

Land-use effects on aquatic macroinvertebrate diversity in subtropical highland grasslands streams

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ABSTRACT

Land-use effects on aquatic macroinvertebrate diversity in subtropical highland grasslands streams

Knowledge of the frequency and occurrence of macroinvertebrates throughout landscapes may clarify the effects of anthropic impacts on aquatic systems and help guide conservation actions for watersheds. We evaluated macroinvertebrate α and β diversity in streams in four different subtropical phytophysiognomies: highland grasslands with arboreal riparian vegetation, highland grasslands without arboreal riparian vegetation, mixed ombrophilous forest and silviculture. We also evaluated how environmental factors influence α diversity. We sampled macroinvertebrate communities (by Surber sampler), litter input (by nets) and physicochemical variables of the water (by multiparameter and chemical analysis) at 10 sites in riparian zones of a highland system. A total of 2124 individuals were sampled representing 41 *taxa*, with high α and β diversity in mixed ombrophilous forest. The results indicate that environmental heterogeneity increases food resource availability, as well as macroinvertebrate diversity. Furthermore, the β diversity was found to increase with distance among the unconnected streams. Areas of silviculture had the highest density values among the sampled areas due high organic material stock in the soil. Therefore, higher leaf litter input may decrease the toxicity effect of secondary compounds released by leaf plant decomposition compared to environments with low systemic leaf litter input, such as highland grasslands. The presence of arboreal riparian vegetation drives canopy openness, water temperature, dissolved oxygen, and orthophosphate in streams, which control the frequency and occurrence of benthic macroinvertebrates. Homogeneous systems, as naturally observed in highland grasslands, plus high canopy openness decrease macroinvertebrate richness and density. The naturally low richness of highland grasslands, although with endemic species, may be an important guideline for legislation on anthropic impacts, management, and conservation of streams in this specific subtropical zone.

Key words: grasses, *Pinus*, landscape mosaic, watershed

RESUMO

Efeito do uso da terra na diversidade de macroinvertebrados aquáticos em riachos de campos de altitude subtropical

Conhecer a frequência e ocorrência de macroinvertebrados nas paisagens pode esclarecer os efeitos dos impactos antrópicos nos sistemas aquáticos e orientar ações de conservação de bacias hidrográficas. Avaliamos a diversidade de macroinvertebrados α e β em riachos de quatro diferentes fitofisionomias subtropicais: campos com mata riparia arbórea, campos sem mata riparia arbórea, floresta ombrófila mista e silvicultura. Também avaliamos como os fatores ambientais influenciam a diversidade

α. Amostramos comunidades de macroinvertebrados (por amostrador Surber), entrada de serapilheira (por redes) e variáveis físico-químicas da água (por análise multiparamétrica e química) em 10 locais em zonas ribeirinhas de um sistema de terras altas. Um total de 2124 indivíduos foram coletados, divididos entre 41 táxons, apresentando maior diversidade α e β em áreas da floresta ombrófila mista, pela maior heterogeneidade ambiental e disponibilidade de recursos alimentares. Os resultados indicam que a heterogeneidade ambiental aumenta a disponibilidade de recursos alimentares, bem como a diversidade de macroinvertebrados. Além disso, a diversidade β aumentou com a distância entre os córregos não conectados. As áreas de silvicultura apresentaram os maiores valores de densidade entre as áreas amostradas devido ao alto estoque de matéria orgânica no solo. Portanto, maior aporte de serapilheira pode diminuir o efeito de toxicidade de compostos secundários liberados pela decomposição de detritos foliares, em comparação com ambientes com baixo aporte de serapilheira no sistema, como campos de altitude. A presença de vegetação ciliar arbórea determina a abertura do dossel, temperatura da água, oxigênio dissolvido e ortofosfato nos riachos, que controlam a frequência e ocorrência de macroinvertebrados bentônicos. Sistemas homogêneos, como naturalmente observados em campos de altitude, somados a alta abertura do dossel diminuem a riqueza e densidade de macroinvertebrados. A riqueza naturalmente baixa de campos de altitude, embora com espécies endêmicas, pode ser uma importante diretriz para a legislação sobre impactos antrópicos, manejo e conservação de riachos nesta zona subtropical específica.

Palavras chave: gramíneas; Pinus; mosaico paisagístico; bacia hidrográfica

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INTRODUCTION

Human activities have negative impacts on inland aquatic systems (Allan, 2004; Rezende et al., 2014b). Some land uses [e.g., native vegetation replacement with pastures, agriculture or urbanization (Firmiano et al., 2021; Jankowski et al., 2021; Rezende et al., 2021)] may decrease stream bed stability (Allan, 2004; Shanafield et al., 2021) and alter both the organic matter dynamic (Burwood et al., 2021; Rezende et al., 2021) and water physicochemical characteristics [e.g., dissolved nutrient input, pH, electrical conductivity, and dissolved oxygen (Chen et al., 2021; Martins et al., 2021; Tonin et al., 2021)]. Anthropogenic land covers may also favor the best competitor, thereby increasing the abundance of a few organisms (Allan, 2004; Shanafield et al., 2021). Therefore, land covers resulting from different uses may affect stream habitats differently, altering the diversity and functioning of the ecosystem and its organisms (Kahirun et al., 2019; Jankowski et al., 2021). Among aquatic organisms that are sensitive to land use changes, macroinvertebrates are perhaps the main indicators of freshwater ecosystem health (Kahirun et al., 2019; Firmiano et al., 2021). In this way, benthic macroinvertebrate communities are central components of freshwater ecosystems and so the analysis of their diver-

sity patterns is an important tool for understanding and conserving aquatic systems (Heino et al., 2015b, 2018).

There are numerous frameworks for investigating biodiversity patterns in ecological studies, such as the use of traditional diversity indices (e.g., Shannon-Wiener, and Simpson) and the partitioning of diversity into alpha (α) and beta (β) components (Magurran, 2001). The latter stands out among the other indices because it is considered easy to apply and comprehend (Anderson et al., 2011; Magurran, 2001), in addition to being considered an efficient metric for detecting impacts of human driven environmental changes (Al-Shami et al., 2013; Wang et al., 2021). The α diversity component (or local diversity) represents the total number of *taxa* of a community, also represented by richness and diversity indices (Magurran, 2001). The β diversity component shows variation in *taxa* composition among sites in the geographic area of interest, describing *taxa* turnover, nestedness, and the ratio between total and mean of number of *taxa* per sample (Anderson et al., 2011; Baselga, 2012; Magurran, 2001; Whittaker, 1960). Finally, the gamma (γ) diversity component describes overall *taxa* diversity in the geographic area of interest (Whittaker, 1960, 1972). Due to the well-known strong influence that environmental characteristics and/or spatial

scale have on aquatic macroinvertebrate communities in stream systems (Clarke et al., 2008; Firmiano et al., 2021; Linares et al., 2021), special attention should be given to α and β diversities (Doretto et al., 2021; Mendes et al., 2021; Rezende et al., 2019a).

α and β diversities may reflect deterministic processes, such as species adaptation to different niches and dispersal capacity (Heino et al., 2013, 2015a). Considering these processes, three patterns of β diversity may be expected: i) uniformity of species composition over large areas (null model); ii) species demographically and competitively equal; and iii) fluctuating at random with species distributions being related to environmental conditions (niche model; Legendre et al., 2005). Several studies on the diversity of aquatic macroinvertebrates corroborate the high importance of environmental characteristics at the local scale (Doretto et al., 2021; Heino et al., 2015a; Rezende et al., 2019a; Mendes et al., 2021). Tropical and subtropical streams run through highly heterogeneous environments, including a vegetation gradient from grasslands to rainforests and elevation ranges from highlands to lowlands (Allan, 2004; Rezende et al., 2021). Among these environments, highland grasslands of subtropical South America harbor high endemism (Barros et al., 2015). However, despite their high endemism, highland grasslands are threatened by high levels of habitat fragmentation and loss in southern Brazil (Barros et al., 2015; Iganci et al., 2011), where more than 50 % of natural grasslands have already been converted into distinct human land uses over last two decades (Magalhaes et al., 2016; Galetti et al., 2020). In this way, examination of the patterns of aquatic macroinvertebrate community composition in relation to environmental conditions and dispersal capacity by means of α and β diversities may be an important tool for evaluating the impacts of anthropogenic land uses on subtropical highland grasslands and for proposing conservation plans (Doretto et al., 2021; Mendes et al., 2021).

Overall, macroinvertebrates are a well-studied group in subtropical streams, with the diversity of the group being (i) negatively affected by anthropogenic land use (Barbola et al., 2011; Galetti et al., 2020; Tonello et al., 2021); (ii) pos-

itively affected by oxygen concentration and high granulometry heterogeneity (Baptista et al., 2014); (iii) influenced by inter-annual temporal factors (Hepp et al., 2021); (iv) negatively affected by the input of leaf litter chemical compounds resulting from anthropogenic land uses (Loureiro et al., 2018, 2021); and (v) highly similar between neighboring areas (i.e., local scale; Hepp & Melo, 2013; Hepp et al., 2021). It is noteworthy that most of these studies focused on forest streams and only a few studied the macroinvertebrate fauna of subtropical grasslands (Baptista et al., 2014; Hepp & Melo, 2013; Hepp et al., 2021; Loureiro et al., 2018, 2021; Tonello et al., 2021), few of which were in highland grasslands (Galetti et al., 2020; Márquez et al., 2015; Montilla et al., 2022; Principe et al., 2015). Similarly, subtropical highland grasslands have been understudied compared to tropical highland grasslands (Callisto et al., 2016, 2021; Castro et al., 2019). In general, areas with highly connected environments tend to have similar physical-chemical habitat characteristics, resulting in the same specific macroinvertebrate composition (Rezende et al., 2014b; Hepp et al., 2021). Furthermore, landscapes with low permeability and a disconnected matrix tend to present high dissimilarity in macroinvertebrate composition, thus increasing β diversity (Rezende et al., 2019a). Therefore, fragmented landscapes tend to select specialized organisms, decreasing α diversity and inflating β diversity (Heino et al., 2018; Firmiano et al., 2021).

Therefore, our objectives were to (i) evaluate α and β diversities of benthic macroinvertebrate communities regarding different land covers (grasslands with arboreal riparian vegetation, grasslands without arboreal riparian vegetation, mixed ombrophilous forest and silviculture) and (ii) evaluate how the environment influences on benthic macroinvertebrate communities in highland areas. We based our hypotheses on the assumptions that (i) resource availability (habitat and food) is greater in natural riparian vegetation compared to the other land covers; (ii) anthropogenic vegetation (silviculture) will favor the best competitor, thereby increasing the abundance of a few organisms; and (iii) the disconnectedness of areas (different micro-basins) determines the

similarity of environmental filters. Our hypotheses are that (i) macroinvertebrate α (richness) and β diversities will be greater in streams in natural forests (mixed ombrophilous forest) compared to the other vegetation types, and greater with the presence of arboreal riparian vegetation compared to areas in which it is absent; and that (ii) *Pinus* silviculture will increase macroinvertebrate density, but decrease macroinvertebrate α (richness) and β diversities, compared to the other land covers.

MATERIALS AND METHODS

Study area

We selected 10 streams (hereafter also referred to as sampling sites) in riparian zones with different land covers distributed along the Wildlife Refuge of Campos de Palmas (17 ha; between 26° 33' 19.07" and 26° 20' 24.82" S and 51° 20' 64" and 51° 43' 8.82" W), in South Brazil (Fig. 1). The study area is part of the Atlantic Forest biome, and its vegetation is composed of *sensu stricto* steppe

(clean field), hygrophilous steppe (wet field), and mixed ombrophilous forest. The arboreal riparian zones of the region possess a dominance of *Psychotria carthagenensis* Jacq., *Daphnopsis fasciculata* (Meisn.), and *Vernonia discolor* (Spreng.) Less. in grasslands with arboreal riparian vegetation systems, as well as high occurrence of *Araucaria angustifolia* ((Bertol.) Kuntze, 1898), *Ilex paraguariensis* A. St. Hil., *Psidium cattleianum* Sabine, *Ocotea porosa* (Nees & Mart.) Barroso, and *Inga uruguensis* Hook. & Arn. in mixed ombrophilous forest systems (ICMBio, 2013).

The climate of the study area is Cfa (c.f. Köppen, Humid subtropical), described as temperate subtropical. The altitude varies between 950 m and 1370 m above sea level. Rainfall and air temperature data were obtained from a meteorological station (number 265 1035) of the National Agency of Waters of Brazil, located at 26° 21' 58.3" S and 51° 51' 58.2" W (available at <http://hidroweb.ana.gov.br>). The mean annual air temperature is 16 °C with mean monthly temperatures ranging from 5 to 27 °C. The mean monthly precipitation throughout the year is

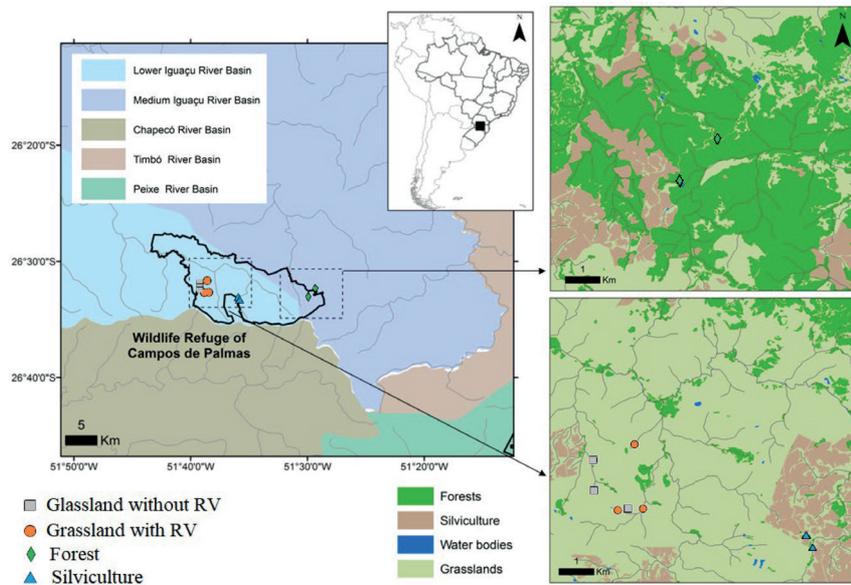


Figure 1. Geographic location of sampling sites with different land covers (mixed ombrophilous forest, silviculture, grassland with arboreal riparian vegetation and grassland without arboreal riparian vegetation - RV) within the Wildlife Refuge of Campos de Palmas, South Brazil. *Localização geográfica dos locais de amostragem com diferentes coberturas do solo (Mata nativa, Silvicultura e Campos com e sem mata ciliar arbórea - RV) no Refúgio de Vida Silvestre de Campos de Palmas, Sul do Brasil.*

142 mm, ranging from 105 to 182 mm, with a yearly total of 1700 mm.

The study was conducted during the spring season, from September to November 2018. The spring is a period during which temperature and rainfall are close to annual averages, with lower rainfall compared to summer, but higher temperatures compared to winter and autumn. Four types of land cover (vegetation) were selected: grassland with arboreal riparian vegetation (hygrophilous steppe; three streams); grassland without arboreal riparian vegetation (hygrophilous steppe; three streams); forest (mixed ombrophilous forest; two streams); and silviculture (*Pinus* sp. monoculture; two streams). The two hygrophilous steppe vegetation types differ by the presence of arboreal riparian vegetation (dominance of *P. carthagenensis*, *D. fasciculata* and *V. discolor*) or the natural absence of arboreal riparian vegetation [with *Agenium leptocladum* (Hack.) Clayton LC, *Andropogon bicornis*, *Axonopus fissifolius* (Raddi) Kuhlm., *Briza calotheca* (Trin.) Hack., *Bulbostylis capillaris* (L.) C.B. Clarke, *Kyllinga odorata* Vahl, *Pterocaulon angustifolium* DC., and *Schizachyrium tenerum* Nees].

Water physical and chemical parameters

At all sampling sites, the following *in situ* measurements were obtained in triplicate per stream: stream depth (m), width (m) and canopy openness [%; digital camera (Nikon D5100) with a 10-mm Fisheye lens (Sigma)], and water flow (cm³/s), velocity (m/s), temperature (°C), pH (pHmeter PHTEK, Curitiba, PR, BR), electrical conductivity (µS/cm; Conductivimeter Quimis, Diadema, SP, BR), dissolved oxygen (mg/L; Jenway 970 Dissolved Oxygen Meter, Staffordshire, OSA, UK), total dissolved solids (g/L) and turbidity (Nephelometric Turbidity Unit, NTU; multianalyzer model 85, YSI Incorporated). The concentrations of nitrate, ammonia (detection limit of 0.05 mg/L) and orthophosphate (detection limit of 0.015 mg/L) in the water were obtained according to Clesceri et al. (1989). Litterfall was collected at each sampling point of streams with arboreal riparian vegetation, using nets (1 m²), and the percentage of organic matter in the litter was estimated according to Graça et al. (2005).

The stock of organic matter of the riparian soil was measured at all sampling sites from litter collected from areas of 1 m² until reaching clean ground, totaling three sub-samples (1 m² each) per stream riparian soil.

Macroinvertebrate collection and processing

To examine the benthic community, three samples (separated by 50 meters) were collected to represent the different microhabitats at each sampling site using a Surber stream-bottom sampler with a sampling area of 1024 cm² and a mesh size of 0.250 µm (Cummins, 1996; Hamada et al., 2014; Rezende et al., 2014b). The collected material was washed on 0.50 mm sieves and screened using a stereomicroscope. Aquatic macroinvertebrates were then collected and identified (to family level) using available and appropriate taxonomic keys (Cummins, 1996; Hamada & Ferreira-Hamada et al., 2014; Holzenthal & Calor, 2017; Keppler, 2012; Sousa & Elmoor-Loureiro, 2019). Also, different studies conducted in the Brazilian Atlantic Forest have consistently demonstrated that ecological assessments based on family-level identifications are suitable to detect the response of macroinvertebrate community by environmental changes (Suriano et al., 2011; Rezende et al., 2019c). Based on this inventory of benthic macroinvertebrate communities, average family richness and density were calculated for each sampling site. This methodology has yielded good results in studies of Neotropical streams (Firmiano et al., 2021; Hamada et al., 2014; Linares et al., 2021; Martins et al., 2021; Rezende et al., 2014b).

Estimation of α and β diversities

The number of invertebrate *taxa* (identified to family level) at all sampling sites was used to estimate α diversity. We estimated β diversity by implementing a multivariate dispersion method (Anderson et al., 2006) using the “*betadiver*” function from the “*vegan*” package of R version 2.0.8; (Oksanen et al., 2013). Multivariate dispersion estimates β diversity as sampling sites average dissimilarity (i.e., distance) from the centroid of their group in multivariate space. Compari-

son among sampling sites was based on the β_{gl} measure of β diversity (Lennon *et al.*, 2001), as proposed by Koleff *et al.* (2003). The β_{gl} value depends on the difference in the number of *taxa* between the two quadrats under consideration and was employed to test whether the other β diversity measures are able to recover patterns in the gradients of the local number of *taxa* (Lennon *et al.*, 2001; Koleff *et al.*, 2003).

Statistical analysis

To verify the independence of sampling sites, the correlation between species composition and geographical distance was tested using the Mantel test (Oksanen *et al.*, 2019). To evaluate the effects of different land covers (grassland without arboreal riparian vegetation, grassland with arboreal riparian vegetation, forest, and silviculture; explanatory variables) on macroinvertebrate richness and density (response variables), we used Generalized Linear Mixed-Effects Models by the “*glmer*” function of the “*lme4*” package of R (Crawley, 2007). The p-values were obtained by likelihood ratio tests (Chi-square distribution) of the full model against a partial model without the explanatory variables. We performed a random effect GLMM analysis considering each sampling sites to account for design imbalance of land cover treatments. To test differences in abiotic variables we used Generalized Linear Models (GLM; by the “*glm*” function of the “*vegan*” package of R; Crawley, 2007). All models were tested for error distribution by the “*hnp*” function from the “*hnp*” package of R and corrected for over or under-dispersion. Therefore, the initial model of macroinvertebrate richness was built using Gaussian distribution (link = identity, test = F), but later corrected to Poisson distribution (link = log, test = F; (Crawley, 2007). All others initial and final models of macroinvertebrate and abiotic variables were built using Gaussian distribution (link = identity, test = F).

A Permutational Multivariate Analysis of Variance (PerMANOVA) was used to estimate the difference in β diversity (different axes related to the distance from the centroid) among scales. PerMANOVA analysis was carried out using a Bray-Curtis distance matrix with 10 000 permu-

tations and pseudo-F by the “*adonis*” function of the “*vegan*” package of R (Oksanen *et al.*, 2008). In this analysis, we tested dispersion differences and not location differences in multivariate space (Heino *et al.*, 2013).

Contrast analysis was used to assess differences among different land covers (explanatory variable) in GLMM and PerMANOVA analyses. In the contrast analysis (orthogonal), the explanatory variable was ordered by increasing input values and pairwise testing (of treatments with the closest values). Stepwise model simplification was performed by sequentially adding treatment values that did not affect the model, and testing against the next variable in the sequence (for more details, see Chapter 9 in Crawley (2007)).

A Redundancy Analysis (RDA) was used to detect variation (Legendre & Legendre, 1998) in aquatic macroinvertebrate community composition among environmental variables (standardized) and different land covers to identify potential environmental requirements of the biological community (Hellinger transformation; “*rda*” function of the “*vegan*” package of R). Abiotic variables were filtered by the variance inflation index (“*vif*” function of the “*usdm*” package of R). The variance inflation index excludes highly correlated variables through a gradual procedure to deal with multicollinearity problems (Dormann *et al.*, 2013). The aquatic macroinvertebrate communities were filtered and those with at least five occurrences at sampling points were considered. The statistical significance of the correlation between environmental characteristics and biotic variables extracted from the RDA was determined by Monte Carlo test based on 5000 permutations ($p < 0.05$).

Finally, an Indicator Analysis (Dufrêne & Legendre, 1997) was used to determine which organisms were characteristic of the respective land covers (the “*indval*” function of the “*vegan*” package of R). This analysis uses the frequency and density of the organisms in the previously defined groups and produces an indicator value ranging from 0 (non-indicator) to 100 (perfect indicator). Significance was tested using Monte Carlo test with 1000 permutations and set to $p < 0.05$. Only significant results are reported.

Table 1. Summary table of Generalized Linear Mixed-Effects Model (GLMM) analysis (α diversity and density) and Permutational Multivariate Analysis of Variance (PerMANOVA; β diversity) evaluating the response of aquatic benthic macroinvertebrate communities to different land covers within the Wildlife Refuge of Campos de Palmas, South Brazil. Land covers are mixed ombrophilous forest (MOF), silviculture (SILV), grassland with arboreal riparian vegetation (GWRV) and grassland without arboreal riparian vegetation (GNRV). *Pos hoc* contrast analysis shows differences among land covers ($p < 0.05$). The values of degrees of freedom (DF), F statistics, and p values [$\text{Pr} (>F)$] are also given for each model. *Tabela resumida da análise de Modelos Lineares Generalizados Efeito-Mistos (GLMM; diversidade e densidade α) e Análise de Variância Multivariada Permutacional (PerMANOVA; diversidade β) avaliando a resposta da comunidade de macroinvertebrados aquáticos bentônicos a diferentes usos do solo no Refúgio de Vida Silvestre de Campos de Palmas, Sul do Brasil. As coberturas do solo são floresta ombrófila mista (MOF), silvicultura (SILV) e campos com mata ciliar arbórea (GWRV) e campos sem mata ciliar arbórea (GNRV). A análise pós-hoc de contraste mostra diferenças entre as coberturas do solo ($p < 0.05$). Os valores dos graus de liberdade (DF), estatística F e valores p [$\text{Pr} (>F)$] também são fornecidos para cada modelo.*

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)	Analysis of contrast
α diversity									
Null model	3	219.83	224.89	-106.91	213.83				
Land cover	6	216.55	226.68	-102.28	204.55	9.27	3	0.0258	GWRV = GNRV < SILV < MOF
Density									
Null model	3	839.51	844.58	-416.75	833.51				
Land cover	6	815.31	825.43	-401.65	803.31	30.21	3	< 0.001	GWRV = GNRV = MOF < SILV
β diversity									
Null model	3	43.43	48.49	-18.71	37.43				
Land cover	6	-13.81	-3.67	12.9	-25.81	63.24	3	< 0.001	SILV < GWRV = GNRV < MOF

RESULTS

Physicochemical parameters

The sampling points had slightly acidic water (pH range from 5.49 to 5.97), with low temperatures compared to tropical systems (range from 14 to 18 °C) and high concentrations of dissolved oxygen (range from 7 to 11 mg/L; Table S1, see Supplementary Information, available at <http://www.limnetica.net/en/limnetica>). Canopy openness was higher for grassland without arboreal riparian vegetation and silviculture (mean = 87 % and 83.97 %, respectively; Table S1), whereas mixed ombrophilous forest and grassland with arboreal riparian vegetation had lower canopy openness (mean = 5 % and 6 %, respectively; Table S1). Stream water had low values for electrical conductivity, total dissolved solids, and nutrients (nitrite, ammonia, and orthophosphate; Table S1). Litter stock was higher for silviculture riparian soil compared to the other land covers (Table S1) whereas litterfall was higher in grassland with arboreal riparian vegetation and mixed ombrophilous forest compared to the other land covers (Table S1).

Macroinvertebrate diversity

A total of 2124 individuals from 41 *taxa* were identified at the sampling sites. (Table S2, see Supplementary Information, available at <http://www.limnetica.net/en/limnetica>). The most abundant macroinvertebrate family was Leptohyphidae, with a mean density of 529 ind/m², followed by Baetidae with 317 ind/m², and Chironomidae with 177 ind/m². Leptophlebiidae, Elmidae and Gripopterygidae also had high density (> 101 ind/m²), while the density of the other *taxa* ranged from 0.97 to 49.80 ind/m². Individuals of the family Chironomidae were present at all sampling sites. The Mantel test did not indicate correlation between geographic distance and species composition ($r = 0.10$, $p = 0.09$).

Macroinvertebrate α diversity was higher for streams of mixed ombrophilous forest, followed by silviculture, while the lowest values were for with and without arboreal riparian vegetation (Table 1a, Fig. 2a). Silviculture had the highest macroinvertebrate density among land covers (Table 1b, Fig. 2b), being responsible for 64.29 % of all specimens collected. Mixed ombrophilous

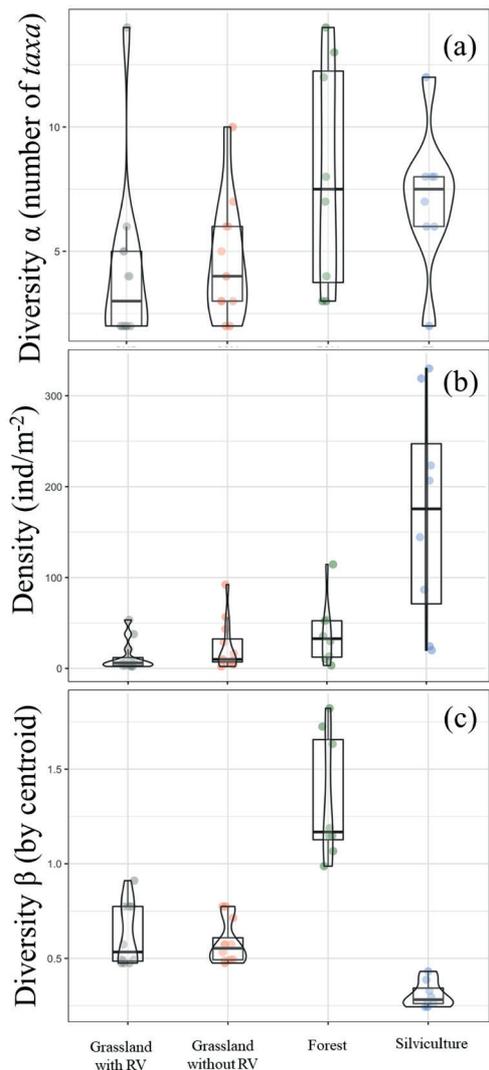


Figure 2. The α diversity (number of taxa; a), density (ind.m^{-2} ; b) and β diversity (by centroid distance; c) of aquatic benthic macroinvertebrate communities in streams in areas with different land covers within the Wildlife Refuge of Campos de Palmas, South Brazil. Land covers are: mixed ombrophilous forest, silviculture, grassland with arboreal riparian vegetation and grassland without arboreal riparian vegetation (riparian vegetation = RV). Violin and box plots with boxes representing quartiles, bold line the median, vertical dashed lines the upper and lower limits. *Diversidade α (número de táxons; a), densidade (ind.m^{-2} ; b) e diversidade β (por distância do centróide; c) da comunidade de macroinvertebrados bentônicos aquáticos entre diferentes coberturas do Refúgio de Vida Silvestre de Campos de Palmas, Sul do Brasil. As coberturas do solo são: campos sem mata ciliar arbórea, campos com mata ciliar arbórea, floresta ombrófila mista e Silvicultura (vegetação ciliar = RV). Gráfico de violino e caixas onde as caixas representam os quartis, a linha em negrito a mediana, as linhas verticais tracejadas os limites superior e inferior.*

forest had the highest β diversity among land covers (Table 1c, Fig. 2c).

Macroinvertebrate community vs. environmental variables

Environmental variables and sampling sites explained 42 % of the inertia (18.58) in the RDA analysis of aquatic macroinvertebrate community structure (total inertia of 43.82; PerMANOVA, inertia = 18.58; $F = 2.45$; $p < 0.001$). Axis 1 of RDA explained 18 % of the inertia (8.21) and Axis 2 explained 10 % (4.56; Fig. 3). The variance inflation index analysis selected nine of the 14 environmental variables that do not show multicollinearity, namely: temperature, canopy openness, electrical conductivity, dissolved oxygen, nitrite, orthophosphate, ammonia, water velocity and stream section. Also, canopy openness, water temperature, dissolved oxygen, and orthophosphate were significantly responsible for structuring the studied macroinvertebrate communities (Table 2). Most forested land covers (silviculture and mixed ombrophilous forest) were positively correlated with Axis 1, whereas grasslands (with and without arboreal riparian vegetation) were mostly negatively correlated with Axis 2 (Fig. 3a). Canopy openness, dissolved oxygen, electric conductivity, orthophosphate, and water velocity were positively correlated with Axis 1 (Fig. 3b), together with Leptophlebiidae, Calamoceratidae, Psephenidae, Oligochaeta, Elmidae, Hirudinida, Hydroptychidae, Simuliidae and Perlidae (Fig. 3c). On the other hand, temperature, stream section, nitrite, and ammonia were negatively correlated with Axis 1 (Fig. 4b), along with Libellulidae, Chironomidae and Caenidae (Fig. 3c).

Finally, indicator analysis (indication value = IV) showed that Leptophlebiidae ($IV = 0.43$; $p = 0.018$), Calamoceratidae ($IV = 0.39$; $p = 0.016$) and Perlidae ($IV = 0.38$; $p = 0.013$) were indicators of mixed ombrophilous forest. On the other hand, Baetidae ($IV = 0.64$; $p = 0.003$), Leptohyphidae ($IV = 0.60$; $p = 0.003$), Elmidae ($IV = 0.54$; $p = 0.011$) and Gripopterygidae ($IV = 0.44$; $p = 0.032$) were indicators of streams in silviculture land cover.

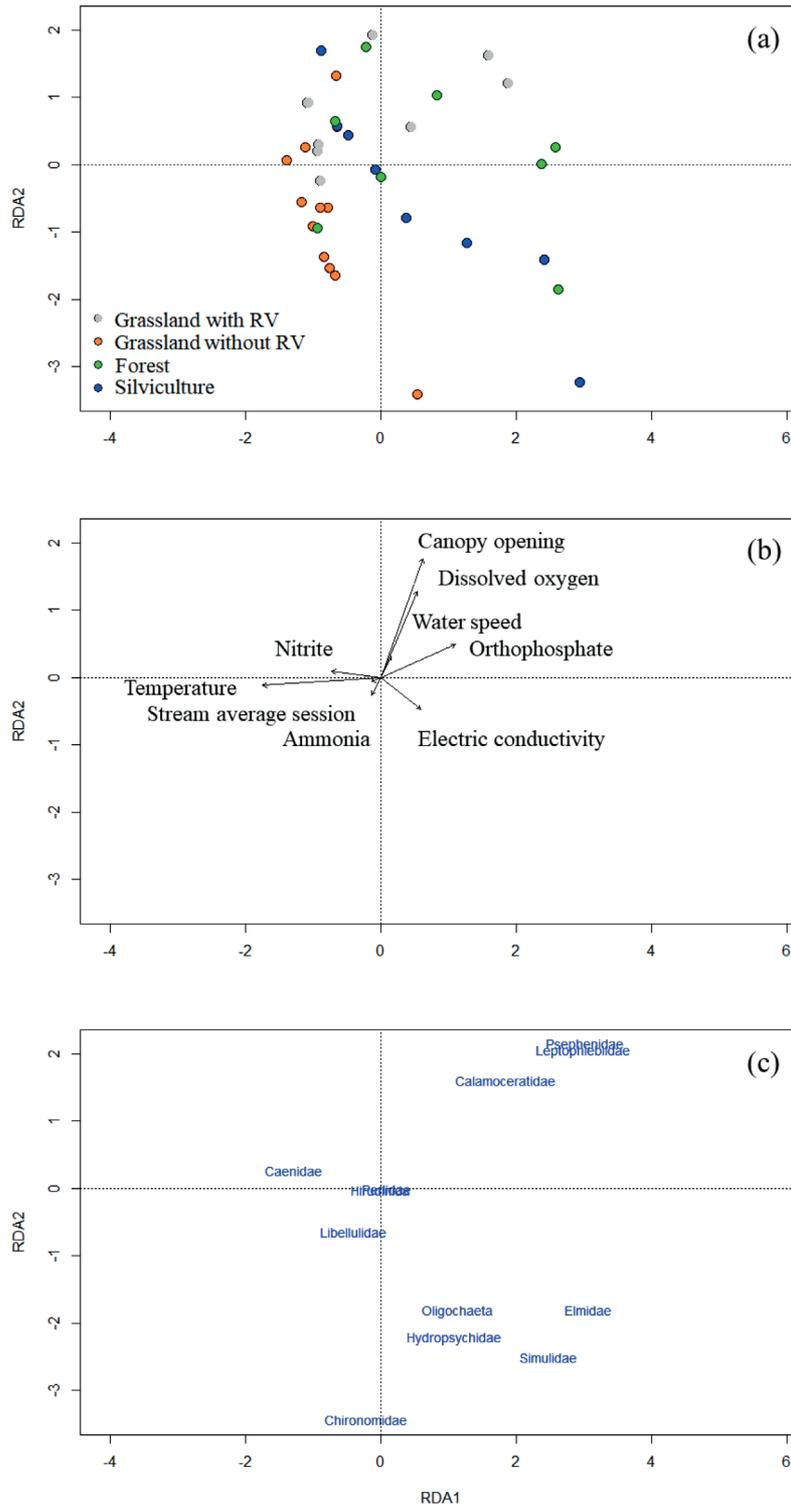


Figure 3. Redundancy analysis among different land covers within the Wildlife Refuge of Campos de Palmas, South Brazil: (a) abiotic variables (b) aquatic macroinvertebrate communities (c). *Análise de redundância entre diferentes coberturas de terra no Refúgio de Vida Silvestre de Campos de Palmas, Sul do Brasil: (a) variáveis abióticas (b) e comunidade de macroinvertebrados aquáticos (c).*

Table 2. Statistical significance [Pr (>r)] of the correlation (r^2) between environmental characteristics extracted from Axis 1 (RDA1) and Axis 2 (RDA2) vectors of the Redundancy Analysis based on the Monte Carlo test. Significant values are shown in bold. Significant values are shown in bold. *Significância estatística [Pr (>r)] da correlação (r^2) entre as características ambientais extraídas dos vetores do eixo 1 (RDA1) e 2 (RDA2) da Análise de Redundância com base no teste de Monte Carlo. Os valores significativos são mostrados em negrito.*

Variables	RDA1	RDA2	r^2	Pr(>r)
Canopy openness (%)	0.25	0.97	0.54	< 0.001
Water temperature (°C)	-1.00	-0.09	0.28	0.002
Electric conductivity mS/cm	0.68	-0.73	0.07	0.272
Dissolved oxygen mg/L	0.29	0.96	0.29	0.002
Nitrite mg/L	-0.98	0.18	0.05	0.385
Orthophosphate mg/L	0.86	0.51	0.15	0.040
Ammonia mg/L	-0.38	-0.92	0.01	0.789
Water flow velocity m/s	0.32	0.95	0.02	0.708
Stream session m	-0.87	-0.49	0.00	0.958

DISCUSSION

Macroinvertebrate diversity

Our results partially support the hypothesis that α and β diversities will be greater in streams in natural forests (mixed ombrophilous forest) compared to the other land cover types studied, as well as with the presence of arboreal riparian vegetation. On the one hand, forested areas, including mixed ombrophilous forest and silviculture, are more diverse (α diversity) in terms of benthic macroinvertebrates than highland grasslands. This finding may indicate that the high amount of litter in silviculture systems may countereffect the negative outcomes of secondary compounds released in litter decomposition by fast leaching of allelopathic compounds in leaves, resulting in a more diverse macroinvertebrate community than that observed for land covers with low amounts of leaf litter, such as highland grasslands systems. On the other hand, mixed ombrophilous forest had greater macroinvertebrate β diversity than the other land covers. The studied mixed ombrophilous forests were located in different sub-basins, and so the disconnection of water flow between them may increase β diversity above that of other land cover streams. Finally, arboreal riparian vegetation drives important environmental characteristics, like canopy openness, water temperature,

dissolved oxygen, and orthophosphate in streams, which in turn control the frequency and occurrence of benthic macroinvertebrates. This may be an important guideline for for legislation, management, and conservation proposals for highland grassland stream areas, since this study showed that these systems are naturally less diverse compared to forested systems, and are part of endemic-rich regions threatened by anthropogenic land changes in the subtropics.

Spatial diversity of macroinvertebrates

Our findings are consistent with the hypothesis that α and β diversities of benthic macroinvertebrates would be higher in mixed ombrophilous forest compared to the other land cover types studied. More specifically, α diversity was high for mixed ombrophilous forest, as well as for silviculture land cover. Forest landscapes increase environmental heterogeneity (Mathers *et al.*, 2021), and thus an increase in habitat quantity and a decrease in community competition are expected (Beisel *et al.*, 1998; Stein & Kreft, 2015). Indeed, the higher incidence of leaves, branches and roots in stream beds in forest landscapes increase the number of micro-habitats and refuges available for the benthic macroinvertebrate community (Nuven *et al.*, 2022). In this way, high environmental heterogeneity allows different species to

coexist, thereby increasing local diversity (Agra et al., 2021; Heino et al., 2015a). Also, Rezende et al., (2019) did not find variation in ecological patterns among different levels of taxonomic resolution (family vs genus) for subtropical macroinvertebrate stream communities in the Neotropics, again consistently demonstrated that ecological assessments based on family-level identifications are suitable to detect environmental changes.

An increase in allochthonous litter input was also observed in areas with dense arboreal riparian vegetation, such as mixed ombrophilous forests, when compared mainly to grassland areas (Rezende et al., 2014a, 2016). High litter input increases the availability of food resources for benthic macroinvertebrates (Rezende et al., 2021; Tonin et al., 2021). Therefore, the high α diversity of benthic macroinvertebrates in mixed ombrophilous forests may be related to an increase in environmental heterogeneity (Nuven et al., 2022; Rezende et al., 2014a) and allochthonous litter input (Rezende et al., 2021; Tonin et al., 2021), which would also explain the high α diversity in silviculture land cover compared to grasslands.

Finally, mixed ombrophilous forest had the highest β diversity, whereas silviculture had the lowest. The streams of the mixed ombrophilous forest areas are in different sub-basins, being disconnected from each other (i.e., they have different water flow directions), explaining the high β diversity. This disconnection may reduce the dispersal of individuals among landscape fragments, as already known for macroinvertebrates with swimming habit (Ellis & Jones, 2013; Rezende et al., 2019a). On the other hand, silviculture land use converts natural habitats into a more homogeneous environment, which explains its low β diversity. In this sense, terrestrial distance (Hepp & Melo, 2013), anthropogenic factors (Agra et al., 2021) and differences between sub-basins (Castro et al., 2019) may explain the differences in β diversity among aquatic benthic macroinvertebrate communities (Brush et al., 2022; Heino et al., 2015a).

The high α diversity of benthic macroinvertebrates found for silviculture land cover, compared to natural grassland areas, was unexpected due to the already known negative effects of silviculture (Bayle, 2019; Rezende et al., 2021). This finding may be related to the higher litter input of silvi-

culture systems compared to grasslands (Table S1). However, silviculture areas may possess high toxicity due to the release of secondary compounds during leaf litter decomposition, thereby simplifying the food web (Carvalho et al., 2019; Gallon et al., 2020; Rezende et al., 2021). Therefore, high α diversity in silviculture systems compared to grasslands can lead to the inference that system litter input was more relevant than secondary compounds released by litter decomposition, because of the fast leaching of allelopathic compounds in leaves (Rezende et al., 2014a).

Our findings also corroborate the hypothesis of higher macroinvertebrate density in silviculture. As the leaching of secondary compounds in litter may occur quickly, leached litter can then be used as food and shelter by adapted organisms (Rezende et al., 2019a, 2021). Also, a thicker stock of litter associated with high canopy openness may allow increased densities of algae, emergent macrophytes and submerged macrophytes (Hilt et al., 2021), a characteristic observed at streams in silviculture areas throughout the study area. The presence of algae and macrophytes may be related to high habitat complexity and shelter and food resources increasing the density and diversity of benthic macroinvertebrates (Rezende et al., 2019b), thus also explaining the high macroinvertebrate density and α diversity in silviculture areas. In this sense, two families of the order Ephemeroptera (Baetidae and Leptohyphidae) that occur with high densities in silviculture streams are mostly of the collector feeding group, which benefit from litter decomposition (Callisto et al., 2021; Firmiano et al., 2021). On the other hand, the family Chironomidae was the most abundant in streams without arboreal riparian vegetation. Therefore, areas with anthropogenic influence tend to filter out some macroinvertebrate families, but those that remain are resistant to local stress and allowed to increase in density (Feio et al., 2021).

Macroinvertebrate community vs. environmental variables

Canopy openness, water temperature, dissolved oxygen and orthophosphate were key factors driving the benthic macroinvertebrate communities.

These factors reinforce the importance of arboreal riparian vegetation to: (i) control the availability of allochthonous resources (Bambi *et al.*, 2017; Tonello *et al.*, 2021); (ii) increase environmental heterogeneity (Hepp & Melo, 2013; Nuven *et al.*, 2022); (iii) decrease water temperature (Arismendi *et al.*, 2012); and (iv) increase dissolved oxygen due to water turbulence (Rezende *et al.*, 2014b) caused by the input of roots and branches onto the stream substrate (Nuven *et al.*, 2022). In this way, high temperatures were inversely correlated with arboreal riparian vegetation due to increased incidence of luminosity (Rezende *et al.*, 2014a), facilitating the occurrence of the families Libellulidae and Chironomidae in high canopy openness areas (Hamada *et al.*, 2014). On the other hand, dissolved oxygen was a positive factor for aquatic macroinvertebrate diversity, justifying its higher association with Leptophlebiidae and Calamoceratidae (Hamada *et al.*, 2014). As observed in silviculture streams, orthophosphate concentration may be influenced by land cover, since there was high input of sediments and litter (Rezende *et al.*, 2014b). Thus, high food availability can explain the correlation between orthophosphate concentration and Oligochaeta and Elmidae, which feed on litter, and Hydropsychidae, which are omnivorous (Hamada & Ferreira-Kepler, 2012; Hamada *et al.*, 2014).

CONCLUSION

We found that environmental characteristics related to land use are important regulating factors of α diversity (by richness), density, and β diversity of aquatic macroinvertebrate communities. Macroinvertebrate communities in streams of silviculture areas (*Pinus* forests) had lower α diversity than only natural forest streams, demonstrating the effects of environmental homogenization (lower diversity and availability of micro-habitats on a regional scale), which in turn favors *taxa* with similar characteristics, as corroborated by the high density in silviculture areas. On the other hand, streams in silviculture areas (*Pinus* forests) had higher α diversity than highland grasslands areas due to high leaf litter input. Also, the preservation of arboreal riparian vegetation increases macroinvertebrate richness due to the high avail-

ability of food resources, thus increasing α diversity. The presence of arboreal riparian vegetation also drives canopy openness, water temperature, dissolved oxygen and orthophosphate of streams, which control the frequency and occurrence of aquatic benthic macroinvertebrate communities. Disconnected streams show the highest environmental fragmentation, which increases dissimilarity and β diversity by distance. Finally, as highland grasslands are naturally more homogeneous, and with high canopy openness, they had the lowest richness and density of macroinvertebrates. The natural low richness of highland grasslands, but with endemic species, may serve as an important guideline for legislation of anthropic impacts, management, and conservation of streams in this specific subtropical zone.

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