Limnetica, 46(1): 000-000 (2027). DOI: 10.23818/limn.46.10

The subterranean break: biodiversity shifts in a cave-intersected neotropical stream

Ana Paula Bueno, Marconi Souza Silva, and Rodrigo Lopes Ferreira*

Universidade Federal de Lavras, Instituto de Ciências Naturais, Departamento de Ecologia e Conservação, Centro de Estudos em Biologia Subterrânea, Caixa Postal 3037, CEP 37200-900, Lavras, MG, Brasil.

* Corresponding author: drops@ufla.br

Received: 03/06/25 Accepted: 04/05/26 Available online: 16/06/26

ABSTRACT

The subterranean break: biodiversity shifts in a cave-intersected neotropical stream

Understanding the dynamics of aquatic ecosystems is essential for biodiversity conservation, as ecological processes and species interactions respond sensitively to environmental variation. It is well established that various environmental factors influence species distribution in aquatic systems, both in surface (epigean) and subterranean (hypogean) environments. However, the absence of light in subterranean habitats can create distinct environmental conditions that may alter the composition and structure of biological communities compared to those in surface habitats. In this study, we analyzed the physicochemical parameters of the water and the structural organization of aquatic benthic invertebrate communities along a 300-meter stream that flows through both epigean and hypogean sections. Environmental variables differed markedly between the two sections, and these differences were reflected in the measured parameters along the sampling gradient. A tributary draining epikarst infiltration influenced the environmental conditions within the main cave passage. The species composition of the aquatic invertebrate community also varied between surface and subterranean sections. However, certain species were dominant in both and were key components of the lotic system. Most species were sensitive to the extreme conditions in the cave, including total darkness and limited food availability. The main cave passage was identified as an ecotonal transition zone, where the interface between hypogean and epigean environments acts as a selective filter along the stream continuum, directly shaping the structure of the aquatic community. The findings of this study offer valuable insights for conservation strategies aimed at preserving biodiversity in hypogean ecosystems with similar environmental characteristics.

KEY WORDS: cave, ecological characteristics, aquatic invertebrates, ecotones.

RESUMEN

El quiebre subterráneo: cambios en la biodiversidad en un arroyo neotropical intersecado por cuevas

Comprender la dinámica de los ecosistemas acuáticos es crucial para conservar su biodiversidad, ya que los procesos ecológicos y las interacciones entre especies suelen estar estrechamente interrelacionados. Está bien establecido que varios factores ambientales influyen en la distribución de especies en sistemas acuáticos, tanto en entornos superficiales (epigeos) como subterráneos (hipogeos). Sin embargo, la ausencia de luz en hábitats subterráneos puede generar condiciones ambientales distintas, lo que puede alterar la composición y la estructura de las comunidades biológicas en comparación con las de los hábitats de superficie. En este estudio, analizamos los parámetros fisicoquímicos del agua y la organización estructural de las comunidades de invertebrados acuáticos a lo largo de un arroyo de 300 metros que fluye a través de secciones epigeas e hipogeas. Las variables ambientales diferían marcadamente entre las dos secciones, y estas diferencias se reflejaron en los parámetros medidos a lo largo del gradiente de muestreo. Un afluente que drena la infiltración del epikarst influyó en las condiciones ambientales dentro del pasaje principal de la cueva. La composición de especies de la comunidad de *invertebrados acuáticos también varió entre las secciones superficiales y subterráneas. Sin embargo, ciertas especies fueron dominantes en ambas y fueron componentes clave del sistema lotico. La mayoría de las especies mostraron sensibilidad a las condiciones extremas dentro de la cueva, incluyendo la oscuridad total y la disponibilidad limitada de alimento. El conducto principal de la cueva fue identificado como una zona de transición ecotonal, donde la interfaz entre los entornos hipogeos y epigeos actúa como un filtro selectivo a lo largo del continuo fluvial, modelando directamente la estructura de la comunidad acuática. Los hallazgos de este estudio ofrecen valiosas perspectivas para el desarrollo de estrategias de conservación destinadas a preservar la biodiversidad en ecosistemas hipogeos con características ambientales similares.*

PALABRAS CLAVE: *cueva, características ecológicas, invertebrados acuáticos, ecotonos.*

INTRODUCTION

Rivers are dynamic ecosystems in which physical and biological characteristics change along their longitudinal gradients (Whitton, 1975). The structure of aquatic communities and the functioning of lotic ecosystems are strongly governed by environmental variables such as light availability, hydrological regime, temperature, and nutrient concentrations (Vannote et al., 1980, Merritt & Cummins, 1996, Allan & Castillo, 2007, Pellegrini et al., 2018, Pellegrini et al., 2020, Pacheco et al., 2021, Martins & Ferreira, 2021). These parameters determine the ecological suitability of habitats and play a critical role in supporting distinct assemblages of aquatic fauna (Hynes, 1974, Deatu, 1989, Strayer & Dudgeon, 2010).

Environmental variables such as flow velocity, substrate composition, temperature, dissolved oxygen, and food availability play a central role in determining the structure and functioning of riverine communities. Spatial and temporal fluctuations in these parameters create habitat heterogeneity that subsequently governs species distribution, community structure, and overall ecosystem resilience (Townsend, 1989, Fovet et al., 2018). Along the river continuum, these factors typically exhibit gradual transitions: flow velocity increases, substrates become progressively coarser, and both thermal and oxygen regimes fluctuate. Such gradients determine distinct ecological zones and drive corresponding shifts in community organisation (Williams & Hynes, 1976, Whitton, 1975, Hart, 1992, Ficsór & Csabai, 2021). Understanding how spatial and temporal heterogeneity modulates ecological processes is therefore essential for interpreting patterns of biodiversity and ecosystem functioning in lotic environments. However, in karstic landscapes, longitudinal continuity in river systems is frequently disrupted by subterranean segments, where surface streams intermittently descend underground before resurfacing downstream. Such discontinuities can profoundly affect habitat conditions and biodiversity patterns along the river course.

When a river runs through a subterranean system, its physicochemical characteristics often differ markedly from those of open-channel sections. The absence of light suppresses autochthonous primary production and shifts energy inputs toward allochthonous organic matter (Simon et al., 2003). Water temperature and dissolved oxygen levels typically become more stable, whereas flow velocity and sediment composition may change as a result of confined channel morphology (Datry et al., 2016). These modifications to the physical and chemical conditions can restructure trophic dynamics and influence the diversity and structure of benthic invertebrate assemblages, which are widely recognized as sensitive indicators of ecosystem integrity.

Benthic invertebrates are highly responsive to environmental gradients and therefore represent effective model organisms for assessing the ecological influence of subterranean environments on riverine ecosystems. Sections of rivers affected by cave passages often support assemblages that differ markedly from those of open-surface reaches, typically favouring taxa adapted to low-flow conditions, fine sediments, and reduced primary productivity (Culver & Pipan, 2019). Simultaneously, the occurrence of subterranean species adapted to life in darkness and nutrient-poor habitats can increase local diversity and contribute to greater functional heterogeneity. Consequently, caves may function both as environmental filters (selecting for specific ecological traits) and as refugia that sustain and enrich biodiversity within fluvial networks.

Despite the ecological significance of transitions between subterranean and surface environments, relatively, few studies have investigated how caves modify river habitats and shape benthic invertebrate communities, particularly within tropical karst systems. Most research on subterranean–lotic interactions has been conducted in temperate regions, resulting in a substantial lack of knowledge regarding biodiversity patterns and ecosystem functioning in tropical environments. In this context, the present study investigates how the passage of rivers through caves alters environmental conditions and influences the composition and structure of benthic invertebrate assemblages. We hypothesize that cave sections exhibit distinct physicochemical conditions and benthic community compositions compared with adjacent surface reaches, reflecting the selective pressures imposed by subterranean environments.

This study aims to evaluate the role of caves as potential barriers within stream ecosystems, with a particular focus on how subterranean passages modify physical conditions and benthic invertebrate diversity. We propose that the distinctive environmental characteristics of caves disrupt the typical longitudinal gradient of streams by substantially altering the physical and chemical properties of water. These alterations restructure biological communities in both surface and subterranean reaches, imposing dissimilarity between communities. Furthermore, variation in cave morphology promotes habitat differentiation, influencing the composition and richness of invertebrate communities, increasing beta diversity and creating an ecotone transition zone, even across relatively short spatial scales.

MATERIAL AND METHODS

Study area

The study was conducted along a first-order headwater stream that flows through the Mandembe quartzite cave, located in the municipality of Luminárias (21°32’38.1” S, 44°47’57.3”W "W, 1141 m asl), Minas Gerais State, Brazil (Pellegrini et al., 2020). According to the Köppen climate classification, the region has a Cwb climate, characterized by wet summers and dry winters (Köppen, 1931), with a mean annual temperature of 19.61°C and an average yearly precipitation of 1530 mm.

The quartzite cave extends 244 meters in horizontal projection with a slope of 18 meters. The allogenic Mandembe stream runs through the cave’s main passage, while a secondary passage receives input from an autogenic tributary fed by infiltration from the overlying soil and epikarst system (Fig. 1; Pellegrini et al., 2020). The stream's surface and subterranean sections feature alternating stretches of slow and fast-flowing water. The streambed primarily comprises sand, pebbles, rock slabs, and organic material. The surrounding vegetation is dominated by riparian forest, which lines the stream along both epigean and hypogean sections (Fig. 1, Pellegrini et al., 2020).

Field and laboratory procedures

To test the hypothesis that the cave acts as a barrier, promoting changes in environmental conditions and the associated benthic invertebrate community, sampling was conducted along three 100-meter stretches: one upstream of the cave, one inside the cave, and one downstream from the cave (Fig. 1). Sampling points were spaced approximately 10 meters apart, resulting in 30 points. In addition, four sampling points were established along an autogenic tributary within the cave.

At each sampling site, the following environmental parameters were recorded: water depth and stream width were measured using a tape meter, and water discharge (m^3/s) was subsequently calculated by integrating both variables with current speed (Maghrebi & Ball, 2006). Additional measurements included pH, dissolved oxygen (expressed as both % saturation and mg/L), electrical conductivity (µS/cm), flow velocity (cm/s), and temperature (°C), all obtained using a U-50 multi-parameter water quality checker (Pacheco et al., 2021, Rachmawati et al., 2021). Light intensity was measured with a digital luxmeter (model LD-400). Benthic invertebrates were sampled

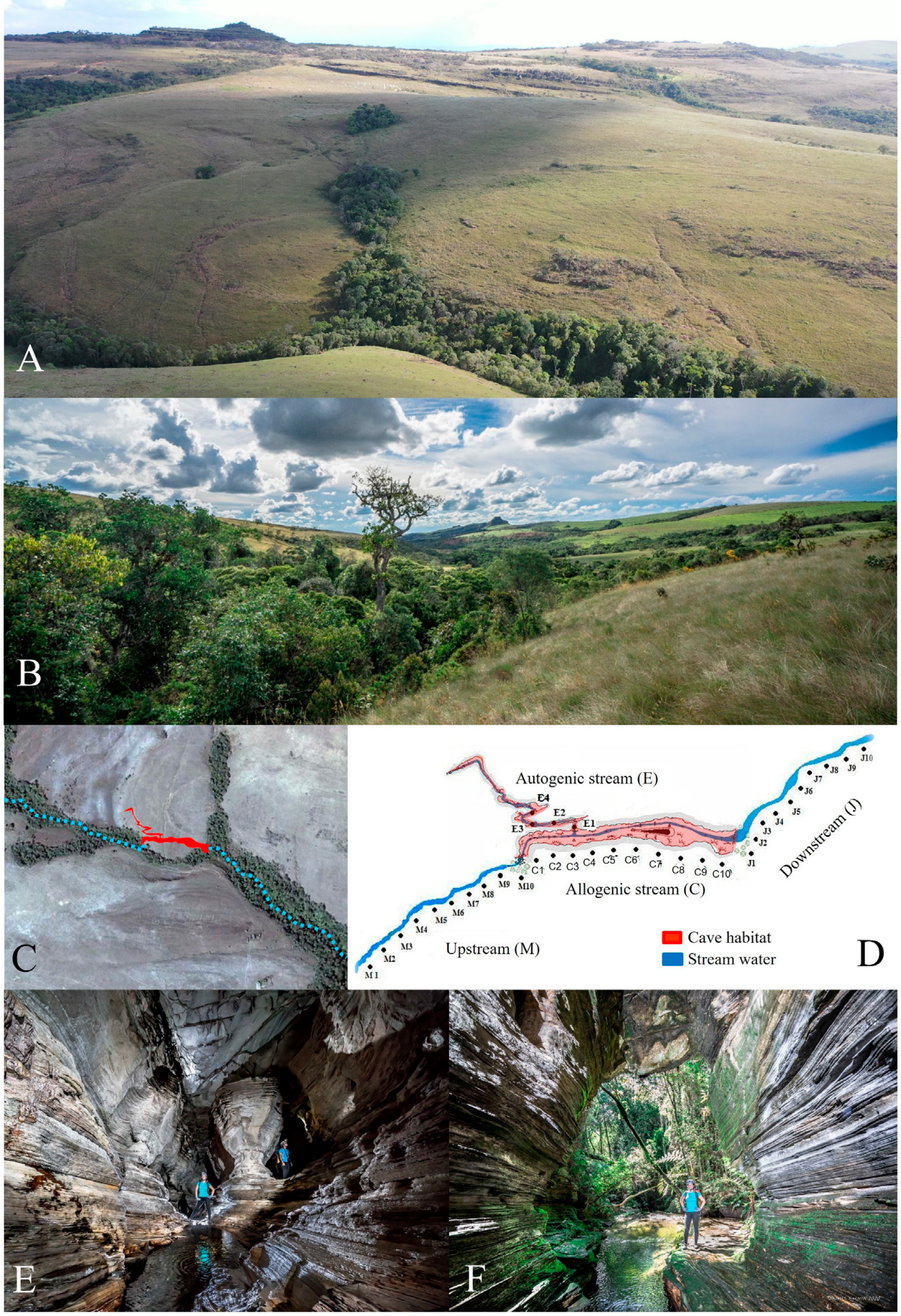

Figure 1. Landscape view of the study area (A, B, C), schematic map showing the sampling station and sections at Mandembe stream (D) and caves (E, F, G). Upstream (M1 to M10), Cave stream with allogenic origin (C1 to C10), cave stream with autogenic origin (E1 to E4), and downstream section (J1 to J10). Photos: Daniel De Stefano Menin. *Vista del paisaje del área de estudio (A, B, C), mapa esquemático que muestra la estación de muestreo y secciones en el arroyo Mandembe (D) y las cuevas (E, F, G). Aguas arriba de la cueva (M1 hasta M10), arroyo de cueva de origen alógeno (C1 hasta C10), arroyo de cueva de origen autógeno (E1 hasta E4) y aguas abajo de la cueva (J1 hasta J10). Fotos: Daniel De Stefano Menin.*

by taking three subsamples at each point using a 30 cm² Surber net with a 250 µm mesh. Samples were placed in labelled plastic bags and preserved in 5% formalin for later analysis (Doretto et al, 2020). Sampling was performed once during the dry season (July).

The samples were washed in a sequence of metallic sieves (from 2mm to 0.250mm) to separate the larger material (large leaves, branches, and rocks) from the smaller particles. After washing, the remaining unconsolidated sediment (mainly sand) was floated off in a supersaturated NaCl solution, facilitating the sorting of the invertebrates under a stereomicroscope. All organisms were preserved in 70% ethanol, identified to the lowest taxonomic level (Pérez, 1988), and grouped into morphotypes (Oliver and Battie, 1996).

Data analysis

We followed the methodology proposed by Prous et al. (2004) to identify the ecotone transition zone. Species in each epigean section (upstream and downstream) were compared based on Jaccard similarity, using equidistant sampling points from the stream's sink and resurgence. Each cave entrance was considered the center of the ecotone, with decreasing similarity values expected as sampling points moved further from the entrance. The boundaries of the ecotone zone were defined as the points where total dissimilarity between epigean and hypogean environments was observed. Beyond this point, it was assumed that no further species overlap occurred between the two adjacent systems. A Principal Component Analysis (PCA) was conducted using the mean values of the physicochemical variables measured at each sampling point. This analysis aimed to identify patterns of environmental variation along the upstream–cave–downstream gradient and in the autogenic stream. The first two principal components derived from the PCA were subsequently correlated with biotic variables through linear regression. As the data did not meet the assumptions of normality, all variables were log-transformed before analysis (Greenacre et al., 2022).

Significant differences in average species richness and physicochemical parameters (pH, oxygen saturation, electrical conductivity, water speed, and temperature) were tested using the Wilcoxon-Mann-Whitney U test (Sprent & Smeeton, 2000). A significance level of $p < 0.05$ was adopted for all statistical analyses.

Species abundance and richness of benthic invertebrates were calculated for each sampling point by summing the three subsamples. A Venn diagram was constructed to assess species overlap among stream sections (Venn, 1880, Heberle et al., 2015). The analyses were performed in R using the ggvenn package. This package is inspired by Venny (https://bioinfogp.cnb.csic.es/tools/venny/index.html).

Invertebrate abundance and richness (alpha diversity) were calculated based on the numbers of individuals and morphotypes per sampling point. Individual-based rarefaction curves were generated to estimate expected species richness in each stream section (Chao & Jost, 2012). The analyses were performed in R software using the package iNEXT (Hsieh et al., 2016). The level of 'completeness' of the sampling effort was achieved by dividing the observed number of taxa by the estimated values calculated by the Jackknife 1 and 2 estimators (Àvila et al., 2019).

A Bray-Curtis similarity matrix, following fourth-root transformation, was used in a one-way analysis of similarity (ANOSIM) with pairwise comparisons to evaluate differences in faunal composition among the upstream, allogenic cave stream, autogenic tributary, and downstream sections (Anderson et al., 2008). The SIMPER (Similarity Percentages analysis) was used to determine the species responsible for sample groupings based on Bray-Curtis dissimilarities (Anderson et al., 2008). Non-metric multidimensional scaling (NMDS), combined with bootstrapping, was performed to visualize dissimilarity patterns in species composition within and between the four stream sections (Anderson et al., 2008). Additionally, the distance-based test for homogeneity of multivariate dispersions (PERMDISP) was employed to assess beta diversity, measuring compositional variation among stream sections (Anderson et al., 2008).

To further investigate the spatial dynamics of environmental factors, we applied Distance-Based Linear Models (DistLM). Jaccard similarity was used for faunal composition (presence/absence data), and Euclidean distance was used for species richness. The forward selection procedure was adopted, with model selection based on the corrected Akaike Information Criterion (AICc) following 999 permutations (Anderson et al., 2008). Distance-based Redundancy Analysis (dbRDA) was then performed to assess the explanatory power of predictor variables on species composition (Anderson et al., 2008).

Finally, we presented a representation of the morphotypes according to the spatial distribution along the stream sections, in a shaded plot. The species composition was re-ordered in cluster analysis using Whittaker's Index of Association (Anderson et al., 2008). The sample points were grouped using a cluster analysis based on the Bray-Curtis similarity index, with clustering by average similarity. The shadeplot was performed in the Plymouth routine in Multivariate ecological research – Primer 7 (http://www.primer-e.com).

RESULTS

Physical attributes of the stream sections

Natural illumination outside the cave ranged from 1550 to 76 700 lux, whereas inside the cave it varied between 0 and 218 lux, with the latter values recorded near the entrances (Table S1, supplementary information, available at https://www.limnetica.com/en/limnetica). In the epigean stream, water depth ranged from 0.5 to 88 cm, whereas in the cave it ranged from 0.6 to 44 cm. Stream width ranged from 1.5 to 80 cm in surface sections and from 1 to 94 cm inside the cave. Water discharge was 0.55 m^3/s upstream, 2.94 m^3/s in the allogenic cave stream, 0.32 m^3/s in the autogenic cave stream, and 7.28 m^3/s downstream (Table S1). The average water temperature, dissolved oxygen, water speed, pH, and electrical conductivity differed significantly among the four stream sections (Fig. 2, Table 1). The statistical significance of these differences is summarized in Table 1. The highest average temperature was recorded in the autogenic stream (20.5 °C, sd = 1), while lower values were observed in the upstream, allogenic cave stream, and downstream sections (15 °C, sd = 0.5). Dissolved oxygen levels were higher inside the cave, particularly in the allogenic and autogenic streams (100% or 8 mg/L). In contrast, lower values were recorded outside the cave (72%, or 5.7 mg/L, sd = 32% and 2.5 mg/L upstream and 51%, or 4.1 mg/L, sd = 34.5% and, 2.4 mg/L downstream). Water speed peaked in the allogenic cave stream section (3.30 cm/s, sd = 2), while the autogenic cave stream exhibited almost stagnant conditions (Table 1, Fig. 2). The average pH values ranged from 4 to 6 (sd = 0.6), with higher averages observed in the autogenic stream (6). Average electrical conductivity ranged from 4 to 6 µS/cm, with the highest values in the downstream section (6 µS/cm) (Table 1).

The first two principal components from the PCA explained 67.8% of the total variance in the environmental dataset (Table 2). Factor 1, accounting for 36.3% of the variance, was primarily characterized by high loadings for conductivity (–0.821) and dissolved oxygen (0.786). Factor 2 explained 31.5% of the variance and was most strongly influenced by pH (0.733), temperature (0.693), and current speed (–0.641). The PCA results revealed a gradient of environmental variation along the stream's epigean–hypogean–epigean course (Table 2). The analysis also indicated that the autogenic stream influenced the ecological conditions along the cave–downstream continuum (Fig. 3A). Specifically, the ecological parameters of the autogenic stream were predominantly associated with Factor 2, which was positively correlated with pH and temperature. In contrast, the central portion of the cave was primarily influenced by Factor 1, which was positively associated with oxygen saturation and negatively with conductivity. Measurements taken near the cave exit were like those from the immediately adjacent downstream section, indicating the continued influence of oxygen saturation and current speed in this zone. Further downstream, however, the impact of Factor 1 diminished, while the effect of increased conductivity became more pronounced.

Benthic invertebrate assemblage attributes

A total of 18 126 specimens, representing 213

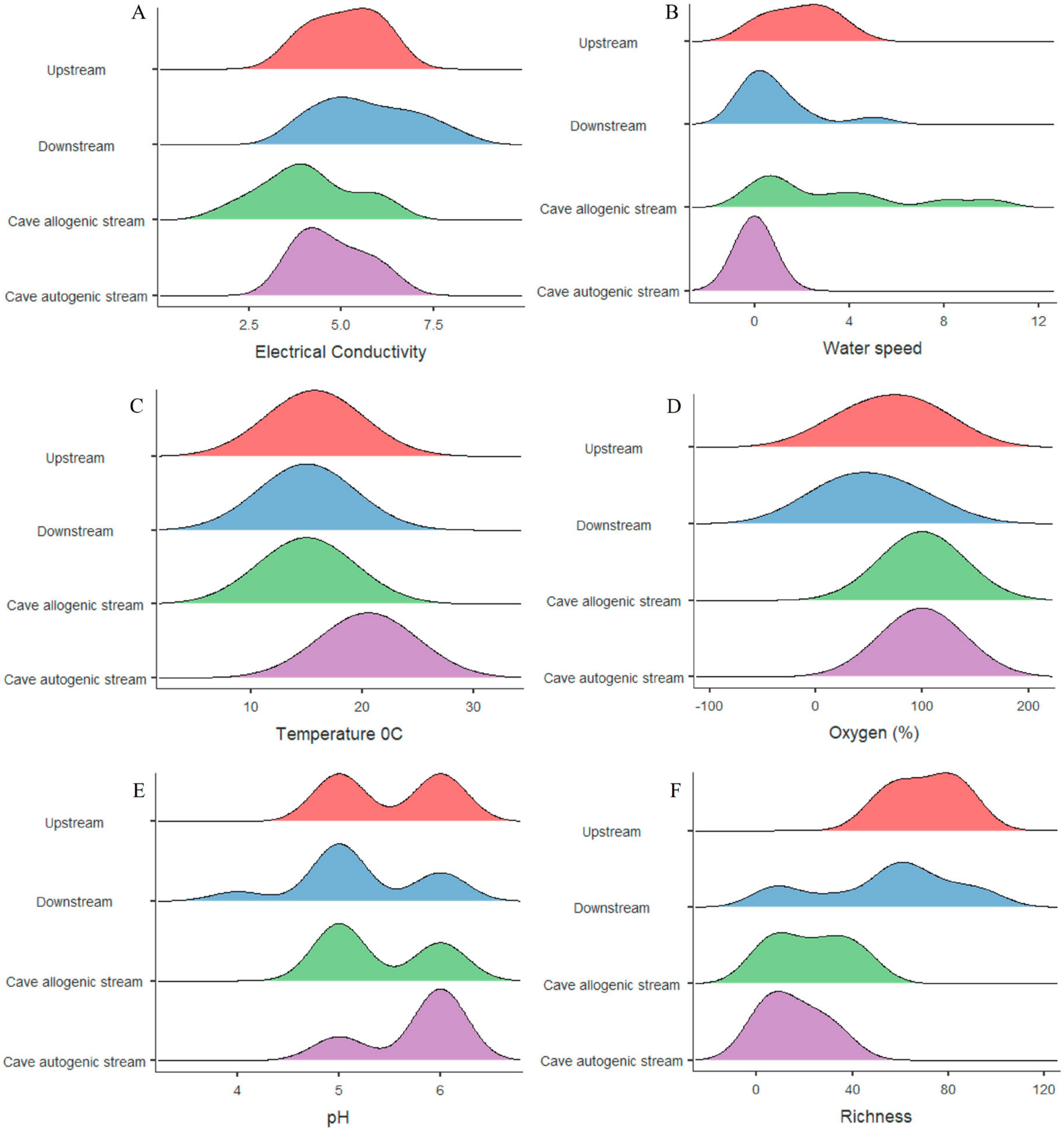

Figure 2. Variations on electrical conductivity (µS/cm), water velocity (cm/s), temperature (°C), oxygen saturation (%), pH, and benthic species richness along four sections of Mandembe stream. Upstream, cave stream, Autogenic stream, and downstream. *Variaciones en la conductividad eléctrica (µS/cm), velocidad del agua (cm/s), temperatura (°C), saturación de oxígeno (%), pH y riqueza de especies bentónicas a lo largo de cuatro secciones del arroyo Mandembe. Aguas arriba de la cueva, arroyo de cueva, arroyo autogénico y aguas abajo de la cueva.*

species, were collected across the four stream sections (Table S2, supplementary information, available at https://www.limnetica.com/en/limnetica). The fauna included representatives of the phyla Cnidaria, Platyhelminthes, Nematoda, Annelida, and Arthropoda, with arthropods comprising 98% of all species. Insects accounted for most species (158), with four orders being particularly well represented: Diptera, Coleoptera, Trichoptera, and Heteroptera. Diptera included 52 species, 27 of which belonged to the family Chironomidae. Coleoptera comprised 39 species, while

Table 1. Significant differences in average species richness and environmental variables of the water in the four sections of the stream. *Diferencias significativas en la riqueza promedio de especies y las variables ambientales del agua en las cuatro secciones del arroyo.*

Variables	**Rank Sum Upstream**	**Rank Sum Cave allogenic stream**	**U**	**Z**	***p*-value**
Temperature	137	73	18	2.381	0.0173
Richness	155	55	0	3.741	0.0002
	Rank Sum Downtream	**Rank Sum Cave allogenic stream**	**U**	**Z**	***p*-value**
Temperature	55	155	0	-3.741	0.0002
Oxygen	55	155	0	-3.741	0.0002
Water speed	78	132	23	-2.003	0.0452
Electrical Conductivity	135	75	20	2.230	0.0257
Richness	138	73	18	2.419	0.0156
Variables	**Rank Sum Downtream**	**Rank Sum Upstream**	**U**	**Z**	***p*-value**
Temperature	55	155	0	-3.741	0.0002
Variables	**Rank Sum Autogenic stream**	**Rank Sum Upstream**	**U**	**Z**	***p*-value**
Temperature	50	55	0	2.757	0.0058
Water speed	14	91	4	-2.192	0.0284
pH	45	60	5	2.050	0.0403
Richness	10	95	0	-2.757	0.0058
Variables	**Rank Sum Autogenic stream**	**Rank Sum Downstream**	**U**	**Z**	***p*-value**
Temperature	50	55	0	2.757	0.0058
Oxygen	50	55	0	2.757	0.0058
pH	46	59	4	2.192	0.0284
Richness	15	90	5	-2.050	0.0403
Variables	**Rank Sum Autogenic stream**	**Rank Sum Cave allogenic stream**	**U**	**Z**	***p*-value**
Temperature	50	55	0	2.757	0.0058
Water speed	12	93	2	-2.474	0.0133
pH	45	60	5	2.050	0.0403

Trichoptera and Heteroptera each contributed 19 species. Mites ("Acari") were also diverse, with 50 species recorded (Fig. 3A).

Total species richness was highest in the upstream section (156 spp.), followed by the downstream section (153 spp.), the allogenic cave stream (90 spp.), and the autogenic cave stream (43 spp.) (Fig. 2). Mean species richness per sampling point was also highest upstream (70.4 spp., sd = 13) and lowest in the autogenic stream (15 spp., sd = 11) (Fig. 2). The estimated species richness suggests that the sampling effort achieved adequate levels of completeness, as the observed richness (213 spp.) corresponded to over 70 to 78% of the estimated richness, according to jacknife 2 and 1, respectively (Fig. 3B and C).

The Venn diagram shows the distribution and overlaps of species among the upstream (156 spp.), downstream (153 spp.), cave allogenic (90 spp.), and autogenic (43 spp.) stream sections (Fig. 4). A total of 52 species (24.4%) were shared among all sections. The upstream and downstream sections exhibited the highest number of exclusive species (38 and 32 spp., respectively), followed by the cave allogenic and autogenic streams (8 spp. each). Additionally, 20 species (9.4%) were shared by three sections (upstream, downstream, and cave allogenic stream).

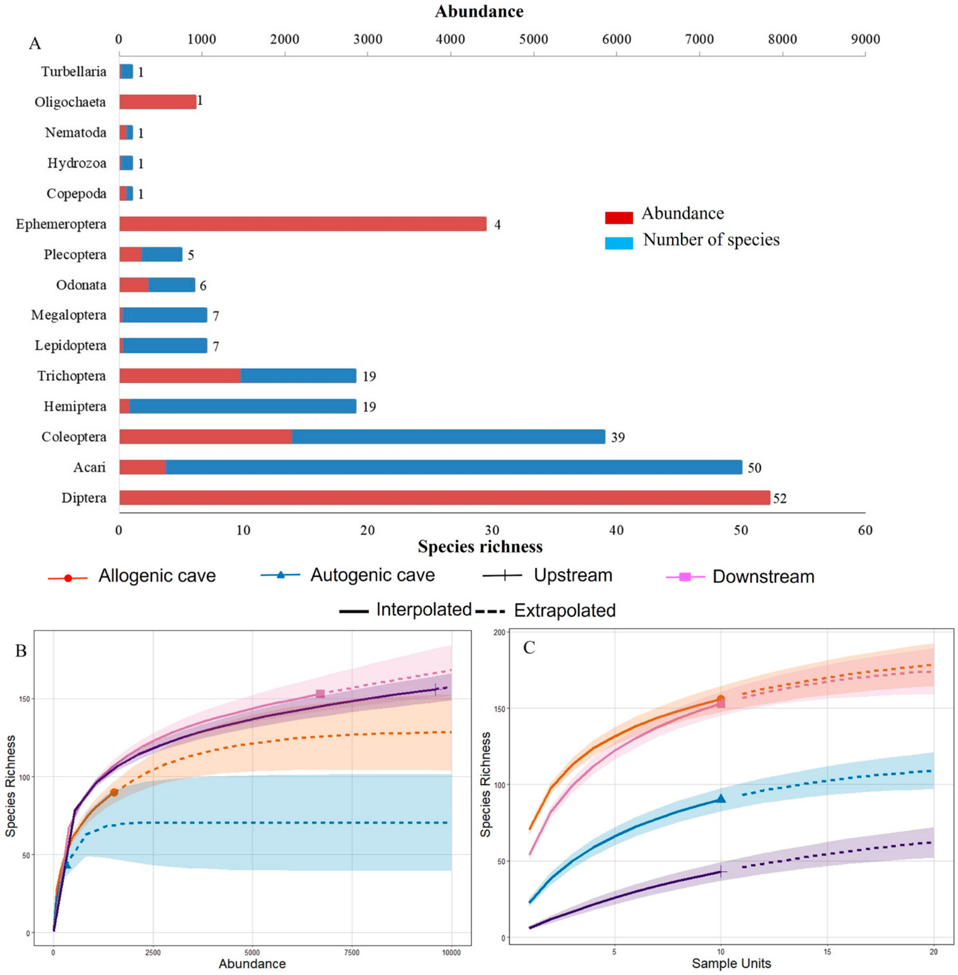

Figure 3. Species richness and abundance (A) of the Mandembe stream and the sample efforts estimation curves for each stream section (B and C). *Riqueza y abundancia de especies (A) del arroyo Mandembe y curvas de estimación del esfuerzo de muestreo para cada tramo del arroyo (B y C).*

These results indicate a substantial core of shared species across the system, with each section also supporting unique assemblages.

The compositional distribution of the most abundant species across the stream sections is shown in Fig. 5. A clear distinction in species composition was observed between hypogean and epigean sections, reflecting environmental filtering and habitat-specific community structuring (Fig. 6A and B).

Ephemeroptera (Baetidae, Leptophlebiidae, Tricorythidae sp1), and Diptera (mainly Chironomidae) were the taxa that most contributed to the disimilarity between the four stream sections (Table S3, supplementary information, available at https://www.limnetica.com/en/limnetica).

Benthic invertebrate assemblage distribution patterns

Similarity values calculated along the upstream and downstream cave gradients indicated the lowest similarity in the central cave region (Fig. 6A), suggesting the greatest divergence in community composition in this area. In the Mandembe Cave, the ecotones at both entrances overlapped, forming a single, extended ecotonal zone along the main passage.

Pairwise comparisons from the ANOSIM test revealed significant differences in faunal composition between several stream sections. Notably, significant dissimilarities were observed between the upstream and cave allogenic stream (Global

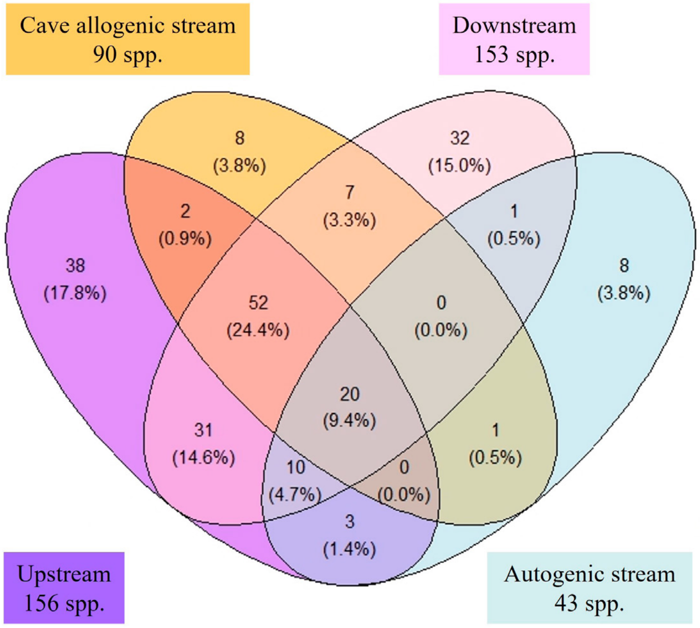

Figure 4. The Venn diagrams between stream sections showing richness and species overlap among the sections of the Mandembe stream. Upstream, cave stream, Autogenic stream, and downstream. *Diagramas de Venn entre secciones del arroyo que muestran la riqueza y la superposición de especies entre las secciones del arroyo Mandembe. Aguas arriba de cueva, arroyo de cueva, arroyo autogénico y aguas abajo de la cueva.*

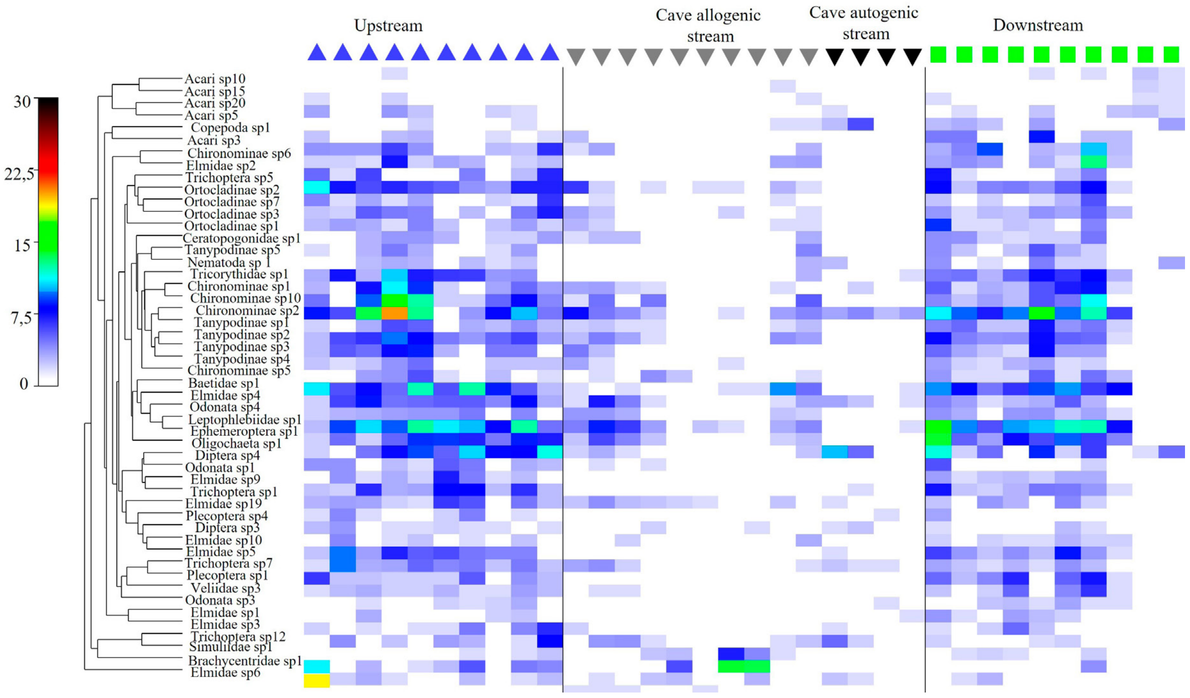

Figure 5. Shadeplot and index of association (left) of the 50 most abundant morphotypes in each stream section. Each line represents a morphotype. Upstream, cave stream, Autogenic stream, and downstream. *Diagrama de sombreado e índice de asociación (izquierda) de los 50 morfotipos más abundantes en cada sección de arroyo. Cada línea representa un morfotipo. Aguas arriba de la cueva, arroyo de cueva, arroyo autogénica, y aguas abajo de la cueva.*

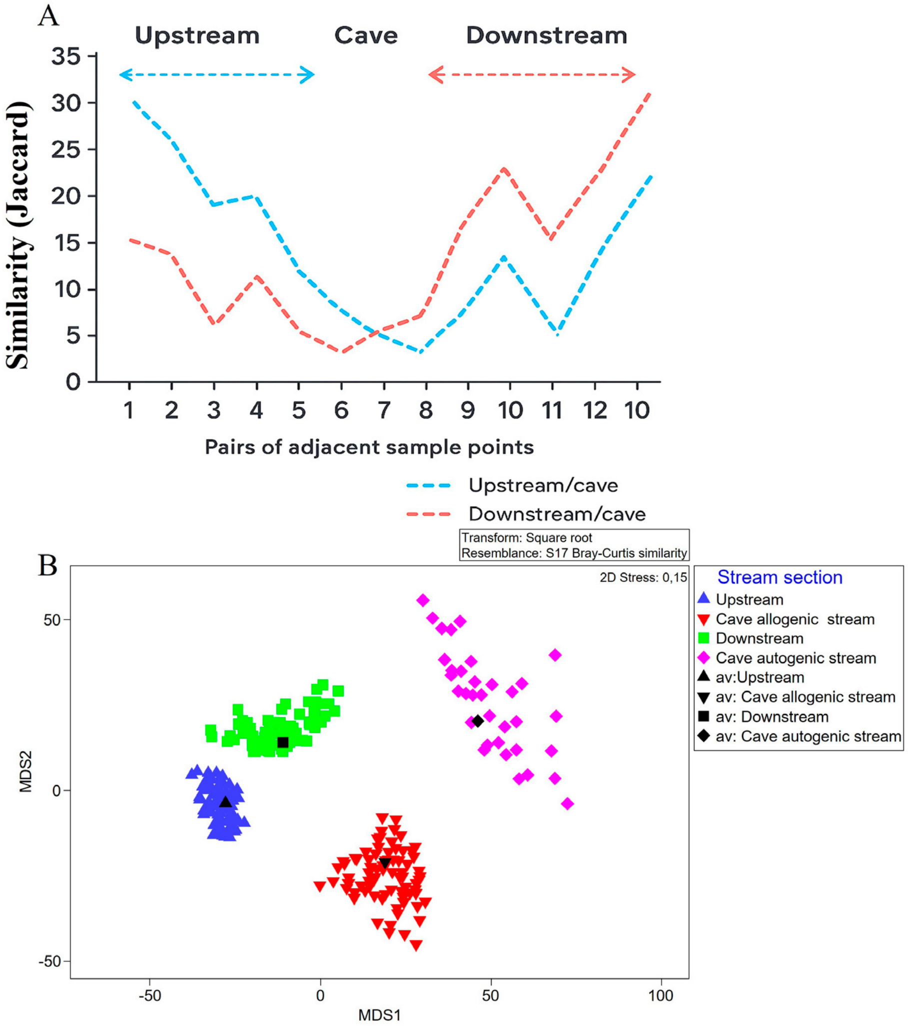

Figure 6. Decrease in similarity to the upstream, cave, and downstream sections (A) and similarity in a Metric multidimensional scaling plot (NMDS) (B). Average (Av). *Disminución en la similitud con las aguas arriba de la cueva arroyo de la cueva y aguas abajo de la cueva (A) y similitud en un gráfico de escalado multidimensional métrico (NMDS) (B). Promedio (Av).*

R = 0.469, p = 0.01), upstream and downstream (Global R = 0.080, p = 0.04), upstream and cave autogenic stream (Global R = 0.940, p = 0.03), cave allogenic stream and downstream (Global R = 0.279, p = 0.02), cave allogenic stream and cave autogenic stream (Global R = 0.309, p = 0.04), and between downstream and cave autogenic stream (Global R = 0.440, p = 0.02).

The non-metric multidimensional scaling (NMDS) analysis and bootstrap overlays demonstrated clear apparent variation in faunal composition between and within the four stream sections (Fig. 6B). Pairwise comparisons using PERMDISP revealed significant differences in beta diversity between the upstream and cave allogenic stream sections (t = 4.937, p = 0.01), and between the upstream and cave autogenic stream sections (t = 3.571, p = 0.03). Environmental predictors of invertebrate community variation differed across stream sections. Distance-based linear modeling (DistLM) indicated that the combination of longitudinal distance (upstream to downstream), temperature, and current speed was the main predictor of changes in benthic invertebrate composition (AICc = 274.65, R^2 = 0.22, p = 0.003). The first two axes of the distance-based redundancy analysis (dbRDA), incorporating all environmental variables, explained 17.3% of the total variation in species composition (Fig. 7A). Vector overlays showed that the first dbRDA axis was strongly associated with current speed and temperature.

Finally, PCA Factor 1, primarily representing dissolved oxygen and conductivity, was positively correlated with invertebrate richness (F(1,32) = 9.75, R = 0.48, $p < 0.038$), indicating that these environmental conditions played a significant role in structuring local biodiversity (Fig. 7B).

DISCUSSION

The environmental and biological patterns observed across the stream sections reveal a gradient of variation along the epigean–hypogean–epigean continuum. The physical and chemical parameters, temperature, dissolved oxygen, current speed, pH, and electrical conductivity differed among the four stream segments. These environmental contrasts were closely associated with changes in species richness and community composition, indicating that habitat-specific conditions play a central role in structuring local biodiversity.

Spatial variation in environmental conditions structured distinct ecological zones along the stream. The autogenic cave reach, characterized

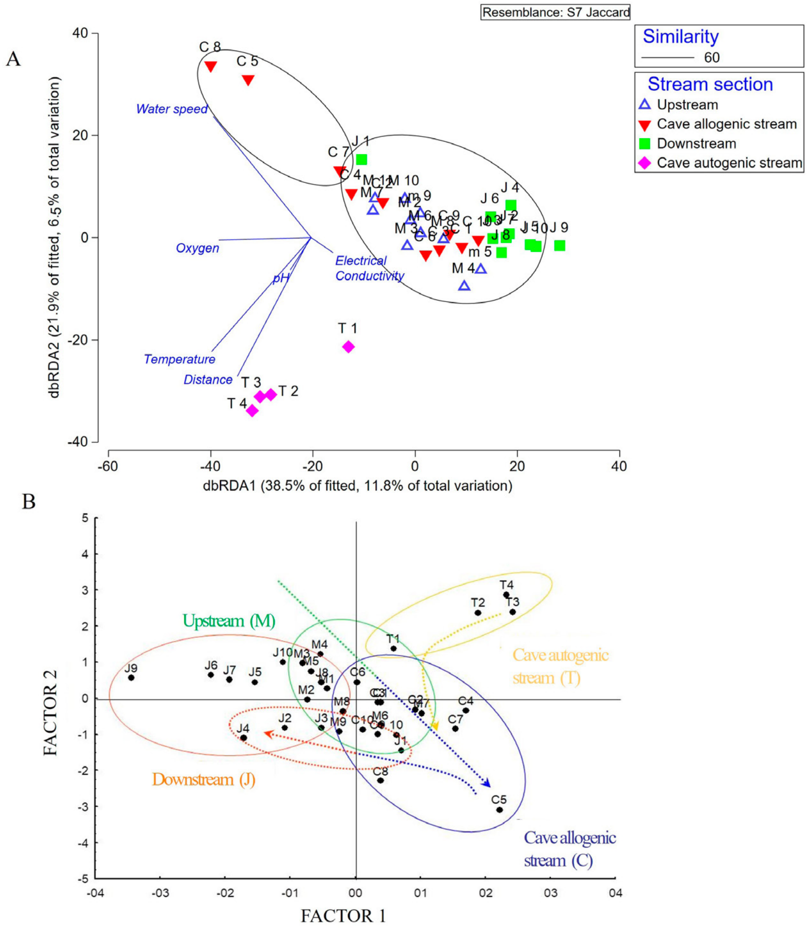

Figure 7. Distance-based redundancy analysis relating species composition with stream environmental variables (A). Plotting of the two factors extracted from the PCA (B). The circles identify stream sections. The colors of the continuous lines indicate the variables along the two axes in each section. Dotted arrows indicate the tendency towards the influence of each factor in the various segments of the water course. *Análisis de redundancia basado en la distancia para evaluar las relaciones entre la composición de especies y un conjunto de variables explicativas (A). Se representan gráficamente los dos factores extraídos del análisis de componentes principales (B). Los círculos identifican secciones del arroyo. Los colores de las líneas continuas indican las variables a lo largo de las secciones. Las flechas punteadas indican la tendencia hacia la influencia de cada factor en los diversos segmentos del curso de agua*.

by higher temperature and pH, influenced downstream conditions, whereas the allogenic reach exhibited higher dissolved oxygen and lower electrical conductivity. These physicochemical gradients shaped benthic invertebrate distributions, with surface segments supporting more diverse assemblages and subterranean sections harboring fewer, more specialized taxa. Patterns of species turnover among stream segments further indicate the combined effects of environmental filtering and spatial separation. Together, these longitudinal changes underscore the importance of hydrological connectivity and microhabitat heterogeneity in structuring aquatic communities in cave-influenced freshwater systems. The following discussion explores how these ecological gradients and habitat transitions shape biodiversity and community dynamics within cave-influenced freshwater systems.

Environmental variables in the stream stretches

The physicochemical parameters recorded in the epigean sections of the Mandembe stream indicate a relatively well-preserved system. Similar studies conducted during the dry season in comparable environments have reported values consistent with those observed here, typical of high-altitude, shallow streams shaded by intact riparian vegetation (Galdean et al., 2000, Moretti et al., 2007). In contrast, studies focusing on the physical and chemical characteristics of subterranean aquatic ecosystems remain scarce compared to those conducted in surface waters (Pellegrini et al., 2018, 2020, Pacheco et al., 2021, Martins & Ferreira, 2021, Rocha-Melo et al., 2025).

A marked decline in water temperature was observed as the stream entered the cave, most likely resulting from the absence of direct solar radiation. The persistence of cooler temperatures downstream appears to be associated with dense riparian vegetation and shaded rocky outcrops that restrict heat input. In contrast, elevated temperatures in the epikarst infiltration zone may be driven by prolonged water–rock contact, whereby the surrounding substrate acts as a thermal buffer that increases water temperature.

Current speed increased sharply at specific points within the cave, particularly in consequence of 18-meter vertical slope. This geomorphological feature likely contributed to higher dissolved oxygen levels within the cave and at specific upstream locations with similarly high current speed. We propose that this pattern resulted from turbulence-driven aeration within narrow cave passages, combined with lower temperatures inside the cave that increased oxygen solubility. Together, these factors likely compensated the absence of photosynthetic oxygen production. Within the Mandembe Cave, previous studies revealed that temperature and water velocity were the two most significant variables influencing ecological assemblage structure (Pellegrini et al., 2018). Together, these two factors accounted for an impressive 53% of the observed variability in community composition. This suggests that both thermal conditions and water flow velocity played critical roles in shaping the distribution and interactions of species within this unique subterranean ecosystem.

Downstream epigean sections, which exhibited higher electrical conductivity, were characterized by slower currents and the accumulation of organic vegetal debris. As previously noted by Esteves (1998), the presence of dissolved substances (dissociated into anions and cations) increases conductivity. Similarly, the autogenic cave stream section displayed slow-flowing water and substantial amounts of organic matter, notably bat guano, which likely contributed to an initial increase in pH values. Although fresh guano deposition can elevate pH, it also stimulates intense microbial activity during decomposition, thereby increasing oxygen demand within the system. In the present system, however, the combined effects of low temperature and turbulent flow appear to maintain dissolved oxygen levels close to saturation.

The PCA results showed that physicochemical parameters in one stream section often correlated with those in adjacent sections, highlighting the interdependence of stream segments. This pattern aligns with the River Continuum Concept (RCC) proposed by Vannote et al. (1980). According to this model, gradients within a river system drive continuous patterns of organic matter transport, storage, and utilization (Minshall et al., 1983, Cummins et al., 1984).

Nevertheless, while our findings acknowledge a degree of environmental continuity as the stream flows through the cave, they also indicate that subterranean stretches introduce disruptions to the expected longitudinal dynamics. Despite partial interdependence among stream sections, the cave environment does not fully conform to models developed for strictly epigean aquatic systems (Datry et al., 2005, O'Sullivan et al., 2022).

Structure of the aquatic invertebrate community

Although allogenic cave streams host fewer species compared to most surface streams, they can maintain a comparable number of feeding groups such as scrapers, collectors, and predators (Simon & Benfield, 2001, Simon et al., 2003, Souza-Silva et al., 2011, Pellegrini et al., 2020, Venarsky et al. 2014). Cave-stream communities can consume nearly all available resources annually (Souza-Silva et al., 2011, Costa et al., 2021, Venarsky et al., 2014). Some previous studies highlight the importance of dissolved organic matter (DOM) in sustaining food webs within cave streams, with energy transfer to higher trophic levels occurring primarily through epilithic biofilms and, to a lesser extent, fine particulate organic matter (FPOM) (Simon & Benfield, 2001, Simon et al., 2003). Bacteria also fulfil a substantial proportion of the energetic demands of many stream invertebrates (Hall & Meyer 1998, Simon et al., 2003). In the present system, invertebrate assemblages in the allogenic stream likely depend primarily on organic matter transported from upstream epigean environments, whereas those inhabiting the autogenic stream appear to rely predominantly on in situ resources such as bat guano and biofilms.

The composition of aquatic invertebrate communities in the epigean sections of the stream resembled that reported for other tropical streams (Galdean et al., 2000, Moulton & Magalhães, 2003, Moretti et al., 2007). Despite the dominance of certain groups, particularly Diptera (Chironominae) and Ephemeroptera, species composition varied across the four sampled sections, reflecting the influence of local environmental conditions on community structure. According to Cummins et al. (2005), many species of Ephemeroptera and Chironomidae function as gathering collectors, feeding on fine particulate organic matter (FPOM) from interstitial spaces in bottom sediments. This feeding strategy may support their persistence even when coarse particulate organic matter (CPOM) is scarce, such as within caves (Costa et al., 2021). In contrast, known predatory species tend to disappear after the water enters the subterranean system. Shredders were generally rare at all sampling points, consistent with findings from other tropical streams, where CPOM decomposition is primarily mediated by microbial activity rather than invertebrates (Moulton & Magalhães, 2003; Moretti et al., 2007), or in lentic cave habitats (Souza-Silva et al., 2013). Nonetheless, some collector taxa may exhibit trophic flexibility and fragment during specific life stages, indicating a predominance of generalist consumers within Neotropical stream food webs (Covich, 1988).

Upstream and downstream sections shared similar community structures, which contrasted significantly with those found inside the cave. Beisel et al. (2000) and Boyero (2003) suggest that habitat heterogeneity is positively associated with higher species richness in temperate lotic environments. Although this study did not quantify organic matter in the substrate, it was visibly more abundant in the epigean sections than in the cave. This likely contributed to the higher species richness and diversity observed in epigean habitats, which offer a broader array of microhabitats and organic resources (Death, 1989).

Some aquatic species appeared to be sensitive to the extreme environmental conditions within the cave, particularly the absence of light and the limited availability of trophic resources (Death, 1989). This filtering effect was evident in the rapid decline in species richness and individual abundance immediately after the stream entered the cave. The cave thus functions as an ecological filter, preventing many upstream species from persisting into the subterranean section (Death, 1989). However, community structure was rapidly reestablished downstream of the cave, as environmental conditions, such as light availability and trophic resources, quickly returned to epigean-like conditions. This suggests that the overall ecological impact of the cave is less disruptive than expected, and that the downstream community resembles that of the upstream section.

Research findings indicate that concentrations of chlorophyll a and phaeopigments in both cave environments and shaded stream habitats decrease markedly as light intensity diminishes. This trend suggests that light availability is a critical factor influencing the growth and activity of Periphyton organisms, which play a vital role in primary productivity within these aquatic ecosystems (Towns, 1981, Death, 1989). Additionally, the benthic fauna demonstrated a significant behavioral and ecological response to variations in incoming light (Death, 1989). Specifically, increased light penetration reduced shaded areas within the cave, thereby facilitating the development of more diverse and abundant communities (Death, 1989).

Costa et al. (2021) investigated the aquatic fauna in some quartzite caves in Brazil characterized by allogenic drainage, focusing on the gut contents of Plecoptera nymphs. A significant proportion (40.5%) had empty guts, and fine particulate organic matter was the most frequently observed item. The high incidence of empty guts may reflect a limited capacity of these nymphs to adapt to the resource-scarce conditions typical of subterranean habitats.

In contrast, Souza-Silva et al. (2011), studying a limestone allogenic cave stream, found that the primary food resources used by aquatic fauna were submerged roots and organic detritus transported into the cave by the stream, supporting a distinct invertebrate community, with higher species richness and denser populations. The aquatic community described by Souza-Silva (2011) thus appears more complex than those typically observed in aquatic cave environments.

Interestingly, the autogenic tributary section within the cave exhibited the highest average diversity among the four sampled areas. This may be due to the presence of a bat colony in the passage through which the tributary flows, providing an additional input of organic matter (e.g., guano) and enhancing habitat suitability for a more diverse invertebrate assemblage. The presence of layered communities in perennial epikarst waters suggests that some populations may be permanent residents of these habitats (Camacho, 1992). In this study, the community associated with the autogenic stream played a key role in maintaining the overall diversity within the Mandembe cave system. The findings also indicate that the main cave passage represents only part of the aquifer's functional structure, and that many species from the epikarst may be passively transported into the main drainage channel, further contributing to the diversity of the subterranean assemblage.

The cave as an ecotone

The main passage of the Mandembe cave functions as a broad transition zone, as evidenced by the overlapping ecotones associated with its two entrances. These ecotones act as selective filters, allowing only species pre-adapted to subterranean conditions to persist (Hansen et al., 1988, Death, 1989). Thus, the Mandembe cave acts as a biological filter within the spatial continuum of the stream, exerting a direct influence on the structure and composition of the aquatic community.

In the study by Prous et al. (2004), ecotone boundaries were defined at points of total dissimilarity, where similarity values between adjacent systems reached zero. However, in the Mandembe cave, complete dissimilarity was not observed. This is likely due to the presence of two entrances, which caused the ecotones to overlap. As a result, after an initial decline in similarity within the cave, similarity values began to rise again toward the central region, reflecting the influence of the second entrance and its proximity to epigean conditions.

Consequently, the entire cave functions as a continuous transition zone. This justifies using the term "filter" to describe its role, as its presence disrupts the typical longitudinal continuum of the drainage system that would exist in the absence of an underground segment. Furthermore, if the cave were longer, with a greater distance between the two entrances, a central stretch would likely exhibit near-total dissimilarity with both upstream and downstream communities. In such a scenario, the cave's filtering effect would be even more pronounced, potentially resulting in a complete decoupling of the aquatic communities on either side of the cave, except for species specifically adapted to subterranean conditions. This would lead to a more marked ecological discontinuity between the epigean sections of the stream.

The occurrence of 16 exclusive, non-stygobitic species within the cave, as shown in the Venn diagram, indicates that subterranean habitats function not only as transitional zones along the fluvial continuum but also as distinct ecological niches. The rapid recovery of epigean macroinvertebrate assemblages downstream underscores the resilience of surface fauna, while simultaneously emphasizing the cave's role as a refuge for specialized communities. Notably, these "specialized" taxa may exhibit strong pre-adaptive traits, suggesting that they belong to lineages particularly suited to colonizing and potentially evolving within subterranean environments, eventually giving rise to cave-restricted descendants. This underscores the broader relevance of studies that identify such specialized but non-stygobitic species, as they may represent early stages in the process of subterranean adaptation and, consequently, warrant special conservation consideration. Collectively, these findings highlight the importance of recognizing caves as integral components of fluvial systems, characterized by unique physicochemical conditions and distinct biodiversity patterns.

The relation between environmental variables and the aquatic invertebrate community

The PCA analysis indicated that the downstream section of the stream is primarily influenced by Factor 1, which is strongly associated with electrical conductivity. The observed positive correlation between Factor 1 and species richness suggests that, in this area, species richness tends to decrease with lower electrical conductivity. Since conductivity reflects the concentration of dissolved substances in water (Esteves, 1998), areas with lower input or accumulation of organic material (particularly plant-derived) may support less diverse communities. However, because organic content was not directly measured in this study, the observed relationship may be a spurious correlation. It is also plausible that higher richness is associated with increased organic input, elevating conductivity.

In addition, Factor 1 captures variation in oxygen saturation, a key variable in the cave's main passage. The positive correlation between richness and Factor 1 indicates that oxygen saturation is critical in sustaining aquatic diversity within the cave. Several studies have reported that habitats with higher oxygen availability tend to support more diverse aquatic communities, reinforcing the ecological importance of this variable in subterranean systems (Vannote et al., 1980, McCafferty, 1981, Allan & Castillo, 2007, Rocha-Melo et al., 2025).

FINAL CONSIDERATIONS

This study showed that the passage of a stream through a subterranean environment substantially alters its conditions, particularly physicochemical variables and benthic invertebrate communities. These findings support the filter effect of cave environments on the dynamic continuum and the longitudinal dynamics of lotic systems. Consequently, traditional models of riverine ecosystems (Vannote et al., 1980, Junk & Wantzen, 2004) should be adapted to account for the unique ecological patterns observed in karst systems (Towns, 1981, Death, 1989, Costa et al., 2020). Beyond this, the study reveals additional environmental implications. Rather than viewing caves solely as biodiversity filters, they should be recognized as complementary habitats that contribute to the overall heterogeneity and conservation value of river networks. This perspective aligns with emerging views on karst systems and further supports the need to revise traditional riverine ecosystem models to incorporate subterranean ecological dynamics.

ACKNOWLEDGEMENTS

We thank Paulo Pompeu, Diego Castro, Rafael Costa Cardoso, Lucas Guimarães, and Érika Taylor for their help with fieldwork and analysis. We thank Daniel De Stefano Menin for providing the photos of the cave in Figure 1. We are also grateful to the institutions that supported the study with funding for scholarships and infrastructure (FAPEMIG and VALE). To the Postgraduate Program in Applied Ecology of the Federal University of Lavras for all the support. APB is thankful to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the grant. RLF is grateful to the CNPq (National Council for Scientific and Technological Development) for the grant provided (CNPq n. 308334/2018-3). MSS is thankful to the CNPq (National Council for Scientific and Technological Development) for the grant provided (CNPq n. 303434/2025-2).

AUTHOR CONTRIBUTIONS

A.P.B.: Responsible for the methodology application, data acquisition, data validation, and prepared the original draft. M.S.S.: Assisted with the methodology application, data acquisition, and was accountable for statistical analysis, reviewing, and editing the manuscript. MSS was also responsible for the manuscript submission. R.L.F.: Responsible for the study conceptualization, analysis, reviewing, and editing of the manuscript. RLF also assisted with the application of methodology and data acquisition.

REFERENCES

Allan, J. D.; Castillo, M. M. 2007. *Stream Ecology: Structure and Function of Running Waters*. Springer, Dordrecht.

Anderson, M. J., Gorley, R. N., & Clarke, K. R. (2008). *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*. PRIMER-E, Plymouth, UK.

Ávila, A. C., Pires, M. M., Rodrigues, E. N., Costi, J.A., Stenert, C., & Maltchik, L. (2019). Drivers of the beta diversity of spider assemblages in southern Brazilian temporary wetlands. *Ecological Entomology*, 45(3), 466–475. DOI: 10.1111/een.12816

Beisel, J. N., Polatera, P. U., & Moreteau, J. C. (2000). The spatial heterogeneity of a river bottom: a key factor determining macroinvertebrate communities. *Hydrobiologia*, 422/423, 163–171.

Boyero, L. (2003). The quantification of local substrate heterogeneity in streams and its significance for macroinvertebrate assemblages. *Hydrobiologia*, 499, 161–168. DOI: 10.1023/A:1017094606335

Camacho, A.I. (1992). *The natural history of biospeleology*. SCIC, Madrid.

Chao, A., & Jost, L. (2012). Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. *Ecology*, 93(12), 2533–2547. DOI: 10.1890/11-1952.1

Costa, B. G., Ferreira, R. L., & Pellegrini, T. G. (2021). Feeding in the dark: are stonefly nymphs good indicators of reference conditions for cave streams? A case study using gut content analysis in Brazilian quartzite caves. *Limnetica*, 40(1), 79-91. DOI: 10.23818/limn.40.06

Covich, A.P. (1988). Geographical and historical comparisons of neotropical streams: biotic diversity and detrital processing in highly variable habitats. *Journal of North American Benthological Society*, 7, 361–386. DOI: 10.2307/1467297

Culver, D. C.; Pipan, T. 2019. *The Biology of Caves and Other Subterranean Habitats*. 2nd ed. Oxford University Press, Oxford.

Cummins, K. W., Merritt, R. W., & Andrade, P. C. N. (2005). The use of Invertebrate Functional Groups to Characterize Ecosystem Attributes in Selected Streams and Rivers in Southeast Brazil. *Studies on Neotropical Fauna and Environment*, 40(1), 69–89. DOI: 10.1080/01650520400025720

Cummins, K. W., Minshall, W. G., Sedell, J. R., Gushing, C. E., & Petersen, R. C. (1984). Stream ecosystem theory. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte SIL Proceedings*, 1922-2010, 22(3), 1818–1827. DOI: 10.1080/03680770.1983.11897580

Datry, T., Malard, F., and Gibert, J. (2005). Responses of invertebrate assemblages to 823 increased groundwater recharge rates in a phreatic aquifer. *Journal of the North 824 American Benthological Society,* 24, 461-477. DOI: 10.1899/04-140.1

Death, R.G. (1989). The effect of a cave on benthic invertebrate communities in a South Island stream. *New Zealand Natural Sciences,* 16, 67–78.

Doretto, A., Bo, T., Bona, F., & Fenoglio, S. (2020). Efficiency of Surber net under different substrate and flow conditions: Insights for macroinvertebrates sampling and river biomonitoring. *Knowledge & Management of Aquatic Ecosystems*, (421), 10. DOI: 10.1051/kmae/2020001

Esteves, F. A. (1998). *Fundamentos de limnologia*. Interciência: FINEP, Rio de Janeiro.

Ficsór, M., & Csabai, Z. (2021). Longitudinal zonation of larval Hydropsyche (Trichoptera: Hydropsychidae): abiotic environmental factors and biotic interactions behind the downstream sequence of Central European species. *Hydrobiologia*, 848(15), 3371–3388. DOI: 10.1007/s10750-021-04602-0

Fovet, O., Humbert, G., Dupas, R., Gascuel-Odoux, C., Gruau, G., Jaffrézic, A., …. Grimaldi, C. (2018). Seasonal variability of stream water quality response to storm events captured using high-frequency and multi-parameter data. *Journal of Hydrology*, 559, 282–293. DOI: 10.1016/j.jhydrol.2018.02.040

Galdean, N., Callisto, M., & Barbosa, F. A. R. (2000). Lotic ecosystems of Serra do Cipó, southeast Brazil: water quality and a tentative classification based on the benthic macroinvertebrate community. *Aquatic Ecosystem Health & Management*, 3, 545–552. DOI: 10.1080/14634980008650691

Greenacre, M., Groenen, P. J. F., Hastie, T. d'Enza, I., Markos, A., Tuzhilina, A., Principal, E. (2022). Principal component analysis. *Nature Reviews Methods Primers*, 2, 100. DOI: 10.1038/s43586-022-00184-w

Hall, R. O., J. B. Wallace, & Eggert. S. L. (2000). Organic matter flow in stream food webs with reduced detrital resource base. *Ecology,* 81, 3445–3463. DOI: 10.1890/0012-9658(2000)081[3445:OMFISF]2.0.CO;2

Hart, D. D. (1992). Community organization in streams: the importance of species interaction, physical factors, and chance. *Oecologia*, 91, 220–228. DOI: 10.1007/BF00317787

Heberle, H., Meirelles, G. V., da Silva, F. R., Telles, G. P., & Minghim, R. (2015). InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. *BMC Bioinformatics*, 16, 1. DOI: 10.1186/s12859-015-0611-3

Hynes, H. B. N. (1974). *The biology of polluted waters*. University of Toronto Press, Toronto.

Hsieh, T. C., & Ma, K. H. (2016). Chao, A. iNEXT: An R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution*, 7(12), 1451-1456. DOI: 10.1111/2041-210X.12613

Junk, W. J., & Wantzen, K. M. (2004). The flood pulse concept: new aspects, approaches and applications – an update. In: *Proceedings of the 2nd International Symposium on the Management of Large Rivers for Fisheries*. FAO Regional Office for Asia and the Pacific, Bangkok, Thailand, 2, 117–140.

Köppen, W. (1931). *Climatologia*. Fondo de Cultura Econômica, Buenos Aires.

Maghrebi, M. F., & Ball, J. E. (2006). New method for estimation of discharge. *Journal of Hydraulic Engineering*, 132(10), 1044-1051. DOI: 10.1061/(ASCE)0733-9429(2006)132:10(1044

Martins, V. M., & Ferreira, R. L. (2021). Environmental factors structuring the assemblage of aquatic Invertebrates in an epigean and hypogean stretch of a Neotropical karst stream. *Marine and Freshwater Research*, 72(7), 1027–1032. DOI: 10.1071/MF20229

McCafferty, W. P. (1981). *Aquatic entomology: the fishermen's and ecologist's illustrated guide to Invertebrates and their relatives*. Jones and Bartlett, Boston.

Merritt, R. W., & Cummins, K. W. (1996). *An introduction to the aquatic Invertebrates of North America* (3rd ed.). Kendall/Hunt, Dubuque, Iowa.

Minshall, G.W. (1967). Role of allochthonous detritus in the trophic structure of the Woodland springbrook community. *Ecology*, 48(1), 139–149. DOI: 10.2307/1933425

Minshall, G. W., Petersen, R. C., Cummins, K. W., Bott, T. L., Sedell, J. R., Cushing, C. E., & Vannote, R. L. (1983). Interbiome comparison of stream ecosystem dynamics. *Ecological Monographs,* 53, 1–25. DOI: 10.2307/1942585

Moretti, M. S., Gonçalves, J. F., Ligeiro, R., & Callisto, M. (2007). Invertebrates colonization on native tree leaves in a neotropical stream (Brazil). *International Review of Hy-*

drobiology, 92(2), 199-210. DOI: 10.1002/iroh.200510957

Moulton, T. P., & Magalhães, S. A. P. (2003). Responses of leaf processing to impacts in streams in Atlantic Rain Forest, Rio de Janeiro, Brazil – A test of the Biodiversity-Ecosystem Functioning Relationship?. *Brazilian Journal of Biology*, 63(1), 87–95. DOI: 10.1590/S1519-69842003000100012

Oliver, I., & Beattie, A.J. (1996). Invertebrate morphospecies as surrogates for species: a case study. *Conservation Biology*, 10, 99–109. DOI: 10.1046/j.1523-1739.1996.10010099.x

O'Sullivan, A. M., Devito, K. J., D'Orangeville, L., & Curry, R. A. (2022). The waterscape continuum concept: Rethinking boundaries in ecosystems. *Wiley Interdisciplinary Reviews: Water*, 9(4), e1598. DOI: 10.1002/wat2.1598

Pacheco, G. S. M., Pellegrini, T. G., & Ferreira, R. L. (2021). Cave lithology influencing EPT (Ephemeroptera, Plecoptera, Trichoptera) assemblages and habitat structure in south-eastern Brazil. *Marine and Freshwater Research*.72(10), 1-7. DOI: 10.1071/MF20359

Pellegrini, T. G., Faria, L. D. B., & Ferreira, R. L. (2020). Temporal diversity patterns of benthic Invertebrates in subterranean streams: a case study in Brazilian quartzite caves. *Hydrobiologia*, 847, 2417–2431. DOI: 10.1007/s10750-020-04262-6

Pellegrini, T. G., Pompeu, P. S., & Ferreira, R. L. (2018). Cave benthic invertebrates in south-eastern Brazil: are there 'key' factors structuring such communities?. *Marine and Freshwater Research*, 69(11), 1762–1770. DOI: 10.1071/MF18025

Pérez, G.R. (1988). *Guia para el estudio de los macroinvertebrados acuáticos del Departamento de Antioquia*. Fondo Fen.Colombia/Colciencias/Universidad de Antioquia, Antioquia.

Prous, X., Ferreira, R. L., & Martins, R. P. (2004). Ecotone delimitation of epigean-hypogean ecotone zone in two limestone caves in south-eastern Brazil. *Austral Ecology*, 29, 374-382. DOI: 10.1111/j.1442-9993.2004.01373.x

Rocha Melo, L. M., Ferreira, R. L. & Silva, M. S. (2025). A review of the factors influencing invertebrate community structure in subterranean habitats. *Community Ecology,* DOI: https://10.1007/s42974-025-00243-8

Simon K. S., & Benfield E. F. (2001). Leaf and wood breakdown in cave streams. *Journal of the North American Benthological Society,* 20, 550–563. DOI: 10.2307/1468087

Simon, K. S., Benfield, E. F., & Macko, S. A. (2003). Food web structure and the role of epilithic biofilms in cave streams. *Ecology*, 84(9), 2395-2406. DOI: 10.1890/02-334

Smeeton, N., Spencer, N., & Sprent, P. (2025). *Applied nonparametric statistical methods*. CRC Press.

Souza-Silva, M., Martins, R. P., & Ferreira, R. L. (2011). Trophic dynamics in a neotropical limestone cave. *Subterranean Biology*, 9, 127–138. DOI: 10.3897/subtbiol.9.2515

Souza-Silva M, Rezende K. S. R., Ferreira R. L. (2013). Detritus processing in lentic cave habitats in the neotropics. *Subterranean Biology,* 11, 3–14. DOI: 10.3897/subtbiol.11.5107

Strayer, D. L., & Dudgeon, D. (2010). Freshwater biodiversity conservation: recent progress and future challenges. *Journal of the North American Benthological Society*, 29(1), 344-358. DOI: 10.1899/08-171.1

Taylor, E. L. S., & Ferreira, R. L. (2012). Determinants on the structure of an aquatic invertebrate community in a Neotropical limestone cave. *Revista Brasileira de Espeleologia*, 2(1), 1-12. https://revistaeletronica.icmbio.gov.br/index.php/RBEsp/article/view/118

Townsend, C. R. (1989). The patch dynamics of stream community ecology. *Journal of the North American Benthological Society*, 8, 36–50. DOI: 10.2307/1467400

Towns, D. R. (1981). Effects of artificial shading on periphyton and invertebrates in a New Zealand stream. *New Zealand Journal of Marine and Freshwater Research,* 15, 185-192. DOI: 10.1080/00288330.1981.9515911

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, 37, 130–137.

Venn, J. (1880). I. On the diagrammatic and mechanical representation of propositions and reasonings. *The London, Edinburgh, and Dublin philosophical magazine and journal of sci-*

ence, 10(59), 1-18.

Venarsky, M. P., Huntsman, B. M., Huryn, A. D., Benstead, J. P., & Kuhajda, B. R. (2014). Quantitative food web analysis supports the energy-limitation hypothesis in cave stream ecosystems. *Oecologia*, 176, 859-869. DOI: 10.1007/s00442-014-3042-3

Whitton, L. (1975). *River ecology*. University of California Press, Berkeley.

Williams, D.D., & Hynes, B.N. (1976). The recolonization mechanisms of stream benthos. *Oikos*, 27, 265–277. DOI: 10.2307/3543905