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SUMÁRIO

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Who´s inside, and who´s outside? Habitat structure determines the richness and composition of invertebrates in quartzite caves and surrounding habitats

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RESUMO: Nos últimos anos, estudos têm buscado compreender como as estruturas físicas e ambientais dentro das cavernas influenciam as comunidades de invertebrados. No entanto, ainda há uma escassez de pesquisas que explorem como a composição do substrato e a heterogeneidade do habitat influenciam as comunidades faunísticas em cavernas de quartzito e em áreas epígeas adjacentes, apesar da importância de entender essas relações ecológicas para embasar estratégias eficazes de conservação. Este estudo avaliou a similaridade das comunidades de invertebrados entre cavernas de quartzito e habitats de superfície circundantes, bem como as variáveis ambientais que influenciam a composição e a riqueza faunística. O estudo foi conduzido em três sistemas cavernícolas de quartzito, com amostragens realizadas no interior das cavernas e em zonas epígeas adjacentes. Os resultados revelaram dissimilaridade na composição faunística entre os ambientes subterrâneo e de superfície, enquanto as comunidades dentro das cavernas apresentaram maior similaridade entre si. A substituição de espécies foi identificada como o principal componente da diversidade βsør total entre os dois ambientes. As composições faunísticas distintas observadas são impulsionadas por filtros ambientais, sendo que as condições restritivas das cavernas limitam a colonização e favorecem invertebrados especializados. Além disso, variáveis distintas de substrato emergiram como fator-chave na estruturação das comunidades, com detritos vegetais influenciando a fauna epígea, e guano e substratos inorgânicos estruturando a fauna cavernícola. Esses achados avançam a compreensão da dinâmica dos ecossistemas subterrâneos, destacando a importância das características do habitat na formulação de estratégias de conservação da biodiversidade.
Palavras-chave: invertebrados cavernícolas; similaridade; heterogeneidade de habitat; Neotrópicos

ABSTRACT: In recent years, studies have aimed to understand how physical and environmental structures within caves influence invertebrate communities. However, there is still a lack of research exploring how substrate composition and habitat heterogeneity influence faunal communities in

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quartzite caves and adjacent epigean areas, despite the importance of understanding these ecological relationships to inform effective conservation strategies. This study evaluated the similarity of invertebrate communities between quartzite caves and surrounding surface habitats, as well as the environmental variables influencing faunal composition and richness. The study was conducted across three quartzite cave systems, with sampling carried out within the caves and in adjacent epigean zones. Results revealed dissimilarity in faunal composition between subterranean and surface environments, while communities within caves exhibited higher similarity. Species turnover was identified as the primary component of total βsør-diversity between the two environments. The distinct faunal compositions observed are driven by environmental filters, with restrictive cave conditions limiting colonization and favoring specialized invertebrates. Additionally, distinct substrate variables emerged as a key driver of community structure, with plant debris influencing epigean fauna, and guano and inorganic substrates structuring cave fauna. These findings advance understanding of subterranean ecosystem dynamics, highlighting the importance of habitat features in informing biodiversity conservation strategies.
Keywords: cave invertebrates; similarity; habitat heterogeneity; Neotropics.

1. INTRODUCTION

Variations in habitat heterogeneity have been widely used to explain species diversity in both natural and human-modified environments (MacArthur & MacArthur, 1961; Tews et al., 2004; Previati et al., 2007; Souza et al., 2014). Heterogeneity, driven by variations in environmental conditions and resources, directly influences species diversity by expanding ecological niche availability (MacArthur & MacArthur, 1961; Pianka, 1966; Magurran, 2013). Additionally, heterogeneity has been instrumental in clarifying community structuring across different taxonomic groups, as its effects can vary based on species-specific habits and traits (MacArthur & MacArthur, 1961; Atauri & Lucio, 2001; Travassos-de-Britto & da Rocha, 2013; Stein et al., 2014). Thus, substrate diversity and habitat heterogeneity play critical roles in facilitating the coexistence of multiple organism groups within a given area (MacArthur & Levins, 1967; May, 1986).

In recent years, numerous studies have explored how substrate diversity and habitat heterogeneity influence fauna composition in cave environments (Prous et al., 2015; Pellegrini et al., 2016; Pacheco et al., 2020a; Souza-Silva et al., 2021; Reis-Venâncio et al., 2022). However, relatively few have examined the contrasting roles of hypogean (subterranean) and adjacent epigean (surface) environments in shaping invertebrate community structures (Mendes-Rabelo et al., 2021; Cardoso et al., 2022; Pacheco et al., 2025).

Different types of substrates and the availability of trophic resources are crucial factors enabling invertebrate colonization in cave environments (Mammola et al., 2016; Zagmajster et al., 2018). Caves are generally more stable than surface environments and are characterized by constant temperature, high humidity, and the absence of light (Barr, 1968; Poulson & White, 1969; Culver, 1982; Badino, 2010; Mammola et al., 2019a). Allochthonous organic resources, primarily plant or animal matter, provide the primary energy source for caves, transported through physical processes like water flow, wind, and gravity or introduced by animals in the form of feces, guano, and carcasses. These resources are vital nutrient sources for cave fauna and play a fundamental role in the trophic dynamics of these ecosystems (Poulson & White, 1969; Simon et al., 2007; Culver & Pipan, 2019; Souza-Silva et al., 2011; Souza-Silva et al., 2013).

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Research on the influence of substrate types and habitat heterogeneity in cave fauna has predominantly focused on limestone caves (Prous et al., 2015; Pellegrini et al., 2016; Pacheco et al., 2020a; Furtado et al., 2022). However, for quartzite caves, there remains a significant research gap regarding the role of these variables in shaping community structures. Studies on quartzite caves to date have addressed trophic ecology, physical characteristics, and the impact of lithology on cave fauna organization (Souza-Silva et al., 2013, 2020a, 2020b). Nonetheless, few studies have investigated how substrate types and habitat heterogeneity influence invertebrate communities within both caves and nearby epigean habitats or examined the similarity between the fauna in these environments (Prous et al., 2004; 2015; Mammola et al., 2016; Oliveira & Ferreira, 2024; Pacheco et al., 2025).

This study aimed to investigate the similarity of fauna between caves and the surrounding epigean habitats. Additionally, we sought to assess the environmental parameters that influence fauna composition and richness in a system comprising three interconnected quartzite caves linked by intermittent drainage and their adjacent epigean habitats.

We hypothesize that the epigean fauna adjacent to the caves will show greater species richness and a distinct composition compared to the cave-dwelling fauna due to the colonization constraints imposed by the trophic and microclimatic conditions within the caves. We expect that the cave fauna represents a subset of the epigean fauna. Furthermore, the distance from the entrance of the first cave to the remaining caves (in the upstream to downstream direction) is anticipated to be a key factor in determining species composition and richness throughout the cave system. Finally, we expect that due to differences in habitat structure, the environmental variables influencing the composition and richness of the epigean fauna near the caves will differ from those affecting the cave fauna.

2. METHODOLOGY

Study area

This study was conducted in Andrelândia, situated in the southern region of Minas Gerais, southeastern Brazil. It focuses on a system of three interconnected quartzite caves (Gruta Cabeça de Vaca, Gruta Barra, and Gruta João Japonês) linked by intermittent drainage (Fig. 1). These caves are located within the Andrelândia Quartzite Speleological Province, part of the Andrelândia Group, which is characterized by rocks belonging to the Andrelândia Megasequence (Corrêa-Neto, 1997). The study area is situated in the Atlantic Forest biome, which has significantly degraded agricultural and pastoral activities. As a result, only a few fragments of forest remain near the three caves, with most of the surrounding land being used for grazing. Gruta Cabeça de Vaca (UTM 23 L: 565534.22 – 7592086.46) has four entrances and a linear extent of 53 meters. Gruta Barra (UTM 23 L: 565557.27 – 7592026.41) has two entrances and a linear extent of 45 meters. Gruta João Japonês (UTM 23 L: 565609.90 – 7591985.42) also features two entrances, with a linear extent of 52 meters (Figure 1).

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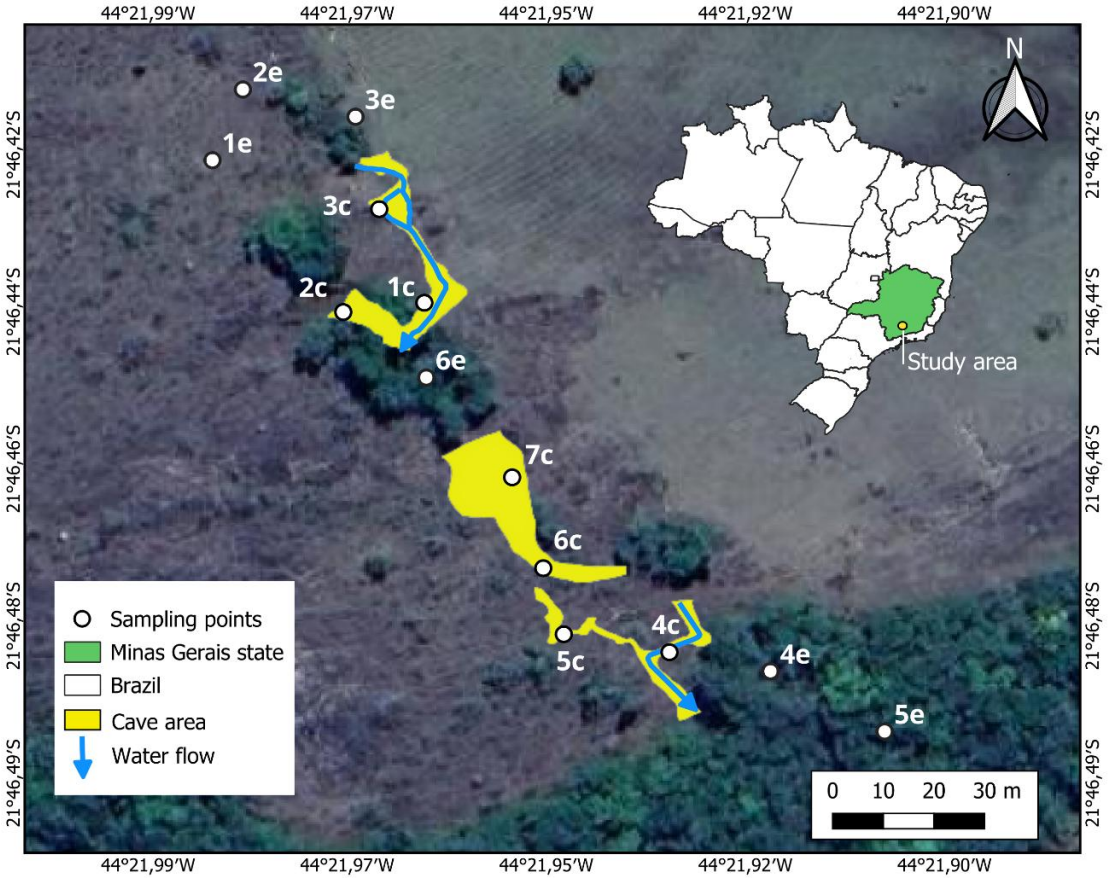

Figure 1. The study area and cave are in the municipality of Andrelândia, Minas Gerais, Brazil. The letters 'e' indicates epigean sampling points, and 'c' indicates cave sampling points.

Sampling

Biotic and abiotic data were collected during a single sampling event on June 16 and 17, 2022. The sampling units were arranged in transects, each measuring ten meters long and comprising three quadrants, each 1 m^2 in size, positioned at least five meters apart. Six transects were sampled in the epigean area adjacent to the caves, encompassing 18 quadrants. In comparison, seven transects were sampled within the caves, resulting in 21 quadrants. Sampling was conducted exclusively within these quadrants.

A modified sampling methodology was adapted from Souza-Silva et al. (2021), Pacheco et al. (2025). Invertebrates were actively collected by hand for 10 minutes per quadrant using forceps and brushes. The collected samples were placed in containers filled with 70% ethanol and are currently deposited in the subterranean invertebrate collection (ISLA) at the Federal University of Lavras (https://www.biologiasubterranea.com.br/pt/).

Invertebrates were sorted, identified to the most accessible taxonomic level, and grouped into morphotypes with the aid of stereomicroscopes, following the methodologies outlined by Oliver & Beattie (1996), Derraik et al. (2002), Derraik et al. (2010), and Pellegrini et al. (2016).

To document the habitat's structural characteristics, we took photographs at chest height, positioning the camera at a 90-degree angle to each quadrant. Subsequently, these photographs were analyzed using ImageJ software (Rasband, 1997) to characterize the substrate types and quantify their coverage area in each quadrant (Pacheco et al., 2020b; Furtado et al., 2022).

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The substrate types analyzed included guano, green plants, plant debris, rocks (250–500 mm), blocks (64–250 mm), fine gravel (2–16 mm), coarse gravel (17–63 mm), sand, silt (0.2–0.05 mm), bedrock, and hardpan.

Data analysis

Abiotic data

To evaluate the differences in substrate types between the epigean area and the caves, we compared the percentage coverage of each substrate within the quadrants using a one-way ANOVA non-parametric test (Kruskal-Wallis). A similarity analysis (ANOSIM) was performed to determine whether there were differences in substrate composition between the epigean and cave environments, utilizing a Bray-Curtis similarity matrix (Clarke, 1993). The Kruskal-Wallis and ANOSIM analyses were conducted using R software (R Core Team, 2025).

Based on habitat characteristics and resources, the substrates were categorized into five variables: Guano, Green Plants, Plant Debris, Shelter (rocks 250-500 mm, blocks 64-250 mm, fine gravel 2-16 mm, coarse gravel 17-63 mm), and Inorganic Substrate (hardpan, sand, silt 0.2-0.05 mm and bedrock). A shade plot was generated to visualize the distribution of these environmental substrate variables in both the epigean area and the caves.

Biotic data

Species richness was assessed by adding the morphotypes identified in each area. We evaluated the normality of the data using the Shapiro-Wilk test ($p = 0.001$) and examined the variance among groups with Levene's test for homogeneity of variances ($p = 0.011$). Since the data did not conform to a normal distribution and the variances between the sampled groups were unequal, we employed the non-parametric Mann-Whitney U test to explore differences in richness between the epigean and cave environments (Levene, 1960; Shapiro and Wilk, 1965; Sprent & Smeeton, 2000). The Mann-Whitney U statistical analysis was performed using Jamovi software (2023), with a significance level set at $p < 0.05$.

To investigate differences in faunal group composition between the epigean and cave environments, we conducted a similarity analysis (ANOSIM) employing the Bray-Curtis similarity index, with the locations (epigean and caves) serving as the grouping factor (Clarke, 1993). Additionally, to compare the composition of faunal groups among the caves, we carried out another similarity analysis (ANOSIM), using the caves as the grouping factor and also utilizing the Bray-Curtis similarity index (Clarke, 1993).

To illustrate variations in faunal similarity between the epigean and cave environments and among the caves we employed non-metric multidimensional scaling (MDS) in conjunction with a Bray-Curtis similarity matrix.

Spatial β-diversity was calculated for each quadrant based on differences among sampling points within each environment using the βsør index. This index was partitioned into its two additive components, turnover (βsim) and nestedness (βsne), ranging from 0 (0%) to 1 (100%), thereby representing their contributions to the total βsør turnover. The turnover component captures species replacement between sites, while nestedness reflects patterns where the species composition of one site constitutes a subset of another.

To evaluate whether turnover constitutes the dominant component of total βsør-diversity, beta regression models were employed, using βsim and βsne as explanatory variables. Statistical significance was determined through Type III Analysis of Variance (ANOVA), and the relative

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effects of each component were visualized using boxplots comparing chi-square (χ^2) statistics associated with turnover and nestedness.

All β-diversity analyses were conducted in RStudio version 4.3.3 (R Core Team, 2025). Type III ANOVAs were performed using the *car* package (Fox et al., 2012), and visualizations were generated with *tidyverse*. The βsør index and its components were calculated using the *betapart* package (Baselga, 2010), while beta regression models were fitted with the *betareg* package (Cribari-Neto & Zeileis, 2010).

To assess how habitat components influence faunal composition in epigean and cave environment, we utilized distance-based linear models (DistLM) (Anderson et al., 2008). The substrate variables considered included Guano (GUA), Green Plants (GP), Plant Debris (PD), Shelter (SH), and Inorganic Substrate (IS). Additionally, we incorporated substrate diversity (SD) for each quadrant, which was calculated using the Shannon Index (H') (Magurran & McGill, 2010). The SD value was derived from all components present in the substrate of the quadrants, allowing for an assessment of substrate diversity in the areas where collections occurred (modified from Pacheco et al., 2020a; Cardoso et al., 2022). Statistical models in DistLM were constructed using the Bray-Curtis species similarity matrix and a forward selection procedure, employing adjusted R^2 as the criterion for model selection, and we performed 999 permutations (Anderson et al., 2008).

The adjustments and explanatory power of the models for the variation in composition data were assessed using distance-based redundancy analysis (dbRDA) (Clarke & Gorley, 2006; Anderson et al., 2008). ANOSIM and DistLM analyses for the faunal data were performed using the statistical software Plymouth Routine in Multivariate Ecological Research - Primer 7 + Permanova Software (Anderson et al., 2008), available at https://www.primer-e.com/. A significance level of $p < 0.05$ was adopted for the analyses.

We employed Generalized Linear Models (GLMs) to evaluate the influence of habitat components on the variation in invertebrate fauna richness in epigean and cave environments. The predictor variables included GUA, GP, PD, SH, IS, and SD. To assess collinearity among predictors, we calculated Spearman's correlation coefficients using the *chart.Correlation* function from the *PerformanceAnalytics* package (Peterson & Carl, 2020). Additionally, we applied Variance Inflation Factors (VIF) to exclude variables with VIF values greater than 3 (Zuur et al., 2013). For the epigean environment model, the variables SD and GP were excluded to avoid multicollinearity, as their pairwise correlation coefficient exceeded 0.70 (Schober et al., 2018). Furthermore, the variable GUA was removed from the analysis due to its absence in the epigean environment, rendering it uninformative for the model.

To ensure independence among variables, we further assessed multicollinearity diagnostics. The model was initially fitted using the Poisson distribution, and we employed the *simulateResiduals* function from the *DHARMa* package to test for overdispersion and the test Zero Inflation function to assess zero inflation in the model (Hartig, 2022). As the Poisson model exhibited overdispersion, we corrected the error structure by fitting a model using the negative binomial distribution.

The adjusted R^2 value was obtained using the *rsq* function from the *rsq* package. A significance level of $P \leq 0.05$ was adopted for all statistical tests. All GLM analyses were performed in R software (R Core Team, 2025).

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3. RESULTS

Substrate characteristics in the caves and epigean habitats

Eleven substrate categories were identified, including plant debris, green plants, block, bedrock, and silt, which were present in both the caves and the surrounding epigean areas. In contrast, guano, rock, fine gravel, coarse gravel, sand, and hardpan were found exclusively within the caves. In the epigean environment, the predominant substrate was "green plants," primarily comprising grasses (70.91%). The most frequently recorded component within the caves was "plant debris," found in 66% of the quadrants. However, the most abundant substrate was "hardpan," accounting for 22.90% of the total substrate collected across all quadrants in the caves (Figure 2A).

The Kruskal-Wallis test indicated a significant difference in substrate occupancy between the epigean and cave areas (KW = 4.64; p = 0.03) (Figure 3A). ANOSIM further revealed significant differences in substrate composition between the two environments, indicating a high degree of separation between the groups (GlobalR = 0.758; p = 0.001).

Among the environmental variables analyzed, the "green plants" category was the most abundant in the epigean environment, comprising 70.91% of the total sampled area, followed by "plant debris," which accounted for 24.07%. In contrast, within the cave environment, the "inorganic substrate" was the most prevalent, representing 50.42% of the total, followed by "shelter," which occupied 30.39% of the cave area (Figure 2B).

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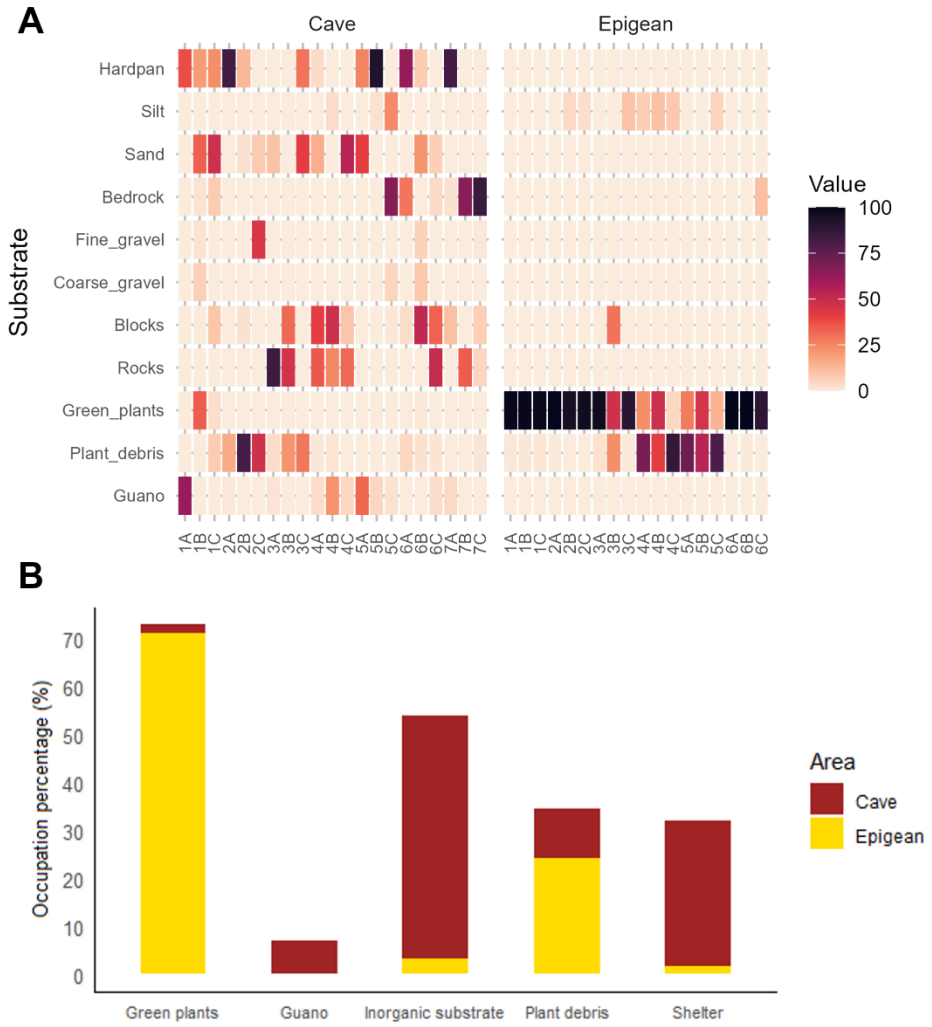

Figure 2. Shade plot showing the distribution and percentage of occupancy of different substrate types at each sampling area. Numbers 1 to 7 denote the collection stations, and letters A, B, and C indicate the quadrants (A). Percentage of substrate variables occupancy in epigean and cave environments, with the x-axis representing the variables and the y-axis representing the occupancy percentage of these variables in the respective regions (B).

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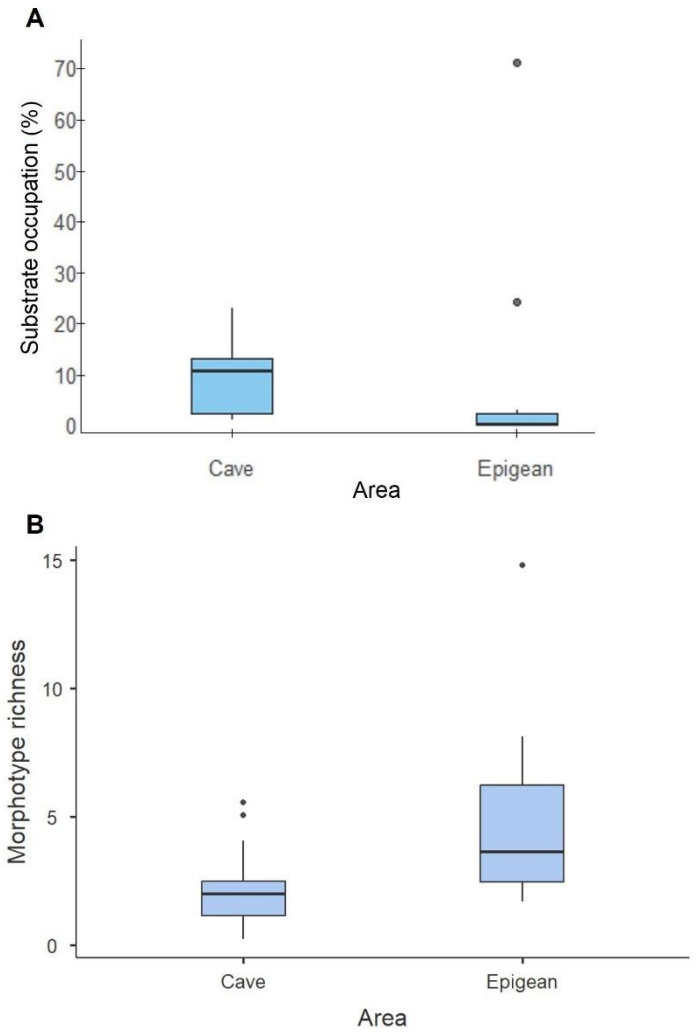

Figure 3. Average substrate occupancy percentage in cave and epigean areas (A). Average morphotype richness in the cave and epigean regions (B).

Invertebrate richness and composition among areas

A total of 310 individuals were recorded, representing 121 species. The most diverse taxa included Hymenoptera (23 species), Araneae (22 species), and Coleoptera (17 species) (Supplementary material).

The epigean environment exhibited greater species richness, with 85 species and 186 individuals, compared to the cave environment, which contained 46 species and 124 individuals. The most diverse groups in the epigean environment were Hymenoptera (23 species), Araneae (16 species), and Coleoptera (12 species). In contrast, the cave environment was dominated by Araneae (8 species), Coleoptera (6 species), and Diptera, Hymenoptera, and Hemiptera, each represented by four morphotypes (Figure 4). A total of 10 morphotypes were found in both environments, including members of the Araneae, Coleoptera, Collembola, Hymenoptera, and Isopoda groups. Additionally, 14 morphotypes were identified in more than one quadrant within the cave, while 24 were recorded across multiple epigean quadrants.

The Mann-Whitney test revealed significant differences in mean richness between the cave and epigean environments ($W = 74.0$; $p = 0.001$). The mean richness in the epigean environment

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was 4.72 (sd = 3.28), whereas the cave environment had a mean richness of 2.19 (sd = 1.42) (Figure 3B).

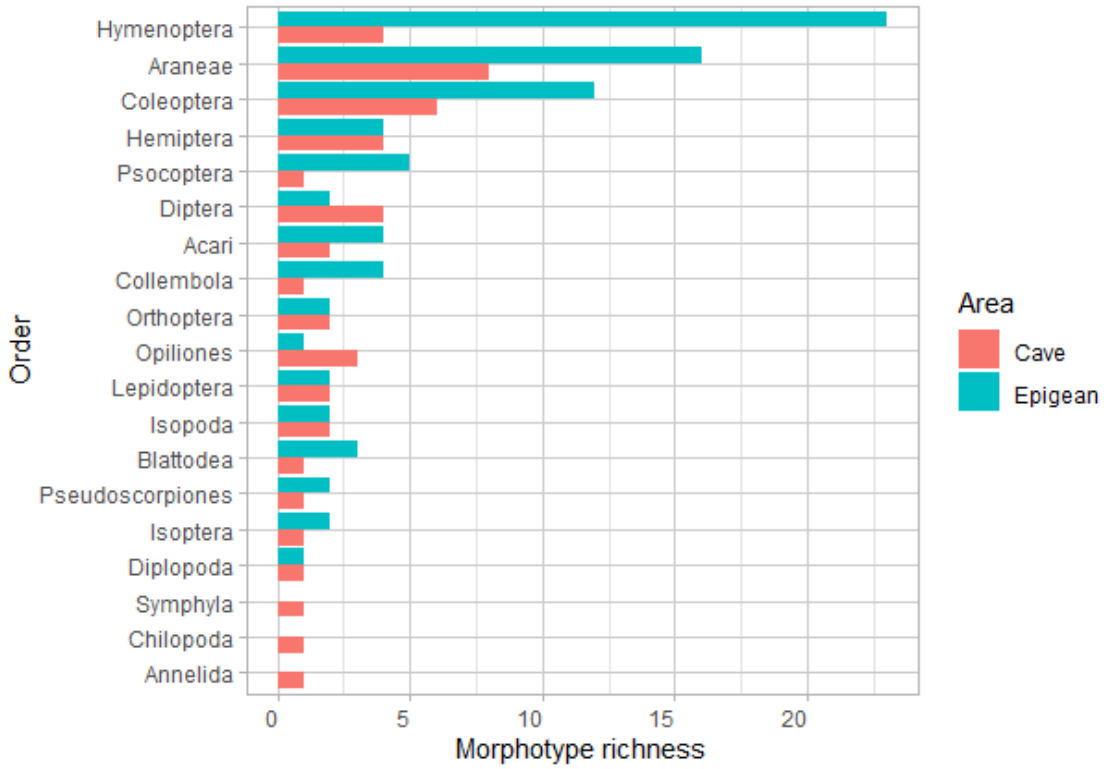

Figure 4. Species richness of terrestrial invertebrates recorded in quartzite caves and adjacent epigean areas.

The ANOSIM analysis revealed significant differences in faunal composition between the cave and epigean areas (p = 0.001; GlobalR = 0.127), highlighting a clustering of cave fauna distinct from that of the epigean environment in the MDS (Figure 5A). In contrast, the similarity analysis of faunal composition among the caves showed no significant differences.

Spatial βsør-diversity across epigean and cave indicate that the turnover was the predominant component ($p < 0.001$; pseudo $R^2 = 0.693$), accounting for approximately 69% of the observed variation. The nestedness component was also statistically significant ($p < 0.001$; pseudo $R^2 = 0.351$), explaining 35% of the variation in total βsør-diversity (Figure 6).

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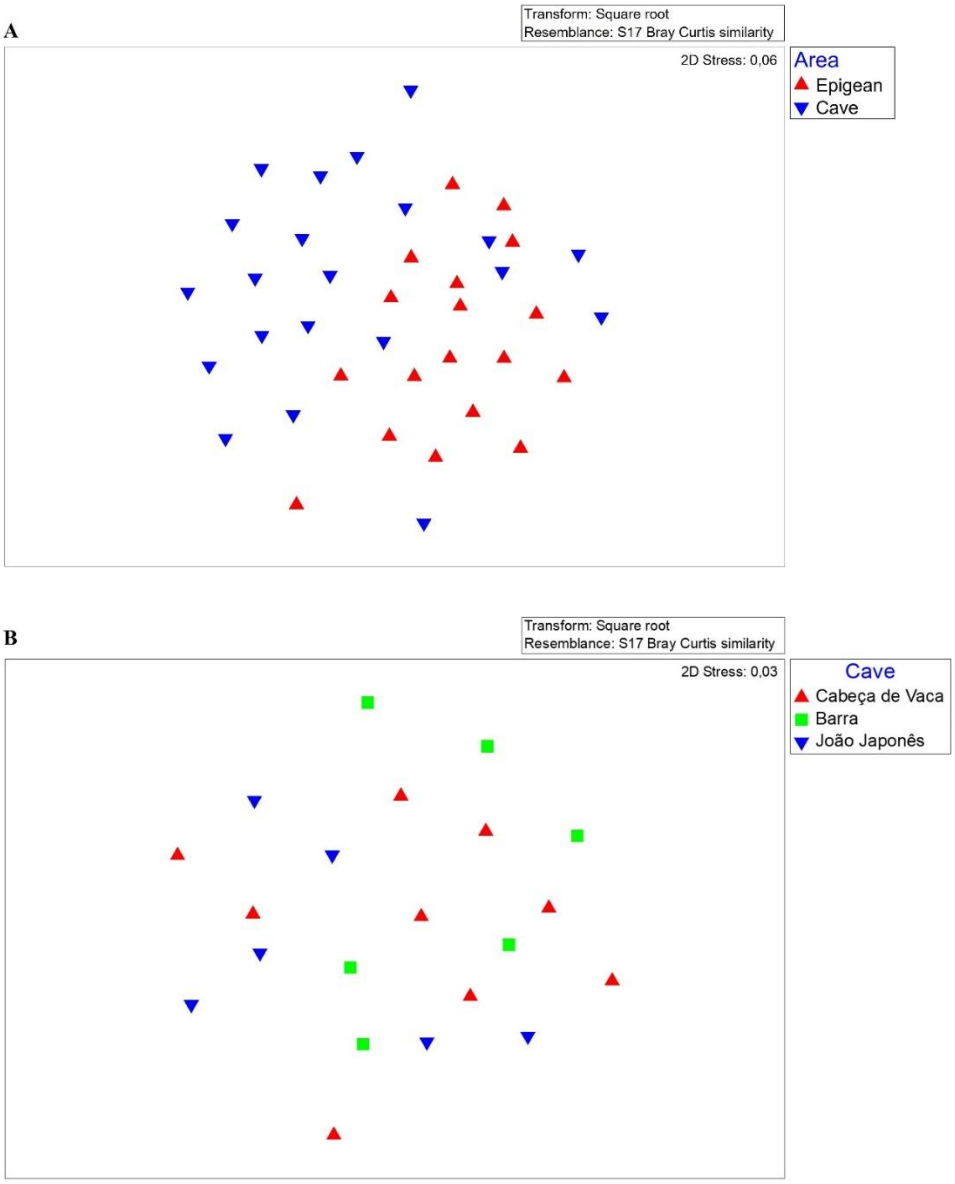

Figure 5. Multidimensional Scaling (MDS) showing the similarity in fauna composition between epigean and cave environments (A); and the among fauna composition at Cabeça de Vaca, Barra, and João Japonês caves (B).

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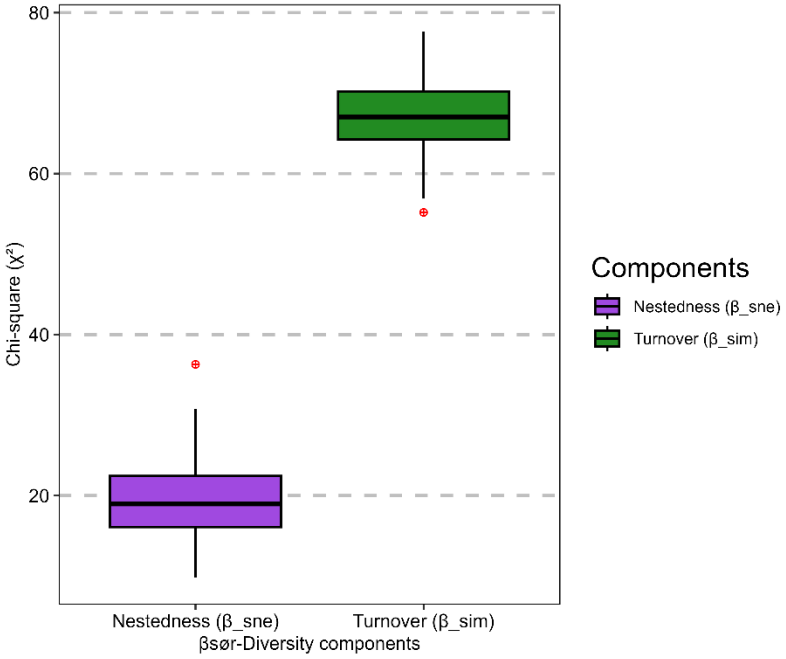

Figure 6. Contribution of β-diversity components to overall β-diversity. Boxplots represent the distribution of chi-square (χ²) values derived from model comparisons, illustrating the contributions of turnover and nestedness components to total β-diversity.

Factors determining the faunal composition and richness

The DistLM analysis indicated that the faunal composition in the epigean environment adjacent to the caves was solely influenced by Plant Debris (p = 0.002; adjusted R^2 = 0.047). Conversely, within the caves, the composition was determined by Guano (p = 0.001; adjusted R^2 = 0.039) and Inorganic Substrates (p = 0.001; adjusted R^2 = 0.080).

The dbRDA analysis demonstrated that the measured variables accounted for16.4% of the variation in faunal composition in the epigean area, while in the cave environment, the model accounted for 17.4% (Figure 7A-B).

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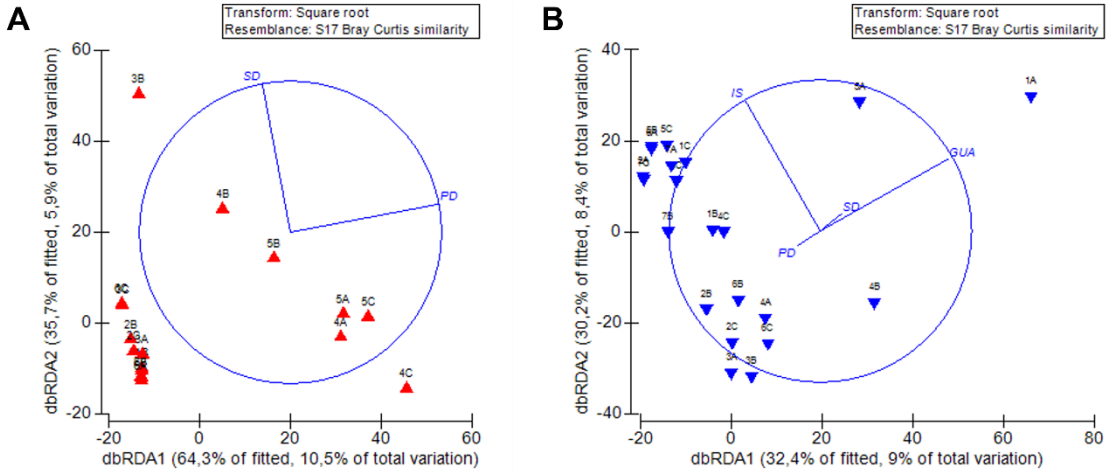

Figure 7. Distance-based redundancy analysis (dbRDA) showing the influence of variables on fauna composition in the epigean (A) and cave (B) environments. Numbers 1-7, labeled with A, B or C, denote the sampled area and quadrant. Variables: Plant debris (PD), Guano (GUA), Inorganic substrate (IS), and Substrate diversity (SD).

In the epigean environment, the variable Plant Debris exhibited a positive and significant relationship with species richness (Table 1 and Figure 8). In contrast, none of the evaluated variables accounted for the variation in species richness in the cave environment.

*Table 1. Model selection for variables. The table shows each predictor's estimated coefficients, standard errors, z-values, and p-values in the final Negative Binomial Generalized Linear Model (GLM.nb). The predictor variables used in the model were: SH (Shelter), IS (Inorganic substrate), PD (Plant Debris). A significance levels of the variables are denoted as (*** $p < 0.001$, ** $p < 0.01$).*

Variable	Estimate (β)	Std. Error	z value	p-value	Significance
Intercept	1.8783	0.1048	17.919	< 0.001	***
SH	0.1086	0.0908	1.196	0.23156	
IS	-0.1182	0.1182	-1.0	0.31737	
PD	0.3026	0.1057	2.862	0.00421	**

B) AIC (97.131); adjusted R^2 (0,2833); Zero-inflation Test (ratioObsSim = 0, p = 1).

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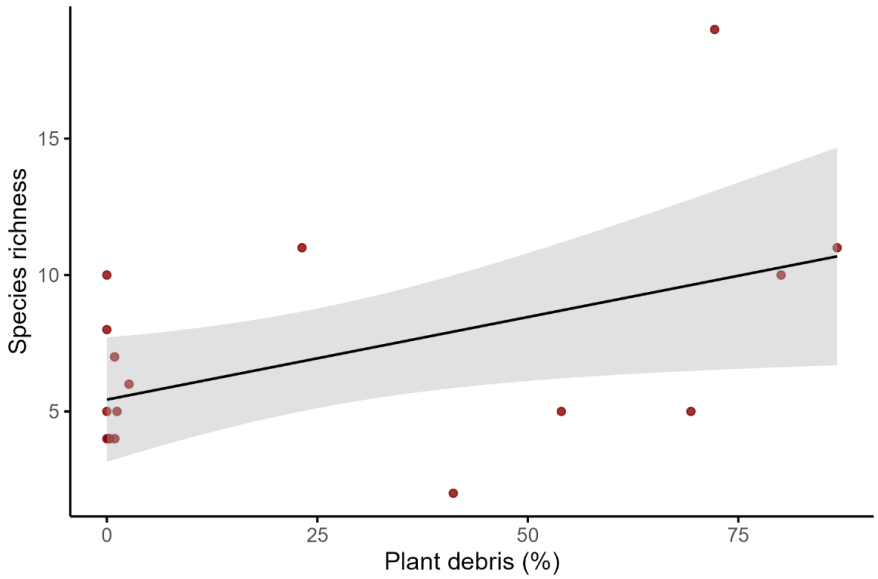

Figure 8. Distribution of fauna richness concerning the percentage of positively significant variable Plant Debris.

4. DISCUSSION

Differences in species richness and composition between areas

Faunal surveys revealed significantly higher species richness in the epigean environment compared to the cave, as evidenced by greater mean richness values. In addition to differences in richness, faunal composition varied markedly between the two environments. Spatial β-diversity was primarily driven by species turnover, with a secondary contribution from nestedness, indicating that both taxonomic replacement and differential species loss occurred across habitats.

These patterns in species richness and composition are likely shaped by the environmental constraints inherent to cave ecosystems. The absence of light, limited trophic resources, and reduced structural complexity act as strong ecological filters, limiting the colonization and persistence of many species (Poulson & White, 1969; Humphreys, 1991). Consequently, cave communities are structured by distinct selective pressures, leading to unique biological assemblages, even when ecological connectivity with adjacent epigean habitats exists (Prous et al., 2015; Mendes-Rabelo et al., 2021).

The composition of cave fauna is closely linked to the regional species pool found in adjacent epigean environments, which serves as a source of potential colonizers of subterranean ecosystems (Mendes-Rabelo et al., 2021). Many of these species are capable of moving between surface and cave habitats, particularly within cave entrance zones, where they often play key ecological roles in the dynamics and structuring of subterranean communities (Prous et al., 2004; 2015). However, their successful entry and persistence within cave systems are governed by environmental filters that selectively permit only certain organisms to establish and survive under the prevailing extreme conditions (Prous et al., 2015; Mendes-Rabelo et al., 2021; Reis-Venâncio et al., 2022).

In contrast, epigean areas typically offer a wider range of habitats and organic resources, supporting a more varied and abundant set of species (Ferreira & Marques, 1998; Loyola et al., 2006; Prous et al., 2015; Mammola et al., 2019b; Cardoso et al., 2022). The observed differences can be understood through various factors, including environmental conditions, climatic

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influences, and trophic interactions. These elements often limit the successful establishment and persistence of different invertebrate groups within specific ecosystems (Loyola et al., 2006). Inside caves such ecological factors may encompass habitat type, availability of resources, and physical conditions such as soil porosity and microhabitat availability (Pacheco et al., 2020a; Souza-Silva et al., 2021). Microclimate conditions could include temperature variations, moisture patterns, and seasonal changes that affect invertebrate cycles in number and composition (Bento et al., 2016; Lunghi et al., 2017; Cardoso et al., 2024).

The environmental conditions within caves play a pivotal role in shaping the composition and diversity of cave-dwelling fauna. These habitats, defined by the absence of light, relatively stable temperatures, and high humidity, give rise to distinct ecological niches that foster the development of specialized biological communities. Consequently, the faunal assemblages found in caves often differ markedly from those in adjacent epigean ecosystems (Souza-Silva et al., 2021; Oliveira & Ferreira, 2024). This pattern was also evident in the present study, which revealed pronounced dissimilarities between invertebrate communities inhabiting cave and surface environments.

Additionally, the oligotrophic nature of cave habitats significantly restricts the variety of species that can thrive within these environments. The low-nutrient condition leads to a reduction in primary production, thereby limiting the availability of food resources. As a result, the food webs within cave ecosystems tend to be less complex and are often characterized by fewer trophic levels (Ferrreira & Martins, 1999; Gibert, 2001). The simplicity of these food webs may increase the ecosystem's vulnerability to changes, as each species contributes significantly in the overall balance of this specialized habitat. (Huxel & McCann, 1998; Schneider et al., 2011).

Therefore, allochthonous resources play an important role in shaping the biodiversity of cave ecosystems (Schneider et al., 2011). These resources, which originate from outside the cave, serve as the main source of nutrients for the various species inhabiting these subterranean spaces. They can include organic matter such as guano, leaves, plant detritus, and other materials that enter the cave from the surface (Ferreira & Martins 1999, Souza-Silva et al., 2011; 2013). Introducing these external nutrients not only supports the growth and survival of cave-dwelling organisms but also influences the overall structure and dynamics of the cave food web (Venarsky et al., 2014; Venarsky & Huntsman, 2018). This interaction highlights the importance of outside environments in sustaining the complex life forms found within caves (Schneider et al., 2011; Venarsky & Huntsman, 2018; Culver & Pipan, 2019).

Sket (1999) proposed three factors that may explain this disparity between caves and external environments: limited habitat access due to the small number and size of cave entrances, habitat homogeneity, and low energy availability. However, it is important to note that the caves sampled in this study have multiple entrances and lack extensive linear development, reducing these limitations compared to other caves. Cave entrances can offer shelter to epigean invertebrates by providing resources and protection and acting as a filter that promotes the colonization of certain species (Prous et al., 2004; 2015). Regarding habitat homogeneity, the epigean environment was more homogeneous than the subterranean environment, with sampling conducted primarily in pasture areas. The third factor Sket (1999) mentioned, related to low energy availability, is corroborated by our findings, which revealed greater availability of trophic resources in the epigean region. This factor might explain the observed results, with significant differences in faunal composition between the two environments.

Our results indicate that cave fauna does not follow a dissimilarity gradient based on distance from the cave entrances, where the watercourse is situated. Despite an intermittent stream

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shared among the caves, which might facilitate species dispersal, no similarity was observed across the quadrants along this drainage down to the cave's furthest downstream point, contrary to initial expectations. This dissimilarity pattern may be attributed to the multiple entrances within the cave system, which enable considerable exchange of fauna and resources between subterranean and epigean environments. This finding further suggests that colonization and dispersal within the subterranean habitats of this system do not depend mainly on the watercourse.

Habitat traits determining patterns of richness and composition

As expected, the findings support the hypothesis that substrate variables influencing faunal composition differ markedly between cave and epigean environments. In epigean environments, fauna mirrored trends observed in previous studies, with "plant debris" emerging as a key resource for community structuring. For cave areas, guano was an important nutritional source for sustaining oligotrophic cave environments (Ferreira & Martins, 1999; Ferreira, 2019). Additionally, the other substrate significant for faunal structuring, the inorganic substrate primarily composed of compacted floor material, is the most common substrate in the study caves.

In epigean environments, the composition and richness of invertebrate communities can be influenced by "key landscape elements," defined as spatial structures that offer resources, shelter, or specific conditions for species (Tews et al., 2004). Thus, the variables affecting fauna in these environments are tied to physical substrate structures, trophic resources, and shelter, emphasizing the importance of such structural factors. Both autochthonous and allochthonous food resources are essential for animal communities across various environments (Marczak et al., 2007; Schneider et al., 2011). In oligotrophic habitats like caves, invertebrate communities depend heavily on allochthonous resources, which positively impact species richness and shape community composition (Ferreira et al., 2009; Schneider et al., 2011; Souza-Silva et al., 2011).

5. CONCLUSIONS

The findings of this study deepen our understanding of how microhabitat variables shape cave invertebrate communities and their adjacent epigean environments. Moreover, the differences in faunal composition between the subterranean and epigean habitats emphasize the critical need for conservation efforts to protect these communities. While distinct, these environments form interconnected and interdependent ecosystems, underscoring the necessity for integrated strategies to conserve and manage these areas.

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Author Contributions

Rodrigo Lopes Ferreira was responsible for the conceptualization, methodology, translation, and English corrections. Gabrielle Soares Muniz Pacheco participated in field activities. Felipe Carvajal was for the statistical analysis and original draft preparation. Gabrielle Soares Muniz Pacheco, Rodrigo Lopes Ferreira, and Marconi Souza Silva were responsible for the artwork, reviewing, and editing. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data supporting this study's findings are available on request from the corresponding author. The Editor-in-Chief has waived the required archiving due to privacy or ethical restrictions.

Conflicts of Interest

The corresponding author confirms on behalf of all authors that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated

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Cave Community Instability in the Tropics: A Case Study on Environmental Filtering and β-Diversity Partitioning of Terrestrial Invertebrates

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RESUMO: Ambientes subterrâneos são caracterizados por condições atmosféricas relativamente estáveis e ausência de luz; no entanto, sua complexidade morfológica pode influenciar a conectividade com a superfície, tornando-os suscetíveis a flutuações ambientais que afetam as comunidades de invertebrados. Neste estudo, examinamos a relação entre temperatura, umidade e distância da entrada da caverna de Salitre, no município de Cordisburgo, Minas Gerais, Brasil, ao longo de duas estações (seca e chuvosa), e seus efeitos sobre a riqueza de espécies, composição e diversidade beta (β-diversidade) de invertebrados terrestres. A riqueza de espécies foi maior durante a estação chuvosa e em áreas mais próximas da entrada. A temperatura influenciou positivamente a riqueza de espécies, enquanto a umidade afetou principalmente a composição da comunidade. A β-diversidade temporal apresentou variação sazonal e foi impulsionada principalmente pela substituição de espécies (turnover). A distância da entrada afetou negativamente a β-diversidade espacial em ambas as estações, enquanto a temperatura teve um efeito positivo significativo apenas durante a estação seca. O particionamento da β-diversidade revelou a substituição de espécies como o principal processo por trás da diferenciação espacial das comunidades cavernícolas. Nossos resultados sugerem que tanto a heterogeneidade ambiental temporal quanto espacial molda a dinâmica das comunidades nesta caverna. Temperaturas elevadas sustentam maior riqueza de espécies, ressaltando seu papel na manutenção da biodiversidade. Além disso, a distância da entrada influencia a distribuição das espécies, refletindo gradientes de composição e riqueza ao longo do ecótono subterrâneo. De modo geral, a substituição de espécies (e não o aninhamento/nestedness) emerge como o principal mecanismo estruturador das comunidades cavernícolas, destacando a sensibilidade ecológica e a instabilidade inerente dos habitats subterrâneos frente às mudanças ambientais.

Palavras-chave: Gradientes ambientais, Substituição de espécies, Filtragem ambiental, Invertebrados neotropicais.

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ABSTRACT: Subterranean environments are characterized by relatively stable atmospheric conditions and the absence of light; however, their morphological complexity can influence connectivity with the surface, making them susceptible to environmental fluctuations that affect invertebrate communities. In this study, we examined the relationship between temperature, humidity, and distance from the entrance of Salitre cave, located in Cordisburgo, Minas Gerais, Brazil, across two seasons (dry and rainy), and their effects on species richness, community composition, and beta diversity (β-diversity) of terrestrial invertebrates. Species richness was higher during the wet season and in areas closer to the entrance. Temperature positively influenced species richness, whereas humidity primarily affected community composition. Temporal β-diversity showed seasonal variation and was mainly driven by species turnover. Distance from the entrance negatively affected spatial β-diversity in both seasons, while temperature had a significant positive effect only during the dry season. Partitioning of β-diversity revealed species turnover as the dominant process underlying spatial differentiation of cave communities. Our findings suggest that both temporal and spatial environmental heterogeneity shape community dynamics in this cave. Elevated temperatures support greater species richness, underscoring their role in sustaining biodiversity. Furthermore, distance from the entrance influences species distribution, reflecting compositional and richness gradients along the subterranean ecotone. Overall, species replacement (rather than nestedness) emerges as the primary mechanism structuring cave communities, highlighting the ecological sensitivity and inherent instability of subterranean habitats to environmental change.

Keywords: Environmental gradients, Species turnover, Habitat filtering, Neotropical invertebrates.

1. INTRODUCTION

Subterranean environments exhibit unique characteristics that distinguish them from surface ecosystems, including the permanent absence of light (except near entrances) and a tendency toward relatively stable atmospheric conditions, particularly in terms of light intensity, temperature and humidity (Mammola et al. 2019; Pipan et al. 2019; Mejía-Ortíz et al. 2020). However, the extent of this environmental stability may vary depending on the morphological complexity of cave conduits (Bento et al. 2016; Mejía-Ortíz et al. 2020; Cardoso et al. 2024). As a result, subterranean ecosystems exhibit varying degrees of connectivity with the surface, which can increase or decrease their susceptibility to external environmental fluctuations, which can directly impact cave-dwelling invertebrate communities (Rendoš et al. 2012; Ferreira et al. 2015; Souza-Silva et al. 2021).

In tropical regions, the wet season often brings an increased influx of organic matter, which influences both the abundance and reproductive activity of subterranean fauna (Souza-Silva et al. 2011; Bento et al. 2016). The food supply available to cave communities is predominantly allochthonous (originating from outside the cave) and supports distinct subterranean food webs (Schneider et al. 2011; Souza-Silva et al. 2011). Due to the environmental heterogeneity within caves, subterranean biodiversity is influenced by multiple factors, including variations in light exposure, the availability of organic resources, air flow, temperature, and humidity (Tobin et al. 2013; Prous et al. 2004, 2015; Mammola, 2019). Caves often exhibit well-defined environmental

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gradients, which provide valuable opportunities to investigate how linear transitions in abiotic conditions shape faunal composition (Mammola, 2019; Reis-Venâncio et al. 2024). Understanding the drivers of these community shifts is crucial for unraveling the biological complexity of distinct ecosystems (Bascompte & Solé, 1995; Körner, 2007; McDonnell & Hahs, 2008; Chen et al. 2011).

It is well established that species inhabiting the twilight zones of caves are typically trogloxenes and troglophiles, while the deeper, more humid, and aphotic zones are predominantly occupied by troglobites (Reis-Venâncio et al. 2024; Souza-Silva et al. 2021). This zonation pattern is commonly attributed to factors such as microclimate (Tobin et al. 2013; Souza-Silva et al. 2021), light intensity (Reis-Venâncio et al. 2024; Silveira et al. 2024), availability of food resources (Souza-Silva et al. 2012; Rendoš et al. 2012), and competitive exclusion dynamics (Silveira et al. 2024; Vaz et al. 2025; Junta et al. 2025).

Caves are among the few natural systems characterized by relatively homogeneous microclimatic conditions, as most subterranean environments maintain nearly constant temperatures throughout the year and exhibit high relative humidity, often approaching saturation in their deeper regions (Badino, 2010). Consequently, variables such as temperature, humidity, and distance from the cave entrance are widely studied in cave invertebrate ecology, with distance from the entrance recognized as a key factor influencing community structure (da Rocha Melo et al., 2025). Cave entrances typically exhibit high environmental heterogeneity, which gradually decreases with increasing depth (Souza-Silva et al. 2021; Reis-Venâncio et al. 2024).

With increasing depth, environmental heterogeneity progressively declines, giving rise to more homogeneous and stable conditions within aphotic zones (Souza-Silva et al., 2021). These permanently dark regions are characterized by relatively constant temperature and humidity levels, coupled with a limited influx of organic matter, factors that exert a strong influence on the composition, structure, and dynamics of subterranean invertebrate communities (Tobin et al. 2013; Mammola et al. 2015).

For non-troglobitic species, cave entrances may serve either as preferred habitats or as transitional zones connecting to surface environments. In contrast, for troglobites and some non-troglobitic species, this ecotonal region can act as a strong environmental filter and represent a source of disturbance (Mammola, 2019; Souza-Silva et al. 2021). Nevertheless, multiple independent colonization events of subterranean habitats have been documented in cave-adapted taxa (Porter, 2007), as well as in surface-dwelling species likely dispersing through superficial subterranean habitats (Růžička, 1999; Culver & Pipan, 2019). These patterns suggest a complex network of ecological connectivity between surface and subterranean systems, enabling various pathways for species to colonize, persist in or retreat from hypogean environments.

This study aimed to assess whether the richness and composition of non-troglobitic terrestrial invertebrates vary in response to climatic and spatial factors within a Neotropical limestone cave. To address this objective, we tested the following hypotheses: (i) species composition varies along the entrance-to-depth gradient in relation to temperature and humidity, as well as between the dry and wet seasons; (ii) species richness increases with temperature and decreases with both humidity and distance from the cave entrance, with higher richness expected during the wet season; and (iii) invertebrate β-diversity is greater during the wet season and responds to variation in temperature, humidity, and distance from the entrance, with species turnover representing the dominant component of β-diversity in both seasons.

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2. MATERIALS AND METHODS

Study area

This study was conducted in Salitre Cave (-19.121918°, -44.351499°), located within the Peter Lund State Natural Monument (MNEPL), a protected area established in 2005 by the government of the state of Minas Gerais (Decree No. 44.120, 2005), in the municipality of Cordisburgo, Minas Gerais, Brazil (Figure 1). Regional geomorphology is characterized by an elongated relief with prominent rocky outcrops, caves, sinkholes, temporary lagoons, and grassland formations (Travassos & Kohler, 2009), and is situated within the Cerrado ecoregion (Olson et al., 2001). According to Köppen's climate classification, the area presents a mesothermal climate (Cwa), defined by hot, humid summers and dry winters. The regional climate is distinctly seasonal, with a wet season from October to March and a dry season from April to September. The annual mean temperature is approximately 22 °C, and annual precipitation ranges between 1,300 and 1,600 mm (Alvares et al., 2013).

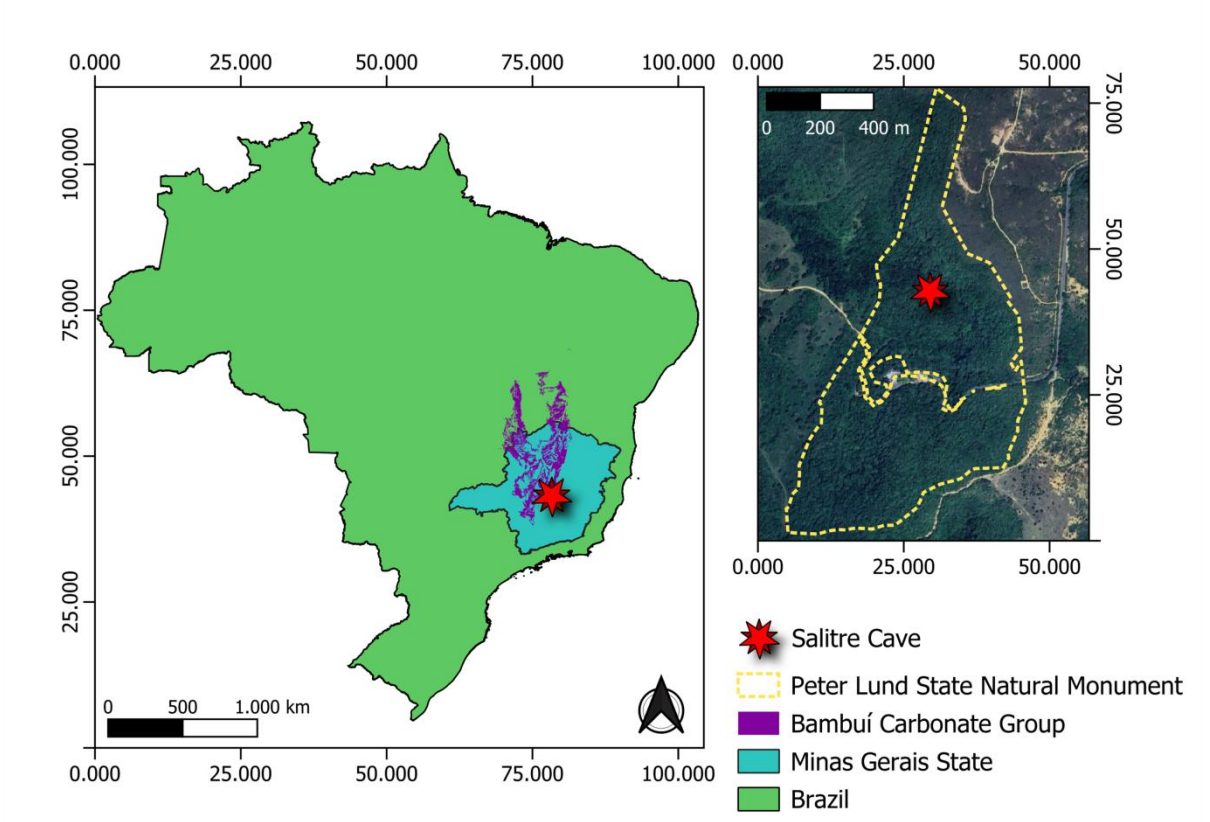

Figure 1. Location of Salitre Cave (red star) in the state of Minas Gerais, Brazil. The cave is situated within the Bambuí Carbonate Group and lies within the boundaries of the Peter Lund State Natural Monument, as outlined by the dashed yellow line.

The karst landscape in the region belongs to the Bambuí Geological Group, which dates to the Upper Proterozoic. Salitre Cave has developed within stratified, folded, and fractured carbonate rocks of the Lagoa do Jacaré Formation. It exhibits a predominantly horizontal profile, extending over 1,908 meters (Silva & Simões, 2002). Seasonal water flow occurs throughout most of the cave galleries, resulting in the accumulation of sediment deposits such as gravel, sand, and silt. The cave entrance is situated on a steep rock face, approximately halfway up the doline wall, with a sinkhole

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at its base where the surface stream disappears. Internally, the cave is traversed by a stream whose flow intensity varies with external precipitation. During the wet season, some low-ceiling passages may become completely flooded (Silva & Simões, 2002). A detailed cave map is provided in Silva & Simões (2002).

S*ampling design*

Sampling was conducted in January (wet season) and July (dry season) along a 750-meter transect extending from the cave entrance to its deeper zones. The transect was divided into 25-meter-long sectors, resulting in a total of 30 sampling points. The methodology followed Ferreira (2004), in which each observed species is assigned a unique identifier, and its occurrences are spatially mapped within the cave. This approach allows for detailed analyses of species distribution and abundance patterns.

Although studies based on a single cave inherently present limitations, particularly in terms of spatial representativeness and the number of sampling events, repeated seasonal sampling, as employed in the present study, is recommended to improve the understanding of temporal dynamics and the structure of subterranean communities (da Rocha Melo et al., 2025).

Sampling of Terrestrial Invertebrates

Terrestrial invertebrates were collected through intensive visual surveys and manual sampling using soft forceps and brushes, following established protocols (Ferreira, 2004; Wynne et al. 2019). All accessible microhabitats were thoroughly examined, including plant debris, guano deposits, spaces beneath rocks, and moist substrates. The sampling team consisted of five experienced researchers, all proficient in manual invertebrate collection and the identification of cave-dwelling fauna.

Collected specimens were preserved in 70% ethanol and identified to the lowest possible taxonomic level, then grouped into morphospecies for subsequent analyses (Souza-Silva et al., 2021) using a stereomicroscope. Troglobitic species (Figure 2) were identified based on the presence of troglomorphic traits, including reduced pigmentation, elongated appendages, and ocular reduction (Culver & Pipan, 2009; Deharveng et al. 2024). Taxonomic specialists (acknowledged in the Acknowledgments section) were consulted to confirm the troglobitic status of morphologically specialized taxa. All voucher specimens were deposited in the Subterranean Invertebrate Collection of Lavras (ISLA), housed at the Center for Studies in Subterranean Biology, Department of Ecology and Conservation, Federal University of Lavras (CEBS-UFLA) (biologiasubterranea.com.br).

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Figure 2. Troglomorphic species found in the Salitre Cave: A) Oxarthrius sp. (B,C) Isopoda, D) Pseudochthonius sp., E) Acari: Parasitengona, F) Spaeleoleptes spaeleus, (G,H) Rhamdiopsis sp.

Sampling of Environmental Variables

Temperature and relative humidity were measured in each sector using a thermo-hygrometer positioned at the center of the sampling area. The instrument had an accuracy of ±1 °C for temperature and ±5% for relative humidity. Readings were recorded after allowing the device to stabilize for approximately 15 minutes. To minimize potential interference with the measurements, the presence of researchers near the instrument was avoided during this period (Furtado-Oliveira et al., 2022).

Data analysis

Seasonal variation in abiotic conditions (temperature and humidity) was assessed using Analysis of Similarities (ANOSIM) based on a Euclidean distance matrix (Clarke et al., 2008). Faunal dissimilarity between seasons was also evaluated using ANOSIM, this time based on a Bray–

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Curtis similarity matrix. Both sets of results were visualized through Metric Multidimensional Scaling (MDS) ordinations. In order to identify the taxa contributing most to seasonal differences in faunal composition, a Similarity Percentage Analysis (SIMPER) was performed (Clarke, 1993).

The influence of environmental variables (temperature, relative humidity, and distance from the cave entrance) on species composition was investigated using a Distance-Based Linear Model (DistLM) with a Bray–Curtis similarity matrix, square root–transformed to reduce the influence of highly abundant species (McArdle & Anderson, 2001). Variable selection followed the forward selection procedure based on the corrected Akaike Information Criterion (AICc) (Anderson, 2008). To assess model fit and quantify the variation in community composition explained by environmental factors, a Distance-Based Redundancy Analysis (dbRDA) was applied (Anderson, 2008). All multivariate analyses were performed using PRIMER 7 software (Plymouth Routines in Multivariate Ecological Research; https://www.primer-e.com/software).

To evaluate the effects of distance from the cave entrance, temperature, and humidity on species richness, a Generalized Linear Mixed Model (GLMM) was applied. Species richness of terrestrial invertebrates was used as the response variable, with distance from the entrance, temperature, and humidity as fixed effects, and season included as a random effect. Prior to model fitting, Spearman's correlation analysis was conducted to assess multicollinearity among predictors. Due to a correlation coefficient exceeding 0.80, humidity was excluded from the model.

The initial model was fitted using a Poisson distribution; however, overdispersion was detected, necessitating the use of a negative binomial distribution. Model adequacy was verified through residual diagnostics, which revealed no significant overdispersion ($p = 0.224$). Multicollinearity was further evaluated using the Variance Inflation Factor (VIF), with all values below 2, indicating an absence of problematic collinearity among predictors.

To test whether species richness differed between seasons, a Generalized Linear Model (GLM) was constructed with season as the explanatory variable. As with the GLMM, the Poisson model exhibited overdispersion and was subsequently replaced with a negative binomial distribution. Residual diagnostics confirmed no significant overdispersion ($p = 0.208$).

Seasonal differences in β-diversity were examined using the Temporal Beta-diversity Index (TBI), based on a presence–absence matrix and the Sørensen dissimilarity index. Statistical significance was assessed through a permutation-based t-test ($n = 999$).

Spatial β-diversity was calculated for each sector by assessing dissimilarities among sampling points within each season separately, using the Sørensen dissimilarity index (βsør). This index was further partitioned into its turnover (βsim) and nestedness (βsne) components, both ranging from 0 (0%) to 1 (100%), indicating their relative contributions to overall βsør. Turnover represents species replacement among sites, while nestedness reflects differences due to species loss, where communities with lower richness are subsets of richer ones.

To assess the influence of environmental variables on βsør, two GLMs were fitted (one for each season) with βsør as the response variable and temperature and distance from the cave entrance as predictors. Given the bounded nature of the response variable (0–1), a beta distribution was applied to the models. To determine whether turnover (βsim) is the dominant component of total βsør, beta regression models were constructed using βsim and βsne as explanatory variables. Significance was evaluated using Type III ANOVA, and chi-square (χ^2) statistics were used to compare the relative influence of turnover and nestedness. Boxplots were generated to visually illustrate the contributions of these components.

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All species richness and β-diversity analyses were conducted in RStudio version 4.3.3 (R Core Team 2025). The GLMM was fitted using the lme4 package (Bates et al., 2015), and negative binomial models were implemented using MASS (Ripley, 2013). Spearman's correlation was calculated with PerformanceAnalytics (Peterson et al., 2018), and residual diagnostics were performed with DHARMa (Hartig, 2022). VIF and Type III ANOVA were computed using car (Fox et al., 2012). Effect graphs were produced using tidyverse (Wickham et al., 2019). The TBI was calculated using adespatial (Dray et al., 2018), while βsør and its components were obtained using betapart (Baselga, 2010). Beta regression models were implemented with betareg (Cribari-Neto & Zeileis, 2010), and GLMs with beta distributions were fitted using glmmTMB (Brooks et al., 2017).

Due to the limited number of troglomorphic species and their confinement to the deeper, aphotic regions of the cave, the influence of environmental filters on their richness and distribution was not evaluated in the present study.

3. RESULTS

Faunal Composition and richness

A total of 3,938 individuals were recorded along the sampled transect of the cave, comprising 142 species assigned to 18 taxonomic orders. Hexapoda was the most dominant group, both in terms of species richness (89 species; 62.7% of the total) and abundance (3,290 individuals; 88.7% of the total).

The most species-rich groups across both seasons were Coleoptera (33 species; 23.2%), Diptera (24 species; 16.9%), Araneae (20 species; 14.0%), Collembola (14 species; 9.8%), Lepidoptera (13 species; 9.15%), and Psocodea (7 species; 4.9%). Other groups included Acari (5 species; 3.5%), Pseudoscorpiones and Hemiptera (4 species each; 2.8%), Hymenoptera, Spirostreptida, and Polydesmida (3 species each; 2.11%), as well as Opiliones, Blattodea, and Trichoptera (2 species each; 1.40%). Ensifera, Archaeognatha, and Pauropoda were represented by a single species each (0.70% of the total). Species richness was higher during the wet season, with 101 species recorded, compared to 68 species in the dry season.

Seven species exhibited troglomorphic characteristics, including two isopods (Styloniscidae), one millipede (Polydesmida), one beetle (*Oxarthrius* sp.), one pseudoscorpion (*Pseudochthonius* sp.), and two harvestmen (*Spaeleoleptes spaeleus* H. Soares, 1966 and *Spinopilar* sp.).

Environmental conditions and faunal similarity between seasons

Temperature and humidity significantly differed between seasons (Global R = 0.106; p = 0.01; Figure 3A), with lower humidity values recorded near the entrance sectors during the dry season. Seasonal variation was also observed in species composition (Global R = 0.244; p = 0.01; Figure 3B). The SIMPER analysis identified *Endecous* sp. 1, *Keroplatidae* sp. 2, *Carabidae* sp. 3, *Hypena* sp. 2, *Lutzomyia* sp. 1, *Entomobryomorpha* sp. 66, and *Plato* sp. 1 as the taxa contributing most to compositional dissimilarity between seasons.

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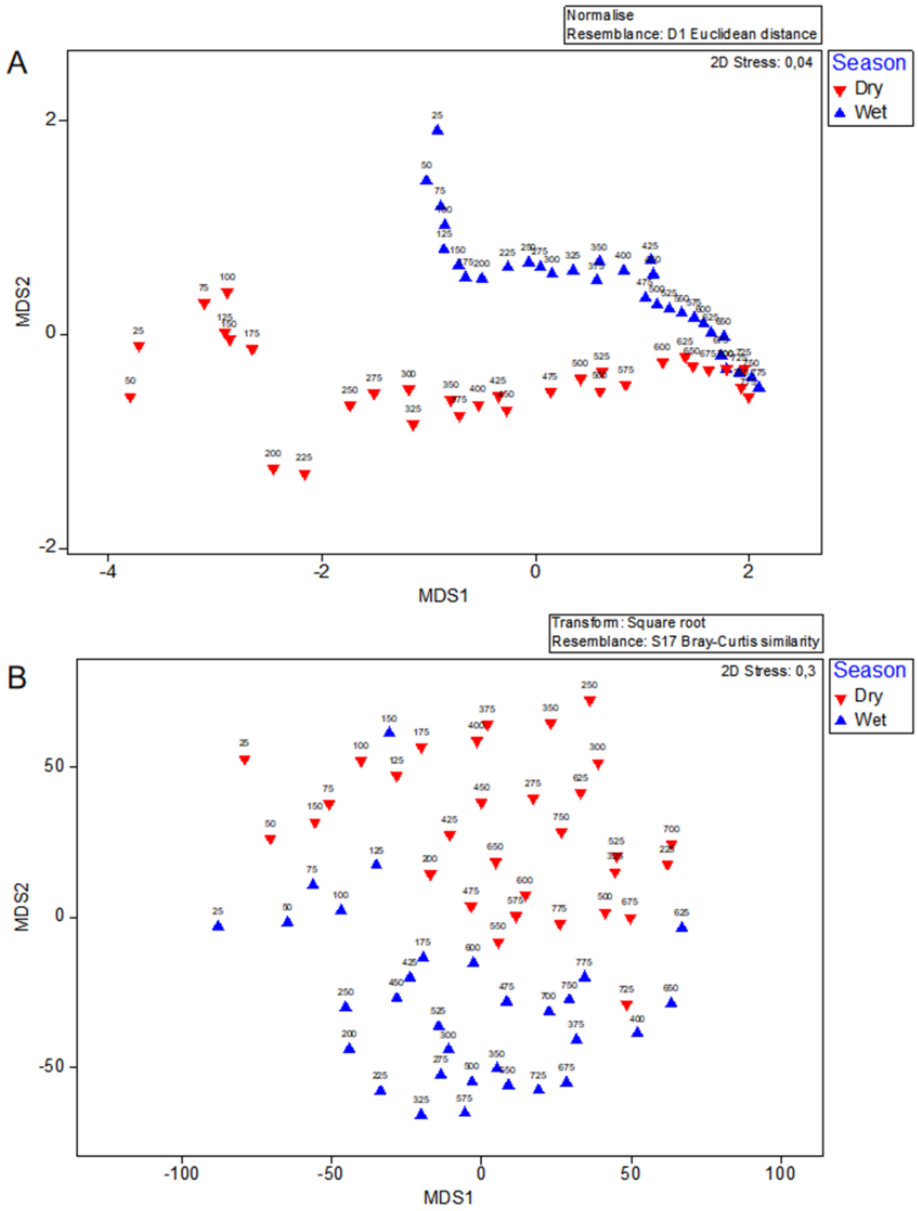

Figure 3. Non-metric multidimensional scaling (nMDS) ordinations showing the variation between dry (red, downward triangles) and wet (blue, upward triangles) seasons. (A) nMDS based on environmental variables using Euclidean distance (normalized data), stress = 0.04; (B) nMDS based on species composition using Bray–Curtis similarity (square root–transformed data), stress = 0.3. Numbers indicate the distance from the cave entrance (in meters).

Environmental influences on faunal similarity and species richness

The DistLM analysis revealed a significant relationship between species composition and both relative humidity and distance from the cave entrance ($R^2 = 0.26$; AICc = 237.74; $p = 0.001$). The dbRDA further indicated that the abiotic variables collectively explained 26.2% of the spatial variation in faunal composition (Figure 4).

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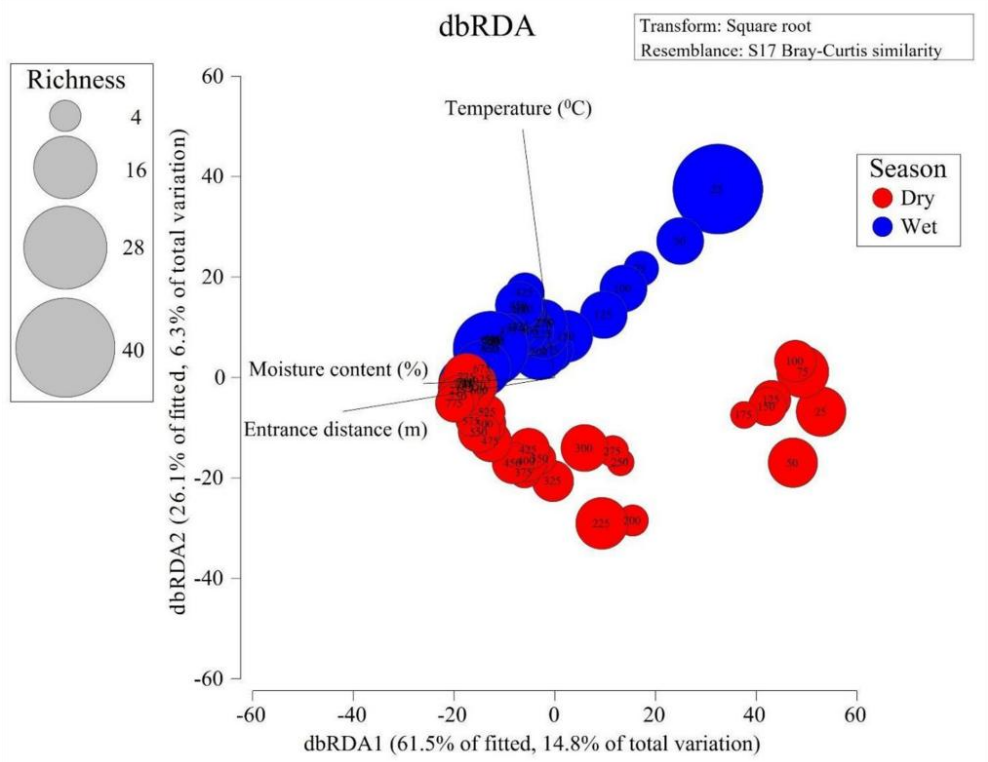

Figure 4. Distance-based redundancy analysis (dbRDA) showing the relationship between species composition and environmental variables across seasons. The analysis is based on Bray–Curtis similarity using square root–transformed species abundance data. Each point represents a sampling unit, with colors indicating the season (red = dry; blue = wet) and point size proportional to species richness. Vectors represent the direction and strength of environmental gradients: distance from the cave entrance (m), moisture content (%), and temperature (°C). The first and second dbRDA axes explain 14.8% and 6.3% of the total variation, respectively.

Species richness increased significantly with temperature ($\chi^2 = 13.730$; $p < 0.001$; Figure 5A) and declined with increasing distance from the entrance, indicating lower richness in the deeper cave sectors ($\chi^2 = 11.969$; $p < 0.001$; Figure 5B). Furthermore, richness varied significantly between seasons, with lower values recorded during the dry season ($\chi^2 = 11.056$; $p < 0.001$; Figure 5C).

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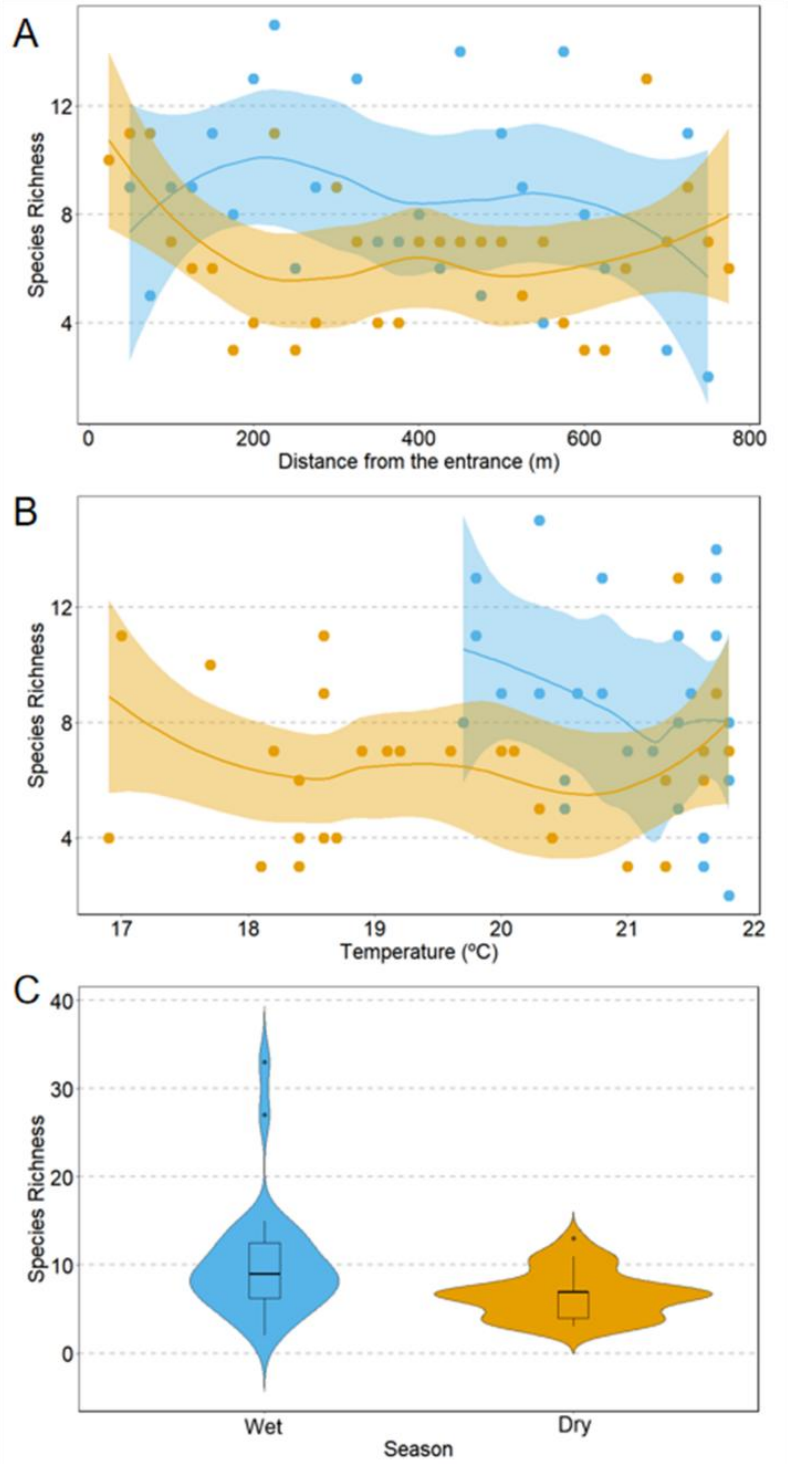

Figure 5. Relationship between species richness and environmental variables. (A) Species richness as a function of distance from the cave entrance (meters); (B) species richness in response to temperature (°C); and (C) comparison of species richness between the dry (yellow) and wet (blue) seasons. (A,B) Lines represent model fits with 95% confidence intervals (shaded areas); the yellow line corresponds to the dry season and the blue line to the wet season.

Variations in temporal and spatial β-diversity

Temporal β-diversity analysis using the TBI revealed distinct patterns in the contributions of turnover and nestedness components to total dissimilarity among sampled sectors (Figure 6). On average, species turnover emerged as the dominant driver of temporal dissimilarity, accounting for 43% of total β-diversity, while nestedness contributed 28%. The relationship between

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components showed that, on average, species turnover accounted for 60.5% of the total dissimilarity, while nestedness contributed 39.5% (Table 1A). A paired *t*-test confirmed the statistical significance of this difference ($t = -2.736$; $p < 0.05$; Table 1B), reinforcing that species turnover plays a greater role in seasonal community shifts than nested species loss or gain (Table S1). Nonetheless, despite the predominance of turnover, some sectors exhibited higher nestedness values, particularly sectors 1C (75 m), 3A (225 m), 5A (425 m), 5C (475 m), 6B (550 m), 7D (700 m), and 8B (750 m) from the cave entrance.

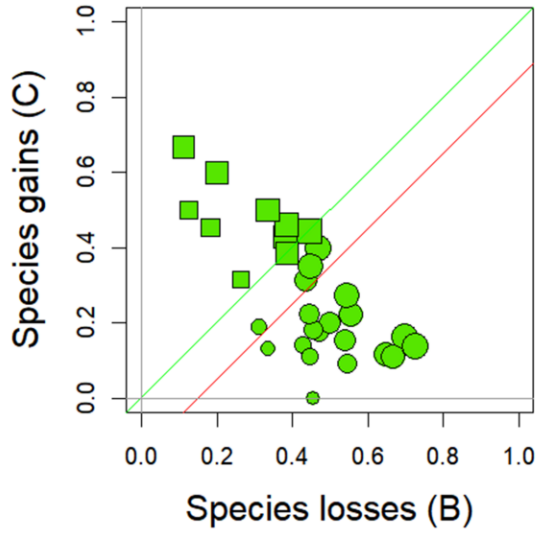

Figure 6. Temporal beta-diversity partitioning between wet and dry seasons based on species presence/absence data. Each point represents a sampling unit comparison, where the x-axis indicates species losses during the transition from dry to wet season (component B), and the y-axis indicates species gains (component C). Circular symbols represent sampling points where species gains exceed losses (C > B), while squares represent points where losses are greater than gains (B > C). The red diagonal line marks the 1:1 ratio between gains and losses, and the green diagonal line highlights deviations where gains are greater than losses.

Table 1. Temporal beta diversity analysis between dry and wet seasons. A) Mean values of the partitioning of total beta diversity (D) into turnover (species replacement) and nestedness (species subset inclusion), with relative proportions calculated in relation to the total number of differing species between seasons. B) Results of the paired t-test comparing the nestedness and turnover components between seasons. The negative mean difference (C - B) indicates a predominance of turnover over nestedness. Significant differences (p ≤ 0.05) are indicated with a ✓ symbol

A) **Summary of Turnover and Nestedness Analysis (TBI)**

Average Turnover (B/den)	Average Nestedness (C/den)	Average Beta Total (D)	Turnover Proportion (B/(B+C))	Nestedness Proportion (C/(B+C))	Global Change
0.4309	0.2814	0.7123	0.6049	0.3951	-

B) **t-test**

Statistic	Mean (C - B)	t-value	p.param	p.perm	p <= 0.05
Paired t-test	-0.1494	-2.7357	0.0105	0.01	✓

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Spatial β-diversity analysis during the wet season revealed a significant negative relationship with distance from the entrance ($\chi^2 = 4.267$; $p < 0.05$; Figure 7A), while temperature had no significant effect ($\chi^2 = 2.216$; $p = 0.136$). In the dry season, distance also had a significant negative effect on βsør-diversity ($\chi^2 = 7.177$; $p < 0.01$; Figure 7B), whereas temperature exhibited a significant positive effect ($\chi^2 = 5.458$; $p < 0.05$; Figure 7C). Partitioning of βsør revealed that species turnover was the dominant component of total β-diversity in both seasons, with highly significant effects in the wet season ($\chi^2 = 24.905$; $p < 0.001$; Figure 7D) and dry season ($\chi^2 = 106.153$; $p < 0.001$; Figure 7E).

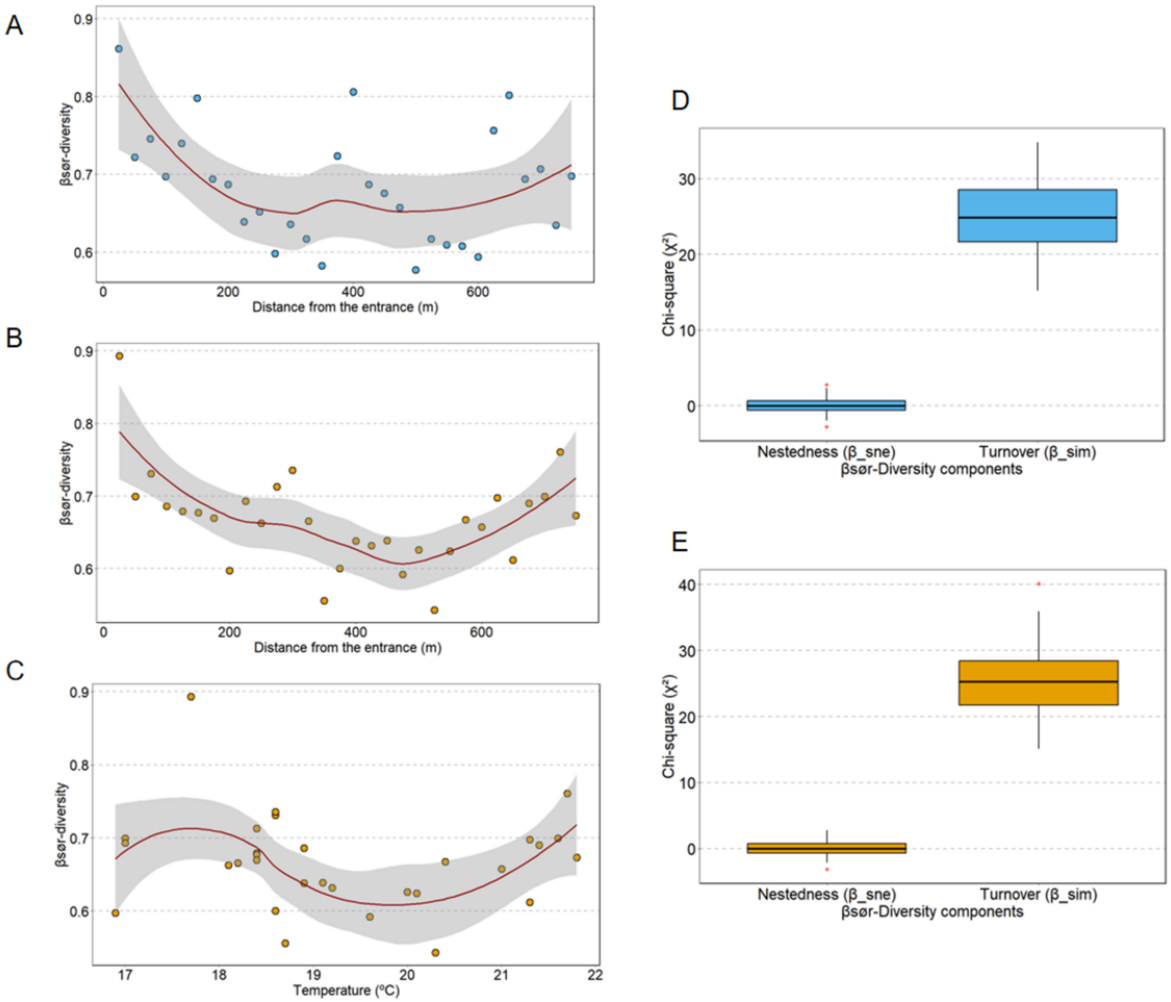

Figure 7. Patterns of β-diversity in relation to environmental gradients and the contribution of its components. (A-C) Relationships between β-diversity and environmental variables during the wet season (blue) and dry season (yellow): (A) β-diversity as a function of distance from the cave entrance (meters) in the wet season; (B) β-diversity as a function of distance from the cave entrance in the dry season; and (C) response of β-diversity to temperature (°C)in the dry season. Lines represent model fits with 95% confidence intervals (shaded areas). (D-E) Contribution of turnover and nestedness to overall β-diversity in each season: (D) wet season and (E) dry season. Boxplots represent the distribution of chi-square values (χ^2) from model comparisons.

4. DISCUSSION

This study demonstrates that species richness was higher during the wet season and in sectors located closer to the cave entrance, suggesting that microclimatic conditions are key determinants in the structuring of subterranean invertebrate communities. Temperature exhibited

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a positive effect on species richness, whereas relative humidity primarily influenced species composition. Temporal β-diversity varied significantly between seasons and was predominantly driven by species turnover, indicating that temporal replacement, rather than nestedness, is the principal mechanism underlying community dynamics. Spatially, distance from the cave entrance had a consistent negative effect on βsør-diversity across both seasons, while temperature was a significant predictor only during the dry season. Partitioning of βsør-diversity further confirmed species turnover as the dominant component driving spatial differentiation among cave sectors.

Seasonal variations influencing cave fauna

Salitre Cave experiences seasonal water flow (Silva & Simões, 2002), which, during the wet season, may facilitate the transport of organic matter into the cave, thereby enhancing the availability of trophic resources (Souza-Silva et al. 2011; Venarsky et al. 2012). This influx benefits subterranean invertebrates, which often exhibit increased abundance and reproductive activity during this period, a pattern also observed in other karst systems (Bento et al., 2016). Consequently, the observed increase in species richness during the wet season may be attributed to enhanced food availability, which promotes habitat colonization and intensifies foraging activity (Schneider et al. 2011; Souza-Silva et al. 2012).

Seasonal variation also influenced temporal β-diversity, with species turnover emerging as the primary mechanism underlying compositional change. This indicates that community shifts are driven largely by species replacement in response to environmental fluctuations. During the dry season, more restrictive microclimatic conditions may lead certain species to reduce their activity levels or seek refuge in more stable microhabitats, such as rock crevices or shallow subterranean zones (Ledesma et al. 2020; Novak et al. 2012). Such behavioral and spatial shifts may result in the exclusion of less tolerant taxa and the appearance of others better adapted to drier conditions, promoting seasonal turnover in the subterranean fauna.

Although deep and shallow subterranean habitats are relatively buffered from surface conditions, they are not entirely stable and can exhibit seasonal fluctuations in temperature and humidity, often with a delayed response to external climatic changes (Tobin et al., 2013). Moreover, surface environments exert a strong influence on subterranean ecosystems, with abiotic factors such as precipitation positively affecting cave species richness, particularly in regions where dry-season rainfall is higher (Mendes Rabelo et al., 2020).

Despite recent advances in understanding subterranean community responses to seasonal variability, significant knowledge gaps remain. Long-term, high-resolution monitoring of community structure (particularly with comprehensive humidity data across cave zones) is essential to deepen our understanding of these dynamics (Tobin et al., 2013). Furthermore, assessing the impacts of extreme climatic events will be critical for developing more effective conservation and management strategies aimed at safeguarding subterranean biodiversity under increasing global environmental change (da Rocha Melo et al., 2025).

Temperature variations influencing cave fauna

Temperature had a significant positive effect on species richness, suggesting that higher temperatures promote increased activity and diversity among cave-dwelling invertebrates. Previous studies have shown that thermal variation can modulate the behavior of subterranean organisms, influencing ecological interactions and shaping community structure (Souza-Silva et al. 2021;

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Mammola, 2019). Our findings are consistent with earlier research demonstrating a positive association between temperature and species richness in subterranean systems (Souza-Silva et al. 2021; Tobin et al. 2013; Junta et al. 2025; Mendes Rabelo et al. 2020). In general, average annual temperature plays a key role in subterranean ecosystems, particularly in Neotropical regions, where species richness tends to be higher in areas with elevated minimum temperatures (Mendes Rabelo et al., 2020).

Conversely, Vaz et al. (2025) did not detect a significant relationship between temperature and species richness, likely due to the presence of a perennial stream running through the entire length of the cave analyzed in their study, which may buffer thermal variability along the subterranean gradient. This contrasts with the conditions in Salitre Cave, where the watercourse is intermittent, allowing for greater thermal amplitude and thereby amplifying the influence of temperature on community structure.

The effect of temperature on βsør-diversity was significant only during the dry season, suggesting that in the wet season, other factors (such as increased humidity and resource availability) may exert a stronger influence on community composition. During the wet season, temperature remained relatively stable (20°C to 22°C), while in the dry season, a broader thermal range (17°C to 22°C) was observed. This increased variability likely contributed to spatial differentiation in community structure, as reflected by elevated βsør-diversity driven by species turnover. Species less tolerant to lower temperatures may have shifted toward warmer microhabitats, while cold-adapted species may have occupied cooler sectors. This redistribution likely reduces interspecific competition for resources and facilitates species coexistence along the thermal gradient, thereby enhancing species turnover and spatial βsør-diversity during the dry season.

Influence of distance from the cave entrance on cave fauna

Distance from the cave entrance was a key factor influencing the structure of the invertebrate community, affecting species richness, β-diversity, and species composition. The entrance zone represents a more ecologically heterogeneous environment, characterized by greater variability in humidity, temperature, and light availability. This environmental complexity promotes the formation of diverse microhabitats, which in turn supports higher species richness (Tamme et al. 2010; Mammola, 2019; Reis-Venâncio et al. 2024). In addition, the entrance region receives a more substantial influx of organic matter from the surface, resulting in increased availability of trophic resources for subterranean fauna (Souza-Silva et al., 2021).

As distance from the entrance increases, the observed decline in βsør-diversity and its effect on species composition indicate a trend toward faunal homogenization in the deeper cave zones. This pattern suggests that reduced environmental heterogeneity and the persistence of oligotrophic conditions limit local diversity, favoring a restricted set of species adapted to these stable, resource-poor environments (Tobin et al., 2013). Similar findings have been reported in previous studies, which have shown that cave communities in deeper sectors typically exhibit lower species richness and greater structural predictability due to limited resource input and reduced environmental variability (Moseley, 2008, 2009).

In addition to reduced connectivity with surface ecosystems, other factors may constrain colonization of deeper cave areas. The progressive loss of light, lower availability of organic material, and the presence of physical barriers hinder both the dispersal of fauna and the transport

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of trophic inputs via gravity, water flow, or air currents (Prous et al. 2015; Souza-Silva et al. 2021). Consequently, there is a marked decline in species richness along the cave depth gradient, an ecological pattern widely documented in subterranean systems across the globe (Culver et al. 2000; Prous et al. 2015; Souza-Silva et al. 2021).

Humidity variations influencing cave fauna

Humidity played a fundamental role in structuring the invertebrate community in Salitre Cave, significantly influencing species composition throughout the cave's spatial gradient. The critical importance of this variable for subterranean fauna has been well documented in the literature (Tobin et al. 2013; Prous et al. 2015; Souza-Silva et al. 2021; Reis-Venâncio et al. 2024; Junta et al. 2025). During the dry season, sectors near the entrance exhibited lower humidity levels, which may have constrained the persistence of hygrophilous taxa and favored species with greater desiccation resistance (Silveira et al., 2024). This microclimatic variability likely contributed to the observed pattern of species turnover, reinforcing turnover as the dominant driver of temporal β-diversity.

Numerous studies have shown that troglobitic species tend to be more abundant in cave sectors characterized by high and stable humidity, whereas taxa with greater desiccation tolerance typically occupy drier, more environmentally variable zones (Martins & Ferreira, 2020; Novak et al. 2021; Moseley, 2008; Souza-Silva et al. 2021). This distribution pattern highlights the role of humidity as a critical environmental filter, selectively favoring species based on their physiological tolerance thresholds. Furthermore, as humidity tends to increase with distance from the cave entrance, it is important to consider this gradient in conjunction with spatial variables when analyzing patterns of species richness and occurrence (Tobin et al. 2013; Prous et al. 2015; Souza-Silva et al. 2021).

The pronounced intolerance to desiccation observed among troglobitic invertebrates is largely attributed to traits such as reduced body size and thinner cuticles, which enhance cutaneous respiration but increase vulnerability to water loss. While these adaptations are advantageous in dark, humid environments, they impose physiological constraints that restrict these species to stable, high-humidity microhabitats. Such microhabitats are typically characterized by low thermal variability, creating narrow but stable ecological niches (Tobin et al. 2013; Lunghi et al. 2014, 2017; Kozel et al. 2019). As a result, troglobitic invertebrates are more frequently found in the deeper, climatically buffered regions of caves, avoiding entrance zones where abiotic conditions are more variable and potentially inhospitable (Novak et al. 2012; Tobin et al. 2013; Lunghi et al. 2017; Mammola & Isaia, 2018).

A clear gradient is also observed in the diversity of non-troglobitic species, which tends to decrease with increasing distance from the cave entrance. This pattern suggests the presence of ecological ecotones along the entrance-to-depth gradient. In the entrance zones, where environmental conditions are less extreme and organic matter input is higher, non-troglobitic species prevail. In contrast, as conditions become more oligotrophic, humid, and thermally stable deeper inside the cave, they increasingly favor the persistence of highly specialized troglobitic species that have evolved to thrive in these extreme subterranean conditions (Novak et al. 2012; Tobin et al. 2013; Kozel et al. 2019; Souza-Silva et al. 2021). This ecological gradient exemplifies how environmental filters and habitat specialization shape the distribution, composition, and coexistence of species in cave ecosystems.

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This study highlights the need for future research to explore the cave's ecological connectivity with its surrounding environments, including investigations of both surface and soil-dwelling fauna. Such studies would provide deeper insights into species interactions across habitats. Additionally, examining seasonal dynamics within these communities could help identify potential migration patterns and behavioral responses to environmental fluctuations.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author contribution: All authors contributed to the study conception and design. Material preparation and data collection were performed by Rodrigo Lopes Ferreira and statistical analysis were performed by Dara Veiga and Marconi Souza-Silva. The first draft of the manuscript was written by Dara Veiga and all authors commented on previous versions of the manuscript. All authors read and approved of the final manuscript.

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Supplementary material

Table S1. Turnover and nestedness components of beta diversity between wet and dry seasons for each sampling site, based on the Temporal Beta Diversity Index (TBI). Values represent turnover (B), nestedness (C), and total dissimilarity (D = B + C) standardized by the Sørensen dissimilarity denominator (2A + B + C). The column "Change" indicates the direction of species replacement: "+" for sites with more species gained than lost (C > B), "−" for more losses than gains (B > C), and "0" for equal turnover and nestedness (B = C).

Sector	Distance (m)	Turnover (B/(2A+B+C))	Nestedness (C/(2A+B+C))	Beta Total (D)
S1A	25	0.6977	0.1628	0.8605
S1B	50	0.2632	0.3158	0.5789
S1C	75	0.1250	0.5000	0.6250
S1D	100	0.3125	0.1875	0.5000
S2A	125	0.3333	0.1333	0.4667
S2B	150	0.4706	0.1765	0.6471
S2C	175	0.5455	0.0909	0.6364
S2D	200	0.6471	0.1176	0.7647
S3A	225	0.3810	0.4286	0.8095
S3B	250	0.5556	0.2222	0.7778
S3C	275	0.5385	0.1538	0.6923
S3D	300	0.4444	0.4444	0.8889
S4A	325	0.5000	0.2000	0.7000
S4B	350	0.4545	0.1818	0.6364
S4C	375	0.5455	0.2727	0.8182
S4D	400	0.4667	0.4000	0.8667
S5A	425	0.3846	0.4615	0.8462
S5B	450	0.4375	0.3125	0.7500
S5C	475	0.3333	0.5000	0.8333
S5D	500	0.4444	0.2222	0.6667
S6A	525	0.4286	0.1429	0.5714
S6B	550	0.1818	0.4545	0.6364
S6C	575	0.6667	0.1111	0.7778
S6D	600	0.4545	0.0000	0.4545
S7A	625	0.4444	0.1111	0.5556
S7B	650	0.7241	0.1379	0.8621
S7C	675	0.3846	0.3846	0.7692
S7D	700	0.2000	0.6000	0.8000
S8A	725	0.4500	0.3500	0.8000
S8B	750	0.1111	0.6667	0.7778

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Microhabitat heterogeneity and organic resources drive invertebrate diversity in a remote Brazilian limestone cave

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RESUMO: Os ecossistemas subterrâneos são caracterizados pela estabilidade ambiental e pela limitação de recursos tróficos, sustentando uma fauna com adaptações especializadas à vida na escuridão. Apesar de sua relevância ecológica, muitas cavernas brasileiras permanecem pouco estudadas, particularmente no bioma Pantanal, restringindo nosso conhecimento sobre a biodiversidade subterrânea. Este estudo investigou os efeitos de variáveis ambientais sobre a riqueza, composição e diversidade taxonômica de invertebrados na remota caverna calcária Ricardo Franco. Foram registradas assembleias de invertebrados, as condições microclimáticas (temperatura e umidade) e a diversidade e disponibilidade de substratos tróficos e de abrigo em duas escalas espaciais: mesoescala (setores de 30 m²) e microescala (quadrantes de 1 m²), ao longo da cavidade. Identificamos 30 espécies, incluindo seis troglóbios: *Potiicoara brasiliensis*, *Megagidiella azul*, *Novamundoniscus* sp., *Pirassununguleptes* sp., *Chernetidae* sp. e *Chelodesmidae* sp. Na mesoescala, a riqueza de espécies diminuiu com o aumento da distância da entrada, mas aumentou com maior heterogeneidade do habitat e disponibilidade de recursos orgânicos; a composição de espécies também foi influenciada pela distância. Na microescala, nenhuma variável explicou significativamente a riqueza ou a composição, embora a diversidade de recursos tróficos tenha mostrado um papel potencial na estruturação das comunidades. A diversidade taxonômica foi principalmente influenciada pela temperatura, umidade e disponibilidade de abrigos, que juntas explicaram mais de 90% da variação. Esses resultados evidenciam como os gradientes ambientais e a complexidade do habitat atuam na organização das assembleias subterrâneas, enquanto a presença de fauna troglóbia na Caverna Ricardo Franco acrescenta um peso adicional ao seu valor ecológico como uma área prioritária para ações de conservação.
Palavras-chave: fauna cavernícola, troglóbios, Pantanal, distinção taxonômica, conservação.

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ABSTRACT: Subterranean ecosystems are characterized by environmental stability and limited trophic resources, supporting fauna with specialized adaptations to life in darkness. Despite their ecological relevance, many Brazilian caves remain poorly studied, particularly in the Pantanal biome, restricting our understanding of subterranean biodiversity. This study investigated the effects of environmental variables on invertebrate richness, composition, and taxonomic diversity in the remote Ricardo Franco limestone cave. Invertebrate assemblages, microclimatic conditions (temperature and humidity), and the diversity and availability of trophic and shelter substrates were recorded at two spatial scales: mesoscale (30 m² sectors) and microscale (1 m² quadrats), along gradients of distance from the entrance. We identified 30 species, including six troglobites: *Potiicoara brasiliensis*, *Megagidiella azul*, *Novamundoniscus* sp., *Pirassununguleptes* sp., Chernetidae sp., and Chelodesmidae sp. At the mesoscale, species richness declined with increasing distance from the entrance but increased with greater habitat heterogeneity and organic resource availability; species composition was also shaped by distance. At the microscale, no variable significantly explained richness or composition, though trophic resource diversity showed a potential role in structuring communities. Taxonomic diversity was mainly driven by temperature, humidity, and shelter availability, which together explained over 90% of variation. These results underscore the influence of environmental gradients and habitat complexity on the structuring of subterranean assemblages, while the presence of troglobitic fauna within Caverna Ricardo Franco further elevates its ecological significance, establishing it as a conservation priority.
K**eywords**: cave fauna, troglobites, Pantanal, taxonomic distinction, conservation.

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1. INTRODUCTION

Subterranean environments are unique ecosystems characterized by stable abiotic conditions, including the absence of light, high relative humidity, constant temperature, and limited trophic resources (Culver & Pipan, 2019). These conditions impose ecological constraints that favor the evolution of highly specialized organisms, such as troglobitic species, which often display morphological traits including loss of pigmentation, eye reduction, and elongation of sensory appendages (Sket, 2008; Deharveng et al., 2024). Despite their relevance for understanding evolutionary, biogeographical, and ecological processes, subterranean faunas remain among the least studied worldwide and are frequently overlooked in conservation planning (Mammola et al., 2019; Cardoso et al., 2022).

In Brazil, research in subterranean ecology has advanced substantially in recent decades, with most studies concentrated in the karst regions of Minas Gerais and Bahia, which host the highest numbers of recorded caves and known species (Souza-Silva et al., 2020; Ferreira et al., 2023; Reis-Venâncio et al., 2024; Rocha Melo et al., 2025). These efforts have revealed complex patterns of species distribution, diversity, and endemism, alongside growing threats from mining, agriculture, and unregulated tourism (Cardoso et al., 2022). However, vast portions of the country (particularly the Central-West, South, and North regions) remain under sampled, limiting our understanding of large-scale ecological patterns. This paucity of data hinders biodiversity assessments and impedes the development of effective conservation and management strategies (Cordeiro et al., 2014).

Among Brazil's neglected subterranean regions, the Pantanal stands out as one of the largest wetland systems on Earth and a biome renowned for its exceptional epigean biodiversity (Alho & Sabino, 2011). In contrast, its subterranean fauna remains largely unknown (Cordeiro et al., 2014). Ricardo Franco Cave, also known as Hell Cave, is located in Forte Coimbra, municipality of Corumbá (Mato Grosso do Sul), within this biome. The cave holds considerable historical significance: it was explored in the 18th century by Colonel-Engineer Ricardo Franco de Almeida Serra during boundary surveys between Portuguese and Spanish territories (Coelho, 2005) and later visited by the naturalist Alexandre Rodrigues Ferreira, who documented it during his expedition to the western regions of the colony (Martins & Abreu, 2022).

Geographically, Ricardo Franco Cave lies within a small, isolated limestone outcrop near the borders of Brazil, Paraguay, and Bolivia. Despite its historical prominence, the cave remains largely unexplored from an ecological perspective. The only biological record comes from a broader survey of subterranean biodiversity in the Serra da Bodoquena region (Cordeiro, 2014), which reported the presence of troglobitic and threatened species. However, no study has yet addressed the role of environmental factors (such as humidity, temperature, substrate diversity and availability, or distance from the entrance) in shaping its cave fauna. This knowledge gap is especially concerning given the evidence of anthropogenic disturbances, including old structural modifications and occasional tourist visitation.

Recent studies have demonstrated that the organization of subterranean invertebrate communities is strongly influenced by environmental gradients, which act as ecological filters shaping species distributions (Prous et al., 2015; Rocha Melo et al., 2025). Key factors include microhabitat availability, substrate heterogeneity, organic matter input, and microclimatic stability (Souza-Silva et al. 2021; Vaz et al. 2025). Species responses to these variables may vary depending on the spatial scale of analysis, highlighting the importance of multi-scale approaches to adequately

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capture patterns of occupancy and diversity (Pacheco et al., 2020; Junta et al., 2025). In general, heterogeneous environments sustain higher species richness by providing a broader range of niches and promoting coexistence (Tews et al., 2004; Pacheco et al., 2020).

In the case of Ricardo Franco Cave, the lack of targeted ecological investigations limits our understanding of how such variables influence the local fauna. Assessing the role of physical, microclimatic, and trophic factors in shaping species richness, composition, and taxonomic diversity is essential for robust environmental evaluations and for developing effective management strategies, particularly in caves subject to anthropogenic disturbance (Souza-Silva et al., 2015; Mammola et al., 2020).

Given this context, the present study aimed to evaluate the influence of environmental variables on the richness, composition, and taxonomic diversity of terrestrial invertebrates in Ricardo Franco Cave. Specifically, we examined the effects of distance from the entrance, relative humidity, temperature, and the diversity and availability of trophic and shelter substrates. We hypothesized that areas closer to the entrance would harbor greater species richness and distinct community composition, driven by higher environmental heterogeneity and increased organic matter input.

2. MATERIAL AND METHODS

Study Area

This study was conducted in Ricardo Franco Cave (417081.38 E; 7800764.80 S), located in the Forte Coimbra region, municipality of Corumbá, Mato Grosso do Sul, Brazil. The cave lies within the Pantanal, a region designated as a National Heritage site in 1988. Covering approximately 160,000 km² (of which about 140,000 km² are within Brazilian territory), the Pantanal is one of the largest wetland systems on Earth (Junk et al. 2006). The regional climate is classified as Tropical Savanna (Aw) under the Köppen system, and the dominant vegetation corresponds to the Cerrado biome. The Pantanal represents about 1.8% of Brazil's total area (IBGE 2019), extending across the states of Mato Grosso and Mato Grosso do Sul, with roughly 65% of its extent in the latter (Coelho, 2005; Cordeiro et al., 2014; Martins & Abreu, 2022).

Ricardo Franco Cave lies on the opposite bank of the Paraguay River, about 200 km from Serra da Bodoquena National Park. It is situated within the boundaries of Forte Coimbra, a fortification established in 1775 to defend the frontier, first contested between Portugal and Spain, and later between Brazil and Paraguay (Figure 1). The fort, now registered by IPHAN, currently houses military facilities and residences associated with the Third Border Company, preserving an important part of regional history (Coelho, 2005). The fact that this cave is located within an area legally protected as historical heritage by IPHAN also facilitates access control and helps maintain its current state of conservation.

Geologically, Ricardo Franco Cave developed in dolomitic and calcitic limestone. Evidence of historical human use is widespread, including marks of cannon fire, inscriptions (e.g., a record of a visit by Marshal Rondon), and several modifications to the cave interior and its surroundings. Notable alterations include remnants of a deactivated lighting system, an unused external generator, and a masonry staircase built prior to 1876, connecting the entrance to the main hall, where a permanent lake is located (Cordeiro et al., 2014).

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Figure 1. The Forte Coimbra region is located in the municipality of Corumbá, Mato Grosso do Sul State, Brazil. A) Aerial view of the landscape of Forte Coimbra and the Paraguay River (the yellow arrow indicates the entrance of the cave); B) Entrance of Ricardo Franco Cave; C) Interior of the Cave, highlighting the lake inside the cave; D) Speleothems present inside the cave. Photos: Rodrigo Lopes Ferreira.

Sampling design

Sampling was carried out at two spatial scales: (i) the mesoscale, represented by 12 sectors measuring 3 × 10 m each, and (ii) the microscale, consisting of 36 quadrats of 1 m² (Figure 2, Figure 3). Within each sector, three quadrats were established, one at each extremity and one in the

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central area. Sampling extended from the cave entrance to the innermost accessible zones, thereby encompassing the main environmental gradient.

Temperature, relative humidity, and distance from the nearest entrance were measured using a thermohygrometer and a laser tape measure. For each sector, the thermohygrometer was placed at floor level in the central area for approximately 15 minutes to obtain microclimatic values (Souza-Silva et al., 2021). Biotic and abiotic data were collected during a single field campaign conducted in September 2022.

Sampling abiotic data

At the sector level (mesoscale), each 3 × 10 m sector was subdivided into ten perpendicular subsections measuring 3 × 1 m. Within each subsection, visual inspection was used to estimate the proportion of the area occupied by different substrate classes. For each class, percentage values from all subsections were summed and divided by 100 to obtain a single value per sector (Pacheco et al., 2020; Souza-Silva et al., 2021).

At the quadrat level (microscale), three representative 1 m² quadrats, located at the beginning, middle, and end of each sector were photographed with the camera positioned perpendicular to the ground (90°) prior to invertebrate collection. The images were processed in *ImageJ* software (Rasband, 1997) to quantify the proportion of each substrate class. As at the mesoscale, the percentage values for each class were summed and divided by 100, resulting in a single value per quadrat (Pacheco et al., 2020; Souza-Silva et al., 2021).

Invertebrate sampling

Invertebrates were collected through exhaustive active visual searches and manual capture using tweezers and brushes. Specimens were preserved in vials containing 70% ethanol, following established protocols (Pacheco et al., 2020; Souza-Silva et al., 2021). To increase the likelihood of detecting troglobitic species, complementary surveys were conducted outside the predefined sectors and quadrats, encompassing a variety of microhabitats throughout the cave. However, only specimens collected within the designated sampling units were included in the statistical analyses, whereas records obtained outside these units were used exclusively to compile the overall species inventory of Ricardo Franco Cave.

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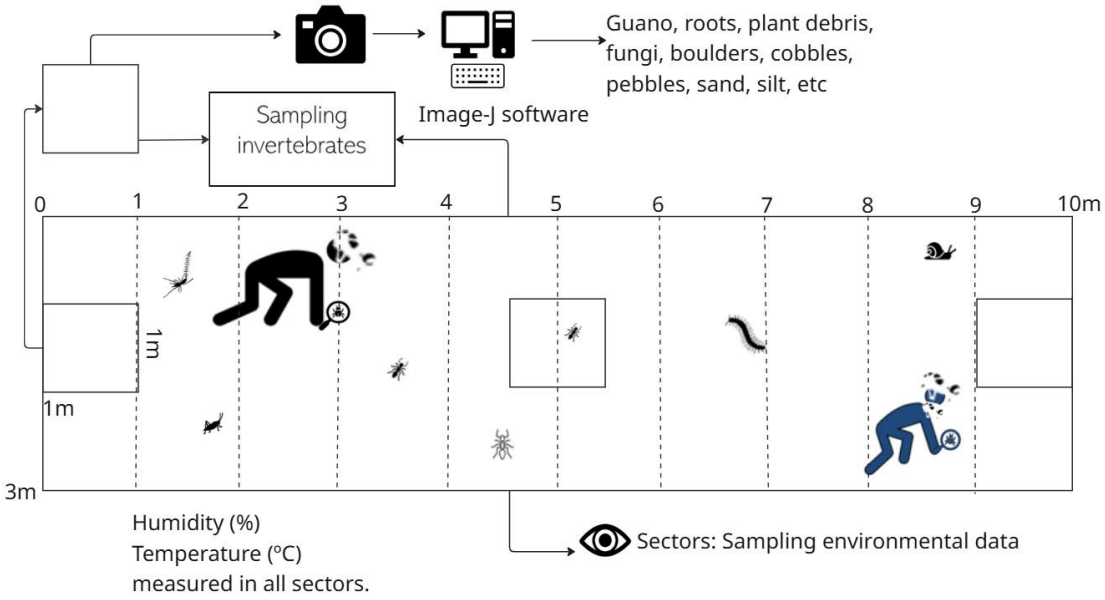

Figure 2. Diagram of invertebrate sampling and abiotic variables in quadrats and sectors inside Ricardo Franco Cave.

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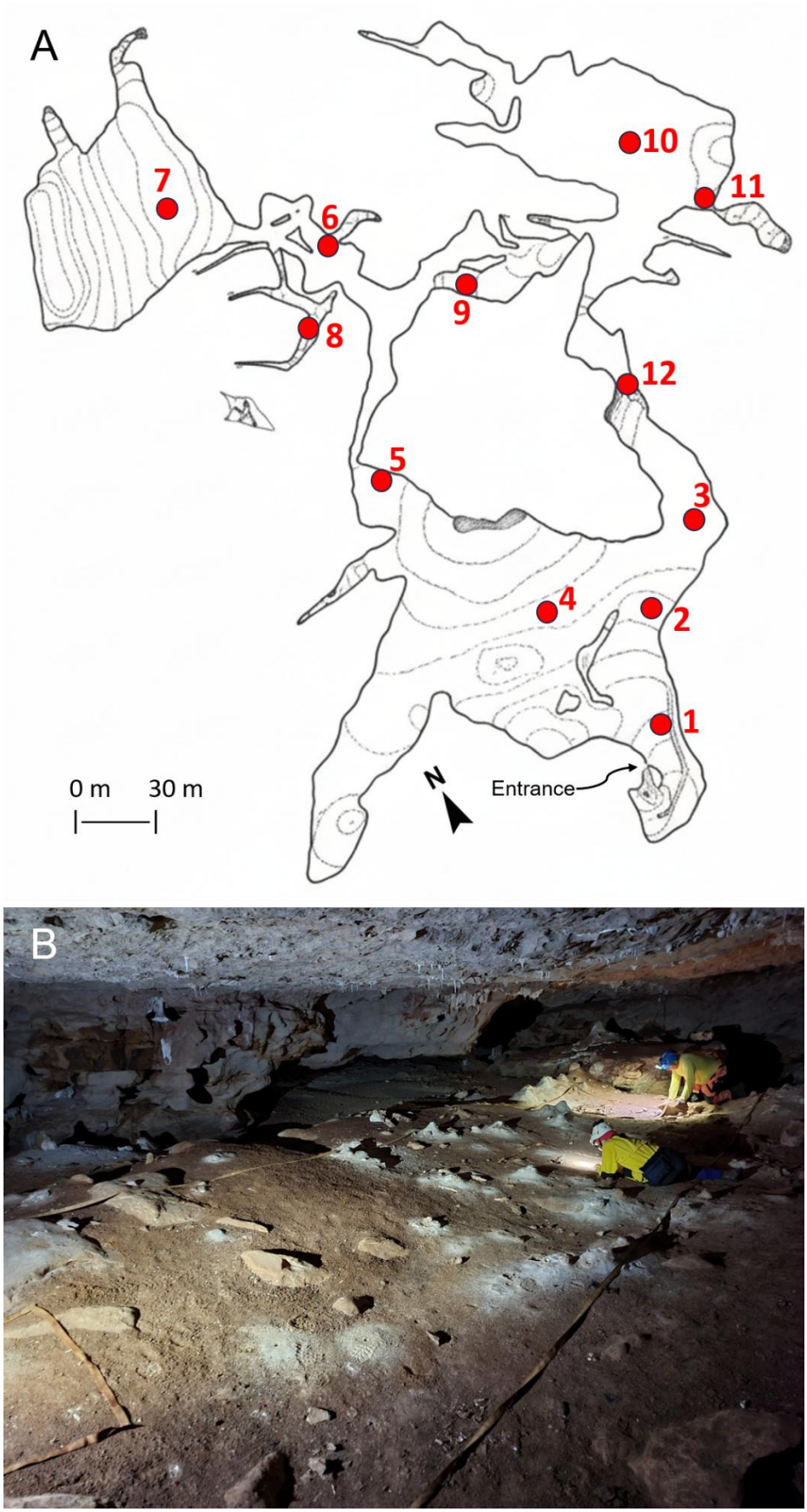

Figure 3. Distribution of sampling points in Ricardo Franco Cave. (A) Cave map showing the twelve sampling sectors (red points). Contour lines represent variations in floor topography, and the arrow indicates the location of the entrance. (B) Sampling activity in one of the surveyed sectors.

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Sorting and identifying invertebrates

Invertebrates were sorted and identified to the lowest possible taxonomic level using available literature (Oliver & Battie, 1996) and with the assistance of specialists in Isopoda, Acari, Orthoptera, and Pseudoscorpiones. Potential troglobitic species were recognized based on troglomorphic traits indicative of evolutionary adaptation to subterranean environments (Culver & Pipan, 2019; Aden, 2005).

All identified morphotypes were deposited in the Lavras Subterranean Invertebrates Collection (ISLA), housed at the Center for Studies in Subterranean Biology, Federal University of Lavras (CEBS–UFLA), Minas Gerais, Brazil (biologiasubterranea.com.br).

Data analyses

Substrates recorded in the sectors and quadrats were classified into three categories according to their ecological functions: *trophic*, *shelter*, and *general*. Trophic substrates included roots (RO), guano (GU), fine twigs (TB), and phanerogams (PH), as these provide direct or indirect food resources for cave fauna. Shelter substrates (those offering potential refuge for invertebrates) included rough rock (RR), boulders (BO), blocks (BL), coarse gravel (CG), fine gravel (FG), and fine twigs (TB). General substrates encompassed both organic and inorganic elements, such as water bodies (WB), sand (SA), silt/clay/mud (SCM), hardpan (HP), undifferentiated inorganic substrate (IS), and speleothems (SP), which, although not directly functioning as food or shelter, contribute to habitat heterogeneity.

Resource diversity (trophic) and shelter diversity were calculated using the Shannon–Weaver index (H'), based on the proportional cover of trophic and shelter substrate types, respectively (Buttigieg & Ramette, 2014). Resource and shelter availability were determined from the summed proportional cover of the substrate classes in each group (Souza-Silva et al., 2021; Reis-Venâncio et al., 2022).

To visualize substrate distribution across transects and quadrats, a shade plot was generated after square-root transformation of the data. Substrate types were grouped through cluster analysis using Whittaker's Similarity Index to identify spatial associations and reorder substrate classes by similarity in floor distribution (Whittaker, 1952; Clarke et al., 2014).

The relationships between the diversity and availability of trophic and shelter resources and their distance from the cave entrance were examined using simple linear models (lm function, *stats* package). Residual normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was checked via diagnostic plots (*graphics* package). The aim was to characterize the spatial distribution of physical and trophic components along the entrance–deep zone gradient. All analyses, including those described in subsequent sections, were performed in R (R Core Team, 2019).

To assess factors influencing cave-dwelling species richness at different spatial scales, we applied generalized linear models (GLMs) and generalized linear mixed models (GLMMs). At the mesoscale (sector level), GLMs with a Poisson distribution were fitted using species richness as the response variable. Explanatory variables included shelter diversity, trophic resource diversity, and distance from the cave entrance. Additional models incorporated combinations of biotic and abiotic predictors, such as temperature, relative humidity, shelter availability, and food resource availability. Model selection followed a stepwise procedure based on the Akaike Information Criterion (AIC). Goodness-of-fit was evaluated by the ratio of deviance residuals to residual degrees of freedom, with values close to 1 indicating adequate fit and absence of overdispersion.

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Residual patterns were further checked via simulations in the DHARMa package (Hartig, 2016) to confirm the suitability of the Poisson distribution.

At the microscale (quadrat level), GLMMs were used with species richness as the response variable. Explanatory variables included resource availability, shelter availability, distance from the entrance, and the diversity of both resources and shelters. Sector was included as a random effect. Poisson GLMMs provided a better fit than negative binomial models, based on AIC values, residual diagnostics from DHARMa, and visual inspection of residual normality and homoscedasticity.

To evaluate the influence of substrate diversity and availability, temperature, humidity, and distance from the entrance on species composition, we applied distance-based linear models (DistLM) using a Bray–Curtis similarity matrix. Model selection followed the corrected Akaike Information Criterion (AICc) as recommended by Anderson et al. (2008). Distance-based redundancy analysis (dbRDA) was then used to jointly assess model fit and quantify the proportion of explained variation (Anderson et al. 2008). Variation in species composition among sampling units was further visualized with non-metric multidimensional scaling (NMDS) combined with bootstrap resampling (Clarke et al. 2014).

Taxonomic Distinction (Δ+) was calculated in PRIMER 7 using 24 of the 30 species identified to at least the family level (Anderson et al. 2008; Souza-Silva et al. 2020). The analysis considered five hierarchical taxonomic levels with progressively decreasing weights: Phylum (100), Class (80), Order (60), Family (40), and "Species" (morphotype – 20). These data generated a species-by-sampling unit matrix from which Δ+ values were computed.

To identify environmental variables significantly influencing Δ+ at each spatial scale, we performed distance-based linear models (DistLM) using an Euclidean distance matrix and the same environmental predictors applied in the community composition analyses. Models were built using a forward selection procedure, starting from a null model and sequentially adding variables with the highest explanatory power (Anderson et al. 2008).

3. RESULTS

Describing Habitat Structure inside the sample units

At the microscale, Spearman correlation analysis revealed significant negative relationships between distance from the entrance and both resource diversity ($Rs = –0.313$, $p < 0.001$) and resource availability ($Rs = –0.286$, $p = 0.0017$), indicating that resource diversity and availability decrease progressively toward the deeper cave zones.

At the mesoscale, Spearman correlations also showed significant negative relationships between distance from the entrance and shelter diversity ($Rs = –0.332$, $p = 0.039$), trophic resource diversity ($Rs = –0.397$, $p = 0.012$), and resource availability ($Rs = –0.390$, $p = 0.014$). These results indicate that habitats closer to the entrance are generally more diverse and provide greater resource availability.

Substrate composition also varied with distance from the entrance across both spatial scales. At the microscale, speleothems, guano, and inorganic substrates were more common in the cave's interior, whereas roots, coarse gravel, and speleothems predominated near the entrance (Figure 4A). At the mesoscale, inorganic substrates and fine gravel dominated interior sectors, while roots and hardpan were more frequent in entrance-proximal areas (Figure 4B).

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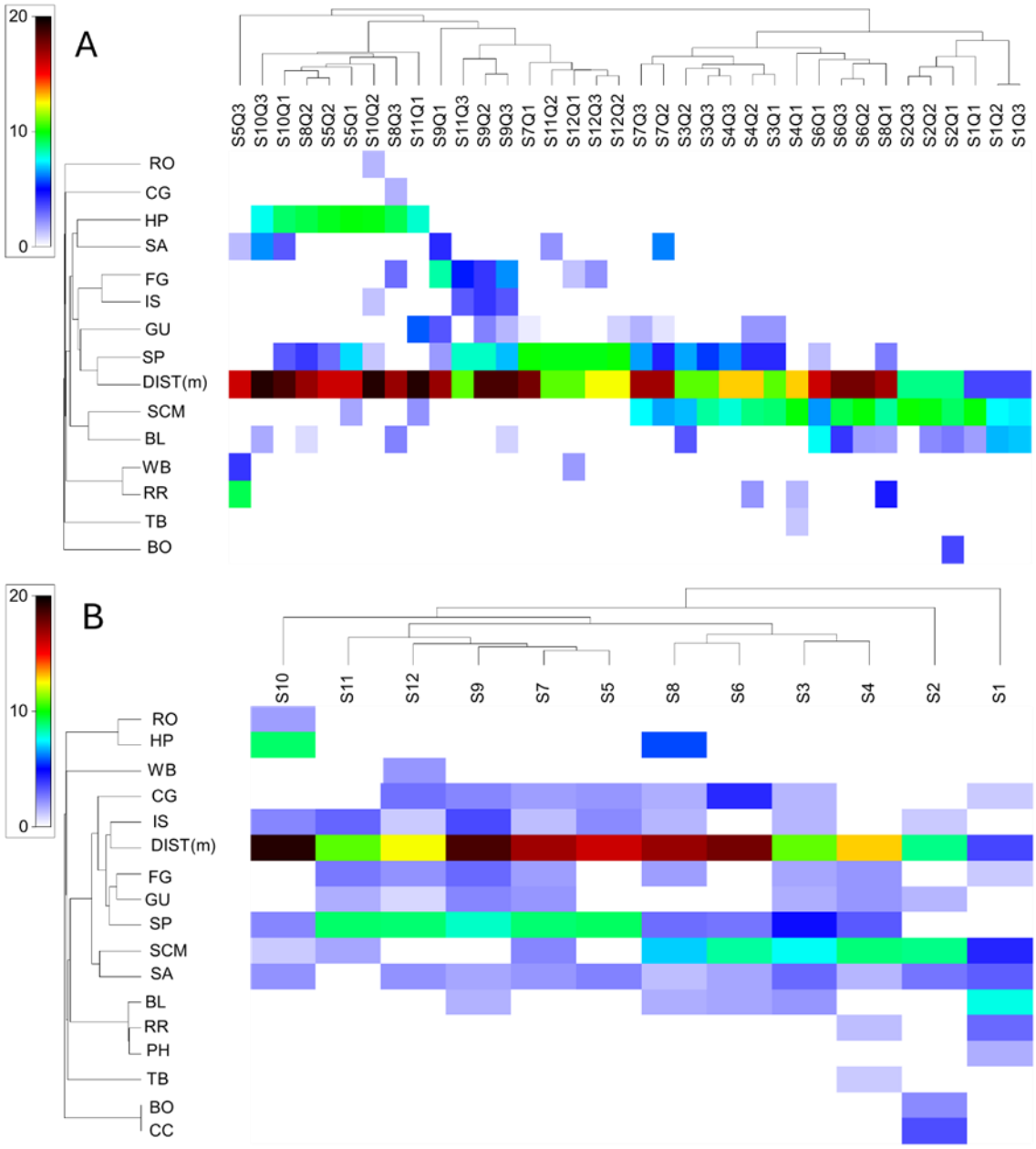

Figure 4. Shadeplot representing the variations in percentage and distribution of each substrate class, as well as the relationship of each class with the distance from the entrance (DIST) in quadrats (A) and sectors (B). The grouping of substrate classes was based on the Whittaker index, and the intensity of the color indicates the amount of each substrate class in a given sector and quadrant. RO: roots; CG: coarse gravel; HP: harpan; SA: sand; FG: fine gravel; IS: inorganic substrate; GU: guano; SP: speleothem; DIST: distance from the entrance; SCM: silt/clay/mud; BL: blocks; WB: water bodies; RR: rough rock; TB: thin branch; BO: boulder; PH: phanerogams; CC: contraction crevice.

Species Composition and richness of cave invertebrates

Considering the sampling effort, a total of 30 species were recorded, with 25 occurring at the mesoscale (sectors) and 17 at the microscale (quadrats). Overall, 15 orders and 24 families of invertebrates were identified. The order Araneae exhibited the highest richness, with six species, followed by Diptera with four species, and Psocodea and Opiliones with three species each.

Five additional species were recorded outside the sampled quadrats and sectors: Tabanidae sp. 1 (Diptera), Chernetidae sp. 2 (Pseudoscorpiones), Gonyleptidae sp. 13 (Opiliones), Chelodesmidae sp. 1 (Polydesmida), and *Tytius* sp. 1 (Scorpiones). Six species displayed

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troglomorphic traits: *Potiicoara brasiliensis* (Spelaeogriphacea: Spelaeogriphidae), *Megagidiella azul* (Amphipoda: Artesiidae), *Novamundoniscus* sp. (Isopoda: Dubioniscidae), *Pirassununguleptes* sp. (Opiliones: Zalmoxidae), Chernetidae sp. 1 (Pseudoscorpiones), and Chelodesmidae sp. 1 (Polydesmida) (Figure 5).

Figure 5. Troglobitic species found in the Ricardo Franco Cave, located in Forte Coimbra. A – Potiicoara brasiliensis (Spelaeogriphacea: Spelaeogriphidae); B – Megagidiella azul (Amphipoda: Artesiidae); C – Novamundoniscus sp. (Isopoda: Dubioniscidae); D – Pirassununguleptes sp. (Opiliones: Zalmoxidae); E – Chernetidae (Pseudoscorpiones); F – Chelodesmidae (Polydesmida). Photos: Rodrigo Lopes Ferreira.

Environmental influence on invertebrate Community Structure

At the mesoscale, distance from the cave entrance was the only significant predictor of reduced species richness ($R^2 = 0.50$, $p = 0.003$; Figure 6). Neither shelter diversity nor organic

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resource diversity showed a significant effect on richness (Table 1). At the microscale, none of the evaluated variables significantly influenced species richness (Table 1).

Table 1. Results obtained from the generalized linear model (GLM) applied at the meso-scale. Statistically significant effects ($p < 0.05$) are indicated with an asterisk (*).

Mesoscale	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	2.573158	0.600590	4.284	1.83e-05 ***
Distance from the entrance	-0.003360	0.001135	-2.960	0.00307 **
Trophic diversity	-0.158400	0.604635	-0.262	0.79334
Shelter diversity	0.074249	0.332297	0.223	0.82319

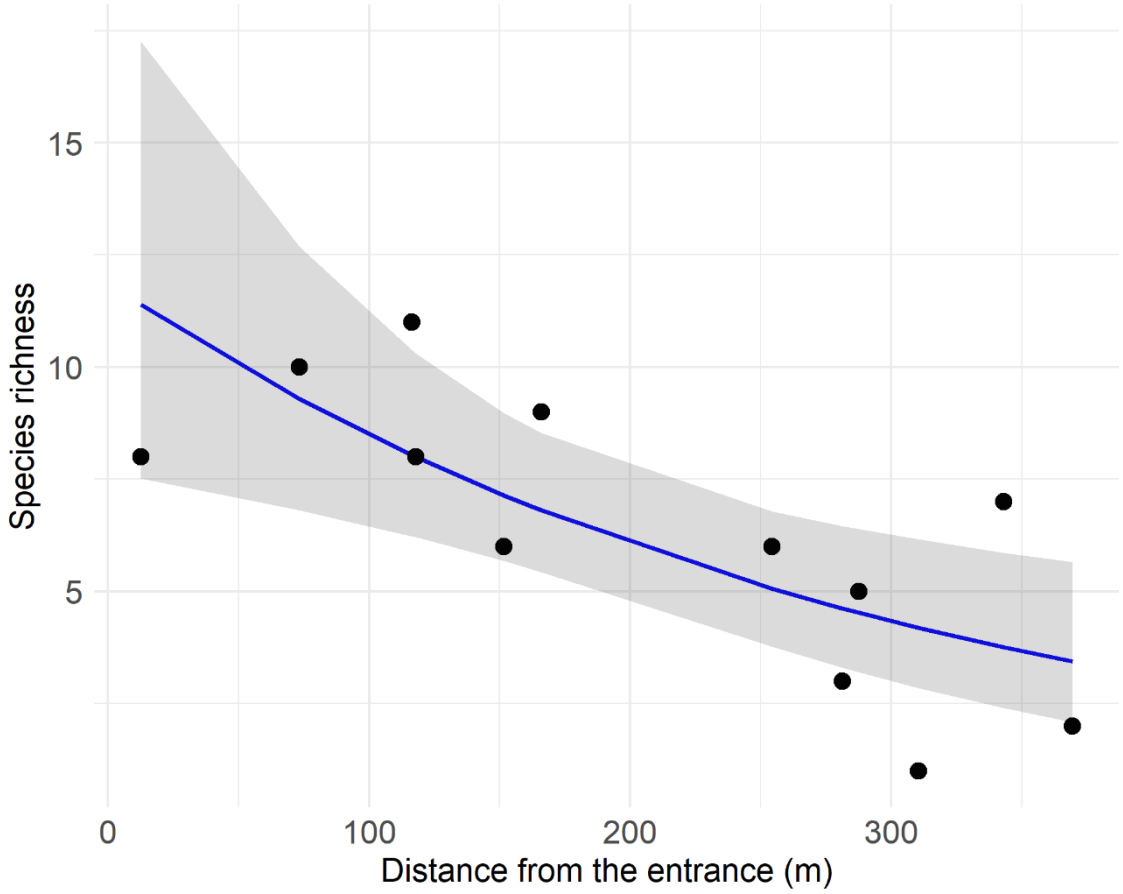

Figure 6. The GLM applied to the mesoscale revealed a negative relationship between species richness and distance from the entrance.

DistLM analysis revealed that, at the microscale (quadrats), none of the environmental variables significantly explained variation in community composition. In contrast, at the mesoscale (sectors), distance from the cave entrance was the only variable that significantly explained species composition, accounting for 21.14% of total variation.

The dbRDA ordination at the mesoscale confirmed that distance from the entrance was the strongest driver of community composition. The first two dbRDA axes together explained 40.9% of the total variation in faunal composition (Figure 7).

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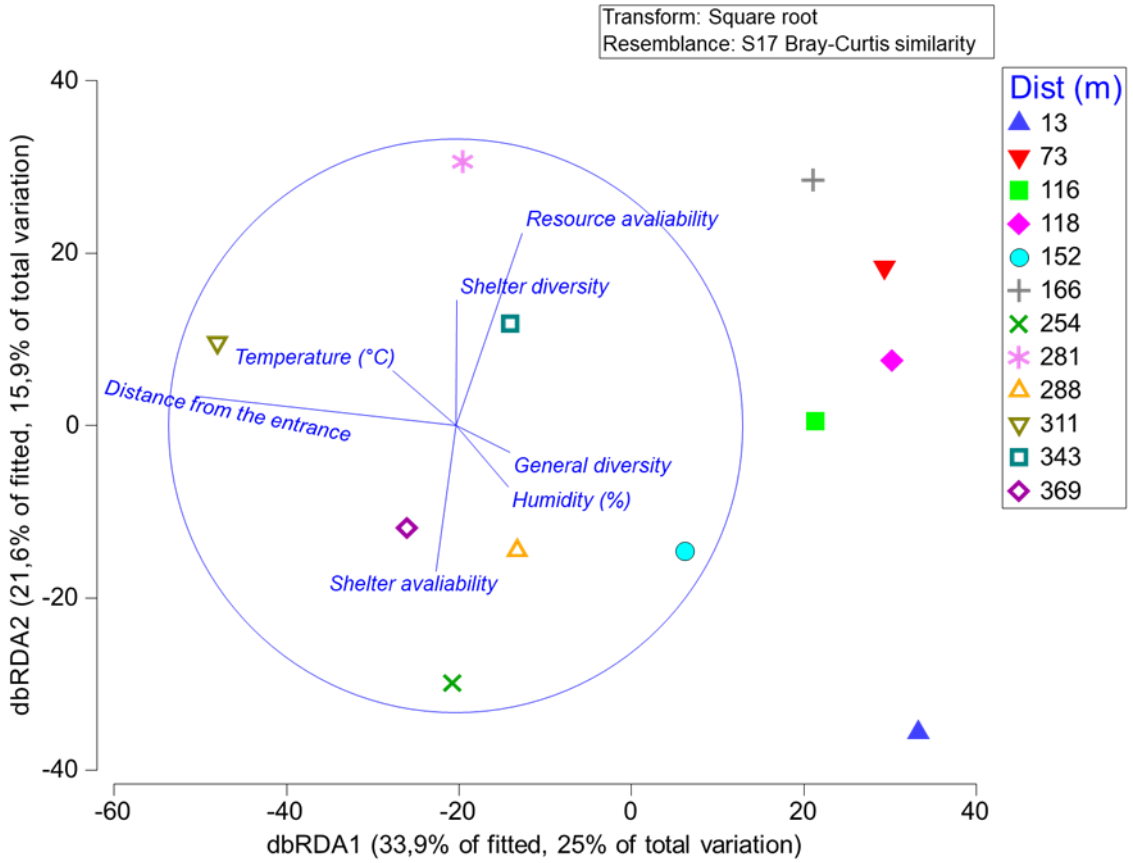

Figure 7. Distance-based Redundancy Analysis (dbRDA), using Bray-Curtis similarity (square root transformation), represents the relationship between environmental variables and subterranean fauna composition. The vectors indicate the direction and magnitude of the influence of physical, microclimatic, and trophic variables: distance from the entrance (m), temperature (°C), relative humidity (%), availability of shelters (disp.shelter), availability of trophic resources (disp.trophic resource), overall substrate diversity (DIVG), and shelter type diversity (DIV.AB). The symbols represent the different sampled sectors, coded according to the distance from the entrance.

Taxonomic distinction on the invertebrate community

At the mesoscale (sectors), sequential tests from the DistLM analysis identified temperature (Pseudo-F = 15.75, p = 0.02, R^2 = 61.17%), relative humidity (Pseudo-F = 5.73, p = 0.039, R^2 = 15.10%), and shelter availability (Pseudo-F = 16.13, p = 0.003, R^2 = 15.86%) as the most influential predictors of taxonomic distinction. The final model, incorporating these three variables, explained 92.13% of the total variation in taxonomic distance between samples. These results indicate that microclimatic factors (temperature and humidity) and habitat structural complexity (shelter availability) play a key role in shaping the taxonomic diversity of invertebrate communities in Ricardo Franco Cave.

At the microscale (quadrats), none of the evaluated environmental variables (including distance from the entrance, resource and shelter availability, and substrate diversity) showed a significant relationship with variation in taxonomic distinction.

4. DISCUSSION

The results from Ricardo Franco Cave indicate that distance from the entrance is a key factor in structuring invertebrate communities, particularly at the mesoscale. Species richness declined significantly with increasing distance from the entrance, reflecting an ecological gradient widely documented in subterranean systems (Culver & Pipan, 2009; Lunghi et al., 2017; Souza-

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Silva et al., 2021). This pattern is consistent with the reduction in environmental heterogeneity and the decline in allochthonous resource availability deeper inside the cave. In addition to richness, species composition also varied significantly along this gradient, indicating turnover likely driven by environmental filtering (Prous et al., 2015). Inner zones, characterized by more stable and oligotrophic conditions, tend to favor more specialized taxa, whereas entrance areas harbor generalist or troglophilic species, which are typically more abundant and diverse (Prous et al., 2015; Souza-Silva et al., 2021).

Substrate distribution within the cave reinforces this interpretation. Organic and heterogeneous substrates (e.g., roots and gravel) were more frequent near the entrance, while fine inorganic materials, guano, and speleothems predominated in deeper zones. This structural shift along the entrance–interior gradient generates greater microhabitat diversity and trophic input in the transition zone between epigean and hypogean environments (Reis-Venâncio et al., 2022).

At the microscale, no clear patterns emerged in richness or composition, suggesting that key variables relevant to this scale may not have been included in the analyses. Processes such as species interactions (predation, competition, mutualisms), succession dynamics, and biological invasions can strongly influence community structure but remain difficult to quantify and are rarely addressed in cave ecological studies (Da Rocha Melo et al., 2025). These findings highlight the need to expand the set of variables considered at fine spatial scales and to incorporate ecological processes that remain understudied in subterranean systems.

The adoption of multiple spatial scales is increasingly recognized as important in ecological research, as different species may respond differently depending on the observational scale and sampling frame (Wiens, 1989; Pacheco et al., 2020; Souza-Silva et al., 2021; Furtado-Oliveira et al., 2022; Vaz et al., 2025). In this study, the strong influence of distance from the entrance at the mesoscale, contrasted with the absence of effects at the microscale, suggests that the entrance–interior gradient and structural heterogeneity of Ricardo Franco Cave become apparent only when broader environmental variation is captured. This distinction underscores that both ecological inference and management recommendations are scale-dependent, emphasizing the importance of multi-scale designs in subterranean biodiversity research (Lovell et al., 2002).

The observed variation in taxonomic distinction (Δ^+) suggests that communities inhabiting sectors with greater structural complexity and microclimatic stability tend to harbor more distantly related lineages, resulting in greater taxonomic distance. The finding that temperature, humidity, and shelter availability together explain more than 90% of this variation indicates that these factors act as ecological filters shaping species composition and taxonomic diversity a pattern consistent with reports from other subterranean systems (Lunghi et al., 2017; Mammola & Isaia, 2018; Souza-Silva et al., 2021).

Climatic stability is widely recognized as a major determinant of biodiversity patterns in both surface and subterranean ecosystems (Tews et al., 2004; Souza-Silva et al., 2021). Among these factors, humidity plays a particularly critical role for invertebrates, whose small body size and thin cuticle make them highly susceptible to desiccation (Strachan et al., 2015; O'Donnell, 2022). In caves, high humidity levels can be maintained through multiple mechanisms, including limited atmospheric exchange with the surface, low thermal variation, condensation of water vapor, percolation and infiltration from soil and rock, and the presence of internal or entrance-proximal water bodies (Buecher et al., 1999). Together, these conditions generate the environmental stability that supports the persistence of specialized cave fauna (Howarth, 1983; Culver & Pipan, 2009; Souza-Silva et al., 2011).

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From a conservation perspective, the results of this study reinforce the urgent need to recognize Ricardo Franco Cave as a priority area for protection and management. The occurrence of troglobitic species, combined with the cave's historical and cultural significance, highlights its status as a natural heritage of exceptional ecological and cultural value, whose preservation is essential. The absence of formal protection, coupled with increasing anthropogenic pressures in the Pantanal including unregulated tourism and potential indirect impacts from landscape alterations intensifies the risk of irreversible biodiversity loss (Mammola et al., 2020; Furtado-Oliveira et al., 2022). In this context, the incorporation of Ricardo Franco Cave into management and conservation programs would represent a crucial step toward safeguarding its ecological and cultural attributes.

Although several studies have addressed strategies to mitigate tourism impacts on cave fauna, evidence shows that without proper planning, tourism may compromise visitor safety, degrade cave integrity, and negatively affect local fauna (Lobo, 2006; Faille et al., 2015; Furtado-Oliveira et al., 2022). The persistence of troglobitic organisms in Ricardo Franco Cave underscores the importance of maintaining key ecological processes, including microclimatic stability and the preservation of minimal resource inputs (Souza-Silva et al., 2021; Vaz et al., 2025). Therefore, modified caves should be subject to continuous monitoring and evaluation, based on biological and ecological criteria such as the presence of endemic species, population trends, and fauna responses to tourism impacts, as recommended in recent conservation frameworks for subterranean ecosystems (Mammola et al., 2019; Cardoso et al., 2022).

Finally, this study contributes to filling critical knowledge gaps on ecological patterns in subterranean environments of the Pantanal, a region still poorly explored from the perspective of cave biology. The multiscale approach employed revealed distinct mechanisms structuring subterranean biodiversity, emphasizing the roles of distance from the entrance, substrate heterogeneity, and microclimatic stability. Future research should broaden the scope of variables considered, incorporating ecological interactions, invasion dynamics, and succession processes, in order to establish a more comprehensive foundation for conserving subterranean biodiversity in this biome.

5. FINAL REMARKS

The multiscale analysis demonstrated that distance from the entrance is the primary factor structuring the invertebrate fauna of Ricardo Franco Cave, influencing both species richness and community composition at the mesoscale. Microclimatic stability and shelter availability also emerged as key determinants of taxonomic distinctness. Together, these findings underscore the importance of incorporating spatial scale and environmental heterogeneity into cave management strategies. The occurrence of troglobitic species, combined with the cave's historical and cultural significance, reinforces its prioritization in conservation programs aimed at preserving ecological integrity and mitigating anthropogenic impacts.

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Beyond the Banks: The Influence of a Subterranean River on the Cave Invertebrate Community, A Case Study in Niquelândia, Goiás, Brazil

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RESUMO: Os cursos d'água presentes no interior de cavernas podem exercer influência significativa sobre a riqueza e a composição das comunidades de invertebrados terrestres. Neste estudo, investigamos se a proximidade com uma drenagem ativa afeta a diversidade de espécies em uma caverna calcária. Para isso, realizamos amostragens comparativas em dois ambientes distintos: as margens de um curso d'água e um conduto superior mais seco. Utilizando metodologia padronizada, delimitamos setores e quadrantes como unidades amostrais, registrando tanto os invertebrados terrestres quanto os substratos do piso da caverna. Os resultados revelaram diferenças marcantes entre os ambientes úmidos e secos: foram registradas 35 espécies próximas ao curso d'água e 27 nas áreas secas, com *Endecous* sp1 destacando-se como o morfotipo mais determinante na variação composicional. A diversidade beta total foi de 0,72, com predominância da substituição de espécies como principal componente. Nesse sentido, a presença dessa drenagem molda de maneira significativa a fauna associada, regulando a umidade local, influenciando padrões de oviposição e atuando como vetor de matéria orgânica para o meio subterrâneo. Esses achados ampliam a compreensão sobre a dinâmica dos ecossistemas subterrâneos e fornecem subsídios relevantes para estratégias de conservação e manejo de ambientes cavernícolas.
Palavras-chave: Drenagem, Invertebrados, Cavernas, Estrutura da Comunidade, Temperatura, Umidade.

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ABSTRACT: Watercourses inside caves can exert significant influence on the richness and composition of terrestrial invertebrate communities. In this study, we investigated whether proximity to an active drainage affects species diversity in a limestone cave. For this purpose, we conducted comparative sampling in two distinct environments: the margins of a watercourse and a drier upper conduit. Using standardized methodology, we delimited sectors and quadrants as sampling units, recording both terrestrial invertebrates and the substrates present on the cave floor. The results revealed marked differences between humid and dry environments: 35 species were recorded near the watercourse and 27 in the dry areas, with *Endecous* sp1 standing out as the morphotype most determinant in compositional variation. Total beta diversity was 0.72, with species replacement as the predominant component. In this sense, the presence of this drainage significantly shapes the associated fauna, regulating local humidity, influencing oviposition patterns, and acting as an important vector of organic matter into the subterranean environment. These findings broaden our understanding of the dynamics of subterranean ecosystems and provide relevant support for conservation and management strategies of cave environments.
Key-words: Drainage, Invertebrates, Caves, Community Structure, Temperature, Humidity.

1. INTRODUCTION

Subterranean ecosystems represent exceptional "natural laboratories" due to their structural simplicity and reduced biodiversity compared to surface environments (Poulson & White, 1969; Mammola, 2019). These ecosystems are typically associated with caves, aquifers, wells, and other subterranean habitats, which are often partially isolated from external influences such as weather fluctuations and anthropogenic disturbances. This degree of isolation gives rise to distinct and stable environmental gradients that are rarely observed in surface systems (Badino, 2010; Souza-Silva et al., 2021; Reis-Venâncio et al., 2024).

One of the most pronounced gradients in subterranean environments is light availability. Proximal to cave entrances or skylights, sufficient light may penetrate to support limited photosynthetic activity, permitting the growth of phototrophic organisms such as vascular plants, lichens, and algae (Monro et al., 2018). However, light intensity decreases sharply with distance from these openings, and the deeper zones are typically completely aphotic, rendering them unsuitable for photoautotrophic life (Souza-Silva & Ferreira, 2022; Reis-Venâncio et al., 2024). In these lightless zones, energy inputs are derived primarily from allochthonous organic matter transported from the surface, supporting communities of heterotrophic organisms (Souza-Silva & Ferreira, 2022). These environments are often classified as oligotrophic due to their low nutrient availability (Simon et al., 2007). Nevertheless, exceptions exist in systems where autochthonous energy sources, such as chemoautotrophic production, sustain specialized faunal assemblages (Sarbu et al., 1996).

Another defining feature of subterranean ecosystems is their pronounced thermal stability. Temperatures within these environments remain relatively constant throughout the year, with minimal fluctuations typically not exceeding a few degrees Celsius (Cardoso et al., 2024). Consequently, subterranean habitats often harbor unique and endemic species, many of which exhibit structures and behaviors shaped by the subterranean environment, including reduced pigmentation, diminished or absent vision, and enhanced non-visual sensory modalities (Howarth, 2023). These traits reflect strong selective pressures imposed by the darkness, high humidity, and environmental constancy characteristic of subterranean ecosystems.

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Despite their apparent simplicity, subterranean ecosystems encompass a wide range of microhabitats, each providing distinct ecological niches that can be exploited by different invertebrate species (Souza-Silva et al., 2021, da Rocha Melo et al., 2025). These microhabitats may vary considerably in substrate type, moisture levels, and organic matter content, creating a complex interplay of factors that shape species distribution and community dynamics (Reis-Venâncio et al., 2022; da Rocha Melo et al., 2025).

Understanding how substrate availability and composition influence the associated fauna has become an increasingly important focus in contemporary ecological research, particularly in the Neotropics. This line of inquiry is highly relevant to the conservation of speleological heritage, as it helps elucidate the ecological processes that sustain subterranean life. Substrates such as guano, plant debris, sand, silt, and clay not only determine which organisms can persist in these environments but also regulate nutrient cycles and contribute to ecosystem stability (Souza-Silva et al., 2012). By clarifying the intricate relationships among substrate characteristics, moisture, nutrient availability, and biodiversity, researchers can support more effective conservation strategies aimed at safeguarding these fragile ecosystems, which often harbor high levels of endemism (Simões et al., 2014).

Research in subterranean environments not only advances the understanding of these systems but also provides valuable insights into evolutionary and ecological processes applicable to more complex ecosystems (Poulson & White, 1969; Mammola, 2019). Factors such as dispersal limitations, climatic variables, and habitat heterogeneity exert strong influences on both alpha and beta diversity, shaping species replacement and loss (turnover and nestedness) in cave-dwelling invertebrate communities (Calderón-Patrón et al., 2016; Souza-Silva et al., 2021; Reis-Venâncio et al., 2022, 2024). In this context, several factors have been recognized as drivers of invertebrate community structure in Neotropical caves, including the presence of drainage systems (Simões et al., 2015). Water bodies play a particularly important role in shaping patterns of dispersal and distribution across multiple animal groups. Flooding events, for instance, can generate disturbances that deeply alter community composition (Simões et al., 2015).

Drainage systems also enhance local humidity in both air and soil, thereby influencing faunal distribution (Simões et al., 2015). Moreover, river margins accumulate large amounts of transported and deposited organic matter, which serve as critical resources for cave-dwelling species (Souza-Silva et al., 2012). These processes highlight the potential importance of rivers in structuring invertebrate assemblages within caves, underscoring the need for detailed investigations to subsidize conservation and management strategies for these ecosystems.

In this context, the present study examines how proximity to a riverbank affects the composition and species richness of terrestrial invertebrates in a limestone cave. Specifically, we test two hypotheses: (i) species richness differs significantly between the regions analyzed, with greater richness expected in areas closer to the drainage; and (ii) faunal composition varies between regions, as distinct environmental conditions act as ecological filters, favoring species able to establish under the conditions of each habitat.

2. MATERIAL AND METHODS

Study site

Terrestrial invertebrates were sampled during a single field campaign conducted in the dry season of 2021. The study was carried out in Gruta da Lapa, located in the municipality of

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Niquelândia, northeastern Goiás state, Brazil (Figure 1), within the Cerrado biome, recognized as one of the world's biodiversity hotspots (Myers et al., 2000). The region is classified as having a tropical "Aw" climate under the Köppen–Geiger system, with a pronounced dry season extending from March to October (Álvares et al., 2013).

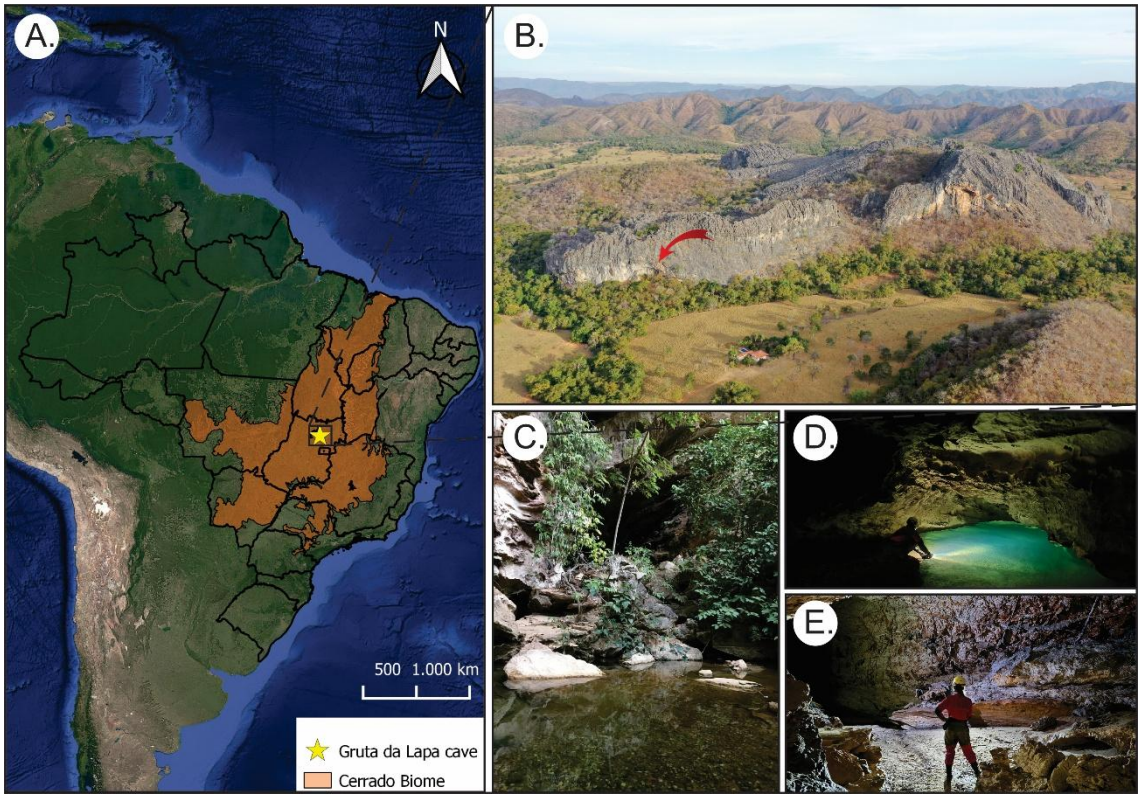

Figure 1. Location of Gruta da Lapa in the state of Goiás, Brazil (A); carbonate outcrop hosting the cave, with a red arrow indicating the cave's entrance (B); cave entrance at the upper portion, with the drainage at its base (C); drainage inside the cave (D); and internal cave conduit (E).

Gruta da Lapa contains an active autogenic drainage system that flows continuously throughout the year. This drainage emerges just below the main cave entrance, forming a spring. The cave has no skylights or secondary entrances, only the principal one. No established bat colonies were observed inside the cave, although small amounts of guano were recorded. The cave features linear conduits with few recesses: a lower conduit running adjacent to the river, and an upper conduit that is completely dry, with no direct contact with the drainage. In the vicinity of the entrance, local residents have constructed a dam to supply water to the surrounding community.

Sampling design

Invertebrate sampling was conducted at two spatial scales: sectors of 30 m² and quadrants of 1 m. Within each sector, three quadrants were evenly distributed on the cave floor - two positioned at the extremities and one centrally - as illustrated by Vaz et al. (2025). Along the entire cave, six sectors (totaling 18 quadrants) were established: three located along the drainage margin, approximately 25m, 60m, and 94m from the entrance, and three situated in a drier upper conduit, at about 120m, 65m, and 60m from the entrance (Figure 2). Sectors were used for compositional analyses and to enhance the cave's faunistic inventory by increasing species detectability. Species composition within each sector was determined by the specimens collected and by aggregating the

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species recorded across its three quadrants.

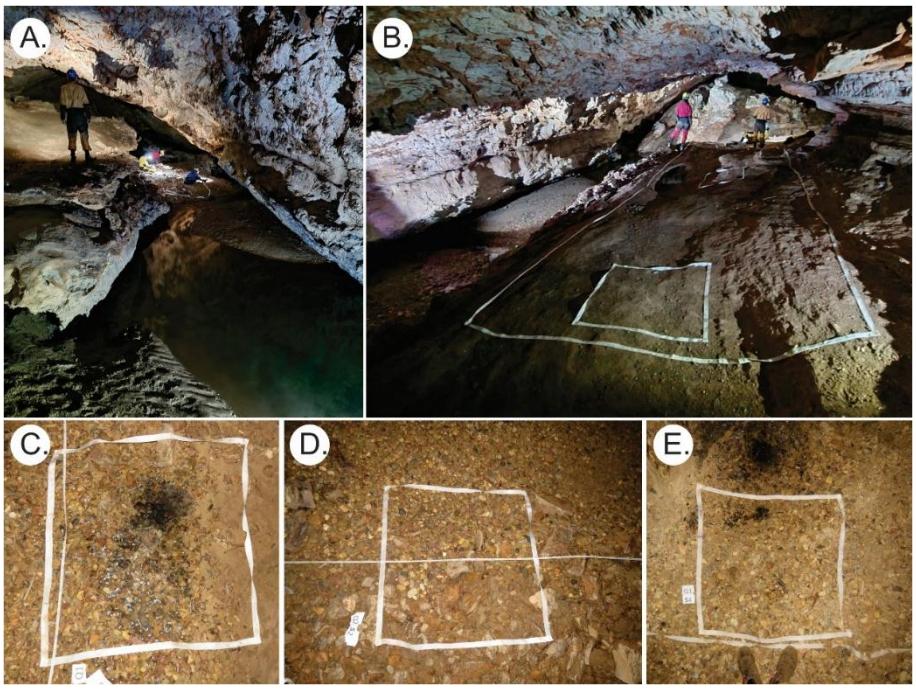

Figure 2. (A) Lower conduct along the drainage margin, highlighting one of the sampling points; (B) Upper passage in the dry-area of the cave, with one of the sampling points indicated; (C–E) Examples of sampling quadrats, illustrating the different substrates measured in the study.

Invertebrate sampling and identification

Terrestrial invertebrates were collected and recorded through visual searches and active manual collection using tweezers and brushes, following adapted methodologies from Ferreira (2004) and Wynne et al. (2019). Four experienced researchers conducted exhaustive sampling, including direct capture and in situ abundance recording within the predefined sectors and quadrants.

All specimens were preserved in labeled containers with 70% ethanol. In the laboratory, they were identified to the highest possible taxonomic level using stereomicroscopes and identification keys, then grouped into distinct morphotypes (Oliver & Beattie, 1966a, b). Finally, all specimens were deposited in the Collection of Subterranean Invertebrates of Lavras (ISLA), affiliated with the Center for Studies in Subterranean Biology at the Federal University of Lavras, Brazil (https://www.biologiasubterranea.com.br/en/).

Habitat structure measurement

To qualitatively and quantitatively characterize soil substrate components, digital photographs of each quadrant were taken prior to collection (several examples are shown in Figure S1), following Souza-Silva et al. (2021). Images were captured with the camera held at chest height and perpendicular to the soil surface to minimize distortion.

In the laboratory, images were analyzed using ImageJ software (Ferreira & Rasband, 2012) to calculate the area occupied by each substrate type within the quadrants. The total image area was standardized to 1, enabling determination of relative substrate proportions ranging from 0 to 1. Substrates were classified into the following categories: guano, leaf litter, thin and medium branches, water, matrix rock, small and medium blocks, coarse and fine gravel, sand, and silt.

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Data Analysis

Influence of Habitat Structuring on Species Richness

Substrate diversity was calculated using the Shannon index (H') (Magurran, 2004), based on the relative proportions of all substrate categories identified per quadrat. In addition, the diversity of organic resources and shelters was estimated separately, also using the Shannon index. Organic resources included guano, branches, and leaf litter, whereas shelters comprised branches, blocks, and gravel (Souza-Silva et al., 2021; Reis-Venâncio et al., 2024; Vaz et al., 2025).

Analyses of species richness were performed exclusively at the quadrat scale. Richness was estimated as the total number of morphospecies recorded per quadrat. To assess the influence of substrate, resource, and shelter diversities on species richness, we applied two independent Generalized Linear Models (GLMs) (Zuur, 2009). The first model included the river-margin quadrats, while the second included only dry-area quadrant. Both models assumed Poisson distribution, appropriate for count data.

Model fit was evaluated in two steps. First, we tested for overdispersion using the *testDispersion()* function in the "DHARMa" package (Harting, 2022). Subsequently, the *check_model()* function from the "performance" package (Lüdecke et al., 2021) was applied to assess homoscedasticity, agreement between predicted and observed values, residual normality, multicollinearity among predictors, zero inflation, and potential outliers.

Differences in mean species richness between river-margin and dry-area regions were tested using the Mann–Whitney U test (Mann & Whitney, 1947). To further explore substrate structure, we performed a cluster analysis based on the Whittaker Association Index (Clarke et al., 2014). Sampling units were grouped using the average linkage method and Euclidean distance, after fourth-root transformation of the data (Figure 3).

Faunal Similarity Between Assemblages

To compare faunal composition between river-margin and dry-area assemblages, a Permutational Multivariate Analysis of Variance (PERMANOVA) was performed on a Bray–Curtis similarity matrix constructed from Hellinger-transformed data (Legendre & Gallagher, 2001). Additionally, a Similarity Percentage (SIMPER) analysis was applied to determine the species contributing most to the dissimilarities between the two groups.

Beta diversity between regions was estimated using the *beta()* function of the "BAT" package (Cardoso et al., 2015), based on an abundance matrix at the sector level. This metric was partitioned into two components: β-replacement, representing compositional turnover between regions, and β-nesting, reflecting species loss (Baselga, 2010).

Species diversity within each region was further assessed using Hill numbers, which incorporate a sensitivity parameter (q) to differentially weight rare versus common species. The parameter varied from 0 to 3, in increments of 0.1. Low values (q = 0–1) emphasize rare species, whereas higher values (q > 1) progressively highlight common species. This approach provides a continuous framework for diversity assessment, unifying traditional indices such as species richness (q = 0), Shannon diversity (q = 1), and Simpson diversity (q = 2). Calculations were performed with the *hill_taxa()* function from the "hillR" package in R (Chao et al., 2014).

3. RESULTS

A total of 51 invertebrate species were recorded, distributed across 16 orders (Figure 2).

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Mean species richness per quadrat was higher in river-margin areas (3.22 ± 1.20 SD) compared with dry, more distant areas (1.80 ± 1.09 SD). The most species-rich orders were Araneae, Diptera, Coleoptera, and Hemiptera (Figure 3). In sectors near the river margin, 228 individuals representing 35 species were recorded, whereas 180 individuals belonging to 27 species were sampled in the dry portion of the cave. Photographs of several sampled species are shown in Figure 4.

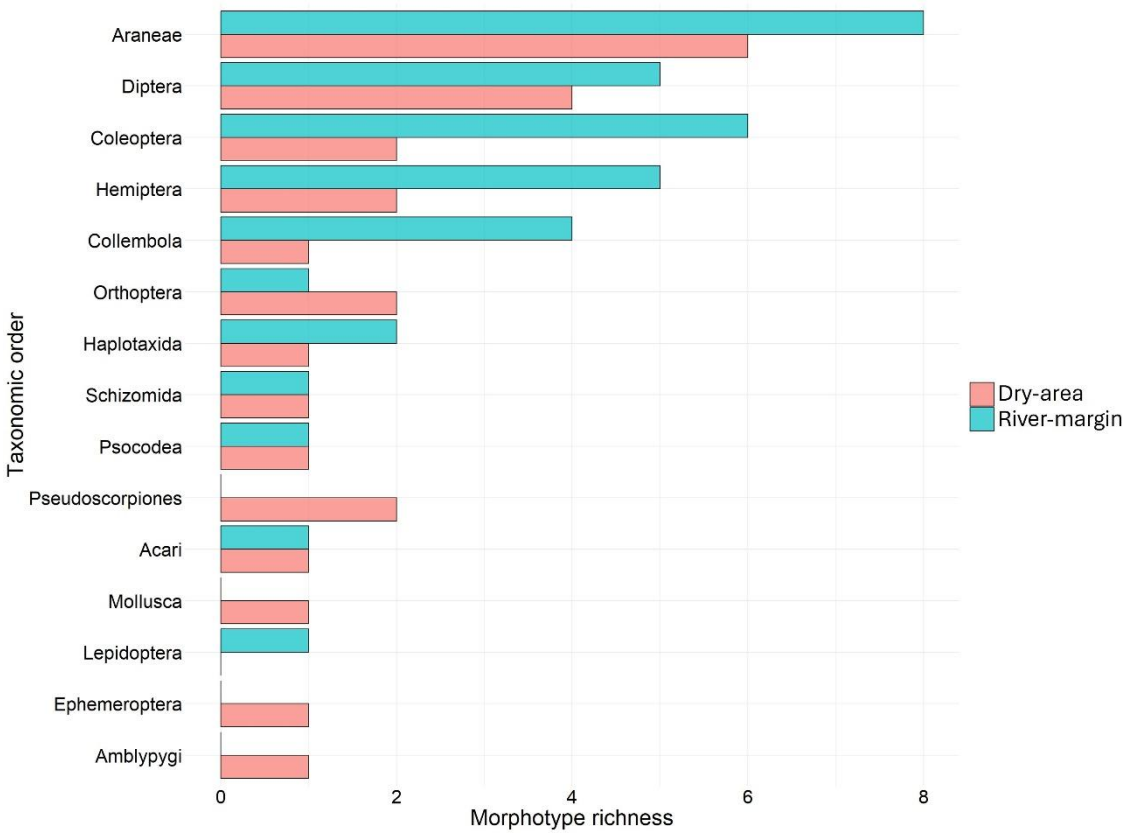

Figure 3. Morphospecies richness by taxonomic order recorded in each of the investigated areas, the river-margin and the dry-area, in Gruta da Lapa Cave, Niquelândia, northeastern Goiás, Brazil.

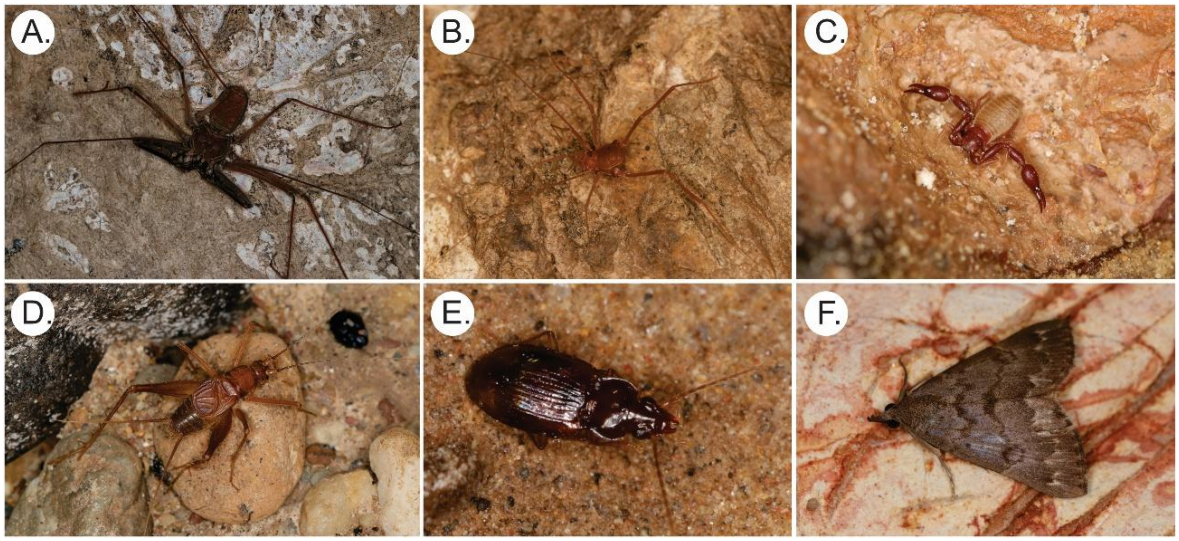

Figure 4. Representative species recorded during the study: (A) Heterophrynus (Amblypygi: Phrynidae); (B) an opilionid Eusarcus (Opiliones: Gonyleptidae); (C) a pseudoscorpion (Chernetidae); (D) Endecous (Orthoptera: Phalangopsidae); (E) a beetle from the subfamily Pterostichini (Carabidae); (F) Hypena (Lepidoptera: Erebidae).

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No significant relationships were detected between the diversity variables tested and morphospecies richness. However, the Mann–Whitney U test revealed a significant difference in mean richness between the two regions (W = 15.5; p = 0.026).

The Whittaker's Association Index indicated that the greatest guano concentration (GA) occurred in quadrat one of sector two (river-margin). The dendrogram revealed a relationship between distance from the entrance and blocks (Figure 5).

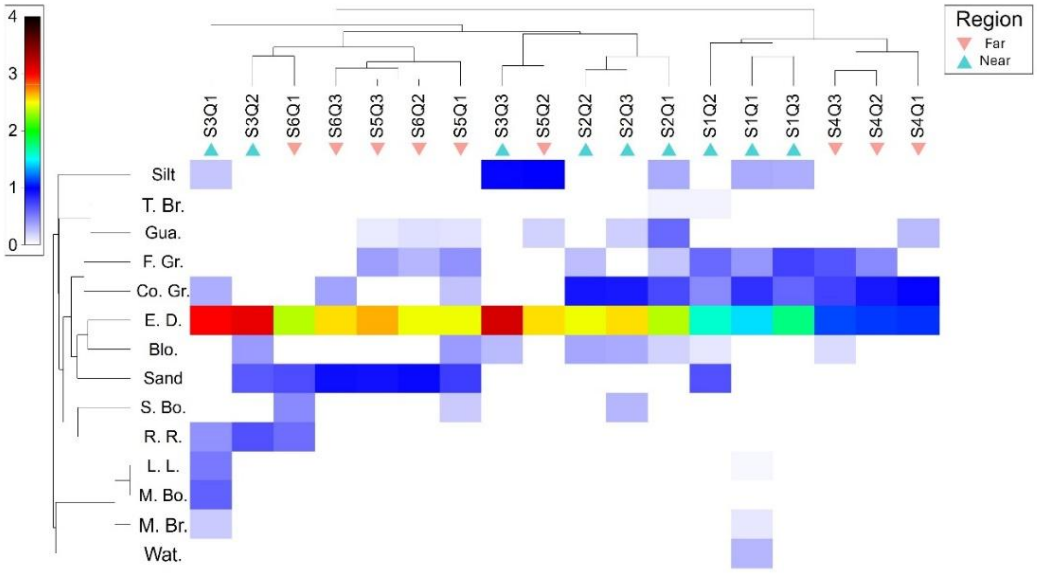

Figure 5. Whittaker Association Index showing the relationships between the quantities of different substrates and the distance from the cave entrance (D.E.). Substrates: T.Br., thin branch; Gua., guano; F.Gr., fine gravel; Co.Gr., coarse gravel; Blo., blocks; S.Bo., small boulder; R.R., rough rock; L.L., leaf litter; M.Bo., medium boulder; M.Br., medium branch; Wat., water.

Faunal composition differed significantly between river-margin and dry-area assemblages (R^2 = 0.114; F = 2.06; p = 0.039). SIMPER analysis identified *Endecous* sp. 1 (p = 0.001) as one of the main contributors to this dissimilarity, with preferential occurrence along the river margin. Beta diversity between regions was βtotal = 0.72, with species turnover contributing most (βreplacement = 0.57) and nestedness playing a smaller role (βnesting = 0.15).

Hill number analysis showed that for *q* values between 0 and 1, diversity was greater in river-margin sectors, indicating the predominance of rare species. For $q > 1$, however, diversity was higher in the dry-areas, reflecting the dominance of more widespread and relatively abundant species across sampling points (Figure 6).

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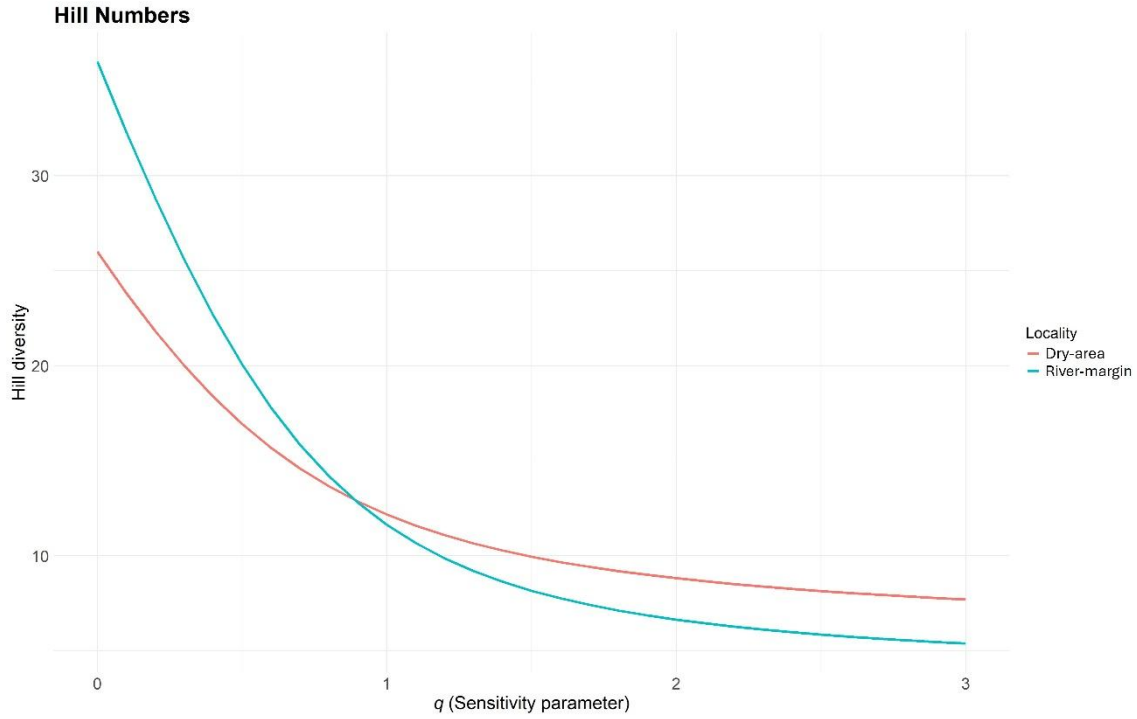

Figure 6. Hill number curve illustrating the variation in Hill diversity as a function of the sensitivity parameter (q). Values of q between 0 and 1 place greater emphasis on rare species, whereas values of q > 1 give more weight to the most frequent and/or abundant species.

4. DISCUSSION

Our findings, demonstrates that assemblages associated with different regions – "river-margin" and "dry areas" - differ significantly in both species' richness and composition. As expected, richness was greater in river-margin and lower in the dry areas. Community composition also varied, reflecting differences in ecological conditions and habitat structure, which appear to filter species according to their affinity for these environments and the specific niches they provide.

Variation in habitat structure is a key factor shaping invertebrate communities in subterranean ecosystems, and several structural parameters have previously been shown to play an important role in determining both species richness and community composition (Reis-Venâncio et al., 2024; da Rocha Melo et al., 2025). In the case of Gruta da Lapa, however, none of the parameters tested as proxies for habitat structure were related to richness or composition. This result may be attributable to the limited number of sampling units within the cave, as well as to the pronounced heterogeneity between the two sampled areas (river-margin and dry areas).

Our results indicate that proximity to the drainage exerts a significant influence on the composition of terrestrial invertebrate species within the cave. The drainage enhances both atmospheric and soil moisture, thereby creating favorable microhabitats that support the establishment of species adapted to such conditions (Chown et al., 2011). The homeostasis of these organisms is primarily regulated by thermoregulatory mechanisms and water balance, and environmental shifts in temperature or water availability commonly trigger adaptive responses (Chown et al., 2011). Notably, some invertebrates possess the ability to absorb water vapor directly from the environment through specialized hygrosensory mechanisms, enabling them to maintain water balance and homeostatic stability (Chown et al., 2011).

Beyond its role in elevating humidity, the river also functions as a key vector of organic

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matter into the subterranean environment, providing essential resources that sustain cave-dwelling communities. Similar dynamics have been documented in several cave systems worldwide (Weinstein, 1994; Souza-Silva et al., 2012). This process is ecologically significant, given that a substantial fraction of organic inputs in caves is of allochthonous origin, transported from external ecosystems. The continuous influx of moisture and resources from the river thus represents a critical driver of community structure.

Other critical environmental parameters, such as fluctuations in temperature and humidity levels, may play a significant role in shaping the distribution patterns of terrestrial invertebrate communities within caves (Souza-Silva et al., 2021). These factors may underline the differences in species richness observed between the analyzed sectors. Hill numbers revealed greater diversity of rare species in the river-margin areas, suggesting a measurable influence of the watercourse. Similarly, a recent study (Junta et al., 2025) demonstrated that habitat alterations can drive changes in species occurrence. The decline in non-troglobitic species (i.e., those not restricted to subterranean environments) with increasing distance from the entrance suggests that invertebrates not adapted to cave conditions are unable to persist due to a series of environmental filters. Taking together with other studies, these findings highlight the importance of watercourses as key structuring elements of subterranean ecosystems (Souza-Silva et al., 2021). The abrupt transition in humidity between the "margin river" and dry cave sectors may account for the high species turnover observed.

Humidity is a key environmental factor that directly influences the reproductive biology of many invertebrates. In females of crickets of the genus *Endecous*, oviposition site selection is strongly determined by substrate moisture (Farias-Martins et al., 2017). This preference likely accounts for the higher concentration of individuals in areas adjacent to rivers, where elevated humidity reduces the risk of desiccation (Castro-Souza et al., 2020). In addition, humidity can influence the acoustic signaling of males in stridulating species such as *Endecous* (Farias-Martins et al., 2017). The intense calls produced by males may serve as cues to females, indicating that the environment provides suitable conditions of moisture for oviposition (Hertl et al., 2001).

In contrast, some invertebrate species commonly recorded in cave environments were observed occupying microhabitats characterized by lower humidity levels, which may function as refuges. Groups such as Lepidoptera larvae, spiders of the family Filistatidae, and psocopterans of the family Psyllipsocidae were found predominantly in the drier cave sectors. Conversely, taxa such as *Endecous* sp.1, Carabidae (Coleoptera), and Veliidae (Hemiptera) were more abundant in moister areas, where high humidity is essential due to physiological constraints, increased foraging efficiency, or specific water requirements at certain stages of the life cycle.

These contrasting habitat preferences highlight the diverse adaptive strategies of cave invertebrates in meeting the demands of their ecological niches. Such patterns are shaped by the interplay between humidity (Souza-Silva et al., 2021) and resource availability (Simões et al., 2015).

In general, the presence of water bodies in caves is considered an important factor for increasing invertebrate richness, not only because they contribute to higher humidity but also because they function as pathways for the import of organic resources into subterranean ecosystems (Souza-Silva et al., 2012). Consequently, more humid environments are expected to harbor greater species richness (Simões et al., 2022). However, it is important to note that the drainage observed in this cave is of autogenic origin, with water flow beginning inside the cave and moving outward. This type of flow passes through multiple physical barriers that act as filters, preventing the passage of coarse organic material into the deeper portions. As a result, only fine

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particles or dissolved organic compounds are transported, which may be less attractive and/or accessible to many of the sampled invertebrates, or may not be detected by the methodology employed.

Overall, our findings highlight the central role of watercourses in shaping subterranean invertebrate communities, both by regulating humidity, influencing reproductive and behavioral patterns, and mediating the supply of organic matter. The marked differences between "river-margin" and "dry" cave sectors demonstrate how localized hydrological and microclimatic variation can strongly influence community assembly and species turnover. From a conservation standpoint, these results indicate that disturbances to natural hydrological regimes (such as groundwater extraction, alterations in surface drainage, or other human-driven impacts) can critically affect cave biodiversity. Safeguarding the natural dynamics of subterranean water systems is therefore essential to preserve the ecological processes that sustain species richness and functional diversity within cave ecosystems.

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Author Contributions

Rodrigo Lopes Ferreira was responsible for conceptualization, methodology, and English revision. Paulo César Reis-Venâncio conducted the statistical analyses, prepared the artwork, constructed the maps, and handled translation and revisions. Ana Laura Alves prepared the original draft. Ana Laura Alves, Paulo César Reis-Venâncio, Rodrigo Lopes Ferreira, and Marconi Souza Silva contributed to review and editing. All authors have read and approved the final version of the manuscript.

Data Availability Statement

The data supporting this study's findings are available on request from the corresponding author. The Editor-in-Chief has waived the required archiving due to privacy or ethical restrictions.

Conflicts of Interest

The corresponding author confirms on behalf of all authors that there have been no involvement that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

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A buried umbrella: historical changes in the landscape surrounding the habitats of threatened troglobitic isopods in Brazil

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ABSTRACT: Impacts on karst environments exert particularly severe effects on species restricted to subterranean habitats (troglobites), as disturbances in surface ecosystems are typically transmitted to subsurface compartments. Given the high habitat specificity of these organisms and the extensive alteration of epigean environments, we investigated the potential impacts and pressures affecting 17 troglobitic isopod species of *Spelunconiscus*, all endemic to the Arcos-Pains-Doresópolis speleological province in southeastern Brazil. To assess temporal landscape changes surrounding the caves inhabited by these species, we delineated 14 drainage areas using the ottobasin methodology. These areas were analyzed based on land-use and land-cover maps derived from Landsat satellite images for the years 1984 and 2019. Subsequently, we ranked the ottobasins according to the degree of anthropogenic impact to guide conservation and management priorities. Among the 14 areas evaluated, 11 exhibited an increase in native vegetation cover. Nevertheless, expansions of mining, agriculture, and silviculture were also detected. The most intense impacts occurred in the upstream sectors of the ottobasins, which exert greater influence on the subterranean environment due to their hydrological connectivity with the caves. The Eden cave ottobasin emerged as the most threatened, combining intense anthropogenic pressure with the highest diversity of endemic troglobites. Prioritizing the protection and conservation of the five most impacted ottobasins would safeguard approximately 60.5% of the troglobitic species recorded in these areas, corresponding to 46% of all troglobitic species in the province. In contrast, extending protection to all 14 ottobasins would ensure the preservation of 76% of the province's troglobitic fauna.
Key words: land use, *Spelunconiscus*, mining, cave, umbrella species.

1. INTRODUCTION

Karst areas cover approximately 20% of the Earth's surface and include distinctive landforms such as dolines, blind valleys, sinkholes, and caves (FORD; WILLIAMS, 2007; GOLDSCHEIDER *et al.*, 2020). These systems are defined by the high solubility of their rocks and drainage patterns that frequently involve subterranean flows (JENNINGS, 1971). As a result, karst landscapes are particularly vulnerable, given their geological attributes that facilitate direct

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hydrological connectivity between surface and subterranean ecosystems (GUTIÉRREZ et al., 2014).

Anthropogenic disturbances in karst areas are especially concerning because pollutants and environmental changes can be rapidly transmitted through permeable rock, often reaching areas distant from their source. This dissemination occurs primarily through subterranean hydrological processes, which are less visible and difficult to monitor directly. Consequently, by the time impacts become evident, ecosystem alterations are typically at an advanced stage (FORD; WILLIAMS, 2007). The main drivers of change in karst landscapes are associated with land-use modifications, particularly urbanization, mining, deforestation, silviculture, agriculture, and livestock activities (VAN BEYNEN, 2012). Such activities impose severe environmental pressures that, in turn, negatively affect local biodiversity.

When pollutants reach subterranean habitats, they frequently alter species' richness and community composition, as caves are fragile ecosystems that often shelter threatened species, many of which are endemic to a single cave (CULVER; PIPAN, 2019). These alterations are particularly alarming because cave fauna play key ecological roles, such as maintaining water quality and supporting groundwater-dependent ecosystems (e.g., rivers and springs). Moreover, groundwater organisms are adapted to resource-limited and environmentally stable habitats, which makes them highly sensitive to anthropogenic disturbances (GRIEBLER; AVRAMOV, 2014). Due to these characteristics, subterranean species are increasingly recognized as promising bioindicators for environmental assessment and management (GRIEBLER et al., 2010).

The Karst Province of Alto São Francisco (KASP), located in southeastern lime and cement industries Brazil, contains the highest cave density in South America, with 2,503 registered caves (CECAV, 2021). However, this number is likely underestimated, as large portions of the region remain unexplored. Despite its outstanding speleological and biological significance, the KASP province is subject to multiple anthropogenic pressures, including limestone mining, lime and cement industries, groundwater extraction for industrial and domestic use, agricultural expansion, pasture establishment with consequent suppression of native vegetation, as well as the installation of cemeteries, solid waste disposal sites, and other contaminating activities (CAVALCANTI et al., 2012). Although the province harbors a remarkable diversity of troglobitic species, many remain undescribed (ZAMPAULO, 2010). Unfortunately, the exceptional biological richness and speleological importance of the area have not prevented increasing threats from intense human activities.

Among the cave-restricted taxa in the KASP province, isopods of the genus *Spelunconiscus* (Styloniscidae) stand out as potential models for the conservation of subterranean biodiversity. These groundwater-dependent organisms have restricted distributions and are therefore highly susceptible to anthropogenic impacts. Notably, the 17 caves in which *Spelunconiscus* occurs encompass 76% of all troglobitic species known from the KASP province. Given their ecological characteristics, these isopods can be regarded as umbrella species, since protecting them would also ensure the conservation of numerous co-occurring taxa (ROBERGE; ANGELSTAM, 2004). According to Lambeck (1997), the umbrella species concept is based on the premise that the ecological requirements of highly demanding species also encompass those of less demanding ones.

In this context, the present study evaluates temporal changes in land use and land cover surrounding caves harboring *Spelunconiscus* populations in the KASP speleological province. Additionally, we propose a ranking of conservation priorities based on the degree of anthropogenic impact within these areas. By assessing land-use dynamics and their potential implications for

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subterranean ecosystems, this study provides insights into the conservation status of caves in this karst region. The findings aim to support decision-making and guide management strategies to safeguard cave-restricted species, many of which face imminent risk of extinction.

2. METHODOLOGY

2.1 Study area

The São Francisco River basin comprises a region where approximately 40% of the caves in the Brazilian territory are found (ICMBIO, 2020). The study area is located in the subbasin of tributaries of the Upper São Francisco River in the state of Minas Gerais, where approximately 54% of the caves in the São Francisco basin are concentrated (Cavalcanti et al., 2012), and covers part of the municipalities of Arcos, Córrego Fundo, Doresópolis, Formiga, Pains and Piumhi (Figure 1).

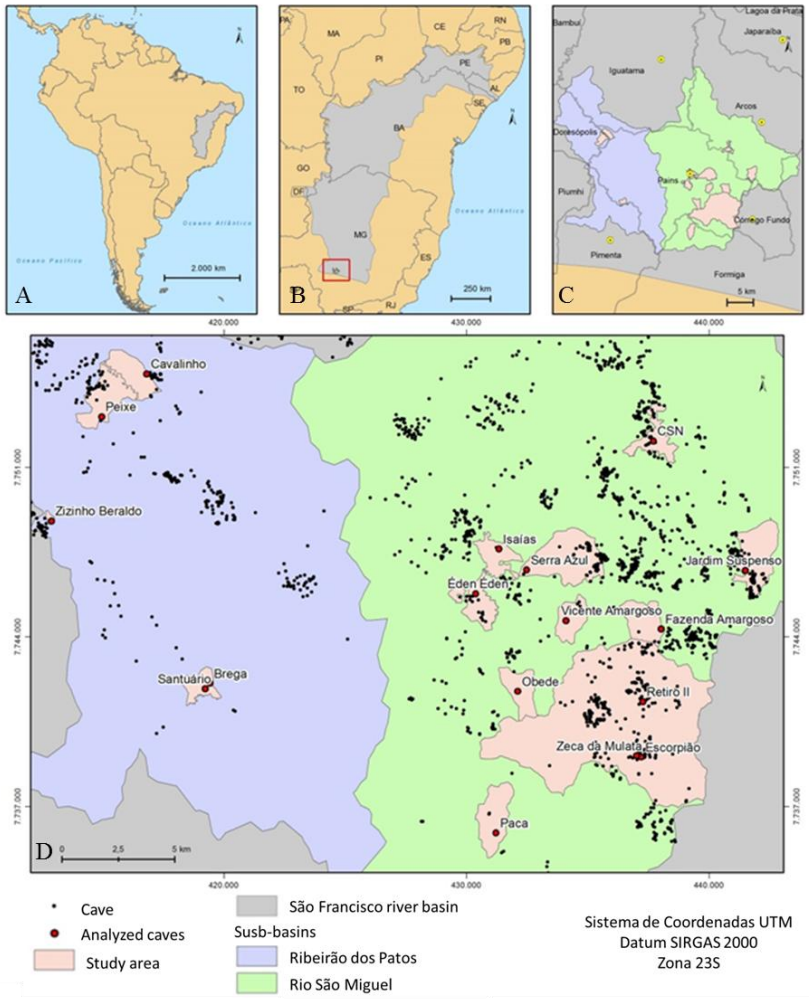

Figure 1. A and B) Location of the São Francisco River basin in Brazil and Minas Gerais. C) São Miguel and Ribeirão dos Patos river basins and ottobasins studied. D) Location of the ottobasins and distribution of the caves in the speleological province.

The caves considered in this study are those harboring populations of *Spelunconiscus* (Figure 2) within the Arcos-Pains-Doresópolis speleological province. All are situated in the São Miguel and Ribeirão dos Patos basins (Figure 1), tributaries on the right margin of the São Francisco River, where karst relief is well developed at both endokarst and exokarst levels. The analyzed region

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encompasses 14 areas surrounding 17 caves (Table 1), located within a karst landscape whose geomorphological compartmentalization includes fluvial plains, limestone outcrops, mountain ranges, and heterogeneous plateaus (MARTINS; RODRIGUES, 2016).

The local hydrographic network is relatively incipient due to the predominance of direct infiltration through limestone fissures and karst absorption features such as sinkholes (MENEGASSE et al., 2002). The regional vegetation is typical of the Cerrado biome (Brazilian savanna), which presents a continuum ranging from shrub-free grasslands (*campo limpo*) to denser arboreal formations, including deciduous and semideciduous seasonal forests, particularly associated with the bases of rocky outcrops (MELO et al., 2013; MENEGASSE et al., 2002).

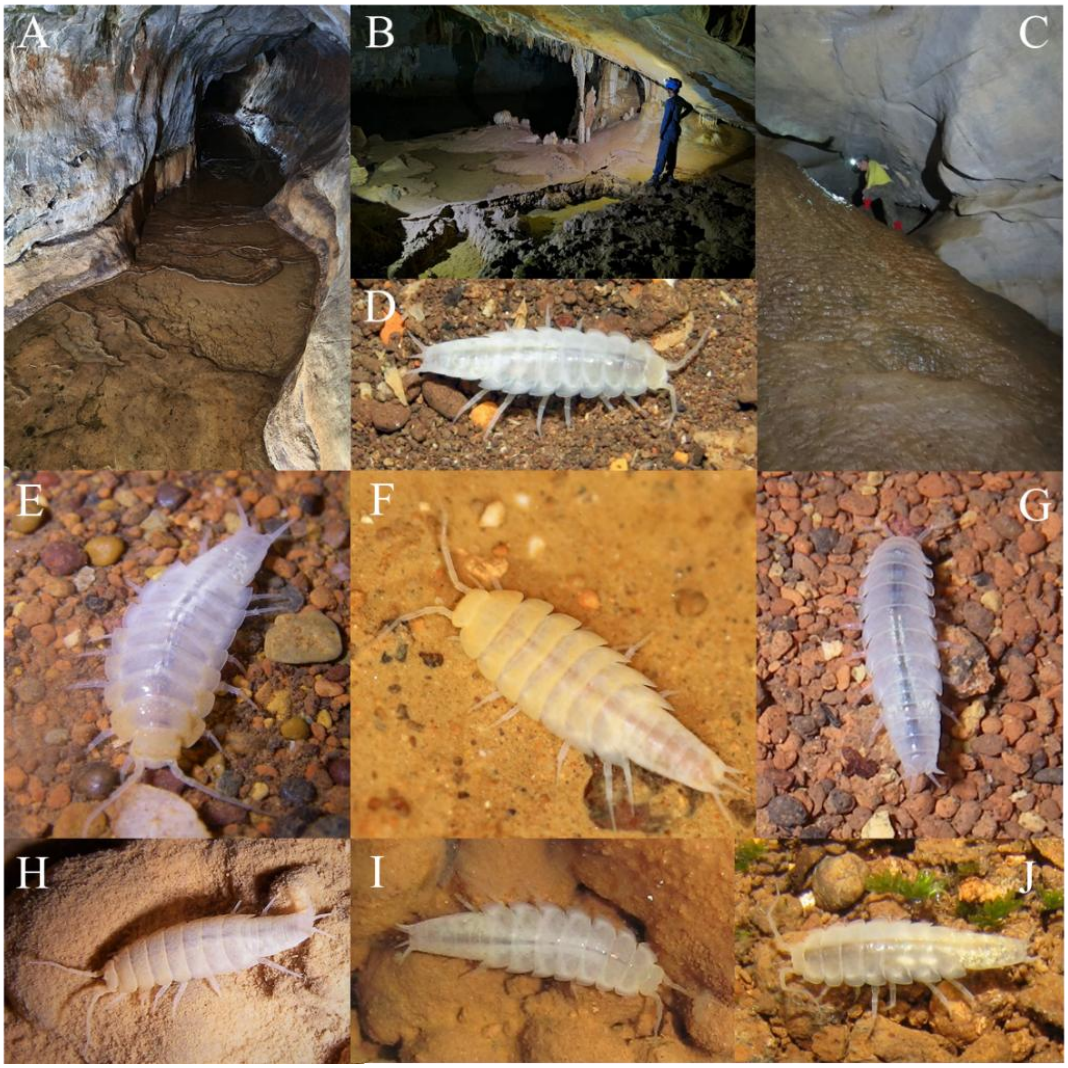

Figure 2. Some species of Spelunconiscus (Isopoda: Styloniscidae) found in the studies caves: A) Microhabitat of Spelunconiscus sp. in Escorpião cave; B) Microhabitat of Spelunconiscus sp. in Santuário cave; C) Microhabitat of Spelunconiscus sp. in Brega cave; D-J) distinct species of Spelunconiscus sp found in the area.

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Table 1: Identification and geographic location of the caves included in the study area, with respective municipalities, geographic coordinates (latitude and longitude in degrees, minutes, and seconds; converted from SIRGAS 2000 / UTM), and altitude above sea level.

Caves	City	Latitude (S)	Longitude (W)	Altitude (m)
Brega	Pains	20° 25' 23.81"	45° 39' 19.41"	708
Cavalinho	Pains	20° 33' 01.71"	45° 40' 38.26"	685
CSN	Pains	20° 30' 57.14"	45° 27' 37.10"	767
Éden	Pains	20° 27' 39.79"	45° 31' 38.88"	709
Escorpião Cave	Pains	20° 21' 16.24"	45° 27' 46.39"	822
Fazenda Amargoso	Pains	20° 26' 04.48"	45° 26' 56.75"	847
Isaías	Pains	20° 28' 53.42"	45° 30' 34.82"	732
Jardim Suspenso	Arcos	20° 28' 09.72"	45° 25' 56.34"	896
Obede	Pains	20° 24' 55.74"	45° 29' 59.03"	726
Paca	Formiga	20° 22' 26.16"	45° 31' 00.84"	772
Peixe	Doresópolis	20° 31' 43.26"	45° 42' 16.66"	697
Retiro II	Pains	20° 24' 17.95"	45° 27' 44.77"	811
Santuário	Pains	20° 25' 12.84"	45° 39' 29.22"	702
Serra Azul	Pains	20° 28' 13.68"	45° 29' 39.20"	771
Vicente Amargoso	Pains	20° 26' 39.43"	45° 28' 47.83"	794
Zeca da Mulata	Pains	20° 21' 20.60"	45° 27' 58.77"	837
Zizinho Beraldo	Pains	20° 29' 59.42"	45° 44' 32.43"	680

2.2 Delimitation of the surrounding areas

To account for the interaction between ecological attributes of the areas surrounding the caves, we adopted the use of ottobasins (LUIZ; FARIA, 2013) to define their perimeters, as an alternative to the fixed 250-meter buffer established by current Brazilian speleological legislation (CONAMA, 2004). Because more than one cave may occur within the same ottobasin, certain regions encompassed two or three caves, as in the cases of the Santuário–Brega and Retiro II–Zeca da Mulata–Escorpião groups.

The delimitation of ottobasins was based on the spatial overlap of cave coordinates (Table 1) with the vector database provided by the Minas Gerais Institute of Water Management (IGAM, 2019). In a GIS environment, the selected polygons were subdivided to include only the slope where the caves were located. For ottobasins lacking active surface drainage that would allow subdivision by slope, the entire ottobasin was considered the study area. To evaluate the influence of anthropogenic activities, we also incorporated urbanized and mining areas intersecting the ottobasin polygons, provided these areas were located upstream of the caves.

2.3 Changes in the landscape

The analysis of the landscape surrounding the caves was conducted in a GIS environment following the methodology of Gomes et al. (2019), adapted for the objectives of this study. Vegetation cover and land-use maps were generated using Landsat orbit–point 219-074 images acquired by the Thematic Mapper (TM, 06/13/1984) and the Operational Land Imager (OLI, 08/01/2019). The classification legend was based on the *Manual Técnico de Uso da Terra* (IBGE, 2013) and included the following classes: Water, Urban Areas, Mining Areas, Cropland–Pastureland, Forestry, Forestland, and Grassland. Image classification was performed in Spring (INPE, 2001) using supervised region-growing with the Bhattacharya classifier and a 99% acceptance threshold. Post-classification procedures were carried out in ArcGIS, where raster data

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were converted into vector format and validated through field surveys, high-resolution imagery (GOOGLE, 2013), and orthoimages.

The reliability of the land-use and vegetation cover maps was tested using the Kappa index. Forty random points were generated per ottobasin in ArcGIS, which were validated through field observations and Google Earth imagery. A confusion matrix was constructed, from which the Kappa coefficient was calculated according to Congalton and Green (2019), as follows:

$$K = \frac{n\sum_{i=1}^{C} x_{ii} - \sum_{i=1}^{C} x_{i+}x_{i}}{n^{2} - \sum_{i=1}^{C} x_{ii}}$$

where K is the Kappa coefficient; xiix_{ii}xii is the value in row i and column i; xi+x_{i+}xi+ is the sum of row i; x+ix_{+i}x+i is the sum of column i; n is the total number of samples; and c is the total number of classes.

To evaluate changes in landscape structure relative to cave position (upstream vs. downstream), we used Alos Palsar radar images (JAXA/METI, 2011), processed in ArcGIS with the surface/contour tool to generate contour lines used for geomorphological compartmentalization. To increase accuracy, Alos Palsar data were integrated with land-use maps using the ArcGIS Zonal Statistics tool, allowing extraction of class-level statistics (pixel by pixel). Landscape metrics were then derived from the V-LATE 2.0 beta extension (Vector-based Landscape Analysis Tools), including number of patches (NP), class area (CA), average class size (ACS), standard deviation of patch size (SDPS), and mean shape index (MSI).

Vegetation-cover change from 1984 to 2019 was further evaluated using the annual deforestation rate (ADRR) proposed by de Barros Ferraz et al. (2009):

$$\text{ADRR} = \left(\frac{MF_n}{MF_1}\right)^{1/(Y_n - Y_1)} - 1$$

where MF_n is the extent of mature forest in year n (ha), MF_1 the extent of mature forest in year 1, Y_n the final year, and Y_1 the initial year, with results expressed as % yr^{-1}.

To assess the impacts of mining activities, recognized as one of the most severe and irreversible threats to caves (BRASIL, 2008), we performed a spatial analysis of mining concessions recorded in the National Mining Agency (ANM, 2021) database and their overlap with the study areas.

2.4 Ranking conservation efforts

To establish conservation priorities for subterranean biodiversity, the ottobasins were categorized based on potentially impacting land uses (Table 2), using the disturbance index proposed by Van Beynen (2005) as a reference. Land-use impacts were classified according to their percentage of spatial occupation within each ottobasin. Additionally, upstream impacts were assigned higher weights, given their disproportionately greater influence on downstream subterranean habitats.

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Table. 2. Indicators used to classify the potentially impacting land use and occupation of the ottobasins.

Land use and occupation	**Classes of use**			**Upstream impact**			
Indicator	**3**	**2**	**1**	**0**	**3**	**2**	**1**
% of area occupied by mining	>66%	34–66%	-33%	None	>20%	10-20%	1-10%
% of area occupied by urbanization	>66%	34–66%	-33%	None	>20%	10-20%	1-10%
% of area occupied by agriculture and livestock	>66%	34–66%	-33%	None	>20%	10-20%	1-10%
% of area occupied by forestry	>66%	34–66%	-33%	None	>20%	10-20%	1-10%

3. RESULTS

Over the analyzed period (1984–2019), there was a general reduction in areas allocated to agricultural activities, accompanied by increases in forest cover, mining, forestry, and urbanization (Table 3). Although mining occupied a relatively small percentage of some ottobasins, its absolute extent reached more than 75 ha in Jardim Suspenso, indicating a high level of impact.

The most pronounced negative changes were observed in the Jardim Suspenso and Retiro II–Zeca da Mulata–Escorpião ottobasins, where mining and forestry expanded considerably (Table 3). Land-use maps (Figures 3–7) highlighted shifts across all land-use and vegetation-cover classes. In the Zizinho Beraldo, CSN, and Fazenda Amargoso areas, forest regeneration declined, whereas in most other areas this class showed an increase. Overall, negative land-use changes were more evident upstream than downstream (Supplementary Material 1).

The highest annual deforestation rates were recorded in Fazenda Amargoso, followed by CSN and Zizinho Beraldo (Table 4). In these ottobasins, the principal drivers of deforestation were forestry, mining, and cropland/pastureland, respectively. According to the classification proposed by Figueiredo and Vieira (2007), the accuracy of the land-use and vegetation maps, assessed using the Kappa index, ranged from "excellent" ($0.8 < K \leq 1.0$) to "very good" ($0.6 < K \leq 0.8$) (Supplementary Material 2).

Landscape metrics for natural vegetation patches revealed a general reduction in the number of fragments across most ottobasins (Table 5). As with land-use changes, negative trends in landscape metrics were more pronounced in upstream than in downstream sectors (Supplementary Material 3).

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Table 3. Changes of land use and vegetation cover in the ottobasins in which caves are present.

Ottobasin	Year	Forest		Crop-livestock		Mining		Urban		Forestry		Water		Natural grasslands	
		%	ha	%	ha	%	ha	%	ha	%	ha	%	ha	%	ha
Zizinho Beraldo	1984	42.33	5.28	57.67	7.19										
Zizinho Beraldo	2019	41.29	5.14	58.71	7.32							0.22			
Jardim Suspenso	1984	32.98	123.38	63.49	237.54	3.53	13.21								
Jardim Suspenso	2019	34.54	129.23	37.07	138.68	20.10	75.20			8.07	30.21	0.22	0.83		
Vicente Amargoso	1984	23.72	41.34	74.06	129.10					2.22	3.87				
Vicente Amargoso	2019	27.29	47.57	71.69	125.00					1.01	1.77				
Santuário e Brega	1984	29.54	38.79	70.46	92.54										
Santuário e Brega	2019	35.94	47.21	64.06	84.15										
Cavalinho	1984	25.58	58.53	74.42	170.30										
Cavalinho	2019	25.71	58.82	71.07	162.60	2.63	6.03								
Peixe	1984	43.50	129.40	46.37	137.90							5.01	14.90		
Peixe	2019	45.66	135.80	46.56	138.50					3.15	9.36	4.07	12.09	0.57	1.68
Obede	1984	44.60	88.45	55.40	109.90										
Obede	2019	57.62	114.30	40.00	79.33					2.38	4.71				
CSN	1984	64.94	117.30	18.38	33.19	16.68	30.12			1.18	46.08				
CSN	2019	57.25	103.40	17.47	31.54	25.28	45.65			9.26	358.44				
Retiro II – Zeca da Mulata – Escorpião	1984	26.55	1033.90	71.36	2757.85							5.12	15.21		
Retiro II – Zeca da Mulata – Escorpião	2019	41.42	1603.88	47.41	1835,77	1.44	55.89			6.37	358.44	0.24	9.18		
Serra Azul	1984	17.57	91.61	82.16	428.5							0.19	1.41		
Serra Azul	2019	32.88	171.5	58.00	302.6	1.78	9.28					0.22	5.05		
Isaías	1984	6.39	11.61	44.73	81.3	0.72	1.98					0.27			
Isaías	2019	14.75	26.800	17.07	31.01	1.78	16.42	48.88	88.83			0.97			
Éden	1984	33.82	73.84	57.26	125.00										
Éden	2019	51.80	112.9	27.21	59.31	7.53	16.42	12.85	28.00	18.24	42.03				
Fazenda Amargoso	1984	35.77	82.45	63.37	146.10	8.92	19.47								
Fazenda Amargoso	2019	31.32	72.19	49.18	113.40	1.27	2.92			0.61	1.33				
Paca	1984	33.50	99.59	66.50	197.70	0.86									
Paca	2019	42.24	125.6	57.76	171.70										

* Percentages may not sum to exactly 100% due to rounding.

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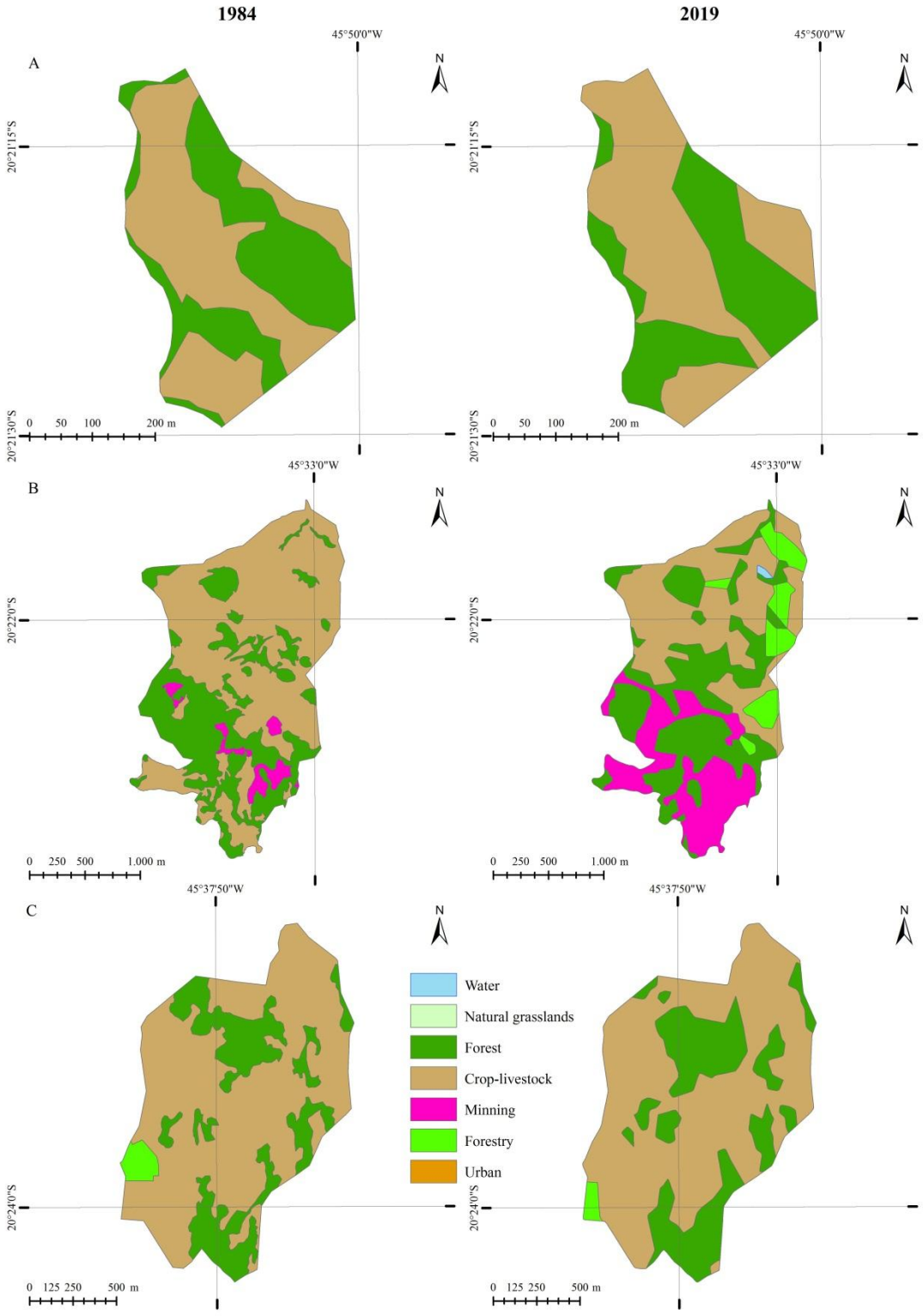

Figure 3. Land use maps of 1984 and 2019 of the ottobasins. A) Zizinho. B) Jardim Suspenso. C) Vicente amargoso.

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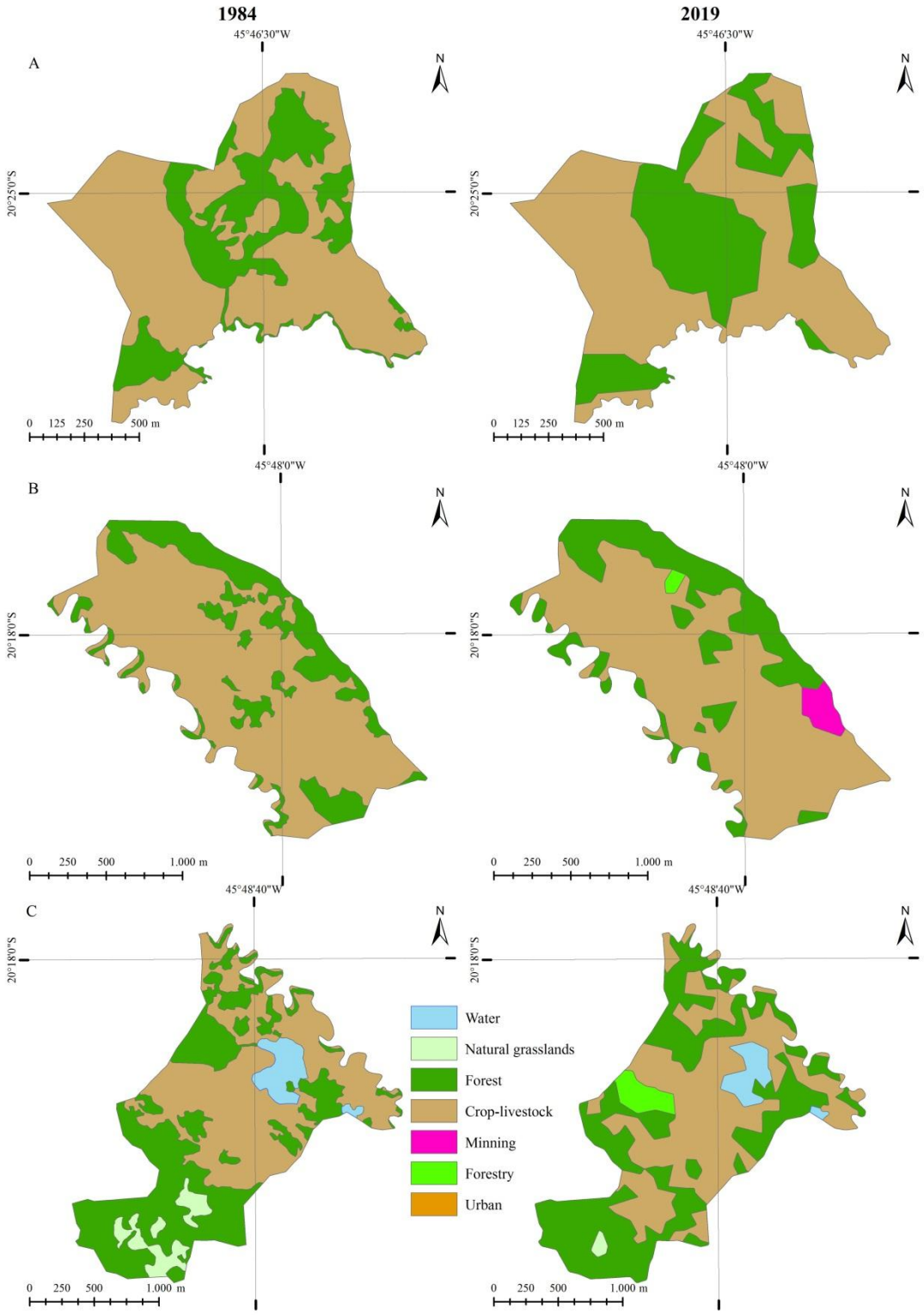

Figure 4. Land use maps of 1984 and 2019 of the ottobasins. A) Santuário-Brega. B) Cavalinho. C) Peixe.

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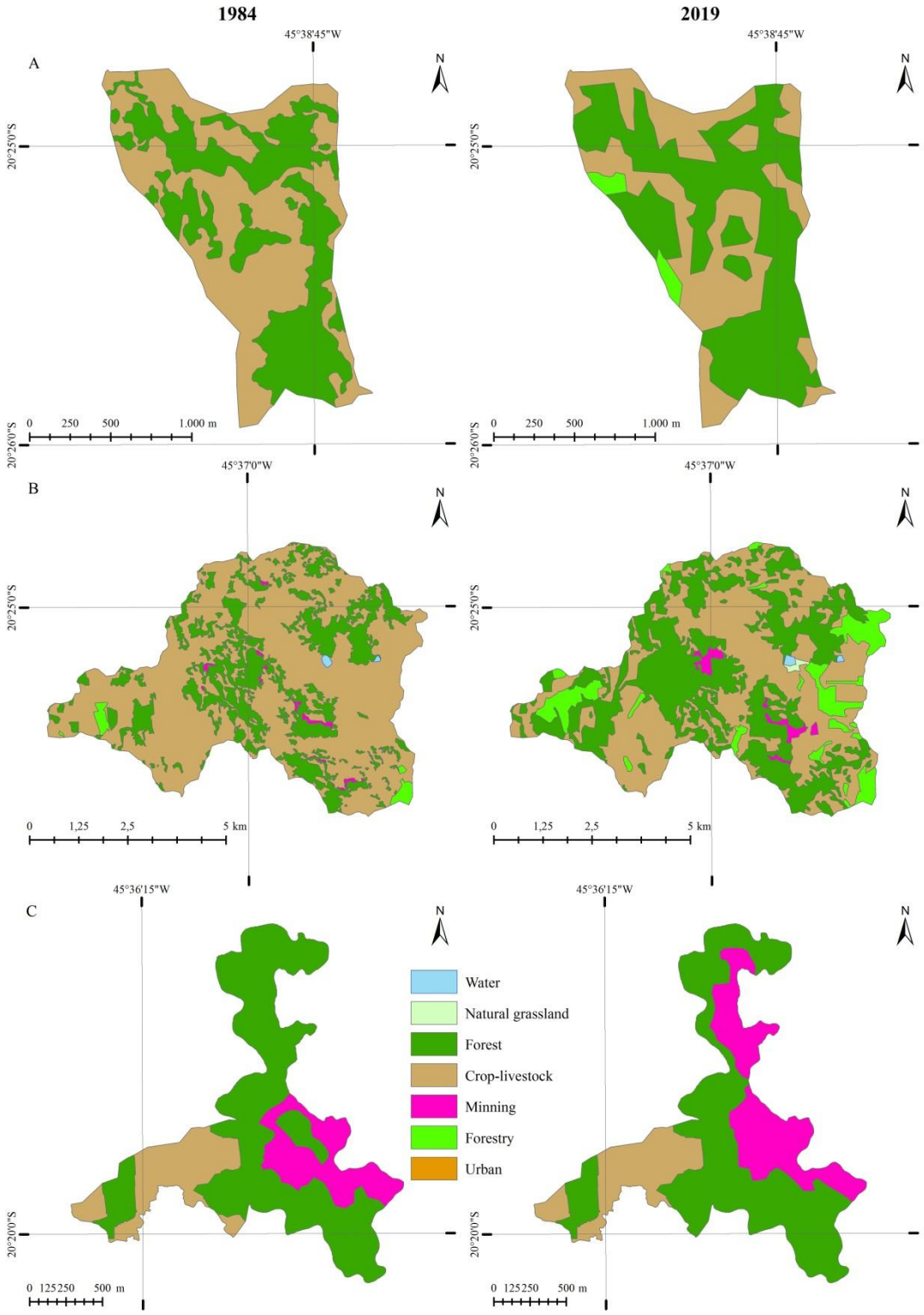

Figure 5. Land use maps of 1984 and 2019 of the ottobasins. A) Obede. B) Retiro II-Zeca da Mulata- Escorpião. C) CSN.

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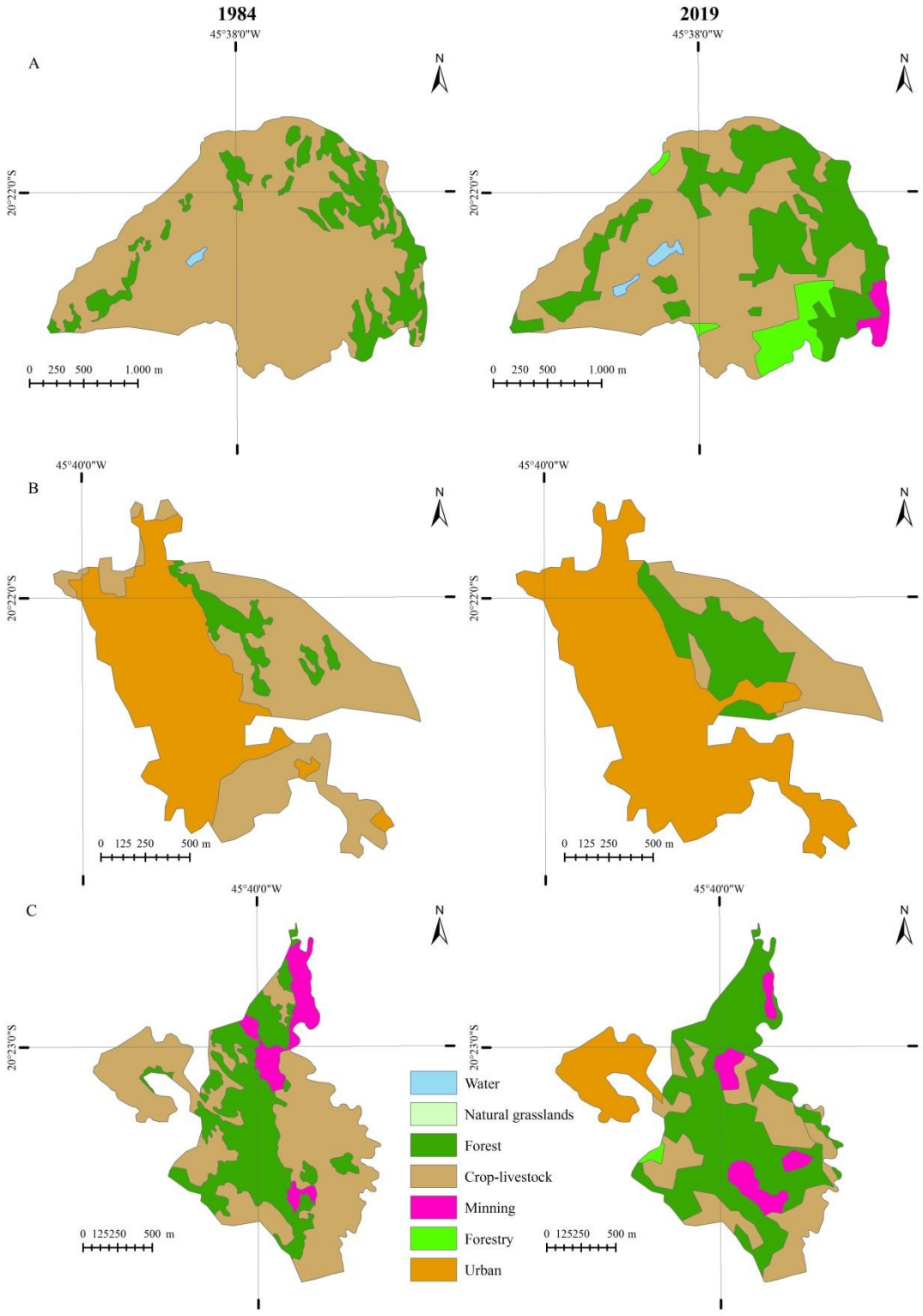

Figure 6. Land use maps of 1984 and 2019 of the ottobasins. A) Serra Azul. B) Isaías. C) Éden

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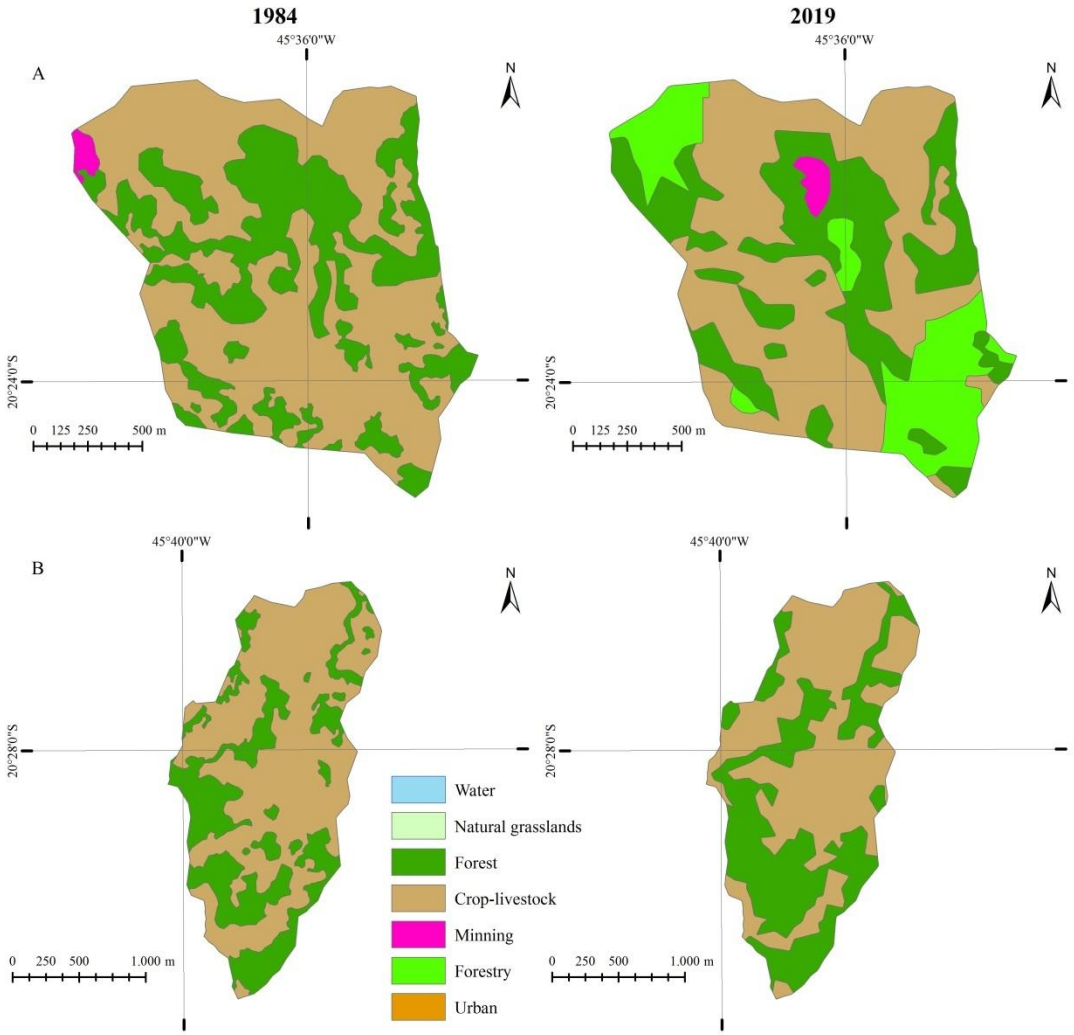

Figure 7. Land use maps of 1984 and 2019 of the ottobasins. A) Fazenda Amargoso. B) Paca.

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Table 4. Annual deforestation/regeneration rate (ADRR) (% year^{-1}) for ottobasins.

Cave		TAD/R
Zizinho Beraldo	Ziz	-0.072%
Jardim Suspenso	JdS	0.132%
Vicente Amargoso	VcA	0.402%
Santuário e Braga	SnB	0.563%
Cavalinho	Cav	0.014%
Peixe	Pex	0.138%
Obede	Obd	0.735%
CSN	CSN	-0.359%
Retiro II-Zeca da Mulata-Escorpião	RZE	1.262%
Serra Azul	SeA	1.808%
Isaías	Isa	2.419%
Éden	Edn	1.220%
Fazenda Amargoso	FzA	-0.379
Paca	Pca	0.665%

Table 5. Landscape metrics for the ottobasins generated for the upstream portion of the caves.

Ottobasins	Year	Classes	NP	CA	ACS	MSDC	TCA	ICA	MSI
Cavalinho	1984	Forest	3	37605.85	12535.28	11960.7	20219.58	53.77	1.574
	2019	Forest	1	29206.66	29206.66	0	16901.11	57.87	2.122
CSN	1984	Forest	2	958582.8	479291.4	422705	850974.7	88.77	2.126
	2019	Forest	1	803321.8	803321.8	0	706804.7	87.99	3.07
Éden	1984	Forest	5	458900.2	91780.05	168869	359268.5	78.29	2.025
	2019	Forest	1	456373	456373	0	383603.9	84.05	3.066
Fazenda Amargoso	1984	Forest	12	108721.3	9060.11	10012.1	62149.2	57.16	1.58
	2019	Forest	9	61200.15	6800.02	5694.19	35824.05	58.54	1.507
Isaías	1984	Forest	4	23391.29	5847.82	5382.48	10761.49	46.01	1.47
	2019	Forest	2	105224.4	52612.2	42183.3	82209.18	78.13	1.599
Jardim Suspenso	1984	Forest	6	383659.5	63943.25	116313	280815.7	73.19	1.798
	2019	Forest	7	243571.4	34795.91	39211.3	183212.1	75.22	1.458
Obede	1984	Forest	7	646392.4	92341.77	127842	524001.1	81.07	1.818
	2019	Forest	4	845780.4	211445.1	320472	735959.2	87.02	1.631
Paca	1984	Forest	10	507015.2	50701.52	51436.1	385376.6	76.01	1.783
	2019	Forest	7	600652.4	85807.48	97709.8	484950.2	80.74	1.617
Peixe	1984	Campo	1	17359.41	17359.41	0	9813.42	56.53	1.717
		Forest	11	216198.6	19654.42	28981.9	159427.7	73.74	1.392
	2019	Forest	9	193637.1	21515.23	15326.5	137881.6	71.21	1.315

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Retiro II Zeca da Mulata	1984	Forest	57	4528165	79441.49	245896	3603618	79.58	1.84
	2019	Forest	20	5903742	295187.1	628576	5092374	86.26	1.977
Serra Azul	1984	Forest	11	752250.5	68386.41	95478.7	567340.1	75.42	1.867
	2019	Forest	9	1464797	162755.2	437551	1269985	88.05	1.559
Santuário-Brega	1984	Forest	6	306604.1	51100.69	79464.4	217560.9	70.96	1.923
	2019	Forest	4	335053.2	83763.3	76773.6	266189.7	79.45	1.81
Vicente Amargoso	1984	Forest	7	238322.2	34046.02	44708.8	157629.8	66.14	1.95
	2019	Forest	8	281535.3	35191.91	49593.5	216925.9	77.05	1.388
Zizinho	1984	Forest	1	21357.08	21357.08	0	16017.35	75	1.096
	2019	Forest	1	19714.35	19714.35	0	14151.4	71.78	1.196

NP: number of patches. CA: class area (m²). ACS: average class size; MSDC: mean standard deviation of the class; TCA: total central area of the class; ICA: index in % of the central area of the class; MSI: mean shape index.

According to the mining processes registered with the DNPM, all analyzed areas have at least part of their territory under applicationfor mining concessions (Figure 8). Among these, Jardim Suspenso, Cavalinho, Peixe, CSN, Retiro II–Zeca da Mulata–Escorpião, Serra Azul, and Fazenda Amargoso already hold active concessions and currently contain quarries. Consequently, both medium- and long-term scenarios indicate that these areas may undergo severe impacts from mining activities.

When considering the ottobasins containing caves with *Spelunconiscus* species, the most threatened is that of the Éden cave, which harbors the highest richness of troglobitic species in the province (Table 6). The five caves identified as conservation priorities in the ranking together comprise 60.5% of the troglobitic richness of the analyzed ottobasins and 46% of the total troglobitic richness known for the entire province.

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*Table 6. Ranking of ottobasins in terms of impacts. *S: Richness*

Ottobasins	Score	Troglobites S	Endemic troglobites
Éden cave	12	15	10
Fazenda Amargoso cave	10	3	1
Cavalinho cave	9	6	1
Serra Azul cave	9	4	1
Isaías cave	9	3	1
Vicente Amargoso cave	8	3	2
Jardim Suspenso cave	7	1	1
Obede cave	7	1	1
Retiro II & Zeca da Mulata caves	6	8	4
Peixe cave	6	2	1
CSN cave	5	1	1
Paca cave	5	2	1
Santuário & Brega caves	5	7	2
Zizinho cave	5	4	1

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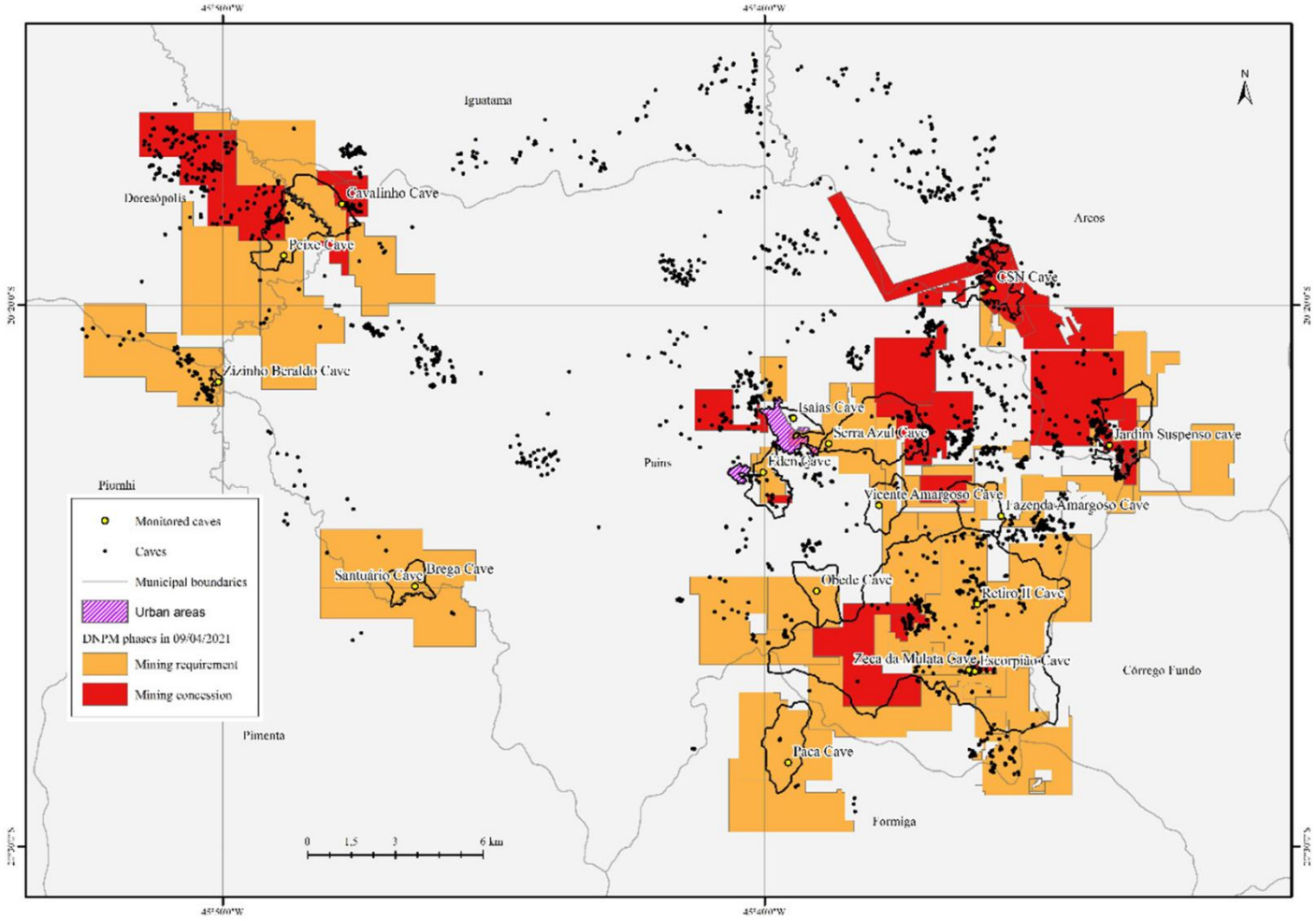

Figure 8. Ottobasins and the process phases in the National Mining Agency (NMA).

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4. DISCUSSION AND CONCLUSION

Across the 14 areas analyzed, there was a general increase in mining, urban expansion, forestry, and cropland/pastureland in several ottobasins. Among these, Zizinho, CSN, and Fazenda Amargoso exhibited a reduction in forest cover over the 35-year period studied (Table 3), involving the conversion of several hectares of native vegetation in each basin. It is important to emphasize that an increase in native vegetation provides numerous ecological benefits, including regulating microclimate, light penetration, airflow, energy input, and water infiltration into subterranean systems (URICH, 2002). Thus, the loss of forest in these regions may directly reduce habitat quality for cave-restricted species.

In the Zizinho Beraldo ottobasin, agriculture–livestock areas increased by 1.04%, corresponding to approximately 0.13 ha. Although this expansion was modest in relative terms, it still represents a measurable alteration of the landscape. Livestock farming is associated with the conversion of native vegetation, changes in soil chemistry and percolating water, erosion, sedimentation, and agrochemical use (URICH, 2002). Agricultural practices in karst systems have strong implications for soil, water, and biodiversity (COXON, 2011). Erosion, for example, increases sediment deposition in both the epikarst and cave environments (GILLIESON; THURGATE, 1999; HARDWICK; GUNN, 1990). The widespread use of fertilizers and pesticides leads to infiltration of chemicals into groundwater, often reaching concentrations lethal to fauna (DI MARZIO et al., 2009). Elevated potassium ion (K^+) concentrations in groundwater from the KASP province (LUCON et al., 2018) are likely linked to fertilizer use. Laboratory studies have shown mortality of subterranean crustaceans at potassium salt concentrations ranging from 0.28 mg/L to 17.99 mg/L (REBOLEIRA et al., 2013). Such values are particularly concerning *Spelunconiscus* species, which depend heavily on percolation water and are thus highly exposed to ionic changes.

Mining activity increased significantly in several ottobasins, including Jardim Suspenso (+16.57%, >75 ha), CSN (+8.6%, ~46 ha), Cavalinho (+2.63%, ~6 ha), Serra Azul (+1.78%, ~9 ha), Retiro II–Zeca da Mulata–Escorpião (~1%, >55 ha), and Fazenda Amargoso, where mining decreased to approximately 3 ha in 2019.Although mining sometimes occupied a relatively small proportion of basin area, its absolute spatial extent often reached tens to more than seventy hectares, indicating a high level of impact. Mining in karst areas generates severe ecological and geomorphological impacts, altering the physicochemical properties of groundwater and degrading macroinvertebrate communities (MILIŠA et al., 2010). In the KASP province, cadmium concentrations in groundwater range from 0.001 µg/L to 15.63 µg/L, exceeding the threshold for human consumption (5 µg/L) in some cases (LUCON et al., 2020). Cadmium toxicity has been demonstrated at values as low as 16 µg/L in Ephemeroptera and even lower in salmonids (MEBANE et al., 2012), suggesting that current concentrations may already threaten subterranean fauna. Amphibious *Spelunconiscus* species, commonly associated with travertine dams, are especially vulnerable to water quality degradation, which can alter their biology and behavior and ultimately cause population declines. Moreover, many of these isopods migrate seasonally to the epikarst during dry periods, a compartment also susceptible to contamination from mining. Thus, even when macro-caves are not directly affected, their connected epikarst habitats may be significantly impacted.

Urban expansion also emerged as a notable driver of environmental degradation, with increases of 48.88% (~89 ha) in Isaías and 12.85% (~28 ha) in Éden. In karst systems, urbanization

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intensifies pollution, reduces groundwater quality, and generates irreversible impacts (DE WAELE; FOLLESA, 2004). According to Decree No. 6.640/2008 (BRASIL, 2008), caves harboring rare troglobitic species are considered of maximum relevance and should not be exposed to irreversible impacts. However, legislation primarily addresses easily measurable impacts, leaving uncertainties about the medium- and long-term consequences of sewage infiltration and soil impermeabilization on subterranean fauna. Previous studies have demonstrated a negative relationship between groundwater pollution and stygobitic richness (MALARD et al., 1996; SIMON; BUIKEMA, 1997).

Forestry expansion also imposes cumulative pressures. In some ottobasins, forestry plantations expanded by tens to hundreds of hectares, particularly in larger upstream basins such as Retiro II–Zeca da Mulata–Escorpião and CSN. By reducing evapotranspiration, tree plantations increase surface runoff, leading to erosion and nutrient leaching (URICH, 2002). Eucalyptus monocultures reduce organic matter input into aquatic systems (ABELHO; GRAÇA, 1999; MOLINERO; POZO, 2004). While they may temporarily alter soil organic carbon levels depending on pedological conditions (MONTERO; DELITTI, 2017), long-term declines in organic matter are expected (DEAN et al., 2017). Such changes reduce the quality and quantity of trophic resources entering subterranean ecosystems, with cascading effects on cave fauna (SCHNEIDER et al., 2011). Given that troglobitic populations are especially dependent on external trophic inputs (HUMPHREYS, 1991), forestry-related impacts may trigger population declines. Although total forest area increased in some ottobasins, upstream zones in Zizinho, Jardim Suspenso, Santuário–Brega, Peixe, Cavalinho, CSN, and Fazenda Amargoso experienced forest losses involving several to dozens of hectares due to anthropogenic expansion. These upstream alterations are particularly concerning, as disturbances propagate rapidly through karst systems and disproportionately affect downstream cave environments (URICH, 2002; VAN BEYNEN, 2011).

Deforestation exacerbates sedimentation, turbidity, nutrient infiltration, and flood pulses in subterranean ecosystems (URICH, 2002; FORD; WILLIAMS, 2007; GILLI, 2011). Because caves lack primary producers, adjacent vegetation is critical in supplying organic matter and energy to subterranean food webs (GIBERT; DEHARVENG, 2002). Reductions in these inputs, even when involving relatively small percentages but large absolute areas, likely intensify interspecific competition, with unknown but potentially severe consequences for *Spelunconiscus* and other troglobitic species. Given their low population densities and restricted distributions, such taxa are especially vulnerable to extinction. Landscape homogenization further amplifies these risks, as environmental heterogeneity is a major driver of biodiversity (SHMIDA; WILSON, 1985) and of cave community composition (PELLEGRINI et al., 2016).

Landscape metrics confirmed degradation of non-anthropogenic soil-cover classes, with upstream zones experiencing the most pronounced declines (Supplementary Material 3). Reductions in forest patch number and area, often involving tens to hundreds of hectares, decrease trophic input and water infiltration while also modifying habitat connectivity. The mean shape index (MSI) revealed increased edge effects, as irregular fragment shapes are more exposed to microclimatic variation (DAVIES-COLLEY et al., 2000). These effects reduce soil moisture and negatively affect soil fauna foraging activity (RIUTTA et al., 2016).

Looking ahead, mining concessions already registered in the National Mining Agency database (Figure 2) indicate that the long-term scenario will likely intensify pressures on karst ecosystems. Mining invariably results in vegetation removal, rock extraction, sedimentation, and

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air pollution, often involving large absolute areas, causing cascading impacts on subterranean biodiversity.

The ranking of ottobasins based on impact levels identified the most threatened areas, highlighting Éden as the most vulnerable site due to its high richness of troglobitic and endemic species. Protecting the seven most threatened ottobasins would safeguard approximately 52% (26 species) of all troglobites known in the province. However, the optimal scenario would involve mitigating impacts across all 14 ottobasins, which collectively shelter 17 undescribed *Spelunconiscus* species and approximately 76% of the province's troglobitic fauna. Given that the KASP province contains over 2,500 registered caves, the conservation of *Spelunconiscus* isopods as umbrella species (STOCH et al., 2009) is a promising strategy, as their protection would ensure the preservation of numerous co-occurring troglobitic, troglophilic, and trogloxenic species.

In summary, this study identified critical sources of anthropogenic pressure in the KASP province and demonstrated their negative consequences for subterranean biodiversity. Although some land-use changes appeared moderate in relative terms, they involved the conversion of extensive areas, often exceeding tens or hundreds of hectares, reinforcing the magnitude of human impacts. The current scenario is unfavorable for the long-term persistence of cave-restricted species, with some at risk of extinction due to their sensitivity and restricted ranges. Nevertheless, targeted conservation measures, particularly in priority ottobasins, could safeguard more than half of the province's troglobitic species. Further studies are urgently needed to clarify the ecological effects of land-use changes on subterranean communities and to guide effective mitigation and conservation strategies.

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Author Contributions Statement

V.M.M: conceptualization, methodology, writing - original draft and visualization. **M.G.:** conceptualization, methodology and formal analysis, **R.L.F.:** conceptualization; methodology, writing – review & editing and supervision.

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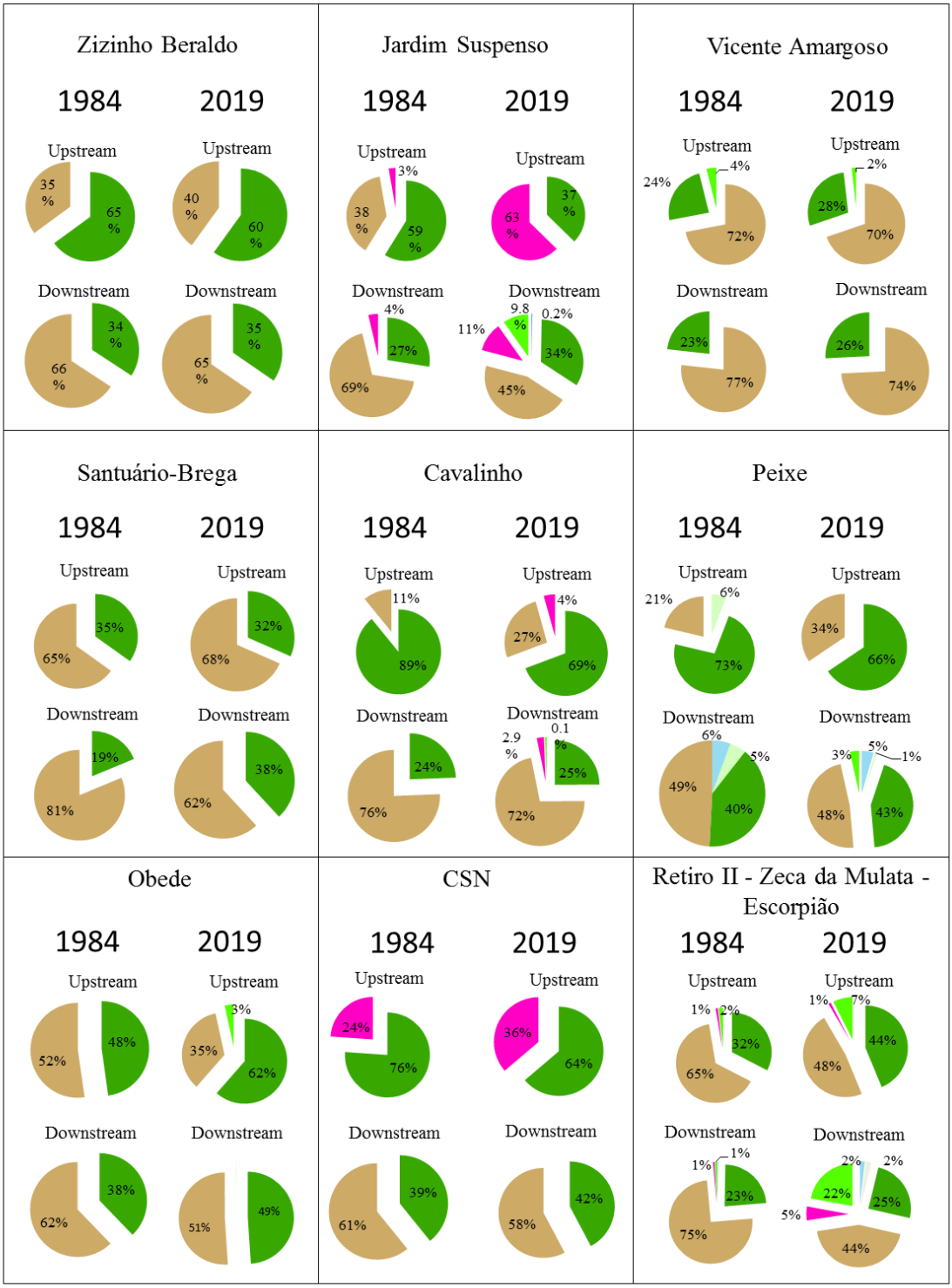
Supplementary material 1

Percentages of land use in each by ottobasin upstream and downstream related to the caves' positions on the landscape.



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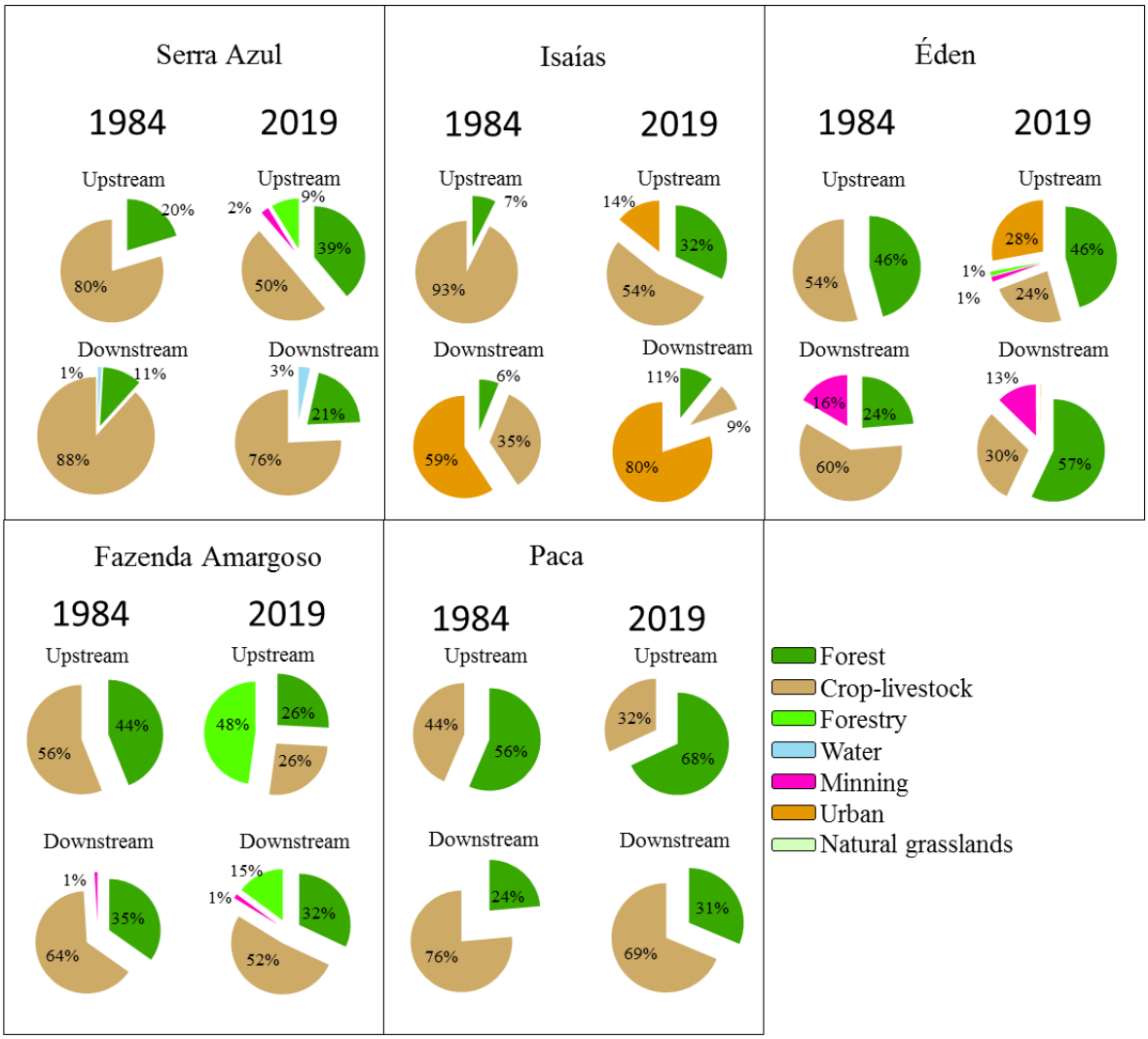

Supplementary material 2

Kappa index generated for each ottobasin studied.

Areas	Kappa
Zizinho Beraldo	0.62
Jardim Suspenso	0.74
Vicente Amargoso	0.70
Santuário e Brega	0.64
Cavalinho	0.62
Peixe	0.69
Obede	0.90
CSN	0.76
Retiro II, Zeca da Mulata e Escorpião	0.85
Serra Azul	0.76
Isaías	0.94
Éden	0.69
Fazenda Amargoso	0.85
Paca	0.76

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Supplementary material 3

Landscape metrics for natural vegetation cover patches in the downstream portion of ottobasins (1984 and 2019).

Ottobasins	Year	Classes	NP	CA	ACS	MSDC	TCA	ICA	MSI
Cavalinho	1984	Forest	25	547699.40	21907.98	60070.30	20219.80	53.77	2297
	2019	Forest	18	559002.60	31055.7	83432.34	429347.20	76.81	1.405
CSN	1984	Forest	2	214009.7	107004.9	19578.37	171738.60	80.25	1.85
	2019	Forest	2	230518	115259	27807.60	186826.90	81.05	1.847
Éden	1984	Forest	9	279511	31056.77	32167.38	186809.70	66.83	1.742
	2019	Forest	3	672520.20	224173.40	297735.60	553982.30	82.37	2.419
Fazenda Amargoso	1984	Forest	25	715766.10	28630.64	103133.40	509451.60	71.18	1.616
	2019	Forest	13	660740.90	50826.22	88455.13	505814.10	76.55	1.647
Isaías	1984	Forest	2	92699.84	46349.92	36950.96	57953.65	62.52	2.317
	2019	Forest	2	162793.70	81396.87	70488.78	124671	76.58	2.214
Jardim Suspenso	1984	Forest	24	850178.50	35424.10	86406.64	600487.20	70.63	1.885
	2019	Forest	13	1048700	80669.26	119580.80	841864.40	80.28	1.743
Obede	1984	Forest	8	238106.80	29763.35	39929.91	155696.30	65.39	1.824
	2019	Forest	5	296946	59389.21	78592.1	237446.70	79.96	1.954
Paca	1984	Forest	22	488854	22220.64	25207.33	316198.40	64.68	1.578
	2019	Forest	11	655154.30	59559.48	72245.1	498707	76.12	1.865
Continuation									
	1984	Natural grasslands	4	134784.40	33696.10	28222.66	91962.01	68.23	1.688

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Ottobasins	Year	Classes	NP	CA	ACS	MSDC	TCA	ICA	MSI
Peixe		Forest	27	1077596	39910.96	107250.50	796547.30	73.92	1.967
	2019	Natural grasslands	1	16806.22	16806.22	0	12012.28	71.48	1.112
		Forest	13	1163916.00	89532.04	124470.60	916437.9	78.74	1.899
Retiro II-Zeca da Mulata-Escorpião	1984	Forest	73	5810803.00	79600.04	222095.60	4435926.00	76.34	1.955
	2019	Natural grasslands	1	91820.69	91820.69	0	72580.73	79.05	1.849
		Forest	23	10135081	440655.70	1153368	8948206	88.29	2.13
Serra Azul	1984	Forest	14	163835.10	11702.51	19702.16	99894.14	60.97	1.538
	2019	Forest	6	302369.10	50394.85	73586.23	228135.70	75.45	1.891
Santuário-Brega	1984	Forest	13	81338.64	6256.82	11292.19	28974.56	35.62	2.305
	2019	Forest	6	137052.40	22842.06	22591.67	92475.94	67.47	1.694
Vicente Amargoso	1984	Forest	4	175085.30	43771.32	53214.18	117726.10	67.24	2.154
	2019	Forest	6	194128.70	32354.79	46511.84	143450.00	73.89	1.706
Zizinho	1984	Forest	5	31395.14	6279.03	5924.56	13289.00	42.33	2.187
	2019	Forest	5	31731.44	6346.29	5627.34	16925.49	53.34	1.535

NP: number of patches. CA: class area (m²). ACS: average class size; MSDC: mean standard deviation of the class; TCA: total central area of the class; ICA: index in % of the central area of the class; MSI: mean shape index.