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Insecticide concentrations in stream sediments of soy production regions of South America

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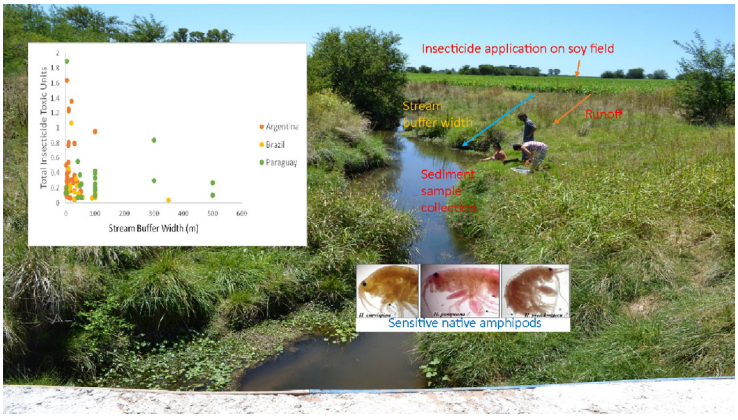
HIGHLIGHTS

- Characterized insecticides in stream sediments of Argentina, Paraguay, and Brazil
- Chlorpyrifos, cypermethrin, lambda-cyhalothrin and endosulfan frequently detected
- Most sediment samples contained multiple insecticides
- Highest toxic units occurred in sediment at sites with stream buffer widths <20 m Pyrethroids found at concentrations toxic to aquatic invertebrates

GRAPHICAL ABSTRACT



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ABSTRACT

Concentrations of 17 insecticides were measured in sediments collected from 53 streams in soy production regions of South America (Argentina in 2011–2014, Paraguay and Brazil in 2013) during peak application periods. Although environmental regulations are quite different in each country, commonly used insecticides were detected at high frequencies in all regions. Maximum concentrations (and detection frequencies) for each sampling event ranged from: 1.2–7.4 ng/g dw chlorpyrifos (56–100%); 0.9–8.3 ng/g dw cypermethrin (20–100%); 0.42–16.6 ng/g dw lambda-cyhalothrin (60–100%); and, 0.49–2.1 ng/g dw endosulfan (13–100%). Other pyrethroids were detected less frequently. Banned organochlorines were most frequently detected in Brazil. In all countries, cypermethrin and/or lambda-cyhalothrin toxic units (TUs), based on *Hyalella azteca* LC50 bioassays, were occasionally > 0.5 (indicating likely acute toxicity), while TUs for other insecticides were <0.5. All samples with total insecticide TU > 1 were collected from streams with riparian buffer width < 20 m. A multiple regression analysis that included five landscape and habitat predictor variables for the Brazilian streams examined indicated that buffer width was the most important predictor variable in explaining total insecticide TU values. While Brazil and Paraguay require forested stream buffers, there were no such regulations in the Argentine pampas, where

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Stream buffers
Aquatic toxicity

buffer widths were smaller. Multiple insecticides were found in almost all stream sediment samples in intensive soy production regions, with pyrethroids most often occurring at acutely toxic concentrations, and the greatest potential for insecticide toxicity occurring in streams with minimum buffer width < 20 m.

1. Introduction

In recent years, soybean production has become a major export crop for multiple countries in South America, including Brazil, Argentina, Paraguay, Uruguay, and Bolivia. Between 1986 and 2010, the total area in soy production in the Americas increased from 37 to 79 million hectares (Mha), and most of this expansion occurred in Argentina, Brazil, and Paraguay (Garrett et al., 2013). Between 1995 and 2011, soy cultivation area expanded by 126% and 209% in Brazil and Argentina, respectively (Castanheira and Freire, 2013). In Paraguay, soy cultivation area increased from 1.3 Mha in 2000–2001 to 2 Mha in 2007–2008 (Garcia-Lopez and Arizpe, 2010). Land use changes caused by expansion of soy cultivation in South America have raised a number of environmental concerns, including reductions in ecosystem complexity, loss of biodiversity, deforestation, increased erosion, adverse effects of agrochemicals, and increased greenhouse gas emissions (Botta et al., 2011; Castanheira and Freire, 2013; Lathuilliere et al., 2014).

A life cycle analysis of the soy-biodiesel crops produced in Argentina for export concluded that the aquatic toxicity impacts from soy-production pesticides were substantially higher than their terrestrial toxicity impacts, with the pyrethroid insecticide cypermethrin being the main contributor (Panichelli et al., 2009). Although application rates of the herbicide glyphosate in the cultivation of genetically modified soy are much higher than those of fungicides and insecticides, the potential toxic impact of glyphosate and other herbicides in aquatic areas near soy production systems of South America are considered to be negligible compared to those of fungicides and insecticides (Nordborg et al., 2014). Insecticide application rates are approximately double those of fungicides, and the insecticides most frequently used in soy production have very high aquatic toxicity (Nordborg et al., 2014).

Insecticides are typically applied several times to each soy crop, and are used primarily to control lepidopteran pests during plant growth, and hemipteran pests during the fruiting stage. Lepidopteran pests are often controlled by applications of chlorpyrifos, an organophosphate, and hemipteran pests by endosulfan, an organochlorine. Pyrethroids, especially cypermethrin, are commonly used for both types of pests, and are often applied at the same time as other pesticides (Di Marzio et al., 2010; OPDS, 2013). In Brazil, diamides and growth inhibitors are becoming more frequently used to control lepidopteran pests, while mixtures of neonicotinoid and pyrethroid insecticides are often used to control hemipteran pests. Contrary to recommendations from pest control advisors, pesticide applications for soy production in Brazil are primarily done prophylactically, with four to six applications per year (Bueno et al., 2011). The same trend is true in Argentina, with cypermethrin often being added to herbicide applications in order to prevent lepidopteran pests from laying eggs (OPDS, 2013). Moreover, the systemic neonicotinoid insecticide imidacloprid is commonly used in Paraguay and Brazil as a seed treatment, and is also applied as a spray later in the season along with pyrethroids, such as lambda-cyhalothrin or cypermethrin.

Multiple studies have detected soy production insecticides in both sediment and water collected from streams in Argentina and Brazil; however, most studies did not include all of the most frequently used insecticides, and data were not always comparable because of the use of variable matrices, methods, and reporting limits (Jergentz et al., 2004a; Mugni et al., 2010; Di Marzio et al., 2010; Marino and Ronco, 2005; Possavatz et al., 2014; Casara et al., 2012; Miranda et al., 2008; Laabs et al., 2002). Several studies in Argentina and Brazil have found associations between stream insecticide concentrations and effects to aquatic invertebrates and/or fish (Jergentz et al., 2004a; Rico et al., 2010; Di Marzio et al., 2010; Mugni et al., 2010; Chelinho et al., 2012); however, no studies of this type have been published on data collected from Paraguay.

Stream buffer width may be one of the most important factors in mitigating transport of pesticides to streams in agricultural areas (Bunzel et al., 2014; Rasmussen et al., 2011), but buffer zone requirements differ substantially among the three countries included in the present study. Riparian buffer zones are required to be maintained in both Brazil and Paraguay, although specific requirements are in flux. For example, in Paraguay, Resolution 485/03 by the Ministry of Agriculture requires a protected zone of 100 m around all water bodies. In Brazil, a new forest code was approved in 2012 (Law No. 12.651/12) establishing that riparian buffer zone requirements should vary with the general use of the land adjacent to the water body, the aquatic environment, the stream width, and the size of the rural property. As a general rule for stream widths of 10 m or less, the legislation requires a buffer width of 15 m of native riparian forest in rural areas or 30 m if in areas newly converted for rural activities. In contrast, in Argentina there are no national requirements for stream buffers. Moreover, stream buffer zones in the Argentine Pampas are generally unregulated, and many small streams in the most intensive soy production regions of the Santa Fe and Cordoba provinces are completely channelized with crops planted right up to the banks (no buffer zones). Some Argentine provinces do prohibit pesticide application within a specific distance from surface water (Chaco: Law 7032 — DR 1567/13; Formosa: Law 1163 — DR 109/02; Río Negro: Law 2175 — DR 769/94).

The objectives of the present study were to: (1) measure and compare insecticide concentrations in sediments collected from streams in four soy production regions: two in the Pampas of Argentina, one in eastern Paraguay, and one in south Brazil; (2) evaluate the potential for acute toxicity of insecticides on sensitive aquatic invertebrate taxa, such as *Hyalella* spp.; and, (3) evaluate the relationship between buffer strip widths and insecticide concentrations in stream sediments, taking into account the influence of other environmental variables.

2. Methods

2.1. Study locations and sampling schedule

The study sites included small streams that flowed through agricultural fields in four soy production regions: two regions in the Argentina Pampa (La Plata-Magdalena and Arrecifes), and one region each in the former Atlantic forest habitat of Brazil and Paraguay (Fig. 1). In the La Plata-Magdalena region, the principal land use was cattle grazing, with scattered plots of soy production and other agriculture. In the three other regions, intensive soy production was the predominant land use. In the La Plata-Magdalena region, five streams were sampled during five monitoring events in the 2011 to 2012 season only, including three sampling sites in one watershed and the remaining sites were located in separate watersheds. In the Arrecifes region, 16 sites were sampled over three years (2012–2014), and all sampling sites were on tributaries of the Arrecifes River. In Paraguay, 17 sites were sampled over two seasons (January and December 2013), and all sampling sites were on tributaries of the Pirapó River in the state of Itapúa. In Brazil, 18 sites were sampled once in November 2013, and all sampling sites were on tributaries of the San Francisco River in the state of

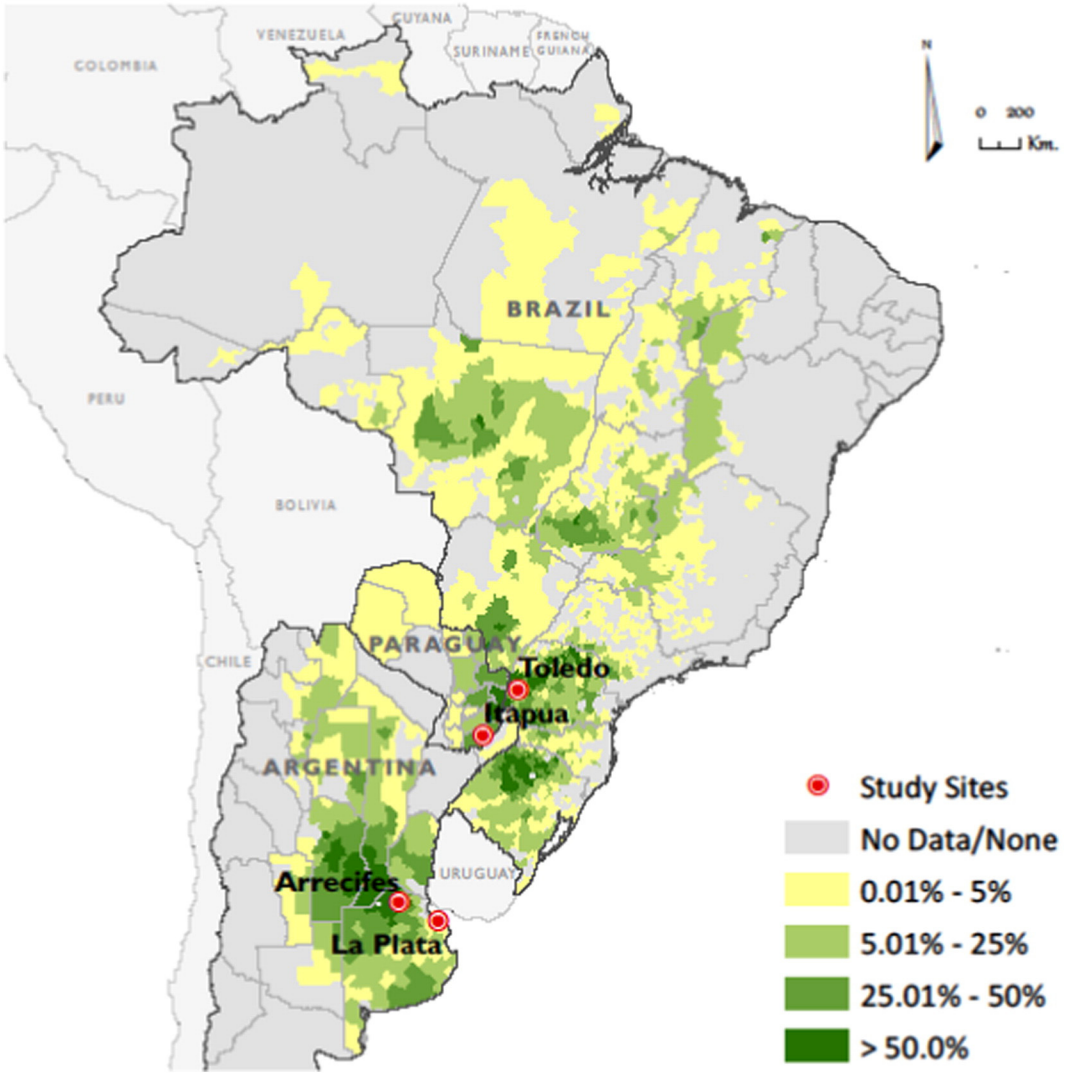

Fig. 1. Study regions and soy production intensity as percent of total land use by province or department in Argentina, Brazil, and Paraguay based on data reported by governments (Argentina: http://www.minagri.gob.ar; Brazil: http://www.ibge.gov.br; Paraguay: http://www.mag.gov.py).

Paraná. All study watersheds were tributaries of the Paraná/La Plata River.

Streams selected for the present study were not channelized, and most had a buffer strip of at least 5 m from the crops (Tables S1, S2). In the Brazil and Paraguay streams, the buffer zones generally contained Atlantic forest remnants and/or introduced tree species. In both Argentina regions, the buffers generally contained grasses and low shrubs with occasional trees. Minimum buffer widths were measured immediately upstream of sampling sites, and confirmed with LANDSAT images in Brazil and Paraguay. However, confirmation with LANDSAT images was not possible in Argentina, because there generally were not forested areas around streams and it was difficult to differentiate herbaceous vegetation from cropland. Catchments were delineated using topographical maps to estimate catchment size, and in Brazil and Paraguay the percent forest and percent agriculture within each catchment were estimated using LANDSAT images. Substrates in streams of both Argentina regions generally consisted of sediment with no rocks and little woody debris, although a few sites in Arrecifes contained some gravel. Substrates in Brazil and Paraguay streams usually contained relatively large amounts of rocks and/or cobble, and tended to have higher gradients and faster velocities than streams in Argentina. Stream depths ranged from about 0.6 m to >2 m (although all except two in the La Plata region were <1 m), and widths ranged from about 3 m to about 25 m (Table S2). While streams in Brazil and Paraguay were generally free of aquatic vegetation, most streams in Argentina included emergent vegetation (e.g. *Typha* spp. and *Scirpus* spp.) and submerged vegetation (e.g. *Potamogeton*, *Ceratophyllum* and *Egeria*), and many in the La Plata-Magdalena region were also characterized by abundant floating vegetation (e.g. *Eichornia*, *Lemna* and *Azolla*).

Stream sampling was timed to coincide with peak insecticide application periods, which varied by region depending on planting time. Soy can either be planted as an early season crop or a late season crop. In the Argentine Pampas, the early season crop was planted in October or November and harvested in February, while in Paraguay and southern Brazil it was planted in September or October and harvested in January. The late season crop was typically planted between December and February and harvested several months later. In the Argentine Pampas, peak insecticide applications for soy production usually occurred in late December to early February, while in Paraguay and southern Brazil they occurred in November and December.

2.2. Field water quality measurements

At each sampling site, pH, conductivity, dissolved oxygen, and temperature were measured with a Yellow Springs Instruments SI 556 multi-parameter probe (Yellow Springs, OH, USA). Turbidity was measured with a portable turbidity meter (Hanna Instruments 93,414, Woonsocket, RI, USA), and maximum and average water velocities

Table 1
Sediment concentrations of the most heavily used insecticides, by sampling event. Mean and standard deviation values were calculated by assigning a value of half the QL for non-detect results and for detections below the QL.

Region and date			La Plata			Arrecifes					Paraguay		Brazil
			Dec 2011	Mar 2012	Apr 2012	Jan 2012	Mar 2012	Apr 2012	Feb 2013	Feb 2014	Jan 2013[a]	Dec 2013	Nov 2013
Method			GC-ECD	GC-ECD	GC-ECD	GC-ECD	GC-ECD	GC-ECD	GC/MS	GC/MS	GC-ECD and GC/MS	GC/MS	GC/MS
Quantitation limit (ng/g dw)			0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.25	0.5 (GC-ECD) 0.25 (GC/MS)	0.25	0.25
Number of samples[b]			7	7[c]	4	6	7	5	12[d]	10[e]	8[f] (GC-ECD) 8[b] (GC/MS)	14[g]	18[h]
Chlorpyrifos	k_{oc}[i]	% samples > 0.5 ng/g dw	29%	57%	100%	86%	100%	20%	100%	75%	56%	77%	83%
	995–31,000	Maximum (ng/g dw)	4.88	7.41	1.42	2.67	3.56	2.02	2.50	2.61	1.26	1.24	1.47
		Mean ± sd (ng/g dw)	1.21 ± 1.79	2.67 ± 2.89	0.92 ± 0.34	1.35 ± 0.83	1.94 ± 0.98	0.69 ± 0.88	1.26 ± 0.54	0.87 ± 0.71	0.50 ± 0.27	0.68 ± 0.26	0.72 ± 0.32
Endosulfan	350–19,953	% samples > 0.5 ng/g dw	29%	14%	0%	57%	43%	60%	8%	25%	13%	0%	0%
		Maximum (ng/g dw)	31.88	4.05	–	1.37	2.12	1.42	1.05	4.42	0.85	0.25	0.49
		Mean ± sd (ng/g dw)	7.71 ± 13.13	0.79 ± 1.44	–	0.69 ± 0.44	0.85 ± 0.84	0.66 ± 0.55	0.19 ± 0.27	0.33 ± 0.37	0.26 ± 0.19	0.13 ± 0.04	0.14 ± 0.09
Endosulfan Sulfate	320,000	% samples > 0.5 ng/g dw	29%	14%	0%	29%	57%	40%	58%	33%	6%	8%	0%
		Maximum (ng/g dw)	155.50	37.64	–	4.98	6.19	1.67	12.03	2.19	0.58	0.52	0.47
		Mean ± sd (ng/g dw)	33.88 ± 61.38	5.59 ± 14.13	–	1.06 ± 1.76	1.48 ± 2.14	0.88 ± 0.74	1.53 ± 0.57	0.60 ± 0.63	0.22 ± 0.12	0.22 ± 0.15	0.21 ± 0.12
Cypermethrin	20,800–503,000	% samples >0.5 ng/g dw	29%	0%	0%	29%	29%	40%	33%	8%	31%	8%	44%
		Maximum (ng/g dw)	1.94	–	–	8.32	4.16	2.68	1.85	0.89	1.18	1.22	4.94
		Mean ± sd (ng/g dw)	0.67 ± 0.72	–	–	1.61 ± 3.01	1.23 ± 1.70	0.86 ± 1.22	0.64 ± 0.64	0.21 ± 0.23	0.45 ± 0.32	0.24 ± 0.22	0.88 ± 1.20
Lambda cyhalothrin	>80,000–182,000	% samples >0.5 ng/g dw		0%	0%		29%	40%	17%	0%	6%	8%	39%
		Maximum (ng/g dw)		–	–		6.09	5.05	0.63	0.42	16.57	1.22	1.32
		Mean ± sd (ng/g dw)		–	–		1.12 ± 2.19	1.45 ± 2.40	0.28 ± 0.20	0.23 ± 0.11	1.22 ± 4.10	0.13 ± 0.26	0.50 ± 0.30

[a] Two different analytical methods were used for this sampling event, and statistics are based on all 16 samples.
[b] Statistics include all samples including those with low surrogate recovery, low MS/MSD recovery, or high RPD.
[c] 2 samples had surrogate recovery < 50%.
[d] MS sample had <50% recovery for endosulfan.
[e] MS samples had <50% recovery and/or RPD was >25% for endosulfan and chlorpyrifos.
[f] 4 samples had surrogate recovery < 50%, and MS samples had <50% recovery for endosulfan and chlorpyrifos.
[g] 10 samples had surrogate recovery < 50%, and MS samples had <50% recovery for endosulfan and chlorpyrifos.
[h] RPD was >25% for chlorpyrifos and cypermethrin.
[i] Range of k_{oc} values reported at http://toxnet.nlm.nih.gov/.

Table 2
Maximum sediment concentrations and detection frequencies of additional compounds analyzed in 2013 and 2014 (GC/MS, quantitation limit 0.25 ng/g dw).

Number of samples	k_{oc}[a]	Arrecifes		Paraguay		Brazil
		Feb 2013	Feb 2014	Jan 2013	Dec 2013	Nov 2013
		12[b]	10[c]	8[b]	14[d]	18[e]
PBO	399–830	3.31 (92%)	2.91 (33%)	1.87 (88%)	1.23 (8%)	11.14 (94%)
Bifenthrin	131,000–302,000	nd	2.96 (17%)	0.37 (38%)	0.63 (31%)	1.44 (44%)
Permethrin	10,471–86,000	0.47 (8%)	0.47 (15%)	2.56 (13%)	nd	2.07 (33%)
Cyfluthrin	3700 to 33,913	<0.25 (8%)	nd	<0.25 (13%)	0.40 (38%)	<0.25 (11%)
DDD	130,600–131,800	nd	7.26 (25%)	nd	nd	3.97 (33%)
DDE	26,300–75,860	nd	nd	nd	1.88 (15%)	5.67 (100%)
DDT	113,000–350,000	nd	0.29 (8%)	nd	0.49 (23%)	1.06 (100%)
Esfenvalerate	5248	<0.25 (8%)	nd	<0.25 (38%)	nd	0.29 (22%)
Endrin Ketone	11,420	nd	nd	<0.25 (13%)	nd	0.34 (6%)
Alpha Chlordane	20,000–76,000	nd	0.33 (8%)	nd	<0.25 (8%)	nd
Deltamethrin	79,000–16,300,000	<0.25 (8%)	nd	<0.25 (13%)	nd	0.87 (6%)
Aldrin	400–28,000	nd	nd	nd	nd	0.42 (11%)
Heptachlor Epoxide	7800	nd	<0.25 (8%)	nd	nd	nd
Gamma Chlordane	20,000–76,000	nd	0.32 (8%)	nd	nd	nd
Endrin	11,420	nd	nd	nd	<0.25 (8%)	nd

[a] Range of k_{oc} values reported at http://toxnet.nlm.nih.gov/.
[b] MS/MSD samples had <50% recovery and/or RPD was >25% for the following pesticides: lindane, endrin, dieldrin, heptachlor, aldrin, chlordane, DDD, DDE, DDT.
[c] MS samples had <50% recovery and/or RPD was >25% for the following pesticides: lindane, endrin, heptachlor, aldrin, chlordane, tefluthrin, DDD, DDE, and DDT.
[d] 10 samples had surrogate recovery < 50%, and MS samples had <50% recovery and/or RPD was >25% for the following pesticides: lindane, heptachlor, aldrin, chlordane, tefluthrin, and deltamethrin.
[e] RPD was >25% for cyfluthrin and deltamethrin.

were measured with a current meter (Global Water FP311, College Station, TX, USA).

2.3. Sample collection

Based on the properties of the insecticides analyzed, streambed sediments rather than water samples were examined. Most insecticides commonly used in soy production in South America have low water solubility, and a high affinity to bind to soil and sediments based on chemical properties, such as k_{oc} (Tables 1 and 2). Moreover, pesticide concentrations in stream water often occur as ephemeral events, and peak immediately following the first rain after application (Schäfer et al., 2011). However, elevated concentrations of the target insecticides can persist longer when they are associated with sediments (Jergentz et al., 2005). In all of the regions studied, precipitation occurs often during the peak pesticide application period. Sampling events in the present study were generally timed to occur within a week after a heavy rainfall during the peak insecticide application season.

Sediment samples were collected with a stainless steel scoop from the top two centimeters, generally from depositional areas depending on depth, access, and availability of sediment. Composite samples were prepared from 3 to 5 locations at each site and placed in pesticide-free amber glass jars with Teflon lids, which were kept in coolers on ice until arrival at the laboratory where they were kept refrigerated until extraction (maximum of 5 d), or frozen for later extraction (maximum of 4 mo). After thoroughly homogenizing each sample in the laboratory, an aliquot was taken from each sample for analysis of total organic carbon by ferrous sulfate titration (USDA, 1996). A separate sample was collected at each location for sediment grain size analysis (Table S2).

2.4. Chemicals

All pesticide standards, internal standards (lindane d6 and chlorpyrifos d10), and the surrogate standard decachlorobiphenyl (DCBP) were purchased from Accustandard and had purities > 93% as reported by Accustandard (New Haven, CT, USA). The solvents used in extractions and analysis were all pesticide grade. Granular copper used in sample extractions was purified by covering with methylene chloride, shaken vigorously, and allowed to dry in the hood for 24 h. During the first 18 months of the project, gas chromatography coupled with electron capture detection (GC-ECD) was used to analyze the insecticides reported to be most frequently used in Argentina on soy crops including cypermethrin, chlorpyrifos, lambda-cyhalothrin, and endosulfan (Table 1).

Throughout the project, information on pesticide use was obtained by interviewing personnel from government agencies, universities, pesticide manufacturers, and grower cooperatives in all three countries studied, and by searching documents from all sources including gray literature. In 2013 and 2014, analysis of organochlorine pesticides was added, because of concerns about their potential illegal application (Table 2). For quantification of the larger analyte list, the more advanced method of a GC coupled with a mass spectrometer (GC–MS) was used. Analysis of additional pyrethroids and the synergist piperonyl butoxide (PBO) was also added when the new method was implemented (Table 2). Although PBO is not present in insecticide formulations sold for use in soy production, it is possible that growers are mixing it with pyrethroid pesticides to increase their efficacy, or it may come from other sources such as tick control in farm animal production.

2.5. Extraction procedure

Extraction procedures followed You et al. (2004b), who demonstrated that sonication provided good recovery for the pesticides of interest (You et al., 2004b; You and Lydy, 2007; You et al., 2008). After each sample was thoroughly homogenized manually, approximately 20 g of sediment (wet weight) was removed, spiked with 100 ng of the surrogate DCBP, and mixed with 4 g of copper and anhydrous Na_2SO_4 in an ice-cooled beaker until the sediment was sufficiently dry. A 50-ml aliquot of a 50:50 mixture of acetone and methylene chloride was added, and the mixture was sonicated for 5 min in 3-s pulse mode using a high-intensity ultrasonic processor at an amplitude of 60 (model VCX 500; Sonics and Materials, Newtown, CT, USA). The extract was decanted and filtered through a Whatman no. 41 filter paper (Whatman, Maidstone, UK) filled with approximately 2 g of anhydrous Na_2SO_4. This procedure was repeated two additional times with a sonication time of 5 min each time. Extracts were combined and decreased to approximately 1–2 ml by evaporation.

2.6. Cleanup of extracts

Prior to cleanup, extracts for the methylene chloride and acetone: methylene chloride mixture were solvent-exchanged to hexane, and the volumes of all treatments were reduced to 0.5 to 1 ml under nitrogen gas. A Envi-Carb II/primary–secondary amine solid phase extraction (SPE) cartridge was connected to a vacuum manifold, adding 1 g of purified sodium sulfate to the top of the sorbent to remove any residual water, then primed with 3 ml of hexane. The extract was then loaded onto the cartridge. Next, 7 ml of a 30:70 methylene chloride/hexane mixture was added to the cartridge, the extract was removed from the vacuum manifold and reduced to a volume of 0.5 to 1 ml under nitrogen gas. The collection vial was then rinsed three times with 0.5 ml of a 0.1% acetic acid in hexane solution and added to the GC vial. The volume was further reduced to 1 ml for analysis. The acidification step was used to minimize isomerization of the pyrethroids (You and Lydy, 2007). Granular copper was added to extracts and placed on a shaker (Lab Rotator model G-2, New Brunswick Scientific Co., NJ, USA) for 2 to 3 h when high residual sulfur was detected in the extracts. Once at final volume, internal standards were added at a concentration of 20 ng/ml (for GC/MS analysis only) and the samples were stored at −20 °C until analysis.

2.7. Analytical methods

2.7.1. Gas chromatograph-electron capture detector

During the 2011 to early 2013 sampling period, analysis of the most commonly used insecticides (Table 1) was performed on an Agilent 6890 series GC equipped with an Agilent 7683 autosampler and a micro- ECD (Agilent Technologies, Palo Alto, CA, USA). Two columns — a HP-5MS (30 m × 0.25 mm × 0.25 μm film thickness; Agilent) and a DB-608 (30 m × 0.25 mm × 0.25 μm film thickness; Agilent) were used to confirm the analytical results. Helium and nitrogen were used as the carrier and makeup gas, respectively. A 2 μl sample was injected into the GC using a pulsed split-less mode. For the DB-608, the oven was set at 100 °C, heated first to 250 °C at 10 °C/min increments, then to 280 °C at 3 °C/min increments and finally held at 280 °C for 23 min. For the HP-5, the oven was set at 100 °C, heated to 190 °C at 5 °C/min increments, then to 214 °C at 6 °C/min increments, then to 280 °C at 6 °C/min increments and finally held at 280 °C for 20 min. The flow rates of carrier gas were 1.7 ml/min and 2.0 ml/min for the HP-5MS and DB-608 columns, respectively. Calibration was based on area using three to six external standards. The standard solutions were made by dissolving 2.5,10, 50, 100, or 250 μg/l of each pesticide and surrogate in hexane. The calibration curves generated were linear within this concentration range. Qualitative identity was established using a retention window of 1% with confirmation on a second column, and quantitation was performed using external standard calibration.

2.7.2. Gas chromatography–mass spectrometry

For the 2013 to 2014 sampling period, a longer analyte list was used, and quantification of the samples was completed on an Agilent 6850 gas chromatograph with a 5975 XL mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). Piperonyl butoxide was quantified in electron impact (EI) mode, while all of the other target pesticides were quantified in negative chemical ionization (NCI) mode. The analytes were separated for both EI and NCI modes on a HP-5MS column (30 m × 0.25 mm, 0.25 μm film thickness, Agilent Technologies) initially set at 50 °C, and heated to 295 °C at 10 °C/min. Inlet, ion source, and quadrupole temperatures were 260, 230, and 150 °C, respectively. A 2.0 μl sample was injected in pulsed splitless mode at 7.59 psi. Helium was the carrier gas and column flow was 1.0 ml/min. Identification of the target pesticides was based on detecting the target and qualifier ions (Table S3) within a retention time window of 1%, and the target pesticides were detected in selected ion monitoring (SIM) mode. Quantification was performed using internal standard calibration.

2.8. Quality assurance–quality control

A matrix spike (MS), matrix spike duplicate (MSD), and laboratory blank were extracted for at least 5% of the samples. A surrogate (DCBP) was added to each sample prior to extraction to verify the performance of the extraction and cleanup processes. Calibration curves were constructed using six levels for each pesticide and surrogate, while the internal standards (for the GC–MS analyses) were kept constant for all levels at a concentration of 20 ng/ml. Quantitation limits (QL) were based on the lowest calibration standard. Each QL was at least three times the method detection limits calculated measuring a low level spike in clean sediment. The QLs are reported instead of the method detection limits to ensure that low sample concentrations are quantitatively accurate. Sample results were considered to meet quality control criteria if the surrogate recovery was between 50–150%, MS recovery for each analyte was between 50–150%, no pesticides were detected above QLs in the laboratory blank, and the relative percent differences in MS/MSDs did not exceed 25%. Exceptions to the quality control criteria were identified for each sample (Tables 1 and 2).

2.9. Toxic unit calculation

Toxic units (TUs) were calculated for all sediment samples. A TU was equal to the sediment concentration normalized to total organic carbon (TOC), divided by the organism 10-d median level lethal concentration (LC50) for each pesticide. The LC50 values for freshwater aquatic invertebrates were identified from the literature for sensitive species (Table 3). Most of the LC50 values used in the present study were for the amphipod *Hyalella azteca*, which is known to be very sensitive to pyrethroids and chlorpyrifos (Weston and Lydy, 2010). Although *H. azteca* does not occur in South America, several closely related species (*H. curvispina*, *H. pampeana*, and *H. pseudoazteca*) are important components of the aquatic invertebrate communities in the region; however, published sediment LC50 values are not available for native species. For endosulfan, the LC50 for the more sensitive *Chironomus tentans* was used to calculate TUs, because it is substantially lower than the LC50 for *H. azteca* (You et al., 2004a). Toxicity of pesticides in sediment is highly dependent on organic carbon content; therefore, the concentrations were normalized for total organic carbon to calculate TU values.

2.10. Statistical analysis

To evaluate the relationship between buffer width and pesticide concentrations after accounting for other landscape and habitat predictor variables, a linear multiple regression analysis was conducted for the Brazil data set, which had the largest number of sampling sites (18). Insufficient data were available to conduct a similar analysis for Argentina, as minimum buffer widths could not be verified with LANDSAT data and the sample size was small (12 sites). The Paraguay data set did not have sufficient variation in buffer widths to run a regression analysis because 8 of the 17 sites had a minimum buffer width of 100 m (the minimum required by law). The following predictor variables were considered based on their potential to affect pesticide concentrations in stream sediments: minimum upstream buffer width; percent fines (clay and silt fraction) in sediment; percent organic carbon in sediment; stream gradient (slope measured upstream of the sampling site); and, catchment size. Collinearity of these variables was evaluated by examining pairwise plots, correlation matrices, and variance inflation factors, and variables with the highest multi-collinearity were eliminated. For the linear regression model (lm function in R), predictor variables were square root transformed and the outcome variable (total insecticide TU) was log transformed. A stepwise process was then performed to select final model variables by comparing the Akaike information criterion (AIC) values, using the R function "step". The lmg metric in the relaimpo (Relative Importance for Linear Regression) package was used to evaluate the relative contribution, or variance explained by each predictor

Table 3
Maximum and mean toxic units (TUs) for each sampling event, for pesticides that had at least one TU value > 0.01. TUs were calculated as the ratio of the carbon-normalized concentration in sediment over the carbon-normalized LC50.

Pesticide	LC50 (ng/g organic carbon)	Statistic	La Plata (Argentina)			Arrecifes (Argentina)					Paraguay		Brazil
			Dec 2011	Mar 2012	Apr 2012	Jan 2012	Mar 2012	Apr 2012	Feb 2013	Feb 2014	Jan 2013	Dec 2013	Nov 2013
Chlorpyrifos	4160[a]	Maximum	0.01	0.02	0.01	0.06	0.16	0.04	0.09	0.08	0.15	0.05	0.02
		Mean	0.00	0.01	0.01	0.03	0.06	0.02	0.03	0.03	0.04	0.02	0.01
Endosulfan	960[b]	Maximum	0.32	0.04	nd	0.14	0.18	0.37	0.01	0.09	0.01	0.04	0.02
		Mean	0.08	0.01	nd	0.07	0.08	0.11	0.00	0.03	0.00	0.02	0.00
Endosulfan Sulfate	5220[b]	Maximum	0.28	0.07	nd	0.08	0.10	0.08	0.12	0.03	0.05	0.01	0.01
		Mean	0.06	0.01	nd	0.02	0.03	0.02	0.02	0.01	0.03	0.01	0.00
Cypermethrin	380[a]	Maximum	0.05	nd	nd	1.15	0.97	0.58	0.38	0.13	0.19	0.27	0.83
		Mean	0.02	nd	nd	0.28	0.27	0.20	0.16	0.03	0.06	0.10	0.11
Lambda-cyhalothrin	450[a]	Maximum		0.02	nd		0.71	0.93	0.23	0.16	1.77	0.61	0.16
		Mean		0.01	nd		0.17	0.26	0.04	0.07	0.12	0.11	0.05
Bifenthrin	520[a]	Maximum							nd	0.36	0.00	0.14	0.13
		Mean							nd	0.04	0.00	0.05	0.03
Permethrin	10830[a]	Maximum							0.00	0.00	0.02	0.01	0.01
		Mean							0.00	0.00	0.00	0.00	0.00
Cyfluthrin	1080[a]	Maximum							<QL	nd	<QL	0.05	<QL
		Mean							<QL	nd	<QL	0.02	<QL
Deltamethrin	790[a]	Maximum							nd	0.00	<QL	nd	0.06
		Mean								0.00	<QL	nd	0.00
Esfenvalerate	1540[a]	Maximum							<QL	nd	<QL	nd	0.01
		Mean							<QL	nd	<QL	nd	0.00
Total pyrethroid TU[c,e]		Maximum	0.05	0.05	0.05	1.15	1.16	1.51	0.45	0.41	1.85	0.77	1.03
		Mean	0.02	0.02	0.03	0.28	0.44	0.46		0.13	0.19	0.28	0.20
Total insecticide TU[d,e]		Maximum	0.66	0.14	0.07	1.23	1.36	1.64	0.96	0.54	1.89	0.84	1.07
		Mean	0.16	0.05	0.05	0.40	0.61	0.60		0.20	0.26	0.34	0.21

[a] LC50 for *Hyalella azteca* from Weston et al. (2013).
[b] LC50 for *Chironomus tentans* from You et al. (2004a).
[c] Total pyrethroid TU values for each sample were calculated by summing the TU values for each pyrethroid.
[d] Total insecticide TU values for each sample were calculated by summing the TU values for each insecticide.
[e] A concentration value of half the QL was assigned for pesticides not detected, or detected < QL.

variable (Grömping, 2006). All statistical analysis was performed with R 3.2.0 (R Development Core Team, 2015).

3. Results and discussion

3.1. Distribution and seasonality of insecticides

3.1.1 Insecticide concentrations and detection frequencies

The most commonly detected insecticides in the three intensive soy production regions were those reported to be the most heavily used: chlorpyrifos, endosulfan (and its degradation product endosulfan sulfate), cypermethrin, and lambda-cyhalothrin (Table 1). Other pyrethroid and organochlorine insecticides were detected occasionally (Table 2). Chlorpyrifos had the highest detection frequency in all regions examined, and for almost all sampling events (57 to 100% detection frequency, with 29 to 100% above the highest QL of 0.5 ng/g dw). Maximum concentrations ranged from 1.24 to 7.41 ng/g dw, with the highest concentration measured in the La Plata region, which included a mix of agricultural crops and grazing lands. Chlorpyrifos, which is used for a wide variety of crops in Argentina (OPDS, 2013) was the only insecticide that was consistently detected in this region; however, this region was studied for only the first season (Dec 2011–April 2012) and only the four insecticides most commonly used in soy production were measured (Table 1).

Endosulfan and its degradate endosulfan sulfate were frequently detected in all three intensive soy production regions (43 to 100% detection frequency, with 0 to 100% above the highest QL of 0.5 ng/g dw), but less frequently in the mixed use La Plata region (0–29%). While the highest concentrations of endosulfan (31.88 ng/g dw), endosulfan sulfate (155.5 ng/g dw) were detected in the La Plata region, it was likely that upstream vegetable greenhouse production contributed to the elevated levels of these compounds, as they were found in spring at the start of the soy planting season. At the time of sampling, endosulfan was commonly applied on many crops in Argentina (OPDS, 2013). Maximum endosulfan concentrations in the three intensive soy regions ranged from 0.25 to 4.42 ng/g dw. Although endosulfan was widely used in soy production in all three countries at the start of the present study, it has since been prohibited (UNEP, 2013). Although the detection frequencies of endosulfan increased in the latter half of sampling rounds, this was most likely because the analytical method changed from GC-ECD to GC/MS-NCI. When we examined frequency of detection above the higher QL of 0.5 ng/g dw, across all sampling events using either method, the frequency of detections above this threshold decreased in later sampling events (Table 1).

Seven pyrethroids were detected in all three intensive soy production regions, with cypermethrin and lambda-cyhalothrin consistently being the most frequently detected insecticides (Tables 1 and 2). Cypermethrin and lambda-cyhalothrin were detected at similar frequencies in the three intensive soy production regions, and at similar frequencies for each sampling event, ranging from 29 to 100% for both insecticides (0 to 44% above the highest QL of 0.5 ng/g dw). Although the detection frequencies of these two pyrethroids increased in the latter half of the sampling rounds, the frequency of detection above 0.5 ng/g dw remained similar across years. Maximum concentrations ranged from 0.89 to 8.32 ng/g dw for cypermethrin, and 0.42 to 16.57 ng/g dw for lambda-cyhalothrin. The pyrethroids bifenthrin, cyfluthrin, esfenvalerate, deltamethrin, and permethrin were occasionally detected at lower concentrations in all three intensive soy production regions (they were not measured in the La Plata region). Tefluthrin was the only pyrethroid analyzed that was not detected during the project. The pyrethroid synergist PBO was detected frequently in the three intensive soy production regions (8 to 92% of samples), with maximum concentrations from 1.23 to 11.14 ng/g dw.

Dichlorodiphenyltrichloroethane (DDT) was the only prohibited insecticide that was detected frequently. DDT and its degradates DDE and DDD were detected in all three intensive soy production regions, but most frequently in Brazil (100% detection frequency for DDT and DDE, with maximum concentrations of 1.06 and 2.53 ng/g dw, respectively).

In the Arrecifes region, the ratio of DDD to DDT was high (4 to 15.1) and DDE was not detected. DDD is most likely to occur under anaerobic conditions, which would be expected in the region because of the low gradient and little riparian cover (Table S2). Other prohibited organochlorinated insecticides that were detected rarely (and usually at or slightly below QLs) included endrin, chlordane, aldrin, and heptachlor epoxide. Banned organochlorinated insecticides that were analyzed, but not detected, included lindane, heptachlor, and dieldrin.

3.1.1. Seasonality and timing

A review of studies conducted within the Arrecifes region of Argentina showed that measured concentrations in sediments were highly dependent on the timing of sampling after pesticide applications. For example, the highest concentrations of endosulfan in the soy production regions in the Argentine Pampa were found by Di Marzio et al. (2010), who sampled within 24 h after aerial pesticide application (maximum concentration of 553 ng/g dw in sediment, compared to a maximum of 4.4 ng/g dw for sites in the same regions sampled during the present study). Marino and Ronco (2005) also studied streams in the Arrecifes watershed and reported higher concentrations of cypermethrin (maximum concentration of 1075 ng/g dw and a mean of 160 ng/g dw) than detected in other studies at the same sites during the same years. Jergentz et al. (2005) measured only 4.4 ng/g dw in suspended sediment collected at the same locations during the same month (Dec 2003), and did not detect cypermethrin in bed sediment samples collected twice the following month. Previous studies in the Arrecifes region by Jergentz et al. (2004a, 2004b) analyzed cypermethrin, chlorpyrifos, and endosulfan in suspended sediment, and only chlorpyrifos and endosulfan were detected in streams samples, although all three pesticides were detected in field runoff samples. Although the present study targeted sampling during peak insecticide application periods, the sampling events may not have captured the highest concentrations occurring immediately after insecticide application and rainfall.

Several other studies in Argentina detected insecticides in water bodies even though they did not sample during the peak soy production season (Bonansea et al., 2013; Agostini et al., 2013; De Geronimo et al., 2014). Regardless, insecticides were detected in all three studies, and Bonansea et al. (2013) found a maximum concentration of cypermethrin of 112.4 ng/l in stream water, which is one of the highest reported detections reported during any season. Although all of these studies included soy production regions, other crops, such as wheat, were grown in soy regions during other seasons, so insecticides may have been applied to control pests in multiple crops.

3.1.2. Comparison to previous studies

The types of insecticides most frequently detected in the present study were generally similar to those detected in most previous studies in the region. In Argentina, most studies on soy production insecticides focused on the Arrecifes region, where they have detected endosulfan (Di Marzio et al., 2010; Jergentz et al., 2004a, 2004b), cypermethrin (Marino and Ronco, 2005; Jergentz et al., 2005), and chlorpyrifos (Jergentz et al., 2004a, 2004b). None of these studies analyzed lambda-cyhalothrin. In Brazil, studies have primarily focused on the Mato Grosso state and the Pantanal region, where endosulfan, chlorpyrifos, and lambda-cyhalothrin were detected (Possavatz et al., 2014; Casara et al., 2012; Miranda et al., 2008; Laabs et al., 2002).

Although the neonicotinoid insecticides were not analyzed as part of the present study because there was little evidence of their use at the start of field work, it is likely that their use in the soy production in South America has increased in recent years, and will continue to increase. In South America, neonicotinoids are often applied in combination with pyrethroids for control of hemipteran pests in soy. In Argentina, there are at least 57 neonicotinoid/pyrethroid mixture formulations registered for this purpose, although not all of them are currently in commercial use (Servicio Nacional de Sanidad y Calidad Agroalimentaria, personal communication, Dec 2013). Recent studies in soy production regions of South America detected imidacloprid in 43% of surface water samples (Argentina; De Geronimo et al., 2014) and thiamethoxam in 100% of surface water samples (Brazil; Rocha et al., 2015).

Pesticide concentrations in soy production areas of South America appear to be similar to soy production areas in the United States, although other pyrethroids were detected more frequently than cypermethrin in the US. A study conducted in 2009 analyzed 14 pyrethroids in sediment samples collected from 13 streams in agricultural areas (primarily soy production) and 23 streams in urban areas throughout the US (Hladick and Kuivila, 2012). Although cypermethrin was not detected in the agricultural streams, and lambda-cyhalothrin was detected at only one site, other pyrethroids (primarily bifenthrin) were detected in 10 of the 13 samples. Pyrethroid concentrations ranged from 0.3 to 180 ng/g dw, and total pyrethroid TUs for *H. azteca* ranged from 0.01 to 2.81. Another study analyzed nine pyrethroids, chlorpyrifos, and 19 organochlorine insecticides in 20 urban streams sites and 49 agricultural (primarily soy and corn) stream sites in Illinois (Ding et al., 2010). Cypermethrin was detected at only two of the agricultural sites (maximum 28 ng/g dw), but other pyrethroids (especially permethrin) were detected more often. Chlorpyrifos was detected in three samples (maximum 35 ng/g dw), while organochlorine pesticides were detected, but only at very low concentrations, and were unlikely to cause acute toxicity. In both studies, pyrethroids were detected more often in urban streams than in agricultural streams, corresponding with previous data from California (Weston and Lydy, 2010).

Previous studies have detected DDT and its degradation products in Brazilian rivers and streams, but at lower concentrations and detection frequencies than those found in the present study. Use of DDT in agriculture has been prohibited in Brazil since 1985, but use for vector control was reported until 1997 (Dores, 2015). In sampling conducted in rivers and streams of the northeastern Pantanal in 1999–2000, Laabs et al. (2002) found DDT and DDE in 79% and 36% of sediment samples, with maximum concentrations of 1.5 and 1.4 ng/g dw, respectively. Lower concentrations (up to 0.6 ng/kg dw) of DDT and DDE were found in a study conducted earlier in sediments of rivers in Parana state (Matsushita et al., 1996). More recent studies have detected DDT only sporadically and DDE occasionally in sediment and water of the Pantanal (Dores, 2015).

3.2. Aquatic toxicity

3.2.1. Toxic units

Although pyrethroid concentrations were similar to other frequently detected insecticides, the TU values for these insecticides were higher because of their higher acute toxicity (Table 3). Lambda-cyhalothrin was the insecticide with the highest TU value (1.77 in Paraguay in January 2013), and TU values above 0.5 were found in four of seven sampling events in the three intensive soy production regions. Maximum cypermethrin TU values were consistently above 0.5 in the Arrecifes region during the three 2012 sampling events, as well as in the 2014 sampling event in Brazil. Bifenthrin had a maximum TU value of 0.36 (Arrecifes Feb 2014), and all other detected pyrethroids had maximum TU values less than 0.1. Endosulfan TU values were always below 0.4, but were generally higher than those of chlorpyrifos. Chlorpyrifos had the highest detection frequency in all regions and during all sampling periods, but always at low concentrations, with a maximum TU value of 0.16 (Arrecifes in March 2012). All TU values for DDT and its degradation products were less than 0.005. In the three intensive soy production regions, pyrethroid TU values contributed more than other insecticides to the total insecticide TU values, while in the mixed use region of La Plata, endosulfan and chlorpyrifos contributed more. The maximum pyrethroid TU for all regions was 1.85 (Paraguay, January 2013), and maximum pyrethroid TU values for each sampling event exceeded 0.5 for all sampling events in the three intensive soy production regions. The

maximum total insecticide TU values ranged from 0.54 to 1.89 in the intensive soy production regions, and from 0.07 to 0.66 in the mixed use La Plata region. In the intensive soy production regions, the maximum pyrethroid TU value contributed 46 to 98% of the maximum total insecticide TUs, while in the La Plata region, it contributed 7 to 71% of the total TUs.

Although maximum total TU values for each sampling event often exceeded one, the mean total TU values for each sampling event were always below 1, and for all regions except for Arrecifes they were always below 0.5. No sampling event had more than two samples with TU values that exceeded one.

3.2.2. Effects of synergists and insecticide mixtures

Of the insecticides found in the present study, the pyrethroids posed the highest potential for acute toxicity to aquatic invertebrates, and toxicity caused by pyrethroids may be exacerbated by the co-occurrence of PBO in streams. The LC50s used to calculate the TU values for most insecticides in the present study were based on toxicity to *H. azteca* (Table 3). Generally, *H. azteca* mortality has been found to increase when the TU of total pyrethroids reaches 0.5, and approaches 100% mortality at a TU of about 10 (Weston and Lydy, 2010). Because PBO inhibits mixed-function oxidase enzymes, it acts as a synergist for pyrethroids, which are detoxified by this pathway. However, PBO can reduce toxicity of organophosphates such as chlorpyrifos, which require activation by mixed-function oxidase enzymes. PBO is often applied with pyrethrins and pyrethroids in mosquito control applications to increase their efficacy, but PBO itself has low toxicity to aquatic organisms (Amweg et al., 2006). Weston et al. (2006) found that PBO applied for mosquito control resulted in water concentrations that were high enough to increase the toxicity of pyrethroids already present in stream sediments. For example, PBO concentrations of 2–4 μg/l nearly doubled the toxicity of sediments to *H. azteca*. Amweg et al. (2006) found that a PBO sediment concentration of 12.5 ng/g and 2.3 μg/l in water almost doubled the toxicity of permethrin to *H. azteca*; however, they did not test the effect of PBO added to sediment only. The PBO concentrations detected in the present study were likely to increase the toxicity of pyrethroids in the sediment to some extent, but with existing information it was not possible to quantify the increase because of the lack of dose response data for PBO synergism with pyrethroids in sediment.

Almost all samples in the three intensive soy production regions contained multiple insecticides from at least two different insecticide classes (Tables 2 and 3), leading to uncertainty in the estimation of toxic effects. While combined effects of insecticides in the same class can be predicted relatively well, combined effects of mixtures of multiple classes are more difficult to predict (Lydy et al., 2004). At the concentrations measured in the present study, it is unlikely that either endosulfan or chlorpyrifos alone would cause significant acute toxicity to most aquatic organisms, but they could contribute to acute toxicity when occurring with other pesticides. While pesticides of similar classes and same mode of action are generally assumed to act via concentration addition, pesticides with different modes of action may act via independent action, antagonistically (less than additive toxicity), or synergistically (more than additive toxicity) (Trimble et al., 2009). In the streams examined in the present study, pyrethroids were likely to contribute more than other insecticides to acute toxicity in aquatic invertebrates, and the concentration addition model (sum of TUs) is reasonably predictive of pyrethroid mixture toxicity (Trimble et al., 2009).

There is mixed evidence on synergy and antagonism among the three classes of insecticides frequently detected together in the present study (pyrethroids, organophosphate pesticides, such as chlorpyrifos, and cyclodiene pesticides, such as endosulfan) (Ahmad, 2009; Belden and Lydy, 2006). Based on available data, the actual toxicity caused by multiple insecticides is not likely to exceed twice the toxicity predicted by the summed TU values (Deneer, 2000).

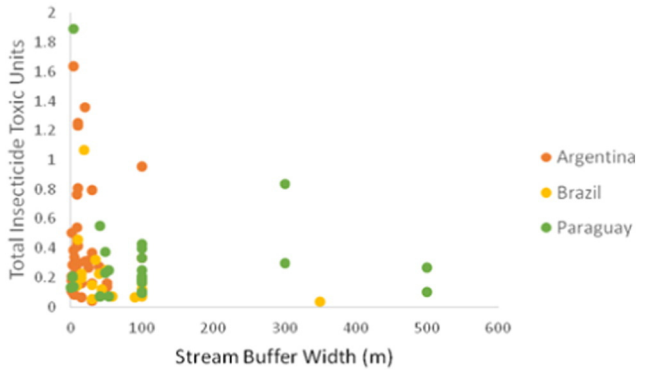

Fig. 2. Relationship between riparian buffer width and total insecticide toxic units for all sites in the three intensive soy production regions studied in Brazil, Paraguay, and Argentina.

3.2.3. Chronic and community level effects

Given that multiple insecticides have been consistently found in stream sediments in the present study and others in the region, it is likely that long-term chronic toxicity to aquatic organisms is occurring in the region. Both acute and chronic effects may result in changes in the invertebrate communities, notably reduction in abundances of the most sensitive taxa and increases in the most tolerant taxa. Van Wijngaarden et al. (2005) reviewed mesocosm and microcosm studies on pesticides and found that for pyrethroids, limited short-term effects tended to occur in the range of 0.01–0.1 TU, while clear and prolonged effects tended to occur in the range of 0.1–1 TU. Schäfer et al. (2012) found effects to relative abundances of sensitive macroinvertebrate taxa at pesticide concentrations lower than 1/1000 of the median effect concentration (EC50) for *Daphnia magna*. Thus, at the range of pyrethroid TU values found in soy production regions in the present study (sampling event means of 0.13 to 0.46, maximums of 0.41 to 1.85) it is likely that there would be widespread chronic and persistent effects on the aquatic invertebrate communities.

3.3. Riparian buffer widths

The highest insecticide concentrations in sediments in all intensive soy production regions occurred when buffer zone widths were 20 m or less. Total insecticide TU values were compared with minimum buffer width measured immediately upstream of each site studied in the three intensive soy production regions (Fig. 2). All samples with total insecticide TU values greater than 1 were collected from sites with minimum buffer widths of 20 m or less.

A stepwise multiple regression for the Brazil data set indicated that buffer width was the predictor variable that had the greatest influence on total insecticide TU. Although variance inflation factors for all predictors variables were low, the correlation matrix showed percent sediment fines to be moderately correlated with three other predictors (correlation 0.45–0.57), and also had the highest variance inflation factor (3.6); therefore, percent sediment fines was dropped from the analysis. As a result of the AIC stepwise regression, catchment size was also eliminated as its contribution was not important in explaining variance in the TU values. The selected model included the following predictor variables: buffer width, percent total organic carbon, and stream gradient ($r^2 = 0.54$; p-value = 0.009). The analysis of relative contribution indicated that buffer width contributed 74% of the explained variance, with percent total organic carbon and stream gradient contributing 9 and 17%, respectively.

The results of the present study corroborate findings from other studies that have found riparian buffer zones to be important in mitigating transport of pesticides to streams. The present study's finding of the highest TU values in streams with buffer widths less than 20 m was

within the range of buffer widths (5 m to 20 m) reported to mitigate pesticide effects on streams (Rasmussen et al., 2011; Di Marzio et al., 2010; Bunzel et al., 2014; Reichenberger et al., 2007). Many factors could affect the buffer width necessary to protect streams from pesticide exposure, including gradient, type of vegetation, soil properties, types of pesticides applied, timing and amount of pesticides applied, and presence of tile drains or drainage ditches that short-circuit the buffer zones (Reichenberger et al., 2007; Bunzel et al., 2014).

Although regulation of pesticide mitigation measures often focuses on application practices, landscape level mitigation measures, such as requiring riparian buffer zones, may be easier to implement and enforce. Bereswill et al. (2014) reviewed the efficacy and practicality of risk mitigation measures for diffuse pesticide entry into aquatic ecosystems, and ranked riparian buffer strips as highly effective for mitigating both spray drift and runoff, with high acceptability and feasibility. However, the implementation and enforcement of new riparian buffer requirements in Brazil has been difficult and controversial, especially in regions with small-scale production where a significant amount of a landowner's productive farmland could be lost with compliance (Alvez et al., 2012).

4. Conclusions

The results of the present study demonstrated that: (1) there was consistency in the insecticides that were most commonly detected in sediment samples from streams in the intensive soy production regions studied in Argentina, Brazil and Paraguay; (2) these insecticides, especially the pyrethroids, persisted in stream sediments at concentrations likely to cause acute and chronic toxicity to aquatic invertebrates; and, (3) acutely toxic insecticide concentrations in bed sediments were most likely to occur in streams with buffer widths less than 20 m. Although frequency of detection differed somewhat between sampling events, the insecticides that were reported to be the most commonly used in soy production were also the ones that were found most frequently in all regions (e.g. chlorpyrifos, endosulfan, cypermethrin, and lambda-cyhalothrin). In addition, the pyrethroid synergist PBO was frequently detected in all three intensive soy production regions, although its use in soy production has not been reported in the literature. These results suggest that the following recommendations should be considered in soy production regions of South America: (1) evaluation and implementation of buffer zones and other management practices to limit transport of pesticides to streams.; (2) field studies focusing on effects to aquatic invertebrate communities; and, (3) continued monitoring that is adapted based on quickly changing pesticide use trends (e.g. increasing use of neonicotinoids).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.12.140.

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