Knowl. Manag. Aquat. Ecosyst. 2020, 421, 5
© M. Arias *et al.*, Published by EDP Sciences 2020
https://doi.org/10.1051/kmae/2019048

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Knowledge &
Management of
Aquatic
Ecosystems

Journal fully supported by Agence française pour la biodiversité

RESEARCH PAPER OPEN ACCESS

# Horticulture affects macroinvertebrate assemblages in adjacent streams (Buenos Aires, Argentina)

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Received: 24 June 2019 / Accepted: 15 December 2019

**Abstract** – The agrochemicals used on crops can reach watercourses, affecting water quality and biologic communities. The aim of this research was to study the effects of horticulture on the water quality and invertebrate assemblages of adjacent streams in Buenos Aires province, Argentina. Four streams draining horticultural basins were compared with another four considered less disturbed: two of the latter located in a Biosphere Reserve and the other two in extensive livestock-raising basins. Pesticides were detected in the horticulture-related streams, while nutrient concentrations were significantly higher than in the less-disturbed streams. The macroinvertebrate assemblages differed: the less-disturbed streams exhibited a significantly higher taxa richness and density. *Hyalella* sp. and *Simocephalus vetulus* were dominant, whereas Entomobryoidea, Dugessidae, and Glossiphoniidae were dominant in the horticulture-associated streams. Ephemeroptera (*Caenis* and Baetidae) were well represented in the less-disturbed streams and rare or absent in the horticulture-adjacent streams. Multivariate analysis indicated that the horticulture-impacted sites contained high nutrient concentrations and tolerant taxa, while the less-disturbed sites corresponded to lower nutrient concentrations and sensitive taxa. We propose *Hyalella* sp. and *S. vetulus* as water-quality indicators in pampean streams and conclude that intensive agrochemical applications in horticulture increase nutrient and pesticide loads affecting the macroinvertebrate assemblages of adjacent streams.

**Keywords:** Land use / agrochemicals / freshwater / communities

**Résumé – L'horticulture affecte les communautés de macroinvertébrés dans les cours d'eau drainants (Buenos Aires, Argentine).** Les produits agrochimiques utilisés sur les cultures peuvent atteindre les cours d'eau, affectant la qualité de l'eau et les communautés biologiques. Le but de cette recherche était d'étudier les effets de l'horticulture sur la qualité de l'eau et les assemblages d'invertébrés des cours d'eau adjacents dans la province de Buenos Aires en Argentine. Quatre cours d'eau drainant des bassins horticoles ont été comparés à quatre autres jugés moins perturbés : deux d'entre eux situés dans une réserve de biosphère et les deux autres dans de vastes bassins d'élevage. Des pesticides ont été détectés dans les cours d'eau liés à l'horticulture, tandis que les concentrations de nutriments étaient considérablement plus élevées que dans les cours d'eau moins perturbés. Les assemblages de macroinvertébrés différaient : les cours d'eau moins perturbés présentaient une richesse et une densité taxonomiques nettement plus élevées. *Hyalella* sp. et *Simocephalus vetulus* dominaient, tandis que les Entomobryoidea, Dugessidae et Glossiphoniidae dominaient dans les ruisseaux associés à l'horticulture. Les éphéméroptères (*Caenis* et Baetidae) étaient bien représentés dans les cours d'eau moins perturbés et rares ou absents dans les cours d'eau voisins de l'horticulture. L'analyse multivariée indique que les sites touchés par l'horticulture contenaient des concentrations élevées d'éléments nutritifs et des taxons tolérants, tandis que les sites moins perturbés correspondaient à des concentrations inférieures d'éléments nutritifs et à des taxons sensibles. Nous proposons *Hyalella* sp. et *S. vetulus* comme

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indicateurs de la qualité de l'eau dans les cours d'eau pampéens et concluons que les applications agrochimiques intensives en horticulture augmentent les charges de nutriments et de pesticides affectant les communautés de macroinvertébrés des cours d'eau voisins.

**Mots-clés :** Utilisation des terres / produits agrochimiques / eau douce / communautés

## 1 Introduction

Horticulture is the main farming activity worldwide following cereal production (FAO, 2013). In Argentina, horticultural production is established mainly in the surroundings of the large cities in order to supply food for urban consumption. The horticultural area around La Plata city is located in the northeast of the Buenos Aires province, where 35% of Argentina's population lives. The area measures 3746 hectares and produces 78,360 tons per year (CHFB, 2005). This high productivity is based on an intensive input-dependent field-cropping system along with greenhouse technology. Fertilizers and pesticides are heavily used to raise crop yields.

Fertilizer consumption in Argentina was 3,121,638 tons in 2014 (CIAFA, 2017), while the annual use of pesticides reached some 336,000 tons in 2011 (CASAFE, 2013). As many as one hundred sixty different pesticides – comprising fungicides, herbicides and insecticides – are used in the area (Sarandón et al., 2015). Endosulfan, cypermethrin, and chlorpyrifos were the most widely used insecticides in Argentina until 2013, when the first of those was banned (SENASA, 2011). At present, cypermethrin and chlorpyrifos represent the most extensively employed insecticides.

Agrochemicals used in crops can reach surface watercourses by spray drift or runoff. The latter transports nutrients (Hart et al., 2004) along with pesticides (Jergentz et al., 2005) from crops to the adjacent streams; the latter input then produces ephemeral toxicity pulses to non-target organisms (Mugni et al., 2011; Schulz, 2001). Several studies have linked pesticide exposure to changes in macroinvertebrate-community structure, most commonly in the form of the disappearance or decreased density of sensitive taxa (Liess et al., 2008; Egler et al., 2012; Schäfer et al., 2012). The differential sensitivity of invertebrates to pesticides could accordingly be used as a biomonitoring tool to assess the condition of aquatic systems (Liess and von der Ohe, 2005). Schäfer et al. (2007) reported that pesticides affected leaf-litter breakdown because the shredders numbered among the sensitive species; thus pesticide exposure can not only impair communities but also affect stream functions.

Agricultural land use affects macroinvertebrate assemblages in regional streams, resulting in the disappearance or decreased abundance of sensitive groups such as Odonata and Ephemeroptera (Jergentz et al., 2004) or Amphipoda (Solis et al., 2016). Agricultural land use has been found to increase nutrient concentrations in pampean streams (Mugni et al., 2013). Enhanced primary production coupled with toxic effects on the consumers would increase eutrophication in horticulture-affected streams (Frieberg Jensen et al., 2003; Relyea, 2004).

The information on pesticide concentrations in horticulture watersheds is scarce (Kreuger et al., 2008; Allinson et al., 2014; Mottes et al., 2017). Only few studies have monitored the levels of those xenobiotic and the consequent toxicity to aquatic organisms in the La Plata horticultural belt (Mac Loughlin et al., 2017; Rimoldi et al., 2018). Solis et al. (2018) compared the assemblage composition in streams influenced by agriculture or livestock and within a reserve. One of the streams considered to be affected by agriculture included areas with adjacent horticulture in the basin. The effect of horticulture on the composition of the non-target resident fauna in the region, however, remained largely unreported.

The aim of this study was therefore to assess the effect of horticultural land use on the macroinvertebrate assemblages of adjacent streams in the Buenos Aires province of Argentina. We hypothesized that the application of agrochemicals altered the macroinvertebrate assemblages of adjacent streams.

## 2 Materials and methods

### 2.1 Study area and sampling design

Eight lowland streams were studied in the Rio de la Plata coastal fringe in the environs of La Plata and Magdalena, Buenos Aires province, Argentina (Fig. 1). The study area is located within the pampa plain, an extensive area of extremely low relief with slopes typically around 0.15% (Hurtado et al., 2006) comprising grasslands with small patches of forest. The baseline stream flow remains imperceptible most of the time except after rainfalls. The streams are shallow (10–50 cm in depth) with beds containing mainly fine sediments (43% silt, 25% clay, and 32% sand), lacking boulders or gravel, and having an abundant litter content. The streams lack forested borders; instead, conspicuous macrophytes and abundant riparian vegetation are developed. The climate is mild-humid with a mean annual air temperature of 16 °C, ranging from 10 °C in July to 23 °C in January. The mean annual precipitation is roughly 1000 mm, being the highest in March (111 mm) and the lowest in June (63 mm; Hurtado et al., 2006).

We sampled four streams – San Juan, Martín, Gato, and Sauce – which drain basins located within the horticultural belt around La Plata city, along with another four that were less disturbed: two, Morales and Destino, run through the Parque Costero Sur, UNESCO Biosphere Reserve (Athor, 2009); the other two, unnamed and Buñirigo, drain basins with extensive livestock on natural grasslands. Solis et al. (2018) had reported the macroinvertebrate-assemblage composition in the reserve and livestock streams as not being significantly different.

The streams were sampled in two successive crop-growing seasons, coinciding with the main agrochemical-application period, from spring (October) to mid-autumn (April). The first period comprised five samplings, in October and December 2014 and January, March, and May 2015; the second consisted of two samplings, in October and December 2015. The sampling was discontinued in January 2016 because of a strong drought.

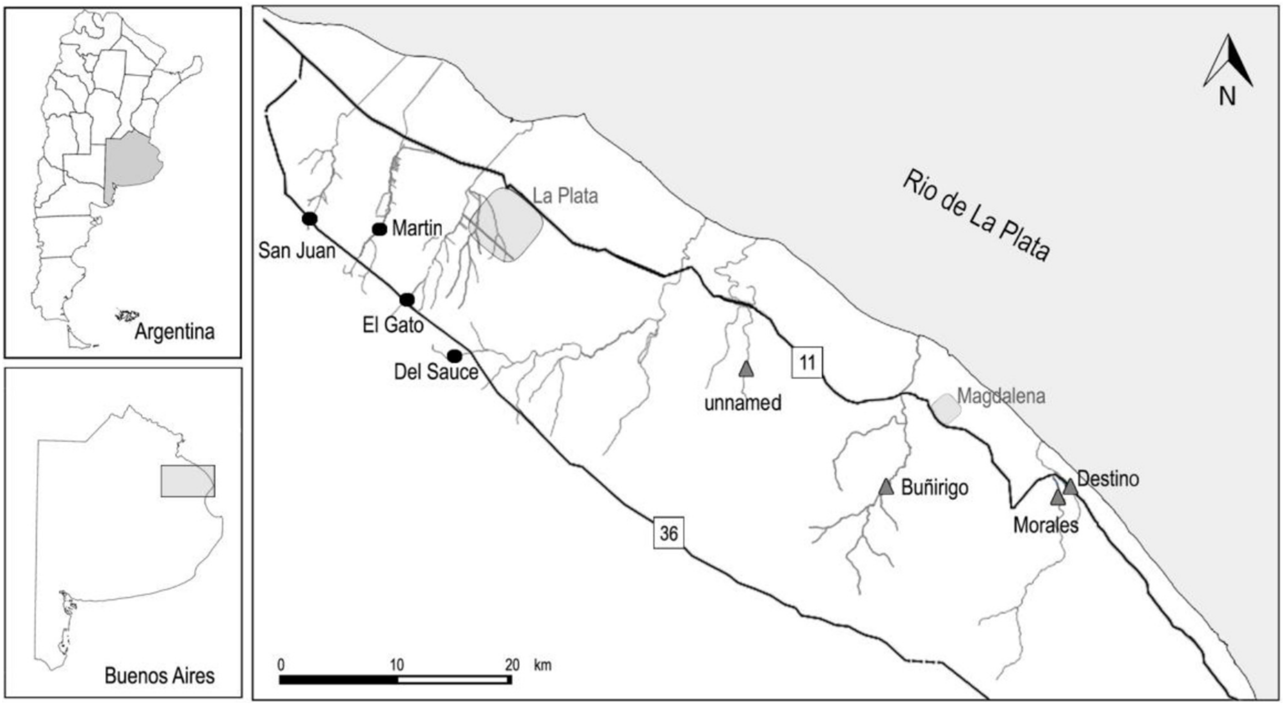


**Fig. 1.** Study area illustrating the location of the sampling sites in relation to La Plata. The *upper inset* indicates the location of the Province of Buenos Aires within Argentina, the *lower inset* the position of the sampling area within the province. Circles, horticulture-associated sampling sites; triangles, less-disturbed sampling sites.

### 2.2 Environmental variables and nutrient concentrations

At each sampling site, the dissolved-oxygen concentration (DO) and temperature (T°) were measured *in situ* with an YSI 51B, the pH with a Hanna Checker, and the conductivity with Hanna Instrument 8733. Water samples were taken in triplicate and transported to the laboratory in coolers covered with ice. In the laboratory, the samples were filtered through a Whatman GF/C filter. The nutrients were then measured in the filtrate: the soluble reactive phosphorus (SRP) was determined by a colorimetric reaction with ascorbic-acid–ammonium-molybdate, the nitrate by hydrazine reduction followed by diazotation, and the ammonium by the indophenol-blue method (APHA, 2012). The suspended matter was estimated as the weight difference of the filter before and after filtration (APHA, 2012). Chlorophyll was acetone-extracted from the dry filters for 24 h, under refrigeration, following Lorenzen (1967).

The sediment samples were taken from the top two centimeters with a stainless-steel scoop and placed in glass vessels. The samples were immediately kept on ice until arrival at the laboratory, where they were then stored at −20 °C until the time of pesticide analysis. The organic carbon in the sediment was assessed by weight loss after drying at 105 °C followed by heating to 550 °C for 2 h (Olivier *et al.,* 2001). The percentage of vegetation cover was estimated visually (Dethier *et al.,* 1993) − in a 1-m$^2$ quadrant, divided into four equal quadrants − before invertebrate sampling.

### 2.3 Pesticides

We assessed the most commonly used pesticides and certain others selected among those previously reported to be found at higher detection frequencies in the region (Hunt *et al.,* 2016; Solis *et al.,* 2017) − namely, the organochlorines α-, ω-, β-, and δ-benzene hexachloride; heptachlor and heptachlor epoxide; aldrin; dieldrin; endrin and endrin aldehyde; methoxychlor; α-, β-, and S endosulfan; the dichlrodiphenyltrichloroethanes (aka DDTs) p, p'-3, 3-dimethyl-4-pentenoic acid methyl ester (DPE), p, p'-dichlrodiphenyldichloroethylene (DDE), and p, p'-dichlrodiphenyldichloroethane (DDD); the organophosphate chlorpyrifos; and finally the pyrethroids λ-cyhalothrin, permethrin, the cypermethrins (mixture of isomers), fenvalerate, and deltamethrin. Because of the hydrophobicity of these compounds, the pesticides are adsorbed onto the particulate fraction of these streams and therefore the analysis was conducted on only the sediment samples in order to increase the detection frequency.

The sediment samples were extracted following You *et al.* (2004) with a mixture of acetone and methylene chloride. A clean-up procedure was carried out through the use of Florisil solid-phase-extraction cartridges.

The sample extracts (1 ml in hexane) were injected for organophosphate-residue determination in a gas chromatograph with nitrogen-phosphorus detection and for organochlorine (OCl) and pyrethroid (Py) residues in a gas chromatograph with an electronic-microcapture detector. The quantitation was performed by comparison with external standards. The organophosphate residues were confirmed by tandem gas chromatography–mass spectrometry, and the OCl and Py residues by injection in a column of a different polarity.

An Agilent 6890 gas chromatograph equipped with an Agilent 7683 autosampler; split/splitless injector; a capillary column, HP 5 (30 m in length, 0.25 mm i.d., 0.25 μm film thickness); and a nitrogen-phosphorus detector was used for the quantitation of the organophosphate compounds (detector temperature, 300 °C; oven-temperature program, 70–240 °C; injector temperature, 250 °C; injection volume, 1 μl; carrier gas, $N_2$). An Agilent 6890N gas chromatograph equipped with an Agilent 7683B autosampler, split/splitless injector and a capillary column, HP 5MS/DB17 (30 m in length, 0.25 mm i. d., 0.25 μm film thickness) with an electronic microcapture detector was used for the quantitation of the OCl and Py compounds (detector temperature, 300 °C; oven-temperature program, 70–240 °C; injector temperature, 250 °C; injection volume, 1 μl; carrier gas, $N_2$). The confirmation was performed by tandem gas chromatography–mass spectrometry (injection system, programmed-temperature vaporizing; injection volume, 5 μl; gas carrier, He). The linearity of the calibration curves, measured by the determination coefficient ($R^2$), was always ≥0.99. The reference standards were from Accustandard (purity >99%) and the quantitation limits 0.7 ng/g dry weight (dw) for OCl, 0.9 ng/g dw for chlorpyrifos, 4.5 ng/g dw for other OPs, 9.1 ng/g dw for cypermethrin, and 1.8–3.6 ng/g dw for other pyrethroids.

### 2.4 Macroinvertebrate assemblages

The sampling was carried out with a 500-μm D-net over aquatic vegetation inside a 1-$m^2$ floating polyvinyl chloride quadrant, which method enabled us to compare the same surface among sampling sites. The samples were taken in triplicate, fixed *in situ* with 96% (v/v) aqueous ethanol, and subsequently processed in the laboratory; where the organisms were counted and identified to the lowest possible taxonomic level with a stereoscopic Leica model EZ4$^{TM}$ microscope and following general (Merrit *et al.,* 2008) and regional (Domínguez and Fernández, 2009) taxonomic keys.

### 2.5 Data analysis

The differences between the horticulture-associated and the less-disturbed sites with respect to environmental variables, nutrients, and the detection frequency of pesticides were tested by the Student *t* test. Whenever normality assumptions were not substantiated, the nonparametric Mann–Whitney *U* test was used. All the statistical analyses were performed by means of the Sigma Plot 12.0 software at a significance level of $p < 0.05$. The samples were grouped according to land use (horticulture-affected or less disturbed) and then compared. The detection frequency of the pesticides was calculated for each stream.

The taxonomic richness (*S*) and density (ind/$m^2$) were calculated for each site at each sampling and then analyzed in the same way as the environmental variables. Differences in the assemblage composition were analyzed through ANOSIM (analysis of similarity) with the factor *land use*, by applying the Bray-Curtis Similarity Index. The density data were transformed by the log ($x$+1) function with those taxa present in only one sample being not considered (Clarke and Warwick, 2001). The SIMPER (percentage similarity) analysis was performed to determine which taxa contributed the most to the differences in the assemblage composition. The analyses were performed with the PRIMER version 6 software (Plymouth Routines Multivariate Ecological Research; Clarke and Gorley, 2001).

The relationships between taxa richness and density, nutrient concentrations, vegetation cover, and chlorophyll were determined by the Pearson correlation analysis; those between the environmental variables and the assemblage composition by the CANOCO version 4.53 software (ter Braak and Smilauer, 1998). The density data were log ($x$+1) transformed. Taxa with total relative abundance lower than 0.5% were excluded from the analysis to reduce the influence of rare species.

The maximum gradient length of macroinvertebrate data was determined by means of the detrended correspondence analysis. The maximum amount of variation in the species-richness data was 1.8, indicating that linear statistical methods were appropriate. Consequently, a Redundancy Analysis (RDA) was carried out. All the variables except pH were normalized, and forward selection was used to determine the variables that significantly explained the distribution pattern of macroinvertebrates at a cutoff point of $p < 0.05$. To subtract the effect of vegetation, a partial redundancy analysis was performed by entering the vegetation cover into the RDA as a covariable. The significance of the RDA axes was assessed by the Monte-Carlo permutation test.

## 3 Results

### 3.1 Environmental variables and nutrient concentrations

Table 1 summarizes the environmental variables and nutrient concentrations measured. No significant differences were observed in the environmental variables when the horticulture-affected and less-disturbed streams were compared. The nitrate, ammonium, and SRP concentrations were, however, significantly higher in the former than in the latter ($p < 0.001$, $p = 0.002$, $p < 0.001$). The mean nitrate concentrations were an order of magnitude higher, while the ammonium and SRP levels were three times higher in the streams adjacent to horticulture. The chlorophyll concentration correlated with both the SRP levels ($R = 0.28$, $p = 0.049$) and the ammonium concentrations ($R = 0.28$, $p = 0.048$).

### 3.2 Pesticides

Insecticides were generally not detected in the less-disturbed streams, except chlorpyrifos and cypermethrin, which compounds were recorded in a single sampling (Tab. 2). Chlorpyrifos was detected at low concentrations (1.5–5.8 ng/g dw) in the less-disturbed Buñirigo Stream and those within the reserve in only December 2014, while cypermethrin was recorded (10–45 ng/g dw) in the Buñirigo and the also less-disturbed unnamed stream in January 2015. The detection frequency was significantly higher ($p = 0.023$) in the horticulture-associated streams than in the less-disturbed streams (Tab. 2).

**Table 1.** Environmental variables and nutrient concentrations in the studied streams. Mean values and (variation range).

| Variables | Studied streams | |
|---|---|---|
| | Horticulture-associated | Less-disturbed |
| *T* (°C) | 19.8 (12–30) | 22.3 (12–29) |
| Conductivity (Cond.) (μS/cm) | 502 (110–853) | 687 (83–1330) |
| Dissolved oxygen (OD) (mg/L) | 6.7 (1.3–19) | 6.8 (2.5–10.6) |
| pH | 7.7 (6.6–9.3) | 7.8 (6.9–9.2) |
| Depth (cm) | 23 (5–50) | 34 (11–63) |
| Suspended matter (SM) (mg/L) | 103 (2–1208) | 105 (13–457) |
| Sediment Organic Carbon (OC%) | 3.9 (1–7) | 3.7 (1–9) |
| Chlorophyll (μg/L) | 45 (4–355) | 24 (3.5–82) |
| Vegetation cover (VEG) (%) | 62 (15–100) | 58 (17–90) |
| SRP (μg/L) | 319 (84–765) | 109 (16–434) |
| Nitrate (μg N-$NO_3^-$/L) | 1183 (20–6808) | 95 (2–367) |
| Ammonium (μg N-$NH_4^+$/L) | 229 (16–1334) | 71 (4–414) |

**Table 2.** Detected pesticides in the studied streams (ng/g dry weight).

| | DF | Chlorpyrifos | L-Cyhalothrin | Cypermethrin | pp'DDT | pp'DDD |
|---|---|---|---|---|---|---|
| HORTICULTURE | | | | | | |
| **San Juan** | **0.29** | | | | | |
| May 2015 | | nd | nd | nd | nd | 1.8 |
| Dec. 2015 | | nd | nd | nd | nd | 2.2 |
| **Martin** | **0.60** | | | | | |
| Dec. 2014 | | 0.9 | nd | nd | nd | nd |
| Jan. 2015 | | nd | nd | 6.4 | nd | nd |
| Oct. 2015 | | nd | nd | nd | nd | 0.8 |
| **Gato** | **0.43** | | | | | |
| Jan. 2015 | | nd | 0.9 | 148 | nd | nd |
| May 2015 | | nd | nd | 199 | nd | nd |
| Dec. 2015 | | nd | nd | nd | 4 | nd |
| **Sauce** | **0.43** | | | | | |
| Jan. 2015 | | nd | nd | 14 | nd | nd |
| May 2015 | | nd | nd | nd | nd | 1.9 |
| Dec. 2015 | | nd | nd | 51 | 2 | nd |
| LESS DISTURBED | | | | | | |
| **unnamed** | **0.2** | | | | | |
| Jan. 2015 | | nd | nd | 18.7 | nd | nd |
| **Buñirigo** | **0.29** | | | | | |
| Dec. 2014 | | 1.5 | nd | nd | nd | nd |
| Jan. 2015 | | nd | nd | 6.6 | nd | nd |
| **Morales** | **0.25** | | | | | |
| Dec. 2014 | | 3.4 | nd | nd | nd | nd |
| **Destino** | **0.14** | | | | | |
| Dec. 2014 | | 5.8 | nd | nd | nd | nd |

DF: Detection frequency; nd: not detected. Samplings without at least one pesticide detected are not shown.

In contrast, cypermethrin evidenced a detection frequency of 20% in the horticulture-adjacent streams, attaining peak concentrations of 148–199 ng/g dw in two consecutive samplings in the Gato Stream, and of 51 ng/g dw in the Sauce Stream. Chlorpyrifos, lambda-cyhalothrin, DDT, and DDD were measured at low concentrations (0.9–6 ng/g dw) only in the horticulture-associated streams.

### 3.3 Macroinvertebrate assemblages

A total of 30,829 specimens were counted and 88 taxa identified, representing 52 families and 18 orders (Supplementary Material, Tab. S1). Amphipoda and Copepoda were present at all the sites. *Simocephalus vetulus* and *S. daphnoides* (Daphniidae) along with the amphipod *Hyalella* sp. were the

**Table 3.** SIMPER analysis showing average abundances (log transformed) of taxa for both land uses and contribution (%) to >30% of dissimilarity.

| Taxa | Av. Abundances | | | |
|---|---|---|---|---|
| | Horticulture-associated | Less disturbed | Contribution (%) | Cum. Cont. (%) |
| *S. vetulus* | 1.1 | 3.6 | 5.0 | 5.0 |
| *Hyalella* sp. | 1.9 | 3.8 | 4.7 | 9.7 |
| Baetidae | 0.1 | 2.2 | 4.0 | 13.7 |
| Dugesiidae | 2.5 | 0.3 | 3.8 | 17.6 |
| *S. daphnoides* | 0.6 | 2.5 | 3.8 | 21.3 |
| Ciclopoidea | 2.0 | 2.5 | 3.5 | 24.9 |
| Entomobryoidea | 3.3 | 1.9 | 3.4 | 28.2 |
| *Caenis* sp. | 0.0 | 1.7 | 3.2 | 31.4 |
| Hirudinea | 2.0 | 1.0 | 2.9 | 34.3 |

most abundant in the less-disturbed streams except for the unnamed one, where cladocerans were dominant followed by Copepoda. The Trichoptera families Hydroptylidae and Polycentropodidae (*Cyrnellus* sp.) and the Decapoda were identified in only the less-disturbed streams. The Ephemeroptera likewise exhibited higher densities in the less-disturbed than in horticulture-associated streams, The Baetidae were rare and *Caenis* sp. was absent at the horticulture-adjacent sites. In contrast, the Collembolans (Entomobryoidea and Poduroidea) were commonly the most abundant taxa in horticulture-influenced streams; while other dominant taxa were the Diptera, the Dugessidae, and the Copepoda.

The taxonomic richness and density were significantly higher in the less-disturbed than in the horticulture-adjacent streams ($p < 0.001$ and $p = 0.025$, respectively). An average of 35 taxa was recorded in the less-disturbed, as against 24 in the horticultur-associated streams. The mean density of the macroinvertebrates in the less-disturbed streams was almost twice that of the in horticulture-adjacent streams (819 ind/m$^2$ *versus* 461 ind/m$^2$, respectively). Among the less-disturbed streams, the unnamed was the one with the highest taxonomic richness (41 taxa) and Morales was the one with the highest density (1286 ind/m$^2$). In contrast, of the 4 horticulture-affected streams, Martín was the one with the poorest abundance parameters at 18 taxa and 172 ind/m$^2$ (Tab. S1). The mean densities of the crustaceans *Hyalella* sp. and *S. vetulus* were significantly higher in the less-disturbed (239 and 136 ind/m$^2$, respectively) than in the horticulture-influenced streams (25 and 13 ind/m$^2$, $p = 0.04$ and $p < 0.001$, respectively).

The vegetation cover was positively correlated with the taxa richness and the macroinvertebrate density ($R = 0.35$, $p = 0.015$ and $R = 0.45$, $p = 0.001$, respectively). The richness was inversely correlated with the SRP ($R = -0.33$, $p = 0.021$) and the ammonium ($R = -0.32$, $p = 0.024$), while the population density was likewise inversely correlated with the SRP concentrations ($R = -0.42$, $p = 0.002$).

### 3.4 Macroinvertebrate assemblages

The assemblage composition of the horticulture-adjacent and the less-disturbed streams was significantly different (ANOSIM: Global $R = 0.45$; $p = 0.001$; factor: "land use"). The SIMPER analysis indicated an average dissimilarity of 68% between the two groups. The taxa that contributed most to the dissimilarity (Tab. 3) were *S. vetulus*, *Hyalella* sp., and the Baetidae – those being well represented in the less-disturbed streams – and *Caenis* because of that genus's absence in horticulture-associated streams. The Dugessidae, Entomobyridae, and Glossiphoniidae contributed to the differences because of their comparatively high abundance in the horticulture-affected streams.

The redundancy analysis revealed that vegetation cover, SRP, DO, conductivity, ammonium, and the percent organic carbon explained 32% of the total variability in the macroinvertebrate-assemblage composition. The global significance test for the first canonical axis and the sum of all axes was significant ($F$ ratio = 6.768; $p = 0.002$ and $F$ ratio = 2.260; $p = 0.002$, respectively). Since the vegetation cover did not differ significantly among the different land uses, that influence was considered separately; a partial RDA analysis was performed involving vegetation cover as a covariable. This RDA explained 23% of the variance. The first and second axes accounted for 75% of the relationship between species and the environment. The first axis was correlated with the SRP (0.60), ammonium concentration (0.41) and the percent organic carbon (0.04); the first two of those variables being higher in the horticulture-associated streams and as such representing an environmental-deterioration gradient. The second axis was positively correlated with DO (0.66) and the percent organic carbon (0.06) and negatively with the conductivity (–0.33). In the diagram of the sampling sites (Fig. 2a), those associated with horticulture were located on the right side of the ordination and were related to high SRP and ammonium concentrations; the less-disturbed sites were located on the left side of the diagram and were accordingly negatively correlated with the SRP and ammonium concentrations. A correspondence between the abundance of certain taxa and the gradients was also observed (Fig. 2, Panel b.). The Entomobryoidea, Poduroidea, Glossiphonidae, Dugessidae, *Paracymus*, *Pomacea*, and *Enochrus* were positively correlated with nutrient concentrations – pointing to agrochemical contamination in the horticulture-adjacent streams – whereas *Hyalella* sp., *Simocephalus* sp., *Caenis*, Baetidae, Ceratopogonidae, and *Tropisternus* were negatively correlated.

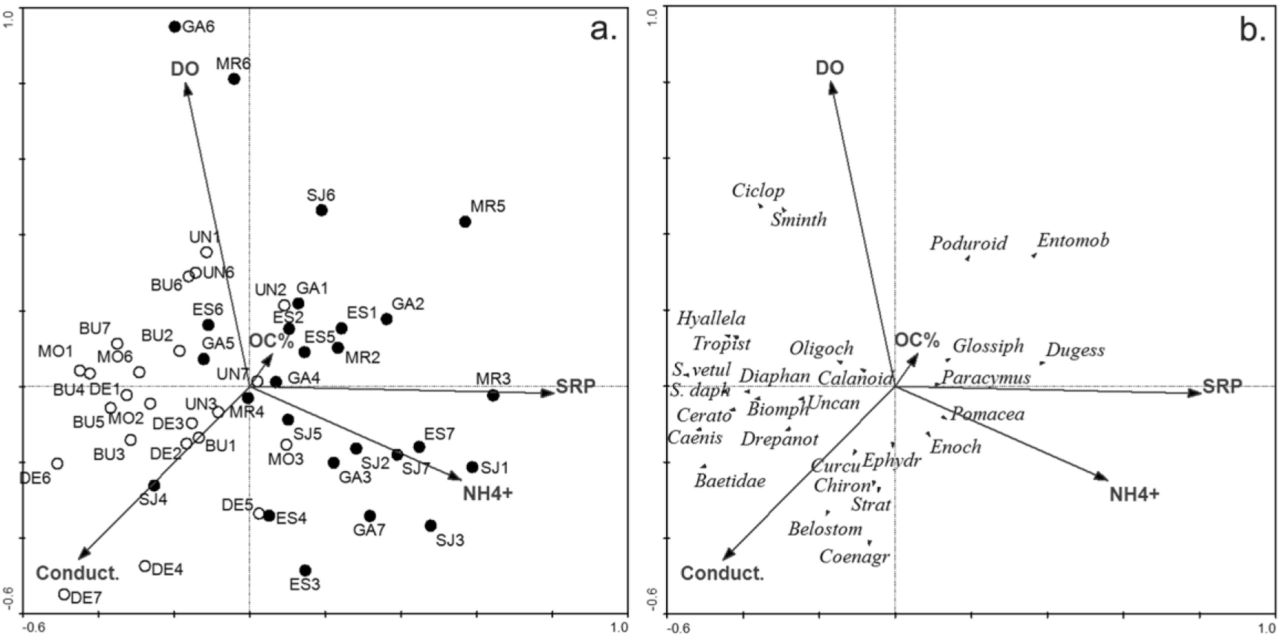


**Fig. 2.** Partial-RDA Analysis. Biplots of environmental data and samples (a) and species (b). Filled circles, horticulture-adjacent streams; white circles, less-disturbed streams. Stream names: SJ, San Juan; MR, Martin; GA, Gato; ES, Sauce; UN, unnamed; BU, Buñirigo; MO, Morales; DE, Destino. Sampling dates: 1, Oct-2014; 2, Dec-2014; 3, Jan-2015; 4, Mar-2015; 5, May-2015; 6, Oct-2015; 7, Dec-2015.Taxa: Baet, Baetidae; Belost, *Belostoma*; Biomph, *Biomphalaria*; Calan, Calanoidea; Cerato, Ceratopogonidae; Chiro, Chironomidae; Ciclop, Ciclopoidea; Coena, Coenagrionidae; Curcu, Curculionidae; Diaph, *Diaphanosoma*; Drepa, *Drepanotrema*; Dugess, Dugessidae; Enoch, *Enochrus*; Entomo, Entomobryoidea; Ephy, Ephydridae; Glossiph, Glossiphonidae; Hyal, *Hyalella* sp.; Oligo, Oligochaeta; Paracy, *Paracymus*; Poduro, Poduroidea; Poma, *Pomacea*; S. daph, *Simocephalus daphnoides*; S. vet, *S. vetulus*; Sminth, Sminthuroidea; Strat, Stratiomyidae; Uncan, *Uncancylus*. Only the variables that significantly explained the macroinvertebrate assemblage are shown.

## 4 Discussion

Higher nutrient concentrations in streams associated with horticulture suggest the occurrence of a leaching of fertilizers applied to the crops from the ground into the adjacent streams. High nutrient concentrations at sites adjacent to agricultural plots have been repeatedly reported. Arbuckle and Downing (2001), studying the relationships between agricultural land use and the N:P ratio in Iowa lakes, found that the TN/TP was positively correlated with agriculture and negatively so with grasslands. Figueiredo *et al.* (2010) demonstrated that the conversion of land use from grasslands to agriculture increased nitrate concentrations in streams where crops bordered the stream margins. Mugni *et al.* (2013) measured higher nutrient concentrations in a stream surrounded by a recently fertilized crop than in the same stream when that crop was not being fertilized. The nutrient concentrations in the present study revealed a wide variability, likewise reported in previous publications (*e.g.*, Mugni *et al.*, 2013; Solis *et al.*, 2016, 2017).

The present results on the detection of insecticides in horticulture-associated streams were consistent with the findings reported the literature. Kammerbauer and Moncada (1998) studied several freshwater systems – *i.e.*, rivers, wells, and lagoons – adjacent to agricultural and horticultural land uses in Honduras; the horticulture-impacted water samples exhibited a higher number of pesticides detected and a greater detection frequency than the agriculture-related samples. Mac Laughlin *et al.* (2017) reported high and frequent concentrations of chlorpyrifos (up to 2258 ng/g dw) and pyrethroids (*e.g.*, lambda-cyhalothrin at 649 ng/g dw) in bottom sediments of the Carnaval Stream located within the horticulture area studied in this work.

The pesticide concentrations measured and the higher detection frequency in the horticulture-associated streams in the present study suggest a contribution from the adjacent crops. The pattern registered was consistent with endosulfan concentrations measured in the air in the same area, reported by Astoviza *et al.* (2016): The maximum air concentrations were recorded in an orchard in Olmos, between the Sauce and Gato streams; while the minimum were reported in a livestock basin in Magdalena, located near the less-disturbed streams sampled in this study. The occasional pesticide detection at low concentrations in the less-disturbed streams is consistent with the long-range atmospheric transportation of those compounds from the application sites that has been previously reported (Weber *et al.*, 2010). The pesticide concentration detected in the less-disturbed streams might conceivably be also contributed by applications associated with cattle-pest control within the livestock areas surrounding the reserve. Cypermethrin and chlorpyrifos are frequently used for such a purpose (Ferré *et al.*, 2018), typically to combat the horn-fly pest in cows (Oyarzún *et al.*, 2008).

The taxonomic richness and density were positively correlated with macrophyte cover. Several authors reported that macrophyte complexity was associated with a high abundance and richness of macroinvertebrates (Walker *et al.*, 2013; Ferreiro *et al.,* 2014). We thus employed a partial-RDA analysis subtracting the effect of vegetation to enable us to refine the assessment of the macroinvertebrate assemblage in relation to land use per se.

The different macroinvertebrate-assemblage composition together with the lower richness and density of the taxa present in the horticulture-associated streams suggested that those changes had resulted from pesticide exposure. *Simocephalus vetulus* and *Hyalella* sp. were the dominant taxa in the less-disturbed streams. The Daphniidae are the most common cladocera used in biomonitoring because of their sensitivity to pesticides (Zhou *et al.,* 2008). *Simocephalus vetulus* – a cosmopolitan cladocera species among the Daphniidae that is associated with aquatic vegetation in eutrophic systems – is extensively used as a model organism for toxicity testing (Willis *et al.,* 1995; Chen *et al.,* 2004; Schroer *et al.,* 2004). Similarly, amphipods of the genus *Hyalella* have repeatedly been used as monitoring organisms (Nebeker and Miller, 1989). Mugni *et al.* (2011) reported toxicity to *Hyalella curvispina* of runoff and the water in the Sauce stream at a site near to the present study sites in coincidence with the first rain following pesticide application to an adjacent crop. Toxicity to *H. curvispina* in runoff water from experimental soy plots after chlorpyrifos, endosulfan, and cypermethrin application followed by rain simulations was likewise documented by Paracampo *et al.* (2012). Mac Laughlin *et al.* (2017) reported sediment toxicity to *H. curvispina* in the Carnaval Creek, where the lowest survival and growth rates were registered in coincidence with the highest insecticide concentrations. The Ephemeroptera and Trichoptera have often been considered in indices and metrics for assessing environmental quality (Barbour *et al.,* 1999), typically being featured in the EPT – Ephemeroptera, Plecoptera, and Trichoptera – index. The taxa *Caenis,* Hydroptilidae, Polycentropodidae, and Decapoda – considered sensitive by Liess and Von der Ohe (2005) – were found in only the less-disturbed streams in the present study. In addition, Suren (1994), in Nepal, recorded a high abundance of Baetidae in grassland-associated streams while being poorly represented in those adjacent to agriculture.

In contrast, the Entomobryoidea, Dugessidae, and Glossiphoniidae were well represented at the present horticulture-affected sites and their presence accordingly correlated with the nutrient concentrations in the multivariate analysis, thus indicating a relation to horticultural land use. The Dugessidae and Glossiphoniidae were considered by Liess and Von der Ohe (2005) to be tolerant to pesticides. Similarly, Egler *et al.* (2012), studying water quality and macroinvertebrate composition in relation to forest, pasture, and intensive agricultural land use in Brazil, recorded a higher abundance of Collembola accompanying elevated nutrient concentrations together with a lower taxa richness in the agricultural streams. Wang *et al.,* (2016) reported that the addition of both organic (soybean cake) and inorganic fertilizers in poplar plantations likewise increased the abundance of collembolans in soil and considered that finding to be a result of increased food availability.

The elevated nutrient concentrations in the horticulture-associated streams might conceivably affect macroinvertebrate assemblages by enhancing the phytoplankton biomass. No significant changes in the chlorophyll content, though, were detected among the different land uses. Water turbidity and/or competition with macrophytes for light and nutrients might, however, limit phytoplankton growth. In the present study, multivariate analysis suggested the combined effect of simultaneous incorporation of nutrients and pesticides through runoff following crop applications to be responsible for the positive correlation of the tolerant taxa and negative association of the sensitive taxa with elevated nutrient concentrations. Malacarne *et al.* (2016), in Brazil, studying the composition of aquatic macroinvertebrates and the kinetics of leaf-litter breakdown in streams with different land uses (*i.e.*, urban, agriculture, and preserved forested areas), documented a higher richness and diversity in the less-disturbed streams. Consistent with our results, sensitive macroinvertebrates were found in only those less-disturbed streams, with that community being significantly different from agriculture- and urban-associated assemblages. Similarly, Fierro *et al.* (2017) – investigating invertebrate assemblages at sites in Chilean rivers that were adjacent to native forest, forest plantation, and agricultural land uses – reported higher nutrient concentrations and a lower macroinvertebrate richness and diversity in the locations associated with agricultural catchments than those affected by other land uses. Because our observations in horticulture-related streams were similar to those reported above in water bodies adjacent to agricultural basins, the agrochemicals employed in both land-use activities in the present work were presumably responsible for the similar effects.

## 5 Conclusions

Macroinvertebrate assemblages and stream-water quality are affected by horticultural land use in the adjacent plots in the Buenos Aires province of Argentina. Fertilizers and pesticides reach nearby streams, increasing nutrient concentrations and altering macroinvertebrate composition. Horticulture-associated streams exhibited assemblages of tolerant taxa along with a lower taxonomic richness and density. *Hyalella* sp., *Simocephalus vetulus*, Baetidae, and *Caenis* sp. – considered taxa sensitive to insecticide exposure – are absent or present at low densities in those horticulture-affected streams, while were well represented in the less-impacted ones. In contrast, Dugessidae, Glossiphoniidae, and Entomobryioidea – considered comparatively tolerant taxa – were dominant in the streams adjoining horticulture. The composition of macroinvertebrate assemblages thus represents a reliable indicator for analyzing land use in lowland streams. We therefore propose *S. vetulus* and *Hyalella* sp. – those being common and abundant macroinvertebrates in the environments of this region that are, for their part, highly sensitive to pesticides – to be reliable indicators of the impact of pesticides on the water bodies in this area.

## Supplementary Material

Supplementary Table S1.
The Supplementary Material is available at https://www.kmae-journal.org/10.1051/kmae/2019048/olm.

*Acknowledgements.* This research was funded by the National Scientific and Technical Research Council (CONICET; PIP 2011 N° 0180) and the Argentine National Agency for the Promotion of Science and Technology (ANPCyT; PICT 2010–0446). We acknowledge the anonymous reviewers for valuable suggestions and comments. Dr. Donald F. Haggerty, a retired academic career investigator and native English speaker, edited the final version of the manuscript.

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**Cite this article as**: Arias M, Scalise A, Solis M, Paracampo A, Indaco M, Fanelli S, Mugni H, Bonetto C. 2020. Horticulture affects macroinvertebrate assemblages in adjacent streams (Buenos Aires, Argentina). *Knowl. Manag. Aquat. Ecosyst.*, 421, 5.