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Periphyton responses to non-point pollution in naturally eutrophic conditions in Pampean streams

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With 6 figures and 4 tables

Abstract: The aim of this study was to examine the effects of different intensities of non-point pollution on water quality and the periphyton community in natural eutrophic environments such as the Pampean streams. Non-point pollution caused by agricultural and farming activities is an important contributor to water quality deterioration. Our hypothesis was that, in spite of the natural water eutrophication in Pampean streams, pollution generated by rural activities promotes changes in water quality modifying nutrient concentrations and promoting or inhibiting the periphyton developed depending on the kind and intensity of land use. The most important periphyton parameters to assess the non-point pollution effect due to rural activities estimated in this study were chlorophyll-*a*, net production and alkaline phosphatase activity. Water quality parameters which proved useful in differentiating rural activities were soluble reactive phosphorus and conductivity. Agriculture caused an increase in autotrophic biomass and hence an increase in net production whereas cattle farming caused an increase in heterotrophic biomass. Chlorophytes were abundant in agricultural zones whereas cyanophytes or euglenophytes were more abundant in cattle farming areas. Thus, periphyton could be used as an indicator of non-point pollution, differentiating not only the types of activity but also the persistence and intensity of rural practices.

Key words: periphyton, non-point pollution, Pampean streams, cattle farming, agriculture.

Introduction

Non-point pollution caused by agricultural and farming activities is an important contributor to water quality deterioration. This type of pollution could increase as a result of weather events (quantity, quality and frequency of rainfall), as well as geographical (geomorphology, slope) and geological (soil type) conditions, so that its impact may differ significantly from one place to another and from one year to the next. The difficulty in assessing and predicting the impacts caused is due to the intermittent characteristics of pollution, spatiotemporal variability, and many physical, chemical and biological processes that influence the response of pollution indicators (Novotny 2003).

All over the world, intensive agricultural practices and outdoor cattle farming are generally regarded as high risk for P and N loss to rivers, because soils are either regularly over-fertilized, recycle large amounts of manure, or are highly vulnerable to soil erosion (Jarvie et al. 2010).

In the Pampean region, high concentrations of phosphorus have historically been registered due to the phosphorus content in the original volcanic material, and the region is therefore considered to have natural eutrophic conditions (Morrás 1999, Feijoó &

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Lombardo 2007). Agricultural and cattle farming activities have led to an increase in eutrophication in the region (Vilches et al. 2011). However, agricultural pollution becomes more important in the months of tilling, seed planting and harvesting because these are the times when fertilizers and biocides are used on the fields and are then washed into the streams as a result of rain events. Otherwise, the likelihood of causing pollution as a result of the increase in organic matter and nutrients by cattle farming is constant throughout the year. Cattle farming reaches register a higher chemical oxygen demand, humic acid and suspended solids whereas agricultural reaches seem to increase conductivity, pH and dissolved oxygen (Vilches et al. 2011). Thus, each of the rural activities may involve different degrees of intensity and therefore different ways of affecting the system.

Periphyton is an important community in Pampean streams because of the considerable primary production involved (Vilches & Giorgi 2010). Thus, periphyton has been considered a relevant disturbance indicator of the whole ecosystem due to the effects of pollution on the community (Sabater et al. 2007). In addition, it responds predictably to changes in environmental conditions on a large range of spatial scales (Gaiser 2009). Because of these features, the periphyton community is sensitive to changes in aquatic ecosystems by non-point pollution (Delong & Bruswen 1992, Steinman et al. 2011) such as pollution derivates from rural activity. The kinds of rural activity (agriculture or cattle farming) and the intensity thereof are essential elements in determining the impact of pollution. Pollution due to agricultural activity produces higher concentrations of chlorophyll-*a* (Urrea & Sabater 2009), whereas cattle farming produces nutrient pollution, causing increases in the biomass of filamentous algae (Schmutzer et al. 2008). Thus, more intensive agriculture (i.e. herbicide use) causes an increase in the abundance of cyanobacteria (Perez et al. 2007) and more intensive cattle farming (i.e. cattle access to watercourses) causes large inputs of animal waste, and therefore algal inhibition by bacterial activity (Toerien et al. 1984).

The aim of this study is to achieve insight into the effects of different intensities of non-point pollution on water quality and the periphyton community in naturally eutrophic environments such as the Pampean streams.

We assume that pollution generated by rural activities promotes changes in water quality modifying nutrient concentrations and promoting or inhibiting the periphyton developed depending on the nature and intensity of land use. In addition, we predict that agriculture causes an increase in autotrophic biomass whereas pollution generated by cattle farming causes an increase in heterotrophic biomass. We likewise expect that chlorophytes will be dominant in agricultural zones whereas cyanophytes or euglenophytes will be more abundant in cattle farming areas.

Material and methods

Study area

The Pampean biome (30–39° S) occupies the eastern plains of Argentina, Uruguay, and the southern tip of the state of Rio Grande do Sul in Brazil. The climate is mild and humid with a mean annual rainfall between 600 and 1,200 mm, and a mean annual temperature of 16 °C (Cabrera & Willink 1980). The region is extremely flat and Pampean streams cross plains with fertile soils formed after loess deposition during the Quaternary. Stream beds are characterized by fine sediments (primarily silt and clay) underlain by hard and homogeneous substrata with high $CaCO_3$ content. Stones and pebbles are absent (Giorgi et al. 2005). The study was carried out on four Pampean streams, three of which, La Choza, Nutrias and Durazno, flow out of the Reconquista river, with the fourth, Las Flores, flowing into the Luján river (Buenos Aires, Argentina). Although these streams are free of industrial pollution, they are subject to a variety of rural disturbances (Fig. 1). Six 100-m reaches were

Table 1. Summary of rural characteristics of the reaches monitored in the Pampean zone; for more detailed information, see Vilches et al. (2011).

Reach	Rural activity	Intensity	Plant coverage	Cultivated period	Signs of activity: trampling/ grazing	Signs of activity: Fertilizers/ Biocides	Signs of activity: Channel morphology
La Choza II	Agriculture	Low	Complete	Seasonal	Absent	Low	Preserved
La Choza III	Agriculture	High	Complete	Continued	Absent	High	Preserved
Nutrias	Cattle farming	High	Discontinued	Absent	High	Absent	Unpreserved
Durazno I	Cattle farming	High	Discontinued	Absent	High	Absent	Unpreserved
Durazno II	Agriculture	High	Complete	Continued	Absent	High	Preserved
Las Flores	Cattle farming	Low	Complete	Absent	Absent	Absent	Preserved

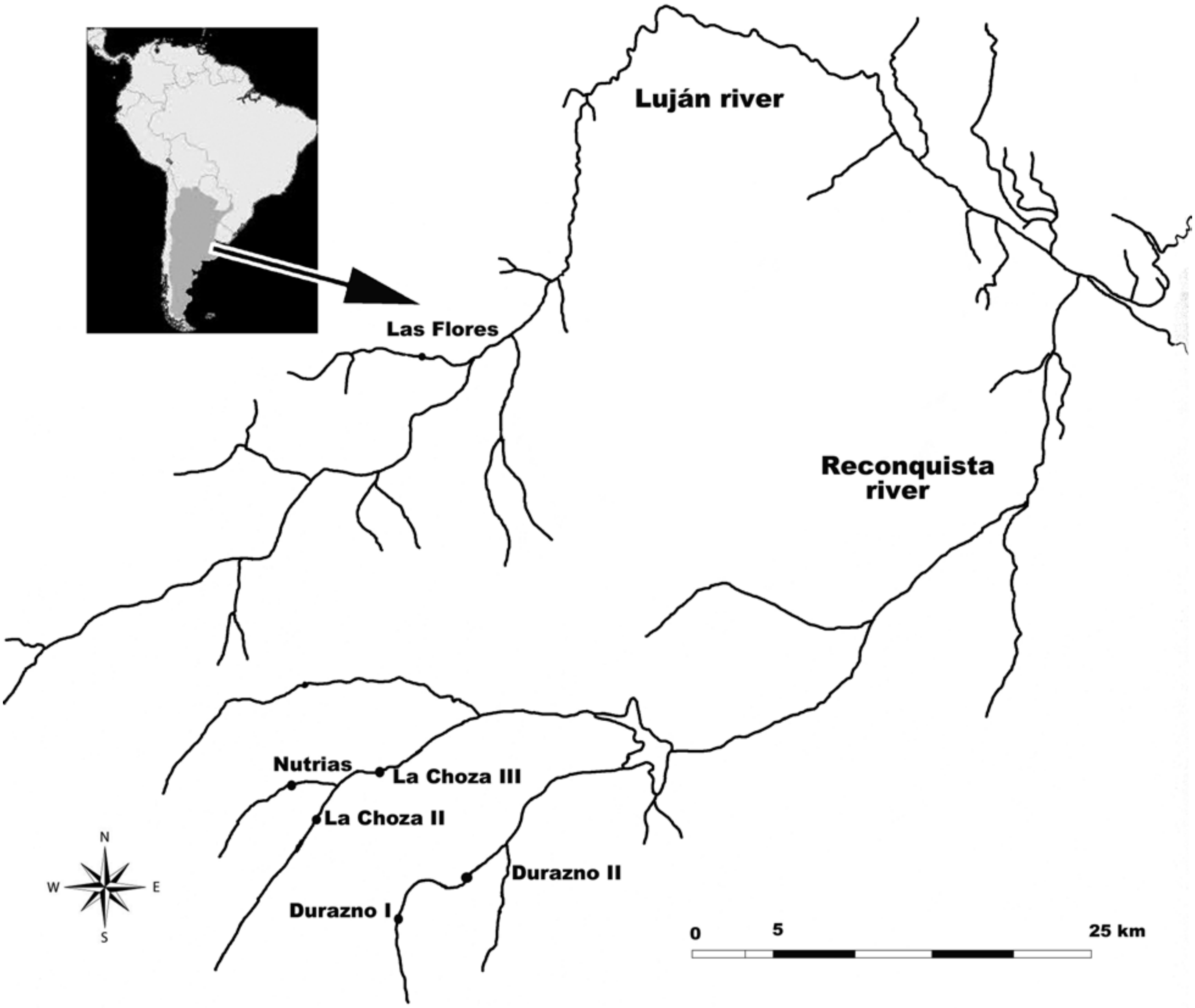

Fig. 1. Map showing the location of each reach studied.

selected in the streams, considering two different intensities of agricultural and cattle farming activities (Table 1). This selection was performed based on the relevant characteristics of each reach in relation to the effects of rural activities in the stream and the riparian zone. Each reach was visited at least once a month over a period of two years to collect information on the farming activity in the area (culture type, fertilizer and biocides used, number of head of livestock, etc.) and reach information (plant coverage on the riparian zone, channel morphology, size of uncultivated zone, etc.). Differences in agricultural intensity were represented by the greater proportion of fertilizer and biocides used and the length of the cultivated period. Differences in cattle farming intensity were represented by the lack of plant coverage on the riparian zone, channel morphology and evident trampling and grazing (Table 1).

Water quality parameters

Three samplings representing the different periods of agricultural activities (tilling, planting and harvesting) were conducted: one in winter (June) 2008, one in spring (October) 2008 and one in summer (February) 2009. It was assumed that cattle farming activities do not vary throughout the year given the mild climate conditions (Viglizzo et al 2011). The following physicochemical parameters were measured at each site and sampling date with Portable Hanna instruments (Woonsocket, USA): dissolved oxygen, conductivity, temperature and pH. We collected stream water samples in triplicate for nutrient analyses in polyethylene bottles and filtered the samples within 2 h of collection using glass fiber filters (pore size = 0.7 mm; Whatman GF/F filters, Maidstone, UK). Water samples were stored at 4 °C and transported to the laboratory for analysis within 24 h. Analyses were conducted according to APHA (2005). Soluble reactive phosphorus (SRP) was measured using the ascorbic acid method, nitrite and nitrate by reaction with sulfanilamide (with prior Cd reduction in the case of nitrate), and ammonium using the phenol-hypochlorite method. All the nutrients were analyzed with a Hitachi U-2001 spectrophotometer (Hitachi Ltd., Tokyo, Japan). In addition, concentrations of organic and inorganic particulate matter were estimated by drying, chlorides by silver nitrate method, humic acids following Lavado et al. (1982), chemical oxygen demand (COD) with the SQ 118

Spectroquant® Merck kit and biological oxygen demand after 5 days incubation at 20 °C (BOD_5). The selected parameters are related to the capability to reflect the water pollution caused by rural activities.

Artificial substrate and periphyton variables

Artificial substrates were utilized to compare the periphyton developing in the different streams without the influence of the kind of macrophyte present. To prevent vandalism and damage by cattle in the stream, a cheap replicable substrate was used to represent the macrophyte stems. In order to achieve these characteristics, black nylon ropes 0.1 cm in diameter tied to stakes fixed on both sides of the streambed were used as artificial substrates for biofilm attachment. Three groups of artificial substrates were placed in the middle of each reach and left there for the 45-day colonization period. A sample was performed on the periphyton developed on 10 cm of rope and three replicates were carried out per site for each determination. Over a span of less than 4 hours, the algal film was removed by scraping the surface of the rope with a soft bristle brush or by repeated sonication over 3-minute periods.

On the same sampling dates, the parameters used to evaluate the response of the biofilm were: total biomass (estimated as organic and inorganic dry weight), chlorophyll-*a*, total nitrogen, total phosphorus and total polysaccharides. Total biomass was measured as dry weight (DW) at 60 °C for 48 h and ash free dry weight (AFDW) as the difference in weight between the DW and weight combusted at 450 °C for 4 h. For chlorophyll-*a* analysis, a 50-ml aliquot was filtered using Whatman GF/F glass fiber filters and immersed in 90 % acetone for 24 h in the dark at 4 °C. The extract was read with a spectrophotometer and chlorophyll-*a* concentration was obtained according to APHA (2005). Additionally, the autotrophic index (AFDW/chlorophyll-*a*), a ratio used in determining the relationship between the biomass of autotrophic (algae) and heterotrophic (bacteria and organic matter) elements of the community, was calculated from these parameters. Values above 200 indicate a high proportion of heterotrophic organisms, no chlorophyll and organic detritus (APHA 2005). Total nitrogen was estimated using the Kjeldahl method (APHA 2005), total phosphorous by the peroxydisulfate digestion method (APHA 2005) and total polysaccharides by phenol/sulfuric acid (DuBois et al. 1956). In addition to this, the algae were studied with samples fixed with formaldehyde (1 %), using a Reichert ® microscope with immersion objectives. A minimum of 300 organisms were identified and a count was taken to differentiate the main algae groups and the number of organisms in each group.

Other parameters evaluated were: metabolism (production and respiration) (Bott et al. 1997) and activity of exoenzymes (β-glucosidase, alkaline phosphatase and cellobiohydrolase) (Romaní & Marxen 2002). Oxygen production and consumption was measured in the laboratory with BOD_5 bottles at constant temperature (20 °C). Production was determined by the variation in oxygen concentration before the bottles were closed and after 1 h exposed to light. In the same way, respiration was determined by the variation in oxygen concentration before the bottles were closed and after 2 h of incubation in the dark. Dissolved oxygen levels in each bottle were measured with an oxygen meter. In order to quantify β-glucosidase (EC 3.2.1.21), cellobiohydrolase (EC 3.2.1.91) and alkaline phosphatase (EC 3.1.3.1–2), exoenzymatic activities were measured using fluorochrome-linked substrates (methylumbelliferyl (MUF)). The biofilm from the rope was sonicated during three 3-min sessions separated by 1-min intervals, in a glass container with 20 mL of tap water. Samples at a final concentration of 300 mmol l^{-1} of MUF (saturation concentration determined for these communities), MUF calibration solutions (0–100 μmol/L) and water controls were incubated on a shaker in the dark for 1 h in a water bath adjusted to the stream water temperature. After incubation, enzymatic cleavage was inhibited by adding 5 mL of 0.05 M glycine buffer (pH 10.4). Activity was determined by fluorescence measurement at 365/455 nm (excitation/ emission for MUF). Exoenzymatic activities were expressed as nmol MUF/h.

Statistical analysis

Statistical analysis was performed using the statistical package Statistica 7.0 ®. Variable normality was checked with the Kolmogorov-Smirnov test, and the variables were adjusted as required. Unless otherwise noted, all the results are reported as mean ± standard deviation (SD). Multivariate analysis using discriminant functions was applied to study the separation between different intensities of rural activities using physicochemical and periphyton variables. One-way ANOVA was carried out for each variable highlighted on the basis of the discriminant analyses of the nature and intensity of the rural activity. All analyses were considered significant at $p < 0.05$.

Results

Water quality

pH, conductivity and temperature, showing seasonal variation. However, the lowest average values were recorded in Durazno I (high cattle farming intensity). The maximum values of BOD_5 and COD (7.1 mg l^{-1} and 98.3 mg l^{-1}, respectively) were also recorded in this reach. The concentrations of organic and inorganic particulate matter were markedly different between reaches. Durazno I (high cattle farming intensity) showed the highest values, whereas Las Flores (low cattle farming intensity) showed the lowest. Las Flores registered the highest SRP concentrations for all seasons, whereas Durazno I recorded the highest ammonium values in fall (Table 2).

In order to compare the reaches with different rural activity a multivariate analysis using all the water quality parameters (dissolved oxygen, conductivity, temperature, pH, SRP, nitrite, nitrate, ammonium, organic and inorganic particulate matter, chlorides, humic acids, COD and BOD_5) was carried out using the reaches as a classification factor. The classification matrix shows a considerable distance between reaches, and highlights Las Flores (low cattle farming intensity) as the most distant. The greatest distance estimated by the Mahalanobis square method (321.15) is between Las Flores and Durazno I (high cattle farming intensity). The reaches used for cattle farming activity

Table 2. Mean concentration and standard deviation (N = 9) of physicochemical parameters for each of the reaches sampled (SS, suspended solids; OSS, organic suspended solids).

	La Choza II	La Choza III	Nutrias	Durazno I	Durazno II	Las Flores
SRP (mgP-PO_4^{-3} l^{-1})	**0.08** (0.08)	**0.14** (0.09)	**0.12** (0.13)	**0.12** (0.13)	**0.08** (0.09)	**0.36** (0.12)
NH_4^+-N (μg l^{-1})	**4.57** (7.12)	**26.36** (21.96)	**7.74** (14.93)	**98.98** (147.44)	**11.28** (9.41)	**33.0** (16.99)
NO_3^-- N (mg l^{-1})	**1.05** (0.70)	**0.74** (0.36)	**0.56** (0.41)	**1.06** (0.98)	**0.16** (0.16)	**3.34** (1.91)
NO_2^--N (μg l^{-1})	**26.02** (23.79)	**14.68** (4.83)	**2.11** (3.36)	**5.87** (8.34)	**2.06** (2.0)	**70.56** (51.2)
Cl^- (mg l^{-1})	**26.17** (13.28)	**62.67** (17.72)	**15.49** (12.83)	**8.25** (7.28)	**24.45** (17.8)	**9.75** (4.38)
Humic acid (Abs)	**0.13** (0.11)	**0.09** (0.07)	**0.25** (0.17)	**0.30** (0.07)	**0.11** (0.08)	**0.05** (0.04)
COD (mgO_2 l^{-1})	**45.67** (24.33)	**34.67** (9.85)	**67.00** (17.68)	**98.33** (22.02)	**32.67** (12.8)	**16.67** (11.8)
BOD (mgO_2 l^{-1})	**1.93** (1.13)	**3.87** (1.94)	**5.03** (1.75)	**7.10** (1.93)	**4.57** (0.96)	**6.87** (2.00)
pH	**8.18** (0.76)	**8.34** (0.49)	**8.19** (1.17)	**7.69** (1.04)	**7.98** (1.09)	**8.07** (0.27)
Temperature °C	**17.97** (6.03)	**14.52** (5.46)	**16.40** (6.33)	**19.07** (6.45)	**21.53** (8.24)	**18.73** (6.85)
Conductivity (μS cm^{-1})	**1246** (435)	**1578** (470)	**1077** (735)	**439** (226)	**1090** (621)	**980** (91)
DO (mgO_2 l^{-1})	**11.05** (4.37)	**10.33** (4.92)	**8.43** (2.98)	**6.08** (3.02)	**12.20** (5.72)	**9.41** (2.98)
SS (g l^{-1})	**38.54** (23.89)	**14.28** (7.56)	**192.13** (168.5)	**366.96** (304.4)	**48.15** (45.3)	**11.54** (11.3)
OSS (g l^{-1})	**7.45** (5.90)	**3.86** (2.22)	**29.15** (27.05)	**40.12** (32.03)	**6.51** (4.91)	**3.91** (2.56)

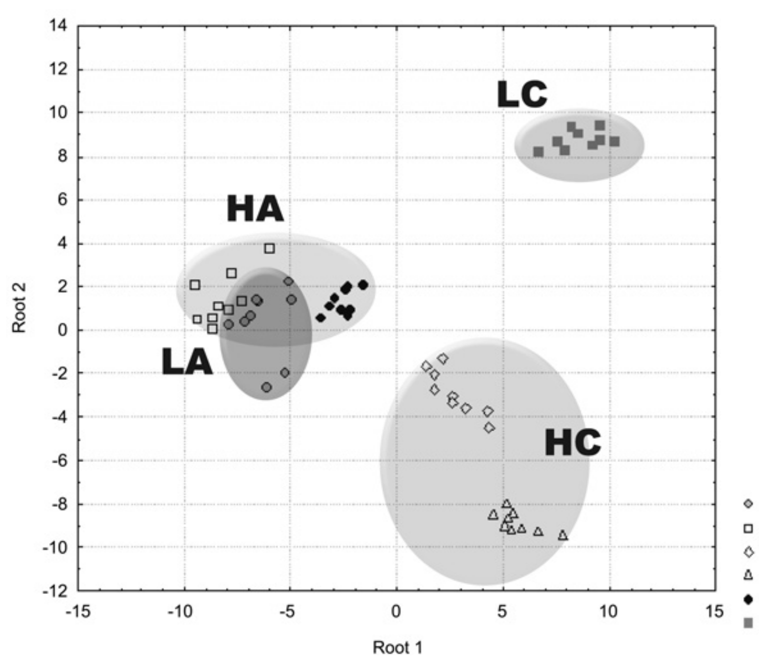

Fig. 2. First two discriminant roots showing the location of the reaches according to the physicochemical parameters. Uses and intensities of rural activities are highlighted in circles. LC, low intensity of cattle farming; HC, high intensity of cattle farming; LA, low intensity of agriculture; HA, high intensity of agriculture.

were distant from those used for agriculture activity. In addition, cattle farming reaches were more scattered than agricultural reaches (Fig. 2). The most important variables for differentiation between reaches located in areas with different rural activities were pH and conductivity, followed by SRP ($p < 0.001$). One-way ANOVA was carried out for these variables. The post-hoc Tukey HSD analysis for conductivity showed that values in high intensity cattle farming reaches were lower and statistically different from those in the agriculture reaches. SRP showed significant differences among low intensity cattle farming areas and the other rural reaches. However, pH was not statistically different from one type of land use to another (Fig. 3).

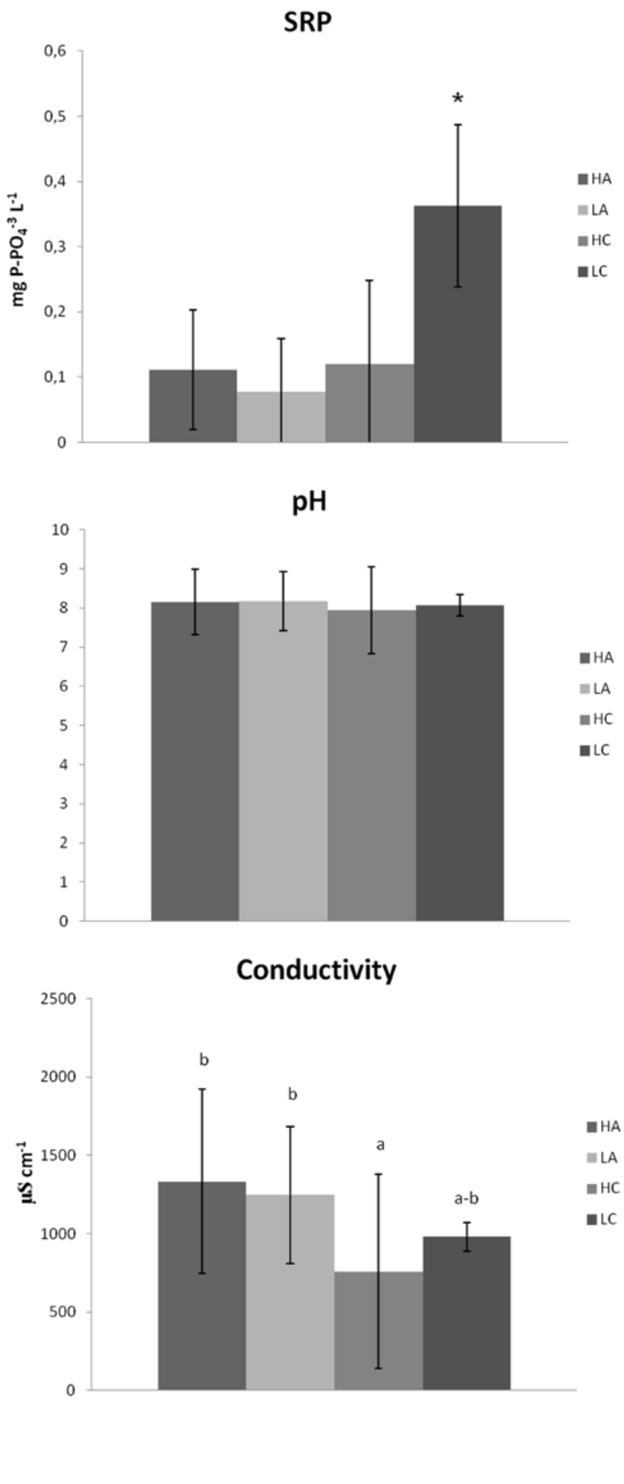

Fig. 3. Mean (± SD) concentrations of soluble reactive phosphorus (SRP), pH and conductivity. *, a–b significant differences, LC, low intensity of cattle farming (Las Flores); HC, high intensity of cattle farming (Nutrias, Durazno I); LA, low intensity of agriculture (La Choza II); HA, high intensity of agriculture (La Choza III, Durazno II)

Biofilm

Table 3 shows the ranks of variation in the structural and functional parameters of periphyton recorded throughout the sampling period. The lowest values of chlorophyll-*a* were recorded in the reaches with high cattle farming intensity. The values in Durazno I (high intensity of cattle farming) never exceeded 17 mg m^{-2}. The autotrophic index (AI) showed heterotrophic values for reaches with high cattle farming intensity, while Las Flores (low cattle farming intensity) showed autotrophic values. Agricultural sites registered AI values in the autotrophic range or near the limit thereof (200). Durazno II (high intensity of agriculture) was the only agricultural reach that showed high heterotrophy. Total polysaccharides were not related to the adjacent land use, although the lowest values were observed at reaches with high cattle farming intensity and likewise in Durazno II. Net production was higher for reaches with high intensity agriculture and low intensity cattle farming. Exoenzyme activities were very variable. No pattern of land use was found for cellobiohydrolase or β-glucosidase. Instead, La Choza II (low agriculture intensity) showed higher values than the other reaches regarding alkaline phosphatase activity (APA).

The percentage composition of the periphyton community in algal groups (Divisions) presented the highest Bacillariophyta values for reaches with high and low agriculture intensity and low cattle farming intensity. Reaches with high cattle farming intensity were represented by similar values of Chlorophyta and Cyanophyta. Euglenophyta in general registered the lowest proportion of the four divisions, although it was very abundant on some dates at the Nutrias reach (high intensity of cattle farming). Bacillariophyta showed a large predominance in fall and spring, whereas Cyanophyta was dominant in the summer regardless of the predominant type of land use (Fig. 4).

Similarly to what was done with the water quality parameters, a discriminant analysis was performed using all the periphyton variables (biomass (Dry weight, AFDW, Chlorophyll-*a*), production, respiration, nitrogen, phosphorous and polysacharides content, exoenzimatic activities of alkaline phosphatase, cellobiohidrolase and β-glucosidase using land use as a classification factor (Fig. 5). The classification matrix showed no great distance between sites. The greatest distance using the Mahalanobis square was 14.65 between reach zones with low and high cattle farming intensity. The most important and significant variables for differentiation were chlorophyll-*a* ($p < 0.001$), net production ($p < 0.05$) and alkaline phosphatase activity (APA) ($p < 0.01$).

One-way ANOVA was carried out for each variable highlighted in the discriminant analysis: chlorophyll-*a*, net production and APA, with significant results ($p < 0.001$) for the three variables. The post-hoc Tukey HSD analysis for chlorophyll-*a* indicated that concentration in reaches with high intensity of cattle farming was lower and statistically different from that in the other reaches. In contrast, net production showed no significant differences between high cattle farming intensity and low agriculture intensity but registered significant differences between low cattle farming intensity and high agriculture intensity. APA showed significant differences between reaches with low agriculture intensity and those given other land uses (Fig. 6).

Table 3. Variation rank (N = 9) of periphyton parameters for each of the sites sampled.

	La Choza II	La Choza III	Nutrias	Durazno I	Durazno II	Las Flores
Chlorophyll-*a* (mg m^{-2})	19.8–402.4	73.7–484.6	2.6–153.3	0.7–16.9	0.9–267.7	72.0–795.4
Pheopigments (mg m^{-2})	0.0–0.0	0.0–4.1	0.0–6.3	0.0–0.0	0.0–135.7	0.0–9.2
DW (g m^{-2})	9.0–198.5	41.6–146.9	20.9–109.5	6.8–24.7	11.9–139.4	18.0–122.8
AFDW (g m^{-2})	6.1–52.3	14.7–63.4	5.8–22.6	3.7–10.0	5.0–51.2	5.7–37.7
Autotrophic index	76.0–312.1	78.2–202.3	101.4–2996.2	280.9–5617.9	46.2–5337.1	29.9–126.3
Polysaccharides (mg ml^{-1})	11.2–62.6	16.1–313.7	5.2–54.8	3.8–45.5	19.2–28.4	4.7–111.1
PT (mg m^{-2})	16.2–110.0	16.2–160.9	2.1–129.8	7.6–112.8	2.4–100.3	20.5–151.9
NT (mg m^{-2})	0.7–108.0	1.1–119.3	0.1–138.8	0.0–739.8	0.0–63.3	1.0–104.6
NP (mgO_2 h^{-1} m^{-2})	–153.4–991.2	31.0–2839.6	–715.4–557.8	–701.7–226.9	–461.7–765.8	–126.1–2259.7
R (mgO_2 h^{-1} m^{-2})	0.0–553.6	0.0–682.8	0.0–448.6	0.0–273.1	0.0–947.6	60.9–600.9
Cellobiohydrolase (nmolMUF h^{-1} cm^{-2})	0.0–69.6	7.0–51.7	0.0–33.8	0.0–208.4	0.0–78.6	0.0–132.3
β-Glucosidase (nmolMUF h^{-1} cm^{-2})	94.3–855.2	0.0–908.9	15.9–741.1	67.4–2553.9	42.8–1600.5	85.3–1965.3
Alkaline phosphatase (nmolMUF h^{-1} cm^{-2})	436.7–3057.4	280.0–1822.0	38.3–1739.2	78.6–2168.9	78.6–1640.8	172.6–1734.8

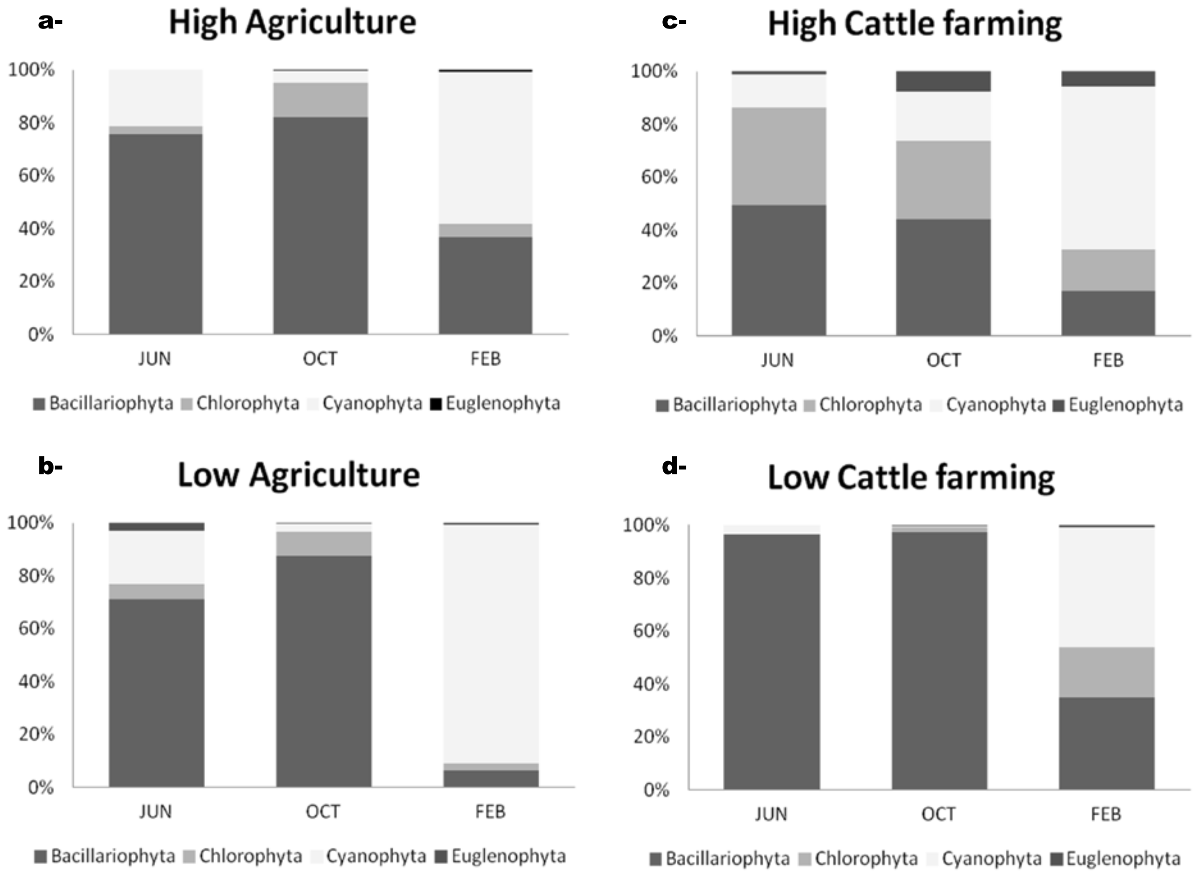

Fig. 4. Percentages of algal groups for each kind and intensity of rural activity. a- High agriculture (La Choza III, Durazno II), b- Low agriculture (La Choza II), c-High cattle farming (Nutrias, Durazno I), d- Low cattle farming (Las Flores)

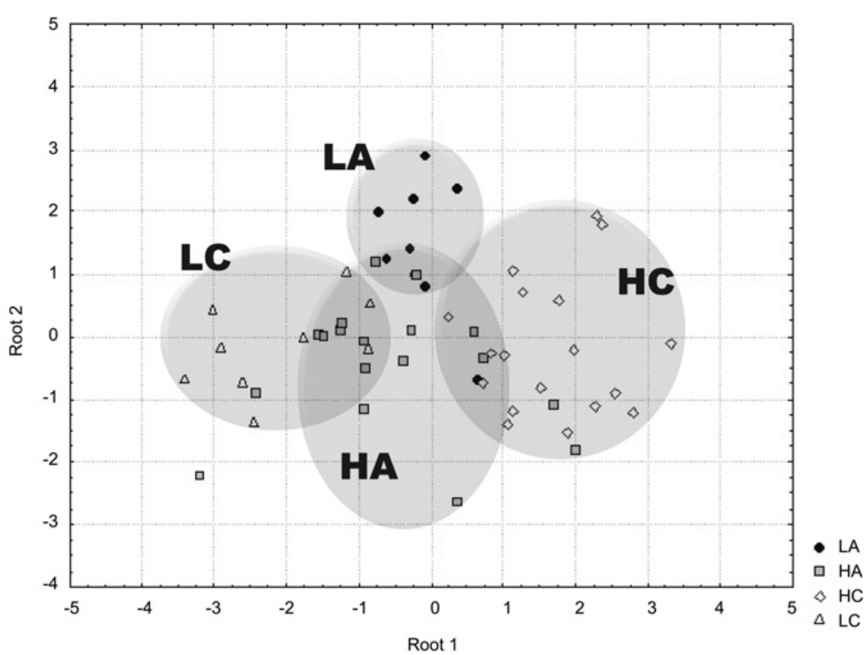

Fig. 5. The first two discriminant roots showing the location of uses and intensities of rural activities according to periphyton parameters are highlighted with circles. LC, Low intensity of cattle farming; HC, high intensity of cattle farming; LA, low intensity of agriculture; HA, high intensity of agriculture

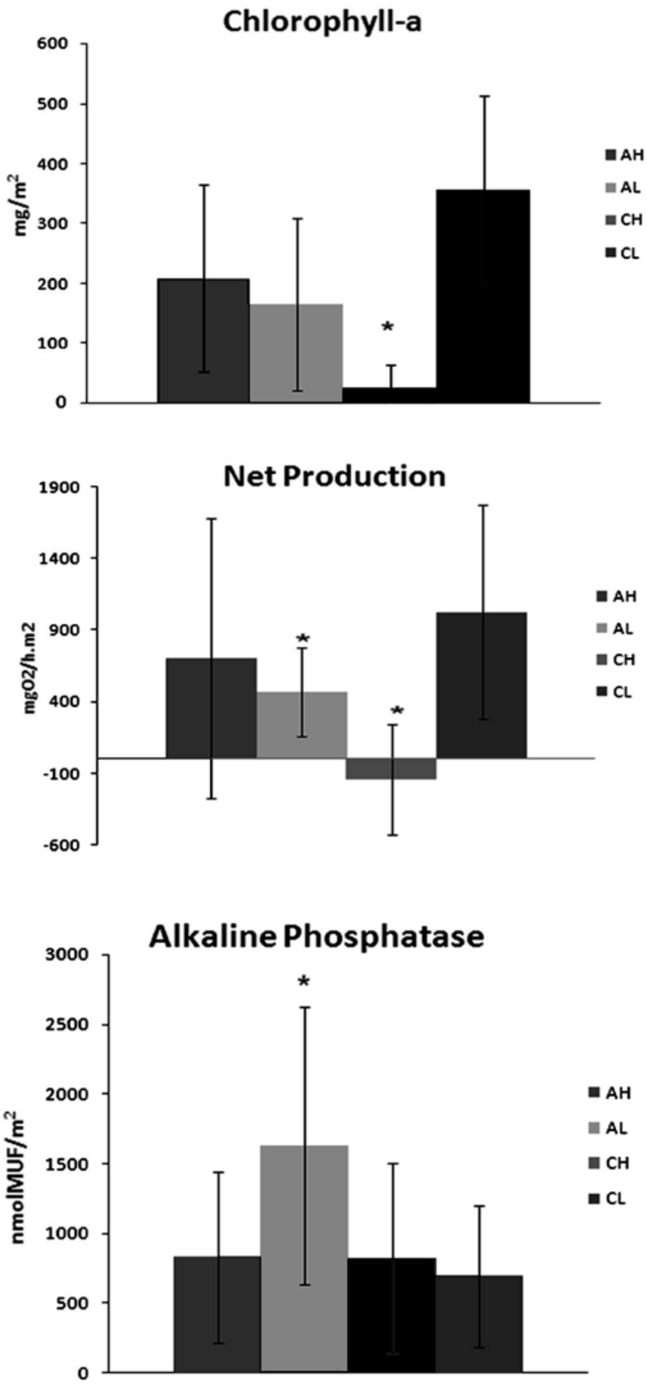

Fig. 6. Mean (± SD) concentrations of chlorophyll, net production and alkaline phosphatase. * significant differences, LC, low intensity of cattle farming (Las Flores); HC, high intensity of cattle farming (Nutrias, Durazno I); LA, low intensity of agriculture (La Choza II); HA, high intensity of agriculture (La Choza III, Durazno II)

The most important and significant variables for differentiation among land uses (based on multivariate analyses) were correlated. SRP was negatively correlative with APA. pH was positively correlative with chlorophyll-*a* and APA. And conductivity was positively correlative with all variables (Table 4).

Table 4. Correlation matrix (N = 50) between physicochemical and periphyton parameters selected from multivariate analyses.

	Chlorophyll-*a*	Net production	Alkaline phosphatase
SRP	n.s.	n.s.	−0.31 $p < 0.05$
pH	0.49 $p < 0.01$	n.s.	0.39 $p < 0.05$
Conductivity	0.74 $p < 0.01$	0.41 $p < 0.05$	0.41 $p < 0.05$

Discussion

Periphyton communities differed depending on whether the streams run through fields where cattle farming or agricultural activities are carried out.

Water quality differences between the sites can be attributed not only to the characteristics of the stream but also to the effect of the kind of activity carried out in the surrounding fields. Glyphosate herbicide, applied to soybean crops, reduces periphyton biomass (Perez et al. 2007). In addition, the possible arrival of herbicides could contribute to reducing the development of periphyton or modifying the dominant algae groups (Vera et al. 2010). In turn, fertilizers used on agricultural reaches increase phosphorus concentration in streams, thus contributing to an increase in periphyton biomass (Jarvie et al. 2010). In this study, similar characteristics in sites having different agricultural intensity can be explained because they all use similar soil tillage (direct seeding, fertilization and herbicides). In contrast, sites with different cattle farming intensity showed greater distance in the set of variables represented in the discriminant analysis (Fig. 2).

A similar response was observed in the discriminant analysis with periphyton parameters (Fig. 5), which differed significantly between the two cattle farming intensities, mainly in chlorophyll-*a* concentration and net production, both of which are correlated with conductivity. Thus, chlorophyll-*a* concentration shows changes when there are variations in algae composition or in physiological conditions. Changes in chlorophyll-*a* may be affected by humic acids concentrations, in which case the increase in chlorophyll-*a* in response to nutrient concentrations would be faster in waters with little concentration of humic substances.

The relative composition of the periphyton community registered a temporal variation with predominance of Bacillariophyta during fall and spring, and of Cyanobacteria during summer at all sites (Fig. 4). However, high cattle farming intensity changed these proportions. Bacillariophyta are favored by their low light saturation threshold and are therefore successful in an environment with a large suspended solids load such as that found in these streams (Gomez et al. 2003), whereas, Cyanophyta became more abundant where conditions were more eutrophic, and registered low flows and high temperatures (Vilalta & Sabater 2005).

High levels of alkaline phosphatase activity (APA) are indicators of phosphorus deficiency in algae (Bothwell 1985, Labry et al. 2005). Our results show the highest APA values in the reach with low agriculture intensity with APA being negatively correlated to SRP concentration and positively to pH and conductivity. Some controversy exists as to the use of APA as an indicator, since it seems to be useful solely in those cases in which phosphorus is the only changing parameter (Newman et al. 2003). In our study, the substrate did not interfere with APA response because it was artificial. However, because the study was carried out *in situ*, factors other than the availability of phosphorus to algae (such as the concentration of nitrogen, pesticides, humic acids, etc.) may interfere in the response. Bacteria and heterotrophic microorganisms in the community could contribute substantially to the synthesis of extracellular phosphatase for the hydrolysis of organophosphoric compounds in water bodies with high trophic level, but probably using the organic fraction as an organic carbon source. This mechanism is known as "induction-repression" (Cao et al. 2010). Cao et al. explain this mechanism by suggesting that this process synthesizes both APA and inorganic P simultaneously, leading to the co-occurrence of high phosphate and APA concentrations. In addition, freshwater microalgae produce extracellular enzymes, especially APA, because this activity is related to the light regime: APA will be highest with the increase in light, since there will be more photosynthetically active algae (Chappell & Goulder 1994). Wetzel (1992) found inhibition of APA by humic substances because these substances form a humic-enzyme complex that reduces enzyme activity. In this way, the complex may be transported downstream and reactivate enzyme activity by selective bacterial degradation of the complex or by physical photolysis by UV.

Humic acids concentration increases in water bodies following rains due to the drag from the surrounding fields (Serrano 1992) and decreases in dry periods due to the photo-oxidation of organic matter (Serrano 1994). Our results matched these statements in the agricultural sites or the sites where cattle farming intensity was low. However, cattle access to streams reduces the availability of light due to constant removal of particulate matter and release of humic substances as a consequence of the trampling produced at high intensity cattle farming reaches. The reduction of light availability has important consequences on community composition and secondary production and thus on photochemical processes, nutrient uptake and periphyton growth conditions (Julian et al. 2008). The results of this study showed that communities developed on artificial substrates were heterotrophic in reaches where intensity of cattle farming is high, which in turn were places where the humic acid concentration is more than two-fold higher (Table 2). Steinberg (2003) asserts that humic substances alter the underwater light climate and consequently the living conditions for autotrophic organisms. In summary, both humic acids and suspended particulate matter, as well as organic matter concentration, would favor the heterotrophic community.

Net production would be disadvantaged by a limited amount of light because of the high concentration of humic substances, or else favored by nutrient inputs. However, this study estimated metabolism in the laboratory under the same light and nutrient conditions for all samples with the purpose of examining the particular response of the community belonging to each reach. Thus, community metabolism was measured including the limitations of the reach studied and therefore any differences recorded were due to the community of origin and not to the experimental conditions. The results showed significantly lower net production at sites with high cattle farming intensity, where the community colonized on the substrates was largely heterotrophic.

Conclusions

The most important periphyton parameters to assess the non-point pollution effect due to rural activities estimated in this study were chlorophyll-*a*, net production and alkaline phosphatase activity. In addition, the water quality parameters which proved useful in differentiating rural activities were SRP and conductivity. Reaches with differing cattle farming intensity showed greater distance in the set of variables represented in the multivariate analysis. In contrast, reaches

with differing agricultural intensity showed similar characteristics.

As predicted, agriculture caused an increase in autotrophic biomass (chlorophyll-*a*) and hence an increase in net production. In addition, non-point pollution generated by cattle farming caused an increase in heterotrophic biomass. Finally, chlorophytes were abundant in agricultural zones whereas cyanophytes or euglenophytes were more abundant in cattle farming areas.

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