Macrophytes, epipelic biofilm, and invertebrates as biotic indicators of physical habitat degradation of lowland streams (Argentina)

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Abstract Our objective was to assess the effect of the physical habitat degradation in three lowland streams of Argentina that are subject to different land uses. To address this matter, we looked into some physical habitat alterations, mainly the water quality and channel changes, the impact on macrophytes' community, and the structural and functional descriptors of the epipelic biofilm and invertebrate assemblages. As a consequence of physical and chemical perturbations, we differentiated sampling sites with different degradation levels. The low degraded sites were affected mainly for the suburban land use, the moderately degraded sites for the rural land use, and the highly degraded sites for the urban land use. The data shows that the biotic descriptors that best reflected the environmental degradation were vegetation cover and macrophytes richness, the dominance of tolerant species (epipelic biofilm and invertebrates), algal biomass, O_2 consumption by the epipelic biofilm, and invertebrates' richness and diversity. Furthermore, the results obtained highlight the importance of the macrophytes in the lowland streams, where there is a poor diversification of abiotic substrates and where the macrophytes not only provide shelter but also a food source for invertebrates and other trophic levels such as fish. We also noted that both in benthic communities, invertebrates and epipelic biofilm supplied different information: the habitat's physical structure provided by the macrophytes influenced mainly the invertebrate descriptors; meanwhile, the water quality mainly influenced most of the epipelic biofilm descriptors.

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Introduction

The type and severity of human-generated pressures affecting the integrity of streams is varied, and the major drivers of these changes can be summarized as: multiple uses (such as fisheries, navigation, and drinking water extraction), nutrient enrichment and organic pollution, acidification, and alteration of hydrology and morphology (Malmqvist and Rundle 2002). The final consequence of these drivers is the

degradation of the aquatic ecosystems. According to González del Tánago and García de Jalón (2004), a river is "degraded" when some deficient aspect in composition, in structure, or in function is present, and as a consequence, the ecosystems lose diversity and functionality. At first, when degradation is low, there are changes in the ecosystem's structure and some sensitive species disappear. However, as soon as degradation becomes severe and exceeds a threshold, the basic ecosystem functions (such as self-purification, biomass production, and decomposition) change, significantly, altering the ecological integrity. The degradation level of aquatic systems is closely related with the demand of food and resources made by human beings in production areas, and this demand is certainly met at the expense of the environment (Foley et al. 2005).

In Argentina, the Pampa ecosystem covers 460,000 km^2, includes the most important urban center, sustains the biggest industrial concentration of the country, and supports increased agricultural and livestock production (Cabrera 1976; Soriano et al. 1991; Burkart et al. 1994). The streams crossing this area are characterized by the lack of riparian autochthonous forest vegetation; a low flow rate because of the minimal slope of the surrounding terrain, absence of dry periods, or extreme temperatures; and the development of dense and rich macrophyte communities, features that make them very peculiar (Giorgi et al. 2005). However, human activities have affected many of these natural characteristics, thus damaging the physical habitat. The construction of artificial irrigation canals and the diversion of natural waterways for increased production are common practices in rural zones. In addition, dredging and channelization of streams are activities that are commonly performed in urban areas to avoid flooding. As a consequence of all these physical interventions, the aquatic systems that cross the Pampean plain have been strongly degraded, mostly affecting the macrophyte communities and the water quality (Gómez et al. 2008; Rodrigues Capítulo et al. 2010; Cortelezzi et al. 2011). We refer to the physical habitat of the streams based on the definition of Jowett (1997), who defined it as the local physical, chemical, and biological features that provide an environment for the in-stream biota. Macrophytes play a key and dual role by being a part of the in-stream biota and, at the same time, being a moderator of the physical condition (Pedersen et al. 2004). Particularly in Pampean streams with silty sediment in the benthos and

reduced rithron, the presence of aquatic plants of diverse architecture is the main generator of environmental heterogeneity (Rodrigues Capítulo et al. 2001; Tangorra 2004; Cortelezzi 2010). Therefore, weed cutting, dredging, and channelization can potentially have cascading effects on in-stream physical habitats and biological communities (Hearne and Armitage 1993; Jacobsen and Sand-Jensen 1994).

Recent studies have addressed the problem of separating the effects of different stressors through the use of a multimetric approach (Barbour et al. 1999; Karr and Chu 1999; Hering et al. 2006; Buffagni et al. 2009; Kail et al. 2009; Sandin 2009). The Council of the European Communities (2000) advocates the use of different organism groups such as benthic diatoms, macrophytes, invertebrates, and fish either singly or together to assess the ecological integrity of stream ecosystems. Similarly in North America, benthic diatoms, macroinvertebrates, and fish are frequently used together to assess the integrity of stream ecosystems (Hering et al. 2006), whereas in South America, few studies have employed together different organism groups in biomonitoring and even less studies about this topic have been carried out in lowland streams (Bauer et al. 2002; Gómez et al. 2008). In this study, we employed the epipelic biofilm, invertebrate, and macrophytes to assess the degradation in different lowland streams. We hypothesized that the habitat degradation in lowland streams, as a consequence of human activities, changes the richness and cover of the macrophytes, thus affecting the structural and functional responses of the epipelic biofilm and the invertebrate assemblage. To address this hypothesis, we explored some physical habitat alterations (mainly the water quality and the channel changes) and its impact on the macrophytes' community, and on the structural and functional descriptors of the epipelic biofilm and the invertebrate assemblages.

This linked approach will allow us to understand the biological responses in degraded lowland streams, and it will facilitate the development and implementation of mitigation and monitoring procedures.

Materials and methods

Study area and site characterization

This study was carried out in three Pampean plain streams called Don Carlos (DC), Martín (M), and El

Pescado (P) which are small tributaries of the Río de la Plata estuary. These streams do not exceed the second order and are located in the surroundings of La Plata city (34°55′17″ S, 57°57′16″ W), the capital of the Province of Buenos Aires (Fig. 1). Three sampling sites were selected in Martín stream (M1, M2, M3) and El Pescado stream (P1, P2, P3), while only two sampling sites were selected in Don Carlos stream (DC1 and DC2). In each sampling site, we selected a reach of 50 m that were sampled four times during 2004 and 2005.

The Martín stream mainly crosses a suburban area: M1was located in an area where the land's main use is rural activity (agriculture), M2 was located in an area of the stream with low urban population, and M3 crossed a recreational area. Sites M2 and M3 have been dredged in the past. The El Pescado is a stream without channel modification and it runs through an area dedicated entirely to extensive rural use. The most relevant physical modifications are related to the "trampling" of cattle and the effect of surface runoff of the agriculture area. Finally, the Don Carlos stream runs through a very urbanized area and has undergone several physical changes: DC1 was exposed to the effluents coming from an industry textile; its streambed had suffered frequent dredging and the banks continued weed cutting. These changes and the intermittent discharge from the textile industry have modified some morphometric and hydraulic characteristics such as the transparency, flow, and granulometric

composition (Table 1). DC2 was influenced by metallurgical industry effluents, and its course had suffered the worst physical alterations such as channelization and the impervious of its bed and bank. Also, both sites are placed in an area densely populated. Morphometric data, granulometric composition, cover of land use, and physical alterations of the sections selected in each sampling site are shown in Table 1. This information was obtained from other studies carried out in these sites (Cortelezzi 2010; Gómez et al. 2008; Sierra and Gómez 2007; Sierra 2009; Hurtado et al. 2006).

Water quality

The following physical and chemical variables were measured at each site: dissolved oxygen (DO, YSI 52 dissolved-oxygen meter), conductivity (Lutron CD-4303), turbidity (Turbidity meter 800-ESD), temperature, and pH (Hanna HI 8633). Water samples that were to be analyzed for dissolved inorganic nutrients were filtered through glass-fiber filters (Whatman GF/C) and, together with the samples for biochemical and chemical oxygen demand, were stored at 4 °C until their arrival at the laboratory. Soluble reactive phosphorus (P–PO_4^{-3}), ammonium (N–NH_4^+), nitrate (N–NO_3^-), nitrite (N–NO_2^-), biological oxygen demand (BOD_5), and chemical oxygen demand (COD) were determined according to Mackereth et al. (1978) and APHA (1998).

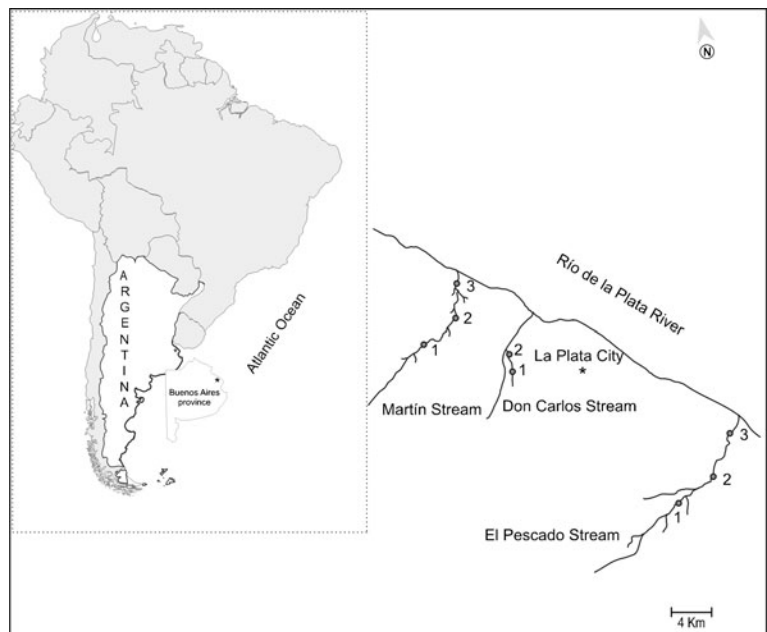Fig. 1 Map of the study area and location of the sampling sites

Table 1 Physical habitat descriptors of each sampling site

	M1	M2	M3	P1	P2	P3	DC1	DC2
Latitude S	34°54′54.00″	34°52′31.36″	34°51′35.47″	35°02′26.11″	35°00′00.45″	34°55′33″	34°53′37.53″	34°52′55.61″
Longitude W	58°04′41.83″	58°04′10.70″	58°03′49.94″	57°48′54.02″	57°47′56.41″	57°45′27″	58°01′23.17″	58°01′33.34″
Morphometric data								
Depth (m)	0.2	0.2	0.3	0.8	1.5	1.5	0.3	0.25
Width (m)	2.6	5.6	8	6.8	15	19.8	0.9	4.90
Flow (m s^{-1})	0.1	0.3	0.2	0.1	0.2	0.5	1.8	0.46
Discharge (m^3 s^{-1})	0.1	0.3	0.3	0.9	10.6	8.2	0.1	0.3
Granulometric composition (%)								
Gravel	6.3	13.3	3	19.1	18.5	17.0	19.5	13.4
Sand	30.9	33.3	35.5	27.5	30.3	50.5	50.1	60.5
Slime	41.0	34.9	46.4	41.7	27.7	24.4	10.9	15.0
Clay	21.8	18.5	15.1	11.7	23.5	8	19.4	11.2
Land uses (%)								
Rural	70	0.00	0	100	100	100	0	0
Suburban	30	58.7	99.4	0	0	0	22.7	27.9
Urban	0	41.3	0.6	0	0	0	77.3	72.1
Physical alterations								
Weed cutting							x	x
Channelization							x	x
Dredging		x	x				x	
Straightening								x

Biological data

Macrophytes

To assess the macrophyte community, a reach of 50 m in each sampling site was selected. A mapping was carried out to record the distribution of patches of species present (Feijoó and Menéndez 2009) and the composition of the present species was determined according to Cabrera and Zardini (1993). The vegetation cover was expressed as a percentage (%VC), and also a classification in function of modes of life (emergent, submerged, and floating free) was performed.

Epipelic biofilm

At each sampling site, 1 cm^2 of the epipelic biofilm was collected by pipetting 5 ml of the bottom's superficial layer (Gómez and Licursi 2001). The samples obtained were kept at 4 °C and in the dark during transportation to the laboratory. Two replicates were taken for chlorophyll-*a* and, for its analysis, two 5-ml aliquats were filtered through Whatman GF/C glass-fiber filters and immersed in 90 % (*v/v*) aqueous acetone for 24 h at 4 °C in the dark. The extract was read with a spectrophotometer and the chlorophyll-*a* concentration was calculated according to Steinman and Lamberti (1996). Two replicates were taken for ash-free dry weight (AFDW) determinations and they were measured as the difference in weight between the mass dried at 60 °C for 24 h and combusted at 550 °C for 4 h (Bourassa and Cattaneo 1998). Five subsamples were fixed with 4 % (*v/v*) aqueous formaldehyde to be used for the study of the community composition of the epipelic biofilm (main taxonomic groups), as assessed through the use of standard keys, and to be quantified in a Sedgwick–Rafter chamber (1 ml) under an optical microscope (Olympus BX 50). The subsamples were diluted according to the amount of suspended solids and the entire chamber was examined at ×400. Each algal cell was counted as a unit except the algal filaments. Portions of 10 μm of length of the filamentous forms were considered as the equivalent of a cell (Gómez et al. 2009). To analyze the diatom assemblage, two subsamples were rinsed with H$_2$O$_2$ and washed thoroughly with distilled water, then mounted on microscope slides with Naphrax and analyzed under the microscope with either interference, phase-contrast, or Nomarski differential-

interference-contrast optics. The proportion of tolerant species to organic pollution and eutrophication were calculated following Lange-Bertalot (1979) and Gómez and Licursi (2001). The consumption of oxygen (O_2 consumption) was measured in triplicate in the laboratory under artificial darkness and constant temperature (20 to 21 °C). Two aliquots were placed in 100-ml glass bottles filled with Whatman-GFC–filtered stream water, sealed airtight, and wrapped in aluminum foil. The oxygen concentration in each bottle was determined with an oxygen meter before closing and again after 1.5 to 2 h of incubation (Sierra and Gómez 2007).

Invertebrates

Triplicate samples of the invertebrates were taken, in both sediment and vegetation. Benthic invertebrates were sampled at each site with an Ekman dredge (100 cm^2). Samples of hydrophytes (emergent and floating plants) were collected with a Plexiglas square (1,300 cm^2) for examination of phytophilous invertebrates. The material was fixed in situ with 5 % (*v/v*) aqueous formaldehyde. After washing the sediment and the hydrophytes over a 250-μm mesh sieve, the invertebrates were separated under a stereomicroscope (Olympus SZ40) and identified through standard keys. The invertebrate abundance was expressed as the average number of individuals per square meter, species diversity was estimated by the Shannon–Weiner Index (H′; Shannon and Weaver 1949), and the richness was estimated as the number of invertebrates' taxa present. The values of density, richness, and diversity are presented as mean (standar error) for the five replicates from each sampling site. The functional feeding groups (FFGs) were identified according to the classification of Merrit and Cummins (1996) and Bonetto and Wais (1995). Finally, an estimate of the proportion of sensitive, tolerant, and very tolerant taxa was made (Barbour et al. 1999; Hilsenhoff 1987; Bode et al. 2002; Rodrigues Capítulo et al. 2001; Ocón and Rodrigues Capítulo 2004).

Data analysis

The ordination of the sampling sites in groups of the physicochemical variables was performed by a principal-component analysis (PCA) and they were represented in a biplot graph (Gabriel 1971); this multivariate approach was selected because it avoids the shortcomings of a bivariate correlation analysis (Van Sickle 2003). Then, to confirm the difference between the groups defined by the PCA, we conducted a linear discriminant analysis. The groups of sampling sites generated from these statistical analyses were considered for the analysis of biological parameters. The Kolmogorov–Smirnov non-parametric test was performed to determine whether the biological variables showed significant differences between sites. In order to examine the relationships between benthic assemblages (biofilms and invertebrates) and macrophytes' richness and coverage, two multiple factor analyses (MFA) were performed (Abdi and Valentin 2007; Greenacre 2010).

Results

Water quality

The ordination of the sampling sites, based on the physical and chemical variables (Table 2), was obtained with a principal component analysis (PCA), and the first two components accounted for 86 % of the total variance (Fig. 2). Reconstruction values of each parameter were obtained, and those physicochemical variables which were not reconstructed at least 50 % (DO, conductivity, pH, turbidity, and temperature), and therefore cannot be explained, were not shown in the biplot and were eliminated for future analyses. The variables that explained the direction of most variability and defined an organic pollution gradient in the first component (PC1) were NO_2^- (+), NH_4^+ (+), BOD_5 (+), COD (+), and PO_3^{-4}. The second component (PC2) was explained mainly by the NO_3^- (+) (Table 3).

In the biplot of the PCA scores, three groups of sampling sites could be distinguished (Fig. 2): the first group included the Martin stream sites which had the lowest levels of organic matter and nutrients (NO_3^-, NO_2^-, and NH_4^+); the second group included the El Pescado stream sites with mean values of organic pollution; and the third group included the sites 1 and 2 of Don Carlos stream which were associated with highest organic pollution and nutrients. The linear discriminant analysis showed that 97 % of the sites were correctly classified ($F=14.71$; $p=0.0001$). Considering this arrangement and the physical perturbations, we grouped the sampling sites in three categories of degradation: low (M1, M2, and M3), moderately (P1, P2, and P3) and highly degraded sites (DC1 and DC2).

Table 2 Physicochemical variables (mean and standard error) of the sampling sites

	M1	M2	M3	P1	P2	P3	DC1	DC2
Temperature (°C)	17.2 (3.2)	22.2 (2.7)	20.5 (3.4)	17.1 (4.2)	16.1 (4.0)	18 (4.6)	19 (0.7)	18.4 (0.9)
DO (mg l^{-1})	4.9 (1.0)	7.9 (0.7)	6.3 (1.2)	5.9 (1.0)	6.7 (1.1)	5.9 (1.5)	3.3 (0.6)	6.7 (3.5)
Conductivity ($\mu S\ cm^{-1}$)	205 (26)	911 (144)	933 (36)	336 (81)	601 (113)	514 (136)	987 (43)	1,081 (32)
pH	7.3 (0.1)	8 (0.1)	8.1 (0.1)	7.4 (0.2)	7.8 (0.2)	7.7 (01)	7.5 (0.1)	7.6 (0.2)
Turbidity (NTU)	30.3 (7.0)	22.2 (6.8)	21.3 (6.1)	56.9 (10.3)	51.6 (8.5)	70.2 (5.1)	23.6 (17.0)	5.1 (1.1)
N–NO$_3^{-}$ (mg l^{-1})	0.2 (0.1)	1.4 (0.2)	0.9 (0.1)	1.2 (0.6)	1.7 (0.8)	2.3 (0.4)	1.1 (0.9)	1.4 (0.7)
N–NO$_2^{-}$ (mg l^{-1})	0.01 (0.01)	0.07 (0.026)	0.04 (0.02)	0.05 (0.01)	0.03 (0.01)	0.05 (0.02)	0.1 (0.08)	0.3 (0.13)
N–NH$_4^{+}$ (mg l^{-1})	0.02 (0.01)	0.07 (0.03)	0.1 (0.03)	0.8 (0.09)	0.5 (0.45)	0.6 (0.44)	0.9 (0.34)	1.1 (0.17)
P–PO$_4^{-3}$ (mg l^{-1})	0.4 (0.03)	0.5 (0.06)	0.7 (0.11)	0.3 (0.06)	0.4 (0.12)	0.4 (0.10)	0.15 (0.05)	0.3 (0.04)
BOD$_5$ (mg l^{-1})	5.7 (1.9)	5.2 (1.7)	4 (1.5)	16.9 (9.6)	8 (2.3)	7 (1.6)	71 (3.1)	88.2 (46.4)
COD (mg l^{-1})	25.5 (5.9)	13.5 (2.2)	12.5 (1.6)	66 (20.4)	57.2 (11.4)	43 (8.8)	141 (37.5)	163.5 (69.9)

Biological data

Macrophytes

The macrophytes had the highest coverage and richness at low degraded sites (Martin stream sites), where *Hydrocleys nymphoides* were the dominant species.

Only at these sites that all modes of life (emergent, rooted floating, and submerged plants) were represented. While at moderately degraded (El Pescado stream sites) and highly degraded sites (Don Carlos stream sites), the vegetation cover was similar, the species richness was the lowest at sites that were highly degraded. Regarding the dominant species,

Fig. 2 Centroids of sampling sites (*black square*) and environmental variables in the first principal plane (biplot)
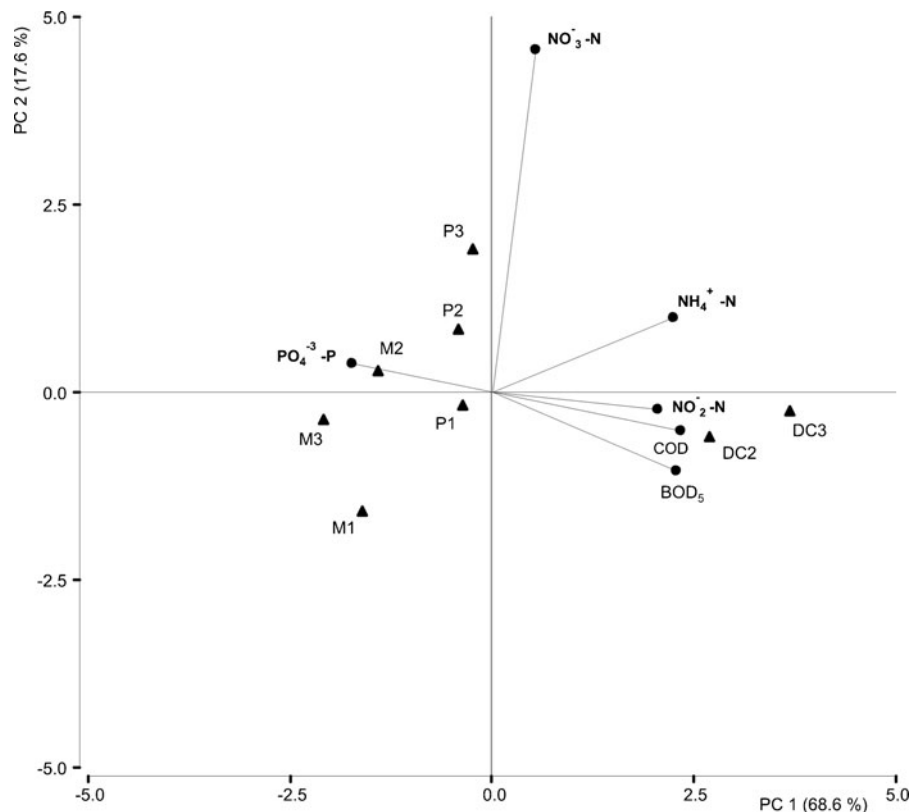
Table 3 Correlations between physicochemical variables and the first two principal components

	PC1	PC2
$N–NO_3^-$	0.23	0.97
$N–NO_2^-$	0.86	−0.05
$N–NH_4^+$	0.95	0.21
$P–PO_4^{-3}$	−0.72	0.08
BOD_5	0.96	−0.22
COD	0.99	−0.11

Schoenoplectus californicus was dominant at moderately degraded sites, while *Typha dominguensis* and *Hydrocotyle ranunculoides* were so at highly degraded sites (Fig. 3; Table 4).

Epipelic biofilm descriptors

Diatoms, euglenoids, and protozoans densities and O_2 consumption of biofilm were significantly higher at highly degraded sites; also, mats of filamentous bacterium *Beggiatoa* spp. were noted covering the streambed and they contributed to the increase in thickness of the biofilm there. On the other hand, at moderately degraded sites, the chlorophytes' density was significantly higher than at low degraded sites while its chlorophyll-*a* concentration was significantly lower (Table 5).

The detailed analysis of the diatoms' assemblage revealed no sensitive species at highly degraded sites and an increase in the proportion of the species most tolerant to pollution, exceeding four to five times the observed in the low and moderately degraded sites (Fig. 4). The main most tolerant species at highly degraded sites were *Diadesmis confervacea, Nitzschia*
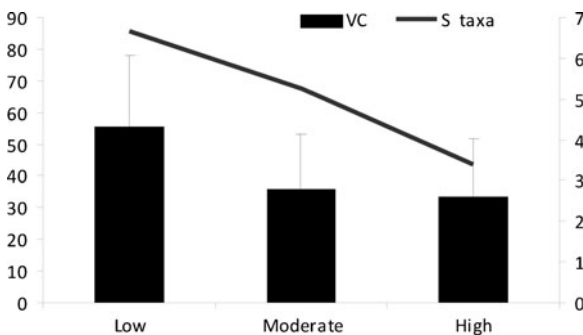
Table 4 List of macrophytes, modes of life, and vegetation cover (* <5 %; ** 5–25 %; *** >25 %) at sites with different levels of degradation

Macrophytes	Types of life	Level of degradation		
		Low	Moderate	High
Sagittaria montevidensis	Emergent	**	*	*
Schoenoplectus californicus	Emergent	*	***	
Typha dominguensis	Emergent	*	**	***
Eleocharis palustris	Emergent	**	*	
Scirpus californicus	Emergent	**		*
Gymnocoronis spilanthoides	Emergent	**	*	
Ludwigia peploides	Emergent	**	*	
Alternanthera philoxeroides	Rooted floating	*		**
Polygonum acuminatum	Rooted floating	*	*	*
Hydrocleys nymphoides	Rooted floating	***	**	
Hydrocotyle ranunculoides	Rooted floating		**	***
Ceratophyllum demersum	Submerged		*	
Egeria densa	Submerged	**		
Potamogeton sp.	Submerged	*		

palea, Nitzschia umbonata, Gomphonema parvulum, Navicula kotchii, Fallacia pygmaea, Navicula submi-nuscula, and *Sellaphora pupula.* The proportion of sensitive, tolerant, and most tolerant species in moderately and low degraded sites was similar, although the species present between these sites were different. At low degraded sites, the most representative species were *Gomphonema gracile* (sensitive specie), *Melosira varians, Cocconeis placentula* (tolerant species), *Navicula cryptocephala,* and *D. confervacea* (most tolerant species); while at moderately degraded sites they were *Achnanthes minutissima, Caloneis bacillum (*sensitive species), *Gomphonema clavatum, Nitzschia linearis* (tolerant species), *Amphora veneta,* and *Pinnularia gibba* (most tolerant species).

Invertebrate descriptors

The taxa richness (S) and the Shannon diversity (H′) diminished following the degradation gradient. In relation to the richness presented, a mean value of 20.6 (SE=4.1) at low degraded sites, of 14.9 (SE=3.0) at moderately degraded sites, and of 11.8 (SE=3.0) at highly degraded sites. The diversity followed the same pattern: low degraded sites presented a mean value of 1.9 (SE=0.4), the moderately degraded sites of 1.9 (SE=0.4), and the highly degraded sites of 1.2 (SE=0.3).

Fig. 3 Percentage of vegetation cover (*VC*) and richness of macrophytes (*S taxa*) at sites with different levels of degradation

Table 5 Mean density and standard error of biofilm taxa at site groups with different levels of degradation

	Level of degradation			Kolmogorov–Smirnov ($p<0.01$)
	Low $n=12$	Moderate $n=12$	High $n=8$	
Cyanophytes (cell cm^{-2})	7.1×10^5 (5.6×10^5)	7.9×10^4 (4.4×10^4)	5.5×10^6 (4.7×10^6)	ns
Diatoms (cell cm^{-2})	4×10^4 (1.6×10^4)	1.3×10^4 (3.5×10^3)	3.2×10^6 (1.8×10^6)	III>II–I
Chlorophytes (cell cm^{-2})	811.1 (689.8)	1.3×10^3 (527)	1.6×10^5 (1×10^5)	II>I
Euglenoids (cell cm^{-2})	266.6 (155.8)	185.89 (63)	4×10^4 (2.2×10^4)	III>II–I
Protozoans (org cm^{-2})	24 (18.3)	36.5 (24.7)	5.5×10^3 (3.4×10^3)	III>II–I; II>I
Chlorophyll-a (mg m^{-2})	284.2 (72.4)	34.9 (6.6)	178.5 (55.6)	II<I
AFDW (g m^{-2})	206.9 (41.4)	67.4 (14.5)	84.7 (21.7)	I>II
O$_2$ consumption (g O$_2$ m^{-2} d^{-1})	1.5 (0.5)	3.0 (0.7)	13.9 (3)	III>II–I

ns non-significant

Regarding the invertebrate assemblages, the Mollusca and microcrustaceans' densities were significantly higher at low degraded sites; Coleoptera and Ephemeroptera presented the highest abundances at moderately degraded sites. Even at these sites, the analysis of the FFGs revealed that the density of predators (e.g., Hirudinea, Odonata, and Hemiptera) and the abundances of gathering collectors (Oligochaeta Tubificinae, Diptera -Chironomidae, Stratiomyidae, Ephydridae, Psychodidae, Syrphidae, and Tipulidae- Ephemeroptera Baetidae) were significantly low, while the detritivores' abundance (Crustacea, Coleoptera) was significantly high. Finally, Diptera's density was the highest at sites with high degradation (Table 6).

In relation to the taxa's tolerance, the highest proportion of sensitive taxa was recorded in moderately degraded sites (Ancylidae *Gundlachia moricandi*, Odonata—*Erytrodiplax* sp., *Perithemis* sp., *Aeshna* sp., *Micrathyria* sp., *Tramea* sp., *Caennis* sp., *Amer-*

icabaetis sp., and *Callibaetis* sp.), while highly degraded sites showed the highest values of very tolerant taxa (Hirudinea, Nematoda, Oligochaeta, Diptera, and Mollusca *Physa* sp.), being sensitive and tolerant species scarce (Fig. 4).

Relationships between the physical habitat and biota

The triplot of Fig. 5 shows the centroids of the three site groups defined by the physicochemical variables analyzed above, in relation with the epipelic biofilm and macrophytes. The first axis (PC1) explained 57 % of the total variation and was defined by the O$_2$ consumption, the density of the taxonomic groups, and the richness of the macrophytes. On the other hand, the second axis (PC2) that explained 25 % of the total variation was defined by the chlorophyll-a, the AFDW, and the vegetation cover. The correlation between the analyzed variables and the two first

Fig. 4 Tolerance of diatom and invertebrate species to eutrophication and organic pollution in the three levels of degradation
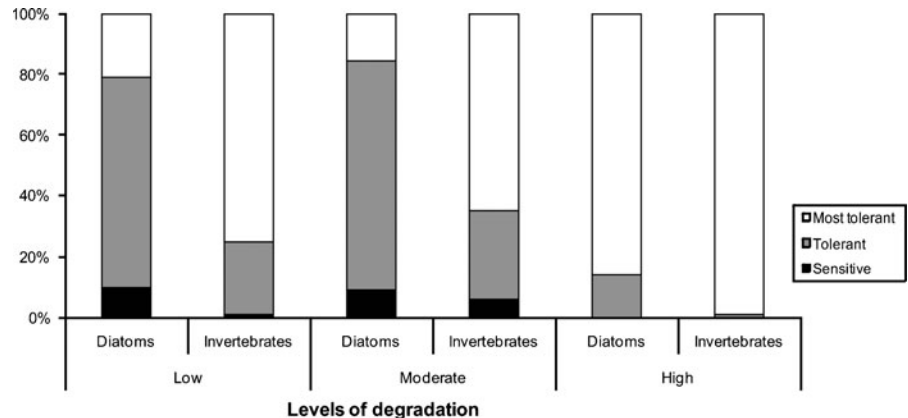
Table 6 Mean density and standard error of invertebrates taxa at sites groups with different levels of degradation

	Level of degradation			Kolmogorov–Smirnov ($p<0.01$)
	Low $n=12$	Moderate $n=12$	High $n=8$	
Nematoda (ind m^{-2})	8,947 (4,060)	806 (430)	5,991 (2,106)	II<I–III
Oligochaeta (ind m^{-2})	4,733 (1,639)	4,935 (2,816)	4,031 (2,410)	ns
Mollusca (ind m^{-2})	5,100 (2,019)	1,357 (619)	192 (89)	I>III
Microcrustacea (ind m^{-2})	8,974 (4,054)	2,683 (1,530)	1,406 (532)	I>II
Macrocrustacea (ind m^{-2})	273 (213)	1,360 (1,429)	66 (24)	ns
Coleoptera (ind m^{-2})	13 (12)	216 (173)	20 (11)	ns
Ephemeroptera (ind m^{-2})	19 (11)	31 (22)	0	I<II
Odonata (ind m^{-2})	253 (149)	115 (80)	7 (6)	ns
Diptera (ind m^{-2})	864 (245)	399 (279)	6,146 (3,337)	III>I–II
Predator (ind m^{-2})	9,397 (4,006)	594 (317)	3,783 (2,134)	I>II
Piercer (ind m^{-2})	1,451 (609)	1,221 (588)	2,706 (1,162)	ns
Gathering collectors (ind m^{-2})	4,939 (1,949)	1,140 (561)	6,633 (3,029)	II<I–III
Filtering collectors (ind m^{-2})	257 (85)	410 (239)	193 (124)	ns
Detritivore (ind m^{-2})	249 (186)	1,398 (1,351)	55 (21)	II>III

ns non-significant

principal components is presented in Table 7. From those results, we noted that the water quality mainly influenced most of the biofilm descriptors, separating the low degraded sites from the highly degraded sites.

Fig. 5 Triplot showing the centroids (*black triangle*) of the three site groups (*I*: low degraded, *II*: moderately degraded, and *III*: highly degraded sites) in relation with the biofilm and macrophytes (*S macrophytes*: richness of macrophytes; *VC*: percentage of vegetation cover; *AFDW*: ash-free dry weight)
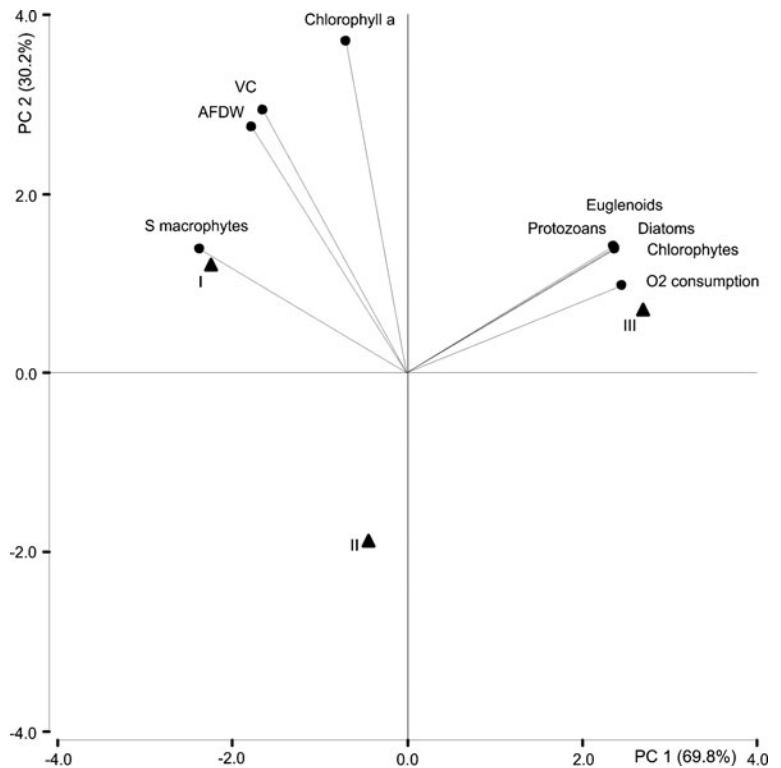
Table 7 Correlations between the biofilm descriptors and the first two main components

	PC1	PC2
Diatoms	0.9	0.3
Chlorophytes	0.9	0.4
Euglenoids	0.9	0.4
Protozoans	0.9	0.4
Chlorophyll-a	−0.3	0.9
AFDW	−0.7	0.7
O$_2$ consumption	0.9	0.2
%VC	−0.6	0.8
S macrophytes	−0.9	0.4

Regarding the invertebrates, the first axis (PC1) of the triplot (Fig. 6) explained 46.3 % of the total variation and was defined by the predators, molluscs, and microcrustaceans densities, the richness and diversity of the invertebrates, the vegetation cover, and macrophyte richness. The second axis (PC2) that explained 27 % of the total variation was associated to the densities of the detritivores, ephemeropterans, nematodes, dipters, and gathering collectors. The correlation between the analyzed variables and the two first principal components is presented in Table 8. These results showed that the richness of the macrophytes and the vegetation cover mainly influenced most of the invertebrate descriptors, separating the low and highly degraded sites.

Discussion

The dynamic physical processes occurring within a river create the habitat's physical structure: the diversity and the dynamics of this habitat's structure are the basis for the river's biodiversity (Harper and Everard 1998). Although retention of physical integrity does not guarantee that the ecological integrity will be maintained, a system with a poor physical structure is almost certain to have a highly degraded ecosystem (Reid et al. 2010). The environmental degradation is being exacerbated by many human activities that are reducing the habitat's physical heterogeneity, simplifying natural disturbance regimes, and homogenizing the species pool worldwide. Streams and rivers arguably have experienced some of the most dramatic forms of habitat simplification in any type of ecosystem (Brookes

and Gregory 1988). The lowland streams in the Pampean plain are not the exception and different reaches of the streams selected for this study have been affected by physical perturbations, such as dredging, straightening of the stream, cleaning activities, and by chemical perturbations, mainly organic matter and nutrient input, thus it is difficult to find sites that are undisturbed by human activity. As a consequence of such physical and chemical perturbations, we differentiated sampling sites with different degradation levels: low, moderate, and high, which were related to different land uses. The low degraded sites were affected mainly by the suburban land use, the moderately degraded sites by the rural land use, and the highly degraded sites by the urban land use. At sampling sites with high degradation, not only have we registered low macrophyte richness and vegetation cover but also low diversity of modes of life of macrophytes and, therefore, the environmental heterogeneity provided by the structural variability of macrophytes also declined. This situation demonstrates the moderating role of the macrophytes in the physical condition of the streams (Pedersen et al. 2004), illustrating that the removal of physical structures eliminates important types of stream habitats (Brook et al. 2002). Furthermore, we observed a higher vegetation cover from the resistant species, such as *T. dominguensis* and *H. ranunculoides*, at the most degraded sites. *T. dominguensis* has been considered a species that increases its dominance with NH_4^+, while the macrophyte richness declines (Craft et al. 2007). On the other hand, *H. ranunculoides* possesses characteristics typical of weed species, including high growth rates, effective vegetative propagation, plasticity in growth response, and high resistance to herbivory (McChesney 1994). Also, the most degraded sampling sites reached the highest levels of ammonium and organic matter (BOD and COD), which concurs with the land's main use in the area surrounding them, the urban land use. Even previous studies detected heavy metal concentrations (Ni, Cu, and Pb) in the streambed at these sites (Gómez et al. 2008; Sierra and Gómez 2010). According to Paul and Meyer (2001), the urbanization increased almost all of the constituents in the urban streams, but consistently with the oxygen demand, conductivity, suspended solids, ammonium, hydrocarbons, and metals.

Aquatic biotic communities associated with watersheds with high urban and agricultural use are generally characterized for having a lower species diversity, a lesser degree of trophic complexity, altered food

Fig. 6 Triplot showing the centroids (*black triangle*) of the three sampling site groups (*I*: low degraded, *II*: moderately degraded, and *III*: highly degraded sites) in relation with the invertebrate and macrophyte descriptors (*S macrophyte*: richness of macrophytes; *VC*: percentage of vegetation cover; *S invertebrates*: richness of invertebrates; *H invertebrates*: Shannon diversity of invertebrates)
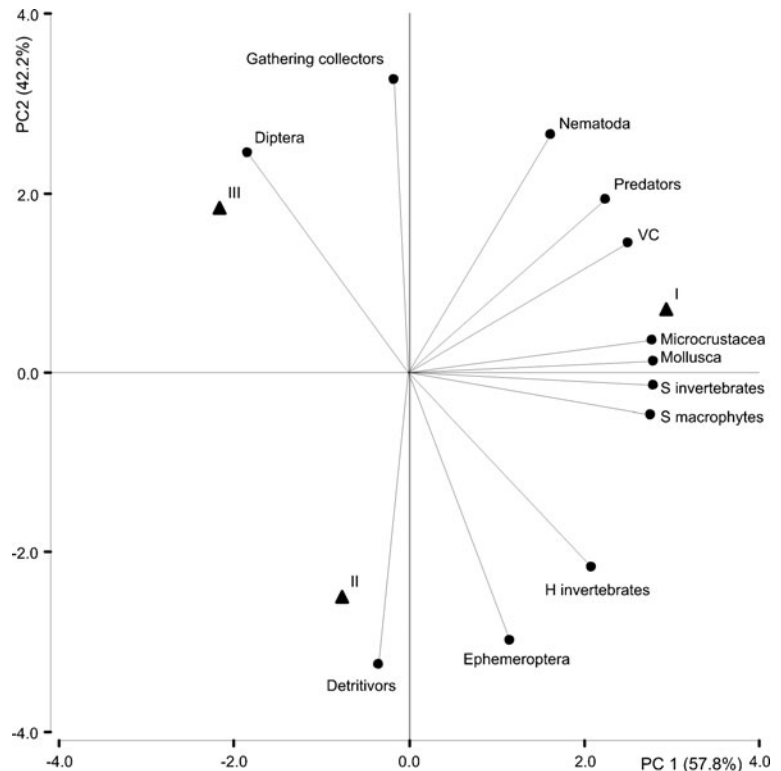
webs, a modified community composition, and a reduced habitat diversity (Dauer et al. 2000). In concordance with this, the macrophyte, biofilm, and invertebrate descriptors analyzed in this study showed some of these changes, reflecting the degradation that the sampling sites have suffered, mainly the sites affected by the urban land use. For example, the

Table 8 Correlations between the invertebrate descriptors and the two first principal components

	PC1	PC2
Nematoda	0.5	0.8
Mollusca	1	0.04
Microcrustacea	0.9	0.1
Ephemeroptera	0.4	−0.9
Diptera	−0.7	0.7
Predators	0.8	0.6
Gathering collectors	−0.06	1
Detritivors	−0.1	−0.9
S invertebrates	0.9	−0.1
H invertebrates	0.7	−0.7
%VC	0.9	0.4
S macrophytes	1	−0.04

biofilm composition was characterized for having a higher proportion of diatoms, cyanophytes, and euglenoids, which was a clear response to the increased levels of nutrients and/or organic matter at the highly degraded sites. Several studies have recorded the reduction of the abundance of diatoms and the increase in the presence of cyanophytes and euglenoids species at highly degraded sites that are rich in organic matter and nutrients (Biggs 1989; Giorgi and Malacalza 2002; Tell and Conforti 1986). However, in our case, the diatoms increased at highly degraded sites and were represented mainly by the taxa tolerant to organic pollution and eutrophication. The diatom assemblage analyses allowed differentiating characteristic species associations at sites with different land uses (Licursi 2005; Licursi and Gómez 2002).

Eutrophic condition is common in the Pampean streams, due to the dissolved phosphorus and nitrogen concentrations that they naturally possess, and which are relatively high compared to other lotic systems in the world (Rodrigues Capítulo et al. 2010). Therefore, it is not surprising that the autotrophic biomass of the epipelic biofilm indicates eutrophic conditions at sites with low and high degradation. At these sites, chlorophyll-*a* exceeded the 150 mgm^{-2}, a value

considered to be a threshold for ecosystem impairment (Dodds et al. 1998; Hill et al. 2010). The high level of chlorophyll-*a* at low degraded sites was probably favored for the high phosphorous concentration at these sites. The high turbidity recorded at moderately degraded sites that was due to erosion and trampling by livestock should have generated the minimum values of chlorophyll and, therefore, a mesotrophic condition. In terms of functional descriptors, the O_2 consumption by the biofilm showed a consistent response to the degradation level. Thus, at highly degraded sites, the O_2 consumption was 8.6× higher than at low degraded sites, and 4.3× higher than at moderately degraded sites. This is in agreement with Bunn et al. (1999) observations, that pointed out that the benthic community respiration increases with increasing disturbance. We thought that the significant increase of O_2 consumption at highly degraded sites was favored by the mats of the sulfur-reducing bacteria, *Beggiatoa* spp., that covered the streambed at these sites. *Beggiatoa* spp. is found in diverse habitats, particularly on the surfaces of organically rich freshwater sediments (Hinck et al. 2007). These bacteria exclude other heterotrophic bacteria and most macrofauna, and have few elements of the infaunal communities that are found in other muddy biotopes and, therefore, would imply changes in the aquatic food webs (Williams and Unz 1989).

Light is a factor that promotes the development of biofilms due to the presence of phototrophic organisms. Light intensity controls photosynthesis, and many authors have reported that there is a range of intensities over which photosynthesis is highly efficient (30–400 umol m^{-2} s^{-1}) with an inhibitory effect above 500 umol m^{-2} s^{-1} in streams with complete and partial shade due to riparian vegetation (Villeneuve et al. 2010). Previous studies carried out in the selected study area showed that the light intensity is not a limiting factor since they recorded high algal density, total biomass and chlorophyll where the light intensity ranged between 170 umol m^{-2} s^{-1} and 2,200 μmol m^{-2} s^{-1} (Sierra 2009; Sierra and Gómez 2010). In our case, those observations were noted particularly at sites with low degradation, in spite of having the highest vegetation cover which causes a shading effect on the benthic communities, we recorded a good development of the epipelic biofilms.

Regarding the invertebrates, the sites with high degradation presented the lowest richness and diversity values, a modified community composition with diminished sensitive taxa (such as Ancylidae, Odonata Aeshnidae, and Ephemeroptera Baetidae), and an increase in abundance and richness of very tolerant families (Diptera Chironomidae, Syrphidae, Culicidae, Stratiomidae, Oligochaeta Tubificinae, etc.). These observations coincide with Moya et al. (2011), who noted that the richness, density, and composition of sensitive groups in the streams of Bolivia are the most affected by environmental degradation. Moreover, they determined that the attributes related to the trophic structure did not show a clear trend towards environmental degradation, which was also noted by us. This last characteristic could be related to non-accurate information available about food categories in South America. Therefore, the classification made in this study, and in most studies about functional feeding groups in South America, comes from information on the organisms of the Northern Hemisphere (cf. Merrit and Cummins 1996). In addition, the flexibility of the life history and mobility that seems to characterize many taxa in the Southern Hemisphere can influence their adaptability in obtaining food sources (Covich 1988). According to Tomanova et al. (2006), this pattern can lead to significant differences in the classification of the FFGs, as some South American taxa cannot eat like most of their counterparts in the temperate zone, and therefore those taxa should not be placed in the same FFG as others. Consequently, the resulting FFG classifications of the neotropical communities based on Merrit and Cummins (1996)—e.g., Poi de Neiff and Neiff 1989; Callisto et al. 2001; Fossati et al. 2001; Buss et al. 2002—may result in FFGs that are biased.

Conclusions

Our results show that the biotic descriptors that reflected the environmental degradation were the vegetation cover and the macrophytes' richness, the dominance of tolerant species (biofilms and macroinvertebrates), algal biomass, O_2 consumption of the biofilm, and richness and diversity of the invertebrates. Also, the results obtained highlighted the importance of the macrophytes in the lowland streams, where there is a poor diversification of abiotic substrates, and where the macrophytes not only provide shelter but also a food source for invertebrates and other trophic levels such as fish. We

also noted that in both benthic communities, invertebrates and epipelic biofilm supplied different information: the habitat's physical structure provided by the macrophytes influenced mainly over the invertebrate descriptors, while the water quality mainly influenced over most of the biofilm descriptors. Finally, we can say that it is adequate that they have been employed together to gather more information about the environmental degradation and to facilitate the interpretation of the data and the development and implementation of mitigating and monitoring procedures.

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