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Influence of land use on benthic macroinvertebrate assemblages in headwater streams of the Jaguari River Basin, Brazil

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ABSTRACT

Headwater streams play a crucial role in the formation of rivers; they boast numerous springs and serve as hotspots for aquatic biodiversity within watersheds. However, human activities frequently compromise the health of these environments. Common disturbances include the removal of riparian vegetation, sedimentation in the streambed, and the introduction of pollutants such as pesticides. This study sought to assess the macrobenthic community in three headwater rivers over a 9-month period, examining variations in water quality and community composition across different land uses. Key water quality variables — electrical conductivity and total suspended solids — were elevated at the Extrema (EX) Stream, likely attributable to pasture management practices. The Toledo (TO) Stream had higher water temperature and phosphate levels, potentially linked to the removal of riparian forest and the use of fertilizers in vegetable cultivation, respectively. Regarding the benthic community, metrics such as dominance (D); the percentage of Ephemeroptera, Plecoptera, and Trichoptera (% EPT); the EPT/Chironomidae ratio; and the Shannon–Wiener diversity index (H') revealed a clear gradient in environmental health among the three study sites. The Monte Verde (MV) stream emerged as the most pristine one, followed by the EX and TO sites. These data underscore the deleterious impact of unsustainable agricultural practices. In conclusion, such agricultural activities exert a negative influence on both environmental quality and benthic macroinvertebrate communities in headwater streams.

Keywords: biomonitoring, Camanducaia River, EPT, riparian vegetation.

Influência do uso da terra nas comunidades de macroinvertebrados bentônicos em rios de cabeceira da Bacia do Rio Jaguari, Brasil

RESUMO

Os córregos e suas muitas nascentes são locais importantes para a formação dos rios e geralmente também são *hotspots* de biodiversidade aquática na bacia hidrográfica. As atividades humanas impactam a saúde desses ambientes, muitas vezes com a retirada da vegetação ciliar e também a entrada de sedimentos no leito do curso d'água, além da carga de poluentes como agrotóxicos. Este estudo teve como objetivo avaliar a comunidade macrobentônica em três riachos de cabeceira, ao longo de nove meses, monitorando a qualidade da água e a composição da comunidade com diferentes usos do solo. As concentrações das variáveis de qualidade da água, condutividade elétrica e sólidos suspensos totais, foram mais elevados no trecho fluvial em Extrema (EX), o que pode estar associado ao manejo da pastagem. No córrego em Toledo (TO), a temperatura da água e o fosfato foram mais elevados, provavelmente devido à retirada de mata ciliar e fertilizantes utilizados no cultivo de hortaliças, respectivamente. Quanto à comunidade bentônica, as métricas Dominância (D), % EPT, EPT/Chironomidae e o índice de diversidade de Shannon–Wiener (H') mostraram claramente um gradiente de saúde ambiental existente nos riachos estudados, sendo o de Monte Verde (MV) o mais preservado, seguido pelos córregos de Extrema (EX) e Toledo (TO). Esses dados evidenciam o impacto deletério de práticas agrícolas insustentáveis. Concluímos que tais atividades agrícolas influenciaram negativamente a qualidade ambiental e a comunidade de macroinvertebrados bentônicos nesses córregos de cabeceira.

Palavras-chave: biomonitoramento, EPT, mata ciliar, rio Camanducaia.

1. INTRODUCTION

Headwater streams play a vital role in nourishing watersheds. They serve as crucial hubs for processing organic matter and recycling nutrients, and they provide sanctuary for aquatic biota against extreme fluctuations in temperature and streamflow. They also provide habitats and refuge for various organisms. These functions are essential for sustaining the overall health of watersheds (Clark *et al.*, 2008; Wohl, 2017). Preserving riparian vegetation is especially critical in headwater areas, as it not only moderates temperature but also regulates soil and sediment transport into the rivers. Additionally, as mentioned previously, it helps regulate base flow, mitigating the impacts of runoff during the rainy season (Tambosi *et al.*, 2015). Moreover, given that headwater streams are often situated in steep terrain, which is more susceptible to non-point source pollution compared with lower, flatter areas of watersheds, conserving their landscape can aid in mitigating the adverse effects of such pollution (Yang *et al.*, 2016). Consequently, headwater streams offer a variety of ecosystem services to watersheds, bolstering riverbed stability and serving as a natural barrier against the influx of fine sediment from soil erosion, nutrients from agricultural fertilizers, and pollutants such as pesticides into water bodies (Pusey and Arthington, 2003). Headwater streams also are crucial for maintaining fish and fisheries, and are closely connected to the basin and its entire area, and consequently, connected to the land uses that occur in this environment (Toth *et al.*, 2024).

In Brazil, the Forest Code mandates a 30-m riparian vegetation buffer to safeguard rivers with widths of less than 10 m. Regrettably, small streams in Brazil are not included in the river monitoring programs of the National Water Agency (ANA), and there is a limited understanding of the water quality of many small rivers, along with a lack of knowledge regarding pollution sources (Cantonati *et al.*, 2012; Figueiredo and Green, 2019).

The quality of river water is directly impacted by the surrounding environment and is influenced by activities within their watersheds. Anthropogenic, geomorphological, biogeochemical, and ecological factors collectively shape water quality and can lead to changes in bed sediment composition and aquatic fauna (Allan, 2004; Poole, 2010). Conversion of forested landscapes to pastures and agricultural areas often results in homogenization of riverbeds due to increased sedimentation, nutrient runoff, and loss of aquatic biodiversity (Allan *et al.*, 1997; Huang *et al.*, 2016). Intensive land use near rivers can disrupt natural processes, altering the longitudinal gradient of freshwater communities as described in the river continuum hypothesis (Vannote *et al.*, 1980; Verbos *et al.*, 2017).

Moreover, aquatic invertebrates play a crucial role in the food web, bridging the gap between primary producers (such as algae and periphyton) and vertebrates (including fish and other aquatic animals) (Bronmark, 1994). A reduction in their biodiversity can have cascading effects on the entire aquatic ecosystem, leading several species to extinction and potentially compromising the ecosystem functions of the aquatic environment (Cao *et al.*, 2018). Parreira de Castro *et al.* (2016) observed changes in the macrobenthic community in a Brazilian Cerrado watershed due to the removal of riparian vegetation in pasturelands, resulting in a shift from specific to more general food niches and increased trophic niche overlap. Given their critical role in reflecting both aquatic ecosystem health and the water supply for many cities in southeastern Brazil, we evaluated the composition and structure of the benthic community in three headwater areas of the Jaguari River Basin under dry and rainy seasons, different land uses and degrees of environmental preservation.

2. MATERIAL AND METHODS

2.1. Study area

In Southeast Brazil, the Jaguari River Basin stands as a vital water resource within the Cantareira Reservoir System, which serves the metropolitan area of São Paulo city. The headwaters of the Jaguari Watershed, nestled in the Serra da Mantiqueira region, have undergone significant land use changes over time. Originally dominated by the Atlantic Forest, these areas have seen extensive conversion to pasturelands for cattle ranching and, more recently, to forestry, primarily for Eucalyptus cultivation (Figueiredo *et al.*, 2020).

The Jaguari River Basin holds particular significance within the Cantareira System, contributing a substantial portion of its water production amounting (45%) with an average annual flow of 25 $m^3 s^{-1}$. Encompassing a total area of 103,243.4 ha, the basin's springs are situated in the municipalities of Camanducaia, Itapeva, Extrema, and Toledo, all located within the state of Minas Gerais (Whatley and Cunha, 2007). Climate of the study area is classified as Cwb, according to the Köppen system, which is characterized as mesothermic, featuring mild summers and cool winters. The average annual temperature is 18°C, with average maximum and minimum of 25.6 and 13.1°C, respectively. The annual average rainfall amounts to 1,477 mm (Antunes, 1986).

The sampling sites were strategically distributed across three small headwater catchments within the Jaguari River Basin, located in the southern region of the state of Minas Gerais, close to São Paulo state borders, as follows: (a) the Monte Verde Stream (MV) (22°51'35" S; 46°02'15" W) situated in a second-order river section draining an area of 767 ha in the district of Monte Verde, municipality of Camanducaia; (b) the Extrema Stream (EX) (22°51'17" S; 46°19'06" W) located in a second-order river section draining an area of 122 ha in the Ribeirão das Posse watershed, in the municipality of Extrema; and (c) the Toledo Stream (TO) (20°10'00" S; 41°49'00" W) positioned in a fluvial section of a first-order stream draining an area of 54 ha on a small farm, in the municipality of Toledo (Figure 1).

Land use and land cover change (LULCC) analysis consisted firstly in obtaining Enhanced Thematic Mapper Plus (ETM+) and Operational Land Imager (OLI) images from Landsat-7 and Landsat-8 data, respectively, available in the "Earth Explorer" catalog from the US Geological Survey (http://earthexplorer.usgs.gov/). The MV site is situated in an area predominantly covered by natural vegetation (90.8% forest). In contrast, the EX site's drainage area primarily consists of pastureland (74.3%), although it is undergoing a reforestation process targeting steep and riparian areas through an environmental services payment program. Lastly, the TO site represents the most degraded environment among the three studied areas; it is predominantly occupied by pastures (62.9%) and vegetable gardens (23.8%). Hence, there is a clear environmental gradient, starting from the most preserved site (MV), transitioning to an area undergoing forest recovery (EX), and, finally, to the most human altered catchment (TO).

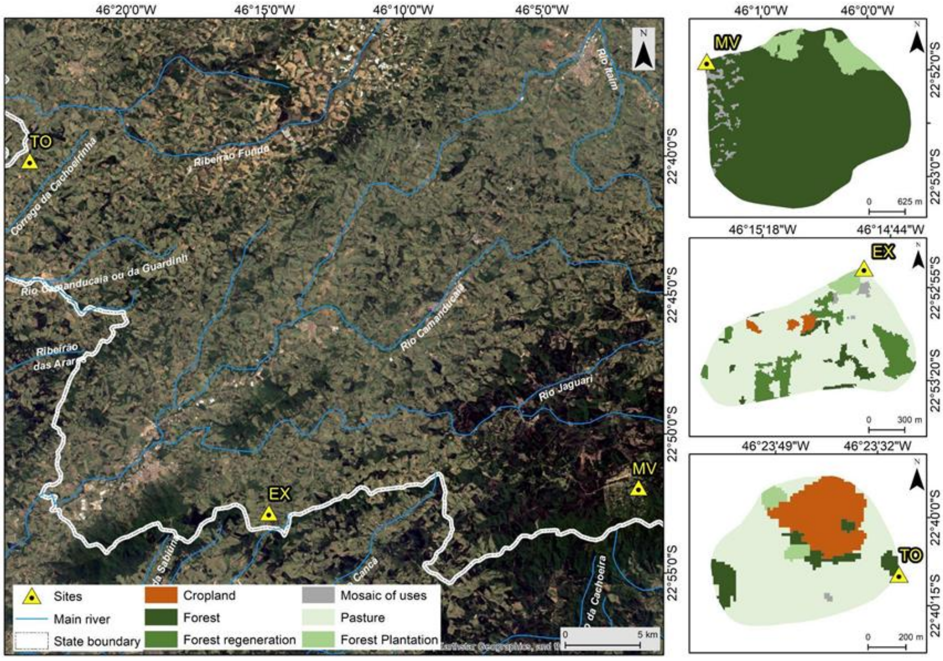

Figure 1. The location of the three studied sites (Monte Verde Stream – MV; Extrema Stream – EX and Toledo Stream – TO) and their respective land use-maps of the Jaguari River Watershed. White line represents the Minas Gerais and São Paulo states border. Maps by Gustavo Bayma.

2.2. Hydrogeochemical characterization

Using a multiparameter probe, we conducted field measurements of several physicochemical variables as dissolved oxygen, pH, electrical conductivity, and water temperature. These measurements were conducted monthly from March to November 2015, during the morning hours. Automatic measurements of these physicochemical variables were carried out once at each sampling point. The equipment itself is calibrated to establish average measurements during the period (1 to 2 minutes) that the sensor is immersed in the river bed. Additionally, as reported by Figueiredo *et al.* (2021), we collected a single sample at each sampling point using pre-washed sample bottles and stored them to take to the laboratory, at Embrapa Meio Ambiente, on the same day. No pseudo-replicates were made. The water samples were stored in a thermal box at approximately 4°C to maintain their integrity during transportation. In the laboratory, these samples were filtered through acetate cellulose membranes (0.45 µm pore size) and have measured the total suspended sediments (TSS) concentrations. After that samples were analyzed for the determination of the concentrations of some cations and anions, total nitrogen (TN) and dissolved organic and inorganic carbon (DOC and DIC). using ion chromatography and elemental analysis according to Figueiredo *et al.* (2021). The mean values of the hydrogeochemical variables at each stream were calculated considering all field campaigns.

2.3. Benthic macroinvertebrate sample and taxonomic identification

During the same monthly campaigns, we collected 20 sediment samples from a predefined 100-m stretch in each stream to represent the macroinvertebrate community of each site. We utilized a Surber sampler with a 250-µm mesh net to collect 20 pseudo-replicates, ensuring a diverse range of substrates such as stones, litter, and sand at each sampling site. Subsequently, all samples were carefully transferred to 1,300-L plastic pots and preserved in 80% alcohol. In the laboratory, the sediment samples underwent washing in sieves with a 250-µm mesh to remove coarse material and to conduct the initial screening of the organisms. Following washing, the substrate was re-immersed in 80% ethanol and placed on transilluminated trays.

The remaining material was meticulously examined under a stereomicroscope at 80× magnification for final screening and taxonomic identification to the lowest possible taxonomic level using Operational Taxonomic Units (OTU) classifications. Taxonomic identification followed established identification keys (Trivinho-Strixino and Strixino, 1995; Simone, 2009; Mugnai *et al.*, 2010). Unfortunately, the samples collected in August and October from the TO site were lost, presumably during storage in the laboratory.

2.4. Data analysis

We considered a variety of community metrics to assess the macroinvertebrate assemblage, including: total density (ind/m^2); the percentage of Ephemeroptera, Plecoptera, and Trichoptera (% EPT); the EPT/Chironomidae ratio; the Shannon–Wiener diversity index (H'); the Pielou evenness index (J); total richness (S); and Simpson dominance. Additionally, we utilized two biotic indices—Biological Monitoring Working Party (BMWP) and average score per taxon (ASPT)—to further evaluate differences among sampling sites. We calculated and analyzed these metrics and indices by using box-whisker charts. Then, we compared the means of these values in each catchment and season by using the Kruskal–Wallis test with Bonferroni adjustment, as described by De Mendiburu (2023).

We conducted hierarchical cluster analysis to assess the similarity between the seasons in different catchments based on environmental and biological data. We employed the Bray–Curtis distance index and the unweighted pair group method with arithmetic mean (UPGMA) technique as the agglomeration method. Moreover, we conducted permutational multivariate analysis of variance (PERMANOVA) using the Bray–Curtis distance index to evaluate the variability of benthic fauna in relation to seasons across different catchments. Finally, we performed principal component analysis (PCA) to investigate the environmental variables (hydrogeochemical variables) that best explained the variation of water quality among sites, as well as for macroinvertebrate taxa and their distribution among sample sites. We used the R software (R Core Team, 2023) for all analyses, with a significance level set to 5%.

3. RESULTS AND DISCUSSION

The dissolved oxygen concentrations measured in both dry and wet seasons were within the acceptable limits of water quality defined established in national legislation by CONAMA for Class 1 rivers (CONAMA, 2005).

Elevated PO_4^{3-} concentrations were observed only at the TO site, indicating an environmental degradation, where significant nutrient sources from the nearby vegetable gardens are present. This inference is supported by the fact that we only detected PO_4^{3-} during the dry season at this location, suggesting a potential association with the use of phosphate fertilizers in the vegetable gardens. The higher concentrations of nitrate and total nitrogen in TO can corroborate this agriculture fertilization. Furthermore, increased cation concentrations (Na+, K+, Ca^{2+} and Mg^{2+}) at EX sites can be attributed to the predominant presence of pastures, where salt is often used in cattle feeding management. The provision of salt leads to increased input of these dissolved cations into the stream bed, thereby contributing to elevated levels (Figueiredo *et al.*, 2020) (Table 1).

Table 1. Mean and standard deviation for hydrogeochemical parameters of stream sites in the Jaguari River Basin, Southeastern Brazil. EX = Extrema; MV = Monte Verde; TO = Toledo; BDL = below the detection limit; TSS = total suspended solids; TH20 = water temperature; %DO = percentage of dissolved oxygen; DO = dissolved oxygen; EC = electrical conductivity; PO4-3 = phosphate; NH4+ = ammonium; NO2- = nitrite; NO3- = nitrate; Ca2+ = calcium; Mg2+ = magnesium; K+ = potassium; Cl- = chlorine; Na+ = sodium; TN = total nitrogen; DBO = biochemical oxygen demand; DOC = dissolved organic carbon; DIC = dissolved inorganic carbon.

Parameter	EX Wet	EX Dry	MV Wet	MV Dry	TO Wet	TO Dry
TSS (mg L^{-1})	26.63 ± 24.92	85.28 ± 74.05	7.43 ± 1.63	51.31 ± 67.68	5.06 ± 3.71	11.60 ± 8.60
T H2O (°C)	20.50 ± 0.95	15.07 ± 0.81	15.43 ±1.68	11.85 ± 1.01	21.65 ± 3.07	18.00 ± 0.18
% DO	76.25 ± 3.30	77.25 ± 6.18	86.6 ± 4.40	80.75 ± 2.22	83.87 ± 4.85	88.00 ± 6.68
DO (mg L^{-1})	6.64 ± 0.50	7.72 ± 0.42	8.59 ± 0.43	8.57 ± 0.11	7.40 ± 0.60	8.40 ± 0.56
EC (µS cm^{-1})	68.22 ± 5.64	67.08 ± 3.65	16.93 ± 0.21	14.62 ± 1.31	28.82 ± 3.43	24.52 ± 2.07
pH	6.51 ± 0.41	6.88 ± 0.12	6.46 ± 0.09	7.04 ± 0.25	6.53 ± 0.48	6.64 ± 0.44
PO_4^{3-} (µM)	BDL	BDL	BDL	BDL	BDL	1.065
NH_4^+ (µM)	0.41 ± 0.15	0.39 ± 0.13	0.957 ± 1.747	0.02 ± 0.04	0.842 ± 1.25	0.44 ± 0.01
NO_2^- (µM)	BDL	BDL	BDL	0.10 ± 0.21	BDL	0.10 ± 0.19
NO_3^- (µM)	1.10 ±	3.50 ± 2,82	4.66 ± 0,54	3.93 ± 0,85	5,25 ± 0,44	5,10 ± 1,06
Ca^{2+} (µM)	45.60 ± 21.20	48.25 ± 13.92	3.45 ± 0.26	2.11 ± 0.86	4.00 ± 0.64	3.61 ± 0.94
Mg^{2+} (µM)	28.47 ± 9.73	27.51 ± 11.43	1.47 ± 0.44	5.11 ± 7.35	7.70 ± 9.97	6.46 ± 7.45
K (µM)	43.61 ± 20.07	51.27 ± 13.10	16.68 ± 6.34	17.31 ± 7.75	40.51 ± 17.56	33.57 ± 7.78
Cl (µM)	38,03 ± 14,18	48,64 ± 19,80	16,33 ± 3,34	14,34 ± 5,43	41,19 ± 9,67	31,81 ± 7,80
Na (µM)	140,84 ± 85,78	196,3 ± 55,48	29,05 ± 16,57	32,99 ± 8,72	51,01 ± 26,47	52,73 ± 6,62
TN (µM)	17.85 ± 7.06	23.2 ± 8.25	23.30 ± 10.53	18.60 ± 11.83	29.31 ± 20.31	30.84 ± 28.21
DBO (mg L^{-1})	1.64 ± 0.71	1.42 ± 0.86	1.25 ± 0.64	1.49 ± 0.94	0.57 ± 0.15	0.54 ± 0.41
DOC (µM)	617 ± 518.00	480 ± 229.00	350 ± 123.65	281 ± 170.00	322.96 ± 158.47	304 ± 61.00
DIC (µM)	476 ± 87,00	443 ± 115,00	323 ± 55,00	345 ± 134,77	601,23 ± 107,55	573,38 ± 160,56

During the rainy season, the EX site presented the highest total suspended solid (TSS) levels compared with the other sites (Table 1). Besides that, the high electrical conductivity at EX site can likely be attributed to the presence of pastures, which in turn promote significant leaching of major cations, like Ca, Mg, Na and K, from the soil into the streams. The elevated TSS levels observed at EX site aligns with previous findings reported by Figueiredo *et al.* (2011) and suggests a potential association between pasturelands in steep areas and elevated TSS levels. Although the municipality of EX has implemented environmental policies and reforestation efforts in riparian forests, we found slightly high concentrations of NH4+ and important high values of EC as shown in Table 1. Figueiredo *et al.* (2021) also highlighted these increases and attributed them to occasional signals of point sources of pollution at the catchment outlet on seemingly random dates and times.

These findings suggest that the initial recovery of riparian forest in the EX area is still insufficient to effectively filter soil nutrient loads from the slopes occupied by pastures, thereby impacting the water quality of the studied stream. This underscores the importance of ongoing efforts to restore riparian vegetation and mitigate the adverse effects of agricultural activities on stream ecosystems.

From March to November, we sampled a total of 39,637 macroinvertebrate individuals, representing 84 OTUs (as detailed in the Supplementary Material). Among these, the five taxa with the highest relative abundances were Chironomidae (43,02%), Simuliidae (17.86%), Hyallelidae (6.19%), Elmidae (6.02%), and Leptoceridae (3.76%). The complete list of collected macroinvertebrates is deposited in the REDAPE database, available at http://doi.org/10.48432/DVFMD8 (Silva and Sonoda, 2015).

The dominance of Chironomidae is consistent with the findings reported by Rezende *et al.* (2022), who noted a similar pattern in both natural and agricultural streams. The prevalence of Chironomidae in impacted locations can be attributed not only to its status as one of the most abundant groups of aquatic insects (Pio *et al.*, 2020; Monteles *et al.*, 2021), but also to its generalist feeding habits. Nevertheless, Sonoda *et al.* (2009; 2018) observed different proportions of functional feeding groups for Chironomidae genera between agricultural and forest areas, where the forest areas presented more shredders than the agricultural areas. According to Sonoda *et al.* (2009), three genera of midge genera (*Rheotanytarsus* spp. (filter-feeder), *Nanocladius* spp. and *Corynoneura* spp. (collector-gatherers) were more abundant at the pasture than at the forest reaches.

Table 2 displays the average values for the macrobenthic metrics across the study sites. The orders of EPT aquatic insects were more abundant at the MV site, followed by the EX and TO sites. The Shannon Diversity Index (H’) was also higher at Monte Verde (MV). Similarly, the total number of individuals was higher at the MV site, along with metrics of diversity, equitability, and the BMWP and BMWP/ASPT biotic indices.

Box-plot analysis revealed that key metrics such as Dominance, % EPT (EPT_p), EPT/Chironomidae, the Shannon–Wiener Diversity Index (H') and the biotic index ASPT were significantly different between sites and seasons, revealing a clear environmental gradient, with the MV site appearing as the most preserved and the TO site as the most degraded. The BMWP and ASPT indices corroborated this pattern, although the BMWP index did not show a significant difference, and the MV site exhibited the highest values for both seasons, as well as for taxonomic richness (S), number of individuals, Margalef richness and Pielou index. Interestingly, although the TO site is considered to be the most impacted, it had a higher BMWP and ASPT scores than the EX site (Figure 2).

Table 2. Metrics of the benthic community at stream sites in the Jaguari River Basin, Southeastern Brazil. Density = number of individuals per area; % EPT = percentage of Ephemeroptera, Plecoptera, and Trichoptera; EPT/Chiro = EPT Chironomidae rate; BMWP = Biological Monitoring Working Party index; ASPT = Average Score Per Taxon; S = taxa richness; Ind = number of individuals; Dom_D = dominance index; H'= Shannon–Wiener Diversity Index; J = Pielou index.

Site Season	MV		EX		TO	
	Dry	Wet	Dry	Wet	Dry	Wet
Density (ind/m^2)	13.38	14.57	13.38	3.11	3.55	3.96
% EPT	23.09	26.97	9.5	10.2	10.12	6.33
EPT/Chiro	0.58	0.72	0.29	0.19	0.17	0.09
BMWP	288	277	246	213	282	219
ASPT	6	6.16	5.59	5.61	5.88	5.76
S	59	53	52	45	62	48
Ind	12038	13109	6735	2795	3191	3568
Dom_D	0.2	0.19	0.28	0.34	0.38	0.52
H'	2.32	2.27	1.76	1.8	1.85	1.3
J	0.57	0.57	0.45	0.47	0.45	0.34

The differences in environmental integrity among the three study sites, as observed at De Mello *et al.* (2018), shows that land uses for pasture and agriculture significantly reduced environmental quality in tributaries of the Tietê River. Overall, these results underscore the critical role of riparian vegetation in maintaining stream ecosystem health and highlight the negative impacts of certain land-use practices on water quality and benthic community composition.

Organism density was notably higher at the MV site compared with the other locations. Taxonomic richness was higher during the dry season across all three sites, with the TO site exhibiting the largest number of taxa. Considering macrobenthic metrics, the greatest taxonomic richness at TO site was unexpected, given that we considered this site to be the most impacted among the study locations. Sonoda *et al.* (2017) observed a greater number of sensitive families, including families belonging to the main orders of sensitive EPT aquatic insects, at the site with the greatest environmental integrity (MV) compared with other sites. Additionally, they noted the absence of families considered tolerant to degradation, such as Hirudinea and Oligochaeta, at the MV site. This suggests that the MV site, with greater environmental integrity, may support a more diverse and sensitive macroinvertebrate community compared with other sites. Nessimian *et al.* (2008) investigated the effects of deforestation on Amazonian streams and found that microhabitats in streams were closely related to land use. They reported significantly lower richness of EPT families in streams impacted by pastures, as well as a distinct community composition compared with reference forest streams. This underscores the importance of considering land use and habitat integrity when assessing the ecological health of stream ecosystems, as different land uses can have significant impacts on macroinvertebrate communities and overall stream biodiversity.

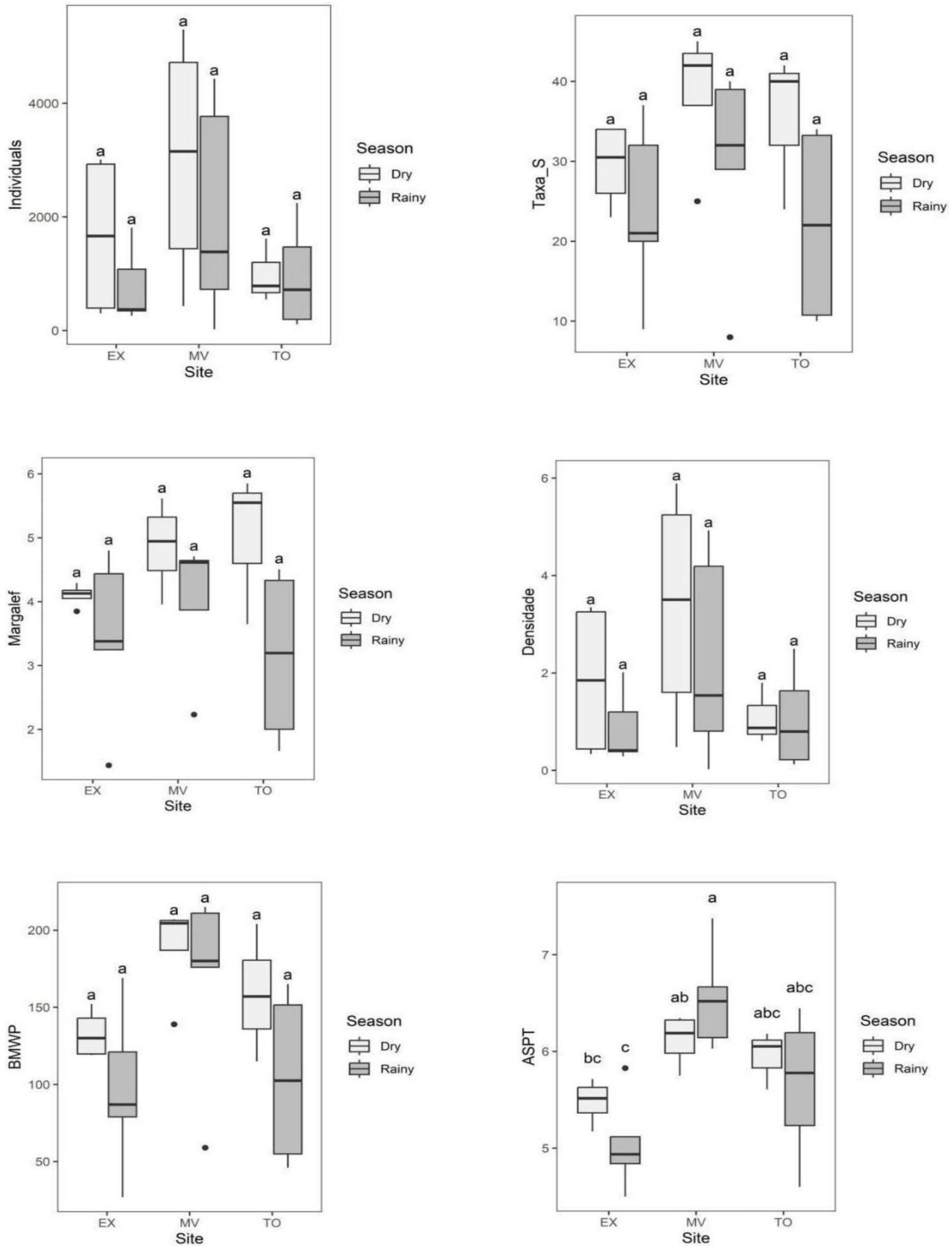

Figure 2. Boxplots of the ecological descriptors for macroinvertebrate assemblages at each site (EX = Extrema; MV = Monte Verde; TO = Toledo) during the rainy and dry seasons. The center line in the box displays the median, and the margins of the box specify the 25th and 75th percentiles. The whiskers extend to the smallest (lower whisker) or the largest (upper whisker) value within the range of 1.5 times the interquartile range.

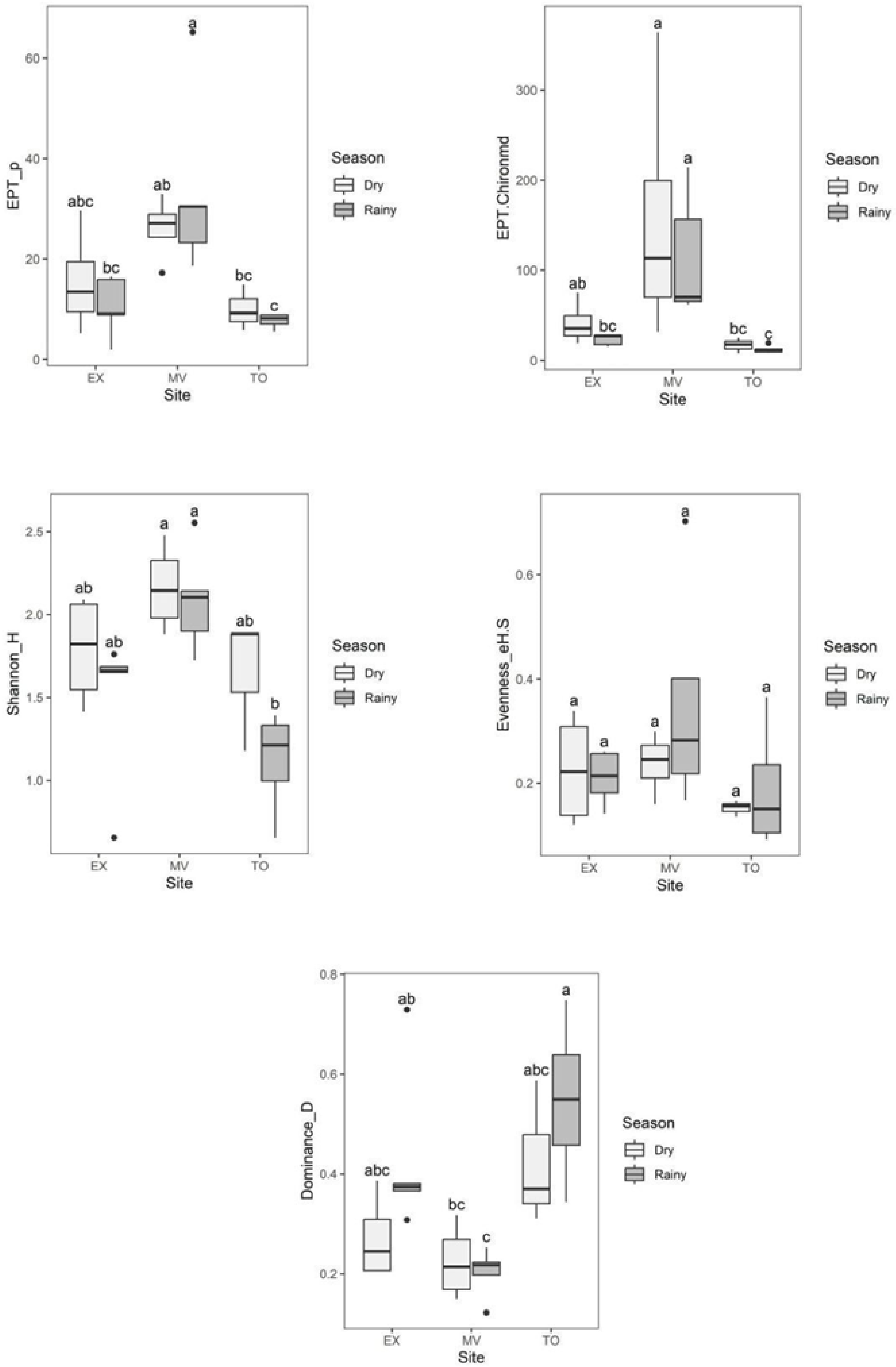

Figure 2 (continued). Boxplots of the ecological descriptors for macroinvertebrate assemblages at each site (EX = Extrema; MV = Monte Verde; TO = Toledo) during the rainy and dry seasons. The center line in the box displays the median, and the margins of the box specify the 25th and 75th percentiles. The whiskers extend to the smallest (lower whisker) or the largest (upper whisker) value within the range of 1.5 times the interquartile range.

It is known that for tropical streams, in general, higher taxa richness and higher relative abundance of macroinvertebrates are observed during the dry season than during the rainy season (Sun *et al.*, 2024). When comparing the dry and rainy seasons, we observed that, in general, the highest % EPT occurred during the dry season. However, there were exceptions, such as dominance at the TO site and % EPT at the MV site, where it was observed to have equivalent values for both seasons. Contrary to these findings, Hepp and Santos (2009) reported a lower number of families during the dry season. Nevertheless, other studies have recommended sampling during the dry season due to the environmental stability it offers, which promotes better structuring of benthic communities. During periods of drought, the environment tends to be more stable, facilitating the establishment and persistence of benthic organisms. In contrast, wet seasons are characterized by increased precipitation, resulting in higher flow rates in streams. This can lead to the displacement of many organisms by the current, affecting benthic community composition and diversity (Barbero *et al.*, 2013). In headwater streams, where environmental conditions are particularly sensitive to changes in flow and precipitation, this effect is especially relevant.

The families that were exclusive to the Monte Verde Stream were: Helicopsychidae, Phylopotamidae, Glossosomatidae, Atriplectidae, Euthyplociidae, and Polymitarcyidae. These EPT families, with the exception of Polymitarcyidae, are demanding in terms of good water oxygenation. The high % EPT observed at the MV site can likely be attributed to the good habitat conditions and preservation of the site. These orders of aquatic insects typically prefer habitats with strong currents and substrate from riparian forests, which provide shelter and food resources. As a result, the abundance of EPT individuals at the MV site suggests good environmental quality and habitat integrity. Similar findings have been reported in other studies (Amaral *et al.*, 2015; Ferreira *et al.*, 2021), further supporting the association between EPT abundance and habitat preservation. While we also observed differences between the sites for the other metrics, they were not as pronounced as the differences in % EPT. Nonetheless, these metrics still provide valuable insights into benthic community composition and environmental quality across the study sites. Overall, the high % EPT at the MV site underscores the importance of habitat preservation and conservation efforts in maintaining healthy stream ecosystems. These findings highlight the need for continuous monitoring and conservation initiatives to safeguard aquatic biodiversity and ecosystem health in headwater streams.

The cluster analysis revealed the formation of distinct groups based on sites rather than seasons. This pattern was particularly evident for the hydrogeochemical parameters, where samples from the same location clustered together regardless of whether they were collected during the dry or rainy season. Similarly, for the benthic macroinvertebrate communities, the clustering pattern was primarily determined by site, with the EX and MV streams showing greater similarity to each other compared with the TO Stream (Figure 3).

These results suggest that site-specific factors, such as habitat characteristics, land use, and local environmental conditions, may have a more significant influence on both environmental parameters and benthic macroinvertebrate communities than seasonal variations. This can facilitate the implementation of water quality monitoring programs at any time of the year.

The separation between the most preserved site (MV) and impacted sites (EX and TO) is evident in the first axis of Principal Component Analysis (PCA). The PCA highlighted two hydrogeochemical variables that significantly influenced the distribution of the data: dissolved inorganic carbon (DIC) and electrical conductivity (EC). Specifically, we observed higher water temperatures and DIC concentrations at TO site and higher electrical conductivity values at EX site, being the hydrogeochemical parameters best explaining the variance in the data. At the MV site, it seems that pH and DO were separated from the other variables precisely because land use would not be affecting their values. Thus, its position in the PCA quadrant does not refer to TO, but to other hydrogeochemical variables. (Figure 4).

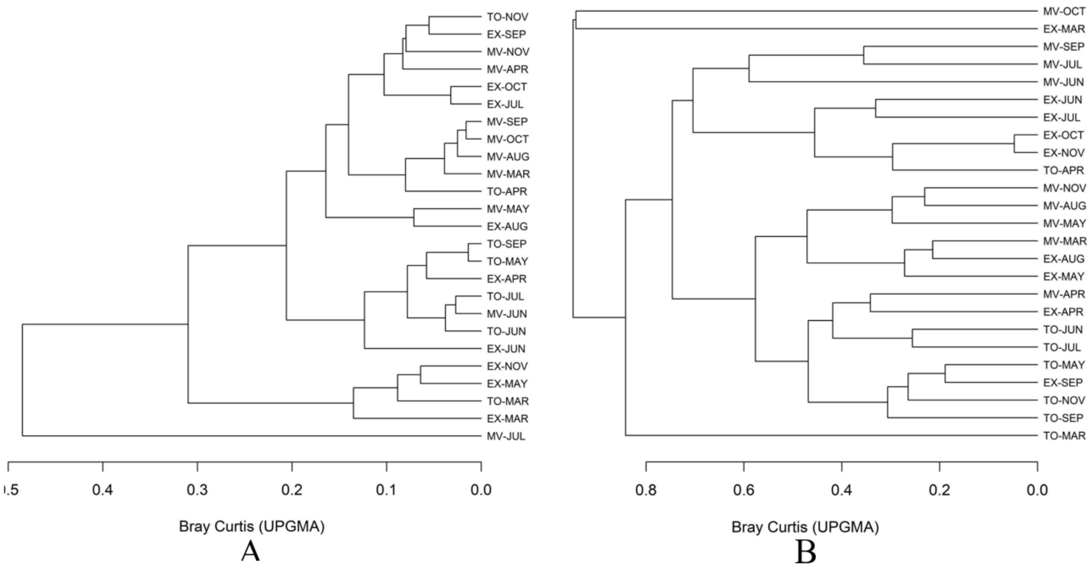

Figure 3. Cluster analysis based on abiotic variables (A) and macroinvertebrate densities (B), using Bray–Curtis index, in the Jaguari River Basin in 2015. EX = Extrema; MV = Monte Verde; TO = Toledo; UPGMA = unweighted pair group method with arithmetic mean.

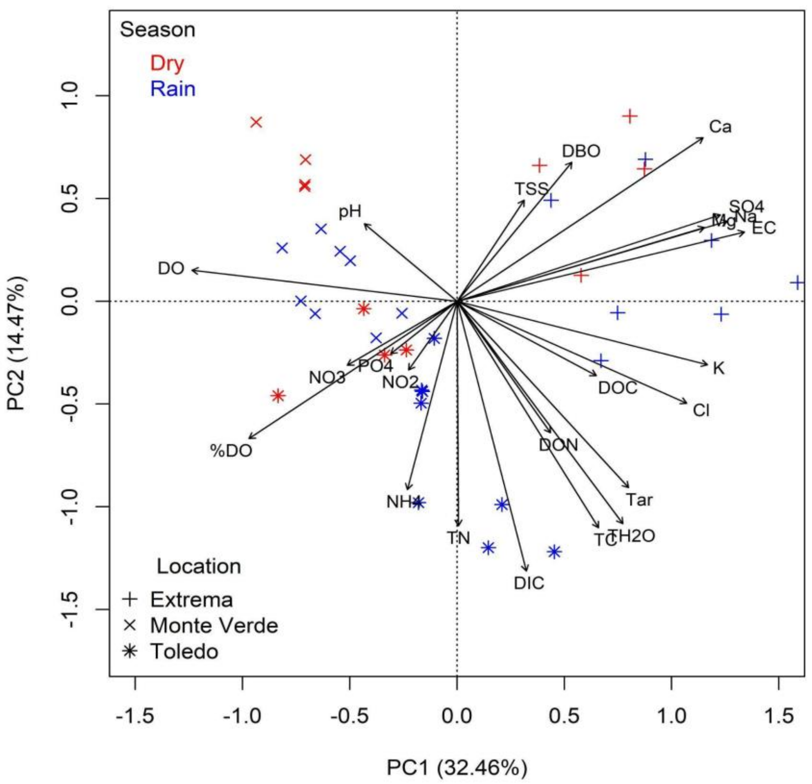

Figure 4. Principal Component Analysis (PCA) diagram showing the ordination of hydrogeochemical variables along the first two axes. EX = Extrema; MV = Monte Verde; TO = Toledo. pH - hydrogen ion potential; DO – dissolved oxygen; %DO – percentage of dissolved oxygen; DBO – biochemical demand of oxygen; NO_3 – nitrate; NO_2 – nitrite; NH_4 – ammonium; PO_4 – organic phosphate; TN – total nitrogen; TC – total nitrogen; DIC – dissolved inorganic carbon; DOC – dissolved organic carbon; DON – dissolve organic nitrogen; Tar: air temperature; TH20 – water temperature; Cl – chloride; K – potassium; Mg – magnesium; Ca – calcium; NA – sodium; EC - electric conductivity; SO_4 – sulfate; TSS – total suspended solids.

Considering the macroinvertebrate community, the PCA suggested that taxa associated with Toledo were: Hirudinea in the rainy season and Bivalvia in the dry season; Elmidae and Psephenidae were related to MV site at the rainy season, and Perlidae at dry season; and for EX site, Oligochaeta was the most associated taxon, especially at the rainy season (Figure 5). Other taxa (not mentioned in the caption of Figure 5), located in the middle of the graph, did not contribute (or contributed very little) to the explanation of the macroinvertebrate ordination.

In general, taxa more tolerant to environmental degradation were in fact found in the most disturbed places on the gradient, such as Toledo (TO) and, to a lesser extent, Extrema (EX). Perlidae is a family of aquatic insects representative of waters of good quality and oxygenation, therefore, their presence and abundance in Monte Verde was expected. On the other hand, Hirudinea, Bivalvia and Oligochaeta are organisms tolerant to higher concentrations of organic matter and suspended solids, in accordance with what was observed for the PCA of limnological variables (Xu *et al.*, 2014).

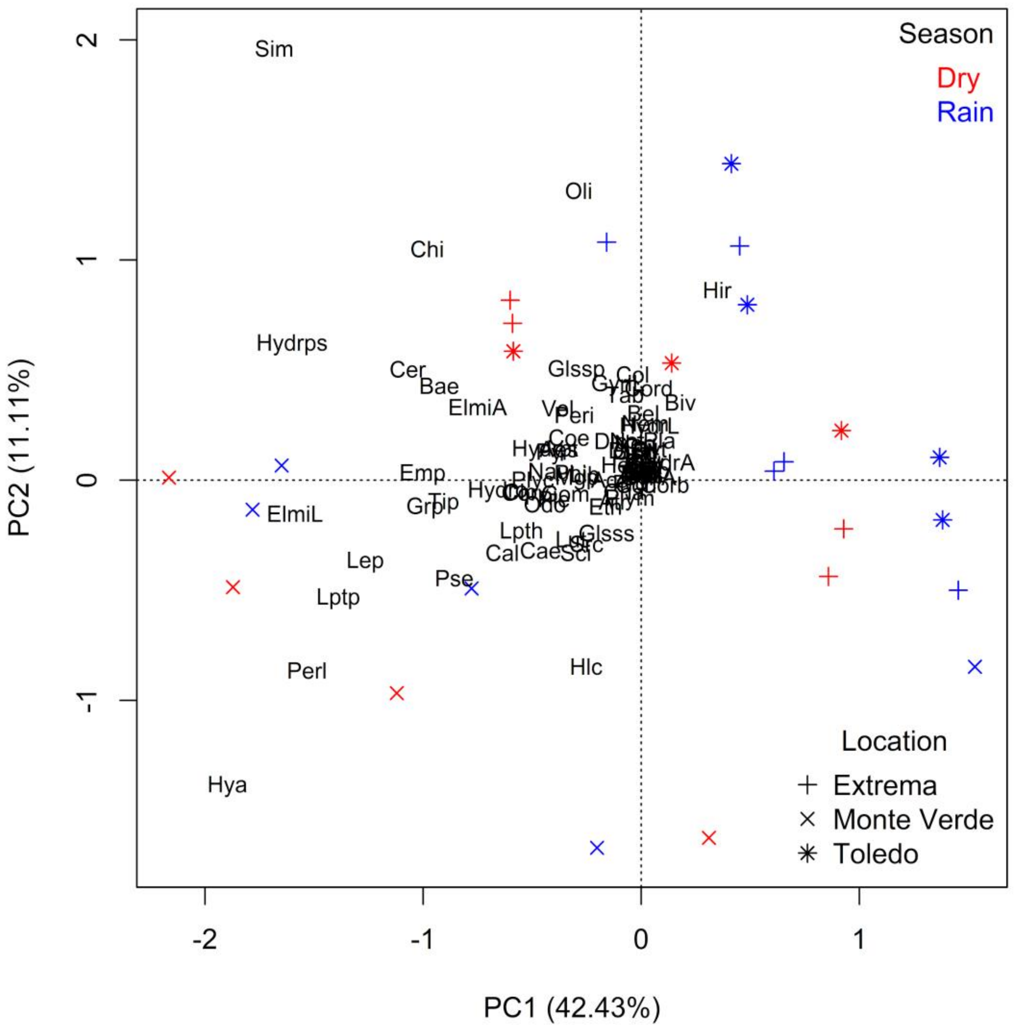

Figure 5. Principal Component Analysis (PCA) diagram showing the ordination of macroinvertebrate taxa along the first two axes. EX = Extrema; MV = Monte Verde; TO = Toledo. Sim - Simulidae; Oli – Oligochaeta; Chi – Chironomidae; Hydrps – Hydropsychidae; Bae – Baetidae; ElmiA – adult Elmidae; ElmiL – larvae Elmidae; Lep – Leptoceridae; Pse – Psephenidae; Hya – Hyalellidae; Perl – Perlidae; Glssp – Glossiphonidae; Emp – Empididae; Grp – Gripopterygidae; Hir – Hirudinea; Biv – Bivalvia; Cer – Ceratopogonidae; Hlc – Helicopsychidae; Coe – Coenagrionidae; Lpth – Leptophlebidae.

4. CONCLUSIONS

In the present study, metrics based on the presence/absence of families, such as the biotic indices BMWP and ASPT, and richness of taxa (Taxa_S), were preferable because they are easier to calculate, and take less time to obtain, since they do not require counting individuals. Choosing metrics based on the presence/absence of families, which are easier to calculate, is preferable for extensive monitoring programs with numerous sampling sites, as well as to guide hydrogeochemical management decisions. The macroinvertebrate assemblage in the Jaguari River Basin corroborated the environmental gradient we observed based on the environmental parameters. Many metrics reflected an increase in the density of tolerant taxa in impacted environments, characterized by little riparian forest or the presence of unsustainable agriculture near water bodies. Conversely, preserved sites such as MV exhibited greater density and diversity of sensitive groups. To address these findings, conservation practices should be implemented to restore riparian forest and to mitigate the input of nutrients and sediments into the headwater streams of the Jaguari River Basin. These measures should aim to facilitate the recovery of benthic fauna composition and to promote overall ecosystem health.

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