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RESEARCH ARTICLE

# Spatial and temporal variation in species composition of ciliates communities (Alveolata, Ciliophora) from tropical urban and rural streams

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**Abstract** – The aim of this study was to investigate the spatial and temporal patterns in species composition of ciliates, in rural streams, affected by agricultural activities, and urban streams, impacted by domestic wastewater. Samplings were taken in two different periods of the year, in the headwater, middle and mouth stretch of ten streams. We recorded 143 species of ciliates, distributed in 14 groups, standing out Hymenostomatia, *Peritrichia* and *Hypotrichia*. Our results showed significant spatial (between rural and urban streams) and, especially, temporal differences (between winter and summer periods) in the ciliates taxonomic composition. Such differences seem to be not related to the organic load that was quite similar among streams and periods sampled. Rather, the changes in ciliates composition are probably driving mainly by other enviromental variables such as resources, determined by the spatial diferences in light availability, and flow water velocity and discharge, which present high temporal dissimilarity.

**Keywords:** Tropical streams / lotic environmental / urban streams / protist

## 1 Introduction

Anthropogenic activities implicate a series of environmental disturbances that directly affect aquatic ecosystem functioning (Segovia *et al.*, 2016; Fañani *et al.*, 2021). Rural and urban watersheds suffer from human activities, which alter the characteristics, the balance and the dynamics of natural resources hindering the supply of good quality water (Kuhl *et al.*, 2010; Debastiani *et al.*, 2016; Harfuch *et al.*, 2019).

The increase in discharge of pollutant (be them pesticides or organic pollution) into water systems and urbanization, has often limited the multiple use of freshwater environments. Likewise, the ecosystem services provided by water bodies also become limited, given the impacts on these ecosystems (Madoni, 2005; Mandaric *et al.*, 2018; Ullah *et al.*, 2018).

These negative effects of urbanization on streams have been so prominent in recent years that Meyer *et al.* (2005) proposed the paradigm of the urban stream syndrome. This syndrome describes a set of symptoms common to lotic ecosystems, including changes in hydrology, increase in concentration of nutrients and contaminants, reduction in species richness with increased dominance of taxa tolerant to environmental disturbances (Walsh *et al.*, 2005).

According Dias *et al.* (2008) physical and chemical characteristics, that have been traditionally used to evaluate the effect of these impacts, isolated from the analysis of the biotic community, do not provide enough evidence to completely evaluate water quality. Therefore, biological data associated with these environmental traits are essential tools to evaluate the water quality of rivers and streams and contribute to controlling the release of organic pollutants in urban lotic systems (Lippert *et al.*, 2019; Martins *et al.*, 2017).

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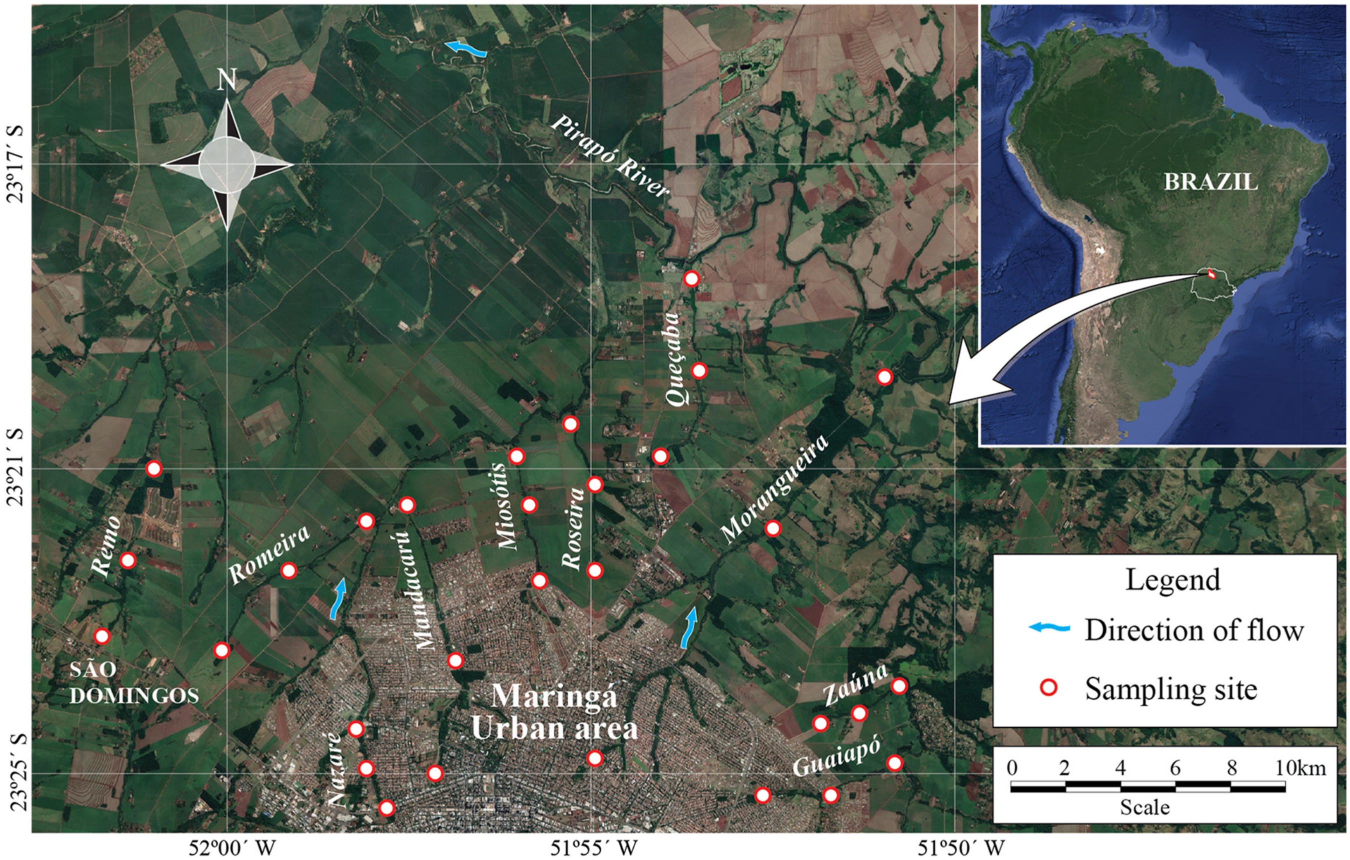


**Fig. 1.** Study area with the location of Pirapó River watershed and studied streams, Maringá County, Paraná State.

Some studies have evidenced that the diversity of ciliates, in both the taxonomic (Bagatini *et al.*, 2013; Dias *et al.*, 2008; Madoni, 2005; Paiva and Silva-Neto, 2004), as well the functional diversity metric (Segovia *et al.*, 2016; Meira *et al.*, 2021), are useful bioindicators for monitoring water quality in tropical impacted streams, since they accurately reflect different anthropogenic disturbances. In addition, according to Camargo *et al.* (2012) it is essential to determine the major factors controlling the structure and dynamics of communities, to subsidize the elaboration of proposals for monitoring, management, conservation and restoration of these ecosystems, which have been extensively modified in the recent decades.

In tropical lotic environments, some protists are primarily controlled by seasonal variation in limnological features, determined by the rainfall regime (Camargo and Velho, 2011). On the other hand, spatially, the water quality, heavily affected by the human impacts from the land use and occupation, can be more important than the seasonality of the rainfall regime on the structure of these communities (Camargo *et al.*, 2012). These authors found that, the water quality seems to be the most important factor for the structure of protists flagellates community composition in tropical streams under direct urban influence.

Although the ecological role of protist ciliates to the aquatic ecosystem metabolism has already been highlighted previously, this community is still little studied, mainly in tropical environments. In the same way, studies concerning ciliates from lotic environments are scarce throughout the world. Nevertheless, we must highlight the studies undertaken by Colzani and Alves (2013), Debastiani *et al.* (2016), Dias *et al.* (2008), Madoni (2005), Madoni and Braghiroli (2007), Mieczan et al. (2013, 2017), Rossi *et al.* (2016), Segovia *et al.* (2016) and Dias *et al.* (2021).

In this context, this study aimed to investigate the spatial and temporal flutuations in species composition of the protist ciliates community in10 urban and rural streams, in two distinct periods of the year (winter and summer). We predicted that: (i) distinct species composition will be found in rural and urban streams, considering the main source of impact of each type of stream (agricultural versus urban) and (ii) temporally, the species composition will change between the two periods of the year, considering the limnological distinctions drove by the temperature and rainfall regime.

## 2 Materials and methods

### 2.1 Study área

This study was undertaken using 10 streams within the urban and rural perimeter of Maringá County, Paraná State (Fig. 1), belonging to the Pirapó River watershed, which is found in the physiographic region of Third Plateau of the Paraná State (22°30′S and 23°30′S; 51°15′W and 52°15′W), with a catchment area of about 487.012 $km^2$ (IBGE, 2021) that drains an area of approximately 5000 $km^2$ (Segovia *et al.*, 2016).

The climate is subtropical, with abundant rain during summer and dry winters, with mean annual temperatures above 20 °C. The average flow of the stream currents varies from 0.13 to 0.32 m/s (Segovia *et al.*, 2016). The region is relatively industrialized and urbanized, and the Municipality of Maringá is the most important urban center in the region, having about 430,157 inhabitants (IBGE, 2020).

In several stretches of these streams there is intense siltation, due to the changes in riparian vegetation, which is caused mainly by anthropogenic activity and irregular use and occupation of the soil. Furthermore, there is wastewater input to most of the streams found in this urban area (personal observation).

Of the 10 studied streams, five streams were considered rural (Queçaba, Remo, Romeira, Roseira and Zaúna), and mainly affected by agricultural effluents, including fertilizers and pesticides (Fig. 1). The Guaiapó, Mandacaru, Miosótis, Nazaré and Pirapozinho streams were considered urban due to the impacts caused by domestic and industrial effluents. However, among these streams, only the Nazaré is totally within the urban area; the Mandacaru has its headwater and middle course in this area, whereas the rest of the streams have only headwater located in the urban perimeter (Fig. 1).

## 2.2 Sampling and periodicity

Samplings of ciliates were performed during two periods of the year: during the winter of 2007 (August), and summer of 2008 (February). Subsurface samplings were taken in triplicate in three stretches of each stream, in the headwater, middle course and mouth, comprising 30 sampling sites and 180 samples.

For each sample, we collected two liters of water using plastic flasks. The samples were kept in thermal boxes until they arrived at the laboratory, where the analysis of species composition was undertaken.

In addition to biological communities, analyzes of limnologic variables were also measured: water temperature (°C), air temperature (°C), pH (Digimed potentiometer DM-23), conductivity (μS/cm; Digimed conductivimeter (DM-3P-E2), dissolved oxygen (mg/L; YSI 550A digital portable oximeter). Further samples were collected to determine the concentrations of total nitrogen (mg/L), phosphate (mg/L), the chemical demand of oxygen (mg O2/L), the biochemical demand of oxygen (mg O2/L) (APHA, 2012). For heavy metal analysis (Pb, Mo and Hg), the samples of water were kept refrigerated, and later sent to a specialized laboratory to quantify the measures and values of these attributes. The sample was centrifuging and after by inductively coupled plasma optical emission (ICP OES) for large and selected oligoelements. The spectrophotometry was done by atomic absorption according to American Public Health Association (APHA, 2012). For the determination of oils and fat, a solvent extraction method was used, according to Best and Ross (1977).

The current velocity measurements for each stream were made with the aid of a JDC Eletronic Flow-meter, model Flowatch (Cunico *et al.*, 2012). The discharge was calculated using the equation: $Q = A \times V$, where $A$ = cross-sectional area of the channel, and $V$ = the current velocity in m/s (Hauer and Lamberti, 2011). The canopy openness was quantified as a percentage of the water not covered by the natural spread of foliage from plants. At each sampling point, 25 measures were taken 10 cm apart using quadrants (0.50 m × 0.50 m), from visual observation to identify shading. The relative frequencies were calculated from the number of measures in which did not occur shading and the total number of measurements taken (Cunico *et al.*, 2012, Segovia *et al.*, 2016).

## 2.3 Laboratory analysis

The analysis of species composition of ciliates was performed *in vivo,* immediately after the samplings, to avoid the loss of cells and alterations in the cell's shape and dimensions. The taxonomic framework followed Adl *et al.* (2019).

Ciliate samples were concentrated to a volume of 100 mL, using a plankton net with a mesh size of 10 μm. Using monochannel pipettes, we analised 20 aliquots of 50 μL, totaling 1 mL for each sample. These aliquots were analyzed on glass slides under an optical microscope. With adequate magnifications (100×, 400× and 1000×), the ciliates were identified at specific levels whenever possible, using a specialized bibliography: Foissner *et al.* (1999), Foissner and Berger (1996) and Lynn (2008).

## 2.4 Data analysis

To characterize the studied environments, we summarized data from physical and chemical water variables in an Analysis of Principal Components (PCA). We used the Broken-Stick criterion (Jackson, 1993) to select the significant PCA axes. Before the analysis, environmental variables were log transformed, except for pH. PCA was performed using the function "prcomp" from vegan package (Oksanen *et al.*, 2018).

The frequency of occurrence of each species (Dajoz constancy index-c) was calculated by the percentage of samples in which each species occurred. Based on their occurrence, the species were classified as constant (present in more than 50% of the samples), accessory (present in 25% to 50% of the samples) or accidental (present in less than 25% of the samples) (Dajoz, 1973).

For synthetize the temporal and spatial distribution of ciliates community, we performed an analysis of homogeneity of multivariate dispersions (PERMDISP; Anderson *et al.*, 2006). This test is based on the average dissimilarities from each sample to the centroid of its group, in a multivariate space built using principal coordinate analysis (PCoA; Anderson *et al.*, 2006). Thus, higher variations in community structure across sites (*i.e.*, beta diversity) are depicted by greater dissimilarities to a group's centroid (Anderson *et al.*, 2006). Four groups were considered, representing the combination of season and landscape type (summer rural (SR); summer urban (SU); winter rural (WR); winter urban (WU)). Statistical significance among group centroids was assessed through 999 permutations. Ordinations were performed on dissimilarity matrices generated using the Jaccard index, calculated from

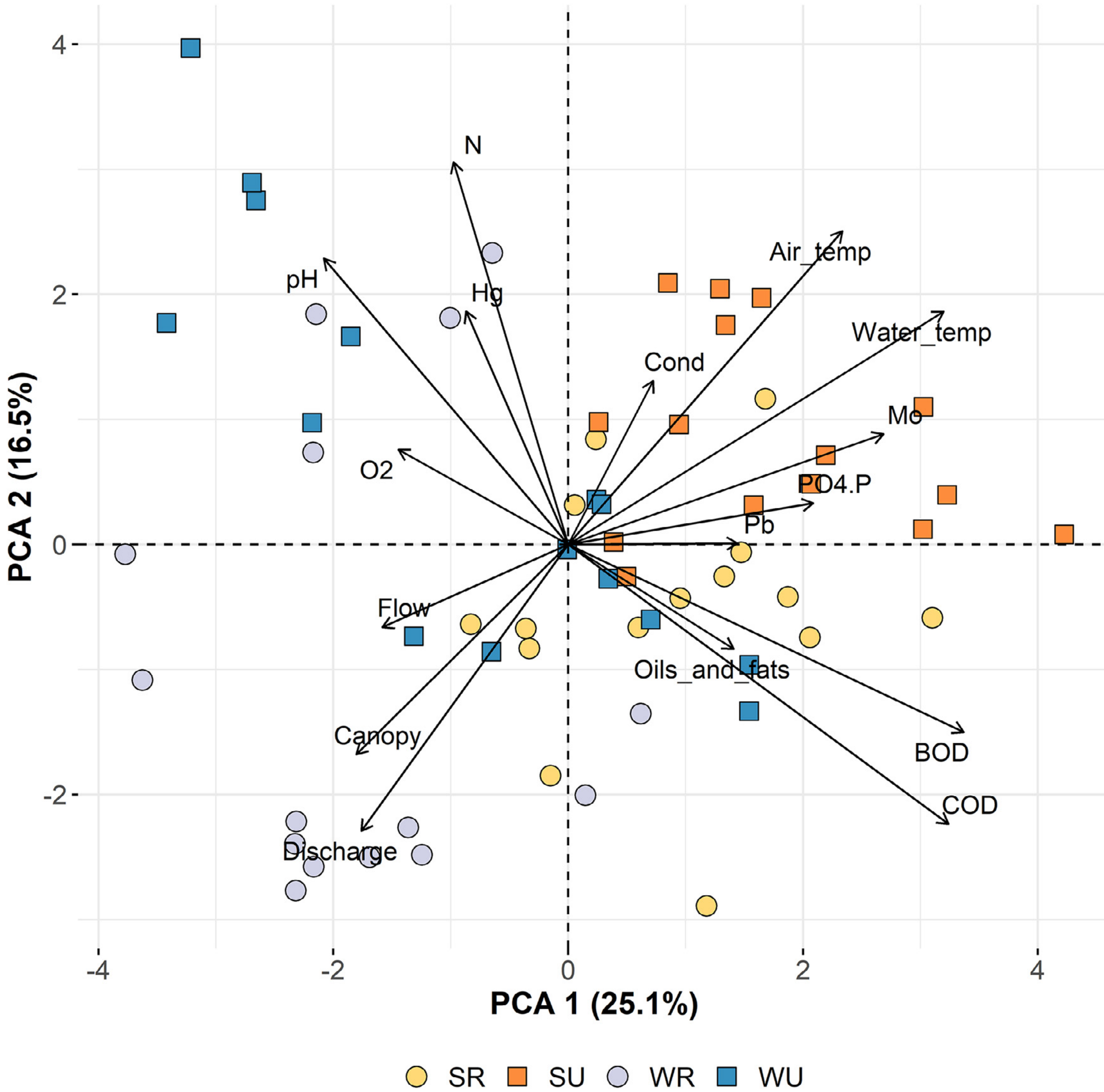


**Fig. 2.** Principal Component Analysis (PCA) results based on the physical and chemical parameters measured in urban and rural streams, during two different hydrological periods (winter and summer).

site-by-species presence-absence data, using the function "betadisper" from vegan package (Oksanen *et al.*, 2018). In addition, we used Moran's I correlograms (Legendre and Legendre, 2012) to check if the control for spatial autocorrelation bias was required, which could somehow inflate the significance of each predictor.

Differences in the composition of the ciliate community between summer and winter seasons, as well as between rural and urban areas, were tested using a Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2005), applied to a species presence-absence matrix, using the function "adonis" from the R package *vegan*. The PERMANOVÁs assumption of homogeneity of dispersion among tested groups was verified with PERMDISP described above. The Jaccard distance was used as a measure of dissimilarity and 9999 permutations to assess the significance of the pseudo-F derived from PERMANOVA. Finally, to verify if differences in Jaccard dissimilarity are related to turnover or nestedness beta-diversity components (Baselga, 2010), we used the function "beta.multi" from the R package *betapart.*

## 3 Results

Results of the Principal Component Analysis (PCA) evidenced a higher temporal than spatial segregation of sampling units. In this way, the first PCA axis, the only significant ($p < 0.05$), discriminated samples from the winter period, negatively correlated to this axis, and characterized by the high values of dissolved oxygen, discharge and water flow, from the summer samples, positively correlated to this axis, and presenting higher values of air temperature, water temperature, BOD, DOC and $PO_4$ (Fig. 2).The ordination also suggested, although not significantly ($p > 0.05$), a spatial segregation of the samples. In this way, most of samples from rural streams are negativily correlated to the second axis of

PCA and characterized by the high values of canopy aperture, while urban streams were, in general, positively correlated with this axis and presented high nitrogen concentations, besides the highest values of conductivity, especially in the summer. More information about the physical and chemical characteristics of each stream sampled can be found in the supplementary material (Supplementary Material, Table S1).

We recorded 143 species of ciliates, belonging to 15 groups, among which *Hymenostomatia* (25 species), *Peritrichia* (15 species) and *Hypotrichia* (15 species) were the most representative (Tab. 1). In addition to these, which stood out in both types of streams, *Pleurostomatida* and *Euplotida* were also importante in urban streams and *Prostomatea* in rural ones (Fig. 3).

At the species level, when comparing the different types of streams, 116 species were reccorded in rural streams and 98 species in urban ones (Tab. 1).

Through the results from Dajoz index, we verified that *Colpoda steinii* Maupas, 1883, *Glaucoma scintillans* Ehrenberg, 1830, *Urocentrum turbo* (Mueller, 1786) Nitzsch, 1827, *Aspidisca cicada* (Mueller, 1786) Claparède and Lachmann, 1858, *Vorticella convallaria* Linnaeus, 1758, *Coleps hirtus* (Mueller, 1786) Nitzsch, 1827, *Urotricha farcta* Claparede and Lachmann, 1859 and *Cinetochilum margaritaceum* Perty, 1849 were the most constant species (Tab. 1). On the other hand, other species, as *Actinobolina smalli* Holt *et al.*, 1973, *Stentor multiformis* (Mueller, 1786) Ehrenberg, 1838, *Paramecium caudatum* Ehrenberg, 1833, *Stylonychia mytilus* Ammermann, 1971, *Opercularia coarctata* (Claparède and Lachmann, 1858) Roux, 1901 and *Urotricha furcata* Schewiakoff, 1892 were found to be accidental (Tab. 1).

In rural streams, 40 exclusive species were recorded, such as *Coleps elongatus* Ehrenberg, 1831, *Cothurnia annulata* Stokes, 1885, *Limnostrombidium* sp., *Loxodes magnus* Stokes, 1887, *Plagiocampa* sp., *Pseudomicrothorax dubius* (Maupas, 1833) Penard, 1922, *Rimostrombidium humile* (Penard, 1922) Petz and Foissner (1992), *Rimostrombidium lacustris* (Foissner *et al.*, 1988) Petz and Foissner, 1992, *Sathrophilus muscorum* (Kahl, 1931) Corliss, 1960 and *Zoothamnium arbuscula* (Ehrenberg, 1831) Ehrenberg, 1838 (Tab. 1). Otherwise, 29 species were registered only in urban streams, such as *Amphileptus pleurosigma* (Stokes, 1884) Foisnner, 1984, *Drepanomonas revoluta* Penard, 1922, *Euplotes* cf. *aediculatus* Pierson, 1943, *Frontonia elliptica* Beardsley, 1902, *Opercularia coarctata, Oxytricha setigera* Stokes (1891), *Podophrya fixa* (Mueller, 1786) Ehrenberg, 1833, *Podophrya* sp., *Trochioides recta* (Kahl, 1928) Kahl, 1931and *Uronema nigricans* (Mueller, 1786) Florentin, 1901 (Tab. 1).

The ordination based on PCoA axis reveal that higher dissimilarities in ciliates species composition occurred between the studied periods (August/winter and February/summer), although spatial segregation of samples had also been evidenced by the analysis (Fig. 4). In this way, the PERMANOVA evidencied significant differences in the species composition of ciliates community between periods (Pseudo $F = 4.70$; $p < 0.001$) and types of streams (Pseudo $F = 2.69$; $p < 0.001$). On the other hand, no pattern was observed for the stream stretches (Pseudo $F = 0.94$; $p = 0.628$). We found that total dissimilarity (0.981) was strongly related to turnover (0.973) than nestedness component (0.001).

PERMDISP results revealed that the magnitude of the dissimilarity was not significantly different among seasons and streams areas ($p = 0.163$) (Fig. 4). The spatial distribution of the sampled sites did not present bias related to spatial autocorrelation structures according to Moran's I correlograms ($p = 0.137$).

## 4 Discussion

The limnological characterization of the streams here investigated, derived from a PCA, evidenced greater environmental changes between the summer and winter samples, than between urban and rural streams. This heterogeneity between seasons was driven especially by temperature, as expected, but also by nutrients and organic matter (BOD and DOC), which showed high values in February (summer), and by O2 and hydrodynamic conditions (water flow and discharge), during the winter. Tropical streams present climatic variations related especially to the seasons, which influence the water regimes controlled by the rains, and which drive the physical and chemical struturation of these ecosystems (Amaral *et al.*, 2015).

Spatially, we expected an expressive distinction between rural and urban streams, especially in terms of the type of impact (agro-industrial pollutants and organic load) and also vegetation cover. However, a weak difference was evidenced between these types of streams. The greater opening of canopy in rural streams is certainly associated with use of the soil for agricultural production (Allan and Castillo, 2007; Thompson and Townsend, 2004), while in the urban ones, at least part of the valley (and their vegetation) is protected by municipal law. On the other hand, the higher conductivity and pH in urban streams suggests an impact of industrial origin in these environments (Daniel *et al.*, 2002; Silva *et al.*, 2012).

In relation to the protist ciliates community, corroborating the trend recorded in other studies about ciliates from rivers and streams (Cleven, 2004; Madoni, 2005; Madoni and Braghiroli, 2007; Reiss and Schmid-Araya, 2008; Debastiani *et al.*, 2016; Negreiros *et al.*, 2017), Hymenostomatia, *Hypotrichia* and *Peritrichia* were the most representative in the present study. Most species of these groups are characterized as interstitial benthic; however, they are commonly found in pelagic zones from continental aquatic environments (Foissner *et al.*, 1999; Cleven, 2004; Mansano *et al.*, 2013; Negreiros *et al.*, 2017; Pauleto *et al.*, 2017; Abamo *et al.*, 2020; Kaur *et al.*, 2021). Among the species identified as constant for rural and urban streams, *Cinetochilum margaritaceum, Urocentrum turbo, Coleps hirtus* and *Glaucoma scintillans* have been considered by other authors to be the dominant taxa in ciliates river communities (Andrushchyshyn *et al.*, 2007; Madoni and Braghiroli, 2007; Segovia *et al.*, 2016).

Corroborating the pattern observed for the physical and chemical characterization of the studied streams, the results of ciliates species composition also showed a more remarkable between winter and summer than spatial change in the organization of the community. Such results evidenced that community of protist ciliates was mainly structured by the greater heterogeneity, responded to the changes in

**Table 1.** Faunistic survey and Dajoz index of ciliate protozoan community in rural and urban streams and respective regions (H = headwater, Mc = middle course, M = mouth), during two hydrological periods (dry and rainy) (+ = accidental, ++ = accessory, +++ = constant).

| Taxa | Rural | | | | | | Urban | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dry | | | Rainy | | | Dry | | | Rainy | | |
| | H | Mc | M | H | Mc | M | H | Mc | M | H | Mc | M |
| **COLPODEA Small and Lynn, 1981** | | | | | | | | | | | | |
| *Colpoda* sp. | | | | | | | + | + | | | | |
| *Colpoda steinii* Maupas, 1883 | +++ | +++ | +++ | | | | +++ | +++ | ++ | + | | +++ |
| *Cyrtolophosis mucicola* Stokes, 1885 | ++ | ++ | + | | ++ | + | | | | | | + |
| **CYRTOPHORIA Faure-Fremiet in Corliss, 1956** | | | | | | | | | | | | |
| *Chamydonella alpestris* Foissner, 1979 | | | | | | + | | | + | | | |
| *Chilodonella uncinata* (Ehrenberg, 1838) Strand, 1928 | + | ++ | ++ | | | + | + | +++ | ++ | | | + |
| *Trithigmostoma cucullulus* (Mueller, 1786) Jankowski, 1967 | | | + | | | | | ++ | ++ | | | |
| *Trithigmostoma steinii* (Blochmann, 1895) Foissner, 1988 | | | | | | | + | + | | | + | |
| *Trochilia minuta* (Roux, 1899) Kahl, 1931 | | + | + | | | | | + | | | | |
| *Trochioides recta* (Kahl, 1928) Kahl, 1931 | | | | | | | | ++ | + | | | |
| *Trochiloides* sp. | | + | + | | | | | | | | | |
| **HAPTORIA Corliss, 1974** | | | | | | | | | | | | |
| *Actinobolina smalli* Holt *et al.*, 1973 | | | | + | | | | | | | | |
| *Askenasia volvox* (Eichwald, 1852) Kahl,1930 | | | | + | | | | | | + | | |
| *Chaenea stricta* (Dujardim, 1841) Foissner *et al.*, 1995 | + | | + | + | | | + | + | | | | |
| *Didinium nasutum* (Muller, 1773) Stein, 1959 | | | | + | | | | | | | | |
| *Enchelys gastenosteus* Kahl, 1926 | + | | | | | | | | | + | + | |
| *Enchelys* sp. | | | | + | | | | | | | | |
| *Lacrymaria olor* (Mueller, 1786) Bory de Saint-Vincent, 1824 | | | | | + | + | | | | | | + |
| *Mesodinium pulex* (Claparède and Lachmann 1859) Stein, 1867 | | | + | +++ | ++ | ++ | | | | + | | |
| *Monilicaryon monilatus* (Stokes, 1886) Jankowski, 1967 | | | | + | | + | | | | | | |
| *Phialina* sp. | | | | | | + | | | | | | |
| *Plagiopyla nasuta* Stein, 1860 | | | | | + | | | | | | | |
| *Protospathidium* sp. | | | | | | + | | | | | | |
| *Trachelius ovum* (Ehrenberg, 1831) Ehrenberg, 1938 | | | | + | + | | | | | +++ | | ++ |
| **HETEROTRICHEA Stein, 1959** | | | | | | | | | | | | |
| *Blepharisma* sp. | | | + | | | | | | | | | |
| *Bryometopus* sp. | | | | | | | | | | | | + |
| *Bryophyllum* sp. | | | | | | + | | | | | | |
| *Caenomorpha uniserealis* Levander, 1894 | | | + | ++ | ++ | +++ | | | | + | | |
| *Spirostomum minus* Roux, 1901 | | + | | | | | | | + | | | |
| *Spirostomum teres* Claparède and Lachmann, 1858 | | | | + | | | | | | | | |
| *Stentor muelleri* Ehrenberg, 1831 | + | | | + | | | | + | | + | | |
| *Stentor multiformis* (Mueller, 1786) Ehrenberg, 1838 | | | | | + | | | | | | | |
| *Stentor roeselii* Ehrenberg, 1835 | + | | | | | | + | + | | | | |
| **HYMENOSTOMATIA Delage and Herouard, 1896** | | | | | | | | | | | | |
| *Colpidium colpoda* (Losana, 1829) Stein, 1860 | | | | | | | | | | + | | |
| *Colpidium kleini* Foissner, 1969 | | | | | + | + | | | | | | |
| *Ctedoctema acanthocryptum* Stokes, 1884 | ++ | + | +++ | + | + | | | | | | + | ++ |
| *Dexiostoma campylum* (Stokes 1886) Jankowski, 1967 | + | + | + | | | | | + | + | + | + | + |
| *Dexiotricha* sp. | | | | + | +++ | +++ | | | | + | | |
| *Epenardia myriophilli* (Penard, 1922) Corliss, 1971 | | + | | | | | | | | | | |
| *Frontonia acuminata* (Ehrenberg, 1833) Buetschli,1889 | | + | | + | | + | | | | | | |
| *Frontonia elliptica* Beardsley, 1902 | | | | | | | | | | | + | + |
| *Frontonia leucas* (Ehrenberg, 1833) Ehrenberg, 1838 | | | | | + | | + | | + | | | |
| *Glaucoma frontata* (Stokes, 1886) Cunha, 1913 | + | ++ | | | + | | | ++ | | | | |
| *Glaucoma scintillans* Ehrenberg, 1830 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++ | +++ | ++ |
| *Kahlilembus attenuatus*(Smith, 1897) Foissner *et al.*, 1994 | | | | + | | | | | | | | |
| *Lembadion bullinum* (Mueller, 1786) Perty, 1849 | + | + | + | | | | +++ | ++ | +++ | | | |
| *Lembadion lucens* (Maskell, 1887) Kahl, 1931 | ++ | ++ | + | ++ | + | +++ | + | | | + | +++ | +++ |
| *Loxocephalus* sp. | | | | ++ | + | | | | | | | + |
| *Ophryoglena* cf. *flava* Ehrenberg, 1833 | | + | | | | | | | | | | |
| *Ophryoglena* sp. | | + | | ++ | + | + | | | | + | | ++ |
| *Paramecium aurelia* (Ehrenberg, 1838) Dujardin, 1841 | + | + | | + | | + | | + | + | ++ | + | + |
| *Paramecium bursaria* (Ehrenberg, 1831) Focke, 1836 | | | | | | | | | | + | | + |
| *Paramecium caudatum* Ehrenberg, 1833 | | | + | | | | | | | | | |
| *Paramecium putrinum* Claparède and Lachmann, 1859 | | | | + | + | | | | | + | + | + |
| *Pseudocohnilembus pusillus* (Quennerstedt, 1869) Foissner and Wilbert, 1981 | | | | | + | | | | | | | |
| *Stokesia vernalis* Wenrich, 1929 | | + | | | | | | | | | | |
| *Tetrahymena pyriformis* (Ehrenberg, 1830) Lwoff, 1947 | | | | ++ | ++ | +++ | | | | ++ | +++ | +++ |
| *Urocentrum turbo* (Mueller, 1786) Nitzsch, 1827 | ++ | + | ++ | + | ++ | +++ | ++ | ++ | + | ++ | + | ++ |
| *Urozona buetschilii* Schewiakoff, 1889 | | | | | | + | | | | | | |
| **EUPLOTIDA Small and Lynn, 1985** | | | | | | | | | | | | |
| *Aspidisca cicada* (Mueller, 1786) Claparède and Lachmann, 1858 | + | ++ | | ++ | +++ | +++ | + | +++ | ++ | +++ | +++ | +++ |
| *Aspidisca lynceus* (Mueller, 1773) Ehrenberg, 1830 | ++ | + | ++ | + | ++ | ++ | | + | + | + | + | |

**Table 1.** (continued).

| Taxa | Rural | | | | | | Urban | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dry | | | Rainy | | | Dry | | | Rainy | | |
| | H | Mc | M | H | Mc | M | H | Mc | M | H | Mc | M |
| *Aspidisca turrita* (Ehrenberg, 1831) Claparède and Lachmann, 1858 | | | | | + | | | | | | | |
| *Euplotes* cf. *aediculatus* Pierson, 1943 | | | | | | | | | | +++ | ++ | +++ |
| *Euplotes moebiusi* Kahl, 1932 | + | + | + | ++ | +++ | + | + | + | ++ | + | + | |
| *Euplotes* sp. | + | | | | | | + | + | + | | | |
| **HYPOTRICHIA Stein, 1869** | | | | | | | | | | | | |
| *Gastrostyla steinii* Engelmann, 1862 | | | + | | | | | | | | | |
| *Halteria grandinella* (Mueller, 1773) Dujardim, 1841 | ++ | + | | + | + | | | | | | + | |
| *Holosticha* cf. *kessleri* (Wrzesniowski, 1877) | | | | | | | | + | | | | |
| *Holosticha monilata* Kahl, 1928 | | + | | ++ | + | | | | | | + | |
| *Holosticha pullaster* (Mueller, 1773) Foissner *et al.*, 1991 | | | | + | | | | + | | | | |
| *Oxytricha chlorelligera* Kahl, 1932 | | + | | | | | + | | | | + | + |
| *Oxytricha haematoplasma* Blatterer and Foissner, 1990 | | | | | | | | | + | + | | + |
| *Oxytricha setigera* Stokes (1891) | | | | | | | | | | | | ++ |
| *Oxytricha similis* Engelmann, 1862 | | | | | | | | | | | | + |
| *Oxytricha* sp. | | | + | | ++ | ++ | | | | + | +++ | ++ |
| *Steinia platystoma* (Ehrenberg, 1831) Diesing, 1866 | | | | + | | | | | | | | |
| *Stichotricha aculeata* Wrzesowski, 1866 | | | | | | | | | | | + | |
| *Stylonychia mytilus* Ammermann, 1971 | | | | | | | | | | | + | |
| *Stylonychia putrina* Stokes, 1885 | | | | + | | | | | | | | |
| *Uroleptus* sp. | | | | | | | | | | | + | + |
| **LOXODIDA Jankowski, 1980** | | | | | | | | | | | | |
| *Loxodes magnus* Stokes, 1887 | | | + | ++ | ++ | | | | | | | |
| *Loxodes rostrum* (Mueller, 1786) Ehrenberg, 1830 | + | | | | + | | + | + | | | | |
| *Loxodes striatus* (Engelmann 1862) Penard, 1917 | | | | + | + | ++ | | | | + | | + |
| **NASSOPHOREA Small and Lynn, 1981** | | | | | | | | | | | | |
| *Chilodontopsis depressa* (Perty, 1852) Blochmann, 1895 | | | | + | | | | | | ++ | + | + |
| *Drepanomonas revoluta* Penard, 1922 | | | | | | | | | | + | | |
| *Leptopharinx costatus* Mermord, 1914 | + | | | +++ | ++ | ++ | ++ | + | | | + | |
| *Microthorax pusillus* Engelmann, 1862 | | | | | | ++ | | | + | | + | + |
| *Microthorax* sp. | | | | | + | + | | | | | | |
| *Nassula picta* Greeff, 1888 | | | | | | | | | | + | | |
| *Pseudomicrothorax agilis* Mermord, 1914 | | | | + | ++ | + | | | | | ++ | |
| *Pseudomicrothorax dubius* (Maupas, 1833) Penard, 1922 | | | | | | +++ | | | | | | |
| **OLIGOTRICHEA Butschili, 1887** | | | | | | | | | | | | |
| *Codonella cratera* (Leidy, 1877) Imhof, 1985 | + | +++ | + | ++ | + | | | + | | | | |
| *Limnostrombidium* sp. | | | | | + | + | | | | | | |
| *Rimostrombidium humile* (Penard, 1922) Petz and Foissner (1992) | | | | | + | + | | | | | | |
| *Rimostrombidium lacustris* (Foissner *et al.*, 1988) Petz and Foissner, 1992 | + | | | + | | | | | | | | |
| *Strobilidium caudatum* (Fromentel, 1876) Foissner, 1987 | | | | | + | ++ | | + | | | | ++ |
| *Tintinnidium* sp. | + | + | + | + | | + | | | | | | |
| **PERITRICHIA Stein, 1859** | | | | | | | | | | | | |
| *Carchesium polypinum* (Linnaeus, 1758) Ehrenberg, 1830 | | + | + | | | | | + | + | ++ | + | + |
| *Cothurnia annulata* Stokes, 1885 | + | | | | | | | | | | | |
| *Epistylis* cf. *entzii* Stiller, 1935 | | | | + | + | + | | | | + | ++ | |
| *Epistylis coronata* Nusch, 1970 | | + | + | | | | | + | + | | | |
| *Epistylis plicatilis* Ehrenberg, 1831 | | | | | | | | + | + | | | |
| *Epistylis* sp. | | | | | | | | | + | | | |
| *Opercularia coarctata* (Claparède and Lachmann, 1858) Roux, 1901 | | | | | | | + | | | | | |
| *Pseudovorticella chamydophora* (Penard, 1922) Jankowski, 1976 | + | ++ | + | + | + | ++ | + | + | ++ | | | |
| *Scyphidia hyalina* Biegel, 1954 | + | | | | | | | | | | | |
| *Vorticella aquadulcis* Stokes, 1887 | | | | + | ++ | ++ | + | ++ | | | | |
| *Vorticella campanula* Ehrenberg, 1831 | +++ | | + | + | +++ | + | +++ | | + | + | | + |
| *Vorticella convallaria* Linnaeus, 1758 | +++ | +++ | ++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| *Vorticella infusionum* Foissner *et al.,* 1994 | | | | | | + | | | | ++ | ++ | ++ |
| *Vorticella octava* (Kahl, 1935) Stiller, 1942 | | | | | | | + | | | | | |
| *Zoothamnium arbuscula* (Ehrenberg, 1831) Ehrenberg, 1838 | | | | | | + | | | | | | |
| **PLEUROSTOMATIDA Schewiakoff, 1896** | | | | | | | | | | | | |
| *Acineria incurvata* Dujardim, 1841 | + | | | | | | + | | | | | |
| *Acineria uncinata* Tulesco, 1962 | ++ | + | + | | | | ++ | +++ | ++ | + | | |
| *Amphileptus claparedii* Stein, 1867 | | | | | | | + | | | | | |
| *Amphileptus pleurosigma* (Stokes, 1884) Foisnner, 1984 | | | | | | | + | ++ | ++ | | | + |
| *Amphileptus punctatus* (Kahl, 1926) Foissner, 1984 | | | + | | | | | | | | | |
| *Litonotus alpestris* Foissner, 1978 | +++ | ++ | ++ | | + | | ++ | +++ | +++ | | + | |
| *Litonotus crystallinus* (Vuxanovici, 1960) Foissner *et al.*, 1995 | + | + | | | | | ++ | +++ | + | | | |
| *Litonotus cygnus* (Mueller, 1773) Foissner *et al.*, 1995 | | | | | | | | | + | | | |
| *Litonotus fusidens* (Kahl, 1926) Foissner *et al.*, 1995 | ++ | | + | | | | + | + | + | | | |
| *Litonotus lamella* (Mueller, 1773 Foissner *et al.*, 1995 | | ++ | + | | | | ++ | ++ | ++ | + | | |

**Table 1.** (continued).

| Taxa | Rural | | | | | | Urban | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dry | | | Rainy | | | Dry | | | Rainy | | |
| | H | Mc | M | H | Mc | M | H | Mc | M | H | Mc | M |
| *Litonotus varsaviensis* (Wrzesniowski, 1986) Wrzesniowski, 1870 | + | + | + | | | | ++ | ++ | ++ | | | |
| **PROSTOMATEA Schewiakoof, 1896** | | | | | | | | | | | | |
| *Balanion planctonicum* (Foissner *et al.*, 1990) Foissner *et al.*, 1994 | + | + | | | | | | + | | | | |
| *Coleps elongatus* Ehrenberg, 1831 | | | | | + | ++ | ++ | | | | | |
| *Coleps* sp. | | | | | + | | | | | | | |
| *Coleps hirtus* (Mueller, 1786) Nitzsch, 1827 | ++ | ++ | +++ | +++ | +++ | +++ | ++ | ++ | +++ | + | ++ | +++ |
| *Holophrya discolor* Ehrenberg, 1833 | | | | + | + | | + | | + | | + | |
| *Holophrya teres* (Ehrenberg, 1833) Foissner *et al.,* 1994 | + | | | | | | | | | | | |
| *Placus luciae* (Kahl, 1926) Kahl, 1930 | | | ++ | + | | ++ | | | | | | |
| *Plagiocampa rouxi* Kahl, 1926 | +++ | + | + | + | + | | + | ++ | + | + | + | + |
| *Plagiocampa* sp. | + | + | + | ++ | | +++ | | | | | | |
| *Prorodon ellipticus* (Kahl, 1930) Foissner *et al.*, 1994 | | | + | | | | | | | | | |
| *Urotricha farcta* Claparede and Lachmann, 1859 | + | ++ | ++ | +++ | +++ | +++ | + | | + | +++ | ++ | ++ |
| *Urotricha furcata* Schewiakoff, 1892 | + | | | | | | | | | | | |
| *Urotricha* sp. | + | +++ | + | | | | + | | | | | + |
| **SCUTICOCILIATIA Small, 1967** | | | | | | | | | | | | |
| *Calyptotricha laguminosa* Penard, 1922 | ++ | ++ | ++ | ++ | ++ | ++ | | | | | | ++ |
| *Cinetochilum margaritaceum* Perty, 1849 | +++ | ++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| *Cyclidium glaucoma* Mueller, 1786 | + | | | | + | | | + | | + | ++ | |
| *Cyclidium heptatrichum* Schewiakoff, 1893 | + | | | ++ | ++ | ++ | | | + | | | |
| *Sathrophilus muscorum* (Kahl, 1931) Corliss, 1960 | ++ | + | + | | | | | | | | | |
| *Uronema nigricans* (Mueller 1786) Florentin, 1901 | | | | | | | + | ++ | | | | |
| **SUCTORIA Claparèdeand Lachmann, 1858** | | | | | | | | | | | | |
| *Podophrya fixa* (Mueller, 1786) Ehrenberg, 1833 | | | | | | | + | + | + | | | |
| *Podophrya* sp. | | | | | | | + | +++ | + | + | ++ | + |

limnological and hydrodynamic conditions. In this way, species or even groups with more or less resistance to the mechanical action of the water flow (Kiss *et al.*, 2009), must predominate in period with more or less water flow velocity.

The temporal influence of hydrodinamic has been registered for several aquatic stream communities (Lampert and Sommer, 1997; Fulone *et al.*, 2008; Cavalheiro and Fialho, 2020). In this way, species or even groups with more or less resistance to the mechanical action of the water flow (Kiss *et al.*, 2009), must predominate in period with more or less water flow velocity. Moreover, while periods with higher water flow and discharge may mitigate environmental impacts due to dilution of total dissolved solids (Ahearn *et al.*, 2004). Periods with reduced water flow, the environments become more susceptible to point sources of pollution (Ramírez *et al.*, 2014) increasing the concentration of nutrients, organic matter and pollutants (Hatt *et al.*, 2004; Yang *et al.*, 2020). Therefore, since the concentration of organic load and pollutants is an important factor influencing the distribution of organisms in streams and rivers (Segovia *et al.*, 2016; Kaur *et al.*, 2021), this may also have contributed to the variation of the Protist ciliates community between the summer and winter samples of the studied streams.

In respect to the spatial variation in species composition, the differences between rural and urban streams seem to be, especially related to the main food resource available in each one. In this way, in the rural streams, we found the riparian vegetation greatly reduced, resulting in greater availability of light. Under such conditions, the primary production is enhanced, favoring algivorous species (Segovia *et al.*, 2016). On the other hand, in urban streams the high input of allochthonous organic matter (from the canopy and the industrial and domestic effluents), as well as the less light penetration due to greater canopy coverage, favor the growth of the bacterial community, and therefore, the predominance of bacterial ciliates (Segovia *et al.*, 2016).

In this way, we observed the occurrence of several ciliates that are characteristically found in environments with a high input of organic matter, such as some streams from the urban zone (like Suctoria). On the other hand, in some rural areas, we found species typical of environments with low organic loading (*e.g.*, species from the Oligotrichea). Moreover, the replacement of *Prostomatea* in rural streams by *Pleurostomatida* in urban ones, suggest an advantage for creeper species in these environments.

In turn, no environmental heterogeneity among stretches (headwater, middle course or mouth) was evidenced by PCA, and this low variability was also expressed by the ciliates community, at least in terms of composition. Thus, any longitudinal variation in limnological conditions, if there is any, seems to be of little importance in driving the organization of ciliate communities, at least in the studied streams.

Considering the components of beta diversity envolved in changing species composition, we found that total dissimilarity of ciliates was explained by turnover component. Using a similar approach to partition beta diversity into the turnover and nestedness components (Baselga, 2010), Soininen *et al.* (2018) observed that the turnover component was clearly more important that the nestedness component in a meta-analysis of 269 data points.

In synthesis, our results showed significant differences in the composition of ciliate communities between the types of

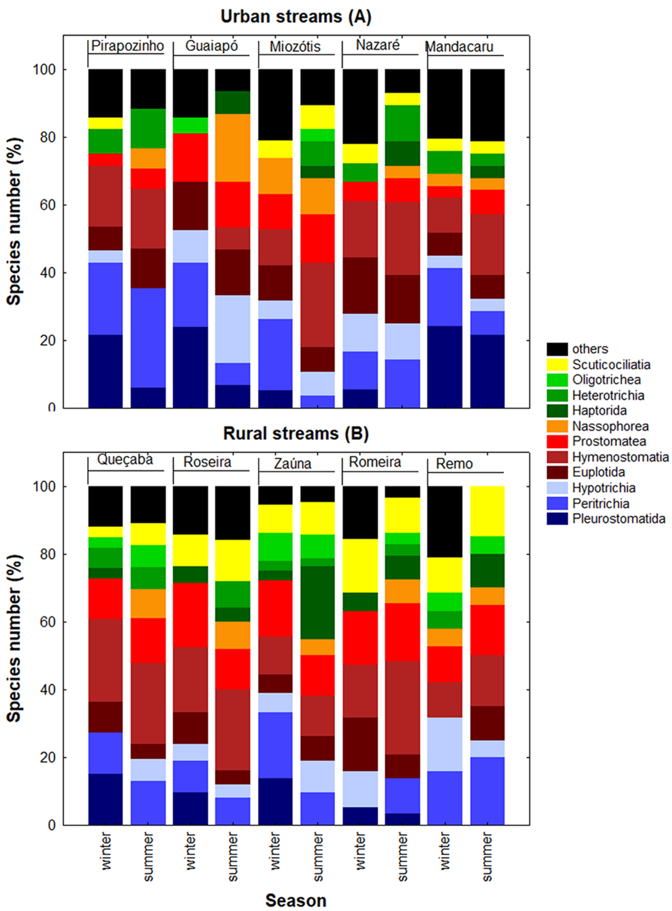


**Fig. 3.** Relative composition of species number by order, for urban streams (A) and rural streams (B) in both summer and winter periods (Pir: Pirapozinho, Gua: Guaiapó, Mio: Miozótis, Naz: Nazaré, Man: Mandacaru, Que: Queçaba, Ros: Roseira, Zau: Zaúna, Rom: Romeira, Rem: Remo).

streams (rural and urban) but, especially, between periods of the year (winter and summer). Such spatial and temporal differences in the ciliates community structure and dynamics are probably driving by the resources, determined by the spatial diferences in light availability, and flow water velocity and discharge, which present high dissimilarity among studied periods.

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### Author contributions

L.F.M.V. and F.A.L.T idealized and coordinated the research. S.F.R.C, G.M.A., F.M.L.T., and L.F.M.V, participated in the samplings, laboratorial and data analysis; B.R.M., G.M.A., S.F.R.C, F.M.L.T., F.A.L.T., F.R.O and L.F.M.V participated in the writing and revision of the manuscript.

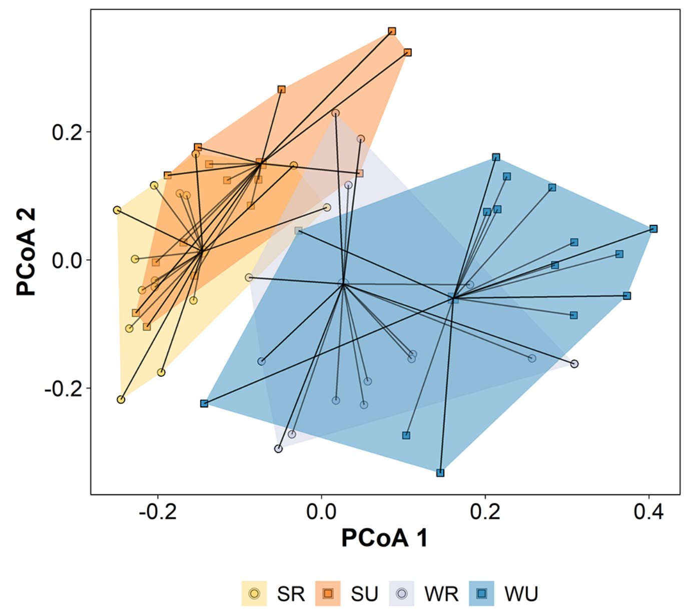


**Fig. 4.** Test for homogeneity of multivariate dispersions (PERMDISP) based on Principal Coordinates Analysis (PCoA) performed from data of species composition of planktonic ciliates in urban and rural streams, in both summer and winter periods.

## Supplementary Material

The Supplementary Material, Table S1 is available at https://doi.org/10.1051/limn/2021022.

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