ECOSYSTEMS

Aquatic insects in subtropical streams: the role of different grassland ecosystems and local environmental descriptors

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Abstract: Organisms can respond to environmental gradients from local to landscape features. Aquatic insects are particularly affected by watershed peculiarities due to their dependence on microhabitat conditions. However, these relationships are poorly understood in lotic ecosystems of subtropical grasslands, limiting water resources management and bioassessment proposals. Here, we investigated how local stream environment and variations in landscape types affect the assemblage structure of a bioindicator insect group, face to the spatial proximity of the sampled locations. We sampled immatures of Ephemeroptera, Plecoptera, and Trichoptera in streams along the Brazilian Pampa biome, recording environmental descriptors in different grassland ecosystem types. The structure of aquatic insect assemblages differed across grassland types, with specific dominant genera associated with each landscape. Spatially-structured water physicochemical descriptors explained a significant amount of variation in assemblage data. Our findings suggest that grassland ecosystem type delimitations capture ecological attributes, influencing watershed features important to EPT assemblage structuration. Moreover, we highlight the importance of niche-based process structuring EPT assemblages along grassland ecosystem types of Pampa biome. In addition, we encourage using aquatic insects in bioassessment of lotic waters to assess local and landscape environmental impacts. We strongly recommend considering the grassland ecosystem schedule for water resources management and bioassessment proposals.

Key words: assemblage structure, ecoregions, Ephemeroptera, Plecoptera, Trichoptera.

INTRODUCTION

The community structure is defined by the multivariate abundance of different taxonomic groups. In lotic ecosystems, the community structure is influenced by environmental filters (e.g. water physicochemical variables, landscape and watershed features) and ecological interactions (e.g. competition, predation) among co-occurring species within a determined local assemblage (Allan & Castillo 2007). Communities are also influenced by large-scale processes, such as dispersion, speciation, and biogeographic history, which determine the regional pool of species available to colonize a habitat (Ricklefs & Schluter 1993). Communities presenting predictable structure require organization through nonrandom processes, rather than arising from chance and dispersal capacity, which may result in ecological patterns alluding to landscape configurations (Allan & Castillo 2007). Therefore, landscape classifications are often used to predict local site-specific environmental conditions. Ecological attributes of aquatic ecosystems are strongly influenced

by watershed characteristics, generating a spatial structure with significant effects on the biota (Hynes 1975, Hawkins et al. 2000, Legendre & Legendre 2012). The relationship between landscape classifications and the structure of biological communities in aquatic ecosystems has been tested (Hawkins & Norris 2000). However, the strength of the relationship between landscape features and local biota is poorly known (Hawkins et al. 2000), especially in the Brazilian Pampa biome, where the knowledge of aquatic insects is incipient and there is a prevalent belief that the grassland landscape is homogeneous.

Grasslands are the main vegetation type in the southernmost part of Brazil, and they are divided in the Highland Grasslands of Atlantic biome and the Pampa Grasslands of Pampa biome. The last grasslands areas constitute the "Río de la Plata grasslands", covering an extensive area that encompass a large portion of Rio Grande do Sul state, central-east Argentine, and all of Uruguay (Soriano et al. 1992, Overbeck et al. 2007, Andrade et al. 2019, Saccol et al. 2022). Although the Pampa is usually regarded as being a relatively homogenous grassland landscape, it comprises several different physiographic ecosystems (Roesch et al. 2009). Researchers have created classifications relating the local vegetation physiognomy with the respective landscape characteristics since the last century (Rambo 1956, Chebataroff 1968, Lindman 1974). Floristic surveys have improved the characterization of the Pampa Grassland types, resulting in refined proposals of distinct ecological systems. These proposals are based on the association of plant communities and the physical environment characteristics such as altitude, slope, geology, soil types, and geological substrates (Boldrini 2009, Boldrini et al. 2010, Hasenack et al. 2023). Thus, recently Hasenack et al. (2023) delimited the Pampa

grassland into ten distinct ecosystems based on biophysical delimitation. According to literature, factors act not only in shaping the changes in taxonomic composition of plant communities in the grassland matrix, but also in modulating vegetation structure, degree of soil cover, and the presence of wood species in the herbaceous matrix (Boldrini et al. 2010).

Ecological system distinction can strongly influence the regional distribution of several animal groups (as seen in examples for birds within the Brazilian Cerrado biome phytophysiognomies in Laranjeiras et al. 2012; and for anuran amphibians within South Brazilian grasslands in Saccol et al. 2022). This influence is seen through the detailed description of the ecological systems that explain the spatial and temporal distribution of biological diversity (Hirzel et al. 2002, Hasenack et al. 2023). However, this effect has rarely been examined for aquatic insects in subtropical grassland ecosystems, except for a recent study with Odonata, which detected a strong influence of ecosystem type on taxonomic composition variation (Renner et al. 2019).

Macroinvertebrates are organisms associated with different aquatic environment substrates and comprise several taxonomic groups, including crustaceans, annelids, mollusks, and a myriad of hexapods (Merrit et al. 2019). These animals are often used as bioindicators of water quality due to their high taxonomic diversity within aquatic ecosystems (Luiza-Andrade et al. 2017, Amaral et al. 2019, Brasil et al. 2020b) as well as their reliance on specific environmental conditions (Crisci-Bispo et al. 2007, Cortezzi et al 2009, Souza et al. 2020, Baptista et al. 2001, Gartner et al. 2013, Brasil et al. 2020b). In addition, macroinvertebrates are widely distributed, have a relatively long-life cycle, and have relatively sedentary behavior, thereby limiting their ability to disperse in

habitats like streams (Bonada et al. 2006, Rosenberg & Resh 1993, Eriksen et al. 2021).

Defining ecological systems for aquatic biota is valuable for managing water resources (which fluctuate according to landscape features such as physiography, geology, soil type, vegetation, and land use). Ecological systems also function as fundamental classification unit for aquatic bioassessment and water quality evaluation using bioindicator organisms (Marchant et al. 1999, Newall & Wells 2000, Hawkins et al. 2000). In this study, we aimed to test the response of potential variations in the structure of insect assemblages (i.e. in the multivariate abundance of genera) of streams of the Brazilian Pampa biome (Ephemeroptera, Plecoptera, and Trichoptera - EPT) to: (i) local environmental predictors (i.e. sampling reach scale) face to the possible influence of geographical proximity among locations, and (ii) different grassland types of the Brazilian Pampa biome (i.e. grassland ecosystems *sensu* Hasenack et al. 2023). For this, we determined which organisms were the most representative within the grassland ecosystem types. Our hypothesis was that the structure of EPT assemblages differs among grassland types since landscape attributes (such as geology, soil classes and fertility, vegetation, relief and climatic characteristics) determine watershed characteristics in grassland ecosystems (Hynes 1975, Allan & Castillo 2007) and the distribution of aquatic insects (Renner et al. 2019). Therefore, we expected high similarity in assemblage structure of EPT for more geographically closer streams as a result of the induced spatial dependence from spatially-structured environmental variables (*sensu* Legendre & Legendre 2012).

MATERIALS AND METHODS
Study area

The study was conducted within the Brazilian Pampa biome (extreme coordinates ranging from -28.52° N; -30.85° S; to -53.19° E; -55.97° W) (Figure 1), located exclusively in the state of Rio Grande do Sul, covering an area of approximately 193,836 km² (IBGE 2019). The Brazilian Pampa covers over half of Rio Grande do Sul (68%) but only 2.3% of the national territory (Hasenack et al. 2023). Its geology consists of diverse lithologies, including granite rocks, sandstone, basalt, and sedimentary deposits (Hasenack et al. 2023). The climate of the studied region varies from subtemperate to temperate, according to the Köppen modified by Maluf (2000). Rainfall is well-distributed throughout the year, without a dry period (although surface water deficit may occur in some regions along the summer, due to the high rate of evaporation of soil moisture exceeding the volume of rainfall); it is marked by low winter temperatures, often dropping below 0 °C, and hot summers with maximum temperatures from 22 °C to over 24 °C (Wrege et al. 2011). Mean annual temperatures range from 12.1 to 23 °C and annual precipitation ranges from 1,200 to 2,400 mm (Wrege et al. 2011). Elevation ranges from flat to steep, and altitude ranges from 0 m to 603 m (Kuplich et al. 2018).

The diversity of grassland ecosystem types in southern Brazil is generated by a combination of climatic (e.g. gradients of temperature and rainfall, continentality), relief (altitudinal and slope gradients), and edaphic factors (soils vary in geological substrates, depth and water holding capacity) (Overbeck et al. 2015). These landscape and climatic variables, as well as the management particularities along the Brazilian Pampa biome, culminate in the formation of distinct plant assemblages that are used to name the grassland ecosystem types in the region (Boldrini et al. 2010). Thus, four distinct grassland
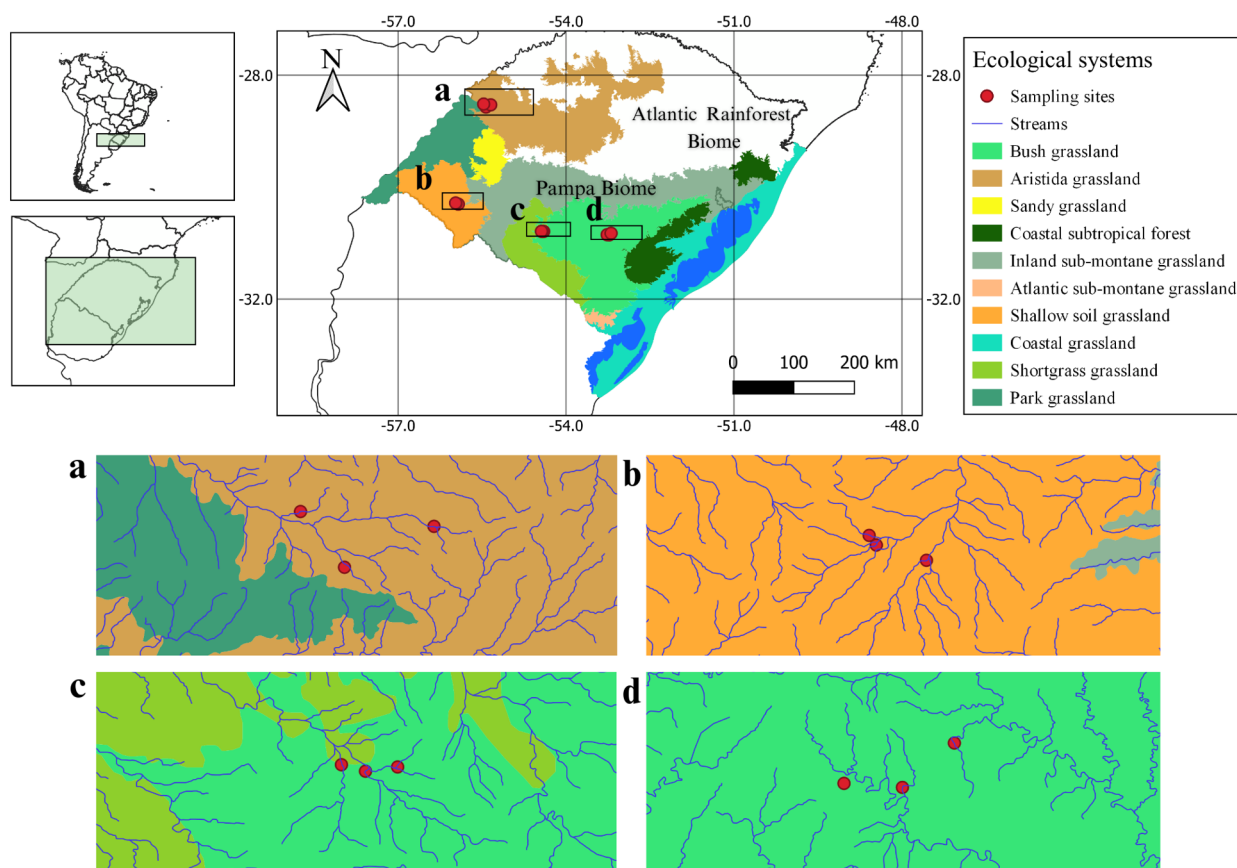
Figure 1. Distribution of grassland ecosystem types in the Brazilian Pampa biome and sampled streams location in the four systems: a) Aristida grassland, b) Shallow soils grassland, c) Bush grassland and d) Shortgrass grassland in the state of Rio Grande do Sul.

ecosystem types were selected to represent the diversity of the Brazilian Pampa biome (Figure 1, and stream general view in the Supplementary Material, Figure S1-S4). The following description is based on the biophysical delimitation of the grassland ecosystem types outlined in Hasenack et al. (2023):

The Aristida grassland (ARG)

Locally known as "barba-de-bode", is characterized by summer cespitose and prostrate species covering the interfluves of the tributaries of the left edge of the upper Uruguay River valley up to the transition with areas of Araucaria and Subtropical Forest, along the main tributaries of the Uruguay and Jacuí rivers. The

elevation ranges from 100 to 500 m, with gentle slopes relief and deep soils with low fertility.

The Shallow soils grassland (SSG)

Characterized by low, mainly erect vegetation, situated on a low-lying basaltic plateau in the far west of the state. The vegetation is associated with very shallow, stony basalt soils with low moisture retention. The water deficit in summer makes this environment a challenging one for living organisms. The elevation ranges from 100 to 300 m, slopes are gentle and the soils very shallow.

The Shortgrass grassland (SHG)

Presents many winter erect and summer prostrate grasses, is dominated by herbaceous species, essentially grassy ones, with a rhizomatous habit, while others present a tufted habit. It is located in the south portion of the state on the colluvium of the Uruguayan-sul-rio-grandense Plateau at an elevation between 100 and 200 m. The slopes are gentle and the soils are deep with high fertility.

The Bush grassland (BUG)

Is characterized by the presence of cactus and woody species, with vegetation divided into strata. The upper stratum is formed by woody species dominated by Asteraceae species. The lower stratum by erect grasses, and cactus species. This ecosystem is found on the Uruguayan-sul-rio-grandense plateau, with elevation between 30 and 500 m, undulating slopes, with both deep and shallow soils with low fertility.

The general affinities among these grassland ecosystem types, considering physical and climatologic profiles (geological substrates, soil classes, soil slope, topography, climate type, air humidity, air temperature, and potential evapotranspiration), can be assessed in both, table data and respective summarized cluster analysis (Supplementary Material, Table SI and Figure S5). This description shows the sampled grassland ecosystem types as grouped mainly by climate type, suggesting some effect of geographical proximity among them.

Sampling design

In each grassland ecosystem, three independent low-order streams (first and second order, following Strahler 1957) from the same watershed were selected within native grassland landscapes that have been historically used for extensive livestock grazing. The selection was carried out within a 5 km x 5 km grid. Sampling sites were selected based on satellite imagery (Google Earth) and consisted of stream reaches bordered by riparian forests with a mean width of 80 m (± 35 m) and a mean cover canopy of 50% (± 20%). Three 50 m reaches were sampled in each stream, and five subsamples were taken from each reach in a single instance (Figure 2a). The streambed substrate general composition of the reaches consisted of 8% of cobble, 12% of gravel, 55% of sand, and 25% of silt, with sporadic occurrence of boulders (Supplementary Material, Figure S1-S4). Subsamples were collected using Surber sampler (with an area of 0.01 m² and mesh size of 0.25mm) from gravel substrate located within riffle areas (Figure 2a). Riffle samples were chosen because this habitat hosts more diverse macroinvertebrates assemblages compared to pools (Baptista et al. 2001, Buss et al. 2004). Furthermore, gravel substrate in riffles supports a range of all other substrate types found in streams, given its high habitat heterogeneity, especially in low-order streams.

The following environmental descriptors were measured within each stream reach: water velocity (m/s) using the float method (Bain & Stevenson 1999), wet width and depth of streams (cm) by using a measuring tape (five measurements by reach); electrical conductivity (mS/cm); turbidity (NTU); dissolved oxygen concentration (mg/L); pH, and water temperature (°C), measured using a Horiba® Model Multiprobe (three measurements per reach).

Sampled streams in ARG presented smaller dimensions, high concentrations of dissolved oxygen, and slightly acid pH. SSG streams presented low dissolved oxygen concentration, turbidity, and water velocity. SHG e BUG streams presented slightly basic pH, and higher values of turbidity, water velocity, larger dimensions, and well-oxygenated water (Table I).

Figure 2. Infographic summarizing methodological approaches employed to assess how grassland ecosystem type and local environmental descriptors affect the assemblage structure of aquatic insects: a) sampling procedure assemblage data and environmental descriptors; b) data analytical schedule.

Sampling was conducted during the spring/summer of 2018/2019 and summer 2020, corresponding to the period of peak abundance of these insect orders in the Southernmost region of Brazil (Spies et al. 2006, Siegloch et al. 2008). Collected samples were fixed in 5% formalin. In the laboratory, they were filtered through a 0.25-mm mesh in the laboratory, sorted and identified under a stereomicroscope, and preserved in 80% ethanol. The EPT taxa were identified at the genus level using taxonomic keys (Wiggins 1996, Pes et al. 2005, 2018, Salles 2006, Salles et al. 2018, Domínguez et al. 2006, Lecci & Froehlich 2011). All voucher material (Sisbio licenses #62168-0, ##62168-1, and ##62168-2) was deposited in the Coleção de Invertebrados Aquáticos da Universidade Federal do Pampa (UNIPAMPA).

Statistical Analysis

The sampling unit used in all analyses was the reach (i.e. each sample was composed of the sum of the five subsamples collected in each stream reach). Therefore, each stream was represented by three samples (= three reaches). The EPT genera abundance matrix was subjected to square root transformation to mitigate the impact of the most abundant genera. Next, the matrix was portrayed as a heat map (shade plot), allowing the visualization of the variation in abundance among different grassland ecosystem types.

Table I. Means and standard deviations (SD) of environmental descriptors of grassland ecosystem types streams (Shallow soils grassland - SSG; Shortgrass grassland - SHG; Bush grassland - BUG; and Aristida grasslands - ARG) in Brazilian Pampa biome.

Environmental descriptors	SSG		SHG		BUG		ARG	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Water temperature (°C)	26.21	0.85	19.81	2.01	26.31	3.15	21.61	1.13
Electric conductivity (mS/cm)	0.22	0.08	0.32	0.03	0.06	0.02	0.11	0.07
Dissolved oxygen (mg/L)	5.00	0.81	7.13	0.97	7.21	0.53	13.05	2.45
pH	7.61	0.36	7.94	0.13	8.31	0.86	5.33	0.90
Turbidity (NTU)	3.76	5.93	20.87	5.14	23.51	6.52	16.84	9.53
Water velocity (m/s)	0.25	0.08	0.52	0.14	0.52	0.18	0.41	0.15
Width (m)	3.38	1.51	2.76	1.42	3.07	0.97	1.94	0.77
Depth (cm)	11.16	2.70	12.07	7.31	13.91	4.46	8.95	1.74

To test the primary null hypothesis of absence of differences in the assemblage structure of EPT among grassland ecosystem types, we employed the PERMANOVA routine based on a one-way design with four levels (i.e. the four grassland ecosystems) (Anderson 2017). PERMAVOVA used a resemblance matrix (zero-adjusted Bray–Curtis index). The statistical significance of the null hypothesis was determined by 9999 permutations. Afterwards, a Bootstrap Averages routine (Clarke & Gorley 2015) was performed to illustrate the similarity within the grassland ecosystems factor. The mean values of each grassland ecosystem group were estimated by bootstrap permutations (150) with resampling. Following that, the random mean values matrix and the region with 95% of this distribution were plotted on a metric two-dimensional space using metric Multidimensional Scaling (mMDS) (Clarke & Gorley 2015). The most representative genera of each grassland ecosystem type were further investigated using similarity percentage analysis (SIMPER) (Clarke & Gorley 2015).

We performed a Distance-based linear model (DistLM) in combination to a distance-based Redundancy Analysis (dbRDA) ordination to explore the null hypothesis that variability in the structure of EPT assemblages cannot be explained by local environmental descriptors face to the intrinsic spatial structure (i.e. geographic distances among the sampled sites). This means that our approach can explicitly examine the proportion of variation in the assemblage data that is explained by the environmental variables over and above the amount explained by the spatial variables alone (Anderson et al. 2008). Therefore, we build two sets of predictor descriptors: (1) a set of environmental variables, and (2) a set of spatial variables represented by orthogonal scores of distance-based Moran's Eigenvector Maps (dbMEM) (Legendre & Legendre 2012), following the original recommendations of Dray et al. (2006). Finally, we used as selection criteria 'Akaike Information Criterion corrected' (AICc) and the model with the *Best* adjustment was depicted multidimensionally using dbRDA ordination.

Environmental descriptors were previously inspected for collinearity by building a correlation matrix (Pearson' index) and, for

necessity of data pre-treatment by using draftsman plots (Anderson et al. 2008). Thus, no descriptor was excluded from the dataset due to high collinearity with other (r>0.70, according to Dormann et al. 2013), but two descriptors were log-transformed (dissolved oxygen concentration and stream wet width).

The dbMEM scores were calculated by using the original geographic coordinates of the sampled sites and the multivariate data from EPT assemblages previously summarized by the first two axes of the Principal Coordinated Analysis (PCoA) (Legendre & Anderson 1999). PCoA was based on the same resemblance matrix used in the PERMANOVA analysis. The truncation distance (i.e. the minimum geographical distance connecting all sampled sites) was automatically calculated as 197.28 km. Only statistically significant dbMEM scores ($p<0.05$) were considered in the following step of analysis (i.e. DistLM and dbRDA ordination).

The DistLM models the relationship of the sets of predictor descriptors (environmental and spatial) with the first two axes of the PCoA of the assemblage (Legendre & Anderson 1999) (see a summary of the analytical approach in Figure 2b). In this analysis, we used the two *Best* selection procedure (AICc criterion) for all possible combinations of predictor variables, for each predictor set separately (Anderson et al. 2008). Next, we performed two DistLM partial tests considering only environmental and spatial predictors sets selected in the previous two selection phases in order to assess the variation partitioning among both predictor sets. Then, in the first DistLM partial tests, we fitted the spatial set first, followed by the environmental set, since our null hypothesis was of no relationship between EPT assemblage structure and the environmental descriptors, given the spatial descriptors (Anderson et al. 2008). Next, in the second DistLM partial test,

we fitted the environmental set first, followed by the spatial set. Additionally, we looked for the permutational *Sequential Test*s in order to verify the statistical significance and to quantify the variation of the following components: [a] the variation independently explained by the spatial descriptor set; [b] the variation independently explained by the environmental descriptor set; [c] the variation shared by both descriptor sets (i.e. the contribution of spatially structured environmental variation); [a+b+c] the total variation accounted by the model, and [d] the unexplained variation (Legendre & Legendre 2012).

Finally, the set of environmental and spatial descriptor selected in previous phases were fitted to a new DistLM in combination to a dbRDA ordination, using *Best* selection procedure (AICc criteria) to find the linear combination of descriptors that accounts for the highest variation in the more parsimonious model, and to examines the variance explained by each environmental/spatial descriptor set, providing pseudo-F statistics and respective p-values associated (Anderson et al. 2008).

Additionally, predictor descriptors that best explained the data were superimposed as biplots representing strength (vector length) and direction of influence (Anderson et al. 2008). For this, DistLM outputs were represented graphically with (dbRDA) two-dimensional (2D) bubble plots of the most representative genera of each grassland ecosystem type.

Environmental descriptors were automatically standardized (by standard deviation) in order to eliminate the influence of different measurement units (Clarke & Gorley 2015). Most multivariate analyses were performed using Primer-E 7.0.23 and Permanova+ software (Anderson et al. 2008, Clarke & Gorley 2015), except db-MEM scores, for which calculation was

obtained using the software Spatial Analysis in Macroecology (SAM) (Rangel et al. 2006).

RESULTS

We recorded a total of 13,583 EPT individuals, distributed in 26 genera within 14 families. The highest EPT abundance was collected in SHG streams, amounting to 6,907 individuals (approximately 51% of total EPT), followed by SSG streams (3,463 individuals; approximately 21%), BUG (2,234 individuals; 16.4%) and ARG (979 individuals; 7.2%) (Figure 3). Five EPT genera were collectively responsible for 81% of the total abundance: *Smicridea* (3,542 individuals; 26%), *Traveryphes* (2,947; 22%), *Americabaetis* (2,204; 16.28%), *Chimarra* (n=1,167; 8.6%), and *Itauara*

(1,148; 8.4%) (Figure 3). Six genera were exclusive to a single grassland ecosystem type: *Tupiara* and *Hydroptila* to SSG streams; *Leptohyphes*, *Askola* and *Helicopsyche* to ARG streams; and *Peltopsyche* to SHG streams (Figure 3).

The PERMANOVA revealed a significant difference in the structure of EPT assemblages among the sampled grassland ecosystem types (Pseudo-F=7.012; p<0.0001). The pairwise PERMANOVA tests also showed significant differences among samples for all pairwise grassland ecosystems comparisons (Table II). The mMDS ordination effectively portrayed this segregation, since EPT samples of the four grassland ecosystems appear segregated in the bi-dimensional space (Figure 4). In addition, the similarity percentage analysis (SIMPER) was
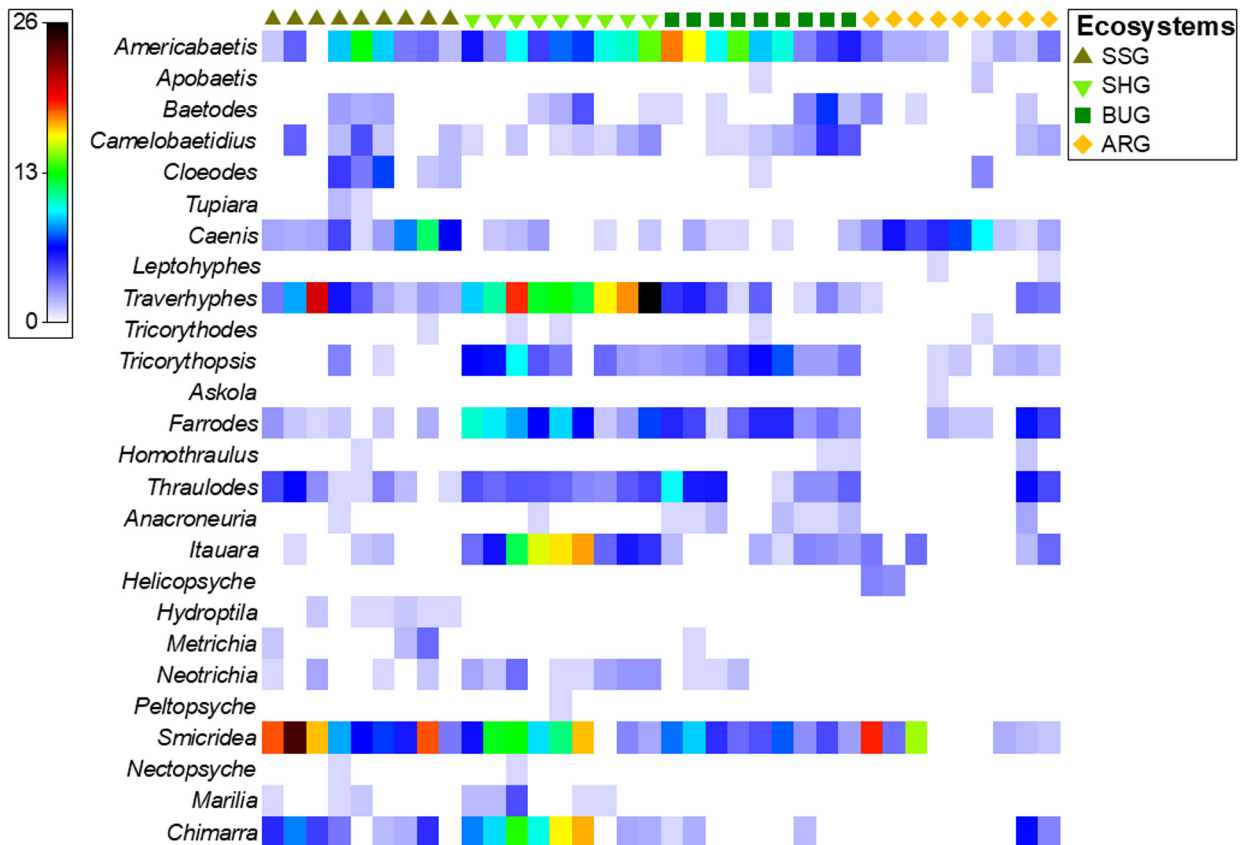
Figure 3. Heat map of EPT genera abundance (shaded plot) from stream in grassland ecosystems of the Brazilian Pampa biome. The color gradient represents abundance using square root-transformed data. (Shallow soils grassland - SSG; Shortgrass grasslands - SHG; Bush grasslands - BUG; Aristida grasslands - ARG).

able to identify the most representative genera of each grassland ecosystem: *Smicridea* in SSG (responsible for 35.43% of within ecosystem similarity), *Traverhyphes* in SHG (26%), *Americabaetis* in BUG (9.35%), and *Caenis* in ARG (46%) (Table III).

The first two axes of PCoA accounted for 55.9% of the data variation in the multivariate structure of EPT assemblages (36.5% in axis 1 and 19.4% in axis 2). Two dbMEM orthogonal scores resulted as statistically correlated with the PCoA axes (p<0.05), the first dbMEM was correlated with PCoA axis 1, and the second dbMEM with PCoA axis 2. In both cases, dbMEMs represented positive autocorrelation associated to short distances (below distance classes of 50 km); as well as negative autocorrelation associated to large ones (within distance classes $\cong$ 250 - 300 km) (Supplementary Material, Figure S6).

Best selection procedures for the DistLM routine retained five variables from the environmental descriptor set (water temperature, electrical conductivity, dissolved oxygen concentration, pH, and turbidity) (AICc=261.3; Pseudo-F=6.46; p<0.01), in addition to two dbMEMs in the spatial descriptor set, (filter 1 and filter 2) (AICc=263.6; Pseudo-F=8.01; p<0.01) in the two most parsimonious models. After the last selection procedure, the variation partitioning accounted 2.0% of pure spatial structure [a]; 17.2% of pure environmental structure [b], and 26.6% environmental spatially structured variation [c]; thus, our explanatory model accounted to 45.8% of the variation in EPT assemblage data [a+b+c], while 54.2% remained unexplained [d].

The Distance-based redundancy analysis (dbRDA) ordination represented the relationship between EPT assemblages and environmental descriptors (since DistLM *Best* selection (AICc = 259.68) retained only the environmental set). The two first dbRDA axes accounted for 41.65% of the total variation of EPT assemblages, and the adjusted model explained 80.3% of the summarized variation in the two first axes (Figure 5). The pH and turbidity were negatively correlated with the first axis, while dissolved oxygen was positively correlated (r = -0.62, -0.47, and 0.56, respectively). Electrical conductivity and water temperature were positively correlated with the second axis, while dissolved oxygen was negatively correlated (r = 0.77, 0.38, and -0.43, respectively). EPT assemblages in SHG and BUG were correlated with higher values of pH and turbidity while ARG samples were more correlated with high dissolved oxygen concentrations, and SSG samples were correlated with higher values of electrical conductivity, and water temperature (Figure 5).

Table II. The difference in the structure of EPT assemblages among grassland ecosystem types in the Brazilian Pampa biome. Pairwise comparisons of PERMANOVA based on Bray-Curtis similarity matrix in a one-factor fixed model. SSG - Shallow soils grassland; SHG - Shortgrass grassland; BUG -Bush grassland; ARG - Aristida grassland. Average similarity percentage (Av.Sim%); t test (t); p-value through permutations (p(perm)); permutatios (perms); Monte Carlo p-value (p(MC)).

Comparisons	Av. Sim%	t	p(perm)	perms	p(MC)
SSG x SHG	42.0	28.92	0.0001	8186	0.0001
SSG x BUG	42.0	25.59	0.0002	8195	0.0005
SSG x ARG	34.0	18.13	0.0129	8185	0.0177
SHG x BUG	48.2	30.62	0.0001	8196	0.0001
SHG x ARG	25.0	32.34	0.0003	8150	0.0001
BUG x ARG	31.0	25.61	0.0002	8122	0.0007

Genus-specific dbRDA bubble plots illustrated the relationship of the most abundant genera with local environmental descriptors (Figure 6). *Smicridea* exhibited higher abundance in samples with higher electrical conductivity and water temperature (Figure 6a). *Americabaetis* e *Traverhyphes* displayed higher abundance in samples with higher pH and turbidity (Figure 6b and 6c), while *Caenis* showed greater abundance in samples with higher dissolved oxygen concentrations, and high temperature (Figure 6d).

DISCUSSION

Our results demonstrated that grassland ecosystem types were important predictors of EPT assemblage structure, with distinct dominant genera being clearly associated to each ecosystem. Moreover, the environmental descriptors set (including their spatially-structured explainability) of stream retained a significant amount of the variation in EPT assemblage data within grassland sampled ecosystems. These findings suggest that the biological communities, including the structure of the EPT assemblages, were influenced by grassland ecosystem types. Therefore, the biophysical delimitation of grassland ecosystems (Hasenack et al. 2023) was able to capture the landscape ecological attributes, influencing watershed features important to EPT assemblage structuration.
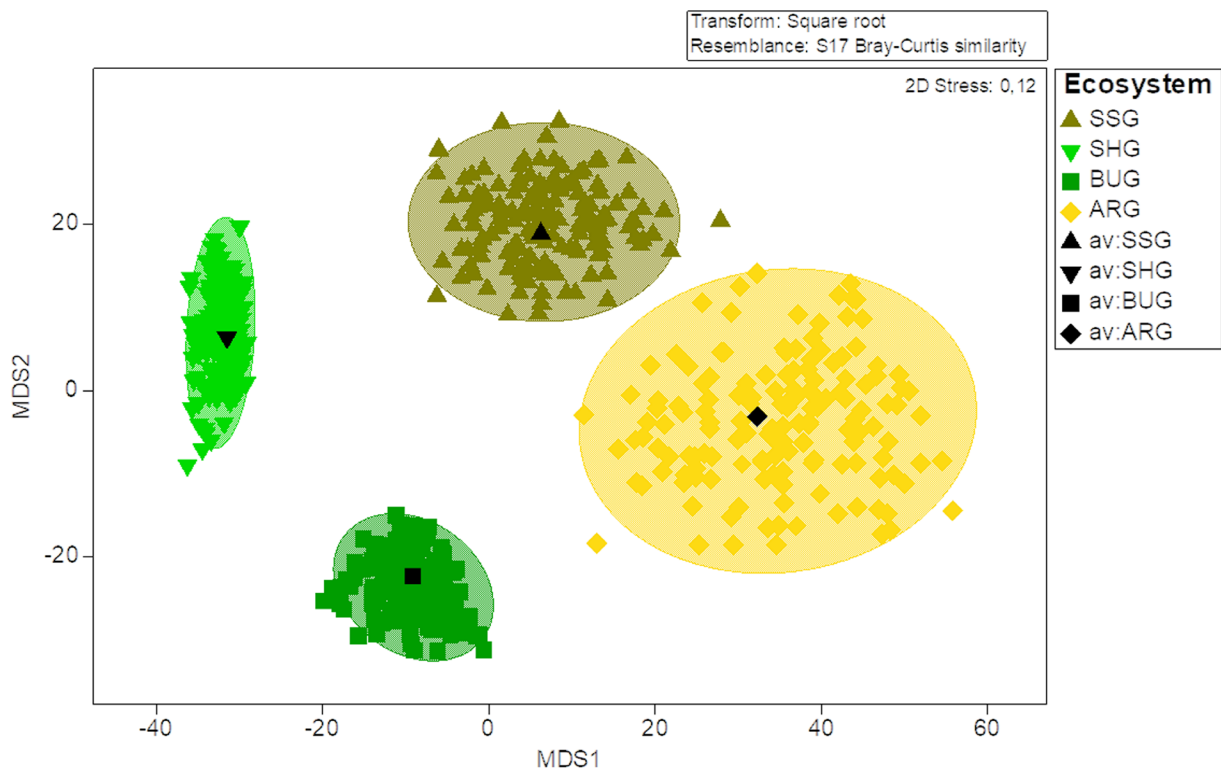
Figure 4. Similarity matrix (Bray–Curtis) plotted on two-dimensional ordination (mMDS) depicting the structure of EPT assemblages in grassland ecosystem type streams (Shallow soils grassland - SSG; Shortgrass grassland - SHG; Bush grassland - BUG; and Aristida grassland - ARG) in the Brazilian Pampa biome. Color-shaded ellipses represent the multivariate standard error (95% of sampling distribution). Black symbols represent the mean values for each ecoregion.

We recorded a clear structuring of EPT assemblages according to grassland ecosystem type. In fact, the approach of subdividing a landscape into different ecological systems based on regional patterns of topography, vegetation, climate and other elements has been widely adopted. In the United States and Australia, ecological systems have been instrumental in shaping water quality monitoring programs using benthic macroinvertebrates (Plafkin et al. 1989, Marchant et al. 2000). Since land features of the landscape are emphasized in general ecological systems, they play an early role in regionalization when it comes to aquatic life, providing a crucial framework for biomonitoring purpose (Marchant et al. 2000). By considering

the factors that influence both land and water, ecological systems allow a more holistic analysis of ecological relationships. Understanding these ecological interactions is essential for the development of adequate strategies for the conservation and management of biodiversity and for the integrity of lotic ecosystems.

The highest EPT abundance recorded in the SHG (51% of total) may be related with a combination of landscape and watershed features. This grassland ecosystem type presents deep and high fertility soil and, availability of gravely substrate of igneous plutonic/metamorphic in the streambed. The vegetation is dominated by herbaceous species, essentially grassy ones, with a rhizomatous

Table III. Most representative EPT genera of assemblage in streams of each grassland ecosystem type in Brazilian Pampa biome. Similarity percentage analysis. Average similarity within samples of each ecoregion, average abundance (AvAbund), average similarity (AvSim.) and standard deviation (SD) per genus, percentage of contribution of the most representative genera (Contrib%), and cumulative contribution (Cum%) in each ecosystem.

SSG	Average similarity			50%	
Genus	AvAbund	AvSim	SD	Contrib%	Cum%
Smicridea	12.12	17.57	2.13	35.43	35.43
Caenis	4.51	6.54	1.15	13.18	48.61
Traverhyphes	5.61	6.16	2.46	12.41	61.02
Americabaetis	4.99	5.96	1.12	12.02	73.03
SHG	Average similarity			70%	
Traverhyphes	15.09	17.44	3.50	26.03	26.03
Americabaetis	8.08	8.78	2.18	13.11	39.14
Itauara	9.55	8.45	2.14	12.61	51.75
Farrodes	6.80	6.78	1.90	10.13	61.88
Chimarra	8.69	6.36	1.13	9.50	71.38
BUG	Average similarity			61%	
Americabaetis	10.01	17.88	2.29	29.35	29.35
Smicridea	5.28	10.17	3.65	16.70	46.05
Tricorythopsis	3.95	7.92	2.73	13.00	59.05
Farrodes	3.86	7.71	2.38	12.66	71.70
ARG	Average similarity			35%	
Caenis	4.48	15.92	1.11	45.94	45.94
Americabaetis	1.91	6.18	1.30	17.83	63.77
Smicridea	4.74	4.76	0.52	13.74	77.51

habit, while others present a tufted habit (Hasenack et al. 2023). According Siegloch et al. (2016), factors that influence the composition of EPT insects in subtropical streams include small changes in riparian forest complexity (e.g. tree and shrub size and top diameter) as well as the composition of inorganic substrate, amount of organic matter, primary production and physicochemical characteristics. These factors influence the availability of food and shelter for organisms, potentially contributing to the dominance of certain genera within these assemblages (Poff et al. 2006, Silva et al. 2014, Brasil et al. 2020a, Luiza-Andrade et al. 2022).

Specific genera of EPT were recovered as dominant for each grassland ecosystem type. A high abundance of the genus *Smicridea* was recorded in SSG, which presents gently undulating slopes and shallow, and stony soils on a basaltic plateau (Hasenack et al. 2023), which result in larger availability of stony

substrate in streambed. Nonetheless, this genus was abundant and widely distributed across all sampled grassland ecosystems. *Smicridea* also showed higher abundance related to samples with higher water temperature and electrical conductivity. Similar relation was demonstrated in Braun et al. (2014). *Smicridea* is a generalist genus, well distributed from headwater to larger rivers, and are typical of stony substrate streams (Flint et al. 1999, Spies et al. 2006, Spies & Froehlich 2009, Salvarrey et al. 2014). Their larvae are collectors/filters, and build shelters from plant fragments and produce capture nets with medium-sized meshes, allowing them to occupy diverse running water habitats (Wiggins & Mackay 1978, Spies et al. 2006). The high abundance at SSG can be attributed to the need of these filter feeders in the thick and stable substrate to fix their nets and shelters (Statzner 2011, Malacarne et al. 2024). Similarly, the relative high abundance of *Chimarra* along grassland
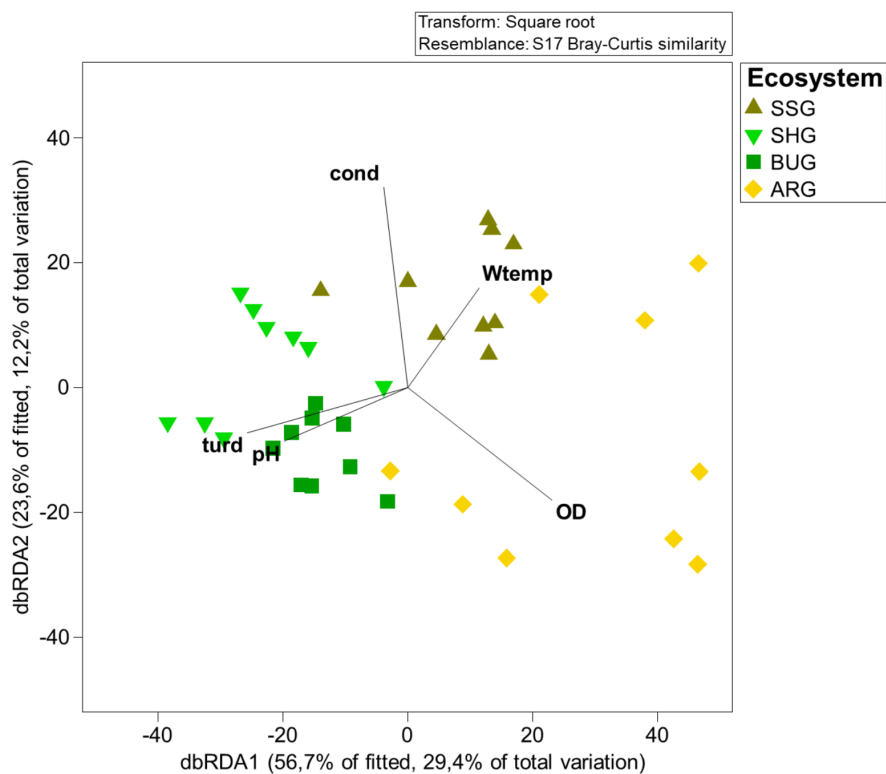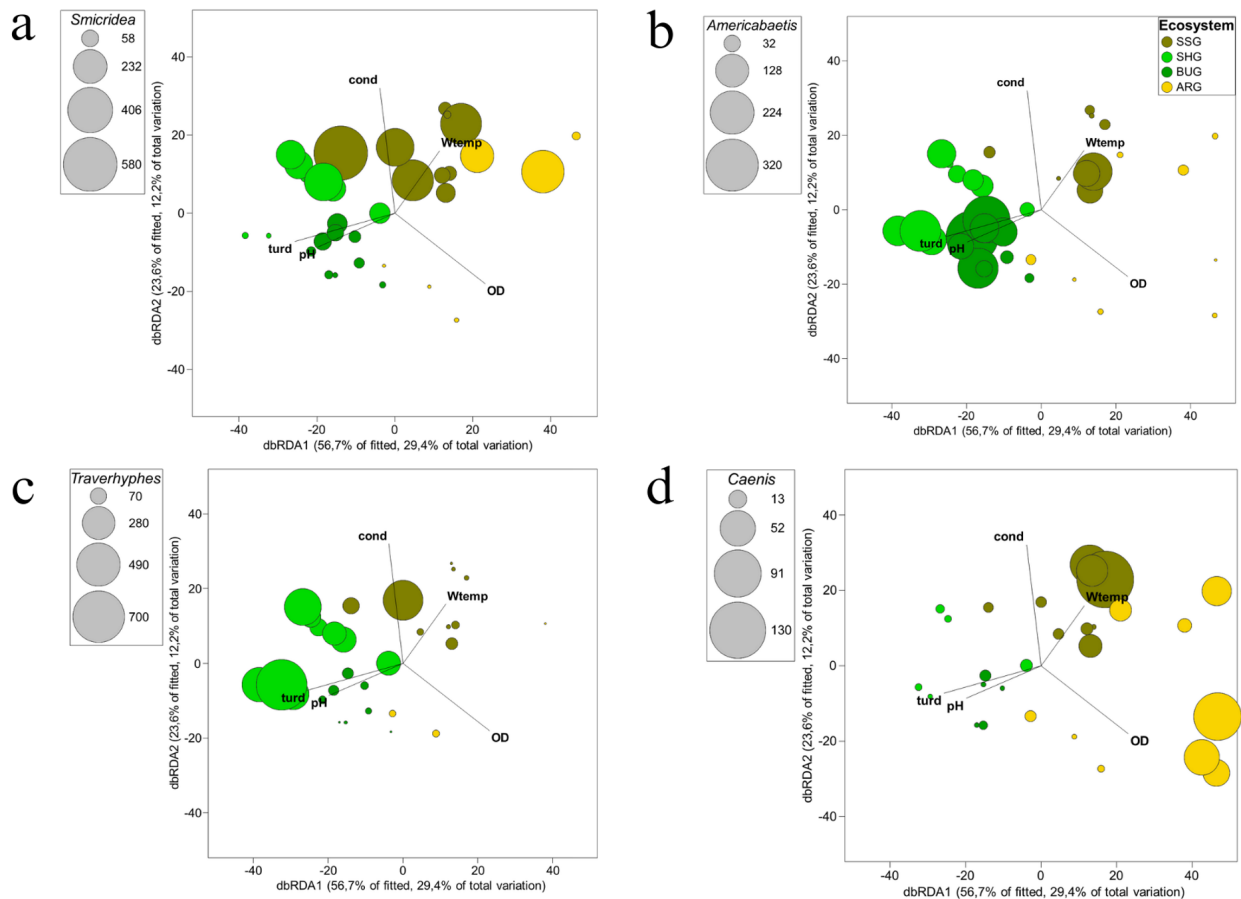

Figure 5. Distance-based redundancy analysis (dbRDA) ordination of local environmental predictors explaining structure of EPT assemblages in grassland ecosystem type streams (Shallow soils grassland - SSG; Shortgrass grassland - SHG; Bush grassland - BUG; and Aristida grassland - ARG) in the Brazilian Pampa biome. Wtemp=water temperature; OD= dissolved oxygen; cond=electrical conductivity; turb=turbidity. The variation accounted and percentage of constrained variation explained by each of the first two dbRDA axes are also shown.

ecosystems could also be related to stability of stony substrate, where their collectors/filters larvae build capture nets with fine-sized meshes (Flint et al. 1999, Merritt et al. 2019).

The genus *Traverhyphe*s was more abundant in SHG streams, this grassland ecosystem type also has gently undulating slopes, along with deep and fertile soils (Hasenack et al. 2023). The streams in this grassland ecosystem type presented high turbidity values. Individuals of this genus have an opercular gill, which allows them to better adapt to environments with sedimentation, such as still water or water with greater turbidity (Espinosa et al. 2023).

This genus is classified as collector-gatherer feeders (Cummins et al. 2005), with the presence of bristles adapted to capture fine sediment particles for feeding. This set of features seems to be a key factor for explaining the abundance of the genus *Traverhyphes* in streams with greater turbidity. The genus *Itauara* was also abundant in the SHG ecosystem. The larvae of this genus are scrapers on periphyton growing over gravel and stony substrates in well sunny are essential food source (Dudgeon et al. 2006, França et al. 2009). High turbidity may be related to the transport of fine particulate organic matter, which contributes to the development

Figure 6. Relationship of the most representative EPT genera of each grassland ecosystem type and local environmental descriptors in the Brazilian Pampa biome. Distance-based redundancy analysis (dbRDA) ordination with bubble overlay of the abundance for EPT genera: a) *Smicridea*, representative of Shallow soils grassland streams; b) *Traverhyphes*, representative of Shortgrass grassland streams; c) *Americabaetis*, representative of Bush grasslands streams; and d) *Caenis*, representative of Aristida grassland.

of periphyton, as well as the transport of algae from it (Allan & Castillo 2007).

Americabaetis presented high representativity of in BUG streams (but also in SHG), and has been associated with higher values of turbidity, pH, and water velocity in the streams in these ecosystems. This group usually have gills and specialized bristles, which allow them to collect fine particles. As a result, the greater flow of water in the rapids carries a greater amount of fine particulate matter, contributing to the establishment of greater richness and abundance of these organisms (Amaral et al. 2019). This genus is widely distributed across habitats, including those impacted by human activities or environmental events (Siegloch et al. 2008). Some species show adaptations to soft-flow aquatic environments and to riparian vegetation (Salles 2006, Siegloch et al. 2014).

Caenis was more representative in ARG streams, but also occurred in high abundance in SSG streams. Abundance of *Caenis* increased with increasing dissolved oxygen and water temperature. This genus is widely found on aquatic habitat and they tolerate variations of water temperature and oxygen levels, and some degree of contamination (Dominguez et al. 2006). Experiments on the effect of the oxygen concentration in water on the survival of a species of *Caenis*, showed decrease in survival at concentrations below 7 mg/L (Puckett & Cook 2004). However, temperature and dissolved oxygen levels can affect several aquatic insects, since warmer waters can potentially accelerate the growth and development of larval stages, enhancing food availability (Gallegos-Sanchez et al. 2022). Meanwhile, high dissolved oxygen levels can improve the metabolic efficiency and reduce stress (Bonacina et al. 2023). The interpretation of particular effects of dissolved oxygen and water temperature on *Caenis* should

be investigated, since responses dependent on species (Bonacina et al. 2023).

A significant amount of multivariate structure of EPT assemblages was related to the water physicochemical descriptors of streams (i.e. the environmental descriptors set, plus the environmental descriptors spatially structured), according to variation partitioning of the most parsimonious model. A high variation explained by spatially structured environmental descriptors was expected due to landscape features that configure grassland ecosystem types as discrete units (i.e. summarized delimitation according to several factors as geology, soil, topography, climate, and vegetation) (Boldrini et al. 2010, Hasenack et al. 2023), which result in high similarity for water physicochemical descriptors of streams within each grassland ecosystem type.

The dbRDA indicated that dissolved oxygen, pH, turbidity, electrical conductivity, and water temperature play an important role in the distribution of EPT genera in the grassland ecosystems of the Brazilian Pampa biome. Electrical conductivity and pH are among the environmental descriptors most related to the distribution of aquatic organisms (Segura et al. 2007, Melo 2009, Braun et al. 2014, Savarrey et al. 2014). Several bicarbonates from carbonate rocks dissolved in weakly acidic water determine the physicochemical characteristics of the water (Allan & Castillo 2007). Fluctuations in electrical conductivity result mainly from the amount of dissolved ions, and it is related to distinct geological characteristics (Allan & Castilho 2007). Higher levels of electrical conductivity exhibited by streams in SHG and SSG, probably were related of geology and soil composition of the watershed (Hutchinson 1957, Melo 2009), since the former present deep soil with high fertility, and the latter, to basaltic shallow soil, both release amounts of ions. SSG streams were

related to a pattern of higher water temperatures associated with lower dissolved oxygen levels. These observations may be interconnected, since higher temperatures can influence the metabolism of aquatic organisms, leading to an increased demand for oxygen. Furthermore, higher temperatures may affect water's ability to efficiently dissolve oxygen (Kleerekoper 1990, Ribeiro et al. 2009). However, other factors can also influence dissolved oxygen levels, such as the amount of decomposing organic matter in the water (Nozaki et al. 2014). Although the relationship between high temperatures and low levels of dissolved oxygen is plausible, the complexity of aquatic systems may imply several interactions.

Overall, our null hypothesis of no difference in the multivariate structure of EPT assemblages was refuted, since we were able to identify that assemblages were structured according to grassland ecosystem type, and that water physicochemical descriptors in streams seem to be regulated by idiosyncratic landscape attributes. The spatially-structured explanatory variables detected here indicate an 'induced spatial dependence phenomenon' (sensu Legendre & Legendre 2012) as the main driver of variation in the studied EPT assemblages. Hence, our findings support the environmental control model predicted by the niche-based processes structuring assemblages (Hutchinson 1957, Legendre & Legendre 2012). Moreover, we reinforce the inadequacy of treating the Pampa as a homogeneous landscape. Recognizing and understanding the multiple scales involved in this biome will lead a better comprehension of ecological relationships. By considering the importance of regionalization and the interaction of local and regional descriptors, we can develop more efficient approaches to preserve and manage biodiversity and ecological integrity. Thus, it is essential to prioritize future studies that include more comprehensive inventories of the diversity and biological distribution of animal and plant species, as well as interactions among them.

REFERENCES

ALVARES CA, STAPE JL, SENTELHAS PC, GONÇALVES JLM & SPAROVEK G. 2014. Köppen's climate classification map for Brazil. Meteorol Zeitschrif 22: 711-728.

ALLAN JD & CASTILLO MM. 2007. Stream Ecology: Structure and function of running waters. Springer, The Netherlands, 436 p.

ANDERSON MJ. 2017. Permutational Multivariate Analysis of Variance (PERMANOVA). In: Balakrishnan N et al. (Eds), Wiley StatsRef: Statistics Reference Online. Wiley, Hoboken, p. 1-15.

ANDERSON MJ, GORLEY RN & CLARKE KR. 2008. Permanova+ for Prime: Guide software and Statical methods. Plymouth: Primer-e, 214p.

ANDRADE BO ET AL. 2019. Classification of South Brazilian grasslands: Implications for conservation Jansen F (Ed). Appl Veg Sci 22: 168-184.

AMARAL PHM, GONÇALVES EA, SILVEIRA LS & ALVES RG. 2019. Richness and distribution of Ephemeroptera, Plecoptera and Trichoptera in Atlantic forest streams. Acta Oecol 99: 103-441.

BAIN MB & STEVENSON NJ. 1999. Aquatic habitat assessment: Common methods. Bethesda, Maryland: American Fisheries Society, 136 p.

BAPTISTA DF, BUSS DF, DORVILLÉ LFM & NESSIMIAN JL. 2001. Diversity and habitat preference of aquatic insects along the longitudinal gradient of the Macaé River Basin, Rio de Janeiro, Brazil. Rev Bras Biol 61: 249-258.

BOLDRINI II. 2009. A flora dos campos do Rio Grande do Sul. In: Pillar VDP (Ed), Campos Sulinos: conservação e uso sustentável da biodiversidade. Brasília: Ministério do Meio Ambiente, p. 63-77.

BOLDRINI II, FERREIRA PPA, ANDRADE BO, SCHNEIDER AA, SETUBAL RB, TREVISAN R & FREITAS EM. 2010. Bioma Pampa: diversidade florística e fisionômica. Porto Alegre, Editora Pallotti, 64 p.

BONACINA L, FASANO F, MEZZANOTTE V & FORNAROLI R. 2023. Effects of water temperature on freshwater macroinvertebrates: a systematic review. Biol Rev 98: 191-221.

BONADA, N, PRAT, N, RESH, VH & STATZNER B. 2006. Developments in aquatic insect biomonitoring: A comparative analysis of recent approaches. Annu Rev Entomol 51: 495-523.

BRASIL LS, LUIZA-ANDRADE A, CALVÃO LB, DIAS-SILVA K, FARIA APJ, SHIMANO Y, OLIVEIRA-JUNIOR JMB, CARDOSO MN & JUEN L. 2020a. Aquatic insects and their environmental predictors: a scientometric study focused on environmental monitoring in lotic environmental. Environ Monit Assess 192: 1-10.

BRASIL LS, DE LIMA EL, SPIGOLONI ZA, RIBEIRO-BRASIL DRG & JUEN L. 2020b. The habitat integrity index and aquatic insect communities in tropical streams: A meta-analysis. Ecol Ind. 116: 106-495.

BRAUN BM, PIRES MM, KOTZIAN C B & SPIES MR. 2014. Diversity and ecological aspects of aquatic insect communities from montane streams in southern Brazil. Acta Limnol Bras 26: 186-198.

BRAUN BM, BERTASO TRN, PIRES MM, SPIES MR & KOTZIAN CB. 2018. Responses of riffle beetle assemblages to deforestation in a semi-deciduous Atlantic Forest remnant. An Acad Bras Cienc 90: 2189-2201. http://dx.doi.org/10.1590/0001-3765201820160853.

BUSS DF, BAPTISTA DF, NESSIMIAN JL & ENGLER M. 2004. Substrate specificity, environmental degradation and disturbance structuring macroinvertebrate assemblages in Neotropical streams. Hydrobiologia 518: 179-188.

CLARKE KR & GORLEY RN. 2015. PRIMER v7: user manual/tutorial, 3rd ed., Primer-E Ltd, Plymouth, 296 p.

CHEBATAROFF J. 1968. Estepes, pradarias e savanas da América do Sul. Bol Geogr 27: 3-17.

CORTEZZI SS, BISPO PC, PACIENCIA GP & LEITE RC. 2009. Influência da ação antrópica sobre a fauna de macroinvertebrados aquáticos em riachos de uma região de cerrado do sudoeste do Estado de São Paulo. Iheringia Sér Zool 99: 36-43.

CRISCI-BISPO VL, BISPO PC & FROEHLICH CG. 2007. Ephemeroptera, Plecoptera e Trichoptera assemblages in two Atlantic rainforest streams, Southeastern Brazil. Rev Bras Zool 24: 312-318.

CUMMINS KW, MERRITT RW & ANDRADE PC. The use of invertebrate functional groups to characterize ecosystem attributes in selected streams and rivers in southeast Brazil. Stud Neotrop Fauna Environ 40: 71-90.

DOMÍNGUEZ E, MOLINERI C, PESCADOR M, HUBBARD M & NIETO C. 2006. Aquatic Biodiversity in Latin America: Ephemeroptera of South America. PENSOF, Sofia, Moscow 2: 1-646.

DORMANN CF ET AL. 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography 36: 027-046.

DRAY S, LEGENDRE P & PERES-NETO PR. 2006. Spatial modelling: a comprehensive framework or principal coordinate analysis of neighbour matrices (PCNM). Ecol Model 196: 483-93.

DUDGEON D ET AL. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol Rev 81: 163-182.

ERIKSEN TE, BRITTAIN JE, SOLI G, JACOBSEN D, GOETHALS P & FRIBERG N. 2021. A global perspective on the application of riverine macroinvertebrates as biological indicators in Africa, South-Central America, Mexico and Southern Asia. Ecol Ind 126: 107-609.

ESPINOSA ACE, CUNHA EJ, SHIMANO Y, ROLIM S, MIOLI L, JUEN L & DUNCK B. 2023. Functional diversity of mayflies (Ephemeroptera, Insecta) in streams in mining areas located in the Eastern Amazon. Hydrobiologia 850: 929-945.

FLINT OSJR, HOLZENTHAL RW & HARRIS SC. 1999. Catalog of the Neotropical Caddisflies (Insecta: Trichoptera). Columbus. Ohio Biol Surv, 239 p.

FRANÇA JS, GREGÓRIO RS, PAULA JD, GONÇALVES-JÚNIOR JF, FERREIRA FA & CALLISTO M. 2009. Composition and dynamics of allochthonous organic matter inputs and benthic stock in a Brazilian stream. Mar Freshw Res 60: 990-998.

GALLEGOS-SÁNCHEZ S, DOMÍNGUEZ E, ENCALADA AC & RÍOS-TOUMA B. 2022. Effects of experimental warming on two tropical Andean aquatic insects PLoS ONE 17(7): e0271256.

GARTNER AF, TUYA PS, LAVERY & MCMAHON K. 2013. Habitat preferences of macroinvertebrate fauna among seagrasses with varying structural forms. J Exp Mar Biol Ecol 439: 143-151.

HAWKINS CP & NORRIS RH. 2000. Performance of different landscape classifications for aquatic bioassessments: introduction to the series. J N Am Benthol Soc 19: 367-369.

HAWKINS CP, NORRIS RH, GERRITSEN J, HUGHES RM, JACKSON SK, JOHNSON RK & STEVENSON RJ. 2000. Evaluation of the use of landscape classifications for the prediction of freshwater biota: synthesis and recommendations. J N Am Benthol Soc 19: 541-556.

HASENACK HH, WEBER EJ, BOLDRINI II, TREVISAN R, FLORES CA & DEWES H. 2023. Delimitação biofísica de sistemas ecológicos campestres no Estado do Rio Grande do Sul, sul do Brasil. Iheringia Sér Bot 78: e2023001.

HYNES HBN. 1975. The stream and its valley. Verh Int Ver Theor Angew Limnol 19: 1-15.

HIRZEL AH, HAUSSER J, CHESSEL D & PERRIN N. 2002. Ecological-niche factor analysis: How to compute habitat-suitability without absence data? Ecol. 83: 2027-2036.

HUTCHINSON GE. 1957. A treatise on Limnology. John Wiley & Sons, New York, 1115 p.

IBGE. 2019. Biomas e sistema costeiro-marinho do Brasil: compatível com a escala 1:250.000. Coordenação de Recursos Naturais e Estudos Ambientais, Rio de Janeiro: IBGE.

KLEEREKOPER H. 1990. Introdução ao estudo da limnologia. 2ª ed. Porto Alegre, Editora da Universidade/UFRGS, 330 p.

KUPLICH TM, CAPOANE V & COSTA LFF. 2018. O avanço da soja no bioma Pampa. Bol Geogr Rio Gd Sul 31: 83-100.

LARANJEIRAS TO, MOURA NG, VIEIRA CG, ANGELILI R & CARVALHO AR. 2012. Bird communities in different phytophysiognomies of the Cerrado biome. Stud Neotrop Fauna Environ 47: 41-51.

LECCI LS & FROEHLICH CG. 2011. Ordem Plecoptera Burmeister 1839 (Arthropoda: Insecta). Identificação de larvas de Insetos Aquáticos do Estado de São Paulo. Disponível em: http://sites. ffclrp.usp.br/aguadoce/guiaonline.

LEGENDRE P & ANDERSON MJ. 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecol. Monogr. 69: 1-24.

LEGENDRE P & LEGENDRE L. 2012. Numerical Ecology. Elsevier, 3rd Edition, 1623 p.

LINDMAN CAM. 1974. A vegetação no Rio Grande do Sul. Itatiaia/ USP, Belo Horizonte/São Paulo, 377p.

LUIZA-ANDRADE A, BRASIL LS, BENONE NL, SHIMANO Y, FARIAS APJ, MONTAG LF, DOLÉDEC S & JUEN L. 2017. Influence of oil palm monoculture on the taxonomic and Functional composition of aquatic insect communities in eastern Brazilian Amazonia. Ecol Ind 82: 478-483.

LUIZA-ANDRADE A, SILVA RR, SHIMANO Y, FARIA APJ, CARDOSO MN, BRASIL LS, LIGEIRO R, MARTINS RT, HAMADA N & JUEN L. 2022. Niche breadth and habitat preference of Ephemeroptera, Plecoptera, and Trichoptera (Insecta) in streams in the Brazilian Amazon. Hydrobiologia 849: 4287-4306.

MALACARNE TJ, MACHADO NR & MORETTO Y. 2024. Influence of land use on the structure and functional diversity of aquatic insects in neotropical streams. Hydrobiol 851(2): 265-280.

MALUF JRT. 2000. Nova classificação climática do Estado do Rio Grande do Sul. Rev Bras Agromet 8: 141-150.

MARCHANT RA, HIRST RH, NORRIS & METZELING L. 1999. Classification of macroinvertebrate communities across drainage basins in Victoria, Australia: consequences of sampling on a broad spatial scale for predictive modelling. Freshw Biol 41: 253-268.

MARCHANT R, WELLS F & NEWALL P. 2000. Assessment of an ecoregion approach for classifying macroinvertebrate assemblages from streams in Victoria, Australia. J N Am Benthol Soc 19: 497-500.

MELO AS. 2009. Explaining dissimilarities in macroinvertebrate assemblages among stream sites using environmental variables. Zool 26(1): 79-84.

MERRIT RW, CUMMINS MBB & BERG MB. 2019 An Introduction to the Aquatic Insects of North America. 5th ed. Kendall: Hunt Publishing Company, USA, 1480 p.

NEWALL P & WELLS F. 2000. Potential for delineating indicator-defined regions for streams in Victoria, Australia. J N Am Benthol Soc 19: 557-571.

NOZAKI CT, MARCONDES MA, LOPES FA, SANTOS KF & LARIZZATTI PSC. 2014. Comportamento temporal do oxigênio dissolvido e pH nos rios e córregos urbanos. Atlas Saúde Ambient 2: 29-44.

OVERBECK GE, MÜLLER SC, FIDELIS A, PFADENHAUER J, PILLAR VD, BLANCO, CC, BOLDRINI II, BOTH R & FORNECK ED. 2007. Brazil's neglected biome: the South Brazilian Campos. Perspectives in Plant Ecology. Evol and Syst 9: 101-116.

OVERBECK GE, BOLDRINI II, BARROTTO MRC, GARCIA EN, MORO RS, PINTO, CE, TREVISAN R & ZANIN A. 2015. Fisionomia dos

campos. In: Pillar VP & Lange O (Eds), Os Campos do Sul. Porto Alegre: Rede Campos Sulinos – UFRGS, 192 p.

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.

PES AMO, HOLZENTHAL RW, SGANGA JV, SANTOS APM, BARCELOS-SILVA P & CAMARGOS LM. 2018. Order Trichoptera. In: Hamada N, Thorp JH & Rogers DC (Eds), Keys to Neotropical Hexapoda, Thorp and Covich's Freshwater Invertebrates. Academic Press, Elsevier, London 3, p. 237-324.

PLAFKIN JL, BARBOUR MT, PORTER KD, GROSS SK & HUGHES RM. 1989. Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish. EPA/444/4-89-001. Office of Water, Washington, DC. US Environ Prot Agency.

POFF NL, OLDEN JD, VIEIRA NK & FINN DS, FINNSIMMONS MP & KONDRATIEFF BC. 2006. Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. J N Am Benthol Soc 25: 730-755.

PUCKETT RT & COOK JL. 2004. Physiological tolerance ranges of larval *Caenis latipennis* (Ephemeroptera: Caenidae) in response to fluctuations in dissolved oxygen concentration, pH and temperature. Tex J Sci 56: 123-130.

RAMBO B. 1956. A fisionomia do Rio Grande do Sul: ensaio de monografia natural. Selbach, Porto Alegre, 471 p.

RANGEL TFLVB, DINIZ-FILHO JAF & BINI LM. 2006. Towards an integrated computational tool for spatial analysis in macroecology and biogeography. Global Ecol Biogeogr 15: 321-327.

RENNER S, PÉRICO E, DALZOCHIO MS & SAHLÉN G. 2019. Ecoregions within the Brazilian Pampa biome reflected in Odonata species assemblies. Austral Ecol 44: 461-472.

RIBEIRO LO, KONIG R, FLORES EMM & SANTOS S. 2009. Composição e distribuição de insetos aquáticos no rio Vacacaí-Mirim, Santa Maria, Rio Grande do Sul. Ciênc Nat 31: 79-93.

RICKLEFS RE & SCHLUTER D. 1993. Species diversity: regional and historical influences. Species diversity in ecological communities. Oxford University Press, Oxford 1: 350-363.

ROSENBERG DM & RESH VH. 1993. Introduction to freshwater biomonitoring and benthic macroinvertebrates. In: Rosenberg DM & Resh VH (Eds), Freshwater biomonitoring and benthic macroinvertebrates. New York, Chapman & Hall, p. 1-9.

ROESCH LF, VIEIRA F, PEREIRA V, SCHÜNEMANN AL, TEIXEIRA I, SENNA AJ & STEFENON VM. 2009. The Brazilian Pampa: a fragile biome. Diversity 1: 182-198.

SACCOL SSA, UCHA JLCD, MADALOZZO B, CECHIN SZ & SANTOS TG. 2022. Influence of land use on the diversity of pond-breeding anurans in South Brazilian grasslands. Biodivers Conserv 31: 21-37.

SALVARREY AVB, KOTZIAN CB, SPIES MR & BRAUN B. 2014. The influence of natural and anthropic environmental variables on the structure and spatial distribution along longitudinal gradient of macroinvertebrate communities in southern Brazilian streams. J Insect Sci 14: 1-23.

SALLES FF. 2006. The order Ephemeroptera (Insecta) in Brazil: Taxonomy and diversity. 313 f. Tese (Doutorado em Ciência entomológica; Tecnologia entomológica) - Universidade Federal de Viçosa, Viçosa.

SALLES FF, DOMÍNGUEZ E, MOLINERI C, BOLDRINI R, NIETO C & DIAS LG. 2018. Order Ephemeroptera. In: Hamada N, Thorp JH & Rogers DC (Eds), Keys to Neotropical Hexapoda, Thorp and Covich's Freshwater Invertebrates. Academic Press, p. 61-117.

SEGURA MO, FONSECA-GESSNER AA & TANAKA MO. 2007. Composition and distribution of aquatic Coleoptera (Insecta) in low-ordem streams in the state of São Paulo, Brazil. Acta Limnol Bras 19: 247-255.

SIEGLOCH AE, FROEHLICH CG & KOTZIAN CB. 2008. Composition and diversity of Ephemeroptera (Insecta) nymph communities in the middle section of the Jacuí River and some tributaries, southern Brazil. Iheringia, Sér Zool 98: 425-432.

SIEGLOCH AE, SURIANO M, SPIES M & FONSECA-GESSNER A. 2014. Effect of land use on mayfly assemblages structure in Neotropical headwater streams. An Acad Bras Cienc 86: 1735-1747. https://doi.org/10.1590/0001-3765201420130516.

SIEGLOCH AE, SCHMITT R, SPIES M, PETRUCIO M & HERNÁNDEZ MIM. 2016. Effects of small changes in riparian forest complexity on aquatic insect bioindicators in Brazilian subtropical streams. Mar Freshw Res 68: 519-527.

SILVA DRO, LIGEIRO R, HUGHES RM & CALLISTO M. 2014. Visually determined stream mesohabitats influence benthic macroinvertebrate assessments in headwater streams. Environ Monit Assess 186: 5479-5488.

SORIANO A ET AL. 1992. Río de la Plata grasslands. In: Coupland RT & Goodall DW (Eds), Ecosystems of the world. Natural grasslands. Introduction and Western Hemisphere. Elsevier, Amsterdam, p. 367-407.

SOUZA FN, MARIANO R, MOREIRA T & CAMPIOLO S. 2020. Influence of the landscape in different scales on the

EPT community (Ephemeroptera, Plecoptera and Trichoptera) in an Atlantic Forest region. Environ Monit Assess 192: 1-12.

SPIES MR, FROEHLICH CG & KOTZIAN CB. 2006. Composition and diversity of Trichoptera (Insecta) larvae communities in the middle section of the Jacuí River and some tributaries, State of Rio Grande do Sul, Brazil. Iheringia Sér Zool 96: 389-398.

SPIES MR & FROEHLICH CG. 2009. Inventory of caddisflies (Trichoptera: Insecta) of the Campos do Jordão State Park, São Paulo state, Brazil. Biot Neotropic 9: 1-8.

STATZNER B. 2011. Mineral grains in caddisfly pupal cases and streambed sediments: assessing resource use and its limitation across various river types. In: Annales de limnologie international journal of limnology. EDP Sciences, p.103-118.

STRAHLER HN. 1957. Quantitative analysis of watershed geomorphology. Trans Amer Geophys Union 38: 913-920.

WIGGINS GB & MACKAY RJ. 1978. Some relationships between systematics and trophic ecology in Neartic aquatic insects, with special reference to Trichoptera. Ecol 59: 1211-1220.

WIGGINS GB. 1996. Larvae of the North American Caddisfly Genera (Trichoptera). Toronto, Univesity of Toronto Press, 457 p.

WREGE MS, STEINMETZ S, REISSER JR C & DE ALMEIDA IR. 2011. Atlas climático da região Sul do Brasil: estados do Paraná, Santa Catarina e Rio Grande do Sul. Embrapa Clima Temperado; Colombo: Embrapa Florestas, Pelotas, 333 p.

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