Environmental Advances 15 (2024) 100511

Contents lists available at ScienceDirect

Environmental Advances

journal homepage: www.sciencedirect.com/journal/environmental-advances

How do different modalities of land use practices impact the environmental features and macroinvertebrates? An assessment of mountain streams from Patagonia, Argentina

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ARTICLE INFO

Keywords:
Intensification
Biodiversity
Agroecosystems
Water quality
Integrity

ABSTRACT

Agricultural intensification is a significant process, transforming the land and modifying stream water quality and biodiversity. Land-use practices are associated with substantial changes in vegetation structure, significant inputs of nutrients, erosion, and increased sedimentation, all of which profoundly impact aquatic biota and ecosystem functioning. The effect of different agricultural practices (horticulture, extensive and intensive livestock) on stream water quality was compared in terms of physicochemical features, bacteria, habitat condition, riparian ecosystem, and macroinvertebrate variables in a temperate rural landscape (Patagonia). The main objective was to identify which variables related to land use were determinants in the configuration of biotic communities. Significantly higher values in water temperature, conductivity, nutrients, and bacteria were found at intensively developed sites than at reference ones. The quality of the habitat and the riparian ecosystem had been profoundly modified. A multidimensional scaling analysis revealed that macroinvertebrate assemblages also differed among land use categories, with the variables: total phosphorous, total nitrogen, bacteria (*E. coli*, mesophilic and total coliforms), water temperature, and oxygen having the highest predictive power. Macroinvertebrate metrics (richness of EPT (Ephemeroptera+Plecoptera+Trichoptera), Plecoptera, shredders and scrapers, and the percentage of scrapers and collectors) proved appropriate bioindicators for discriminating intensive land use impacts and highlighted a trend of biodiversity loss. Plecoptera richness, a taxa group with a high level of endemism, responded significantly to habitat condition degradation. These results confirm that the intensification of land use leads to increased nutrient levels and suppressed aquatic-terrestrial interactions involved in the provision of allochthonous detrital input, shade, and habitat structure for larval stages and adults. The Habitat Condition Index appears to be a useful tool for rapid stream assessment and providing early warnings. Several ecological services are threatened in these Patagonian agroecosystems. Restoring riverbank heterogeneity and improving management practices that ameliorate nutrient and sediment inputs are crucial to enhance biodiversity.

1. Introduction

Land use practices in a catchment can significantly impact environmental features and the integrity of aquatic resources (Allan and Castillo, 2007; Feld et al., 2016). The depth of the impact on freshwater biodiversity and ecosystem functioning will depend on the nature and intensity of related human actions. For instance, urbanization, industries, and intensive agricultural activities can increase erosion and sediment deposition, input nutrients in the forms of nitrogen and phosphorus, and might also alter the hydrology and substrate composition of flowing waters (Damanik-Ambarita et al., 2018; Villeneuve et al., 2018; Waite et al., 2019). Those land uses that modify tree cover to transform lands for pasture production or the development of extensive stock-farming, for example, can result in changes to water temperature and alteration to the input of allochthonous detritus (Richardson et al., 2010; Effert-Fanta et al., 2019). The lack of riparian vegetation can increase the amount of light that reaches the stream bottom, stimulating the growth of primary producer communities (e.g. periphyton,

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https://doi.org/10.1016/j.envadv.2024.100511
Received 3 November 2023; Received in revised form 9 February 2024; Accepted 27 February 2024
Available online 29 February 2024

macrophytes) (Niyogi et al., 2007; Principe et al., 2015).

The Patagonia region is associated with both extensive and intensive modalities of cattle breeding. Recently, following the national trend in the intensification process of agricultural practices (Oesterheld, 2008), intensive or confined animal production systems are gaining importance in Patagonian agriculture. The impact of these different production systems on the aquatic environment and the biota has been addressed in several studies worldwide (Quinn et al., 1997; Belsky et al., 1999; Price and Leigh, 2006; Hughes et al., 2016). Most scientists agree that domestic cattle grazing along streams have negative consequences on stream bank stability due to trampling and altered vegetative cover (Magner et al., 2008). Livestock water consumption frequently occurs directly in the stream channel, producing drier and hotter conditions (Belsky et al., 1999). The progressive increase of nutrients inputted by livestock through excretes (urine and faeces) deteriorates water quality (Cox et al., 2005; McKergow et al., 2012), ultimately leading to eutrophication (Epele and Miserendino, 2015; Chen et al., 2017). These effects are significant in confined production systems or those under intensive management (Chen et al., 2017; Horak et al., 2020). Cattle breeding can strongly modify the quality of the riparian corridor because stock grazing and trampling are typically concentrated around water sources (Arnaiz et al., 2011). The condition of instream habitats is further deteriorated by increased embeddedness and sediment deposition on bottom substrates (Connolly et al., 2016; Conroy et al., 2018; Horak et al., 2019).

Catchments dominated by agricultural lands are a significant source of phosphorus and nitrogen that contribute to nutrient enrichment at receiving waters (Castaldelli et al., 2015; Tanaka et al., 2016). The primary mechanism of nutrient mobilization is runoff from cultivated lands (Vondracek et al., 2005; Solis et al., 2018), and using fertilizers further exacerbates this phenomenon. Another pervasive effect on arable lands is the increase in total suspended solids (Cox et al., 2012). Among geomorphological changes, the loss of stream bank sinuosity and complexity are frequently documented (Shields et al., 2010). Agriculture can alter the riparian zone's vegetation structure by clearing or modifying the existing forest. These actions can impact stream ecosystem functions (for instance, leaf litter decomposition) and the attributes of associated biotic communities (Tanaka et al., 2015).

Macroinvertebrates have been successfully utilized in biomonitoring programs to assess the impacts of different land use practices on streams (Sovell et al., 2000; Choi et al., 2015; Boyer-Rechlin et al., 2016; Connolly et al., 2016; Weiss et al., 2023). Freshwater stream communities are highly responsive to processes occurring in the basin, and significant responses in terms of composition, structure, and functioning have been documented in different studies (Utz et al., 2009; Tanaka et al., 2016; Solis et al, 2018).

Earlier studies in Patagonia have revealed that several watercourses are disturbed due to the activities conducted in their watersheds (Miserendino and Masi, 2010; Miserendino et al., 2011). These disturbed streams display significant signs of deterioration and changes in water quality, with sensitive invertebrate groups (e.g. Plecoptera, Ephemeroptera, and Trichoptera) and fish either absent or diminishing in number at most of the affected reaches (Di Prinzio et al., 2009; Brand and Miserendino, 2015). New modalities of land use practices are consistently growing in western Patagonia (Miserendino et al., 2016; Williams-Subiza et al., 2022). Some of the region's land is dedicated to horticulture and fruticulture, for example, vineyards, tulips and peonies, strawberries, and carrots. All these practices require irrigation and are often adjacent to streams and rivers. Recently, Horak et al., (2019, 2020) documented several environmental problems related to water quantity and quality at streams draining intensive production systems, the study also reported substantial changes in aquatic communities.

As previously highlighted, some basins in northwestern Chubut are facing an intensification of land use. This intensification is considered one of the most significant drivers for the increasing rate of biodiversity loss and impairment of ecosystem functions. Aquatic resources in Patagonia provide important ecosystem services: water supply, regulation, and maintenance, they are also environments of high recreational value and support most of the economic activities. This ecoregion is remarkable in terms of its endemism, featured by unique flora and fauna, which in this scenario could be increasingly threatened. Through comparison with reference sites, this paper analyzes how different land use practices - extensive pastures, intensive livestock, and horticulture - alter environmental features, water quality, and the structure and function of macroinvertebrate communities. The main changes occurring in the riparian corridor and habitat conditions are also investigated. The hypothesis is that intensive land use practices will have a higher impact on stream integrity and biotic communities than other land uses. Changes in the composition and structure of macroinvertebrate communities in comparison to reference sites are predicted, with sensitive species eliminated at more impacted study sites (Ephemeroptera, Plecoptera, Trichoptera group) and an assemblage replacement by more generalist taxa (i.e., collector-gatherers). The question of which variables related to land use are determinants in shaping structural and functional aspects of biotic communities is addressed. This information is vital to maintaining the integrity of the regiońs water resources and improving the management of agroecosystems in temperate areas.

2. Material and methods

2.1. Study area and site selection

The streams are situated in the Andean Humid Biozone (del Valle et al., 1998), characterized by a high rainfall (West-East decreasing gradient: 3000 to 800 mm/y) and a mean annual air temperature ranging from 6 to 11°C. Most streams and rivers display a bimodal regime discharge, coinciding with the rainfall period (winter) and the snowmelt (spring) (Coronato and del Valle, 1988).

Vegetation in the area corresponds to the Subantarctic Forest, characterized by perennial (*Austrocedrus chilensis, Nothofagus dombeyi*, and *Maytenus boaria*) and deciduous tree species (*N. pumilio* and *N. antarctica*). The shrub and herbaceous strata are composed mainly of *Chusquea culeou, Berberis microphylla, Fuchsia magellanica, Aristotelia chilensis, Oenothera odorata, Potentilla chiloensis*, and *Geranium* sp. The riparian corridor in some watercourses has been invaded by *Salix fragilis* (Oyarzabal et al., 2018). The dominant soils in the basins are Molisols and Inceptisols, which have good permeability and drainage (Panigatti, 2010).

The study was conducted from March to December 2021 at 12 sites in streams in the Piedmont of northwest Chubut Province (Patagonia). For site selection, images of the area were inspected using Google Earth Software. This procedure was employed to avoid other potential sources of disturbance (e.g., urbanization, forestry industries, etc.) different from the land uses selected for the study. Existing data sources from different governmental administration offices (DPByP Dirección Provincial de Bosques y Parques, INTA Instituto Nacional de Tecnología Agropecuaria) were accessed. Information related to the animal load per unit area, offer of natural forage, and breeding modalities was obtained. Data regarding features of horticulture developments was obtained from the municipal administration (Secretaría de Producción de la Municipalidad de Trevelin). Additional information about the management practices was obtained from landowners' interviews (e.g. use of fertilizers, etc.). Three sampling sites were situated in non-impacted streams (Coihues, Fontana, and Bagillt); these sites were established as reference sites (R) to test for disturbances produced by different land use: horticulture (H), extensive livestock (E), and intensive livestock breeding (I) (Fig.1).

Three sites were situated on streams with horticulture developments (Nant y Fall, Rifleros and Enna). The main productive activities on the land immediately surrounding the stream sites include the cultivation of carrots, flowers (tulips), strawberries, and vineyards. Some sites, such as Rifleros and Nant y Fall, also displayed some signs of livestock activity.

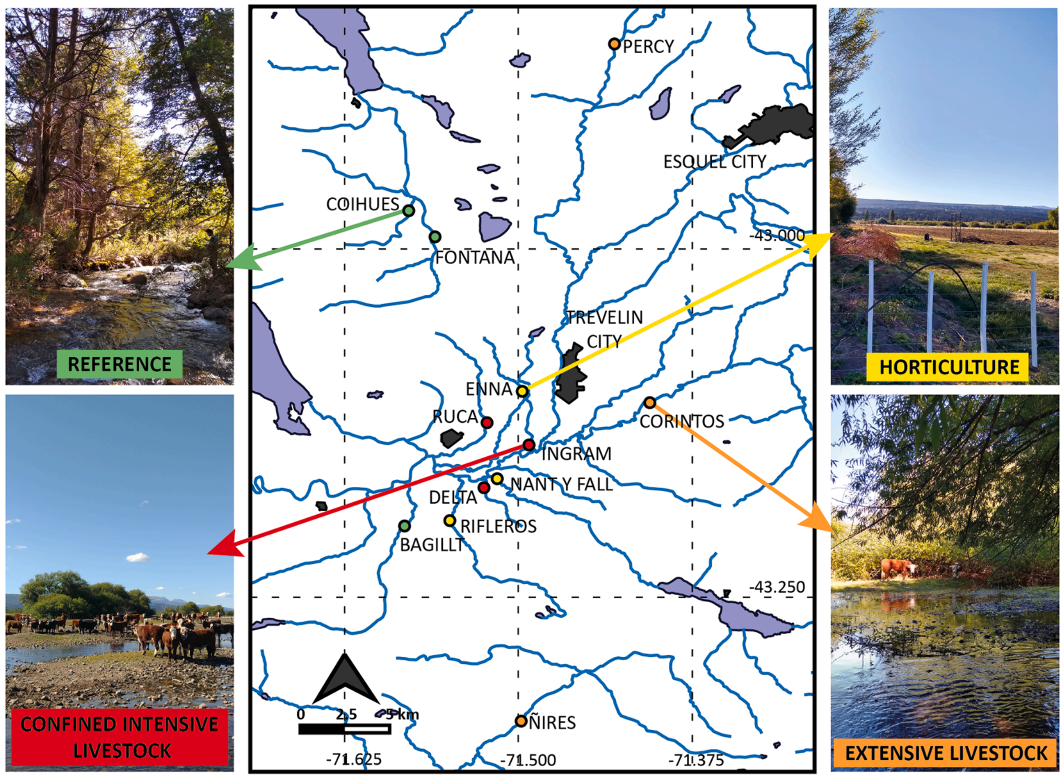

Fig. 1. Location of sampling sites during the study (Norwest of Chubut Province, Patagonia, Argentina). Picture represents different land use practices assessed. Colours represent land use types as follows: green: reference, yellow: horticulture, orange: extensive livestock, and red: intensive livestock.

These sites have historically been used as extensive pastures but have recently been converted to horticulture. For extensive livestock sites, three reaches in streams cleared of native Nothofagus forest and grazed extensively (Frío, Corintos, and Percy) were selected. Pasture sites currently support livestock consisting mainly of cows and sheep (Appendix A). Three stream sites were selected in intensive cattle breeding settlements: Ingram, Ruca, and Delta streams. These are developments with high loads (>10 animals per hectare) in confined settlements with unrestricted stream access (Iglesias et al., 2015).

2.2. Environmental characterization

For a thorough environmental characterization study, sites were visited four times (March, August, September, and December of 2021). Daily rainfall records and mean air temperature data were obtained from the National Institute of Agricultural Technology (Instituto Nacional de Tecnología Agropecuaria - INTA).

Percentage cover of boulder, cobble, gravel, pebble, and sand in the reach was estimated at each site using a 1 m^2 grid. Current speed was measured mid-channel on three occasions by timing a float (average of three trials) as it moved over a distance of 10 m (Gordon et al., 2004). Average depth was estimated from five measurements along one transversal profile across the channel with a calibrated stick. Discharge was obtained by combining depth, wet width, and current velocity as in Gordon et al. (2004).

On each sampling occasion, water temperature (°C), electrical conductivity (μS_{20}/cm), pH, dissolved oxygen (mg O_2/L) and oxygen saturation (% O_2) were obtained with a multi-parameter probe (Hach HQ40D). Water samples (2 L) were collected below the water surface and kept at 4 °C until further analyses in the laboratory. Analyzed nutrients include ammonium (NH_4^+), nitrate plus nitrite nitrogen (NO_3^--NO_2^-) and soluble reactive phosphorus (SRP). All determinations were made with a spectrophotometer and based on standard methods (APHA, AWWA, WEF, 2012). Total suspended solids (TSS) (mg/L) were estimated by filtering a known volume of water with pre-weighted fiberglass filters, drying (110 °C, 4 h), and re-weighing (APHA, AWWA, WEF, 2012).

For microbiological studies, water samples were collected under aseptic conditions and placed in sterile, screw-capped glass bottles, taking into consideration the standard methods of handling water samples. Bacteriological analysis of water samples was undertaken within 24 h of collection. The bacteriological examination of water samples included the colony-forming units (CFU) of presumptive total coliforms, thermotolerant coliforms, and *Escherichia coli* (CFU/100 mL water). Lauryl Tryptose Broth of double and simple concentration was the culture medium used to detect total coliforms in the presumptive stage. Brilliant Green Bile Lactose broth (BGBL) was used in the confirmatory stage following American Water Works Association methods (APHA, AWWA, WEF, 1998).

Pigment analyses were performed to estimate algal biomass. Measurements were conducted in March, August, and December 2021. Stream samples were taken from rock surfaces, and each sample consisted of scraping an area (20 cm^2) on the exposed surface of three randomly selected rocks. For each site and occasion, measurements were performed in triplicate. The content was preserved in water from the site and cooled while transported in dark containers to the laboratory, where they were filtered through GF/FF filters. All samples were frozen until analysis. Chlorophyll *a* was extracted from filters in 90 % acetone, and measured spectrophotometrically with correction by phaeopigments, according to standard methods (Wetzel and Likens, 1991; APHA, AWWA, WEF, 1998).

2.3. The habitat condition index and the quality of riparian ecosystem index

Two indexes frequently employed for watercourses in the hills and mountains of Patagonia were applied at each sampling site to assess any possible disturbance produced by in-stream conditions and riparian

corridors. These techniques are also promoted and validated by the National Biomonitoring Network (REM-AQUA) and available protocols (Giorgi et al., 2022). The Habitat Condition Index (HCI) was applied to evaluate habitat quality using the assessment procedure for the high gradient streams of Barbour et al. (1999). This method computes a score for ten river channel features (e.g., epifaunal substrate availability, frequency of riffles) from 0 to 20. A total score of 200 points means the river is natural, pristine, and in its best possible condition (range: 150–200). This index assesses the ability of the stream's physical habitat to support a specific fauna and then measures the spatial heterogeneity of the watercourse (Castela et al., 2008).

The complexity and attributes of the riparian vegetation in a 100-m reach were examined using an adaptation of the QBR index (Riparian Corridor Quality Index, Munné et al., 1998) for Patagonian streams: the QBRp (Riparian Ecosystem Quality Index for Patagonia, Kutschker et al., 2009). This index combines data from four additive metrics: total cover (proportion of the riparian area covered by trees and shrubs), structure (proportion of riparian vegetation composed of trees and shrubs separately), complexity and naturalness of vegetation (number of trees or shrub species and absence of introduced species, and other human impacts in riparian vegetation), and the degree of channel naturalness (e.g., bank modifications, dredging). The index also evaluated differences in the geomorphology of the river from its headwaters to the lower reaches. The total QBRp score ranges from 0 (extreme degradation) to 100 points (excellent quality, natural riparian forest).

2.4. Macroinvertebrate sampling and analysis

Quantitative macroinvertebrate samples were obtained with a Surber sampler (0.09 m^2; 250 μm mesh size) on one occasion during the summer (March 2021). According to previous studies, summer (low water period) appears the most appropriate time to assess the effect of land use in benthic communities (Miserendino et al., 2011; Horak et al., 2019). In each reach, three samples were taken from riffles and three from pools ($n = 6$). Samples were fixed *in situ* with 4 % formaldehyde and sorted in the laboratory under at least 5x magnification. Macroinvertebrate species were identified using available keys (Fernández and Domínguez, 2001). Functional feeding groups were assigned using available references, knowledge of feeding modes (mouthpart morphology and behavior), and analysis of gut contents (Merritt et al., 2008).

Macroinvertebrate total density (TD), the total richness of taxa (TR), the richness of Ephemeroptera, Plecoptera and Trichoptera (EPT R, and ER, PR, TR), the richness of insect families, diversity (H: Shannon Wiener diversity index) and dominance (Berger-Parker dominance index) were calculated per site. Species richness and relative abundance of shredders (S), grazer/scrapers (GSc), collector filterers (CF), collector gatherers (CG), and predators (P) were also obtained (Ludwing and Reynolds, 1988). The regional biotic index BMPS was also calculated (Miserendino et al., 2022b).

2.5. Statistical approach

Differences in physicochemical parameters and macroinvertebrate metrics across land use modalities were tested using Kruskal-Wallis ANOVA by Ranks test, as these variables did not fulfill the assumptions regarding normality and homogeneity of variances. This is a nonparametric alternative to the between-groups one-way analysis of variance. Land use was used as the main factor, and multiple measurements of physicochemical variables within each site were employed as replicates. The location of significant differences was assessed using multiple comparisons of mean ranks (two-sided significance levels with a Bonferroni adjustment) for all groups in post-hoc test (Siegel and Castellan, 1988). Seasonal patterns in physicochemical and bacteriological variables were analyzed using means and standard errors in the configurations.

A Pearson correlation matrix (distance matrix) based on quantitative macroinvertebrate data was performed, and this matrix was employed to produce a non-linear ordination using the Multidimensional Scaling (MDS) method with 50 random restarts to calculate the minimum stress of the 2D scatter plot. Before analysis, a log (x+1) transformation of macroinvertebrate data was applied. The MDS goodness of fit was estimated with a stress function (which ranges from 0–1), with values close to zero indicating a good fit. A stress of 0.2 or less was considered low stress, and therefore supported a strong pattern in the community analyzed. This procedure is an alternative to the factorial method, but has no assumptions concerning distributions and represents samples as points in low-dimensional space. The main objective is to display significant distances among investigated objects (Ludwing and Reynolds, 1988). Associations between the MDS ordination scores and the environmental variables were tested using Spearman rank correlations. Bivariate relationships (scatterplots) were performed to examine macroinvertebrate metrics response to those significant environmental variables. These procedures were performed using the Statistica package 10 Stat soft Inc. USA.

3. Results

3.1. Environmental features

Physicochemical stream characteristics and some biological features were similar among the studied sampling sites, mostly in terms of elevation (range: 343–723 m.a.s.l), depth (range: 0.11–0.37 m), substrate composition, pH, and chlorophyll *a* (Table 1). All sites displayed good oxygenation; nevertheless, some of them showed unexpectedly high values of BOD_5 during summer (>7 mg/L, Corintos, Fontana, Rifleros). Water temperature differed significantly among land uses (R<H, E, I, $p<0.05$). Reference sites were colder than the rest, and this pattern was consistent during the year, especially during spring (Fig 2 a). Electrical conductivity differed among land uses (R< H, E, I, Kruskal Wallis (KW) test $H_{(3,\ n=47)} = 16.6$, $p<0.0009$). Conductivity values at R and I sites were constant throughout the year, though E sites displayed a significant decreasing pattern in conductivity from summer to autumn (Fig. 2 b).

Signs of sedimentation were evidenced by significantly higher levels of TSS at E and I than at R sites (KW test: $H_{(3,\ n=47)} = 15.57$, $p < 0.001$) and by a higher level of sand deposition at some reaches. No marked differences occurred among seasons, except for winter when a notable increase was seen due to intensive use (Table 1, Fig. 2 c).

As expected, mean annual nutrient values differed markedly at I sites compared to the R and H ones, with values of SRP (KW test: $H_{(3,\ n=47)} = 16.7$, $p < 0.0008$), NH_4^+ (KW test: $H_{(3,\ n=47)} = 16.4$, $p < 0.0009$), and TP (total phosphorous) (KW test: $H_{(3,\ n=47)} = 18.3$, $p < 0.0003$) being significantly higher at I sites (Fig. 2 d, f, h). NO_3^-–NO_2^- and TN (total nitrogen) only differed between I and R sites (I>R, KW test: $H_{(3,\ n=47)} = 12.9$, $p < 0.004$). Patterns of NH_4^+ and NO_3^-–NO_2^- were quite low throughout the year at land uses H, R, and E and very variable at I sites (Fig. 2 d, e). Seasonal trends revealed that the level of SRP decreased from summer to winter and increased from winter to spring at I sites (Fig. 2 f). A decreasing pattern of TN from summer to the following spring occurred at H sites, whereas at E sites, lower values of TN occurred in winter (Fig. 2 g). Levels of TP increased from summer to winter at R sites (Fig. 2 h).

3.2. Bacteria

Significant differences among land uses occurred in mesophilic bacteria levels (R<H<I sites and E<I, KW test: $H_{(3,\ n=47)} = 32.6$, $p < 0.0001$). The relationships observed among the different land uses regarding total coliforms (KW test: $H_{(3,\ n=47)} = 27.5$, $p < 0.0001$) and E. coli (KW test: $H_{(3,\ n=47)} = 29.6$, $p < 0.0001$) were I>R, E, and H (Fig. 3 a, c). Seasonal trends of mesophilic bacteria revealed that maximum values

Table 1
Environmental characterization of 12 sampled sites at streams in Patagonia (Argentina) during 2021. Land use codes: R: reference, H: horticulture, E: extensive livestock and I: confined intensive livestock. Data are: mean value or range (min-max) (n=4). Substrate codes: Bo: boulder, Co/Pe: cobble-pebble, Gra: gravel and Sa: sand.

Stream name	Land use	Elevation (mas l)	Water temperature (°C)	Depth (m)	Water velocity (m/s)	Dry width (m)	Wet width (m)	Discharge (m^3/s)	Chlorophyll a ($\mu g/cm^2$)	pH	BOD_5 (mg O_2/L)	Dissolved oxygen (mg/L)	Total suspended solids (mg/L)	Dominant Substrate type
COIHUES	R	641	3.8-7	0.23	0.69-1.23	7.47	5.13	1.21	0.25	8.44	1-2	11.6-12.6	0.24	Bo/Gra
FONTANA	R	642	3.4-9.8	0.21	0.49-1.28	7.85	5.62	1.16	0.15	8.7	0-8	10.8-12.7	0.28	Bo/Co/Pe
BAGILLT	R	376	3.6-12.5	0.35	0.87-1.39	15	8.44	3.10	0.57	8.23	0-5	10.5-13	0.20	Bo/Gra
RIFLEROS	H	372	3.9-16.2	0.22	0.27-0.42	10.26	7.92	0.52	0.49	8.93	1-8	11.4-13.2	1.64	Gra/Co/Pe
NANT Y FALL	H	343	5.8-14.3	0.30	0.58-0.77	9.60	6.89	1.47	0.98	8.61	0-1	9.6-12.1	0.70	Gra/Co/Pe
ENNA	H	367	3.9-12.6	0.22	0.13-0.40	4.32	5.75	0.89	0.72	8.68	0-4	10.3-13.1	1.67	Bo/Gra
CORINTOS	E	555	6.5-13.7	0.28	0.11-0.99	21.36	8.27	1.81	0.71	8.72	1-9	8.8-12.1	1.70	Gra/Co/Pe
FRIO	E	646	6.4-19.8	0.19	0.47-1.07	9.93	4.91	0.80	0.41	8.63	0-6	9.9-12.3	3.97	Co/Pe/Gra
PERCY	E	723	6.9-17.4	0.37	0.76-2.13	49.62	12.68	6.42	0.24	8.79	1-4	9.2-11.3	2.34	Bo/Gra
INGRAM	I	351	11.7-25	0.13	0.04-0.29	34.06	23.24	0.37	1.74	8.62	0-7	9.7-15.2	29.2	Co/Pe/Sa
RUCA	I	351	4.5-14.2	0.13	0.18-0.74	9.32	3.36	0.22	0.72	8.58	0-3	9.5-12.6	0.98	Co/Pe/Sa
DELTA	I	350	4.7-16.1	0.11	0.37-0.5	7.58	3.13	0.16	0.38	8.17	1-4	8.7-12.2	2.95	Co/Pe/Sa

at H sites corresponded to spring, while at I sites, peaks occurred in summer and autumn (Fig. 3 b). For I land uses, the lowest mean values of E. coli and total coliforms occurred in summer.

3.3. QBRp and HCI

Quality judgment classes of the riparian ecosystems in question varied from extreme degradation at I sites (mean value: 18.3 points) to good quality at R sites (mean value: 71 points) (Fig. 4 a). Substantial riparian ecosystem alteration was found at H sites (mean value: 39 points), though it was also very variable (range 15–63). At E sites, the riparian ecosystem was also considerably altered (mean value: 26 points). Exotic species were well represented at H, E, and I sites (Appendix A). Signs of disturbance detected included browsing (Fontana stream), the presence of faeces, and trampled streambanks (Nant y Fall, Corintos, Frío, Ingram, Ruca, and Delta).

Habitat condition at R sites was optimal (mean value: 190 points) and suboptimal at H sites (mean value: 122.3 points), indicating that some functional attributes or structuring components were missing. As expected, the most degraded sites corresponded to land use E (mean value: 86.6 points) and I (mean value: 39 points), with judgment classes being marginal and poor, respectively (Fig. 4 b).

3.4. Macroinvertebrate communities

A total of 110 macroinvertebrate taxa were recorded in the study (Appendix B), and the mean density per site varied between 601.8 ind/m^2 (Fontana) and 10436 ind/m^2 (Frío). The total richness of taxa ranged from 20 (Coihues) and 44 (Rifleros) (Table 2). Richness was notably variable for Plecoptera (0–7 taxa), Trichoptera (2–10 taxa) and Ephemeroptera (2–8). The highest degree of dominance occurred at Ruca (74 %), and the highest diversity occurred at Bagillt (H index=2.6).

Several macroinvertebrate metrics displayed significant differences among land uses. EPT richness allowed I sites to be split from R and H sites (Table 3). Plecoptera richness was significantly higher at R, H, and E sites than at I ones. Among functional feeding groups, I sites stood apart from the others when it came to the shredder richness, with values being significantly lower. A higher degree of grazers/ scrapers richness occurred at H and E sites than at I and R sites. The percent of grazers/ scrapers was significantly higher at I sites than at E ones, whereas the percent of collectors were markedly lower at R, H, and E sites than at I ones (Table 3).

The MDS analysis (d-had stress: 0.08) based on macroinvertebrate species abundance clearly grouped I sites towards the positive end of dimension 1 (Fig. 5). Interestingly, H sites were grouped in the middle section of the ordination, whereas R sites occurred in the top left quadrant. The E sites were positioned to the negative end of dimension 1. When identifying variables associated with dimension 1, water temperature, nutrients (mostly SRP, TN, and TP), and bacteria (total coliforms, mesophilic, and E. coli) were positively correlated and proved to be significant parameters in explaining the ordination of sites (Appendix C). Other significant variables were dissolved oxygen, water velocity, and habitat condition, which negatively correlated with dimension 1.

The structure of functional feeding groups differed among land uses (Fig. 6, Table 2). The high contribution of collectors at I sites was notable (>64 %), whereas the participation of shredders was extremely low (<1.2 %), as were grazer/scrapers (<33 %). On the other hand, shredders' contribution at R sites ranged from 13 %–32.7 %.

Correlation between macroinvertebrate metrics that significantly responded to land uses and physicochemical variables revealed that Plecoptera richness increased with higher site habitat condition scores (Fig. 7). The habitat was the most significant variable.

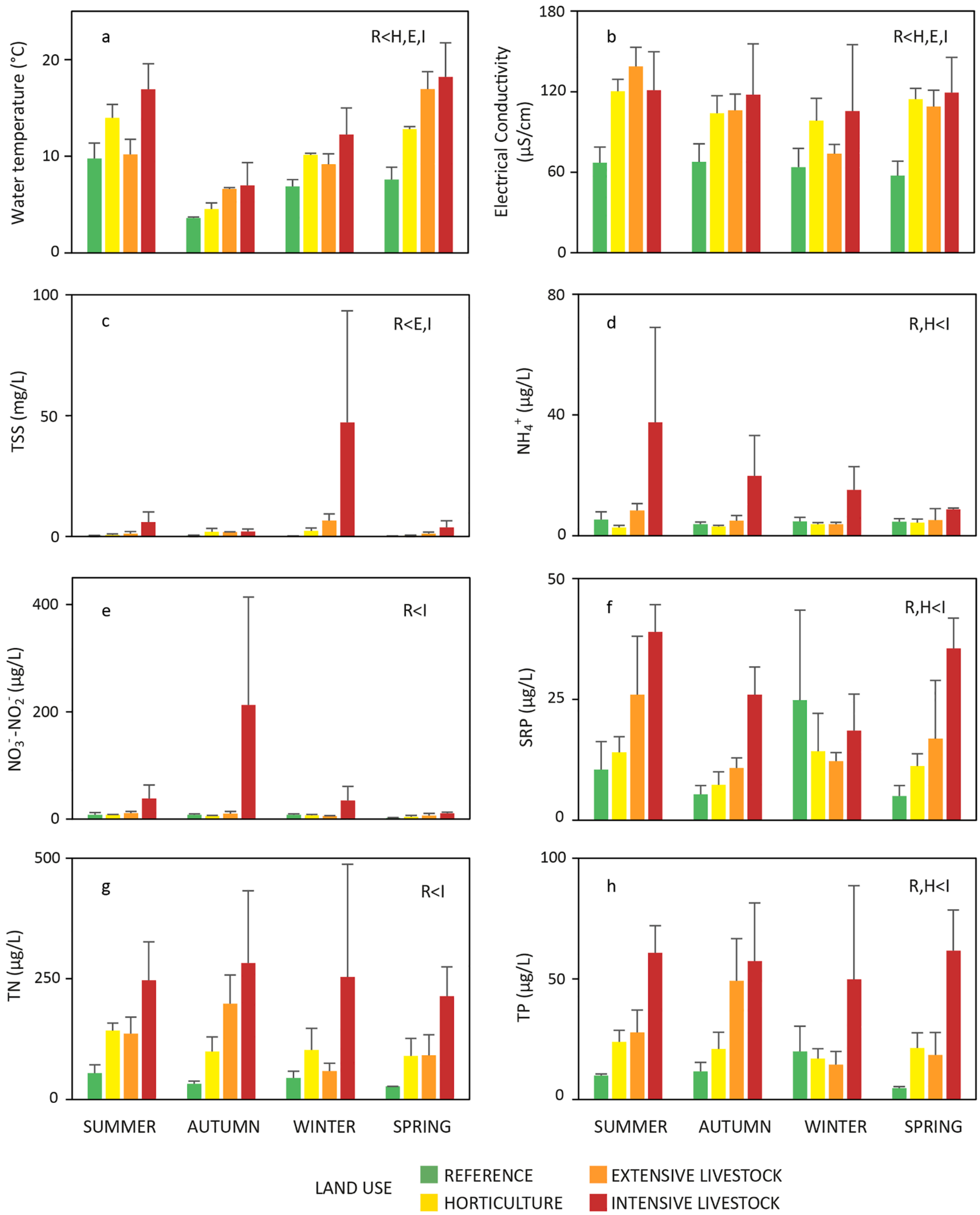

Fig. 2. Seasonal trends of environmental variables assessed per land uses. a) Water temperature, b) Electrical conductivity, c) Total suspended solids, d) Ammonia, e) Nitrate nitrite, f) Soluble reactive phosphorous, g) Total Nitrogen and h) Total phosphorous. Data are mean values (n=3) and bars indicate +SE. Significant differences are marked according to multiple post-hoc comparison after Kruskal-Wallis test (two-sided significance levels with Bonferroni adjustment, $p<0.05$).

4. Discussion

4.1. Environmental effects of land use in Patagonian streams

This work supports the prediction that significant changes in environmental features were associated with the agricultural practices occurring in the area. The most significant modifications at the sites were in terms of conductivity, nutrients, and bacteria; however, the observed pattern varied according to land use type. For example, reference sites differed from the rest with regard to water temperature and conductivity. Stream reaches in the forest are frequently less open, more shaded, and colder than those at developed basins (Connolly et al., 2016), while high electrical conductivity is associated with watercourses dominated by pasture and narrower riparian forests (Tanaka et al., 2016; Horak et al., 2019) or with those draining production systems (Raitif et al., 2019). Several nutrients (TP, NH_4^+, and SRP) and total suspended solids were significantly higher at intensive and extensive livestock sites than at horticulture and reference sites. These findings partially fit the main expectations because horticulture sites did not differ from reference sites with regard to those variables. In previous work, Horak et al. (2020) observed increased values of nitrogen compounds resulting from the application of fertilizers in fields, suggesting that they probably entered the stream by runoff.

Similarly, other authors (Cox et al., 2012; Arias et al., 2020) have

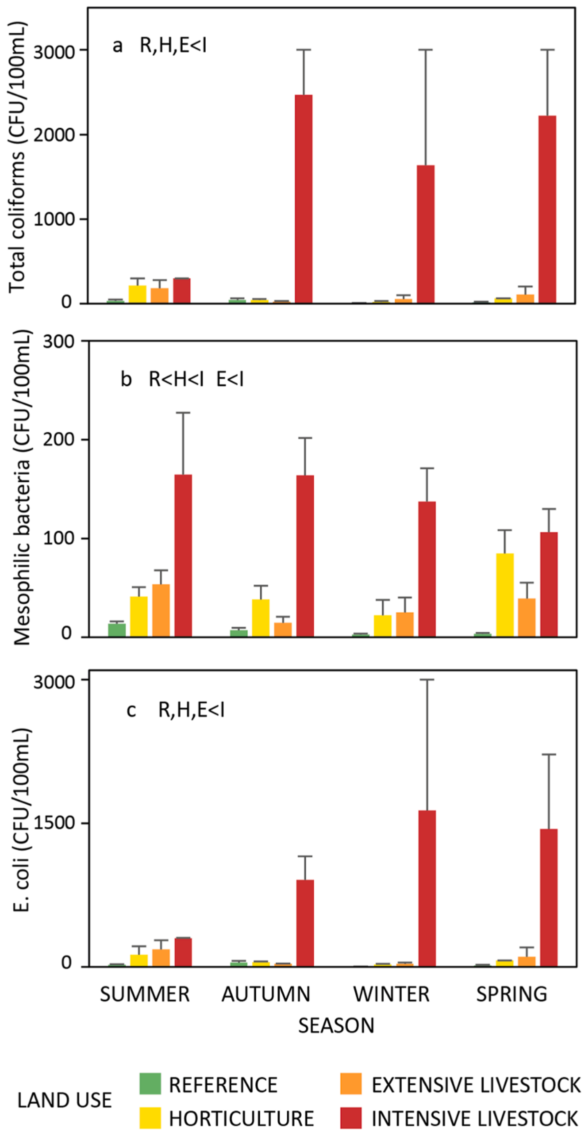

Fig. 3. Seasonal bacteriological trends per land use during the study period. Data correspond to a) Total coliforms, b) Mesophilic bacteria, and c) *Escherichia coli*. Graphs display mean values (n=3) and bars indicate +SE. Data are mean values (n=3) and bars indicate +SE. Significant differences are marked according to multiple post hoc comparison after Kruskal-Wallis test (two-sided significance levels with Bonferroni adjustment, $p<0.05$).

reported high levels of TSS and TN found in surface drainage from different horticultural production sites. As expected, main result demonstrates that sites at intensive livestock productions were the most impacted. These effects could be linked to the heavy livestock activity in the adjacencies (i.e. deposition of feces, urine) (McKergow et al., 2012), runoff, and trampling (Herbst and Kane, 2009; Mesa, 2010) that results in severe environmental problems for water quality due to increased levels of nitrogen, phosphorous, and suspended solids (Volk et al., 2009; O' Sullivan et al., 2019).

Regarding the most nutrients and bacteria (total coliforms, mesophilic and E. coli), the values detected in areas of intensive livestock exceeded those found in areas of extensive livestock land use. Likewise, the free access of livestock to streams was a pollution pressure that resulted in significant ecological degradation of the sites (Conroy et al., 2018; OSullivan et al., 2019), which is similar to that reported by Horak et al., (2019, 2020) in previous studies in the area. Some temporal trends were also highlighted. For example, at horticulture sites, mesophilic bacteria reached their peak during spring, probably due to runoff. The mean values were significantly higher compared to those documented at reference sites. Similarly, at intensive livestock sites, E. coli peaked during winter (rainy season), coincidently with the highest cow load (winter breeding) (Horak et al., 2019). This observation aligns with findings from other studies that indicate that heavy rainfall episodes can increase stream bacteria counts (Scanes, 2018; Xue et al., 2018).Another significant observation at intensive livestock sites was an ammonia peak during summer, coincidently with the low water period. Low discharges and warmer temperatures probably favored lower oxygen levels and ammonification processes (Hefting et al., 2013). Instead, the level of TP remained quite similar among seasons at these sites. At extensive land-use sites, the levels of TP and TN reached their peak during autumn, likely associated with the leaching from the plant-soil system and coinciding with high levels of dissolved oxygen in the water. A peak in SRP was also observed at reference sites during winter, probably as a consequence of runoff episodes during the rainy season (Edwards et al., 2000).

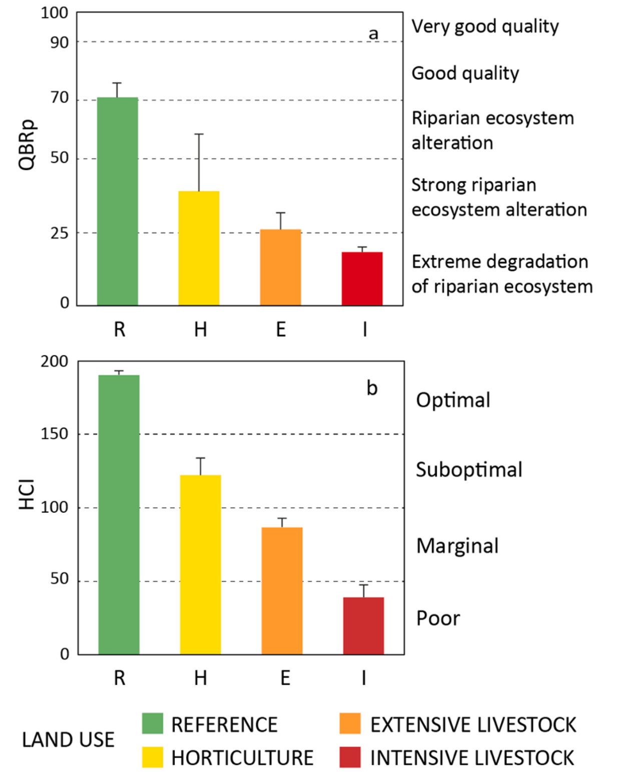

Fig. 4. Quality judgment criteria per land uses during the study a) QBRp (Riparian Ecosystem Quality Index for Patagonia), b) HCI (Habitat Condition Index). Values are mean (+1SE) (n=3). R: reference, H: horticulture, E: extensive livestock, and I: intensive livestock.

4.2. *Impacts on riparian ecosystem and habitat conditions*

As documented here, all land use practices modified the riparian components (width of the buffer strips, the contribution of exotic species, one or more missing vegetation stratum, and structure) and resulted in habitat degradation. Horticulture produced moderate impacts; however, these impacts were very variable among different sites. The riparian corridor was markedly modified at the Enna site (carrot production) because plowing occurred very close to the stream. The reach displayed a highly unstable bank, low complexity, and poor structure in the vegetation. At the same time, the site obtained low scores for habitat condition, which is predictable given the connection between the two parameters, QBRp and HCI. In these piedmont streams, a poor, degraded riparian corridor will fail in preventing erosion, retaining sediments,

Table 2
Macroinvertebrate metrics (abundance, richness, diversity and trophic measures) and biotic index (BMPS) at 12 sampled sites at streams in Patagonia (Argentina) during March 2021. Land use codes: R: reference, H: horticulture, E: extensive livestock and I: confined intensive livestock.

Stream name	Land use	Mean density (ind/ m^2)	Taxa richness	EPT richness	Plecoptera richness	Ephemeroptera richness	Trichoptera richness	Insects family	H (Shannon-Wiener)	% Dominance	Shredders richness	Grazers/ Scrapers richness	Collectors richness	Predators richness	% Shredders	% Grazers/ Scrapers	% Collectors	% Predators	BMPS
COIHUES	R	1673.9	20	14	7	2	5	13	1.6	50.9	8	4	5	3	32.7	59.8	3.0	4.4	95
FONTANA	R	601.8	22	14	4	6	4	12	2.2	27.7	5	7	7	3	13.9	65.2	7.1	13.8	91
BAGILLT	R	1570.2	27	15	5	5	5	16	2.6	16.4	6	7	9	5	20.5	38.4	39.1	2.0	116
RIFLEROS	H	9752.7	44	21	5	6	10	19	2.3	32.8	6	13	15	10	34.4	27.8	35.8	2.0	146
NANT Y FALL	H	4671.8	32	14	1	6	7	13	2.0	44.5	3	10	12	7	14.9	22.4	59.9	2.7	110
ENNA	H	1875.7	30	15	2	5	8	12	2.1	31.6	4	10	10	6	0.5	58.8	35.9	4.7	91
CORINTOS	E	2457.2	30	12	2	4	6	12	1.8	55.2	3	10	10	7	1.4	71.8	23.3	3.4	94
FRIO	E	10436.0	39	18	2	8	8	17	2.1	43.0	3	11	12	13	2.9	72.2	23.8	1.2	126
PERCY	E	1281.4	29	12	3	3	6	16	2.1	43.6	3	9	10	7	5.3	61.6	31.1	2.0	107
INGRAM	I	7397.4	32	5	0	2	3	12	1.9	46.1	1	6	12	13	1.2	4.8	77.2	16.8	96
RUCA	I	4334.8	24	4	0	2	2	14	0.9	74.0	2	4	8	10	0.6	0.8	97.6	1.0	80
DELTA	I	2188.7	34	13	1	2	10	15	1.8	33.8	3	6	11	9	0.3	33.4	64.7	1.6	107

Table 3
Results of non-parametric Kruskal Wallis ANOVA by ranks test H $_{(df=3,12)}$ for effects of land use on macroinvertebrates metrics. Significant differences are in bold, relationships in post-hoc comparisons are consigned. Land use codes: R: reference, H: horticulture, E: extensive livestock and I: confined livestock production.

	$H_{(df:\ 3,\ n=12)}$	*P*	Post-hoc test
Total density	0.86	0.49	
Taxa richness	2.62	0.12	
EPT richness	3.78	**0.05**	R, H>I
Plecoptera richness	8.24	**0.04**	R, H, E>I
Ephemeroptera richness	6.67	0.08	
Trichoptera richness	6.51	0.08	
Insects family richness	1.33	0.72	
H Shannon-Wiener diversity index	4	0.26	
% Dominance	1.33	0.72	
Shredders richness	9.25	**0.02**	R, H, E>I
Grazers/Scrapers richness	12	**0.007**	H, E>R, I
Collectors richness	3.77	0.28	
Predators richness	6.51	0.08	
% Shredders	6.1	0.10	
% Grazers/Scrapers	8.69	**0.03**	I<E
% Collectors	8.12	**0.04**	R, H, E <I
% Predators	1.46	0.69	
BMPS	1.39	0.70	

and supplying woody material. These aspects are crucial to configuring and enhancing the in-stream habitat.

Intensive and extensive livestock land use systems produced the most dramatic changes in riparian ecosystems and habitat conditions. These changes have marked implications for the integrity of the aquatic ecosystem and the biota that inhabit the stream (Schepker et al., 2020; Cole et al., 2020). Animals were not kept out of the watercourses at any of the studied sites, and this was exposed by a marked instability of banks and the degree of channel alteration. Several studies have shown that the removal or exclusion of cattle can significantly increase bank stability, riparian vegetation complexity (Herbst et al., 2012; Muller et al., 2016, Cole et al., 2020), the biomass of instream vegetation, and benthic detritus (Scrimgeour and Kendall, 2003; Masi and Miserendino, 2009).

Stream restoration measures should include the exclusion of livestock from the riverbank and the establishment of buffer strips, a task that is not too easily achieved if landowners are not involved or incentivized through financial support (Carline and Walsh, 2007; Cole et al., 2020). However, according to Muller et al., (2016), these are successful measures in healthy watersheds with a relatively short agricultural history and low-intensity land use, where cattle exclusion has brought about positive and rapid effects on stream water quality. The development of intensive land use practices in the Patagonian northwest is very recent (Iglesias et al., 2015; Epele and Miserendino, 2015; Horak et al., 2019, 2020), and the implementation of some mitigation measures might hopefully prevent a significant deterioration in ecosystem integrity.

4.3. *Intensification of land use practices and compositional and structural changes in macroinvertebrate communities*

In line with other studies (Raitif et al., 2019; Horak et al., 2019; Cole et al., 2020), the main results of this research emphasize that agricultural practices impact aquatic biodiversity, with some sensitive species being extirpated from stream reaches and a significant increase seen in more tolerant or generalist species. For example, a marked increase in collector contribution occurred at intensive sites, which is similar to what has previously been documented at grazed streams in California (Herbst et al., 2012), Ireland (Conroy et al., 2018), New Zealand (Quinn et al., 1997) and other Patagonian streams in the northwestern area (Miserendino et al., 2011; Horak et al., 2019). As predicted, a decrease in the EPT group occurred at disturbed sites; this is a faunistic group

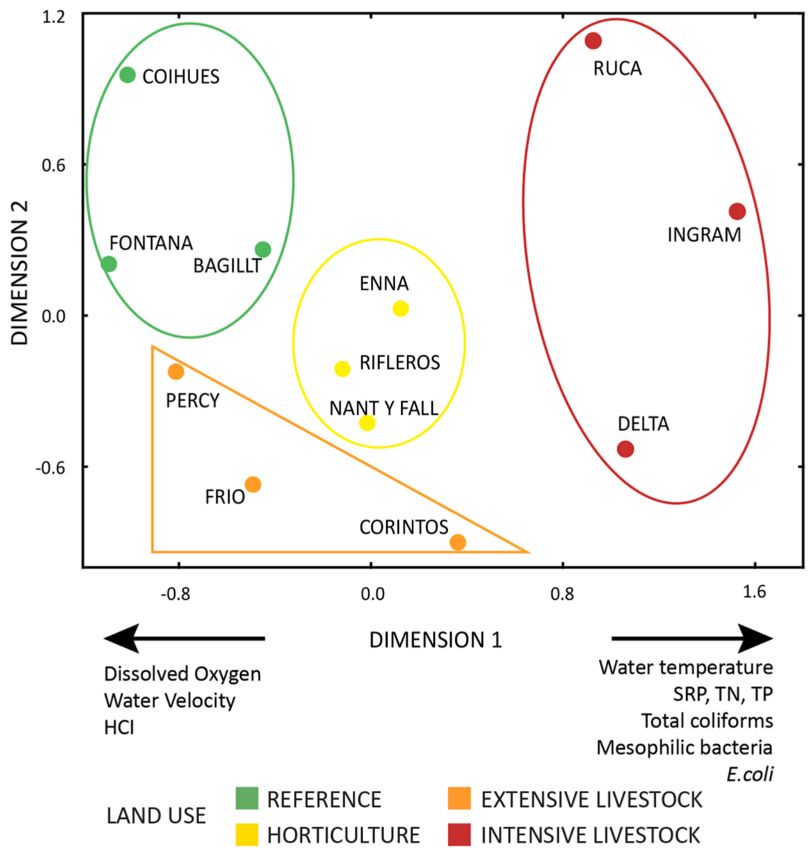

Fig 5. Multidimensional scaling analysis of macroinvertebrate community at 12 sampling sites from rivers subjected to different land uses (Chubut, Argentina). Land use types are indicated with different colours as follows: green: reference, yellow: horticulture, orange: extensive livestock, and red: intensive livestock. Significant correlation coefficients (Spearman rank) with dimension 1 are displayed (r and p values are given in Appendix C).

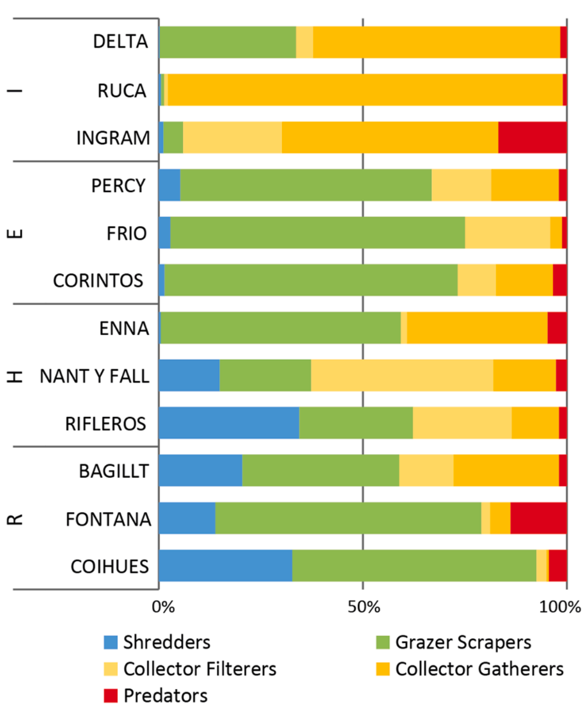

Fig 6. Functional feeding structure of macroinvertebrate community per sites (land use types are consigned). Data express a relative abundance of shredders, grazers/scrapers, collector filterers, collector gatherers, and predators.

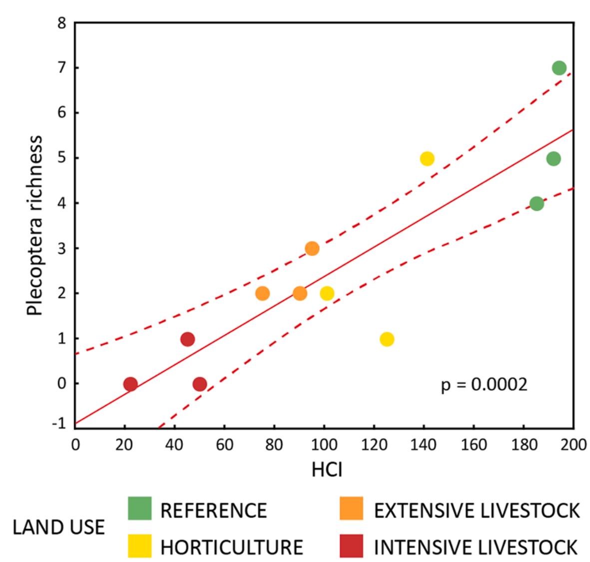

Fig. 7. Scatterplot showing significant relationships between Plecoptera richness and Habitat Condition Index (HCI) scores. (r^2=0.77, solid line: linear fit, dashed line indicates confident level: 0.95).

susceptible to pollution (Miserendino et al., 2016), represented by many endemic species in the Patagonia ecoregion. In a recent study, Horak et al., (2020) found that the overriding stressor explaining community changes in intolerant taxa (EPT) was ammonium, which is toxic to intolerant aquatic insect larvae. Above all, Plecoptera appeared to be

highly intolerant to disturbances resulting from different land use practices. Stoneflies are highly sensitive to geomorphological alterations (De Castro-Català et al., 2020) and water temperature changes (Hollmann and Miserendino, 2008).

Interestingly, a strong relationship between stonefly richness and the Habitat Condition Index was documented. This index included several parameters that are informative of the integrity of the ecosystem, such as the type of substrate and their availability, signs of sedimentation, the combination of flow velocity and depth gradients, the availability of shade, and the allochthonous detritus (woody debris) present. Shredder species are an important part of this group (Plecoptera). Shredders rapidly disappear when the riparian forest is modified (forest to pasture), eliminated, or replaced by exotics (Miserendino and Masi, 2010). The significant decrease in shredder richness at intensive livestock sites can probably be attributed to a combination of factors, including water quality degradation, increased water temperature, and the loss of riparian forest integrity.

Water temperature, nutrients, bacteria, dissolved oxygen, water velocity, and habitat condition were the environmental variables most involved in shaping changes in macroinvertebrate assemblages among sites. These patterns are consistent with those observed by Fierro et al. (2021), who documented that chemical oxygen demand, pH, total coliforms, nitrites, elevation, and water temperature explained 95 percent of the macroinvertebrate assemblages at basins with the largest degrees of agricultural activities in Chile. Miserendino and Masi (2010) compared macroinvertebrate communities among streams subjected to different land use practices and found that the main environmental variables with explanatory powers over community composition were related to detritus availability (wood and leaf biomass) and impairment (TP and sand percent). Similarly, Shilla and Shilla (2011) compared the composition of macroinvertebrates across different land uses (pasture, bush and urban), and the explanatory variables were: electrical conductivity, water temperature, turbidity, nitrate, and dissolved oxygen. In the same way, nutrients, conductivity, and dissolved oxygen have been shown to be the most important parameters in determining macroinvertebrate assemblages impacted by intensive agrochemical applications in streams draining horticultural production sites in Argentina (Arias et al., 2020).

The present study confirmed that the intensity of land use plays a significant role in alterations to the structure and function of the aquatic invertebrate community. These alterations are primarily due to the highly interactive nature of streams with their surroundings (Goss et al., 2014; Miserendino et al., 2022a). For example, most of the extirpated fauna were insects with aerial stages, and the loss of oviposition sites and habitat for adult insects can critically impact the stream's macroinvertebrate diversity.

The importance of preserving freshwater biodiversity in agricultural headwater streams has previously been highlighted by Moore and Palmer (2005). They postulated that decreasing macroinvertebrate biodiversity has significant ecosystem-wide consequences since invertebrates can influence the rates of primary production, decomposition, and resource acquisition. Raitif et al. (2019) have remarked on the role of aquatic insects in agricultural landscapes; they are involved in several ecosystem functions, such as pollination, soil fertilization, and crop pest control. All of these aspects are relevant, considering that the production of flowers (tulips and peonies), fruits (berries), and vineyards – all of which could benefit from such ecosystem services - is gaining a foothold in the area.

5. Conclusion

This comparative work demonstrates that the intensification of land use, predominantly livestock-confined production, and to a lesser degree, extensive livestock and horticulture, impact freshwater stream-s'environmental conditions and aquatic biodiversity. This result was predictable because streams in the area featured a poor nutrient content (oligotrophic) and a highly endemic biota that evolved in these conditions. In this research, compositional changes in the community were linked to different parameters, such as water quality indicators (nutrients, bacteria, dissolved oxygen), or were related to changes to the riparian corridor, bank conditions, and erosion symptoms (e.g. water temperature, sedimentation), and ultimately, with habitat degradation. At present, valuable ecological services are threatened, and this paper highlights some concerning aspects of the evolution of Patagonian mountain agroecosystems. Crucial tasks to enhance biodiversity should include restoring riverbank heterogeneity, maintaining the riparian corridor (e.g., replanting native riparian vegetation) (Kutschker et al., 2009; Cole et al., 2020), and improving management practices (e.g., livestock exclusion by electric fences, drinking water points). These actions could mitigate nutrient and sedimentation inputs (Epele and Miserendino, 2015; Horak et al., 2020). Other appropriate measures include the treatment of animal wastes (i.e., farm ponds) and the reinforcement of the riverbank at crossing areas (with cobble/gravel). However, the success of all of these measures ultimately lies in the active involvement of the landowner in conservation actions (Miserendino et al., 2022a). The present study was limited to a set of streams on the mountain in the Chubut province, and future studies should include other locations to validate or expand the results reported here.

CRediT authorship contribution statement

C.N. Horak: Data curation, Methodology, Visualization, Writing – original draft. **Y.A. Assef:** Conceptualization, Investigation, Writing – review & editing, Supervision. **C.P. Quinteros:** Investigation. **W.M. Dromaz:** Investigation. **M.L. Miserendino:** Conceptualization, Formal analysis, Writing – original draft, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We thank the staff of estancias Los Ñires, La Ponderosa, La Elena, El Delta, Nant y Fall and La Juliana, who facilitated access to some of the sampling sites. We are grateful to Gwion Elis-Williams for English language revision and helpful comments. Thanks to Dr. C. Brand for their valuable collaboration. Finally, thanks to the anonymous reviewers for valuable comments that greatly improved the manuscript. This is contribution No 172 LIESA.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.envadv.2024.100511.

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