

# Influence of vegetation cover on the functional diversity of macroinvertebrates associated with leaf litter in a stream of the tropical dry forest

## Influencia de la cobertura vegetal en la diversidad funcional de macroinvertebrados asociados a la hojarasca en un arroyo del bosque seco tropical

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### Abstract

Vegetation is a fundamental element for the maintenance of macroinvertebrates functional diversity and, therefore, essential to aquatic trophic net preservation, being particularly relevant in threatened ecosystems such as tropical dry forests. Our aim is to determine the relationship between vegetal cover conservation and macroinvertebrates functional diversity associated with litter, in a stream from a tropical dry forest. In La Avería stream (Huila, Colombia), we sampled sites with different vegetation cover, estimated their physicochemical variables, and calculated leaf litter inputs (vertical, side, and drift) for each station for a whole year. Subsequently, we recorded the macroinvertebrates associated with litter from common vegetal species using leaf traps. We performed a PERMANOVA, added to multiple linear regression models and a redundancy analysis to correlate our environmental variables with changes in functional diversity. We observed changes in the functional parameters related to sampling zones and time. On one hand, functional richness and distance, are associated with dry seasons when resource availability and environmental conditions are stabilized. On the other hand, the functional evenness value diminishes in areas with higher pH due to the loss of individuals. We found evidence of a relationship between physicochemical variables and functional diversity. pH and precipitation changes were directly associated with changes in litter supply and therefore define the dimensionality of the functional traits in the ecosystem. Our work emphasizes the idea that the macroinvertebrate diversity carries great potential as a tool for decision-making in the preservation and environmental management of aquatic and riparian systems.

**Keywords:** conservation, Colombia, functional richness, Huila, QBR index, tree cover

### Resumen

La vegetación es fundamental para el mantenimiento de la diversidad funcional de macroinvertebrados y, esencial para la preservación de la red trófica acuática, siendo relevante en ambientes amenazados como bosques secos tropicales. Nuestro objetivo fue establecer la relación entre la conservación de la cobertura vegetal y la diversidad funcional de macroinvertebrados asociados a hojarasca, en una quebrada de bosque seco tropical. En la quebrada La Avería (Huila,

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Colombia), muestreamos sitios con diferentes coberturas vegetales y variables fisicoquímicas, y calculamos los aportes de hojarasca (vertical, lateral y deriva) para cada estación durante todo un año. Posteriormente, registramos los macroinvertebrados utilizando trampas de hojas. Realizamos un PERMANOVA, sumado a un modelo de múltiples regresiones lineales y un análisis de redundancia para correlacionar nuestras variables ambientales con los cambios en la diversidad funcional. Se observaron cambios en los parámetros funcionales relacionados con zonas de muestreo y tiempo; la riqueza y distancia funcional se asociaron a épocas secas cuando la disponibilidad de recursos y las condiciones ambientales se estabilizan y el valor de uniformidad funcional disminuyó en las zonas de mayor pH debido a la pérdida de individuos. Adicionalmente, se observó que los cambios de pH y precipitación están directamente asociados con los cambios en el suministro de hojarasca y, definen la dimensionalidad de los rasgos funcionales en el ecosistema. Nuestro trabajo enfatiza la idea de que la diversidad de macroinvertebrados conlleva un gran potencial como herramienta para la toma de decisiones en la conservación y gestión ambiental de sistemas acuáticos y riparios.

**Palabras clave:** cobertura arbórea, conservación, Colombia, Huila, índice QBR, riqueza funcional

## INTRODUCTION

Tropical Dry Forest (TDF) are environments located in altitudes lower than 1,200 m.a.s.l., with an annual precipitation lower than 2,500 mm, and fertile soils that promote an extensive tree cover (DRYFLOR, 2016; Halffter, 1992). Dry forests also have low resilience to perturbations that, added to their longer dry seasons, cause them to change into xerophytic areas when human disturbance is high (Pennington et al., 2000). Currently, according to the Alexander von Humboldt Institute, more than 90% of the TDF tree cover areas have been modified in Colombia. Additionally, the National System of Protected Areas records that less than 5% of the dry forest is located in some type of protected area, being the most threatened environment in Colombia (Linares & Fandiño, 2009; Pizano & Garcia, 2014). Due to the above, the environmental protection projects for the TDF have focused on the conservation of the hydric and riparian ecosystems to promote dry forest restoration. Nevertheless, these ecosystems have deteriorated due to demographic and agricultural growth in recent years (Melo et al., 2017; Romero-Duque et al., 2019). For this reason, recent studies have highlighted the quality of the riparian habitat as a key element in ecosystem conservation (Galeano Rendón et al., 2017; Rincón et al., 2017). Thus, it is an important variable to consider when defining the restoration processes of aquatic environments (Acosta et al., 2009; Suárez et al., 2002).

Riparian habitats are defined as the vegetation that develops in proximity to a stream and therefore has a strong connection to it (Granados-Sanchez et al., 2006). The riparian vegetation has multiple

functions, including its value as a buffer against external conditions that stabilize the hydrological and physicochemical characteristics of the stream (Gregory et al., 1991; Lowrance et al., 1997). The riparian vegetation also serves as a shield that limits the impact of solar radiation on the stream, since the riparian vegetation is mostly composed of trees (Cory et al., 2014; Granados-Sanchez et al., 2006; Kibichii et al., 2007; Suárez et al., 2002). The importance of the riparian forest focuses on the energy and nutrient input that it provides to the aquatic ecosystem. This directly influences changes in the dynamics of trophic networks and the assembly of organisms in the system (Fajardo et al., 2000; Moulton & Wantzen, 2006; Munné et al., 2003; Segura et al., 2003). Changes in the riparian forest can also affect aquatic biota (Valle et al., 2013; Wilkins et al., 2015). In the case of macroinvertebrates, the disappearance of forest cover and replacement of native species reduces the input of organic matter and supply of available resources, which generates variations in the community functionality (Berger et al., 2018; Kominoski et al., 2013). These variations in the aquatic organisms dynamics are related to the state of conservation of the ecosystem, especially in threatened environments (Casotti et al., 2015; Classen-Rodríguez et al., 2019).

The functional value of macroinvertebrates for the ecosystem is described by biological traits that express their ability to adapt to environmental conditions (Pearson et al., 2017; Tomanova et al., 2006). Therefore, it is important to highlight that functional diversity (FD) is understood quantitatively, based on the value of their traits, measured according to their role in ecological

processes (Boersma et al., 2016). The FD measures are made from its components: functional richness (Fric), which is the amount of space of each functional trait that is occupied by each species; the functional distance (Fdis) which is understood as the species abundance distribution that maximizes the functional traits divergence; and functional evenness (Feve) explained as the homogeneity in the abundance distribution of functional traits (Cordova-Tapia & Zambrano, 2015; Mason & De Bello, 2013).

Observations of functional diversity are a specific methodology to understand how the organisms dynamics are related to the ecosystem conservation state, especially in threatened environments (Casotti et al., 2015; Classen-Rodríguez et al., 2019). In recent years, the conservation of aquatic and riparian systems has been promoted as the central axis of regional projects for TDF restoration in Colombia (Galeano Rendón et al., 2017; Rincón et al., 2017). However, despite its relevance, the specific effects of modifications in the riparian system on the macroinvertebrates functional diversity have been poorly studied. Therefore, in this paper, we establish this research question: What is the relationship between the state of riparian cover forest conservation and the macroinvertebrates functional diversity associated with litter, in a stream located within a remnant of a Tropical Dry Forest? We propose the following hypotheses: (1) the diversity of leaf litter species is related to macroinvertebrates functional diversity, and (2) changes in riparian and stream quality are related to variations in macroinvertebrates functional diversity.

## MATERIALS AND METHODS

### Study area

The study area is located in the municipality of Paicol in the department of Huila, within a remnant of tropical dry forest in the hydrographic basin of the Upper Magdalena River Valley from Colombia. This region registers an average rainfall of 1469 mm per year, temperatures between 19 and 25 °C, and a monomodal regime with a rainy season that goes from November to March, an intermediate season between April to July, and a dry season from August to October (IDEAM, 2013). The study was carried out in “La Avería” stream, which is a third-order fluvial environment belonging to

the Huila western area. This stream arises at an altitude of 922 m.a.s.l., within a conserved remnant of the riparian forest with a rock-type substrate, and covers a length of approximately 4.5 km through areas where the forest has been disturbed by urban dumping, rice crops, and cattle ranching with sand and leaf litter substrates to finally flow into the Páez River at an altitude of 805 m.a.s.l. We defined three sampling zones along the stream according to changes in riparian vegetation: 1) The upper zone (2° 26' 22.3" N 75° 46' 31.4" W, 922 m.a.s.l.) corresponds to the area where the native forest is still preserved, dominated by *Zygia longifolia* (Willd.) Britton and Rose. 2) The middle zone (2° 26' 31.8" N 75° 46' 5.9" W, 813 m.a.s.l.) is located in a sector where the riparian cover disappears as a consequence of the presence of crops and wastewater discharges from the nearby urban center. 3) The lower zone (2° 26' 57.2" N 75° 45' 24.6" W, 805 m.a.s.l.) is a riparian forest dominated by *Guazuma ulmifolia* Lam. with the presence of livestock in the fluvial system surroundings. We established an approximate distance of 1.5 km between each sampling zone. At the same time, and taking into account the rain regime, we established three sampling seasons: the dry season (July to September 2020), the intermediate season (November 2020 to January 2021), and the rainy season (March to May 2021).

### Environmental characterization

The climatic data for the study area—precipitation (mm) and temperature (°C)—were taken from the records of the meteorological stations belonging to the Institute of Hydrology, Meteorology and Environmental Studies (IDEAM), located in the Paicol urban area and the Páez river. Stream physicochemical and hydrological characteristics were recorded in triplicate with a HANNA HI98194® multiparameter probe: conductivity ( $\mu\text{S cm}^{-1}$ ), dissolved oxygen ( $\text{mg l}^{-1}$ ), total suspended solids ( $\text{mg l}^{-1}$ ), pH, and water temperature (°C). We also measured the flow of the stream ( $\text{m}^3 \text{s}^{-1}$ ) according to the method proposed by Eloegi and Sabater (2009), which multiplies depth (m), width (m), and water velocity ( $\text{m s}^{-1}$ ), measured using a flowmeter JDC Electronics 94356 FS. We determined the vegetation cover in each station by quantifying the state of conservation of the riparian forest with the QBR (*qualitat del bosc de ribera*) index adapted for Colombia. At each station, we made 10 transects of 30 m each (five on

each side of the stream) to average the values and interpret our data with theoretical levels (Galeano Rendón et al., 2017; Munné et al., 2003; Posada & Arroyave, 2015; Suárez et al., 2002). Additionally, in the same transects used for the QBR index, we recorded the input of solar radiation ( $W\ m^{-2}$ ) with a LI-COR LI-210R® lux meter. We also calculated the density of the forest cover using a spherical densitometer according to the methodology of Lemmon (1956).

### Organic matter inputs

The contribution of organic matter was determined from vertical inputs (fall), lateral inputs (on the stream edges), and drift inputs, following the method of Elozegi and Sabater (2009). Three leaf litter traps were installed at input entrance sites, distributed every 10 m within each sampling area. The catchment area of the vertical and lateral meshes was  $0.25\ m^2$ , while that of the drift meshes was  $0.045\ m^2$ . Each mesh had a pore size of 0.2 mm. To analyze the vertical and lateral samples, we collected material twice for each sampling season at 31 and 63 days, respectively. We collected material from the drift traps every 12 hours for 24 hours.

All organic material captured by the traps was processed according to the methodology set forth by Elozegi and Sabater (2009). The organic matter was taken to a desiccator oven at  $80\ ^\circ C$  for 72 h, then weighed on an analytical balance with a precision of 0.001 g. After recording weight, we calcined the litter in a muffle at  $120\ ^\circ C$  for four hours to obtain the Ash Free Dry Mass (AFDM) in mg. Finally, the vertical and lateral inputs of organic matter were calculated according to the following equation proposed by Elozegi and Sabater (2009):

$$E_{mo} = \frac{AFDM}{t * A}$$

Where  $E_{mo}$  is the density of organic material coming from the vertical or lateral inputs,  $t$  is the exposure time of the net in hours, and  $A$  is the area of the intake net mouth in  $m^2$ . For the analysis of organic material from drift, we used the equation of Benke et al. (1999):

$$Dd = \frac{AFDM}{t * vel * A_s}$$

Where  $Dd$  is the density of material coming from

the drift,  $t$  is the exposure time of the net in hours,  $vel$  is defined as the velocity of the water flow, and  $A_s$  is the submerged area of the net intake. We repeated the capture of organic matter from each type of trap three times for each station during each sampling season, seeking to measure variations according to the hydrological cycle of the region.

### Macroinvertebrates collect traits

We collected the macroinvertebrates using leaf litter package traps according to the methods in Elozegi and Sabater (2009). The litter was collected from three tree species: *Guadua angustifolia* (Kunth), *Guazuma ulmifolia* Lam. and *Zygia longifolia* (Willd.) Britton and Rose, which previous studies have shown as abundant species along the stream in the study area (Cuéllar-Cardozo et al., 2022). It is important to clarify that the plant material was directly collected from multiple trees located in the study area during the humid season, maintaining the leaves in a healthy and homogeneous state. This material was taken to the laboratory to be dried in an oven at  $40\ ^\circ C$  for 48 h, weighed (4.5 ~ 5.0 g), and enclosed into mesh bags (25 x 10 cm) with a 5 mm mesh opening. In total, 135 packages were placed throughout the experiment, 45 packages (15 per plant species) for each of the three sampling periods in each of the established stations. The organization of the packages within the stream was carried out randomly, choosing points in a map, covering the greatest possible heterogeneity in the environment and maintaining a minimum distance of 5 m between each one of them.

Subsequently, three leaf litter packages were removed on days 7, 14, 28, 56, and 63 after the experiment had elapsed in each of the stations and the macroinvertebrates were removed from the leaf litter and placed in 70% ethyl alcohol.

The categorization of functional traits was based on the method proposed by Rojas et al. (2020) (Table 1). The functional traits of biomass, head width, and body length were selected according to what was suggested by Erős et al. (2015) and Rojas et al. (2020). In the case of Mollusca specimens, the width and length of the shell were measured. The measurement of the morphometric variables was carried out on a scale with photographs of the specimens, employing the

software *ImageJ*® (Gonzalez, 2018). The biomass was determined according to Elozegi and Sabater (2009), Baumgärtner and Rothhaupt (2003), Benke et al., (1999); Burgherr and Meyer (1997) taking measurements of at least 30 individuals per morphotype. Finally, the Functional Feeding Groups (FFGs) selection was established according to the classification of Tomanova et al. (2006) and

was assigned according to the information provided by Aguiar et al. 2018; Bojsen and Jacobsen, 2003; Chará-Serna et al. 2012; Domínguez and Fernández, 2009; Ferreira et al. 2017; Iñiguez-Armijos et al. 2018; Lourenço-Amorim et al. 2014; R. W. Merritt et al. 2017; Miserendino and Masi, 2010; Mosele Tonin et al. 2018; Sonoda et al. 2018; Tomanova et al. 2007; Wantzen and Wagner, 2006.

**Table 1.** List of functional traits, with their respective categories and codes, considered for the present study. FFGs were defined according to Tomanova et al. (2006)

Functional trait	Categories	Code
Head width (mm) or Shell width (mm)	<0.1	A1
	0.10-0.5	A2
	0.51-1.0	A3
	1.01-1.5	A4
	1.51-2.0	A5
	2.01-2.5	A6
	>2.51	A7
Biomass (mg)	<0.1	B1
	0.10-0.5	B2
	0.51-1.0	B3
	1.01-1.5	B4
	1.51-2.0	B5
	2.01-2.5	B6
	>2.51	B7
Functional Feeding Group	Collector-Filterer	C-F
	Collector-Gatherer	C-G
	Predator	Pr
	Piercer	Pi
	Scrapper	Sc
	Detritivore	Dt
	Shredder	Sh
Body length (mm) or shell length (mm)	<0.5	L1
	0.51-1.5	L2
	1.51-2.50	L3
	2.51-3.5	L4
	3.51-4.5	L5
	4.51-5.5	L6
	>5.51	L7

### Statistical analysis

We tested whether the data follow a normal distribution using the Kolmogorov-Smirnov test in the whole environmental variables (climatic data, physicochemical variables, and QBR index) and organic matter input. We found significant differences in the environmental variables according to the sampling area and season through multiple Kruskal-Wallis analyses.

About the functional analysis, a fuzzy matrix was constructed with each of the trait categories, establishing a non-exclusive rating: 0 (no link), 1 (weak link), 2 (moderate link), and 3 (strong link); this is due to the phenotypic plasticity of macroinvertebrates that allows them to link to more than one category per trait (Chevenet et al., 1994). Subsequently, this fuzzy matrix was multiplied by the abundance of the morphotypes to obtain a matrix of functional traits by zone, season,

and plant species of the litter fall during the entire sampling, using Gower's distance to reduce the variability of the data (De Bello et al., 2013). The estimation of functional diversity descriptors such as functional richness (*Fric*), functional distance (*Fdis*), and functional evenness (*Feve*) was carried out using the “FD” package, through the R v4.3.1 software.

We tested the first hypothesis by checking for differences in the functional diversity of invertebrate communities that colonized the leaf litter bags for each sampling area, season, and leaf litter species using a permutational multivariate analysis of variance with two factors (PERMANOVA two-way), and a Monte Carlo randomization (999 permutations; *p-value* < 0.05). The null hypothesis tested was that there was no difference in composition and structure of the benthic community among sites and, still, that there is no interaction between sites and exposure time of the bags containing the leaves.

For the second hypothesis, we examined relations between environmental data, organic matter inputs, and macroinvertebrate functional diversity using multiple linear regression models (MLRM). For this analysis, we are assuming that specimens

found in each bag are directly related to leaf litter species. Before the definition of explanatory variables in MLRM, a redundancy analysis (RDA), due to the data distribution linearity, with a Monte Carlo randomization (999 permutations; *p-value* < 0.05) was used as a variable reduction technique to combine highly intercorrelated variables with independent predictors. The whole database was Ln-transformed and the whole data analysis was performed in R v4.3.1 software.

## RESULTS

We observed significant changes in the stream environmental variables, according to the Kruskal-Wallis analysis ( $k = 27$ ; *p-value* < 0.038). According to the sampling area, there are differences in solar radiation, conductivity, dissolved oxygen, total solids, pH, water flow, and water temperature explained spatially by the three sampling points along the stream. On the other hand, solar radiation, precipitation, air temperature, conductivity, total suspended solids, pH, and water flow had significant changes among seasons (Table 2). About the QBR index, the stream upper area as the natural conditions of the riparian forest registered a score of 95. In contrast, the lower zone had a medium value of 68

**Table 2.** Mean and standard error of the environmental data present in each sampling zone and season in the La Avería stream. Significant values (*p-value* < 0.05) are in bold.

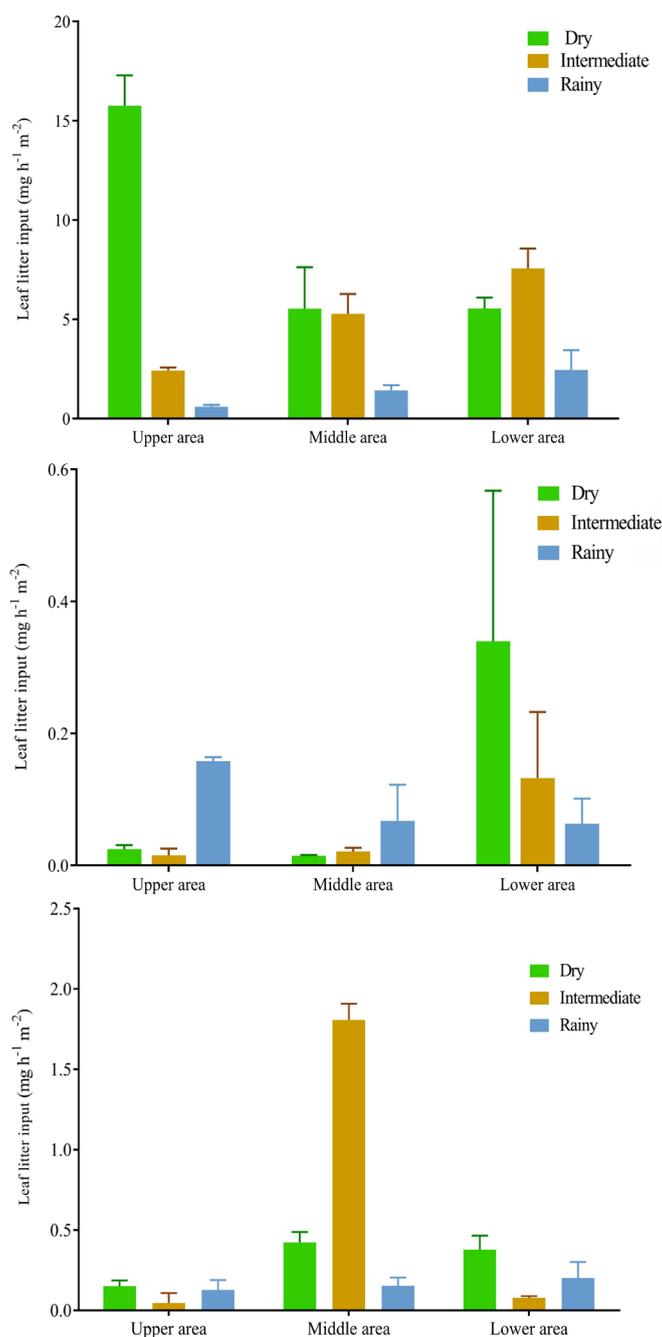
Parameter	Upper Zone	Middle Zone	Lower Zone	Dry	Intermediate	Rainy
Solar Radiation (W m <sup>-2</sup> )	0.345 ± 0.078	<b>0.69 ± 0.162</b>	0.196 ± 0.087	0.493 ± 0.146	<b>0.205 ± 0.048</b>	0.533 ± 0.143
Environmental temperature (°C)	24.46 ± 0.124	24.46 ± 0.124	24.46 ± 0.124	<b>24.202 ± 0.13</b>	<b>24.472 ± 0.085</b>	<b>24.707 ± 0.12</b>
Precipitation (mm)	80.156 ± 10.979	80.156 ± 10.979	80.156 ± 10.979	<b>36.867 ± 4.296</b>	<b>80.5 ± 6.003</b>	<b>123.1 ± 9.403</b>
Water temperature (°C)	23.341 ± 0.237	23.489 ± 0.256	<b>21.674 ± 0.27</b>	22.511 ± 0.348	22.633 ± 0.221	23.359 ± 0.346
Dissolved oxygen (mg l <sup>-1</sup> )	8.324 ± 0.298	7.93 ± 0.416	<b>13.819 ± 0.639</b>	10.565 ± 0.675	10.296 ± 0.751	9.211 ± 0.93
Total Solids (mg l <sup>-1</sup> )	62.963 ± 2.385	62.167 ± 2.506	<b>49.185 ± 3.227</b>	<b>65.315 ± 2.244</b>	56.667 ± 3.761	52.333 ± 2.257
Conductivity (µS cm <sup>-1</sup> )	125.963 ± 4.778	142.815 ± 4.962	<b>96.13 ± 6.47</b>	<b>129.963 ± 4.495</b>	112.463 ± 7.849	104.481 ± 4.597
pH	7.854 ± 0.047	<b>7.364 ± 0.066</b>	7.847 ± 0.042	7.621 ± 0.082	<b>7.842 ± 0.058</b>	7.601 ± 0.074
Water flow (m <sup>3</sup> s <sup>-1</sup> )	1.646 ± 0.563	1.182 ± 0.282	<b>0.81 ± 0.132</b>	<b>0.763 ± 0.147</b>	1.429 ± 0.204	1.446 ± 0.595

due to the livestock impact. Finally, the middle zone scored a low value of 45 as a result of the near disappearance of the vegetation cover.

Both drift and vertical organic matter inputs showed significant differences ( $p\text{-value} = 0.032$ ) by sampling area and season (Figure 1). In detail, the highest values of drift were presented by upper (15.76 mg h<sup>-1</sup> m<sup>-2</sup>) and middle areas (5.54 mg h<sup>-1</sup> m<sup>-2</sup>) during the dry season. In contrast, the lower zone obtained the highest value (7.56 mg h<sup>-1</sup> m<sup>-2</sup>)

during the rainy season. For the vertical inputs, we see its maximum records during the dry period in the upper (0.15 mg h<sup>-1</sup> m<sup>-2</sup>) and lower (0.38 mg h<sup>-1</sup> m<sup>-2</sup>) areas. The middle area (1.81 mg h<sup>-1</sup> m<sup>-2</sup>) obtained its highest record during the intermediate period. On the other hand, there were no significant differences in lateral organic matter inputs.

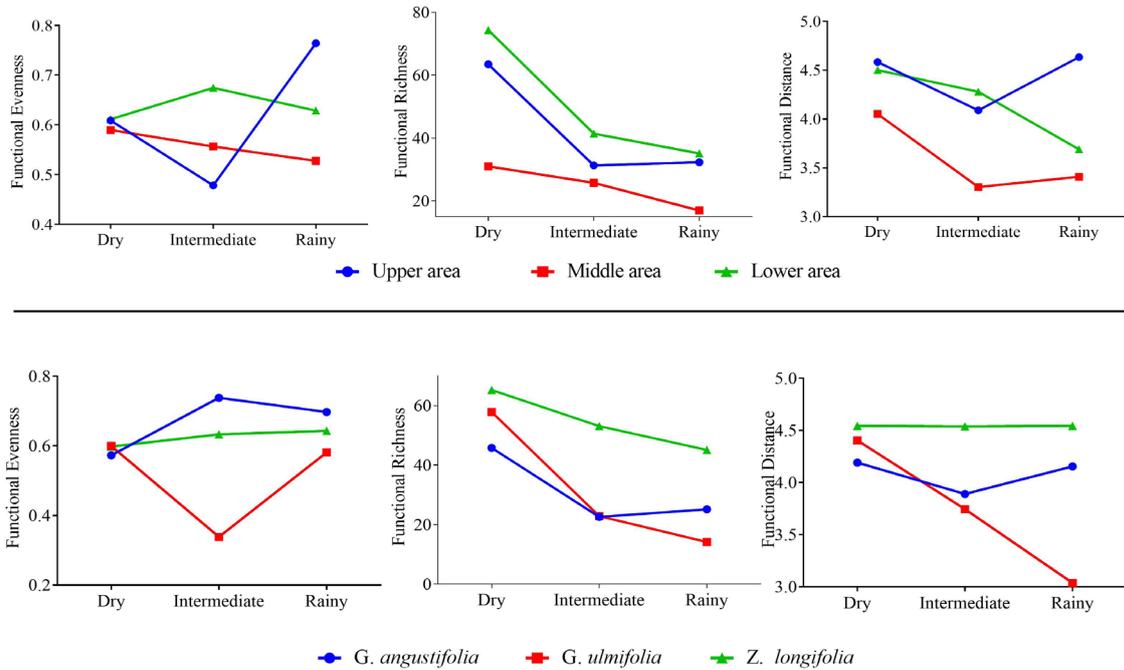
Regarding the functional diversity values (Figure 2), our ANOVA showed significant differences ( $p\text{-value} < 0.05$ ) between sampling areas. In



**Figure 1.** Mean and standard deviation of organic matter inputs collected in the (A) drift, (B) lateral, and (C) vertical traps according to sampling area and season in La Avería stream.

particular, for *Feve*, the lower zone had the highest value during the rainy season, while in the intermediate period, this same place had the lowest value. On the other hand, higher numbers of *Fric* were obtained for the high and low zones during the dry season. Concerning *Fdis*, abrupt changes were recognized, where the middle

zone had the lowest records, regardless of the sampling season. On the other hand, no significant differences were found in the functional diversity descriptors about the plant species used in the litter bags. However, *Feve* changes were observed during the intermediate period, only to end up again with similar records at the end of the wet



**Figure 2.** Functional diversity components across sampling seasons, defined for the macroinvertebrate assemblage, according to (A) climatic season and (B) litter type.

season. Regarding *Fric*, a decreasing trend was recorded for all leaf litter species. Finally, *Fdis* changed abruptly for each plant species, except for *Z. longifolia* which did not show any variation throughout the experiment.

According to two-way PERMANOVA (Table 3), there were significant differences in the functional values of macroinvertebrates by sampling zone and time. Nevertheless, we found no significant

differences in macroinvertebrate functional diversity according to the leaf litter plant species used in the experiment.

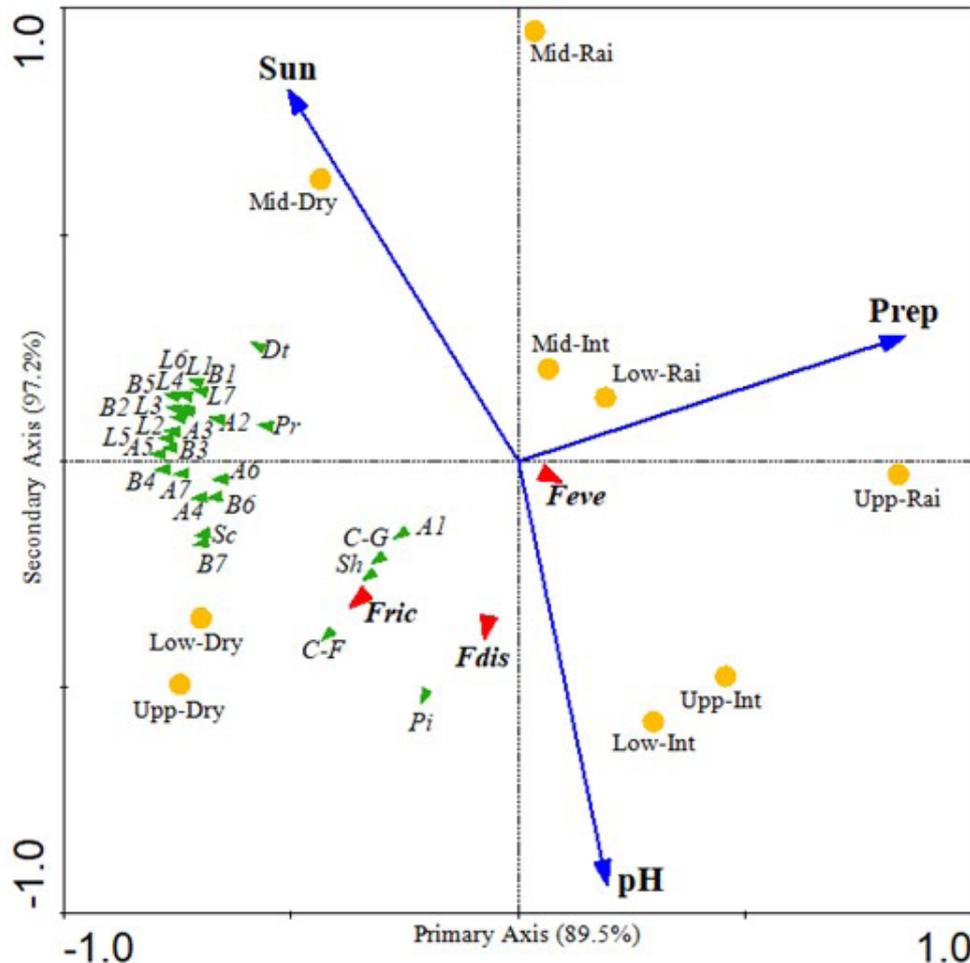
The Monte Carlo test showed significant positive correlations between Ln-transformed functional diversity with precipitation ( $F = 11.407$ ;  $p\text{-value} = 0.001$ ), solar radiation ( $F = 4.519$ ;  $p\text{-value} = 0.005$ ), and pH ( $F = 2.376$ ;  $p\text{-value} = 0.047$ ). According to the RDA (Figure 3), the primary

**Table 3.** Results of PERMANOVA models performed between treatments (Ln-transformed sampling zone, time, and leaf litter). Significant values ( $p\text{-value} < 0.05$ ) are bold

Treatments	d. f.	Sum square (%)	F-value	p-value
Sampling Zone	2	<b>65.09</b>	<b>2.124</b>	<b>0.049</b>
Sampling Time	2	<b>304.88</b>	<b>9.949</b>	<b>0.001</b>
Leaf Litter	2	43.71	1.426	0.234
Sampling Zone and Time	5	50.12	1.635	0.176
Sampling Zone and Leaf	5	20.41	0.666	0.535
Sampling Time and Leaf	5	11.71	0.382	0.827
Sampling Zone, Time and Leaf	8	8.69	0.284	0.928

axis explains 89.5% while the secondary axis explains 97.2% of the data variation implying a negative relation between the rainy season and traits presence. This analysis correlated the *Feve* with the pH and intermediate season. On the other hand, *Fric* and *Fdis* are negatively related to solar radiation.

The MLRM evaluated the relationships between functional diversity components with environmental variables, showing relatively strong and significant relations (Table 4). Functional richness showed a significant negative correlation with precipitation, which is variable and associated with ecosystem seasonality. On the other hand,



**Figure 3.** RDA between the functional diversity of macroinvertebrates and the environmental data in La Avería stream. Prep = Precipitation. Sun = Solar radiation. Feve = Functional Evenness. Fric = Functional Richness. Fdis = Functional Distance. Traits Names= Table 1.

Functional Evenness and Functional Distance did not show a significant connection with the environmental variables selected by the Monte Carlo test.

## DISCUSSION

The results showed a relationship between quality habitat and physicochemical stream variables with the macroinvertebrates functional diversity. In detail, we observed significant differences in the functional descriptors by sampling zone and season. Higher *Fric* records were obtained in high and low zones during the dry season caused by changes in

pH and litter supply that define variations in the trait's dimensionality in the ecosystem. Besides, *Feve* and *Fdis* also showed variations by sampling zone and season, but their variances differences were not significant. Similarly, we did not record significant differences in any functional parameter according to leaf litter type.

Regarding the riparian quality index, the upper zone had a higher value in the QBR index as expected given its higher degree of conservation, which suggests that the natural state of the stream corresponds to a water course surrounded by a riparian cover composed largely of native

**Table 4.** Results of multiple linear regression models performed between parameters (Ln-transformed data). Significant values (p-value < 0.05) are in bold

Parameters		Estimate	Standard error	df	t-value	p-value	R <sup>2</sup>
Functional Evenness	Intercept	0.686	1.152	14	0.595	0.557	0.021
	pH	-0.029	0.144	14	-0.199	0.844	
	Solar Radiation	-0.064	0.138	14	-0.496	0.645	
	Precipitation	0.003	0.006	14	0.480	0.636	
Functional Richness	Intercept	-7.916	12.564	14	-0.630	0.535	0.214
	pH	1.568	1.570	14	0.999	0.328	
	Solar Radiation	0.607	1.501	14	0.405	0.689	
	Precipitation	<b>-0.015</b>	<b>0.007</b>	<b>14</b>	<b>-2.186</b>	<b>0.039</b>	
Functional Distance	Intercept	1.574	1.909	14	0.825	0.418	0.179
	pH	0.029	0.238	14	0.124	0.902	
	Solar Radiation	-0.225	0.228	14	-0.987	0.334	
	Precipitation	-0.001	0.001	14	-1.223	0.234	

vegetation such as *Z. longifolia* (an evergreen species that does not limit its leaf drop to a special season) (Vargas, 2015). These results correspond to our data—in the upper zone, we recorded the highest values of vertical entry and drift of organic matter, both of which are variables influenced by the immediate riparian forest (Carvalho & Uieda, 2010; Zhang et al., 2019).

In contrast, in the middle zone, and to a lesser extent the lower zone, human influence (represented by structures such as viaducts and agricultural and urban dumping areas) consistently generated an increase in conductivity, dissolved solids, pH, and solar radiation, and corresponded to low values in the QBR index. These observed characteristics are typical of highly disturbed environments where riparian cover has been almost eliminated (Cooper et al., 2013; García-Velázquez & Gallardo, 2017; Ometo et al., 2000).

As a principal results, we refuted the first hypothesis because we observed no significant changes in the functional diversity of macroinvertebrates according to plant species within the litter traps. This is consistent with the hypothesis described by Boyero et al. (2011; 2012) which illustrates how macroinvertebrates from neotropical environments are generalists with no preference for particular leaves type since litter here has a lower nutritional value compared to plant species from temperate areas.

We highlight that our results agree with the second hypothesis because there are some variations in macroinvertebrates functional diversity related to changes in the riparian and stream habitat due to anthropic activities from La Avería stream. According to functional diversity records, the parameters (*Feve*, *Fric*, and *Fdis*) changed, in a different way, between sampling areas due to local environmental variables associated with water physicochemical and habitat quality (Belmar et al., 2019; Colzani et al., 2013; Malacarne et al., 2023). Regarding *Feve* value, we found that is lower in areas with higher pH, due to the loss of individuals and functional trait space in these stations (Bae & Park, 2016; Ferreira et al., 2017; Mouillot et al., 2013; Rojas et al., 2020; Tamaris-Turizo & Rodríguez, 2015). In detail, *Fric* is directly related to environmental variables. In the first place, the high and low zones with higher functional richness values present a higher dimensional range that allows the expression of traits with a lower degree of redistribution between taxonomic groups (Colzani et al., 2013; Rojas et al., 2020). At the same way, *Fdis* plus functional traits, were variables directly associated with lower values in precipitation due to dry seasons stabilizing the availability of resources and environmental conditions, which allows a wider dimensionality in the expression of the traits, as a way to reduce competition in the face of a greater leaf litter supply (Cadotte et al., 2011; Rodríguez-Romero et al., 2021; Rojas et al., 2020; Villéger and Mason, 2008). Likewise, the

higher record of organisms is related to a greater record of predators that present larger individuals, causing greater records in biomass and body length during the dry zone (Cory et al., 2014; Rojas et al., 2020; Stewart & Downing, 2008). Finally, there is a reduction of *Fric* and *Fdis* during the rainy season due to abrupt changes in water flow, which alters macroinvertebrate communities and the leaf litter supply in the ecosystem (Bae & Park, 2016; Belmar et al., 2019).

In conclusion, we found evidence for a relationship between stream quality habitat and physicochemical variables with the macroinvertebrates functional diversity. We observed that pH and precipitation are the environmental characteristics that best define changes in functional diversity, since changes in these variables are directly associated with changes in litter supply and therefore define the dimensionality of the functional traits in the ecosystem. For future studies, we recommend including the presence of exotic vegetation in evaluations of the riparian habitat, and adding data on the chemical and nutritional composition of the leaves in the litter packs to better understand leaf litter degradation and how macroinvertebrates affect this process.

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## AUTHOR CONTRIBUTION

Both authors contributed equally to the production of the article. José A. Cuéllar-Cardozo: participation in the methodological phase and in the final drafting of the paper. Hakan Bozdoğan: interpretation of the results, discussion and in the final drafting of the paper.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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