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Agriculture affects functional diversity of aquatic insects in Subtropical Atlantic Forest streams

Agricultura afeta a diversidade funcional de insetos aquáticos em riachos Subtropicais de Mata Atlântica

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Abstract: Aim: We evaluated the effects of native vegetation and agricultural activities on functional characteristics of aquatic insects' assemblages in Atlantic Forest streams southern Brazil. **Methods:** We collected information on land uses, riparian zone structural characteristics, and limnological variables of ten streams to characterize their environmental quality. In the same streams, we collected aquatic insects (Ephemeroptera, Plecoptera, and Trichoptera) and determined their functional characteristics (e.g. food habits, mobility, shape, and body size). The information for each trait category was quantified using a fuzzy code technique. To test the environmental conditions on aquatic insect functional attributes, we used an RLQ analysis. **Results:** In our study, only five environmental variables showed variation in streams (temperature, pH, DO, native vegetation, and agriculture use). We collected a total of 2591 organisms distributed in 21 EPT genera. We observed that the functional attributes of EPT formed distinct groups in relation to the stream categories defined by the measured environmental variables. We observed that shredders and larger and flattened organisms were associated with natural streams. These attributes were associated with the genera *Phylloicus*, *Anacroneuria*, *Tupiperla*, and *Farrodes*. On the other hand, we observed greater proportions of scrapers, with spherical bodies and shelter builders in streams impacted by agriculture. The most frequent genera in these streams were *Itaura*, *Wormaldia* and *Helicopsyche*. **Conclusions:** The modification of natural landscapes by agricultural areas caused significant functional changes in the aquatic invertebrate communities. Since aquatic insects participate effectively in ecological processes, alterations in the functional characteristics of these communities can cause changes in the streams' functioning.

Keywords: biological traits; environmental quality; ecological integrity.

Resumo: Objetivo: Neste estudo avaliamos os efeitos da vegetação nativa e atividades agrícolas sobre as características funcionais de assembleias de insetos aquáticos em riachos de Mata Atlântica, sul do Brasil. **Métodos:** Coletamos informações sobre usos do solo, características estruturais da zona ripária e variáveis limnológicas de dez córregos para caracterizar sua qualidade ambiental. Nos mesmos riachos coletamos insetos aquáticos (Ephemeroptera, Plecoptera e Trichoptera) e determinamos as características funcionais (e.g., hábitos alimentares, mobilidade, forma, tamanho do corpo). A informação para cada categoria de característica foi quantificada usando uma técnica de código fuzzy. Para testar as condições ambientais nos atributos funcionais de insetos aquáticos, utilizamos uma análise RLQ. **Resultados:** Em nosso estudo, apenas cinco variáveis ambientais apresentaram variação entre os riachos (temperatura, pH, DO, vegetação nativa, uso agrícola). Coletamos um total de 2591 organismos distribuídos em 21 gêneros das ordens EPT. Observamos que os atributos funcionais do EPT formaram grupos distintos em relação às categorias de riachos definidas pelas medidas das variáveis ambientais. Observamos que fragmentadores e organismos maiores e achatados estiveram associados a riachos naturais. Esses atributos foram associados aos gêneros *Phylloicus*, *Anacroneuria*, *Tupiperla* e *Farrodes*. Por outro lado, em riachos impactados pela agricultura, observamos maiores proporções de raspadores, com corpos esféricos e construtores de abrigos. Nesses riachos, os gêneros mais frequentes foram *Itaura*, *Wormaldia* e *Helicopsyche*. **Conclusões:** A modificação das paisagens naturais por áreas agrícolas causou mudanças funcionais significativas nas comunidades de invertebrados aquáticos. Como os insetos aquáticos participam efetivamente dos processos ecológicos, alterações nas características funcionais dessas comunidades podem causar mudanças no funcionamento dos riachos.

Palavras-chave: características biológicas; qualidade ambiental; integridade ecológica.

1. Introduction

The expansion of agricultural frontiers has been necessary for the planet's food supply, but it has been considered one of the main forms of human intervention in nature (Paz et al., 2020; Tanentzap et al., 2015). It is estimated that about 40% of the planet's surface is occupied by agricultural activities (Graeber et al., 2015). However, the agricultural expansion reflects in the fragmentation of natural landscapes, water resources degradation, and aquatic diversity decrease (Paz et al., 2020; Huiñocana et al., 2020; Rovani et al., 2020).

Riparian vegetation has numerous ecological functions, such as bank stability, carbon stock, thermal regulation, and habitat for numerous vertebrate and invertebrate species, among others (Medan et al., 2011; Ribeiro et al., 2019; Luke et al., 2019). In addition, riparian vegetation is a source of organic matter for headwater streams influencing the functioning of aquatic environments (Tonello et al., 2021). More specifically, in agricultural landscapes vegetation maintenance helps in the retention of chemical elements used in crops (e.g. pesticides and metals) (Loureiro et al., 2021; Loureiro & Hepp, 2020). The riparian vegetation removal alters the habitats availability within streams, decreasing the diversity and composition of benthic macroinvertebrates communities (Cornejo et al., 2019; Huiñocana et al., 2020; Meza-Salazar et al., 2020). In summary, the presence of riparian vegetation is important for the maintenance of environmental quality and aquatic biodiversity (Dala-Corte et al., 2020).

Aquatic insect assemblages show marked changes in their structure and composition in environments affected by agricultural practices (Huiñocana et al., 2020). In streams surrounded by agriculture, an abundance increase of tolerant organisms is observed, but there is a decrease in taxonomic richness and evenness of assemblages (Breda et al., 2020; Hepp et al., 2010; Sensolo et al., 2012), reflecting on functional attributes of aquatic insect communities (Gomes et al., 2018; Conceição et al., 2020).

The functional characteristics of aquatic insects are important for the functioning of ecosystems (Milesi et al., 2019) and are influenced by variations in the streams' environmental conditions (Díaz et al., 2008; Gomes et al., 2018). Several studies report the existence of clear relationships between the stream insect functional attributes and landscape changes (Ferreira et al., 2017), substrate (Milesi et al., 2016), spacial filters (Lamouroux et al., 2004) and environmental quality gradients (Ippolito et al., 2012; Gomes et al., 2018; Malacarne et al., 2023).

Aquatic insects have an intrinsic relationship with the environments where they live and develop different abilities to maximize their fitness (Hershkovitz & Gasith, 2013). Studies have observed that organisms with small body sizes, multivoltine life cycles and filter feeding habits are observed in greater proportions in places with higher levels of environmental disturbances (Díaz et al., 2008;

Gomes et al., 2018). On the other hand, in natural sites, large body size organisms, semi-voltinism, and the presence of different reproductive strategies were also evidenced (Díaz et al., 2008). Thus, in disturbed environments, functional characteristics associated with organisms' resistance and resilience are observed, favoring their maintenance in these sites (Conceição et al., 2020). In impacted sites, organisms have functional abilities to adapt to adverse conditions based on changes in the community or in the ability to recover after disturbance (Hershkovitz & Gasith, 2013). However, reliable information on how organisms functionally respond to impacted environments is incipient. In this sense, studies are necessary to elucidate the strengths and weaknesses of the functional approach for biomonitoring purposes, which depend on spatial scales, interrelationships and the species pool of the sites (Saito et al., 2020).

In this study, we evaluated the possible effects of replacement of riparian native vegetation with agriculture land use on the functional characteristics of aquatic insect assemblages in Atlantic Forest streams, southern Brazil. We expect functional traits in organisms to reflect environmental impacts observed in streams. We evaluated the functional attributes of body size, feeding habits, morphology, and locomotion of aquatic insect assemblages of the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) along an environmental gradient caused, mainly, by agricultural land use in small streams. Our hypothesis is that the EPT assemblages in streams where riparian vegetation is removed will be composed of (i) smaller organisms, (ii) predominantly collector and filter feeder, (iii) cylindrical shape, and (iv) swimmers. Our expectation is based on the resistance capacity of these organisms in impacted environments (Huiñocana et al., 2020). As they are organisms with little tolerance to disturbed environments, they will present functional attributes associated with abilities that contribute to the acceleration of the reproductive process, greater capacity for dispersion and consumption of indigenous or primarily processing resources.

2. Material and Methods

2.1. Study area

We studied streams located in watersheds belonging (i.e. Dourado, Cravo, Suzana and Campo Rivers) to the upper portion of the Uruguay River Basin in southern Brazil (Figure 1). The region has altitudes ranging from 400 to 750 m a.s.l., average annual temperature of ~18ºC, and average annual precipitation of ~1800 mm (Alvares et al., 2013). The region is inserted in the Atlantic Forest biome with vegetal transition between Mixed Ombrophile Forest and Seasonal Deciduous Forest formations (Oliveira-Filho et al., 2015).

The plant species that occur in the region is *Araucaria angustifolia* (Bertol.) Kuntze, but other

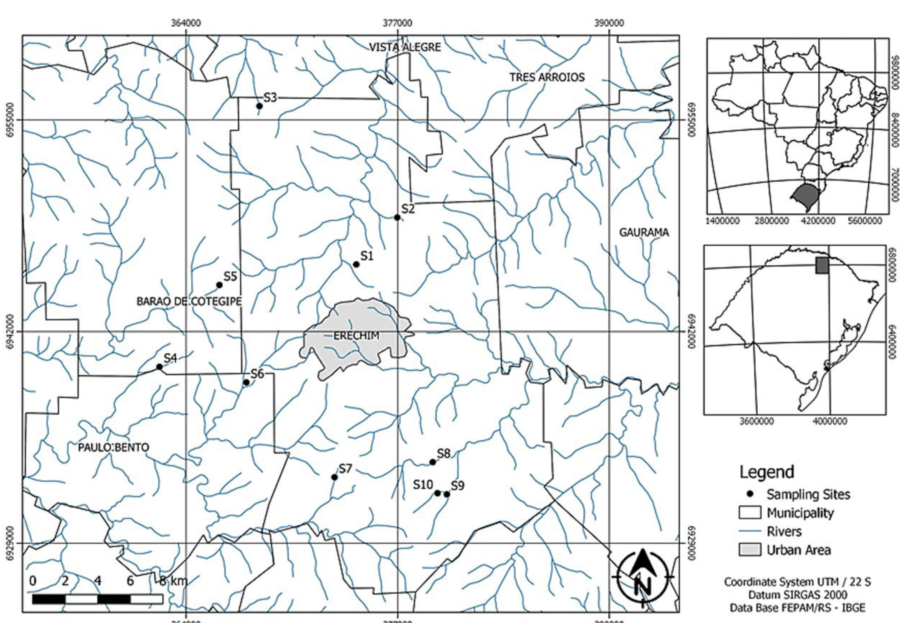

Figure 1. Location of streams studied (S1 to S10) in southern Brazil. Natural streams (S1, S2, and S3), intermediate streams (S4, S5, S6, and S7), and impacted streams (S8, S9, and S10).

species like *Nectandra megapotamica* (Spreng.) Mez., *Cedrela fissilis* Vell., *Cupania vernalis* Cambess., *Campomanesia xanthocarpa* (Mart.) O. Berg e *Sebastiania commersoniana* (Baill.) L.B. Sm. & Downs are common (Loregian et al., 2012). We selected ten streams (Strahler stream order < 3rd) with similar morphological characteristics in the region. The streams width ranged from 1 to 3 m and ~0.4 m in depth. The substrate is basically composed of stones and sediment deposits, and leaves are common in several stream stretches.

2.2. Environmental variables

For each stream, we marked the geographic coordinates, and from this point, we defined the drainage area of each stream. We analyzed the drainage areas following the cartographic parameters for selecting the listed points and water dividers. To quantify the land uses, we used MapInfo 8.5 and Idrisi 32 software. For each drainage area, we classified the land uses using the method of Supervised Classification by Maximum Likelihood (maxlike) in SIG IDRISI 32. We quantified the land use and land cover classes in the drainage areas as follows: agriculture, pasture, and native arboreal vegetation.

In each stretch, we analyzed *in situ* the variables: water temperature, turbidity, electrical conductivity, pH, dissolved oxygen, and total dissolved solids using a multiparameter analyzer Horiba® U51. In the same stretches, we collected water samples and analyzed the nitrite and total phosphorus concentrations in laboratory using spectrophotometric method. Finally, we filtered the samples with 0.45 µm porosity filters and analyzed dissolved organic carbon and total dissolved nitrogen concentrations in a TOC Shimadzu® Analyzer.

2.3. Aquatic insects

We collected aquatic insects with a Surber sampler (250 µm mesh and 0.09 m2 area) in the stony substrate. In each stream stretch, we collected six sub-samples that were grouped, fixed with 80% ethanol, and taken to the laboratory for screening and identification. In the laboratory, we separated insect larvae into orders Ephemeroptera, Trichoptera, and Plecoptera (EPT) and carried out identifications up to the genus taxonomic level according to Fernandez & Dominguez (2001) and Mugnai et al. (2010). After identification, we classified the genera functional attributes according to information from the literature, preferably with data from studies carried out in the Neotropical region (Tachet et al., 2002; Tomanova et al., 2008; Milesi et al., 2016). We used the four functional attributes: body size, feeding habits, morphology, and locomotion described into 14 functional categories (Table 1). We chose these functional attributes because they are characteristics directly associated with environmental changes. The information for each trait category was quantified using a *fuzzy code* technique. This technique determines the affinity of each taxon belonging to each trait category (Chevene et al., 1994). Values from 0 to 3 are assigned, where 0 corresponds to no affinity, 1 corresponds to weak affinity, 2 to moderate affinity, and 3 to strong affinity. This approach helps compensate for for each taxon's different information levels (Chevene et al., 1994).

2.4. Data analysis

Initially, we grouped the streams according to three categories (i.e. natural, intermediary, and impacted), using scores from the first and second principal component (PC1 and PC2) of a Principal Component Analysis (PCA). We performed this ordering based on limnological variables and land use and land cover standardized based on the maximum value among the sampling units. The categorization of streams was based on the relationships between the scores of the first two main components and the values of the limnological and land use and occupation variables. As expected, the highest dissolved oxygen concentrations, native vegetation percentage in riparian zone, and neutral pH were the main predictors of streams with natural conditions. On the other hand, the impacted streams had higher percentages of agricultural use in the riparian zone and higher water temperatures (Table 2). After stream categorization, we

Table 1. Functional attributes and their categories used for the Ephemeroptera, Plecoptera and Trichoptera organisms in streams studied.

Functional atributes	Categories
Feeding habit	Filter feeder
	Collector
	Shredder
	Scraper
	Predator
Body size	<0.25 mm
	25-0.50 mm
	0.50-1 mm
	>1 mm
Body form	Cylindrical
	Hydrodynamic/Flat
Locomotion	Creeper
	Swimmer
	Fixed

Table 2. Pearson correlations between the measured environmental variables and the scores of the first two main components of the PCA. Values in bold: p<0.05.

Variables	PC1	PC2
Water temperature (ºC)	**-0.75**	0.40
pH	0.50	**-0.66**
Electrical conductivity (mS.cm^{-1})	**-0.84**	-0.31
Dissolved oxygen (mg.L^{-1})	0.01	-0.08
Total dissolved solids (mg.L^{-1})	**0.81**	-0.05
Turbidity (NTU)	**-0.81**	-0.56
Nitrite (µg.L^{-1})	**-0.82**	-0.54
Total Phosphorous (µg.L^{-1})	**-0.81**	-0.56
Dissolved organic carbon (mg.L^{-1})	0.22	-0.21
Total dissolved nitrogen (mg.L^{-1})	**-0.89**	-0.22
Vegetation land use (%)	**0.62**	**-0.70**
Agricultural land use (%)	-0.33	**0.90**
Pasture land use (%)	0.40	-0.01

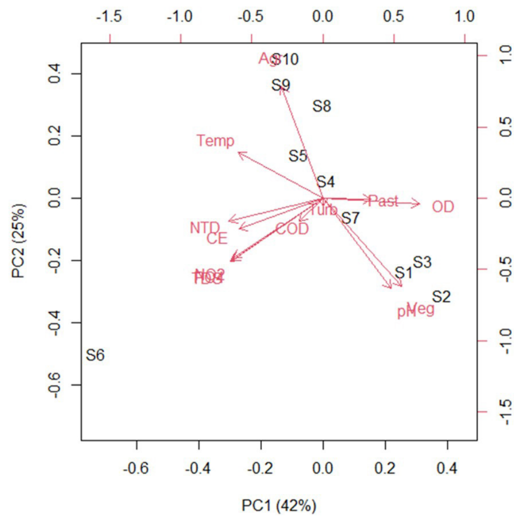

Figure 2. Principal Components Analysis of environmental variables measured in streams studied in southern Brazil. Veg: natural vegetation; Agr: agriculture; Past: pasture; Temp: water temperature; NTD: nitrogen total dissolved; CE: electrical conductivity; TDS: total dissolved solids; NO2: nitrite; OD: dissolved oxygen; Turb: turbidity; COD: dissolved organic carbon.

compared the limnological variables between the three stream categories using an ANOVA with a posteriori Tukey-HSD test. We performed the same procedure to assess differences between EPT abundance (log-transformed) and genera richness between stream categories.

To test the environmental conditions on aquatic insect functional attributes, we used an RLQ analysis based on matrices with environmental variables (R), insect abundance (L), and insect functional attributes (Q). The RLQ uses an inertia analysis to verify the relationships between functional attributes and habitats with the insects that occur in these sites. Based on this association between the three matrices, RLQ provides a simultaneous ordering between biological and environmental information (Dolédec et al., 1996). We used model 2 to perform the analyses, which randomizes the environmental matrix to relate it to the functional attributes' matrix (Dray et al., 2014). We tested the significance of randomization based on a Monte-Carlo test ($p < 0.05$). We used the *rlq* function in the 'ade4' package (Dray & Dufour, 2007). All analyses were performed using the R software (R Core Team, 2017).

3. Results

3.1. Environmental variables

The environmental characteristics observed in the streams promoted a PCA ordination where we classified the sites according to three categories associated with their quality (Figure 2, Table 2). In our study, the 10 studied streams were classified according to the level of impact as natural (n=3; S1, S2, and S3), intermediate (n=4; S4, S5, S6, and S7), and impacted (n=3; S8, S9, and S10). In addition, only five environmental variables showed variation between the impact types (Table 3 and Table 4). The water temperature in natural streams was lower (15.9±0.50 ºC) compared to intermediate (17.9±0.26 ºC) and impacted (17.8±0.32 ºC) streams (Figure 3A). The pH ranged from neutral to slightly acidic between natural (7.10±0.05) and impacted (6.43±0.03) streams (Figure 3B). Dissolved oxygen concentrations were similar between natural and impacted streams (8.7±0.2 mg.L^{-1} and 7.6±0.2 mg.L^{-1}, respectively) and higher than in intermediate streams (6.4±0.3 mg.L^{-1}) (Figure 3C). The native tree cover was higher in natural streams (69.0±6.2%), gradually decreasing in intermediate (46.5±8.0%) and impacted (16.4±5.9%) streams (Figure 3D). The inverse pattern was observed for agricultural use with the highest percentages in impacted streams (75.9±7.5%), followed by intermediate (39.3±7.1%) and natural (20.8±5.8%) (Figure 3E). The other environmental variables measured were similar between the three categories of streams (Table 3 and Table 4).

3.2. Aquatic insect assemblages

We collected 2591 organisms distributed in 21 EPT genera (Table 5). Of these, we collected 1275 organisms (49.2%) in impacted streams, 714 in natural streams (27.5%), and 602 in intermediate streams (23.2%). The abundance of organisms was greater in impacted streams and

Table 3. Environmental variables measured in streams in southern Brazil. Temp (water temperature), DO (dissolved oxygen), pH (pH), Turb (turbidity), CE (electric conductivity), DOC (dissolved organic carbon), TDN (dissolved total nitrogen), NO2 (nitrite), PO4 (phosphate), veg (native vegetation land use), agr (agriculture land use), past (pasture land use).

Streams		Environmental variables											
		Temp (ºC)	pH	CE ($mS.cm^{-1}$)	Turb (NTU)	DO ($mg.L^{-1}$)	NO2 ($\mu g.L^{-1}$)	PO4 ($\mu g.L^{-1}$)	DOC ($mg.L^{-1}$)	TDN ($mg.L^{-1}$)	Veg (%)	Agr (%)	Past (%)
S1	Natural	16.7	7.2	77.0	2.5	8.33	10.023	54.925	1.051	0.78	72.8	26.1	0
S3	Natural	15.0	7.0	75.0	3.1	8.75	2.220	73.675	2.072	1.519	56.1	27.1	16.1
S2	Natural	16.2	7.1	68.0	5.1	9.10	14.358	66.175	1.442	0.86	76.3	9.2	14.4
S5	Intermediate	18.35	6.7	90.0	4.7	5.96	2.220	49.925	0.353	2.229	43.1	45.5	8.5
S4	Intermediate	17.7	7.0	80.0	4.2	6.87	2.220	68.675	1.103	2.032	40.3	54.2	5.2
S6	Intermediate	18.4	6.7	233.0	6.3	5.76	235.700	968.000	3.6	3.808	32.9	28.8	4.1
S7	Intermediate	17.3	6.5	34.0	29.5	7.24	13.780	58.675	5.68	1.119	69.5	25.4	4.9
S8	Impacted	17.7	6.4	45.0	4.5	7.42	9.445	47.425	3.055	1.379	27.2	60.3	11.8
S10	Impacted	18.5	6.4	122.3	4.8	8.02	9.730	60.500	2.421	1.357	7.1	82	9.4
S9	Impacted	17.4	6.5	106.0	4.4	7.37	22.451	79.925	0.474	2.027	14.8	83.2	1.9

similar between natural and intermediate streams ($F_{2,7}$=5.7, p=0.03). In turn, the richness of genera observed between natural and impacted streams was higher than the intermediate ones ($F_{2,7}$=27.6, p<0.001), but similar to each other. In this way, we performed a rarefaction to correct the effect of abundance on the richness of genera and observed that the richness in natural streams was higher than the others ($F_{2,7}$=5.2, p=0.04), while the richness in impacted and intermediate streams was similar.

The genus *Smicridea* (Hydropsychidae, Trichoptera) was the most abundant (30.6% of the total) and present in all streams, the same as *Caenis* (Caenidae, Ephemeroptera, 24.8%) and *Itaura* (Glossossomatidae, Trichoptera, 20.3%). The genera *Cernotina* (Polycentropodidae, Trichoptera) and *Nectopsyche* (Leptoceridae, Trichoptera) were exclusive from natural streams. On the other hand, *Marilia* (Odontoceridae, Trichoptera) and *Wormaldia* (Philopotamidae, Trichoptera) and *Tricorythopsis* (Leptohyphidae, Ephemeroptera) were exclusive from impacted streams (Table 5).

We observed that the functional attributes of EPT formed distinct groups in relation to the stream categories defined by the measured environmental variables (RLQ, p = 0.006, Figure 4). This ordering was summarized in 56% by the first RLQ axis and 33% by the second RLQ axis. Thus, along the first axis we observe the distinction between streams according to the proposed classification (i.e., natural, intermediate and impacted). While natural streams were ordered by the highest percentages of riparian vegetation, impacted streams had higher percentages of agricultural use. In turn, the intermediate streams presented a dispersion along the second axis of the RLQ, corresponding to limnological characteristics associated with nitrogen and DOC concentrations.

Table 4. Results of analysis of variance performed for each environmental variable measured between natural, intermediate, and impacted streams.

Variables	F-value (df = 2, 7)	p-value
Water temperature (ºC)	9.17	**0.011**
pH	15.23	**0.003**
Electrical conductivity ($mS.cm^{-1}$)	0.30	0.747
Dissolved oxygen ($mg.L^{-1}$)	14.99	**0.003**
Total dissolved solids ($g.L^{-1}$)	0.7	0.528
Turbidity (NTU)	0.95	0.433
Nitrite ($\mu g. L^{-1}$)	0.58	0.587
Total Phosphorous ($\mu g. L^{-1}$)	0.67	0.541
Dissolved organic carbon ($mg.L^{-1}$)	0.38	0.695
Total dissolved nitrogen ($mg.L^{-1}$)	2.18	0.184
Vegetation land use (%)	11.93	**0.005**
Agricultural land use (%)	14.85	**0.003**
Pasture land use (%)	0.548	0.601

df: degree freedom.

We observed larger organisms in natural streams, with flattened bodies, tarsal claws, and shredder feeding habits. These attributes were associated with the genera *Phylloicus* (Calomoceratidae, Trichoptera), *Anacroneuria* (Perlidae, Plecoptera), *Tupiperla* (Gripopterygidae, Plecoptera), and *Farrodes* (Leptophebiidae, Ephemeroptera). In impacted streams, we observed smaller organisms with scraper feeding habits, cylindrical bodies, and organisms that lives attached to substrate or build shelters. In these streams, the most frequent genera were *Itaura*, *Wormaldia*, and *Helicopsyche* (Helicopsychidae, Trichoptera). The impacted streams have a higher agricultural use percentage in their drainage area, acidic pH, and higher water temperatures.

4. Discussion

In this study, we evaluated the effects of riparian vegetation and agriculture in the buffer

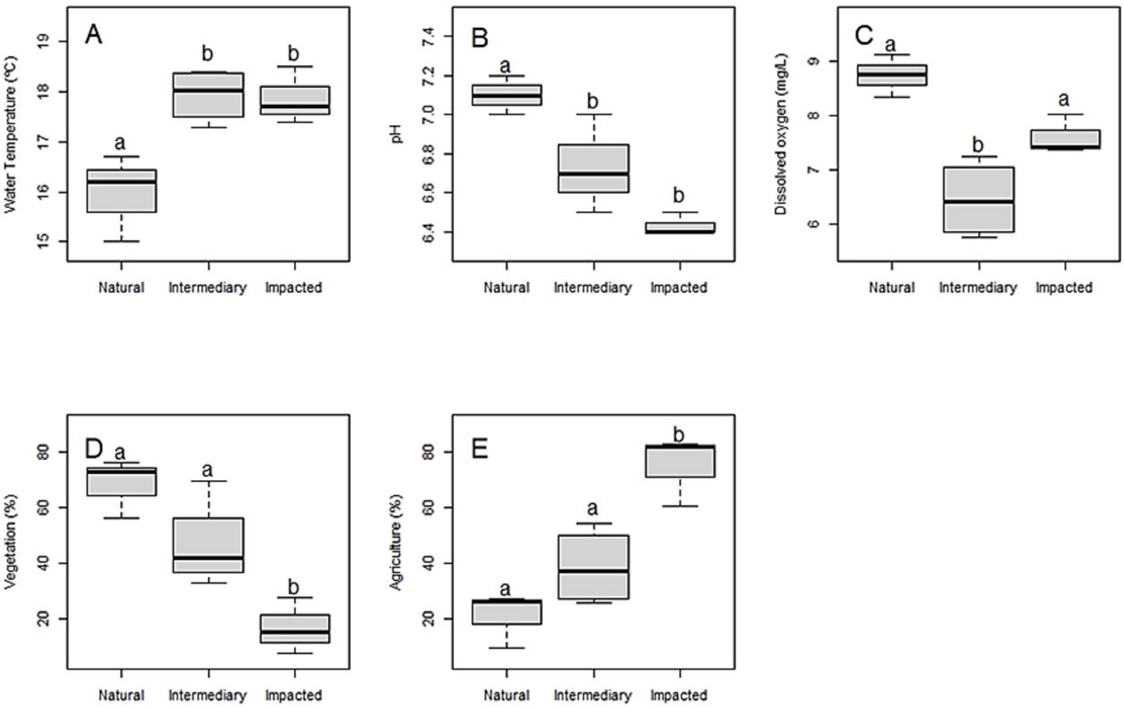

Figure 3. Boxplot for the values (median) of the environmental variables that showed a significant difference (different letters, $p < 0.05$) between the categories of streams (natural, n=3; intermediate, n=4; and impacted, n=3).

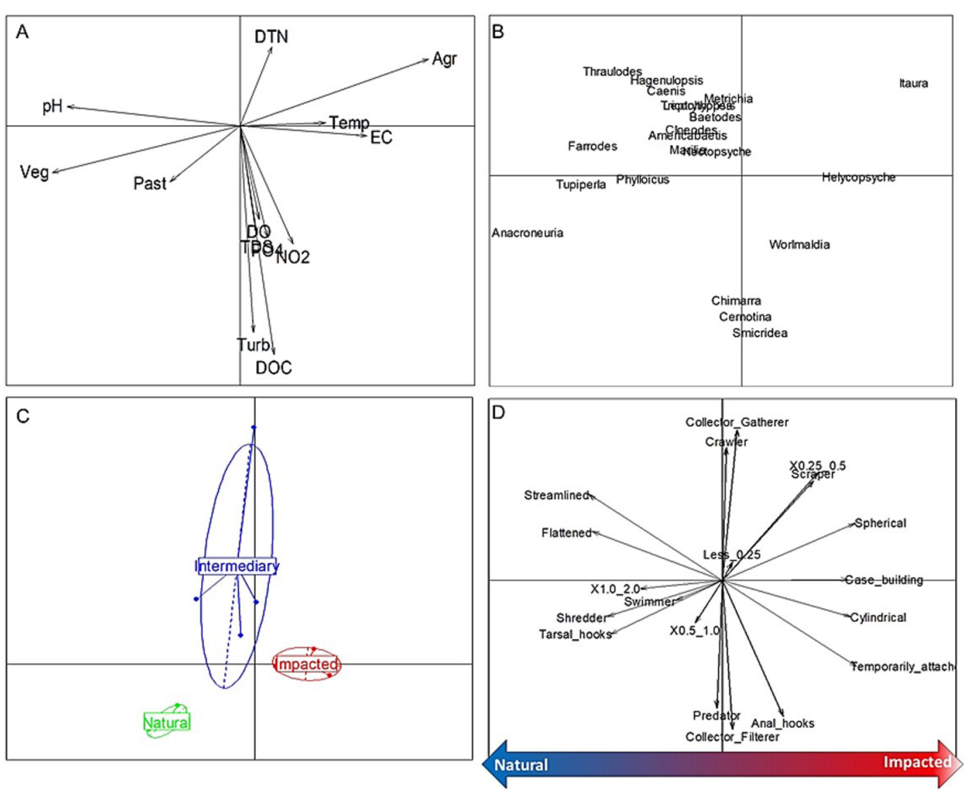

Figure 4. Results of RLQ analysis performed with invertebrate community composition, functional attributes, and environmental variables in streams of southern Brazil. (A) Environmental variables ordination. (B) Organism's composition ordination. (C) RLQ ordination. (D) Functional attributes ordination in natural-impacted streams gradient.

Table 5. Abundance of Ephemeroptera, Plecoptera and Trichoptera larvaes sampling in streams in southern Brazil.

Genera	Streams									
	S1	S3	S2	S5	S4	S6	S7	S8	S10	S9
Ephemeroptera										
Baetidae										
Americabaetis	7	6	16	31	0	2	0	13	22	9
Baetodes	24	1	4	23	4	3	3	3	39	2
Cloeodes	0	1	0	0	0	0	0	6	0	0
Caenidae										
Caenis	30	85	37	218	25	0	17	83	17	133
Leptohyphidae										
Tricorythopsis	0	0	0	0	0	0	0	1	2	0
Leptohyphes	1	1	0	0	0	0	0	1	0	1
Leptophlebiidae										
Farrodes	10	2	47	0	27	0	3	4	11	3
Thraulodes	30	19	0	0	0	0	0	3	5	2
Hagenulopsis	4	10	2	0	0	0	0	1	0	4
Plecoptera										
Gripopterygidae										
Tupiperla	0	4	3	5	1	0	2	6	2	6
Perlidae										
Anacroneuria	6	0	11	0	0	0	0	0	1	0
Trichoptera										
Calamoceratidae										
Phylloicus	41	27	9	5	6	0	1	33	3	8
Helicopsychidae										
Helicopsyche	1	0	1	0	0	1	0	0	0	0
Hydropsychidae										
Smicridea	13	61	118	30	44	31	98	54	202	143
Hydroptilidae										
Metrichia	0	0	1	1	0	0	0	0	1	0
Leptoceridae										
Nectopsyche	1	0	0	0	0	0	0	0	0	0
Philopotamidae										
Chimarra	0	0	1	0	5	0	0	9	2	0
Worlmaldia	0	0	0	0	0	0	0	1	2	2
Polycentropodidae										
Cernotina	2	1	0	0	0	0	0	0	0	0
Glossossomatidae										
Itaura	28	34	14	5	3	3	5	54	168	212
Odontoceridae										
Marilia	0	0	0	0	0	0	0	1	0	0

zone of small streams on the functional diversity of Ephemeroptera, Plecoptera, and Trichoptera assemblages. We observed that the insect functional attributes, body size, feeding habits, morphology, and locomotion reflected the streams' environmental variability. In the present study, we standardized numerous environmental characteristics that can naturally generate functional variability in communities (e.g. size of streams, type of substrate, geological basis, among others). In this way, we observed clear associations between the functional characteristics of the organisms and the environmental variables measured, including characteristics related to the landscape of areas adjacent to the streams (i.e. riparian vegetation, agriculture). Thus, we can see that changes in streams adjacent areas (e.g., land use) are important environmental predictors in the structuring of aquatic insect communities.

Thus, it is expected that changes in land use in the stream drainage area will affect the limnological water quality and, consequently, the aquatic biota (Breda et al., 2020; Castro et al., 2021). In this study, we observed that the vegetation removal was decisive for the change in limnological variables such as water temperature, pH, and dissolved oxygen. Vegetation removal facilitates the light incidence increasing the stream water temperature (Tonello et al., 2021). In addition, removal of riparian vegetation facilitates the sediment input from agricultural areas containing chemical substances (e.g. pesticides and metals) that acidify the water and reduce the dissolved oxygen concentration in streams (Loureiro et al., 2018).

The insect abundance was approximately two times greater in impacted streams, which resulted in a genera richness was greater in to the natural streams. However, the greater abundance in impacted environments was due to the dominance of three genera: *Smicridea*, *Itaura*, and *Caenis*, representing 83% of all collected insects. The dominance of few taxa is a common pattern in biological communities; however, the dominance of some taxa is more evident when the environment is impacted. Studies demonstrate that changes in areas adjacent to streams can cause chemical changes in the water (for example, through the transport of sediments or chemicals) favoring the dominance of tolerant organisms (Hepp et al., 2010; Huiñocana et al., 2020; Hepp et al., 2021). Among the Trichoptera genera, *Smicridea* and *Itaura* are widely observed in streams with agricultural use (Breda et al., 2020), while *Caenis* has been reported as an Ephemeroptera genus tolerant to anthropogenic disturbances (Arimoro & Muller, 2010). In addition, our results clearly demonstrated the effects of organism abundance on richness. The richness of genera observed between natural and impacted streams was similar, however, when we corrected these values based on a rarefaction based on the abundance of organisms, it was possible to observe that the richness in natural streams was higher. In this sense, it is important to highlight that we must be cautious when interpreting the results, based only on some assemblage metrics.

The EPT assemblages in streams responded significantly to streams' environmental quality. The distinction between functional attributes, mainly comparing natural and impacted streams assemblages demonstrates that the effects of environmental filters (at regional or local scales) act decisively on aquatic communities. Local environmental factors are fundamental for establishing the functional composition of aquatic insects, as they can act as environmental filters (Nicacio et al., 2020). In turn, local environmental changes are related to changes in the streams' drainage area (i.e., vegetation replacement by agriculture or urbanization) (Sitati et al., 2021). Thus, riparian vegetation is important for maintaining the integrity of small streams, acting as impacts mitigation observed in drainage areas (Luiza-Andrade et al., 2017; Huiñocana et al., 2020).

The predominant functional attributes observed in impacted streams are characteristic of insects' resistance strategies. Small body size organisms are common in impacted environments (Gomes et al., 2018). Organisms generally develop life history strategies that result in abilities to withstand adverse environmental conditions (Hershkovitz & Gasith, 2013). In impacted locations, communities tend to have smaller organisms, as a possible physiological response to environmental changes, however, there is no consensus in the literature on whether this relationship between disturbance and individual size is really clear (Gomes et al., 2018; Lundquist & Zhu, 2018; Malacarne et al., 2023; Collyer et al., 2023). Furthermore, resistant organisms to disturbed environments tend to feed on fine or dissolved particles. In streams with riparian vegetation replaced by agriculture, the input of fine sediments is common, which facilitates the establishment of scrapers, collectors, and filter feeders (Tonello et al., 2021). On the other hand, the presence of riparian vegetation in natural streams provides the input of organic material which is used as a food resource by shredders (Tonin et al., 2018). Substrate homogenization alters stream current flow making the environment more suitable for organisms that have anchoring structures (e.g., claws or house building) or less hydrodynamic (e.g., cylindrical larvae) (Milesi et al., 2019). Furthermore, streams with intermediate impact levels did not represent a clear pattern in the functional characteristics of EPT assemblages. This occurs precisely because of the transitory environmental characteristics between natural and impacted environments.

5. Conclusion

Aquatic environments functioning depends on complex interactions between species and the environments in which they occur. The conservation of riparian vegetation in small streams is essential for maintaining the functions of these ecosystems and supporting aquatic communities. Aquatic insects can tolerate or re-establish themselves in adverse environmental conditions (Restello & Hepp, 2020). This occurs because insects's have functional characteristics that facilitate their resistance or resilience to these environments. Our study demonstrated an intrinsic relationship between land use and land cover (especially native riparian vegetation and agriculture) and the aquatic insects functional diversity. Furthermore, we demonstrate that changes in the streams' water quality are associated with changes in the adjacent areas of these environments. This was possible because we studied streams with a similar structure, which allowed us to infer more directly about the effects of variables on a local (limnological) and regional (land use) scale. The functional attributes reflected the impact showing that they are useful for checking the environmental quality. Also, resistance functional attributes (e.g., body size, feeding habits, morphology and locomotion) were sensitive to agricultural uses. Finally, the use of functional attributes can complement the taxonomic information of aquatic communities, especially when the objective of biological assessment is detecting the effects of diffuse impacts (e.g. agriculture).

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