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Influence of agricultural practices on the composition of macroinvertebrate assemblages in the Neuquén River, Patagonia (Argentina)

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Abstract. Aquatic species in agricultural areas are often exposed to concentrations of pesticides and fertilizers that can affect the entire community. This study aimed to monitor the presence of nutrients and pesticides (organophosphates and carbamates) and evaluate their effects on the composition of macroinvertebrate assemblages at two sites in the Neuquén River, both upstream (UNR) and downstream (DNR) of an area of intensive cultivation of pome fruit trees. Physicochemical parameters in water samples from UNR and DNR were not significantly different, except for nitrate (NO_3^-). Pesticides analyzed were below detection limits in water and sediment samples. Insects constituted 52.76% of the total individuals in UNR and, among them, Ephemeroptera was the most abundant taxon (n=419). On the other hand, 67.67% of the individuals in DNR were insects, and most of them belonged to the Order Diptera (n=1156). The abundance of Diptera and Ephemeroptera individuals was significantly different between sites. In addition, the temporal variation of richness within each site showed significant differences (P<0.05). There were 15 shared species and a Jaccard's abundance-based index indicated 37.8% taxonomic similarity. In summary, there were no significant differences in abundance and diversity between UNR and DNR. However, there were differences in taxa richness and composition, with a low percentage of similarity between sites. The above can be observed in the canonical correspondence analysis, which shows a clear relationship between environmental variables and the distribution pattern of macroinvertebrate assemblages.

[Keywords: aquatic invertebrates, pesticides, fertilizers, aquatic pollution]

Resumen. **Influencia de las prácticas agrícolas en los ensambles de macroinvertebrados en la Patagonia (Argentina).** Las especies acuáticas de las zonas agrícolas suelen estar expuestas a concentraciones de plaguicidas y fertilizantes que pueden afectar a toda la comunidad. El objetivo del estudio fue monitorear la presencia de nutrientes y plaguicidas (organofosforados y carbamatos) y evaluar los efectos sobre la composición de los ensambles de macroinvertebrados en dos sitios del río Neuquén, aguas arriba (UNR) y aguas abajo (DNR) de un área de cultivo intensivo de frutales de pepita. Los parámetros físicoquímicos en las muestras de agua de UNR y DNR no fueron significativamente diferentes, excepto para el nitrato (NO_3^-). Los plaguicidas analizados estuvieron por debajo de los límites de detección en las muestras de agua y sedimento. Los insectos constituyeron el 52.76% del total de los individuos en UNR y, entre ellos, Ephemeroptera fue el taxón más abundante (n=419). Por otro lado, el 67.67% de los individuos en DNR fueron insectos, y la mayoría de ellos pertenecieron al orden Diptera (n=1156). La abundancia de individuos de Diptera y Ephemeroptera fue significativamente diferente entre los sitios. Además, la variación temporal de la riqueza dentro de cada sitio mostró diferencias significativas (P<0.05). Hubo 15 especies compartidas y el índice de Jaccard basado en la abundancia indicó un 37.8% de similitud taxonómica. En resumen, no se encontraron diferencias significativas en la abundancia y la diversidad entre UNR y DNR. Sin embargo, hubo diferencias en la riqueza y la composición de las taxa, con un bajo porcentaje de similitud entre los sitios. Esto último puede observarse en el análisis de correspondencia canónica, que muestra una relación clara entre las variables ambientales y el patrón de distribución de los ensambles de macroinvertebrados.

[Palabras clave: invertebrados acuáticos, plaguicidas, fertilizantes, contaminación acuática]

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Introduction

Agricultural practices can modify the overall health of the aquatic ecosystem through organic pollution and eutrophication (Yang et al. 2008; Matthaei et al. 2010) and negatively affect non-target species such as macroinvertebrates, that inhabit agricultural streams (Barmentlo et al. 2019). Agricultural streams receive the nutrient load after fertilization, as well as suspended material and other components that drain from the fields (Schäfer et al. 2011; Stefanidis et al. 2015). Pesticides can also reach surface waters through atmospheric drift after their application, by surface runoff or by seepage of contaminated groundwater (Phillips and Bode 2004; Gärdenäs et al. 2006; Loewy et al. 2011). Therefore, multiple stressors, which rarely operate in isolation, can alter an entire ecosystem's functioning, and the combined effects of these stressors are complex and difficult to predict (Matthaei et al. 2010; Côté et al. 2016; Barmentlo et al. 2019). Moreover, these stress factors can modify the physicochemical and biological properties of water, whilst also contributing to other stress factors that modify the structure of macroinvertebrate assemblages, reducing species richness (Zacharia 2011; Stefanidis et al. 2015; Zhang et al. 2021).

Macroinvertebrates are highly sensitive to contamination and play important ecological functions through energy flow, nutrient cycling and the decomposition of organic matter (Wallace and Webster 1996). They are constantly exposed to fertilizers and a wide array of pesticides (Barmentlo et al. 2018; Macchi et al. 2018) to which they are particularly susceptible (Rico and Van den Brink 2015) since they cannot easily avoid exposure by moving to uncontaminated areas, especially when pesticides are water-soluble. Macroinvertebrate assemblages have been extensively used to assess stream integrity since they possess valuable characteristics compared to other groups of organisms (Miserendino et al. 2008; Infante et al. 2009). They are ubiquitous and diverse, exhibit different feeding habits, are sedentary, have life cycles ranging from a few weeks to a few years, exhibit a tolerance range to contaminants and are also of a convenient size for field examination, storage and transportation (Bonada et al. 2006).

Several previous studies demonstrated that macroinvertebrates can respond negatively to increased nutrient loads, which affects species richness in streams (Ouyang et al. 2018) by reducing the number of taxa and causing a shift in the assemblages' structure by increasing the dominance of tolerant taxa and removing sensitive taxa (Gafner and Robinson 2007; Cornejo et al. 2019). However, it is not clear how nutrients shape a particular biological assemblage (Paisley et al. 2011).

Nitrogen and phosphorus derived from fertilizers can have a subsidy effect on macroinvertebrate response variables at low levels but can act as stressors at high levels (Wagenhoff et al. 2012). Nutrient enrichment was shown to increase the diversity and abundance of periphyton, thus taxa that feed on the periphyton might benefit from nutrient inputs (King and Richardson 2007). Juvigny-Khenafou et al. (2020) and Piggott et al. (2015) observed physical growth in most taxa exposed to high nutrient content, as well as an increase in taxa abundance and a decrease in diversity. Another important effect reported by these authors is the increased drift of taxa when nutrient load increases. Negative correlations between sensitive taxa, pesticides and soluble reactive phosphorus were recently highlighted by Marrochi et al. (2020) in Pampean streams.

The effects of pesticides on macroinvertebrate assemblages have been widely studied, with the research highlighting the disappearance of sensitive species and a decrease in abundance (Liess and Von Der Ohe 2005; Schäfer et al. 2007; Beketov et al. 2013). Beketov and Liess (2008) showed that pesticides can initiate the drift of macroinvertebrates a few hours after contamination at concentrations that cause no mortality at short-term pulse exposures. Although the present study also reported a negative correlation between the pesticide chlorpyrifos and the richness and abundance of macroinvertebrates in irrigation and drainage channels, little is known about the physicochemical conditions of the Neuquén River and the composition of the assemblages (Macchi et al. 2018).

Pome fruit production is an important economic activity in Argentina's northern Patagonia, and is characterized mainly by the production of apples and pears. Agriculture in this area requires a network of irrigation channels that derives from the Limay, Neuquén and Negro rivers. A drainage network system then returns excess irrigation water into nearby rivers. Pesticides and nitrogen fertilizers are the most important agrochemicals required for this activity (Kohlmann et al. 2018).

The most commonly used insecticides for *Cydia pomonella* control, a principal pest of apple and pear trees in the region have been the organophosphates and carbamates (Cichón et al. 2015) whose residues are found in groundwater and irrigation channels in the study area (Loewy et al. 1999; Loewy et al. 2003; Loewy et al. 2006; Tosi et al. 2009; Loewy et al. 2011; Macchi et al. 2018). Moreover, chlorpyrifos, azinphos-methyl and carbaryl were found in the Neuquén River, in concentrations ranging from trace values to a maximum of 0.032, 0.19 and 0.120 μg/L, respectively (AIC 2012). The applications of these insecticides to control *Cydia pomonella* were significantly reduced by restrictions on their use on pome fruits (Villarreal et al. 2010). Azinphos-methyl application in Argentina was definitely banned in 2017 (SENASA 2016) and, more recently, chlorpyrifos was banned (tinyurl.com/yujj2f3y). However, vegetable pests in the area continue to be controlled by these types of pesticides, in particular chlorpyrifos, which has been detected both in crops where this insecticide is registered and those in which it is not (Zanetta 2012; Sánchez et al. 2019). Therefore, the aim of this study was to monitor the presence of nutrients and pesticides (organophosphates and carbamates) and evaluate their possible effects on the composition of macroinvertebrate assemblages in two sites of the Neuquén River, upstream (UNR) and downstream (DNR) of an area of intensive cultivation of pome fruit trees.

Materials and Methods

Site description

The study area was located in easternmost Neuquén province, specifically between the towns of Vista Alegre, Centenario and Neuquén. The climate varies from dry sub-humid to arid, with an average annual temperature between 10 and 14 °C (FAO 2015). During December and January, the monthly average temperatures is 24 °C, with maximums of 40 °C, while in July, the average temperature is 7 °C. The strong winds that characterize the area usually range between 20 and 120 km/h in a west-southeast direction. There is very little

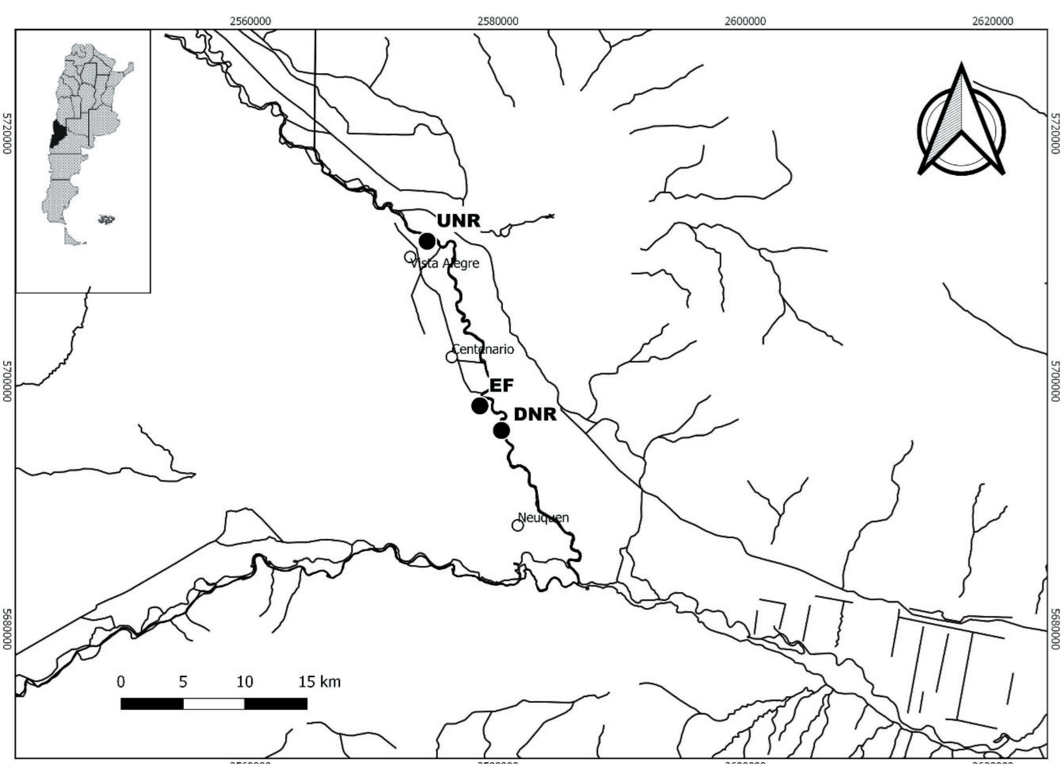

Figure 1. Sampling sites in the Río Negro and Neuquén Valley (Argentina). UNR (upstream) and DNR (downstream) of agricultural area. Black circles indicate the sampling sites and the white circles show the locations.

Figura 1. Sitios de muestreo en el Valle de Río Negro y Neuquén (Argentina). UNR (aguas arriba) y DNR (aguas abajo) del área agrícola. Los círculos negros indican los sitios de muestreo y los círculos blancos muestran las localidades.

rain in the whole region, and mean annual precipitation oscillates between 100 and 350 mm in most of the area (Labraga and Villalba 2009). Because of this, agricultural production (mainly apples and pears) requires a network of irrigation and drainage channels that flow directly to the Neuquén River.

Sampling of macroinvertebrates, water and sediments was performed at the same time at UNR (38°51'18.73'' S - 68°6'38.19'' W) and DNR (38°52'58.84'' S - 68°04'29.69'' W) (Figure 1). Between the two sampling sites, ~15 km apart, sits a drainage channel (EF) with a history of high pesticide contamination. Moreover, chlorpyrifos was detected in sediments (23 μg/kg) from this drainage in 2015. The river has clear waters with low-bottom vegetation, flow rates of between 70 and 130 m^3/s and 60 m of mean width. The substrate is mostly composed of medium-to-large sized stones and sand. The predominant riparian vegetation includes poplars and willows.

Water sampling and analytical methodology

Before water sampling, temperature (°C), pH, electrical conductivity (μS/cm) and dissolved oxygen (mg/L) were measured with multiparametric equipment (PASCO). Additionally, water flow (m^3/s) data was provided by the Interjurisdictional Watershed Authority (hereon referred to as AIC - Autoridad Interjurisdiccional de Cuencas).

Samples were collected during two crop production seasons, November 2014 and February, October and December 2015 (spring-summer period). Water samples for nutrient determination (0.5 L) were filtered with cellulose acetate filters (0.45 μm pore size) within 2 h of collection and frozen for later analysis. Soluble reactive phosphorus (SRP) was determined by the molybdate blue, ascorbic acid method (Golterman 1978). Nitrate (NO_3^-) (cadmium column reduction followed by diazotization method) and nitrite (NO_2^-) (diazotization) followed the methodology set out by APHA (1992). Ammonium (NH_4^+) (indophenol-blue method) was analyzed according to the method outlined by Mackereth (1979).

Water samples for pesticide organophosphates (chlorpyrifos and azinphos-methyl) and carbamates (carbaryl) (1 L) analyzes were collected in amber glass and plastic bottles, respectively. Samples were kept refrigerated during transport to the laboratory and until their chemical determinations. Water samples for pesticide determination were filtered and analyzed by solid-phase extraction (EPA 3535A with modifications), with a reverse phase polymer cartridge (StrataTM Phenomenex). After drying under a nitrogen stream, the cartridges were eluted with hexane and methylene chloride. The extracts were concentrated to dryness and 0.25 mL of hexane was added. The extracts were analyzed by gas chromatography with a nitrogen-phosphorus detector (GC-NPD). Identification and quantification of the compounds were performed by external calibration curves. Confirmation was performed by gas chromatography coupled to a mass spectrometer (GC-MS). The recovery percentages ranged between 70 and 110%, with a variation coefficient lower than 12%. Linearity was measured by R^2 coefficient for the individual pesticide calibration curves, always resulting ≥0.99. Each set of samples was analyzed by duplicate, simultaneously with a laboratory blank. The limits of detection (LOD) and quantitation (LOQ) were 0.07 and 0.10 μg/L, respectively.

Sediment sampling and analytical methodology

Sediment samples were collected with a stainless-steel scoop in glass jars (0.5 kg) and kept refrigerated during transport to the laboratory. Insecticide extraction from sediment samples was carried out in small columns assisted by ultrasound (SAESC). Approximately 10 g of soil, dried and sieved, were placed in 20 mL polypropylene columns, conditioned with glass fiber filters (GMF) and anhydrous sodium sulfate. Eight mL of ethyl acetate was added to each column and sonicated in an ultrasonic bath for 15 minutes. The columns were then brought to the filtration system and the liquid extracts collected in glass-graduated tubes at atmospheric pressure, allowing a slow and steady trickling. After all the liquid was collected, 8 mL of ethyl acetate was added and the procedure was repeated. Finally, 2 mL of solvent was added to each final rinse column and allowed to filter completely. The extracts were concentrated under a nitrogen stream to a volume of 10 mL and transferred to a vial. Finally, they were injected into a gas chromatograph equipped with a nitrogen and phosphorus detector (NPD).

The recovery percentages ranged between 75 and 120%, with a variation coefficient lower than 20%. The limits of detection (LOD) and quantitation (LOQ) were 7 μg/kg and 20 μg/kg dried weight, respectively. The quantification of pesticides from the extracts followed the same methodology as water samples.

Macroinvertebrate sampling and assemblage characterization

To characterize the macroinvertebrate assemblages, three replicates of macroinvertebrate samples per sampling date (n=12) were collected from each site at the same time as water and sediment collection took place, with a Surber net sampler (0.09 m^2, 500 μm mesh size), fixed *in situ* with 4% formaldehyde and sorted in the laboratory under at least 5x magnification. Macroinvertebrate samples were identified to the lowest possible taxonomic level using regional keys (Lopretto and Tell 1995; Fernández and Domínguez 2001; Domínguez and Fernández 2009). A set of biological variables such as taxon richness (number of taxa), abundance (number of specimens), Shannon diversity index and similarity index were determined.

Statistical analyses

Physicochemical variables were analyzed using the non-parametric Mann-Whitney U test. Temporal variation in mean abundance within sites and biological variables such as order abundance, richness and macroinvertebrate diversity between sampling sites were compared using one-way ANOVA and *post-hoc* Tukey.

In addition, estimates of total species richness via multiple non-parametric richness estimators were generated using EstimateS 9.1.0 (Colwell 2013). These estimates were used to evaluate sampling completeness and compare species richness across sampling periods. The abundance-based estimators ACE (Abundance Coverage-based Estimator), Chao (Chao and Ma 1993) and Chao 1 (Chao 1984) together with the incidence-based estimators, Chao 2 (Chao 1987) and Bootstrap (Smith and van Belle 1984) were calculated with 100 randomizations without replacement and a default value of 10 for the upper limit for rare taxa. Sampling effort was evaluated by species accumulation curves produced, while sampling efficiency was calculated as the quotient between Observed species (Sobs) and Estimated species (Sest). A series of species accumulation curves, which graph the cumulative number of observed species as a function of sampling effort, was developed using the software EstimateS (Colwell et al. 2004). The abundance-based Jaccard, which adjusts the Jaccard similarity index for the effect of unseen shared species (Chao et al. 2006), was selected to estimate similarity between sites using the software EstimateS.

The relationship between macroinvertebrate assemblages and environmental variables was examined with a Canonical Correspondence Analysis (CCA) with down-weighting of rare species carried out using CANOCO (ter Braak and Smilauer 1999). Average seasonal values (means of three samples per site) were used in the analysis. All variables (except pH) and species densities were transformed (log x+1) prior to analysis. Variables that were strongly intercorrelated with others (those with an inflation factor <20) in the initial analysis were removed (conductivity, pH, and SRP) and a further analysis was carried out with the remaining environmental variables. A Monte Carlo permutation test was used to verify the significance of the models (ter Braak and Smilauer 1999).

Results

Physicochemical analysis

Physicochemical water parameters (Table 1) were not significantly different between sites, except for NO_3^- (U-Mann-W, P=0.029). Nutrient concentrations were highly variable across sampling dates, especially at DNR (Figure 2) where the maximum concentrations of SRP (17.40 μg/L), NO_3^- (27.10 μg/L) and NH_4^+ (65.00 μg/L) were recorded in November, and the maximum concentrations of NO_2^- (0.012 μg/L) in October. Ammonium concentration was high at DNR in October (64.00 μg/L). On the other hand, the lowest nutrient concentrations at DNR were recorded in February. The organophosphates (chlorpyrifos and azinphos-methyl) and carbamates (carbaryl) analyzed were not detected, neither in the water nor in the sediments.

Macroinvertebrate assemblages and their relationship with environmental variables

The total number of macroinvertebrate individuals from UNR consisted of 1325 individuals (mean 110 individuals, n=12) belonging to 8 orders and 23 taxa. At DNR, the total sample comprised 1769 individuals

Table 1. Mean concentration ± SD of physic-chemical parameters. P-value (Mann-Whitney U test).
Tabla 1. Concentración media ± SD de los parámetros físico-químicos. P-valor (prueba U de Mann-Whitney).

Parameter	UNR	DNR	P-value
Temperature (°C)	19.67 ± 5.33	19.97 ± 2.70	0.68
Water Flow (m^3/s)	93.70 ± 34.35	94.42 ± 33.57	0.99
pH	7.90 ± 0.60	7.50 ± 0.30	0.20
Conductivity (μS/cm)	0.27 ± 0.02	0.28 ± 0.020	0.68
Dissolved oxygen (mg/L)	9.86 ± 2.70	10.00 ± 1.80	0.68
SRP (μg/L)	2.70 ± 1.60	7.22 ± 7.19	0.48
NO_3^- (μg/L)	1.87 ± 1.32	12.05 ± 10.31	0.029*
NO_2^- (μg/L)	1.00 ± 0	1.75 ± 1.50	0.68
NH_4^+ (μg/L)	6.52 ± 6.15	34.45 ± 34.72	0.34

* significant difference

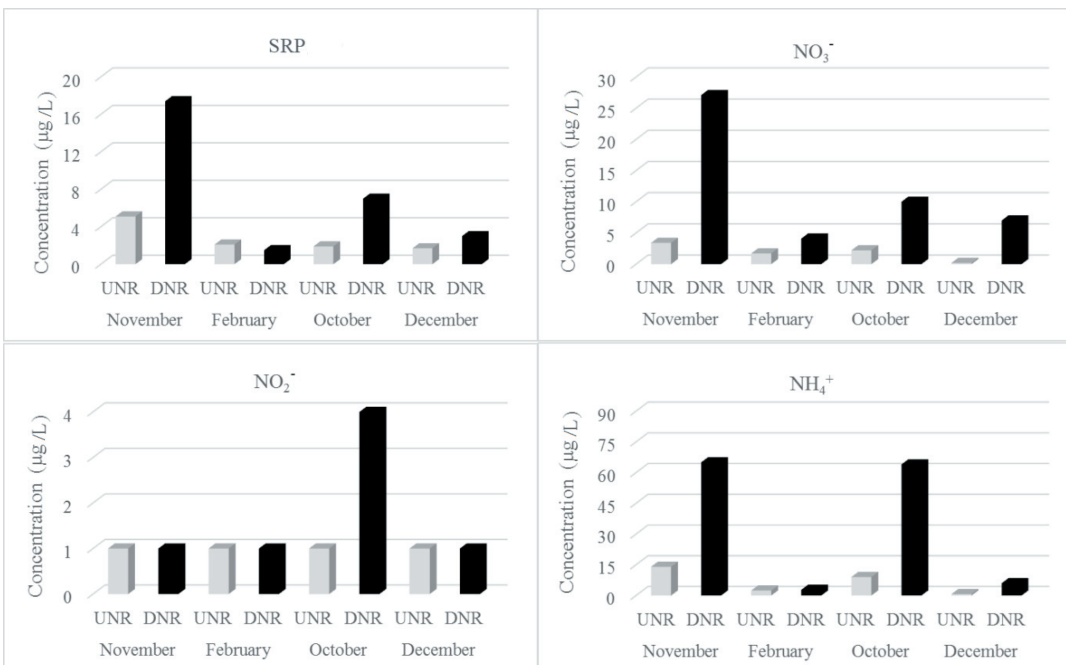

Figure 2. Nutrient concentrations between sites and sampling dates.
Figura 2. Concentraciones de nutrientes entre sitios y fechas de muestreo.

(mean 148 individuals n=12) belonging to 11 orders and 33 taxa (Table 2). The mean abundances of total individuals at UNR and DNR were not significantly different to each other (ANOVA, $F_{1,22}$=0.58; P=0.45). Insects comprised 52.76% of total abundance at UNR and among them, Ephemeroptera was the most abundant taxa (n=419) represented by *Meridialaris laminate*, *Americabaetis alphus*, *Baetodes* sp. and *Caenis* sp. (Table 2, Figure 3). On the other hand, 67.67% of individuals at DNR were insects, and the majority of them belonged to the Order Diptera (n=1156), with 10 taxa (*Simulium* sp., *Tanytarsus* sp., *Polypedilum* sp., *Chironomus* sp., *Cricotopus* sp., *Paracricotopus* sp., *Paratrichocladius* sp., *Dicrotendipes* sp., *Empididae* sp. and *Tipulidae* sp.). The abundance of individuals from Diptera (ANOVA, $F_{1,22}$=9.9, P=0.0045) and Ephemeroptera (ANOVA, $F_{1,22}$=9.1, P=0.0061) was significantly different between sites.

Macroinvertebrate richness at UNR (23 taxa) was lower than at DNR (33 taxa) although mean richness between sites was not significantly different (ANOVA. $F_{1,22}$=1.35, P=0.25). Species belonging to the orders Coleoptera (*Dytiscidae* sp., *Elmidae* sp. and *Berosus* sp.), Odonata (*Cyanallagma interruptum* and *Ischnura fluviatilis*), Hemiptera (*Sigara* sp.) and Lepidoptera (*Paraponyx* sp.) were identified only at DNR as singletons (occurring

Table 2. Mean values (n=12) ± standard deviations of macroinvertebrate abundance in UNR and DNR in the Neuquén River.

Tabla 2. Valores medios (n=12) ± desviación estándar de la abundancia de macroinvertebrados en UNR y DNR en el Río Neuquén.

Taxa	UNR	DNR
Platyhelminthes		
Dugesiidae		
Girardia tigrina	6.83 ± 10.2	12.08 ± 21.9
Annelida		
Enchytraeidae	0.83 ± 2.3	
Naididae		
Dero sp.	0.16 ± 0.5	
Tubifex sp.		11.25 ± 14.5
Lumbriculidae		
Lumbriculus variegatus	8.75 ± 10.8	2.83 ± 6.7
Glossiphoniidae		
Helobdella sp.	1.16 ± 3.45	
Mollusca		
Gastropoda		
Hydrobiidae		
Heleobia hatcheri	5.08 ± 11.8	
Heleobia parchapii	1.5 ± 2.3	2.58 ± 4.9
Chilinidae		
Chilina gibbosa	2.66 ± 5.7	1.33 ± 2.6
Lymnaeidae		1.33 ± 2.7
Lymnaea viatrix	0.08 ± 0.3	
Bivalvia		
Sphaeriidae		
Musculium sp.		8.16 ± 14.6
Corbiculidae		
Corbicula fluminea	3.16 ± 5.16	2 ± 2.9
Arthropoda		
Amphipoda		
Hyalella curvispina	21.66 ± 40.3	4.66 ± 8.3
Decapoda		
Aeglidae		
Aegla sp.	0.25 ± 0.86	
Ephemeroptera		
Leptophlebiidae		
Meridialaris laminata	13.08 ± 24.5	
Meridialaris diguilina		0.25 ± 0.86
Penaphlebia chilensis		0.33 ± 1.15
Baetidae		
Americabaetis alphus	21 ± 36	1.83 ± 4.06
Baetodes sp.	0.25 ± 0.86	0.08 ± 0.28
Caenidae		0.91 ± 2.3
Caenis sp.	0.58 ± 1.7	
Odonata		
Coenagrionidae		
Cyanallagma interruptum		0.16 ± 0.38
Ischnura fluviatilis		0.08 ± 0.28
Aeshnidae		
Rhionaeschna sp.		0.08 ± 0.28
Hemiptera		
Corixidae		
Sigara sp.		0.08 ± 0.28
Coleoptera		
Dytiscidae		0.16 ± 0.38
Elmidae		0.08 ± 0.28
Hydrophilidae		
Berosus sp.		0.08 ± 0.28
Diptera		
Simuliidae		
Simulium sp.	0.16 ± 0.6	3.66 ± 11.1
Muscidae sp.	0.16 ± 0.6	
Chironomidae		
Tanytarsus sp.	0.25 ± 0.45	4.33 ± 8.9
Polypedilum sp.		2.91 ± 6.2
Chironomus sp.		1.5 ± 3.06
Cricotopus sp.		6.08 ± 12.3
Paracricotopus sp.		42.58 ± 118.7
Paratrichocladius sp.	10.16 ± 22	23.08 ± 24.2
Dicrotendipes sp.		11.83 ± 25.8
Empididae		
Hemerodromia sp.		0.083 ± 0.28
Tipulidae sp.		0.25 ± 0.86
Trichoptera		
Glossosomatidae		
Mastigoptila sp.	0.41 ± 1.4	
Hydropsychidae		
Smicridea annulicornis	8.41 ± 14.6	0.5 ± 1
Hydroptilidae		
Metrichia neotropicalis	3.75 ± 11.45	0.16 ± 0.38
Lepidoptera		
Crambidae		
Parapoynx sp		0.08 ± 0.28

Table 2. Continuation.

Tabla 2. Continuación.

only once) or doubletons (occurring twice). On the other hand, the freshwater crabs *Aegla* sp. (Decapoda) were only found at UNR.

To estimate total macroinvertebrate species richness, accumulation curves were constructed using multiple non-parametric richness estimators. The estimated taxa richness (Chao1, Chao2, Jackknife 1, ACE, Bootstrap) in both sites were similar to the observed number of taxa (Table 3). The species accumulation curves of the estimated richness

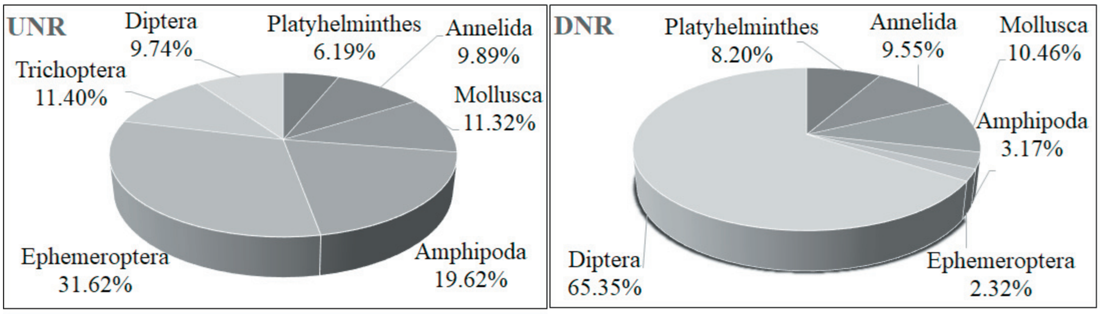

Figure 3. Abundance of macroinvertebrates groups on the four sampling dates from UNR and DNR (macroinvertebrates groups comprising less than 1% of the total were not included).

Figura 3. Abundancia de grupos de macroinvertebrados en las cuatro fechas de muestreo de UNR y DNR (no se incluyeron los grupos de macroinvertebrados que comprenden menos del 1% del total).

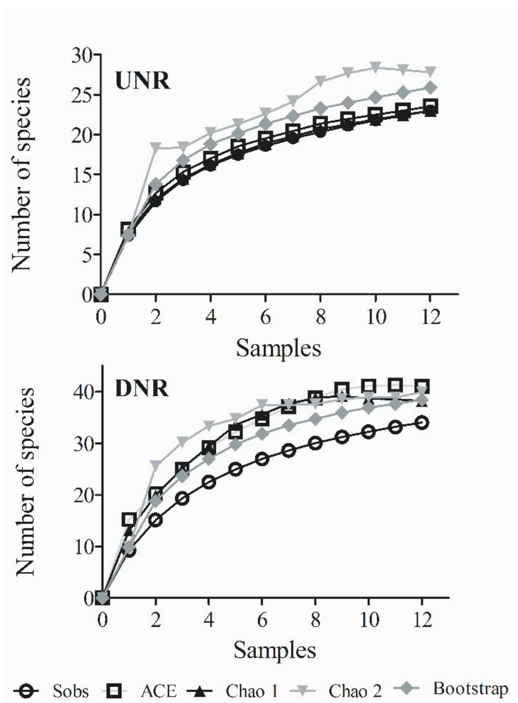

Figure 4. Species-accumulation curves for observed richness (Sobs) and richness estimators (ACE, Chao 1, Chao 2 and Bootstrap).

Figura 4. Curvas de acumulación de especies para la riqueza observada (Sobs) y estimadores de riqueza (ACE, Chao 1, Chao 2 y Bootstrap).

for benthic invertebrate samples from UNR and DNR were close to reaching an asymptote with 25 and 40 species respectively (Figure 4). The Shannon diversity indices for UNR (1.40±0.44) and DNR (1.40±0.55) were not statistically significant (ANOVA, $F_{1,22}$=0.0007, P=0.97). This indicates similar species diversity among the sites. Finally, there were 15 shared species between sites and the abundance-based Jaccard index indicated a taxonomic similarity of 37.8%.

Regarding temporal variations, mean abundances and the Shannon diversity index showed no significant differences within each site (P>0.05). In turn, an examination of the temporal variation of richness per sampling site showed that UNR was significantly different between February and October (ANOVA, $F_{3,8}$=5, P=0.037). Richness in DNR was different in October when compared to November (ANOVA, $F_{3,8}$=10.3, P=0.025), February (ANOVA, $F_{3,8}$=10.3, P=0.013) and December (ANOVA $F_{3,8}$=10.3, P=0.035 (Table 4).

The CCA ordination showed the species, the environmental variables and the sampling sites on the four sampling dates in November, February, October and December. UNR data were clustered in the right quadrants (top and bottom) of the biplot, while DNR data were clustered in the left quadrants. The first two axes of the CCA explained 54.1% of the cumulative variance of the data set. The first axis (eigenvalue CCA1: 0.52) was significant (P<0.02) and represented an environmental gradient mainly defined by nitrates and ammonia and secondarily by nitrites. The second axis (eigenvalue CCA2: 0.35) represented the relationship between oxygen concentration and water temperature (Figure 5a).

The species-environment correlations were 0.99 and 0.98 for the two first axes, and an unrestricted Monte Carlo permutation test indicated that all the axes were significant (F=1.47, P<0.03). This indicates a strong relationship between macroinvertebrate abundance and the measured environmental variables. The CCA species ordination plot illustrates the position of macroinvertebrate taxa along the same gradients (Figure 5b). *Americabaetis alphus*, *Metrichia neotropicalis*,

Table 3. Observed and estimated species, and percentage of efficiency of the estimators.

Tabla 3. Especies observadas y estimadas, y porcentaje de eficacia de los estimadores.

				Richness estimators						% Efficiency				
Site	Taxa	S	D	Obs. taxa	Chao 1	Chao 2	Jack-knife 1	ACE	Boots-trap	Chao 1	Chao 2	Jack-knife 1	ACE	Boots-trap
UNR	1325	1	3	23	23	28	29	24	26	100	82.7	78.1	97.7	88.9
DNR	1769	7	4	33	38	40	43	41	38	86.3	82.7	76.4	80.4	85.9

S = singletons; D = doubletons

Table 4. Mean values and standard deviation of the temporal variations within the sites for abundance, richness and Shannon Diversity.

Tabla 4. Valores medios y desviación estándar de las variaciones temporales dentro de los sitios para la abundancia, riqueza y diversidad de Shannon.

		Richness (S)	Abundance (N)	Diversity Shannon
UNR	November 2014	8.6 ± 1.5 ab	137.7 ± 98	1.6 ± 0.4
	February 2015	3.6 ± 2.8 a	41.6 ± 14.6	0.91 ± 0.62
	October 2015	9 ± 1.7 b	131.7 ± 65.52	1.57 ± 0.04
	December 2015	8 ± 1 ab	130 ± 88	1.5 ± 0.22
DNR	November 2014	8.3 ± 2.8 a	232 ± 264	1.19 ± 0.36
	February 2015	7.3 ± 2.5 a	77 ± 43.28	1.18 ± 0.13
	October 2015	16.3 ± 3.5 b	229 ± 103.6	2.25 ± 0.21
	December 2015	5 ± 1 a	51.3 ± 11	0.98 ± 0.18

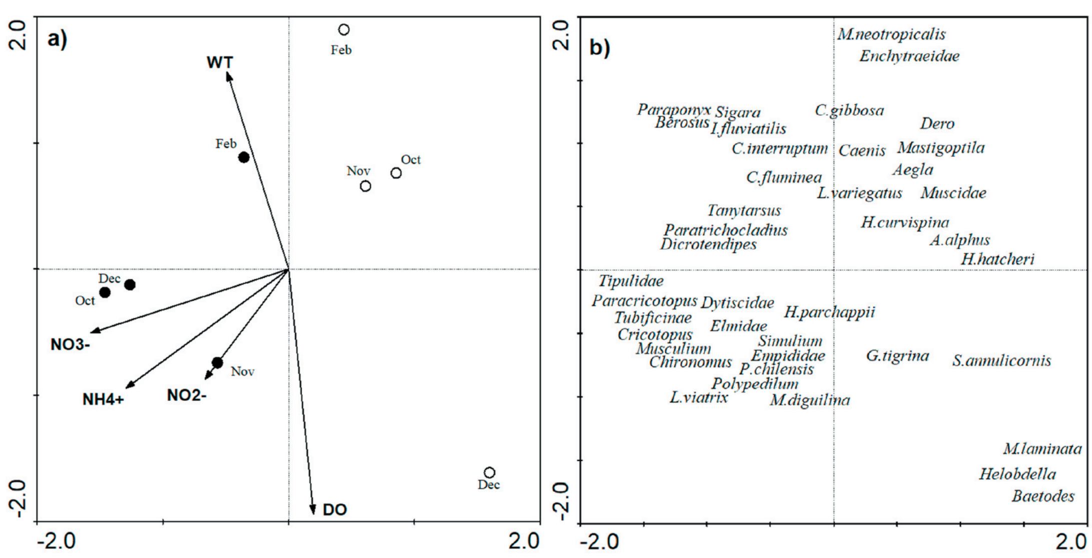

Figure 5. a) CCA ordination diagram for UNR (empty circles) and DNR (black circles) sites, and environmental relationships based on abundance data for 41 macroinvertebrate taxa in November (Nov), December (Dec), February (Feb) and October (Oct); b) Ordenación de especies de macroinvertebrados en los dos primeros ejes ambientales del CCA.

Figura 5. a) Diagrama de ordenación CCA para sitios UNR (círculos vacíos) y DNR (círculos negros) y relaciones ambientales basadas en datos de abundancia para 41 taxones de macroinvertebrados en noviembre (Nov), diciembre (Dec), febrero (Feb) y octubre (Oct); b). Ordination of macroinvertebrate species on the first two environmental axes of the CCA.

Hyalella curvispina, *Aegla* sp., *Heleobia hatcherii* and *Lumbriculus variegatus* exhibited maximum density in November, October and February (upper right quadrant) and were taxa that were characteristic of the UNR. *Meridialaris laminata*, *Baetodes* sp., *Smicridea annulicornis* and *Helobdella* sp. were more abundant at the same site in December (lower right quadrant). The species recorded at the DNR site (which recorded a higher concentration of nutrients) —more abundant in October, November and December— primarily consisted of Chironomidae (*Paracricotopus* sp., *Cricotopus* sp., *Tanytarsus* sp., *Chironomus* sp. and *Polypedilum* sp.), Tipulidae, *Simulium* sp., *Muscullium* sp. and Tubificinae (upper left quadrant). Finally, other Chironomidae (*Paratrichocladius* sp., *Tanytarsus* sp. and *Dicrotendipes* sp.), along with *Corbicula fluminea* and *Cyanallagma interruptum*, were associated with DNR site in February.

Discussion and Conclusions

This study on pesticides and nutrient content in the Neuquén River and their effects on macroinvertebrate assemblages has indicated an absence of pesticides. However, nutrient concentrations can be seen to have contributed to the observed differences in assemblage composition between the sampling sites.

As is well known, the sources of nutrients in surface waters can be both natural and anthropogenic (Khatri and Tyagi 2015). In this vein, agriculture is one of the anthropogenic sources that most contributes to nutrient enrichment in aquatic bodies as diffuse pollution. Levels of inorganic phosphorus and nitrogen are generally increased in surface waters near agricultural sites because of fertilizer applications (Manuel 2014; Marrochi et al. 2020).

The results of this study show no large variation in nutrient levels at the UNR site. Conversely, at the DNR site, nutrients reach higher concentrations during spring (October and November) and are at their lowest at the end of the production season (February). These results reflect the pattern of fertilizer use in agriculture since most of the spraying of organophosphate pesticides takes place between these months (Macchi et al. 2018).

Various studies infer that a higher nutrient load can produce direct effects in macroinvertebrate communities. Alonso and Camargo (2006) reported acute toxicity of nitrite (LC_{50} [96 h] 2.09-60.0 mg/L) on several macroinvertebrate species. Other authors have recommended biological thresholds for total N and P in stream waters ranging from 2.4-3.1 mg/L and 0.084-0.2 mg/L, respectively (Hinsby et al. 2012; Liang et al. 2014).

In the present work, the maximum concentrations determined were always lower than those reported above, including those previously reported by Kohlmann et al. (2018) in the same agricultural area of study. However, the CCA analysis showed a clear relationship between nutrient content and the surveyed species at each site. At higher NO_3^-, NH_4^+ and NO_2^- concentrations, the more tolerant groups prevailed. In contrast, at lower nutrient content levels and higher dissolved oxygen concentrations, the associated groups were some of the most susceptible. Consistent with this, Paisley et al. (2011), Stefanidis et al. (2015) and Zhang et al. (2018) reported that the most susceptible groups (Ephemeroptera, Plecoptera, Trichoptera) and those most tolerant to nutrient enrichment (Annelida, Mollusca, Diptera) were strongly associated with the sites which showed the lowest and highest levels of nutrient loading (NO_3^-, NH_4^+ and, PRS), respectively.

As expected, in the present study macroinvertebrate assemblages at UNR showed a higher abundance and richness of taxa sensitive to organic contamination such as Trichoptera (Glossosomatidae and Hydropsychidae) and Ephemeroptera (Leptophlebiidae, Baetidae and Caenidae) than at DNR. These groups are widely accepted as bioindicators of good water quality (Brasil et al. 2014; Kubendran et al. 2017; Thamsenanupap et al. 2021) and are highly sensitive to agricultural pollution, including nutrients and pesticides (Schulz et al. 2002; Beketov 2004; Thiere and Schulz 2004; Beuter et al. 2019; Vilenica et al. 2020; Rojas-Peña et al. 2021).

Several authors highlighted a decrease in sensitive taxa in watersheds with high agricultural land-use, as well as an increase in some tolerant, non-insect taxa such as nematodes and oligochaete worms (Gerth et al. 2017) and insects such as dipteran (Overmyer et al. 2005; Sánchez-Bayo et al. 2016). In the present study, Diptera comprised 65.35% of the total individuals in DNR; among them, Chironomidae was the family with the highest taxa richness (7 taxa) and made up 96% of dipteran individuals. The family Chironomidae is considered to be tolerant to water pollution, including organic and inorganic compounds (Ossa-López et al. 2018). Particularly, the *Chironomus* genus present in DNR has been associated with highly contaminated sites (Rodrigues-Capítulo and Gómez 2004; Serra 2016). Moreover, the higher abundance of macroinvertebrates in DNR than in UNR could be explained by the extremely high abundance of chironomids, especially *Paracricotopus* sp. and *Paratrichocladius* sp. Similarly, other authors have reported that chironomids were the most abundant taxa in streams with high levels of nutrient enrichment (Camargo et al. 2004; Gafner and Robinson 2007).

The above indicates an indirect effect of nutrient enrichment that may influence macroinvertebrate assemblages (Zhang et al. 2021). When nutrient loads in aquatic ecosystems exceed the capacity for their assimilation by the system, several impacts such as noxious and toxic algal blooms, increased turbidity, oxygen deficiency, disruption of ecosystem functioning, loss of biodiversity, and shifts in food webs may occur (Rabalais 2002; Castillo 2010; Abell et al. 2011; Egler et al. 2012). In addition, the decomposition of organic matter causes an increase in oxygen consumption, favoring the conditions for the development of taxa that live with little oxygen (Stefanidis et al. 2015).

Some orders such as Decapoda, Hemiptera, Lepidoptera, Coleoptera and Odonata were only represented by one or a few species, and not more than four specimens per species (Table 2). Although species from the order Odonata are typically associated with high-quality habitat (Galbrand et al. 2007; Villalobos-Jiménez et al. 2016), in the present study odonates were only found at DNR (*Ischnura fluviatilis* and *Cyanallagma interruptum*). Similar results were observed by Osborn (2005) for the genus *Ischnura*, which was associated in that study with higher levels of ammonia, conductivity, pH and lower levels of oxygen.

Despite reports of previous contamination with pesticides in the Neuquén River (AIC 2012), in this study, neither organophosphates nor carbamates were detected. This could be attributed to the fact that the upper Neuquén valley has been undergoing the displacement of the rural population for several years due to the development of non-agricultural productive activities such as real estate development and the exploitation of petroleum (Sánchez et al. 2016; Catoira 2017; Reggiani 2018). Concordant with this and according to the annual statistical reports of the National Food Safety and Quality Service (SENASA), the productive area of pome fruit in the province of Neuquén has decreased considerably in the last ten years (SENASA 2020).

Previous research conducted in the agricultural area near the study sites reported the presence of organophosphates and carbamates. In 2008, maximum concentrations for chlorpyrifos, azinphos-methyl and carbaryl were 1.16, 22.48 and 45.7 μg/L, respectively (Loewy et al. 2011).

The authors of the present study monitored pesticides in irrigation and drainage channels in the same area from 2008 to 2011, observing a decrease in the range of concentrations for these pesticides from non-detected (ND) to 1.45, 1.02 and 11.21 μg/L, respectively (Macchi et al. 2018). Likewise, during 2011-2012, the same authors found that the maximum concentration detected in water was 0.019 μg/L for chlorpyrifos and 0.26 μg/L for azinphos-methyl, while carbaryl was only found at trace level. On the other hand, sediment concentrations of pesticides were higher, chlorpyrifos ranged between 1 and 38.5 μg/L, and azinphos-methyl, between 1.8 and 2.1 μg/L (Lares 2014). In these studies, the effects on macroinvertebrate assemblages were also evaluated, with differences in abundance and a decrease in taxa richness attributed to organophosphate contamination. It has been established that taxa richness and macroinvertebrate abundance in freshwater ecosystems can decrease as pesticide concentrations increase (Overmyer et al. 2005; Beketov et al. 2013).

Since the implementation of integrated pest management in the upper valley of Río Negro and Neuquén for the control of *Cydia pomonella* in 2006, treatments with organophosphates and other conventional pesticides have been reduced by half (Villarreal et al. 2010; Cichón et al. 2013a) and progressively replaced by other types of insecticides such as neonicotinoids (Cichón et al. 2013b). In turn, over the past few years, anthranilic diamides such as chlorantraniliprole have become the most frequently used insecticide in the area of study (Maero and Anguiano 2018). Thus, insecticides other than organophosphates and carbamates could be reaching the Neuquén River through drainage channels.

In conclusion, the present study demonstrates the impact of agricultural practices on the macroinvertebrate assemblage composition of the River Neuquén. The results show that the higher nutrient content originating from the fertilization of adjacent crops contributes to the observed differences between sampling sites. Both UNR and DNR demonstrated a low percentage of similarity of species between sites, mainly due to the elimination of susceptible species and a higher abundance of more tolerant species in DNR. Considering that one of the limitations of this work was the lack of resources to detect pesticides currently in use, future studies are needed to monitor the presence of pesticides such as neonicotinoids and anthranilic diamides.

The increased monitoring of the physicochemical and biological quality of the study area's water resources in the study area will prove essential in detecting contamination, especially considering the advance of urban occupation over rural areas. Finally, it is also important to consider the socio-political and economic changes that have impacted land-use change in this region in recent years, to understand the environmental impacts in all their complexity. This would contribute to a comprehensive management of water resources, guiding the development of public policies on water resources through reconciliation between economic and social development and the protection and sustainable stewardship of the region's freshwater ecosystems.

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