using ELISA *Chemosphere* 82:1155–1160.
51. Environmental Fate of Imidacloprid. Juanita Bacey. [cited 2017, June 2]. Available from: www.cdpr.ca.gov/docs/emon/pubs/fatememo/imid.pdf.

52. Bonmatin JM, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, Marzaro M, Mitchell EAD, Noome DA, Simon-Delso M, Tapparo A. 2015. Environmental fate and exposure; neonicotinoids and fipronil. *Environ Sci Pollut Res* 22:35–67.
53. Tennekes H. 2011. The systemic insecticides: a disaster in the making. ETS Nederland BV. pp 90.
54. Sánchez-Bayó F, Hyne RV. 2014. Detection and analysis of neonicotinoids in river waters – Development of a passive sampler for three commonly used insecticides. *Chemosphere* 99:143–151.
55. Van Dijk TC, Van Staalduinen MA, Van der Sluijs JP. 2013. Macro-invertebrate decline in surface water polluted with imidacloprid. *PLoS ONE* 8:62374.
56. van der Sluijs JP, Simon-Delso N, Goulson D, Maxim L, Bonmatin JM, Belzunces LP. 2013. Neonicotinoids, bee disorders and the sustainability of pollinator services. *Curr Opin Environ Sustain* 5:293–305.
57. Comission Implementing Regulation (EU) No 485/2013. Off J European Union 139:12-26.
58. Iturburu FG, Zömisch M, Panzeri AM, Crupkin AC, Contardo-Jara V, Pflugmacher S, Menone ML. 2017. Uptake, distribution in different tissues and genotoxicity of imidacloprid in the freshwater fish *Australoheros facetus*: Imidacloprid uptake, distribution and genotoxicity in fish. *Environ Toxicol Chem* 36:699-708.
59. Rodrigues ET, Lopes I, Pardal MA. 2013. Occurrence, fate and effects of azoxystrobin in aquatic ecosystems: A review. *Environ Int* 53:18-28.

60. Toni C, Ferreira D, Kreutz LC, Loro VL, Barcellos LJG. 2011. Assessment of oxidative stress and metabolic changes in common carp (*Cyprinus carpio*) acutely exposed to different concentrations of the fungicide tebuconazole. *Chemosphere* 83:579-584.
61. Rampoldi EA, Hang S, Barriuso E. 2011. The fate of glyphosate in crop residues. *Soil Sci Soc Am J* 75:553–559.
62. Pérez DJ, Okada E, De Gerónimo E, Menone ML, Aparicio VC, Costa JL. 2016. Can an aquatic macrophyte bioaccumulated glyphosate?: A watershed scale study using a non-target hydrophyte *Ludwigia peploides*. Proceedings, VI Argentinian Congress of the Society of Environmental Toxicology and Chemistry, Ciudad de Córdoba, Córdoba, Argentina, October, 11-14, 2016), pp 40.
63. Giaccio GCM, Laterra P, Aparicio VC, Costa JL. 2016. Glyphosate retention in grassland riparian areas is reduced by the invasion of exotic trees. *Phyton-Int J Exp Bot* 85:108-116.

Figure 1. Study area of El Crespo watershed and sampling sites. Site 1: Agricultural lands (37° 44´ 17.44” S; 58° 21´02.81” W); Site 2: Natural grassland (37° 34´ 3,82” S; 58° 02´44,47” W). Arrow indicates the flow direction.

Figure 2. Spatial and temporal trends of individual pesticides (mean ± SE) in surface water of El Crespo stream. (A): Glyphosate, (B): Aminomethylphosponic acid (AMPA); (C): Atrazine; (D) Hydroxy-atrazine (At-OH). Capital letters indicate statistically significant differences between sites for the same season ($p < 0.05$). Lowercase letters indicate statistically significant differences between season at the same site (for Site 1 normal lowercase letters and for Site 2 italic lowercase letters) ($p < 0.05$).

Figure 3. Spatial and temporal trends of pesticide mixture (A) and stream discharge (B) (mean ± SE) in surface water of El Crespo stream. Capital letters indicate statistically significant

differences between sites for the same season ($p < 0.05$). Lowercase letters indicate statistically significant differences between season at the same site (for Site 1 normal lowercase letters and for Site 2 italic lowercase letters) ($p < 0.05$).

Table 1: Description of cultivated crops and pesticides used in El Crespo watershead during 2014-2015 campaign.

Cultivated crops	*Cultivated area in km^2 (%)*[a]	*Pesticides used*[b]	
		Fallow	Growing
Soybean (*Glycine max* L.)	166.29 (45.28)	Glyphosate, 2,4-D, metsulfuron methyl	Glyphosate, imidacloprid, tebuconazole, epoxiconazole, pyraclostrobin, carbendazim
Sunflower (*Helianthus annus* L.)	64.30 (17.51)	Glyphosate, 2,4-D	Flurochloridone, metolachlor, lamdacialotrine
Wheat (*Triticum aestivum* L.)	55.53 (15.12)	Glyphosate, metsulfuron methyl, dicamba	2,4-D, metsulfuron methyl, dicamba, tebuconazole, azoxystrobin, picloram
Maize (*Zea mays* L.)	36.58 (9.96)	Glyphosate, atrazine, acetochlor	Glyphosate, atrazine, 2,4-D, acetochlor, picloram, tebuconazole, epoxiconazole, pyraclostrobin, azoxystrobin
Barley (*Hordeum vulgare* L.)	23.25 (6.33)	Glyphosate	Metsulfuron methyl, dicamba, tebuconazole, epoxiconazonle, pyraclostrobin, azoxystrobin
Potato (*Solanum tuberosum* L.)	8.52 (2.32)	Glyphosate	Metribuzin, metolachlor, acetochlor, paraquat, imidacloprid, chlorpyrifos, dimetoato, cypermetrin, abamectina metalaxyl, manzoceb, azoxystrobin
Sorghum (*Sorghum spp*. L)	6.93 (1.89)	Atrazine, acetochlor	Atrazine, epoxiconazonle, pyraclostrobin
Oats (*Avena sativa* L.)	3.55 (0.97)	Glyphosate, metsulfuron methyl, dicamba	n.i.a.
Rape (*Brassica napus* L.)	2.30 (0.63)	Glyphosate	Dicamba, chlorpyrifos, cypermetrin, azoxystrobin, cyproconazole
Total cultivated area	367.25 (100)		

[a] Estimated from the Argentinean Agroindustry Ministry data base.

[b] Data obtained from surveys to farming producers.
n.i.a.: no information available.

Table 2: Sampling dates, storm events, rainfall and stream regime for each season at El Crespo watershed during the study period.

Season	*Sampling date*	*Storm event* [a]	*Rainfall (mm)* [b]	*Stream regime*
Spring 2014	October 7	October 3-5	41.4	Storm flow
	October 29	October 26-28	39.0	Storm flow
	November 19	November 18	29.6	Storm flow
Summer	December 22			Low base-flow
	December 30	December 29	16.0	Low base-flow
	January 14			Low base-flow
	January 29	January 25-27	16.3	Low base-flow
	March 10			Low base-flow
Fall	April 15	April 9-15	24.2	Low base-flow
	June 10			Low base-flow
Winter	July 20			Low base-flow
	August 3	July 28 - August 1	23.6	Low base-flow
	August 10	August 5-10	158.2	Flooding-Overflow
	August 20			Low base-flow
Spring 2015	September 21			Low base-flow
	October 19	October 19	31.1	Low base-flow

[a] Information obtained from EEA INTA Balcarce Weather Station.
[b] Accumulated rainfall during the storm event before sampling date, only considering rainfall higher than 10 mm.

Table 3: Description of UHPLC- MS/MS conditions for selected pesticides in surface water and sediments.

Compound	*Type*	*Surface water (ng/L)*[a]		*Sediment (µg/Kg)*		*RT (min)*	*Q transition*	*Cone (V)*	*C. E. (eV)*	*q transition*	*C. E. (eV)*
		LOD	*LOQ*	*LOD*	*LOQ*						
Glyphosate	Herbicide	100	500	0.5	1.0	1.99	392 > 88	20	25	392 > 214	15
AMPA	Gly-Metabolite	100	500	0.5	1.0	2.48	334 > 179.1	20	15	334 > 156	15
Atrazine	Herbicide	0.09	0.04	0.1	0.3	6.92	215.9 > 173.9	28	18	215.9 > 95.9	23
At-OH	At-Metabolite	2.6	9.0	42.9	152.1	4.10	198 > 156	28	18	198 > 85.9	23
At-DPP	At-metabolite	2.0	6.0	3.5	12.7	3.45	173.8 > 95.8	25	18	173.8 > 103.7	23
At-DT	At-Metabolite	0.4	1.2	0.5	1.8	4.70	187.8 > 145.8	25	18	187.8 > 103.8	25
Alachlor	Herbicide	16.0	53.0	12.4	43.0	5.54	265.9 > 220	11	5	265.9 > 173.9	15
Acetochlor	Herbicide	3.0	8.0	0.5	1.6	8.54	269.9 > 147.9	10	20	269.9 > 224	8
Metolachlor	Herbicide	0.1	0.4	0.1	0.3	8.58	284 > 252	25	15	284 > 176	25
Dimethoate	Herbicide	1.0	3.2	0.3	1.0	4.21	229.8 > 198.9	28	10	229.8 > 124.8	23
Metribuzin	Herbicide	0.2	0.7	0.3	1.1	5.95	215 > 187	23	20	215 > 83.9	20
2,4-D	Herbicide	5.0	15.0	10.6	35.2	6.93	218.8 > 160.8	19	18	218.8 > 124.8	6
Metsulfuron methyl	Herbicide	0.2	0.6	0.1	0.3	6.11	381.9 > 166.8	18	18	381.9 > 198.8	22
Imazapir	Herbicide	1.0	4.0	0.9	3.3	3.75	261.9 > 217	25	20	261.9 > 148.8	25
Imazapic	Herbicide	1.0	3.5	0.7	2.5	4.74	276 > 162.9	33	28	276 > 231.1	20
Imazetapir	Herbicide	0.1	0.4	0.8	2.7	5.60	290 > 176.9	30	27	290 > 245.1	20
Imazaquin	Herbicide	0.1	0.4	0.8	2.7	5.60	290 > 176.9	30	27	290 > 245.1	20

Table 3: Description of UHPLC- MS/MS conditions for selected pesticides in surface water and sediments (continue).

Compound	*Type*	*Surface water (ng/L)*[a]		*Sediment (µg/Kg)*		*RT (min)*	*Q transition*	*Cone (V)*	*C. E. (eV)*	*q transition*	*C. E. (eV)*
		LOD	*LOQ*	*LOD*	*LOQ*						
Picloram	Herbicide	15.0	50.0	35.1	130.4	2.50	240.8 > 222.9	18	13	242.8 > 224.9	13
Fluorochloridone	Herbicide	0.20	0.60	0.2	0.5	7.14	311.8 > 291.9	35	23	313.8 > 293.9	23
Chlorimuron ethyl	Herbicide	0.30	0.70	0.2	0.6	8.03	414.9 > 185.9	23	20	414.9 > 212.9	18
Metobromuron	Herbicide	0.20	0.40	0.1	0.5	6.85	258.8 > 169.8	20	20	260.8 > 171.8	20
Chlorpyrifos	Insecticide	3.30	11.00	0.2	0.8	10.10	349.7 > 96.8	25	30	351.7 > 96.8	32
Fipronil	Insecticide	0.80	3.00	1.8	6.0	8.71	434.8 > 329.9	23	15	436.8 > 331.9	15
Imidacloprid	Insecticide	2.50	8.00	1.7	5.9	3.75	255.9 > 175	22	20	255.9 > 209	15
Kresoxim methyl	Fungicide	0.50	0.15	4.1	13.6	8.83	314 > 206	15	8	314 > 115.9	15
Triticonazole	Fungicide	0.10	0.40	0.1	0.4	8.46	318 > 70	23	18	318 > 124.9	28
Metconazole	Fungicide	0.20	0.60	0.1	0.5	9.20	320 > 70	35	25	320 > 124.8	33
Epoxiconazole	Fungicide	0.06	0.20	0.5	1.7 5	8.51	329.9 > 120.9	25	23	329.9 > 122.9	23
Tebuconazole	Fungicide	0.20	0.50	0.2	0.6	8.97	308 > 70	32	20	310 > 70	20
Metalaxyl	Fungicide	0.10	0.40	0.2	0.7	7.14	280 > 220	23	15	280 > 192	18

[a] Values expressed in ng/L for surface water (note that the unit used differ to the one used in the manuscript).
LOD: Limit of detection
LOQ: Limit of quantification
RT (min): Retention time
Q transition: Transition of the major ion product
q transition: Transition of the secondary ion product
Cone (V): Cone Voltage

C. E. (eV): Colision Energy
AMPA: aminomethylphosphonic acid
Gly-Metabolite: Glyphosate metabolite
At-OH: Hydroxy-atrazine
At-DPP: Desisopropyl-atrazine
At-DT: Desethyl-atrazine
At-Metabolite: Atrazine metabolite

Table 4: Summary of pesticide residues that had detection frequency above 40% in surface water samples of El Crespo stream.

Compound	*Detection frequency (%)*	*Mean concentration (maximum value)*[a]
Glyphosate	84.61	0.78 (2.90)
AMPA	84.61	0.32 (2.00)
Atrazine	96.66	0.02 (0.11)
At-OH	96.66	0.56 (2.65)
2,4-D	83.87	0.09 (0.99)
Metolachlor	83.87	0.03 (0.36)
Acetochlor	45.16	0.03 (0.48)
Metsulfuron methyl	60.00	0.005 (0.044)
Fluorochloridone	70.97	0.002 (0.01)
Imidacloprid	93.33	0.04 (0.56)
Chlorpyrifos	45.16	0.001 (0.003)
Tebuconazole	90.32	0.004 (0.025)
Epoxiconazole	41.93	5×10^{-4} (0.004)
Metalaxyl	45.16	6×10^{-4} (0.003)

[a] Values expressed in µg/L
AMPA: aminomethylphosphonic acid
At-OH: Hydroxi-atrazine

Table 5: Annual pesticide losses at El Crespo stream during October 2014 – October 2015.

Compound	*Pesticide annual loss* [a]	
	Site 1	*Site 2*
Glyphosate	1.38	0.32
AMPA	0.71	0.11
2,4-D	0.35	0.05
Atrazine	0.03	0.01
At-OH	0.48	0.18
Metolachlor	0.049	0.01
Acetochlor	0.048	0.01
Metsulfuron methyl	0.01	0.004
Fluorochloridone	0.005	0.0005
Imidacloprid	0.05	0.03
Chlorpyrifos	0.002	0.0009
Tebuconazole	0.008	0.002
Epoxiconazole	0.001	0.0001
Metalaxyl	0.001	0.0001
Total pesticide losses	3.11	0.72

[a] Values expressed in g/ha/yr
AMPA: aminomethylphosphonic acid
At-OH: Hydroxi-atrazine

Table 6: Summary of detected pesticide residues in sediment samples of El Crespo stream.

Compound	*Detection frequency (%)*	*Mean concentration (maximum value)*[a]
Glyphosate	78.94	3.85 (18.50)
AMPA	96.49	6.18 (47.50)
Atrazine	9.62	0.12 (2.00)
At-OH	11.54	24.04 (157.00)
Metolachlor	50.00	1.65 (15.00)
Acetochlor	55.77	9.17 (61.00)
Chlorpyrifos	3.85	0.007 (0.20)

[a] Values expressed in µg/Kg
AMPA: aminomethylphosphonic acid
At-OH: Hydroxi-atrazine

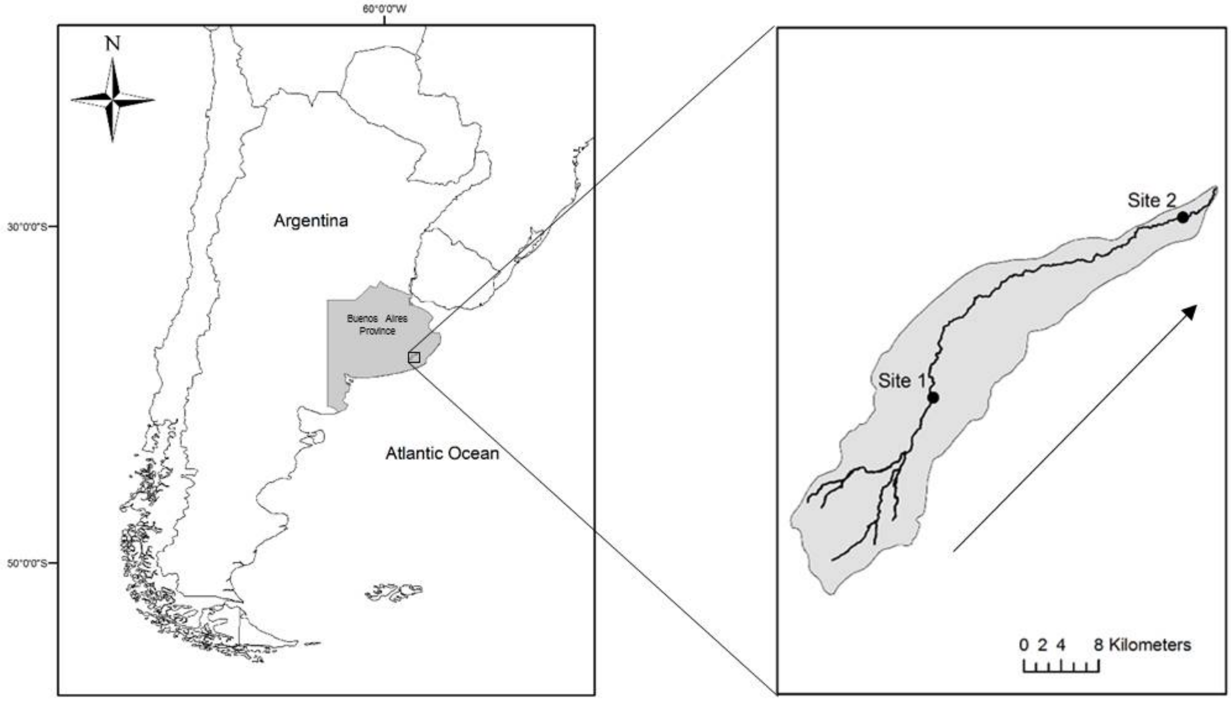
60°0'0"W
N
30°0'0"S
50°0'0"S
Argentina
Buenos Aires Province
Atlantic Ocean
Site 2
Site 1
0 2 4 8 Kilometers

Figure 1

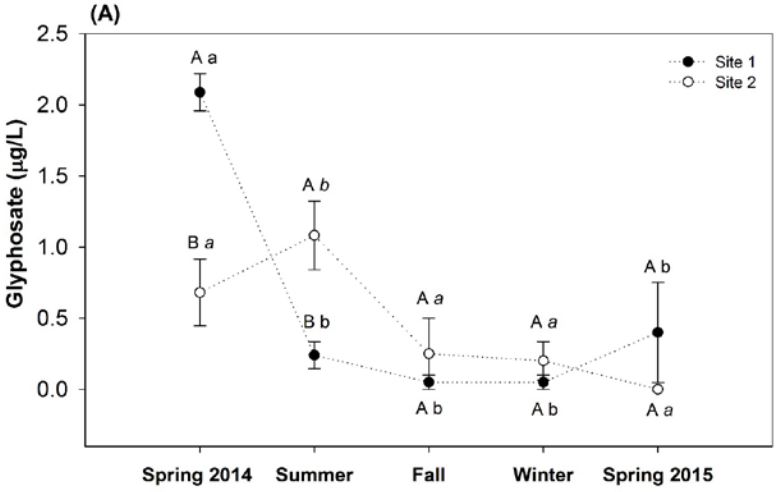
(A)
Glyphosate (µg/L)
2.5
2.0
1.5
1.0
0.5
0.0
Site 1
Site 2
A a
B a
A b
B b
A a
A b
A a
A b
A b
A a
Spring 2014
Summer
Fall
Winter
Spring 2015

Figure 2(a)

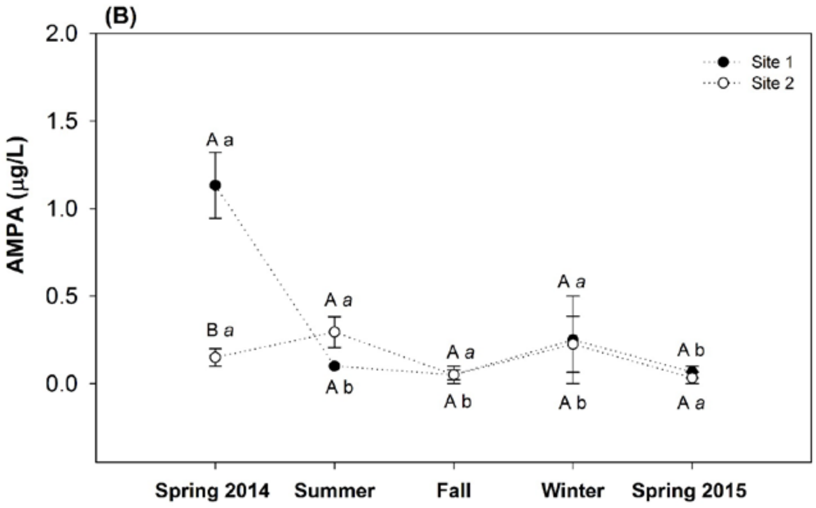
(B)
AMPA (μg/L)
2.0
1.5
1.0
0.5
0.0
Site 1
Site 2
A a
B a
A a
A b
A a
A b
A a
A b
A b
A a
Spring 2014
Summer
Fall
Winter
Spring 2015

Figure 2(b)

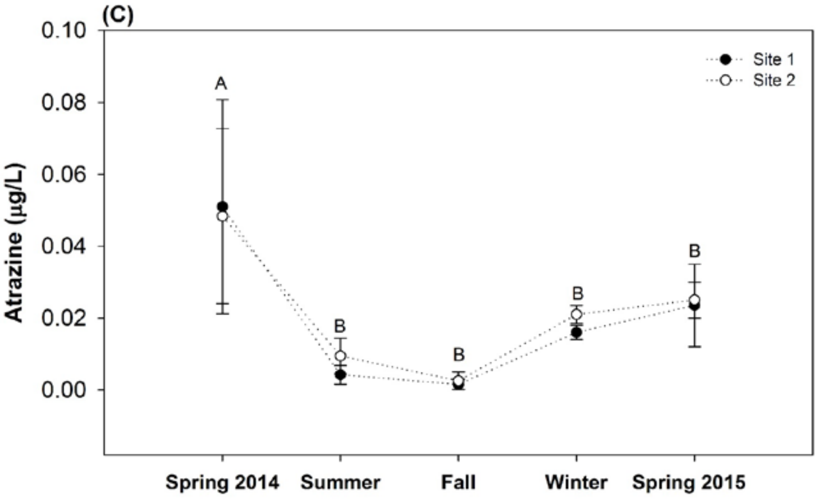
(C)
Site 1
Site 2
Atrazine (µg/L)
0.10
0.08
0.06
0.04
0.02
0.00
A
B
B
B
B
Spring 2014
Summer
Fall
Winter
Spring 2015

Figure 2(c)

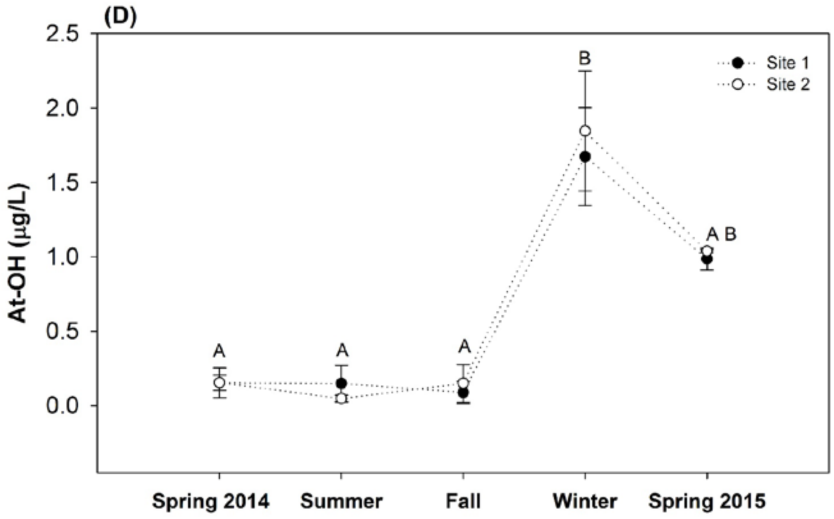
(D)
2.5
2.0
1.5
1.0
0.5
0.0
At-OH (μg/L)
Site 1
Site 2
A
A
A
B
A B
Spring 2014
Summer
Fall
Winter
Spring 2015

Figure 2(d)

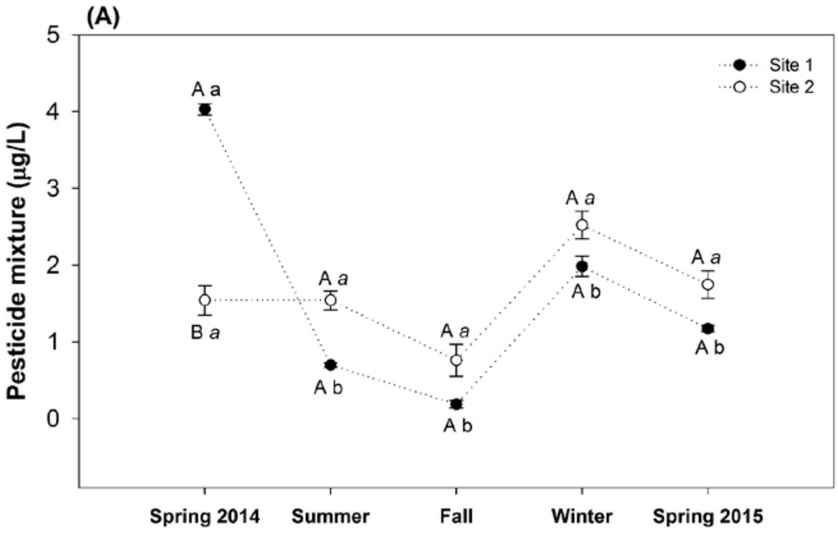
(A)
Site 1
Site 2
Pesticide mixture (µg/L)
5
4
3
2
1
0
A a
B a
A a
A b
A a
A b
A a
A b
A a
A b
Spring 2014
Summer
Fall
Winter
Spring 2015

Figure 3(a)

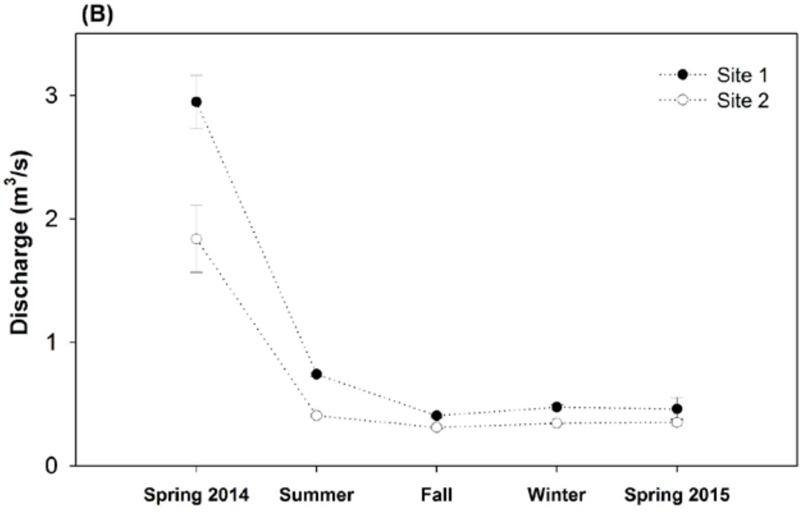
(B)
Site 1
Site 2
Discharge (m^3/s)
0
1
2
3
Spring 2014
Summer
Fall
Winter
Spring 2015

Figure 3(b)