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RESEARCH ARTICLE

Nestedness of insect assemblages in agriculture-impacted Atlantic forest streams

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Abstract – Agricultural land use causes habitats fragmentation and riparian vegetation removal, driving variability in the composition of aquatic insect assemblage in streams. We explored the effect of agriculture on the beta diversity of insect assemblages of Ephemeroptera, Plecoptera and Trichoptera (EPT) in Atlantic forest streams. We measured water physical and chemical variables and quantified the land cover of 10 stream catchments to determine the environmental integrity of the streams. The percentage of vegetation range was ~6% to ~47%, and agricultural land use range was ~24% to ~88%. We collected a total of 2632 individuals distributed in 30 genera. Trichoptera was the most abundant order (57%), followed by Ephemeroptera (41%) and Plecoptera (2%). The abundance was influenced by the higher agriculture practices in the streams adjacent areas. On the other hand, the oxygenated waters and higher percentages of riparian vegetation influenced the EPT rarefied richness. The beta diversity of EPT assemblages was structured by nestedness due to the influence agricultural activities. The variation in the agricultural intensity that occurs in the streams generated more intense limnological variability, which caused the nestedness of EPT insects as well as the reduction of taxonomic richness. Thus, the streams with low environmental integrity had EPT assemblages nested in streams of high environmental integrity.

Keywords: beta diversity / Ephemeroptera / Plecoptera / Trichoptera / stream integrity

1 Introduction

Demographic expansion and the demand for food cause an increase of agricultural activities all over the world (Hooke *et al.*, 2012). As a consequence, large areas with native vegetation have been deforested (Le Polain de Waroux *et al.*, 2016). In cultivated areas, there are important aquatic ecosystems with innumerable ecosystem services (*e.g.* habitat provision, water, thermal regulation), which are essential for the maintenance of aquatic and terrestrial biodiversity (Vidal-Abarca and Suarez, 2013).

Many studies report the negative effects of agriculture on small streams. One of the most important effects consists in changes in the water chemical composition, related to nitrogen and phosphorus input, from agricultural soil fertilization (Rhodes *et al.*, 2001; Bu *et al.*, 2014). Nutrients increase primary productivity in rivers and the consumption of oxygen by decomposer microorganisms (Munn *et al.*, 2010). Agricultural impact can decrease the water quality (Bu *et al.*, 2014), leading to the extinction of sensitive aquatic organisms (Selvakumar *et al.*, 2014). Besides, the deforestation of riparian vegetation by agricultural practices increases the temperature of the water and facilitates the input of agricultural chemicals into the streams, affecting the processing of pollutants and the habitat structure, increasing the sedimentation of the margins, and, consequently, causing the elimination of trophic groups of aquatic invertebrates (Hamada *et al.*, 2014). Riparian zones degradation usually leads to a decrease in the diversity and dominance of some benthic invertebrate groups less tolerant to anthropogenic changes (Arnaiz *et al.*, 2011; Selvakumar *et al.*, 2014; Valente-Neto *et al.*, 2015). Notably, the diversity and abundance of sensitive aquatic insects, especially the orders Ephemeroptera, Plecoptera and Trichoptera (EPT), are altered by riparian deforestation in streams (Kasangaki *et al.*, 2008; Ferreira *et al.*, 2017). Insects of the EPT orders are widely used in biomonitoring programs as part of biological indices for water quality assessment (Nessimian *et al.*, 2008; Biasi *et al.*, 2008).

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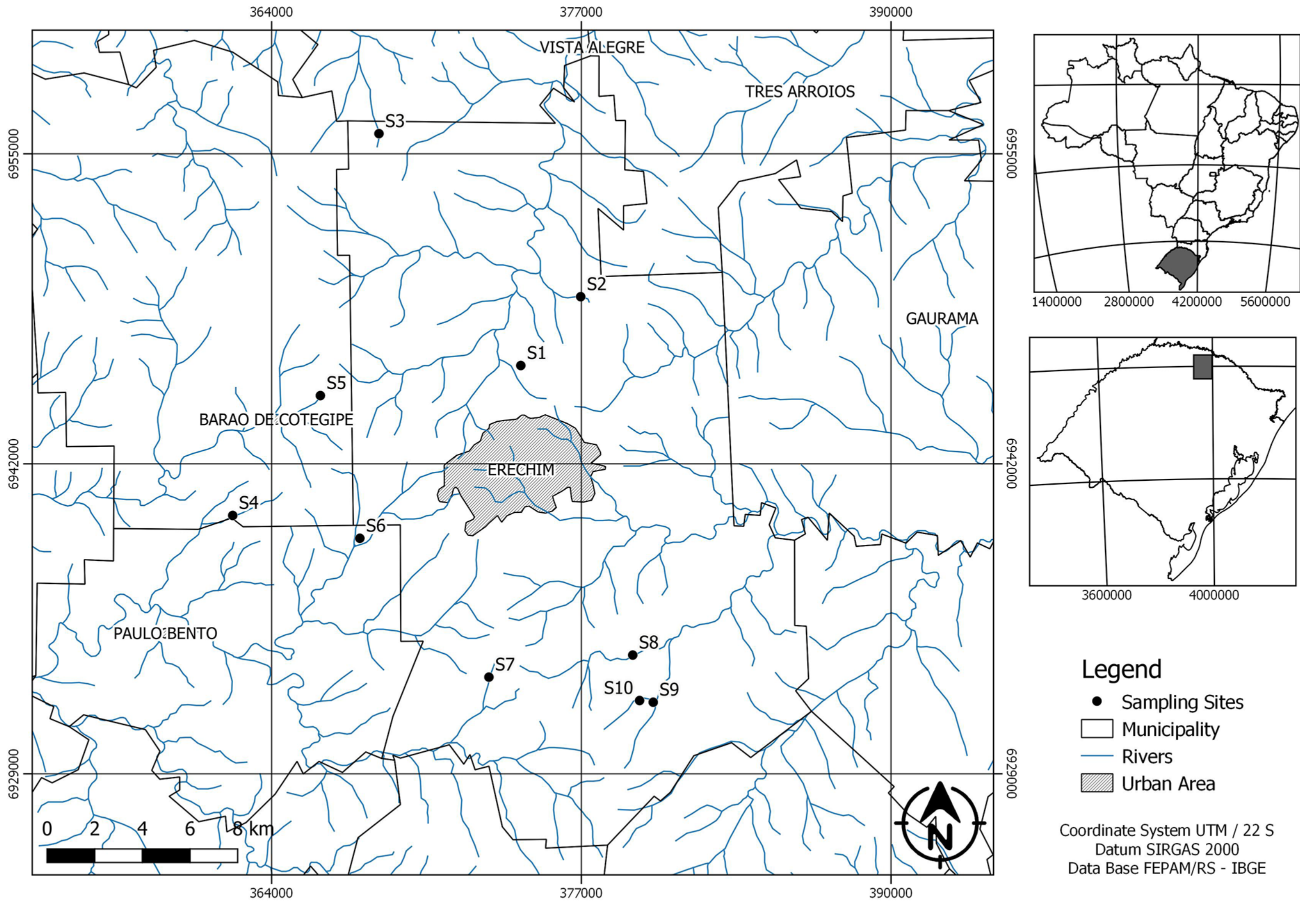

Fig. 1. Location of the streams studied in southern Brazil.

Beta diversity is a component of gamma diversity (Whittaker, 1960) and can be defined as the variation in species composition in a community between different locations or time periods (Anderson *et al.*, 2011). Two mechanisms can manifest biological beta diversity: (i) turnover, which is the substitution of species between communities, or (ii) nestedness, which occurs when the observed community is part of a larger community (Baselga, 2010). Both patterns can be analysed along a spatial, environmental, or temporal gradient (Korhonen *et al.*, 2010; Gianuca *et al.*, 2017). The nestedness pattern can occur due to many factors, among which the environmental conditions stand out (Pinha *et al.*, 2017), this act as filters that cause extinction and selective colonization of species (Wright *et al.*, 1997).

In recent years, an increase in ecological research on beta diversity occurred, especially because of its usefulness in the establishment of conservation areas (Scolar *et al.*, 2016; Bergamin *et al.*, 2017). Approaches to studies with beta diversity are effective in assessing the effect of habitat fragmentation (Hu *et al.*, 2011), among other negative effects caused by anthropisation. In this study, we evaluate if agricultural activities can generate nestedness patterns of EPT assemblages in subtropical streams. The EPT assemblages are sensitive to human disturbances (Theodoropoulos and Iliopoulou-Georgudaki, 2010), and the dissimilarity of the assemblages increases with the degradation of riparian zones. The EPT insects are excellent beta diversity study models because they are distributed in numerous aquatic environments, especially streams, and are very sensitive to disturbances, directly reflecting changes in the structure and composition of the stream insect communities (Ferreira *et al.*, 2017). Still, we believe that the streams assemblages most affected by agriculture will be nested assemblages of the less degraded streams. In addition, we evaluated whether local (*i.e.* physicochemical variables) and/or regional factors (*i.e.* land cover) influence beta diversity.

2 Material & methods

2.1 Study area

We collected water samples, aquatic insects, and landscape information in 10 small-order streams (≤3rd order) located in Southern Brazil (Fig. 1). The climate is classified as humid subtropical, with an average precipitation of 1781 mm and one mean annual temperature of 18.7 °C (Biasi *et al.*, 2008). The streams are located in the Atlantic Forest biome, and the vegetation is characterized as a mix between semi-deciduous seasonal forest and mixed ombrophilous forest (Oliveira-Filho *et al.*, 2015). The region is predominantly agricultural being approximately 70% of the landscape has agricultural use. The most developed crops are soy and corn. The few forest remnants are located in areas with greater slope and on the banks of water bodies (Rovani *et al.*, 2019).

2.2 Collection of aquatic insects

We collected the insects with a Surber sampler (area: $0.09 \, m^2$; mesh: 0.25 mm) in riffle stream with stony substratum (three subsamples) and leaf litter substratum (three subsamples) during November 2016. We chose these two substrate types, as they are predominant in small streams in the study region (Biasi *et al.*, 2008). We obtained a total of 60 sample units (10 streams $\times$ 2 types of microhabitat $\times$ 3 samples in each). We fixed the samples in the field with 96% ethanol and performed the screening of the organisms under a stereomicroscope. We identified the larvae of EPT up to the genus taxonomic level according to Olifiers *et al.* (2004), Pes *et al.* (2005), Mugnai *et al.* (2010), and Segura *et al.* (2011). After identification, the organisms were stored in glass pots containing 70% ethanol and deposited in the Aquatic Invertebrate Collection of the Regional Museum of Alto Uruguay (MuRAU) of Universidade Regional Integrada do Alto Uruguai e das Missões, Brazil.

2.3 Limnological variables

In the streams, we measured *in situ* the variables of water temperature, pH, electrical conductivity, dissolved oxygen, and turbidity using a HORIBA® multiparameter analyser. Also, we collected water samples to determine the concentrations of total dissolved nitrogen (TDN) and dissolved organic carbon (DOC) using a TOC Analyser (SHIMADZU®) and orthophosphates by spectrophotometry. For all measured limnological variables, we performed 3 measurements in each sampled stretch to characterize the stream in multivariate analyzes. All methodologies followed the recommendations described in Standard Methods (APHA, 2012).

2.4 Land cover classification

We quantified the land cover classes in tree vegetation, agriculture, and pasture in the drainage area of each of the 10 streams studied. The classification of land uses followed the method of the supervised classification by maximum likelihood (maxlike). For demarcation of the land uses of riparian zones, we used the Brazilian Environmental Legislation (Brazilian Law #12,651/2012) and the Environmental Conflicts Charter. The calculation of the drainage areas and the classification of land uses were performed through the software MapInfo 8.5 and Idrisi 32. The cartographic base was used in a scale of 1:35,000, composed of articulated leaves and an ETM + sensor image of Landsat 7 satellite from the year 2016, with spatial resolution of 15 m; spectral bands 3, 4, 5; and a panchromatic view.

2.5 Data analysis

Initially, we standardized the environmental variables using "decostand" function (range: 0 to 1) and then a principal component analyzes (PCA) were performed to order the streams according to their limnological characteristics and land cover variables. Due to the high variability in the abundance of larvae in each subsample, we cumulated the quantities of larvae collected in both substrates in each stream. We calculated the rarified richness for each stream based on the lowest abundance found. We evaluated the environmental effects on the abundance and richness of EPT using the first two principal components scores as predictor variables, from the application of linear regressions. We define the first two principal components from a Broken-Stick analysis. The Broken-Stick analysis is recommended as a stopping rule in principal component analysis (Jackson, 1993), provided that the observed eigenvalues are greater than the corresponding random components. Also, we performed a Canonical Correspondence Analysis (CCA) to observe the relationships between environmental variables and the abundance of EPT genus.

To evaluate the existence of the nested pattern, the "nestedtemp" function was used, which calculates the nesting by means of a temperature matrix with presence and absence data set (Rodríguez-Gironés and Santamaría, 2006). The total of unexpected absences and presences is calculated as the temperature statistic (T), with values ranging from $T = 0$ (perfectly nested pattern) to $T = 100$ (non-nesting) (Rodríguez-Gironés and Santamaría, 2006). To evaluate if the value of nested differs from the expected by chance, we use null models ("oecosimu" function). We used SIM1 that produces a simulated model matrix in which all the arrangements are equally distributed. All the data analyses were performed using 'vegan' package (Oksanen *et al.*, 2017) R statistical software (R Core Team, 2018).

3 Results

3.1 Environmental variables

In the streams drainage area, we observed a percentage of vegetation that varies from ~6% to ~47% (Tab. 1) and a predominance of agricultural land use (from ~24% to ~88%). In streams with a higher percentage of agricultural occupation (S9 and S10), we observed higher values of electrical conductivity and nitrite concentrations (Tab. 1). The stream S6 showed greater influence of organic contamination, distinguishing itself from the other streams. Streams S4 and S5 did not show marked variations compared to the other streams. On the other hand, in streams with a higher percentage of natural vegetation (S1 and S2), we observed neutral pH values (pH ~7) and high oxygenation ($>8 \, mg \, L^{-1}$) (Tab. 1).

In the PCA made with the environmental variables, the first two principal components (PC1 and PC2) explained 73% of the total variation of the streams environmental data (Fig. 2). The PC1 explained 42% of the data variability and was positively associated with vegetation ($r = 0.74$, $p = 0.01$) and pasture ($r = 0.84$, $p < 0.01$) land cover, dissolved oxygen ($r = 0.78$, $p < 0.01$) and pH ($r = 0.81$, $p < 0.01$). The second principal component (PC2) explained 32% and was associated positively with agriculture land cover ($r = 0.74$, $p = 0.1$) and negatively with TDN ($r = –0.68$, $p = 0.03$), nitrite ($r = –0.83$, $p < 0.01$), orthophosphates ($r = –0.85$, $p < 0.01$) and electrical conductivity ($r = –0.78$, $p < 0.01$). The streams ordination showed a clear pattern defined by land uses in drainage area. The S1 to S3 were associated with the high percentages of riparian vegetation and water oxygenation. On the other hand, S7 to S10 were ordered by the high percentages of agriculture practice in the drainage area. The S6 was isolated from the others, being ordered by high concentrations of nutrients from residues generated by animal breeding (Fig. 3).

Table 1. Limnological and land cover variables of studied streams in southern Brazil. Veg: vegetation; Agri: agriculture; Past: pasture; Temp: temperature; DO: dissolved oxygen; EC: electrical condutivy; DTN: dissolved total nitrogen; DOC: dissolved organic carbon; PO_4: orthophosphates; NO_2: nitrite.

Strems	Depth (m)	Width (m)	Temp (°C)	pH	EC ($\mu S\,cm^{-1}$)	Turbidity (NTU)	DO ($mg\,L^{-1}$)	NO_2 ($\mu g\,L^{-1}$)	PO_4 ($\mu g\,L^{-1}$)	DOC ($mg\,L^{-1}$)	DTN ($mg\,L^{-1}$)	Vegetation (%)	Pasture (%)	Agriculture (%)
S1	0.2	3.1	16.7	7.2	77.0	2.5	8.3	10.0	54.9	1.0	0.7	47.3	9.5	33.9
S2	0.15	2.5	16.2	7.1	68.0	5.1	9.1	14.3	66.1	1.4	0.8	43.8	31.8	24.3
S3	0.18	3.8	15.0	7.0	75.0	3.1	8.7	2.2	73.6	2.0	1.5	29.5	17.5	45.1
S4	0.21	4.5	17.7	7.0	80.0	4.2	6.8	2.2	68.6	1.1	2.0	18.9	5.7	73.0
S5	0.2	2.7	18.35	6.7	90.0	4.7	5.9	2.2	49.9	0.3	2.2	31.8	6.6	58.8
S6	0.28	4.6	18.4	6.7	233.0	6.3	5.7	235.7	968.0	3.6	3.8	27.7	5.1	33.2
S7	0.24	2.6	17.3	6.5	34.0	29.5	7.2	13.7	58.6	5.6	1.1	17.1	5.5	77.4
S8	0.23	4.2	17.7	6.4	45.0	4.5	7.4	9.4	47.4	3.1	1.3	15.7	11.4	72.5
S9	0.15	3.6	17.4	6.5	106.0	4.4	7.3	22.4	79.9	0.4	2.0	8.6	3.5	87.4
S10	0.19	2.9	18.5	6.4	122.3	4.8	8.0	9.7	60.5	2.4	1.3	5.9	5.3	88.0

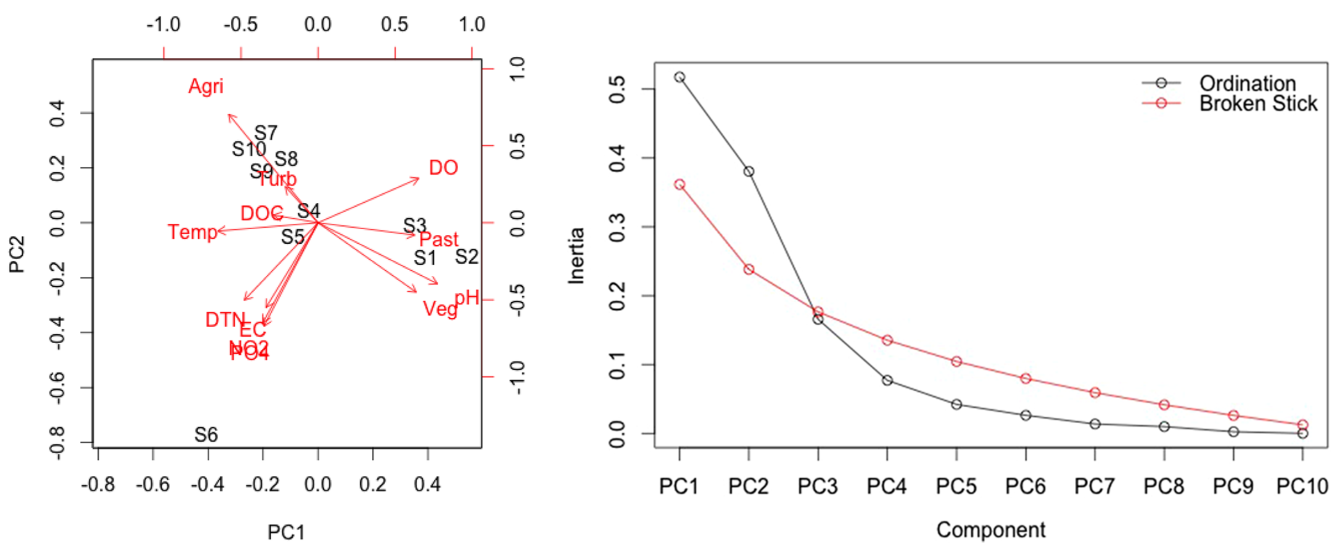

Fig. 2. Principal Components Analysis and Broken-Stick analysis of the physicochemical and land cover variables obtained in the streams studied in southern Brazil. Veg: vegetation; Agri: agriculture; Past: pasture; Temp: temperature; DO: dissolved oxygen; EC: electrical conductivity; DTN: dissolved total nitrogen; DOC: dissolved organic carbon; Turb: turbidity; PO_4: orthophosphates; NO_2: nitrite.

3.2 Aquatic insects

We collected a total of 2632 individuals distributed in 30 genera, being 15 genera belonging to the order Ephemeroptera, 13 genera to the order Trichoptera, and two genera to the order Plecoptera (Tab. 2). Trichoptera was the most abundant order (57% of the total), followed by Ephemeroptera (41%) and Plecoptera (2%). The most abundant genera were *Smicridea* (Trichoptera, 30.2%), *Caenis* (Ephemeroptera, 24.5%) and *Itaura* (Trichoptera, 20%). On the other hand, the least abundant genera were *Marilia* (Trichoptera), *Nectopsyche* (Trichoptera), *Askola* (Ephemeroptera), and *Callibaetis* (Ephemeroptera), with only one specimen collected.

The abundance was influenced by the PC2 scores ($F_{1,8} = 7.1$, $p = 0.02$; Fig. 3), which synthesize the variation generated by the higher percentage of agriculture in the stream adjacent areas. On the other hand, the rarefied richness was influenced by the PC1 scores ($F_{1,8} = 10.1$, $p = 0.01$; Fig. 3).

We observed a distribution of organisms based on the environmental quality of the streams. The first two axes of CCA explained 57% of the data variation (Fig. 4). The *Smicridea*, *Tricorythopsis*, *Worwaldia* and *Callibaetis* were associated with agricultural practices and higher concentrations of nitrite, orthophosphates and electrical conductivity. On the other hand, *Massartela*, *Tupiara*, *Phylloicus* and *Thraulodes* were associated with streams with higher amount of riparian vegetation, greater oxygenation and pH near neutrality. Finally, *Perissoplebiodes*, *Askola*, *Nectopsyche* and *Cyrnellus* presented smaller quantities and were exclusive of stream S1 (larger amount of riparian vegetation).

3.3 Nestedness of EPT assemblages

We observed that the nestedness of EPT assemblages was observed on more impacted streams ($T = 16.7$, $p = 0.01$; Fig. 5). The main environmental factors of nestedness were

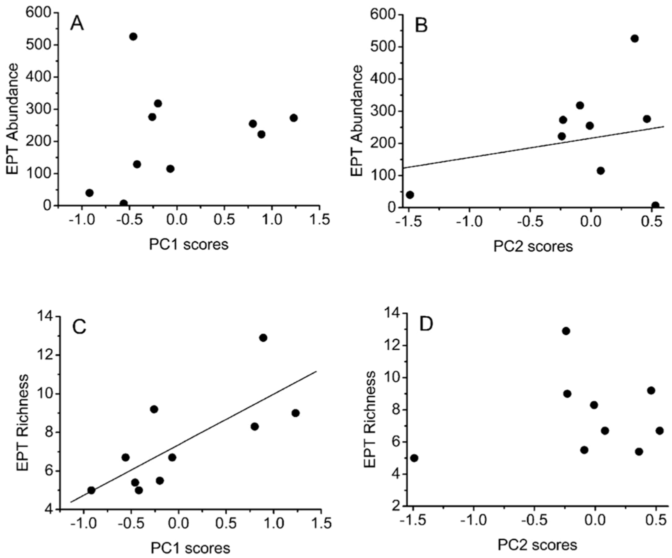

Fig. 3. Linear regressions between the first principal component (PC1) and second principal component (PC2) generated by the environmental variables and land cover of the studied streams with the abundance and rarefied richness of Ephemeroptera, Plecoptera and Trichoptera assemblages.

agricultural uses, riparian vegetation and dissolved oxygen. EPT assemblages of streams with higher percentage of agriculture and/or lower oxygen concentration (S4, S5, S6, S7, S9 and S10) were nested on assemblage of streams with higher percentage of vegetation and/or higher concentrations of dissolved oxygen (S1, S8, S3 and S2). In addition to these environmental variables, higher nutrient concentrations (*i.e.* nitrite and orthophosphate) led to lower taxonomic richness in S6. In the stream site (S6) we observed low taxa richness (5 genera) and abundance (40 specimens), highlighting the low tolerance of EPT insects in impacted environments.

4 Discussion

In our study the hypothesis was corroborated being the EPT assemblages significantly nested, this pattern was caused by the replacement of native vegetation by agriculture crops. In addition, the input of anthropogenic organic matter (*e.g.* agricultural waste, nutrients) in some streams has potentiated this negative effect on aquatic insect communities. This result coincides with others where aquatic insect nested patterns were evaluated in headwater streams and environmental gradients (Heino *et al.*, 2010; Bertaso *et al.*, 2015). The nested pattern can be originated by habitat nesting, where unusual habitats are nested; consequently, the species adapted to those habitats will also be nested (Cook, 1995). In addition, habitat fragmentation is also the cause of nestedness, where there is a generation of patches with new environmental characteristics, local extinction of specialized species and the persistence of more generalized species, which will represent the highest part of the species assemblage (Rocha *et al.*, 2008; Gimenez *et al.*, 2015).

Aquatic invertebrates strongly respond to the water quality and physical habitats, especially EPT insects (Merovich and Petty, 2010). Some studies show that anthropogenic activities such as agriculture can modify stream water chemistry, and consequently the EPT assemblage structure (Egler *et al.*, 2012; Copatti *et al.*, 2013; Selvakumar *et al.*, 2014). The EPT are organisms sensitive to anthropogenic disorders, such as high concentrations of nutrients in water, excessive sedimentation and low oxygenation (Copatti *et al.*, 2013; Ongaratto *et al.*, 2018). On larger scales, the replacement of vegetation by agriculture can generate environmental changes that contribute to the reduction of EPT abundance and richness (Ferreira *et al.*, 2017). The positive effect observed between PC1 and EPT richness is explained by the favorable ecological functions of riparian zones to aquatic systems. The sensitivity of these organisms can cause their disappearance from streams with marked changes in water quality. Water oxygenation is a key point for these insects in their immature phases. In addition, they have low tolerance to changes in nutrient concentrations. The regulation of temperature, well oxygenated water, low nutrients and the supply of allochthonous organic matter generate favorable conditions for the establishment of these organisms that are sensitive to environmental disturbances (Kasangaki *et al.*, 2008; Bertaso *et al.*, 2015; Bruno *et al.*, 2014). In contrast, streams with adjacent areas impacted by agricultural land use present increases in the input of chemical fertilizers like total nitrogen and losses in the input of allochthonous organic matter (*i.e.* organic matter supply by riparian vegetation, especially leaves), also generate habitat simplification (Riseng *et al.*, 2011; Bu *et al.*, 2014). Likewise, riparian deforestation negatively affects the abundance of intolerant aquatic insects (Kasangaki *et al.*, 2008; Nessimian *et al.*, 2008). According to Bertaso *et al.* (2015) the conversion of native vegetation by agriculture causes greater sediment input into streams, negatively affecting the occurrence of sensitive organisms (*e.g.* EPT). The decrease in taxonomic richness is the main feature of community nestedness mechanisms (Baselga, 2010). The studied streams are inserted in a predominantly agricultural landscape (Rovani *et al.*, 2019). Thus, the anthropogenic pressure that these small streams suffer is very strong, causing the species decrease (Ferreira *et al.*, 2017).

Still, even the results are not so clear the association of land use in the streams surrounding area causes changes in the water physicochemical conditions and habitat physical characteristics. The intensity of agricultural activity associated with water alterations and the natural vegetation loss led to a probable nestedness of habitat. The streams assemblage in places with poor limnological conditions represents part of the assemblages that are in places with greater limnological conditions (Hylander *et al.*, 2005). In our case, for example, *Perissoplebiodes*, *Askola*, *Nectopsyche* and *Cynellus* appeared only in one stream. The same was observed for *Phylloicus* and *Anacroneuria* that were sampled in streams with better limnological conditions and greater species diversity (Ferreira *et al.*, 2017). Oppositely, they were almost absent in streams with poor limnological conditions. These genera were affected by habitat modifications expressed in riparian forest loss, low water oxygenation, high total nitrogen concentration and low allochthonous organic matter.input. We observed that *Smicridea, Tricorythopsis, Worwaldia* and *Callibaetis* showed greater sensitivity to adverse conditions. This sensitivity may be associated to the feeding habits of these organisms. The

Table 2. Abundance of Ephemeroptera, Plecoptera and Trichoptera genera collected in stone and leaf substrates in streams of southern Brazil. The values correspond to the sum of the abundances of the 6 subsamples performed.

	Streams									
Taxa	S1	S2	S3	S4	S5	S7	S6	S8	S9	S10
TRICHOPTERA										
Helicopsychidae										
Helicopsyche	1	1	0	0	0	0	1	0	0	0
Calamoceratidae										
Phylloicus	41	9	27	6	5	1	0	33	8	3
Hydropyichidae										
Smicridea	13	118	61	44	30	98	31	54	143	202
Glossosomatidae										
Itaura	28	14	34	3	5	5	3	54	212	168
Philopotamidae										
Chimarra	0	1	0	5	0	0	0	9	0	2
Wormaldia	0	0	0	0	0	0	0	1	2	2
Hydroptilidae										
Ocrotrichia	0	3	0	0	0	0	0	0	0	0
Neotrichia	0	0	0	0	0	0	0	0	0	0
Metrichia	0	1	0	0	1	0	0	0	0	1
Odontoceridae										
Marilia	0	0	0	0	0	0	0	1	0	0
Hydrobiosidae										
Atopsyche	6	5	0	0	0	0	0	2	0	1
Nectopsyche	1	0	0	0	0	0	0	0	0	0
Polycentropodidae										
Cernotia	2	0	1	0	0	0	0	0	0	0
Cyrnellus	2	0	0	0	0	0	0	0	0	0
EPHEMEROPTERA										
Caenidae										
Caenis	30	37	85	25	218	17	0	83	133	17
Leptophlebiidae										
Massartella	4	1	1	0	0	0	0	0	0	0
Farrodes	10	47	2	27	0	3	0	4	3	11
Hagenulopsis	4	2	10	0	0	0	0	1	4	0
Thraulodes	30	0	19	0	0	0	0	3	2	5
Askola	1	0	0	0	0	0	0	0	0	0
Gen. 4	4	0	1	0	0	0	0	0	0	0
Perissoplebiodes	4	0	0	0	0	0	0	0	0	0
Baetidae										
Tupiara	3	0	1	0	0	0	0	1	0	0
Cloeodes	0	0	1	0	0	0	0	6	0	0
Callibaetis	0	0	0	0	0	0	0	0	1	0
Americabaetis	7	16	6	0	31	0	2	13	9	22
Baetodes	24	4	1	4	23	3	3	3	2	39
Leptohyphidae										
Leptohyphes	1	0	1	0	0	0	0	1	1	0
Tricorythopsis	0	0	0	0	0	0	0	1	0	2
PLECOPTERA										
Gripopterygidae										
Tupiperla	0	3	4	1	5	2	0	6	6	2
Perlidae										
Anacroneuria	6	11	0	0	0	0	0	0	0	1
Total Abundance	222	273	255	115	318	129	40	276	526	478

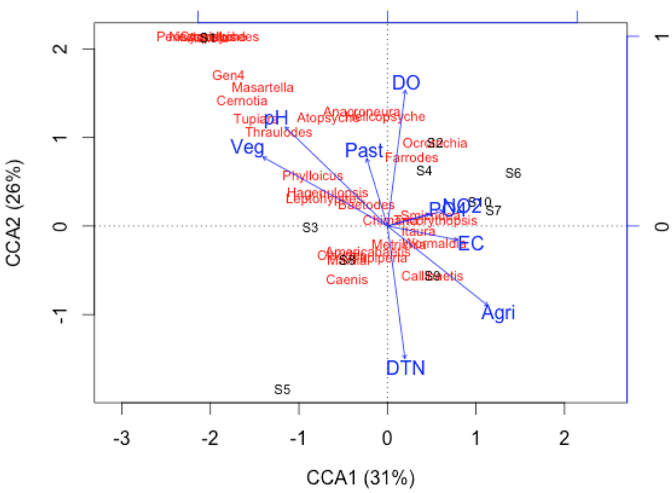

Fig. 4. Canonical Correspondence Analysis of the physicochemical and land cover variables and Ephemeroptera, Plecoptera and Trichoptera abundance in the streams studied in southern Brazil. Veg: vegetation; Agri: agriculture; Past: pasture; DO: dissolved oxygen; EC: electrical condutivy; DTN: dissolved total nitrogen; PO_4: orthophosphates; NO_2: nitrite.

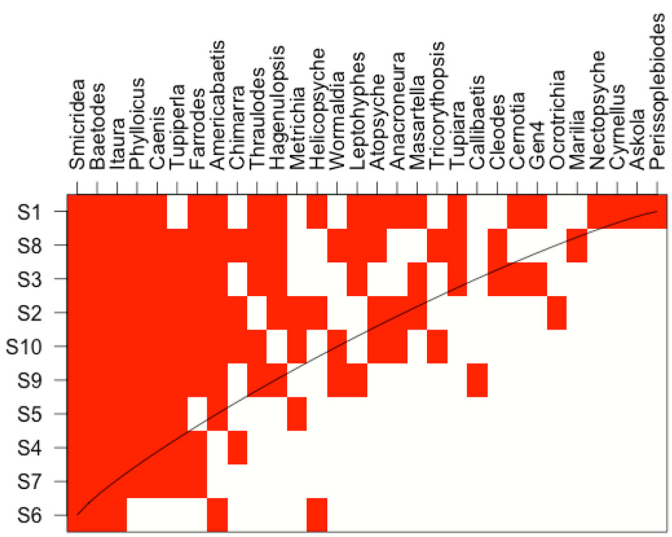

Fig. 5. Incidences matrix from nestedness analysis on Ephemeroptera, Plecoptera and Trichoptera assemlages in studied streams (S1-S10) in southern Brazil. Red blocks (taxa presence), white blocks (taxa absence). The curved line shows isoclines of prefect nestedness.

way these insects obtain food can facilitate the establishment of them in streams with worse environmental conditions. Collectors and scrapers organisms are more common in places without riparian vegetation and high amount of fine organic matter (Ferreira *et al.*, 2017).

Small streams are characterized by their high complexity and dendritic hierarchical structure. This increases the complexity of these environments, resulting in greater species diversity. Thus, stream size can be an important factor in structuring aquatic communities. Heino *et al.* (2009) observed a relationship between stream size and nested patterns of aquatic insects. According to the authors, this relationship occurs due to the effect of disturbances intensity in these environments and the speed of organism reestablishment. In addition, Milesi and Melo (2014) cited that the position of small streams in the watershed contribute to the establishment of nested patterns, since confluence positioned in lower portions of the watershed support larger numbers of species. These arguments support the hypothesis that the structure of aquatic insect communities depends not only on water quality, but also on the watershed physical characteristics (Merovich and Petty, 2010).

In summary, the perfect nestedness occurs when a poor species assemblage was a subset of a richer species assemblage (Baselga, 2010). In addition, the streams with lowest environmental integrity had EPT assemblages nested in streams of the highest environmental integrity. Thus, we conclude that agricultural activities cause changes in the streams environmental integrity. These environmental changes led to the nestedness of EPT assemblages in the studied streams. Thus, agricultural activities are an important factor for the management of water resources. In conservation actions on aquatic environments, managers should carefully observe the intensity of agricultural activities in the small streams drainage area. We further conclude that the water physicochemical variables are altered by variations in land use. This causes environmental changes affecting organisms that are sensitive to pollution and fragmentation, such as EPT. These anthropogenic changes influence the structure of the EPT assemblages (*e.g.* reducing abundance and richness), as well as altering its trophic (Ferreira *et al.*, 2017) and taxonomic composition (Ongaratto *et al.*, 2018). Future studies should cover a larger spatial scale to test the decay of similarity of aquatic insects assemblages within a fragmented landscape. Studies about the theory of intermediate disturbance in beta diversity and the effect of forest patches size and the distance between them could clarify the patterns of extinction and colonization of insects of the EPT orders located in fragmented environments related to agriculture practices.

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References

Anderson MJ, Crist TO, Chase JM, *et al*. 2011. Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. *Ecol Lett* 14: 19–28.

APHA. 2012. Standard Methods for the Examination of Water and Wastewater. Washington, DC: American Public Health Association.

Arnaiz OL, Wilson AL, Watts RJ, Stevens MM. 2011. Influence of riparian condition on aquatic macroinvertebrate communities in an agricultural catchment in South-eastern Australia. *Ecol Res* 26: 123–131.

Baselga A. 2010. Partitioning the turnover and nestedness components of beta diversity. *Glob Ecol Biogeogr* 19: 134–143.

Bergamin RS, Bastazini VAG, Vélez-Martin E, *et al*. 2017. Linking beta diversity patterns to protected areas: lessons from the Brazilian Atlantic Rainforest. *Biodivers Conserv* 26: 1557–1568.

Bertaso TRN, Spies MR, Kotzian CB, Flores MLT. 2015. Effects of forest conversion on the assemblages structure of aquatic insects in subtropical regions. *Rev Bras Entomol* 59: 43–49.

Biasi C, Milesi SV, Restello RM, Hepp LU. 2008. Ocorrência e distribuição de insetos aquáticos (Ephemeroptera, Plecoptera, Trichoptera) em riachos de Erechim/RS. *Perspectiva* 32: 171–183.

Bruno D, Belmar O, Sachez-Fernandez D, Guareschi S, Millan A, Velasco J. 2014. Responses of mediterranean aquatic and riparian communities to human pressures at different spatial scales. *Ecol Indic* 45: 456–464.

Bu H, Meng W, Zhang Y, Wan J. 2014. Relationships between land use patterns and water quality in the Taizi River basin, China. *Ecol Indic* 41: 187–197.

Cook RR. 1995. The relationship between nested subsets, habitat subdivision, and species diversity. *Oecologia* 101: 204–210.

Copatti CE, Ross M, Copatti BR, Seibel LF. 2013. Bioassessment using benthic macroinvertebrates of the water quality in the Tigreiro river, Jacuí Basin. *Acta Sci Biol* 35: 521–529.

Egler M, Buss DF, Moreira JC, Baptista DF. 2012. Influence of agricultural land-use and pesticides on benthic macroinvertebrate assemblages in an agricultural river basin in southeast Brazil. *Braz J Biol* 72: 437–443.

Ferreira WR, Hepp LU, Ligeiro R, *et al*. 2017. Partitioning taxonomic diversity of aquatic insect assemblages functional feeding groups in neotropical savanna headwater streams. *Ecol Indic* 72: 365–373.

Gianuca AT, Declerck SAJ, Lemmens P, De Meester L. 2017. Effects of dispersal and environmental heterogeneity on the replacement and nestedness components of β-diversity. *Ecology* 98: 525–533.

Gimenez BCG, Lansac-Tôha FA, Higuti J. 2015. Effect of land use on the composition, diversity and abundance of insects drifting in neotropical streams. *Braz J Biol* 75: 52–59.

Hamada N, Nessimian JL, Querino RB. 2014. Insetos aquáticos na Amazônia brasileira: taxonomia, biologia e ecologia. Editora do INPA Manaus.

Heino J, Mykrä H, Muotka T. 2009. Temporal variability of nestedness and idiosyncratic species in stream insect assemblages. *Diversity Distrib* 15: 198–206.

Heino J, Mykrä H, Rintala J. 2010. Assessing patterns of nestedness in stream insect assemblages along environmental gradients. *Ecoscience* 17: 345–355.

Hooke RL, Martín-Duque JF, Pedraza J. 2012. Land transformation by humans: a review. *GSA Today* 22: 4–10.

Hu G, Feeley KF, Wu J, Xu G, Yu M. 2011. Determinants of plant species richness and patterns of nestedness in fragmented landscapes: evidence from land-bridge islands. *Landsc Ecol* 26: 1405–1417.

Hylander K, Nilsson C, Gunnar Jonsson B, Göthner T. 2005. Differences in habitat quality explain nestedness in a land snail metacommunity. *Oikos* 108: 351–361.

Jackson DA. 1993. Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecology* 74: 2204–2214.

Kasangaki A, Chapman LJ, Balirwa J. 2008. Land use and the ecology of benthic macroinvertebrate assemblages of high-altitude rainforest streams in Uganda. *Freshw Biol* 53: 681–697.

Korhonen JJ, Soininen J, Hillebrand H. 2010. A quantitative analysis of temporal turnover in aquatic species assemblages across ecosystems. *Ecology* 91: 508–517.

Merovich GT, Petty JT. 2010. Continuous response of benthic macroinvertebrate assemblages to a discrete disturbance gradient: consequences for diagnosing stressors. *J N Am Benthol Soc* 29: 1241–1257.

Milesi SV, Melo AS. 2014. Conditional effects of aquatic insects of small tributaries on mainstream assemblages: position within drainage network matters. *Can J Fish Aquat Sci* 71: 1–9.

Mugnai R, Nessimian JL, Baptista DF. 2010. Manual de identificação de macroinvertebrados aquáticos do estado do Rio de Janeiro: para atividades técnicas, de ensino e treinamento em programas de avaliação da qualidade ecológica dos ecossistemas lóticos. Technical Books Editora, Rio de Janeiro. 174p.

Munn M, Frey J, Tesoriero A. 2010. The influence of nutrients and physical habitat in regulating algal biomass in agricultural streams. *Environ Manag* 45: 603–615.

Nessimian JL, Venticinque EM, Zuanon J, *et al*. 2008. Land use, habitat integrity, and aquatic insect assemblages in Central Amazonian streams. *Hydrobiologia* 614: 117–131.

Oksanen J, Blanchet FG, Kindt R, *et al*. 2017. Vegan: community ecology package. https://cran.r-project.org, https://github.com/vegandevs/vegan

Oliveira-Filho AT, Budke JC, Jarenkow JA, Eisenlohr PV, Neves DRM. 2015. Delving into the variations in tree species composition and richness across South American subtropical Atlantic and Pampean forests. *J Plant Ecol* 8: 242–260.

Ongaratto R, Loureiro RC, Restello RM, Hepp LU. 2018. Effects of land use and limnological variables on the dissimilarity of common and rare aquatic insects in Atlantic Forest streams. *Rev Biol Trop* 66: 1223–1231.

Pes AMO, Hamada N, Nessimian JL. 2005. Chaves de identificação de larvas para famílias e gêneros de Trichoptera (Insecta) da Amazônia Central, Brasil. *Rev Bras Entomol* 49: 181–204.

Pinha GD, Tramonte RP, Bilia CG, Takeda AM. 2017. Differences in environmental heterogeneity promote the nestedness of Chironomidae metacommunity in Neotropical floodplain lakes. *Acta Limnol Bras* 29: e118.

R Core Team. 2018. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Rhodes AL, Newton RM, Pufall A. 2001. Influences of land use on water quality of a diverse New England watershed. *Environ Sci Technol* 35: 3640–3645.

Riseng CM, Wiley MJ, Black RW, Munn MD. 2011. Impacts of agricultural land use on biological integrity: a causal analysis. *Ecol Appl* 21: 3128–3146.

Rocha CFD, Hatano FH, Vrcibradic D, Vansluys M. 2008. Frog species richness, composition and β-diversity in coastal Brazilian restinga habitats. *Braz J Biol* 68: 101–107.

Rodríguez-Gironés MA, Santamaría L. 2006. A new algorithm to calculate the nestedness temperature of presence-absence matrices. *J Biogeogr* 33: 924–935.

Rovani IL, Santos JE, Decian VS, Zanin EM. 2019. Assessing naturalness changes resulting from a historical land use in Brazil South Region: an analysis of the 1986–2016 period. *J Environ Protec* 10: 149–163.

Scolar JB, Gilroy JJ, Kunin WE, Edwards DP. 2016. How should beta-diversity inform biodiversity conservation? *Trends Ecol Evol* 31: 67–80.

Segura MO, Valente-Neto F, Fonseca-Gessner AA. 2011. Chave de famílias de Coleoptera aquáticos (Insecta) do Estado de São Paulo, Brasil. *Biota Neotrop* 11: 394–412.

Selvakumar C, Sivaramakrishnan KG, Janarthanan S, Arumugam M, Arunachalam M. 2014. Impact of riparian land-use patterns on Ephemeroptera community structure in river basins of the southern Western Ghats, India. *Knowl Manag Aquat Ecosyst* 412: 1–11.

Theodoropoulos C, Iliopoulou-Georgudaki J. 2010. Response of biota to land use changes and water and water quality degradation in two medium-sized river basins in southwestern Greece. *Ecol Indic* 10: 1231–1238.

Valente-Neto F, Koroiva R, Fonseca-Gessner AA, Roque FO. 2015. The effect of riparian deforestation on macroinvertebrates associated with submerged woody debris. *Aquat Ecol* 49: 115–125.

Vidal-Abarca MRG, Suarez MLA. 2013. Which are, what is their status and what can we expect from ecosystem services provided by Spanish rivers and riparian areas? *Biodivers Conserv* 22: 2469–2503.

Whittaker RH. 1960. Vegetation of the Siskiyou Mountains, Oregon and California. *Ecol Monogr* 30: 279–338.

Wright DH, Patterson BD, Mikkelson GM, Cutler A, Atmar W. 1997. A comparative analysis of nested subset patterns of species composition. *Oecologia* 113: 1–20.

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