



Aquatic macroinvertebrate assemblages are affected by insecticide applications on the Argentine Pampas



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ABSTRACT

Agriculture intensification in Argentina has increased agrochemicals consumption in the last decades and might represent an environmental risk for adjacent water bodies. The objective of the present work was to assess the effect of land use on water quality and invertebrate assemblages in the Argentine Pampas streams. Eight streams were sampled on 4 occasions during the 2013/14 growing season. Three streams are located within a biosphere reserve, two drain basins with extensive livestock fields, and three run through intensively cultivated plots; one of them contained a 30 m wide uncultivated grass-covered strip between the crop and the stream. Macroinvertebrates were sampled from emergent vegetation by means of a D-net with a 500 µm pore size, and 30 cm diameter.

Higher nutrient concentrations were measured in the agricultural streams. Endosulfan was measured in sediments of the agricultural streams, concentrations being significantly lower in the stream with the buffer strip. Invertebrate assemblages in the cropped streams were significantly different from those in the livestock and reserve streams, those in the latter not being different from each other. Ampullaridae (*Pomacea canaliculata*) and Planorbidae (*Biomphalaria peregina*) were the taxa best represented in the agricultural streams. Hyalellidae (*Hyalella curvispina*), Zygoptera and Planorbidae (*B. peregina*) were the taxa best represented in the reserve and livestock streams. Present evidence suggests that the observed differences in the invertebrate composition in the agricultural streams were related with the impact of agrochemicals and that buffer strips represent a useful attenuation practice. Cattle breeding on natural pastures represented a land use with low impact on the invertebrate assemblages.

1. Introduction

The Pampas plain covers 60 million hectares and represents the main agricultural area of Argentina, accounting for 60% of total crop production (CASAFE, 2009). Over the last decades crop production has increased due to progressive agricultural intensification. Traditionally, a mixed system of livestock raising and crop production, mainly wheat and corn, was the main land use. Genetically modified soy, resistant to glyphosate, was introduced on the Argentine market in 1996. Soy production steadily increased since then to become the main crop. The area sown with soy increased from 37,000 to 19 million hectares between 1970 and 2015 (MAGyP, 2016). Argentina became the world's third largest producer, after the US and Brazil (Aizen and Harder, 2009). Soy was accompanied by large scale husbandry changes. Most of the soy production is carried out under the so called "no-till" system which avoids disturbing the soil but increases herbicide consumption for the chemical fallow. Wheat and soy varieties with a short growing period allowed two harvests per year – wheat followed by soy. Overall,

pesticide consumption increased from 6 million kilograms in 1992 (Pengue, 2000) to 32 million kilograms in 2012 (CASAFE, 2013). The same trend was observed in fertilizer application, which increased at an annual rate of 18% from 1991 to 2007, reaching 3.2 million tons in 2013 (CIAFA, 2016).

Repeated agrochemicals applications represent an environmental risk to adjacent water bodies. Runoff represents an important non-point contamination source in agricultural watersheds (Liess, 1994). The exposure scenario of the resident fauna to agrochemicals is difficult to assess because of the ephemeral nature of peak concentrations attained in coincidence with runoff events occurring after field applications (Jergentz et al., 2004). Biological monitoring is an alternative approach to assess the environmental impact of agricultural practices. Changes in the invertebrate assemblage in response to agrochemical loads have repeatedly been reported (Leonard et al., 1999; Castillo et al., 2006; Schäfer et al., 2007; Liess et al., 2008). Moreover, shifts in the invertebrate community were used to assess agrochemical contamination (Johnson et al., 1993). Changes in species richness and abundance

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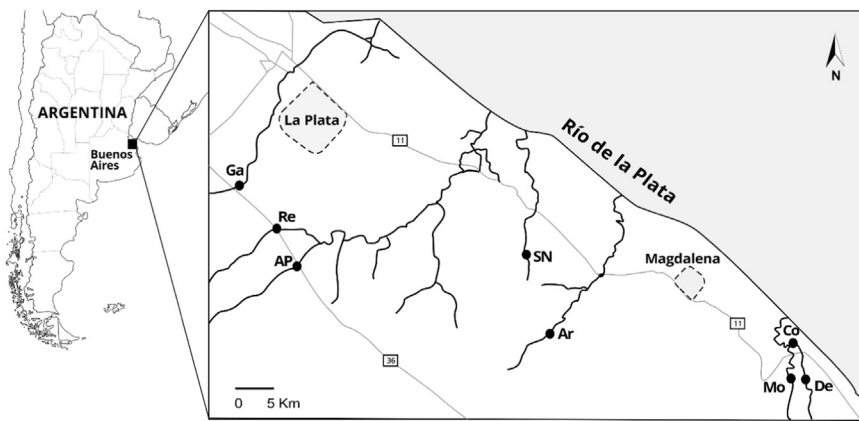


Fig. 1. Studied area and sampling sites.

represent a signal of impairment and might be used to register environmental degradation (Egler et al., 2012). The presence of insecticides in streams and sediments in the Pampas agricultural area has been reported (Jergentz et al., 2005; Marino and Ronco, 2005; Mugni et al., 2011; Aparicio et al., 2013; De Gerónimo et al., 2014; Lupi et al., 2015; Hunt et al., 2016). However, the effect of pesticide exposure on the resident fauna remains largely unreported. To our knowledge only Solis et al. (2016) described the composition of the invertebrate assemblages in streams with different land use in their watersheds, based on a limited number of studied streams. The objective of the present contribution is to further assess the effect of application of agrochemicals to crops on the invertebrate assemblages of adjacent streams on the Argentine Pampas plain.

2. Materials and methods

2.1. Study sites

Eight streams were studied in the Rio de la Plata coastal strip in Buenos Aires province, Argentina (Fig. 1). The streams run in a west-to-east direction towards the Río de la Plata. Stream depths ranged from 0.2 to 0.5 m and widths from 4 to 14 m. The climate is mild and humid with mean monthly temperatures range from 9.9 °C in July to 22.4 °C in January. Mean annual rainfall is 1060 mm with small seasonal variations (Hurtado et al., 2006).

The streams were sampled 4 times during the 2013–14 growing season in December 2013 and January, February and March 2014. The Remes (Re) (35°1'31.87''S; 57°59'39.6''W), Afluente Pescado (AP) (35°3'13.08''S; 57°58'35.3''W) and Gato (Ga) (34°58'53.8''S; 58°3'12.1''W) drain agricultural basins. Remes and Afluente Pescado streams are located close to each other and join to form the Pescado stream (Fig. 1). Next to Remes, the adjacent plot was cultivated up to a couple of meters from the stream, while at Afluente Pescado an uncultivated grassland strip was left between the crop and the stream. Corn was harvested on the plots adjacent to both streams. The strip was 30 m wide on the right bank and 80 m wide on the left bank. The Gato stream drains a basin with intensive horticultural farming, mainly of tomato and lettuce. The streams Arregui (Ar) (35°7'22.1''S; 57°41'11.6''W) and Sin Nombre (SN) (35°2'22.8''S; 57°42'40.5''W) drain basins in which the main land use is extensive livestock raising on natural pastures. The Confluencia (Co) (35°7'53.10''S; 57°24'1.47''W), Destino (De) (35°8'15.35''S; 57°23'40.2''W) and Morales (Mo) (35°8'17.54''S; 57°23'57.7''W) streams run through the “Parque Costero Sur” UNESCO Biosphere Reserve (Athor, 2009). The landscape is grassland with small patches of forest.

The Argentine Pampas streams lack forested borders. Conspicuous macrophyte growth is a common feature. Remes and Sin Nombre contained the largest macrophyte biomass, covering almost the whole water surface, composed mainly of *Typha dominguensis* and *Ludwigia*

peplodes, accompanied in Sin Nombre by *Sagittaria montevidensis* and *Gymnocoronis spinlantoides*. The Morales and Destino streams also showed an important macrophyte covering; in Destino *L. peplodes* and *Myriophyllum aquaticum* were dominant, accompanied by *Hydrochelis nymphoides*, while in Morales *Azolla filiculoides* and *M. aquaticum* were dominant. Confluencia and Arregui streams showed sparse mats of *Schoenoplectus californicus*, often accompanied by *A. filiculoides* and, in Arregui stream, by *Elodea* and mucilaginous algal mats. In the Gato stream the dominant macrophyte were *T. dominguensis* and dense stands of *L. peplodes*; occasional algal mats were observed. The sampled site at Afluente Pescado showed the lowest macrophyte cover, represented mainly by *G. spinlantoides*.

2.2. Environmental variables

Dissolved oxygen, temperature, conductivity and pH in stream water were measured in situ with Yellow Spring Instruments SI 556 multiparameter equipment. The manufacturer's calibration procedures were followed. Dissolved oxygen calibration was carried out in water samples of known concentration as determined by Winkler titration (APHA, 2012); conductivity and pH were calibrated with certified standards from Merck, 1 mS/cm for conductivity and buffer solutions of 4, 7 and 9 for pH calibration. Stream width and depth were assessed with a measuring tape.

2.3. Nutrients

Water samples were filtered through Whatman GF/C filters, and carried in coolers to the laboratory. Dissolved nutrients were determined in the filtrate. Soluble reactive phosphorus (SRP, molybdate-ascorbic), nitrite (NO₂, diazotization), nitrate (NO₃, hydrazine reduction followed by diazotization), and ammonium (NH₄⁺, indophenol blue) were determined following APHA (2012).

2.4. Pesticide analysis

Sediment samples were collected with a stainless steel scoop from the top two centimeters, and placed in amber glass jars. The samples were kept in coolers on ice until arrival at the laboratory where they were kept refrigerated until extraction (maximum 5 d). Sediments were extracted with a mixture of acetone and methylene chloride following You et al. (2004). Cleanup procedures were carried out using Florisil solid phase extraction (SPE) cartridges (USEPA, 2007). Endosulfan in bottom sediments was determined because it was the pesticide found in highest in the studied area in previous reports (Hunt et al., 2016; Solis et al., 2016). Endosulfan was measured following USEPA 8081A (1996); the sample extracts were injected into a GC-ECD (Thermo Scientific 1300), equipped with a HP5 column, 30 m and 0.32 ID, N₂ carrier, ramp and detector temperatures were: 100 °C (3 min), 15 °C/

min to 300 °C, hold 3 min. Solvents used were J. T. Baker for pesticide analysis. Endosulfan Certified Standards utilized for calibration were obtained from AccuStandard Inc. (USA). Detection limit was 0.5 ng/g dw.

2.5. Macroinvertebrate sampling

Macroinvertebrates were sampled from emergent vegetation by means of a D-net with a 500 µm pore size, and 30 cm diameter. At each site, 3 sweeps were collected, covering an area of approximately 1 m² per sample. Samples were preserved with 80% ethanol (Barbour et al., 1999; Merritt et al., 2008) and taken to the laboratory for sorting. All invertebrates were later identified under a stereoscopic microscope. Taxa were identified to family or genus following Merritt et al. (2008) and Domínguez and Fernández, (2009).

2.6. Statistical analysis

Environmental measurements for the different land uses (reserve, livestock, and crops) were compared by means of the Kruskal-Wallis non parametric ANOVA on ranks test because studied variables did not fulfill required assumptions for the use of parametric models. When significant differences were detected Dunn's method of multiple comparison procedures were applied. The same procedure was used to compare endosulfan concentration in bottom sediments. Whenever the pesticide determinations turned out not to be detectable, the detection limit was used in the statistical comparison. All statistical test were done at a significance level of $p < 0.05$. The analyses were performed using the Sigma Stat 3.2 program.

Macroinvertebrate assemblage data were analyzed using the multivariate statistical package PRIMER version 5 (Clarke and Gorley, 2001). Total abundance for each taxon was estimated and reported in terms of number of individuals per surface area (ind/m²).

Data were transformed by a $\log(x + 1)$ function to reduce the contribution of the most abundant groups. Data were analyzed using the similarity index of Bray and Curtis (1957). With the object of assessing habitat delimitation as a function of land use, a cluster analysis was performed. Differences among groups were assessed by means of the analysis of similarity (ANOSIM). The percentage of similarity (SIMPER) was calculated to assess the taxa that contributed most to the differences in the assemblages among groups (Clarke and Warwick, 2001).

3. Results

3.1. Environmental variables

No significant differences were detected in the measured environmental variables among different land uses. Conductivity ranged from 60 to 3900 µS/cm depending on whether the periods were of drought or rainy. Water pH range was 7.1–8.3 and that of the temperature 19–28 °C. Oxygen concentrations were generally high in all streams (5–7 mg/l). However, low oxygen concentrations (0.5–2 mg/l) were occasionally measured when high temperatures at the sites coincided with a conspicuous macrophyte cover and organic matter accumulation on the bottom surface.

3.2. Nutrients

Nitrite, ammonium and PRS concentrations were significantly higher in the streams adjacent to agricultural plots than in the streams located in the reserve (Fig. 2). Nitrate concentrations were higher in the agricultural streams than in the streams adjacent to livestock plots. No significant differences were detected between livestock and reserve streams. Nitrate concentrations in the Remes agricultural stream decreased steadily throughout the sampled period from 1102 µg N/L in

December 2013 to 117 µg N/L in March 2014. The nearby Afluente Pescado, containing the buffer strip, showed comparatively modest nitrate concentrations (103–194 µg N/L) without any recognizably temporal trend.

3.3. Insecticides

Endosulfan concentrations were significantly higher in the agricultural than in the reserve and livestock streams (Table 1). Endosulfan was not detected in the Confluencia, Morales and Arregui reserve and livestock streams at any time during the present study. Endosulfan was detected at low concentrations (0.9–1.3 ng/g dw), in only one sampling (March 2014), in the Destino and Sin Nombre, reserve and livestock streams, respectively. By contrast, in the Remes and Gato streams, adjacent to agricultural plots, endosulfan was detected in all samplings, within a range of 3.6–35.8 ng/g dw. Within the cropped basins, concentrations were significantly lower in the Afluente Pescado stream, possessing a grass-covered buffer strip between the stream and the crop, than in the nearby Remes stream. Concentrations in Afluente Pescado stream ranged around detection limits in 3 of the 4 samplings to attain a comparatively modest maximum of 5.6 ng/g dw (Table 1).

3.4. Macroinvertebrate assemblages

A total number of 4688 specimens were identified, belonging to 51 taxa, 38 families and 9 orders. Hyalellidae (*H. curvispina*) and Planorbidae (*B. peregrina*) were the taxa best represented in the reserve streams. Chironomidae often showed high densities in the reserve and livestock streams, attaining a maximum of 428 ind/m² in the Destino reserve stream on March 2014, and were absent, or present at low densities (1–2 ind/m²), in the agricultural streams. Planorbidae (*B. peregrina*), Dytiscidae (*Bidessini*, *Laccophilus*, *Laccodytes*) and Ceratopogonidae were well represented in livestock streams; the latter was absent from agricultural streams. Ephemeroptera (Baetidae and Caenidae) had a mean abundance range of 13–19 ind/m² in the reserve and livestock streams, a range of 0–1 ind/m² in the Remes and Gato agricultural streams, and amounted to 8 ind/m² in Afluente Pescado, the agricultural stream with a buffer strip. Dugesidae, Culicidae and Ampullariidae (*P. canaliculata*) were the taxa best represented in agricultural streams, followed by Hyalellidae (*H. curvispina*), which attained lower abundances than in the reserve streams.

The cluster analysis recorded differences among the invertebrate assemblages at sites with different land uses (Fig. 3). A small group containing only samplings in streams adjacent to agricultural plots was segregated within group 1. Group 2 was divided in 4 subgroups 2A–2D. All the other samplings from the agricultural streams were joined together within the 2A group. The reserve and livestock streams, which were not segregated from each other, were mixed together in the 2B–2D groups.

The ANOSIM analysis showed significant differences among streams with basins of contrasting land use (Global R = 0.403, $p < 0.001$, Table 2). The assemblage composition in the agricultural streams was significantly different from those in the reserve and livestock streams ($p < 0.001$). No significant differences were recorded between livestock and reserve streams.

The SIMPER analysis identified the taxa with the highest contribution to the assemblage composition for each land use (Table 3). Ampullariidae (*P. canaliculata*) and Planorbidae (*B. peregrina*) were the taxa that most contributed to the assemblage composition in agricultural streams. Hyalellide (*H. curvispina*), Zygoptera and Planorbidae (*B. peregrina*) were the taxa that most contributed to the composition in the reserve and livestock streams.

4. Discussion

Present results showing higher nutrient concentrations in streams

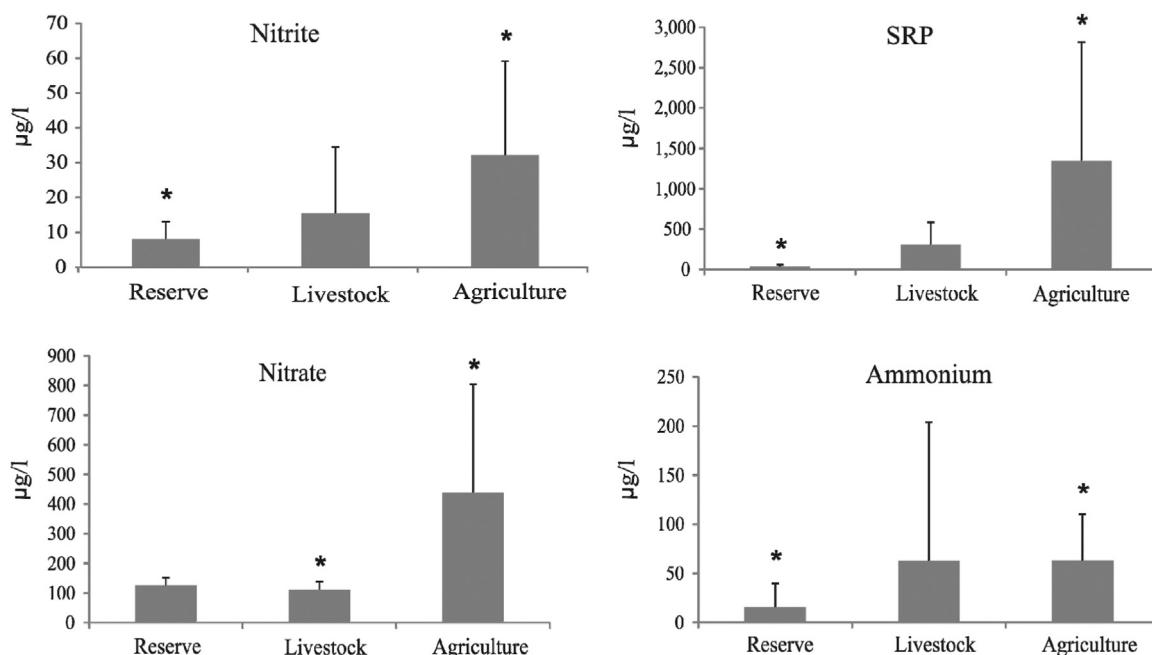


Fig. 2. Nutrient concentrations in streams in basins with different land uses. Significant differences are marked with asterisks.

Table 1

Endosulfan concentration in bottom sediments of the studied streams, in ng/g dw. Detection limit was 0.5 ng/g dw.

Land Use	Reserve ^a			Livestock ^a		Cropped ^b			
	Stream	Confluencia	Destino	Morales	Arregui	Sin Nombre	Af. Pescado [*]	Remes [*]	Gato
Dec. 2013		nd	nd	nd	nd	nd	5.6	24.6	5.3
Jan. 2014		nd	nd	nd	nd	nd	nd	3.7	3.6
Feb. 2014		nd	nd	nd	nd	nd	0.6	32.7	8.2
Mar. 2014		nd	0.9	nd	nd	1.3	0.7	35.8	3.8

Land uses with the same letter are not significantly different. Streams with an asterisk are significantly different.

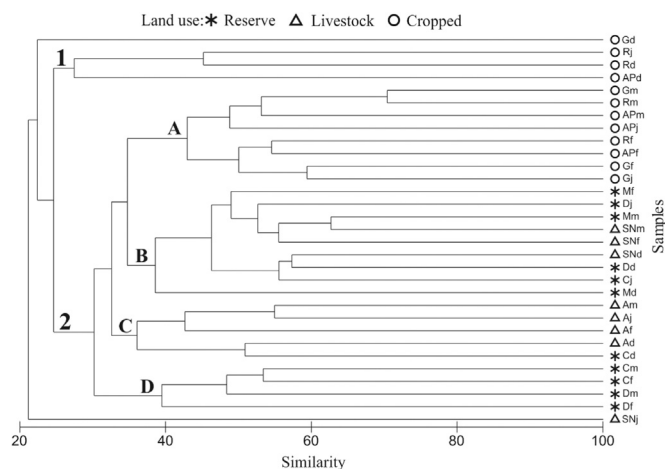


Fig. 3. Cluster of the invertebrate assemblages through the studied period. R: Remes; G: Gato; AP: Afluente Pescado; A: Arregui; SN: Sin Nombre; M: Morales; D: Destino; C: Confluencia, d: December, j: January, f: February, m: March.

draining agricultural basins are consistent with the literature and have repeatedly been reported (Lenat and Crawford, 1994; Meybeck, 1998; Song et al., 2009; Egler et al., 2012; Mugni et al., 2013). In the present study, in Remes agricultural stream, nitrate concentrations decreased steadily from December to March, a trend consistent with corn fertilization performed at the sowing in the adjacent plot, in November. Higher nitrate concentration in runoff after corn fertilization was

Table 2

ANOSIM correlation values and significance levels among streams adjacent to different land uses.

Compared land uses	R	Significance
Cropped vs. reserve	0.431	p < 0,001
Cropped vs. Livestock,	0.364	p < 0,001
Reserve vs. Livestock	0.159	p < 0,035

reported at the same site by Mugni (2009). This trend was not observed in the nearby Afluente Pescado stream, where corn was also harvested on the adjacent plot, suggesting the attenuation effect of the buffer strip.

Macrophytes contribute high organic matter loads to the bottom sediments. During dry periods, in summer, small water flux and high temperatures resulted in quite low oxygen concentrations irrespective of the adjacent land use.

In the present study endosulfan was regularly detected in the bottom sediments of the agricultural streams, suggesting the contribution from the adjacent crops. Endosulfan was determined in runoff water after pesticide applications on experimental crops (Paracampo et al., 2012). Afluente Pescado concentrations were significantly lower than those of Remes, both sites being adjacent to corn plots. The differences were likely due to retention in the buffer strip of Afluente Pescado. Pesticide retention in buffer strips has been documented (Hunt et al., 2016; Reichenberger et al., 2007; Bereswill et al., 2013). A vegetated buffer strip of about 20 m width is expected to retain a large

Table 3

Taxa identified by the SIMPER analysis with the highest contribution to the assemblage composition for each land use.

Reserve	Livestock		Cropped					
	Contrib. %	Accum. %	Contrib. %	Accum. %				
<i>Hyaellidae (H. curvispina)</i>	24.1	24.1	Zygoptera	20.2	20.2	Ampullariidae (<i>P. canaliculata</i>)	20.4	20.4
Planorbidae (<i>B. peregrina</i>)	11.8	35.9	<i>Hyaellidae (H. curvispina)</i>	19.2	39.4	Planorbidae (<i>B. peregrina</i>)	17.5	37.9
Belostomatidae (<i>Belostoma</i>)	11.1	47.0	Dystiscidae (<i>Bidessini</i>)	7.4	46.8	<i>Hyaellidae (H. curvispina)</i>	16.4	54.2
Chironomidae	8.8	55.7	Baetidae	5.7	52.5	Zygoptera	11.4	65.6
Zygoptera	6.9	62.7	Corixidae (<i>Corixini</i>)	4.3	56.8	Hydrophilidae (<i>Enochrus</i>)	9.0	74.6
Baetidae	6.5	69.1	Ancylidae (<i>Gundlachia</i>)	4.2	61.1			
			Hydrophilidae (<i>Tropisternus</i>)	4.2	65.3			
			Dytiscidae (<i>Laccophilus</i>)	4.2	69.4			

part of the highly sorbed pesticide load in runoff, consistent with the observed differences between endosulfan concentration in Remes and Afluente Pescado in the present study. Unfortunately, only a single stream with an attenuation strip was recorded. Further research is needed on this subject.

Hyaella curvispina was often the taxon best represented in the reserve and livestock streams. This species was proposed as a sentinel organism because of its wide distribution, high abundances and sensitivity to insecticide exposure (Mugni et al., 2013).

The comparatively low densities of *H. curvispina* in the Remes agriculture stream, where the highest endosulfan concentrations were measured, might be explained by the effect of insecticide exposure. Toxicity pulses to *H. curvispina* in stream and runoff water on the occasion of the first rain after pesticide application in the adjacent crop was determined in the Remes (Mugni et al., 2011). Toxicity persistence in runoff was observed up to one month after endosulfan application to experimental crops (Paracampo et al., 2012). The absence or low densities (1–2 ind/m²) of Chironomidae recorded in the agricultural streams while being well represented in the reserve or livestock streams might be interpreted in the same way. *Chironomus tentans* is more sensitive to endosulfan than *H. Azteca* (You et al., 2005). Likewise, decreased Ephemeroptera abundance in the agricultural streams in the present study can be explained by the effect of insecticide exposure. Decrease Ephemeroptera abundance in relation with endosulfan application to cotton was reported by Leonard et al. (1999) in the watershed of the Namoi River, Australia, and by Castillo et al. (2006) in streams draining banana plantations in comparison with nearby streams draining forested watersheds in Costa Rica. Similarly to the present study, multivariate analyses showed that the macroinvertebrate assemblages at the banana plantation sites were significantly different from those at the reference sites.

Liess and Von der Ohe (2005) studied the effect of pesticide contamination on the stream invertebrate assemblages of Germany. Species were classified in two groups: SPEAR (Species at Risk), the more sensitive ones, and SPENotAR (Species not at risk) those less sensitive. It is interesting to note that most taxa that were well represented in the agricultural streams in the present study belong to the SPENotAR group (Planorbidae, Ampullariidae, Glossiphoniidae, Dugesidae) while most taxa poorly represented in the agricultural streams but abundant in the livestock and reserve streams belong to the sensitive SPEAR group (Caenidae, Ceratopogonidae, Hyaellidae).

Nutrient concentrations were not significantly different in the livestock and reserve streams. Endosulfan was occasionally detected at low concentrations, close to the detection limit, in both land uses. The macroinvertebrate assemblages in livestock and reserve streams were brought together in the same CLUSTER groups, and did not emerge as significantly different in the ANOSIM analysis. It is therefore suggested that breeding cattle at low densities on natural pastures represents a land use activity with no evident impact on the Pampasic streams.

5. Conclusion

Land use in the basin affects the invertebrate composition in Pampasic streams. The invertebrate assemblages at sites adjacent to cultivated plots were significantly different from those located in a reserve and in livestock basins. Higher endosulfan and nutrient concentrations were detected at the sites adjacent to agricultural plots. Present evidence suggests that pesticide exposure contributed to the observed differences in the assemblage compositions observed in the agricultural streams. Available information suggests that the ongoing agricultural intensification is producing a change toward ruder macroinvertebrate assemblages in the Argentine agricultural basins. The multivariate analysis of the macroinvertebrate assemblages represents a valuable contribution to the assessment of ecological effects and consequences of crop production on the adjacent streams.

The agricultural stream provided with a natural grassland strip contained a significantly lower endosulfan concentration in bottom sediments. It is suggested that leaving a buffer strip represents a useful managerial practice for attenuation the agricultural impact.

Extensive livestock breeding on natural pastures represents a land use with no discernible effect on the invertebrate assemblages of the adjacent streams.

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