



Spatial and temporal factors determining the structure of ciliate protist communities in neotropical streams

Fatores espaciais e temporais que determinam a estrutura das comunidades de protistas ciliados em riachos neotropicais

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Abstract: Aim: In this study, we focused on patterns of ciliate protist community composition in urban and rural streams. **Methods:** We analyzed 12 urban and rural streams during two different periods of the year (rainy and dry seasons) across three different stream mesohabitats (riffles, runs, and pools). We predicted that the species composition of ciliates would differ between types of environments (rural and urban), between basins (Pirapó and Ivaí), between mesohabitats (riffles, runs, and pools), and between seasonal periods. We also expected that ciliate species composition would be similar between streams within the same basin. **Results:** Contrary to our expectations, our results showed that the richness and abundance of ciliates were higher in urban streams than in rural streams. In the analysis of beta diversity, we observed that this measure was higher in rural environments, but only during one period (dry season). Among the mesohabitats, no significant alterations in ciliate species composition were noted, contrary to our predictions. **Conclusions:** The results obtained in this study demonstrated the effect of multiple spatial (basin, type of environment) and temporal (rainy and dry seasons) factors on the structure of ciliate protists in the water column of neotropical streams. No differences in ciliate attributes were found among the mesohabitats analyzed in this study.

Keywords: streams; urbanization; agriculture; ciliates; mesohabitat.

Resumo: Objetivo: O objetivo deste estudo foi investigar os padrões de composição da comunidade de protistas ciliados em riachos urbanos e rurais próximos a um grande centro urbano. **Métodos:** Analisamos 12 riachos entre urbanos e rurais durante dois períodos diferentes do ano (estação chuvosa e seca) em três diferentes meso-habitats dos riachos (rápido, remanso e corredeira). Previmos que a composição de espécies de ciliados seria diferente entre os tipos de ambientes (rural e urbano), entre as bacias nos quais os riachos estavam inseridos (Pirapó e Ivaí), entre meso-habitats (rápido, corredeira e remanso) e entre períodos sazonais. Também era esperado que a composição de espécies de ciliados



fosse semelhante entre riachos dentro de uma mesma bacia. **Resultados:** De forma oposta, os resultados mostraram que a riqueza e abundância de ciliados foram maiores em riachos urbanos do que em riachos rurais. Na análise da diversidade beta, observamos que essa medida foi maior em ambientes rurais, mas apenas durante um período (estação seca). Entre os meso-habitats não foram observadas alterações significativas na composição de espécies de ciliados, contrariando o predito inicialmente. **Conclusões:** Os resultados obtidos neste estudo demonstraram o efeito de múltiplos fatores espaciais (bacia, tipo de ambiente) e temporais (estações chuvosas e secas) na estrutura dos protistas ciliados na coluna d'água de riachos neotropicais. Não foram encontradas diferenças nos atributos dos ciliados entre os meso-habitats analisados neste estudo.

Palavras-chave: riachos; urbanização; agricultura; ciliados; mesohabitat.

1. Introduction

Streams are considered lotic ecosystems, characterized physically by a constant water flow and high current velocity (Townsend, 1980; Jeffries & Mills, 1990). This type of environment exhibits multiple levels of water discharge and nutrient loading, promoting the mixing of water layers and the stability of bottom sediments (Scoarize et al., 2024). The combination of these factors results in varied attributes along stream sections, classifying them according to the speed of water flow into mesohabitats such as riffles, pools, and rapids (Pessoa et al., 2021).

These mesohabitats (riffles, pools, and rapids) play distinct ecological roles. Riffles, characterized by shallow and fast-flowing waters, are crucial for water oxygenation (Pessoa et al., 202). Conversely, pools are deeper areas with slow hydrological flow, providing refuge for a wide variety of aquatic organisms during periods of environmental stress (Brown & Brussock, 1991). Rapids, defined by turbulent water flow and high hydraulic force, are specialized mesohabitats for species adapted to extreme conditions (Pessoa et al., 2021).

Considering the presence of these mesohabitats, the place where they are located can define their biotic and abiotic characteristics. Urban and rural streams are subject to different impacts resulting from anthropogenic activities (Velho et al., 2021), which alter the physical, chemical, and biological characteristics of these environments, influencing the dynamics of the communities inhabiting these areas (Dias et al., 2008; Segovia et al., 2016; Velho et al., 2021; Savić et al., 2022). Urban streams are usually prone to deterioration due to the constant growth in population density, the construction of avenues, roads, and urban centers, the discharge of untreated sewage, and the improper disposal of domestic waste in areas near these streams (Lippert et al., 2019; Pessoa et al., 2021; Katayama et al., 2024). In contrast, rural streams are mainly degraded by the removal of

riparian vegetation and the expansion of agriculture and livestock, which alter land use (Segovia et al., 2016; Pessoa et al., 2021; Velho et al., 2021). Consequently, agrochemicals used in agriculture frequently runoff into water bodies (Kühl & Rocha, 2010; Debastiani et al., 2016; Santos et al., 2024).

In addition to isolated anthropogenic effects, these environments may undergo natural alterations resulting from changes in seasonal periods (e.g., rainy and dry seasons). Environmental variability in time and space is recognized as a determining factor in the distribution of organisms, influencing their interactions and adaptations (Mapurunga et al., 2024). In monitoring these effects, the use of biological communities coupled with the analysis of physicochemical parameters is an essential tool for estimating environmental effects (Walsh et al., 2005; Segovia et al., 2016; Lippert et al., 2019; Savić et al., 2022), as changes in abiotic variables impact the communities within the ecosystem. Thus, possible changes in the structure, diversity, and dynamics of biological communities are a reliable way to assess the effect of anthropogenic impacts (Madoni, 2005).

One way to measure changes in biological communities across spatial and temporal gradients is through the analysis of beta diversity (β), an important component of biodiversity that can be defined as the dissimilarity in species composition between locations, including both small and large spatial scales (Whittaker, 1972; Anderson et al., 2006; Guimarães Durán et al., 2024). The responses obtained from beta diversity analysis effectively capture biodiversity dynamics compared to more traditional measures such as species richness (Canella, 2016; Wang et al., 2012; Oliveira et al., 2024).

Among aquatic microbiota, ciliate protists are among the first levels of trophic webs, being highly diverse organisms with a wide distribution in terrestrial and aquatic ecosystems (Arndt et al., 2000; Vargas et al., 2015; Segovia et al., 2016; Adl et al., 2019; Sieber et al., 2020). These are small-sized

organisms with short life cycles and high metabolic and reproductive rates (Munn et al., 2002; Paiva & Silva-Neto, 2004; Madoni, 2005; Zingel, 2005; Dias et al., 2008; Payne, 2013; Segovia et al., 2016). They have the capacity to detect environmental changes on a short temporal scale (Dias et al., 2021; Wang et al., 2021), as they have specific requirements related to the characteristics of their habitat, such as dissolved organic matter, temperature, pH, electrical conductivity, and oxygen concentration (Lippert et al., 2019). Due to these characteristics, they perform important ecosystem functions that are well-established in ecological literature, such as their fundamental role in aquatic food webs, the transfer of matter and energy, and nutrient cycling (Fenchel, 1987; Weisse & Sonntag, 2016), their use as bioindicators in water quality monitoring (Foissner & Berger, 1996; Munn et al., 2002; Dias et al., 2021; Kulaš et al., 2021), and for various other environmental aspects (Primc-Habdija, 1988; Primc-Habdija et al., 1998; Madoni & Bassanini, 1999; Madoni & Braghiroli, 2007; Payne, 2013; Risse-Buhl et al., 2015; Geisen et al., 2017). Studies have shown that both urbanization and agricultural activities can significantly impact the biodiversity of urban and rural streams, altering community composition and species richness in these areas (Velho et al., 2021; Savić et al., 2022), although these results are more abundant for other groups such as fish and macroinvertebrate assemblages (Weaver & Garman, 1994; Wang et al., 2000; Walsh et al., 2001; Wang & Kanehl, 2003; Luo et al., 2018; Gál et al., 2019; Ortega et al., 2021; Paredes del Puerto et al., 2021; Moi & Teixeira de Mello, 2022).

Therefore, the objective of this study was to evaluate changes in the composition and structure of ciliate communities in urban and rural streams within different watersheds that are subject to distinct anthropogenic stressors. The hypothesis tested was that mesohabitat (riffles, pools and runs), environment type (rural and urban), period (rainy and dry), and basin (Ivaí and Pirapó) will determine the patterns of ciliate richness and abundance, as well as their composition and beta diversity patterns. It was predicted that: (i) higher values of these attributes will be found in environments with pool characteristics and lower in environments characterized as riffles, as environments with higher current velocity do not favor the establishment of planktonic organisms; (ii) species richness will be higher in rural areas, while an opposite pattern will be observed for abundance, which will be higher in urban areas; (iii) the rainy period

will show the highest richness values due to the contribution of species from other compartments (littoral and benthic), while in the same period, lower abundance values will be recorded due to the dilution of organisms in a larger volume of water; (iv) differences in ciliate species composition between urban and rural streams will be observed due to the different types of environmental impacts that select species tolerant to them; (v) there will be changes in species composition according to the analyzed period (dry and rainy), considering that seasonality leads to environmental differences that select species with different requirements; (vi) streams in different watersheds will show differences in ciliate species composition due to differences in their geomorphological characteristics; (vii) streams located in urban areas will show less variability in species composition compared to rural streams, especially during the rainy period, due to the entry of pollutants from urban areas into these streams.

2. Material and Methods

2.1. Study area

The study was conducted by collecting water samples from 12 first-order streams, including 6 urban and 6 rural streams, belonging to two distinct watersheds: the Pirapó River Basin and the Ivaí River Basin. These basins encompass the urban perimeter and areas adjacent to the city of Maringá, the most important urban center in the region, located in the northern part of the state of Paraná, Brazil (Camargo, 2009). The city of Maringá serves as a watershed divide between the two basins and contains several small watercourses that are directly influenced by anthropogenic impacts, with most of the headwaters located within urbanized areas (Figure 1).

The Pirapó River watershed (22°30'S to 23°30'S; 51°15'W to 52°15'W) is located in the Third Paraná Plateau region, in the state of Paraná, Brazil (Peruço, 2004). This basin has a drainage area of approximately 5,076 km², and the streams categorized as rural are Alto Alegre, Jaborandi, and Atlântico, while the urban streams are Morangueira, Guaiapó, and Maringá, based on their location within the study area (Figure 1). The Ivaí River basin extends across the Second and Third Paraná Plateaus in the southern region of Brazil (S 22°56'17" to 25°35'27" and W 50°44'17" to 53°41'43") and drains an area of 36,587 km² (Pessoa et al., 2021). The streams analyzed in this basin were Jaçaná, Jaguaruna, and Colombo (rural), and Borba Gato, Pinguim, and Moscados (urban) (Figure 1). In general, the streams analyzed in both basins are under

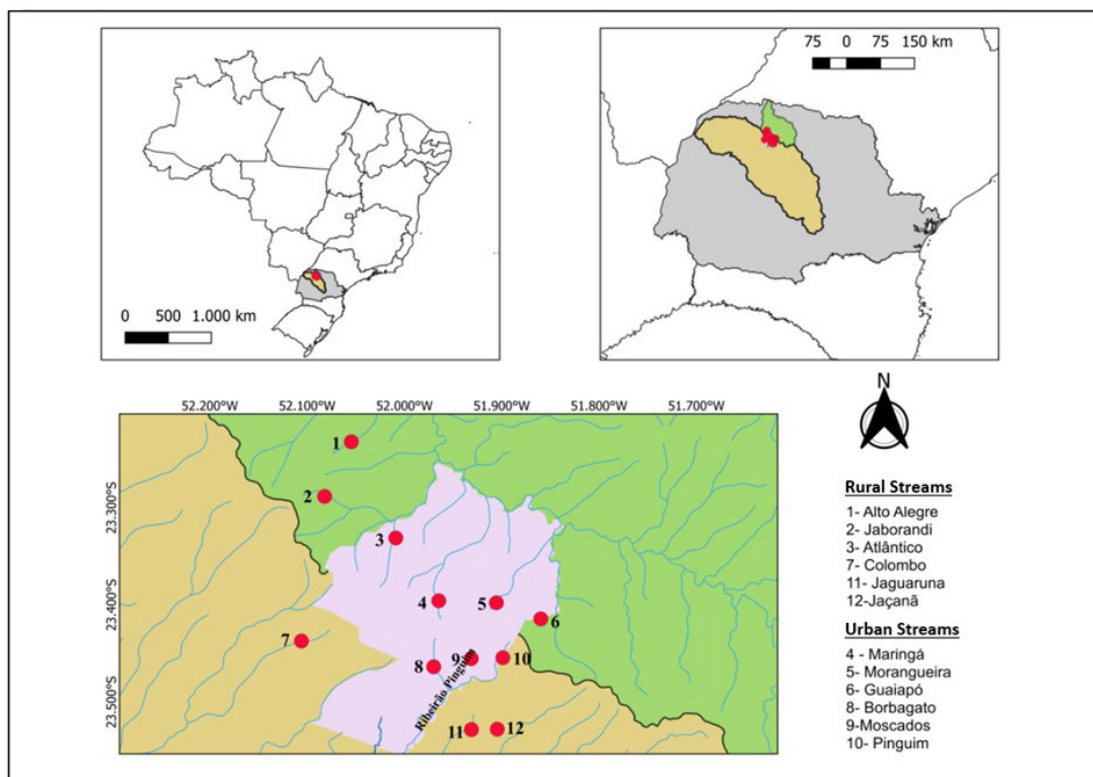


Figure 1. Location of sampling sites in rural and urban streams within the Pirapó (represented in the image by the color green) and Ivaí River (represented in the image by the orange color) located in the municipality of Maringá, Paraná, Brazil (represented in the image by the light lilac color in the middle area).

the influence of the municipality of Maringá (Peruço, 2004), with sections significantly modified by artificial structures (such as construction materials, plastic bottles, tires, etc.), within among other alterations.

2.2. Data collection and laboratory analysis

Sampling was conducted during two distinct hydrological periods: the rainy season (March 2020) and the dry season (September 2021). Samples were collected from the subsurface in three mesohabitats of each stream, defined as riffle, run, and pool, within an area of approximately 50 meters of the stream. The mesohabitats were visually designated in sequence: riffles had fast and turbulent waters, with substrates composed of large, worn, and rounded rocks; runs were characterized by consistent waters with less turbulent and deeper flow than riffles; pools were deep areas where water flow was slow, allowing the deposition of fine sediments on the substrate (Pessoa et al., 2021).

A total of 72 samples were obtained in each campaign (36 for each period (i.e. March and September (2 campaigns x 12 streams (6 pirapó e 6 ivaí) x 3 mesohabitats = 72). In each mesohabitat, 5 liters of water were collected by filtering 50 liters in the field using a graduated bucket and a

plankton net with a mesh size of 10 μm . The samples were stored in 5-liter polyethylene plastic jugs and kept in insulated coolers with ice until arrival at the laboratory, where qualitative and quantitative analyses of the ciliate community were performed.

In the laboratory, the 5 liters of sampled water were further concentrated to 100 mL using a 10 μm mesh net. The counting and identification of ciliates were conducted *in vivo*, to avoid cellular deformations resulting from fixation processes, using an Olympus CX41 optical microscope at 100 $\times$ and 400 $\times$ magnifications (Weisse, 1991). The abundances (individuals/L) of the organisms were quantified from 1 mL of sample, divided into 10 aliquots of 100 μL each, mounted on slides following the method proposed by Madoni (1984). Additionally, to record rare species or those in low abundance, a qualitative analysis was performed by identifying ciliates in 1 mL of sample using a Sedgewick-Rafter chamber. Specialized literature was used for the identification of organisms (Foissner & Berger, 1996).

2.3. Data analysis

Multifactorial analyses of variance (ANOVA-four way) were used to test differences in ciliate

abundance and richness among mesohabitats, environment types, periods, and watersheds (predictions i-iv). When necessary, Tukey's HSD post hoc test was used for multiple comparisons. To verify differences in species composition between periods (rainy and dry) (prediction v) and between watersheds (prediction vi), non-parametric permutational analysis of variance (PERMANOVA) was performed. To calculate the temporal and spatial beta diversity of the ciliate community (prediction vii), a permutational multivariate analysis of dispersions (PERMDISP; Anderson et al., 2006) was conducted. All analyses were performed using R software version 3.1.3 (R Core Team, 2013).

3. Results

3.1. Abundance and species richness

Ciliate species richness was significantly higher in urban environments compared to rural streams (ANOVA, $F=26.72$, $p<0.01$; Figure 2). When

comparing the two basins, higher richness values were observed in the Ivaí River Basin (ANOVA, $F=12.3$, $p<0.01$). Additionally, richness values differed significantly between sampling periods, with higher values recorded during the dry period (September 2021; List available on <https://data.scielo.org/dataset.xhtml?persistentId=doi:10.48331/scielodata.FDRK2P>) compared to the rainy period (ANOVA, $F=15.03$, $p<0.01$). However, no significant differences in richness were found between mesohabitats (riffle, run, and pool) when comparing urban versus rural streams (ANOVA, $F=1.05$, $p=0.31$), between basins (ANOVA, $F=0.27$, $p=0.75$), or between periods (ANOVA, $F=0.004$, $p=0.99$).

A significant difference in ciliate abundance was observed between periods, with higher values recorded in March (rainy period) for urban environments (ANOVA, $F=4.59$, $p=0.037$; Figure 2). An interaction between environment type (urban vs. rural) and basin was also found (ANOVA, $F=7.13$, $p=0.010$), with differences observed only between

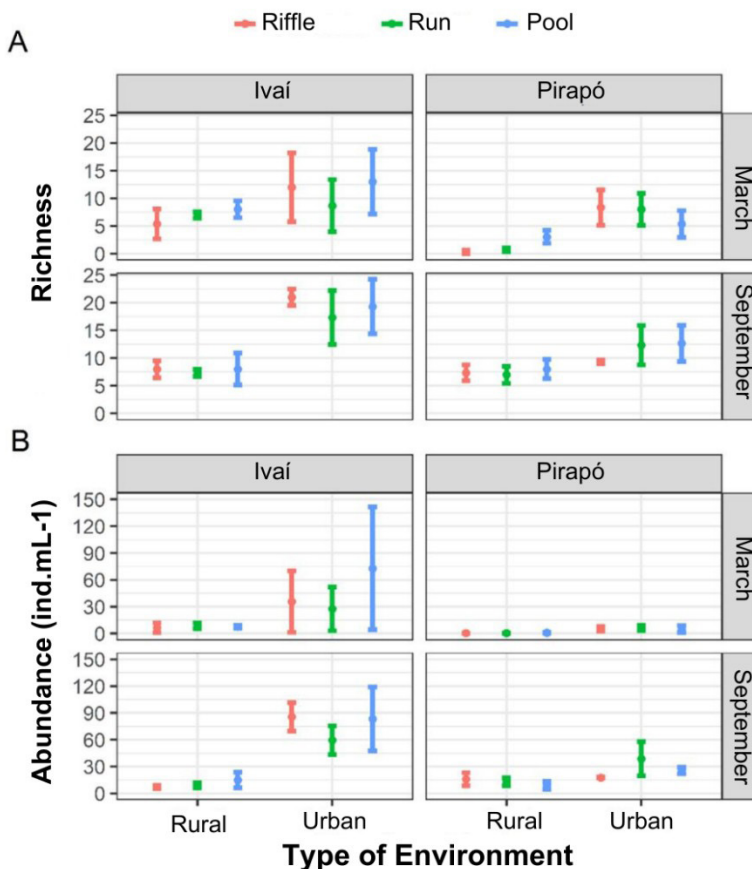


Figure 2. Variation in richness (A) and abundance (B) of the ciliate protozoan community across each mesohabitat (riffle, run, and pool), stream types (urban and rural), sampling periods (March and September), and watersheds (Ivaí and Pirapó). The central point denotes the mean value, and the bars represent the standard error.

rural and urban streams in the Ivaí River Basin. No significant differences in abundance were found between mesohabitats (ANOVA, $F=0.30$, $p=0.75$).

3.2. Species composition

Regarding species composition, PERMANOVA revealed significant interactions between environment type and watershed ($F=2.28$, $p<0.001$) and between environment type and period ($F=1.65$, $p=0.031$) when considering presence-absence data of species (Figure 3). In the analysis based on abundance, a significant interaction was observed between environment type, watershed, and period ($F=1.80$, $p=0.004$). These interactions indicate that the effects of environment type (urban vs. rural) on species composition depend on both watershed and period, rather than suggesting independent main effects of watershed or period. The analysis of species presence-absence data (Figure 3) reveals clustering along the principal coordinates analysis (PCoA), indicating similarity in species composition across environment types, watersheds, and periods.

The results of the permutational multivariate analysis of dispersions (PERMDISP) showed an interaction between environment type and period ($F=4.73$, $p=0.034$) when evaluating presence-absence data, and significant values for watershed ($F=4.48$, $p=0.039$). For abundance data, a significant interaction between environment type and period was recorded ($F=17.59$, $p<0.001$; Figure 4).

4. Discussion

In lotic aquatic systems, both urban and rural, hydrodynamic and geomorphological differences among mesohabitats influence the distribution of various species within the channel (Alexandre et al.,

2010; Wolff & Hahn, 2017; Huang et al., 2019; Pessoa et al., 2021). Despite the differences in flow among the mesohabitats of the studied streams, no clear patterns of variation in the structure of the ciliate protozoan community in the water column were observed. This is likely because these organisms are continuously transported by the hydrodynamic flow, preventing their establishment in a specific location (Meira et al., 2021). Consequently, the current speed carries ciliates from one mesohabitat to another, resulting in no significant changes in ciliate communities between areas with different hydrodynamic patterns in lotic environments (Horvath & Lamberti, 1999).

Contrary to predictions, species richness and abundance were higher in urban environments compared to rural ones. Despite the urbanization process and its impacts on streams (Velho et al., 2021; Lippert et al., 2019; Pessoa et al., 2021), richness values were higher in urban streams during the analyzed period. Pristine streams are not favorable to high ciliate diversity due to factors such as flow velocity, low concentration of suspended nutrients, and consequently low productivity (Segovia et al., 2016). Thus, urban environments, with higher amounts of organic matter, may support increased ciliate richness and diversity due to higher organic matter addition (Segovia et al., 2016). The results suggest that urbanization and land use may have different effects on lotic and lentic environments, being more critical for low-flow environments. In these habitats, due to slower water flow, there is greater accumulation of organic matter, leading to decreased oxygen levels and consequently reduced biodiversity (Savić et al., 2022). Thus, it can be suggested that the impacts of urbanization are less pronounced in lotic ecosystems, at least in terms of their diversity.

Given that organism distribution is determined by their interactions with the environment, anthropogenic activities lead to a variety of impacts directly affecting ecosystem functioning (Segovia et al., 2016). Consequently, these impacts result in severe consequences such as biodiversity loss and high species dominance, selecting those most tolerant to different types of environmental disturbances (Bourdeau & Treshow, 1978). Urban and rural streams are subjected to different types of anthropogenic impacts. Urban streams primarily experience increased organic loading due to untreated sewage discharge. This high organic load provides carbon for bacterial growth in these environments, possibly selecting ciliate

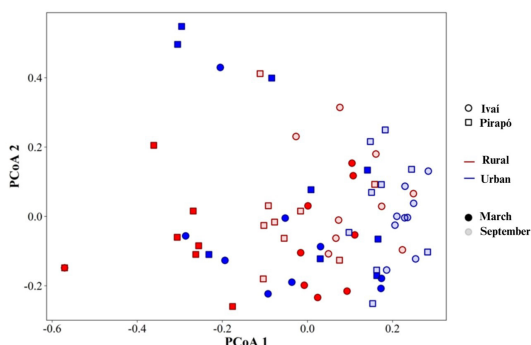


Figure 3. Principal Coordinates Analysis (PcoA) highlighting the interactions between environment type (urban and rural) and watershed (Ivaí and Pirapó), as well as between environment type and period (March and September).

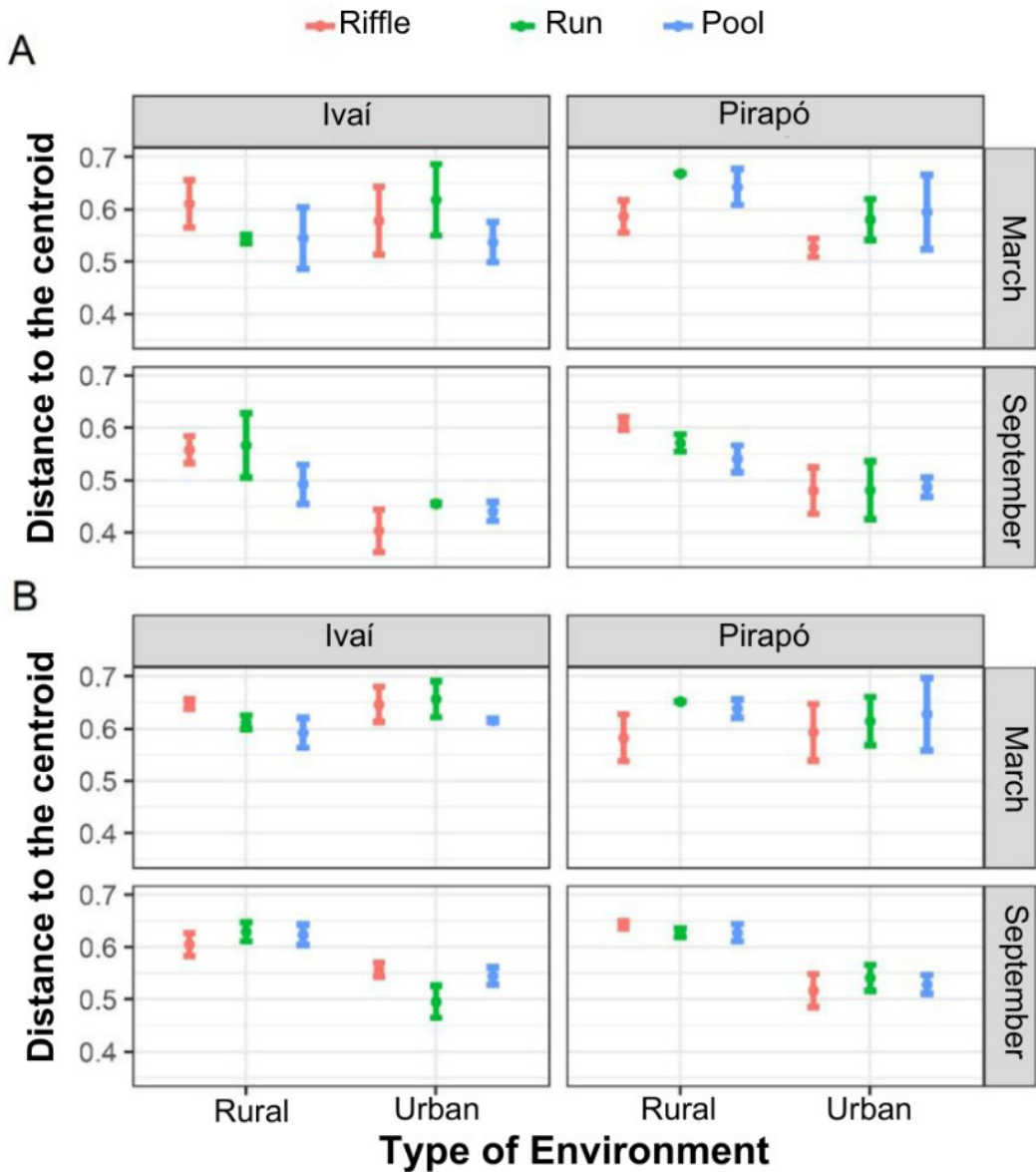


Figure 4. Variation in beta diversity (quantified as distance to the centroid) of the ciliate protozoan community across each mesohabitat (riffle, run, and pool), stream types (urban and rural), sampling periods (March and September), and watersheds (Ivaí and Pirapó). The central point denotes the mean value, and the bars represent the standard error. Abundance (ind.mL⁻¹).

species that consume these resources (Madoni & Braghiroli, 2007). Rural streams face impacts such as riparian vegetation removal, leading to increased nutrients and heavy metal runoff from agrochemicals. The removal of riparian vegetation can increase light intensity in these environments, promoting the growth of autotrophic organisms such as phytoplankton and their consumers (Segovia et al., 2016). Regarding the watersheds, as expected, streams within the same watershed exhibited similar species compositions, regardless

of being rural or urban. These environments are closer and more connected, and they have more similar geomorphological and environmental characteristics, facilitating the dispersal, colonization, and selection of similar species (Cunico et al., 2006; Helfman et al., 2009; Souza & Klepka, 2012). The observed interaction between environment type and period for beta diversity, indicates a difference in species composition between rural and urban streams; however, this is only true for September (dry season) in rural environments. In rural lotic

systems, different land uses, crop variations, pesticide applications, and agrochemical inputs appear to create more distinct aquatic systems in their environmental characteristics (Kühl & Rocha, 2010; Debastiani et al., 2016). These impact variabilities have diverse effects on the communities of these streams. In contrast, in urban systems, the impact seems to produce a consistent and similar trajectory among them (sewage discharge, litter, etc.) (Lippert et al., 2019). Therefore, the environmental filters and, consequently, the species selected are generally those tolerant of these continuous impacts.

Another point to consider is the spatial distribution of streams. Rural streams are usually farther from urban centers and tend to be more diverse compared to urban streams, which are all located in the urban area of the municipality. Thus, due to the spatial proximity of urban streams, ciliate composition may be more similar, while rural streams are more heterogeneous. This pattern was not observed during the rainy season, as elevated flow, volume, and runoff during this period seem to increase similarity between different types of environments and their diversity (Segovia et al., 2016; Velho et al., 2021; Savić et al., 2022). It is noteworthy that a possible limitation of this study is the lack of analysis of physical-chemical factors, trophic guilds, and functional diversity of ciliates, as these are important components for understanding the observed patterns and would certainly aid in providing a more comprehensive and pluralistic view of the ecological processes discussed in this work.

5. Conclusion

The results obtained in this study highlighted the effect of multiple spatial and temporal factors on the structuring of ciliate protozoa in the water column of Neotropical streams. Among the factors analyzed, both the type of environment and the watershed determine differences in the abundance and diversity of this group of protists. Additionally, the observed patterns are further influenced by rainfall seasonality, which greatly alters the hydrodynamics of these systems. Contrary to expectations, at a local scale, the hydrodynamic patterns considered here as mesohabitats did not affect the community attributes analyzed—namely, richness, abundance, and composition—possibly due to the continuous and unidirectional transport of organisms between the different mesohabitats.

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Data availability

All research data analyzed in the research is available in Acta Limnologica Brasiliensia's Dataverse in SciELO Data. Access is open for all researchers, without restrictions. It can be accessed in <https://data.scielo.org/dataset.xhtml?persistentId=doi:10.48331/scielodata.FDRK2P>.

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