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SPATIO-TEMPORAL PATTERNS OF ARTHROPOD LEAF-LITTER COMMUNITIES IN PROTECTED MEXICAN TROPICAL DECIDUOUS FORESTS REVEALED BY METABARCODING

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Abstract: Leaf-litter arthropods represent an essential component of most terrestrial ecosystems, yet comprehensive studies of their species richness and composition remain considerably scarce due to the Linnean shortfall, i.e. the vast number of undescribed species that hampers accurate biodiversity assessments. Here, we explored the spatial and temporal diversity and structure of arthropod leaf-litter communities inhabiting two protected areas of tropical deciduous forest in Mexico using metabarcoding: the Chamela–Cuixmala Biosphere Reserve and Huatulco National Park. As this biome is characterized by marked seasonality, leaf-litter sampling was conducted during both the rainy and dry seasons. A total of 18,885,578 high-quality reads of 418 bp were obtained for the animal DNA barcoding region. Clustering at 97% similarity and subsequent taxonomic assignment yielded 1238 MOTUs assigned to Arthropoda, with diversity estimators indicating that ~66% of the total expected biodiversity was sampled. Insecta was the most species-rich class (~69% of sampled MOTUs), followed by Arachnida (~23%). Formicidae (Hymenoptera), Cecidomyiidae (Diptera) and Chrysomelidae, Curculionidae and Staphylinidae (Coleoptera) were the most species-rich insect taxa, whereas Araneae and Acari dominated Arachnida, highlighting their key functional roles in decomposition, nutrient cycling, and predation in tropical deciduous forest leaf-litter communities. Significant differences in MOTU richness were found between the two regions, with higher diversity during the rainy season in both. Community composition varied more strongly than species richness, indicating that intra-annual variation primarily reflects species turnover rather than net gains or losses. Around 36.5% of the MOTUs detected were also recovered in a previous metagenetic study of these forests using Malaise trap sampling. Our results thus underscore the complementary nature of ground and aerial sampling and the influence of seasonality and spatial heterogeneity in shaping arthropod assemblages. These findings also emphasize the urgent need to expand metabarcoding reference libraries and baseline datasets for leaf-litter arthropod communities, both to overcome the Linnean shortfall and to strengthen long-term biodiversity monitoring and conservation in tropical deciduous forests.

Key words: leaf-litter arthropods, species turnover, tropical deciduous forests; Linnean shortfall; insect diversity.

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Introduction

Tropical forests are among the most biodiverse biomes worldwide, harboring more than half of Earth's known terrestrial plant and animal species (Trejo & Dirzo, 2002; Lewis, 2006; Pillay et al., 2022). This remarkable diversity arises as a combination of environmental conditions, resource availability, and a complex vegetation structure (Trejo & Dirzo, 2002; Pérez-García et al., 2005; Dirzo et al., 2011; Takano-Rojas et al., 2023). Tropical deciduous forests, which extend from equatorial to subtropical zones, account for nearly 42% of all tropical forests and are characterized by their marked seasonality, high species richness and elevated levels of endemism (Eamus & Prior, 2001; Trejo & Dirzo, 2002; Pillay et al., 2022). However, they are also among the most threatened worldwide, having experienced a significant range reduction over the past three decades, primarily due to land-use change, urbanization and tourism (Janzen, 1988; Trejo & Dirzo, 2000; Miles et al., 2006; Sánchez-Azofeifa & Portillo-Quintero, 2011; Hansen et al., 2020). In Mexico, these forests mostly cover extensive areas along the Pacific coast, with remarkable vegetation variability even across short geographic distances (Trejo & Dirzo, 2002; Pérez-García & Meave, 2005; Durán et al., 2006; Dirzo et al., 2011), which reflects their ecological complexity and supports a wide variety of habitats and ecological niches, underpinning their extraordinarily high biodiversity.

The pronounced seasonality of tropical deciduous forests can create harsh and challenging conditions for many species (Janzen, 1988; Sánchez-Azofeifa & Portillo-Quintero, 2011; Pillay et al., 2022). Such seasonality leads to the accumulation of abundant organic matter on the soil surface due to leaf fall in the dry season, which provides shelter, food resources, and refugia against climatic extremes, making leaf litter a particularly vital habitat in these forests (Giller, 1996; Wardle et al., 2004b; Decaëns et al., 2006). Leaf litter serves as a critical interface between above- and below-ground dwelling communities (Bardgett & Wardle, 2010), providing habitat for organisms that initiate organic matter decomposition and play essential roles in nutrient and nitrogen recycling, carbon balance, and ecosystem fertility, therefore influencing both edaphic and plant community structure (De Deyn et al., 2004; Wardle et al., 2004a; Giweta, 2020; Sayer et al., 2024).

The capacity of leaf litter to support ecological processes and maintain ecosystem stability is highly dependent on climate (Aerts, 1997), litter chemistry (Li et al., 2012), and vegetation composition (Li et al., 2013; Schilling et al., 2016), as well as on the diversity and density of arthropods and other decomposer invertebrates and microbes (Bardgett & Van Der Putten, 2014; David, 2014; Liu et al., 2023). Consequently, declines in arthropod diversity are often associated with reduced resilience and slower post-disturbance recovery in ecosystems (Allison & Martiny, 2008; Downing et al., 2012). The composition of leaf litter arthropod communities is primarily shaped by both spatial (*e.g.*, habitat type, geographic region) and temporal (*e.g.*, seasonality) factors, which together drive diversity and turnover patterns across multiple scales (Pérez-Toledo et al., 2020; de Vasconcelos et al., 2024; Villaseñor-Amador et al., 2024).

Overall, the diversity, community composition, and responses to environmental variation in tropical deciduous forests remain poorly documented, with most studies focusing on specific groups of above-ground-dwelling arthropods (*e.g.* Andersen, 2008; Zaldívar-Riverón et al., 2010; Rocha-Ortega and Castaño-Meneses et al., 2015; Corona-López et al., 2017). The recent trend of global declines in arthropod populations (Hallmann et al., 2021; Lister & García, 2018; Seibold et al., 2019; Owens et al., 2020) underscores the urgency of studying their communities and diversity dynamics. Recently, Benites et al. (2025) published the first metabarcoding study on entire insect communities in tropical deciduous forests, analyzing above-ground biodiversity in two regions in Mexico and providing a broad taxonomic perspective on this ecosystem. In contrast, leaf-litter arthropod assemblages–along with other below-ground communities–have been comparatively overlooked in these forests and in other habitats more generally (*e.g.* Levings & Windsor, 1985; Wolters, 2001; Cole et al., 2016), a bias that is also evident in metabarcoding studies (*e.g.* Andújar et al., 2015; Arribas et al., 2016).

Challenges such as sampling difficulties, the minute size of many taxa (Bestelmeyer et al., 2000; Bardgett, 2002; Decaëns, 2010; Arribas et al., 2016; Young et al., 2022), and the taxonomic impediment (Engel et al., 2021) have hindered progress in understanding the species diversity, community structure, and spatio-temporal dynamics of leaf litter and soil arthropods. Consequently, much of this diversity remains undescribed. Metabarcoding represents a robust approach for rapidly revealing the largely unknown fauna of soil and leaf litter, thereby helping to address the Linnean shortfall–i.e., the profound lack of knowledge about the planet's biodiversity (Brown and Lomolino, 1998). This whole-community DNA sequencing technique has proven effective for species identification in mixed and complex samples, such as bulk arthropod collections from megadiverse ecosystems (*e.g.* Andújar et al. 2015; Arribas et al. 2016; Young et al. 2022; Arribas et al. 2022). Cytochrome oxidase I (COI) fragments, clustered into molecular operational taxonomic

Units (MOTUs), serve as species proxies that enable the assessment of biodiversity, community-level structure, and species turnover, revealing hidden diversity and ecological patterns even when reference inventories are limited (Benites et al., 2025). The taxonomic, biogeographic, and molecular information generated through this approach therefore provide essential baseline data to help close the vast gap in our knowledge of Earth's biodiversity.

Here, we characterized the diversity and community structure of arthropods in leaf litter samples from two protected Mexican tropical deciduous forests reserves—the Chamela-Cuixmala Biosphere Reserve in Jalisco, and the Huatulco National Park in Oaxaca–which are located at opposite ends of the Pacific lowlands morphotectonic subprovince and are separated by about 1200 km. These tropical deciduous forest regions show strong seasonality in rainfall and temperature, which may profoundly affect leaf litter arthropod assemblages, as previously seen with above-ground insect communities' assemblages (Benites et al. 2025). By applying DNA metabarcoding to samples collected during both the rainy and dry seasons, we aimed to: (1) estimate the richness and taxonomic composition of leaf litter arthropods, (2) assess differences in diversity and community structure between regions and seasons, and (3) evaluate patterns of species turnover across spatial and temporal scales.

We characterized arthropod community biodiversity using both pairwise beta and zeta diversity metrics (Baselga, 2010; Hui & McGeoch, 2014), evaluating turnover and the contribution of both rare and common species. Our results revealed high levels of diversity and endemicity in both communities, which is expected for such complex habitats, though with considerably low species overlap. Our findings provide new insights into the enormous hidden diversity of tropical deciduous forest leaf-litter arthropods, generating taxonomic and molecular baseline databases for the studied regions that greatly contribute to a better understanding of the structure and dynamics of their communities.

Materials and Methods

Study sites and field sampling

The study consisted of a comprehensive survey of arthropods present in leaf litter samples from two protected tropical deciduous forest regions in México: the Chamela-Cuixmala Biosphere Reserve in the state of Jalisco, and the Huatulco National Park in the state of Oaxaca (hereinafter referred to as Chamela and Huatulco, respectively). Both reserves are located at opposite edges of the lowlands adjacent to Pacific coast within the Sierra Madre del Sur physiographic province (Quiñones, 1987; Krasilnikov, 2013), specifically in the Sierras de las Costas de Jalisco y Colima (Chamela) and the Costas del Sur (Huatulco; INEGI 2004, 2017) subprovinces. The local climate in both regions is warm (20-29º C), with annual precipitation ranging from 600 to 1200 mm and a marked dry season lasting from five to eight months: from October to May in Chamela and from November to April in Huatulco (García, 2004; Takano-Rojas et al., 2023).

Leaf litter sampling was conducted during both the rainy and dry seasons, with one event per season between 19 April and 4 May and between 12 August and 11 September 2022, totaling four collecting events, two per study region. Five collecting sites were established in primary forest in each region (Figure 1), located at an average distance of 653.5 ± 78 m from one another. At each site, a 20-m transect was laid out, along which ten 1-m² quadrants, spaced 1 m apart, were sampled following the protocols of Moreira et al. (2008) and Pérez-Toledo et al. (2020). Leaf litter and the superficial soil layer were manually collected from the edges to the center of each quadrant, and the material was then filtered through a 0.5-cm mesh sifter. Each transect yielded ten subsamples that were preserved in cloth bags and kept in the shade until further processing. Sampling resulted in 100 m² of leaf litter collected in an estimated area per site of 17.6 ha (Chamela) and 36.05 ha (Huatulco).

The contents of each cloth bag corresponding to a transect were transferred to mini-Winkler funnels, totaling ten funnels per transect, and left to process for three days. During this period, organisms migrate downward as the sample dries from top to bottom, ultimately falling into a container filled with 95% ethanol at the end of the funnel (Moreira et al., 2008). The technique is particularly effective for retrieving arthropod specimens, especially ants and small beetles (Bestelmeyer et al., 2000; Sabu et al., 2010), with recovery rates of nearly 95% of the species originally present after three days (Bestelmeyer et al., 2000). Following extraction, all 10 ethanol containers with organisms from a single transect were pooled and transferred to Falcon tubes, which were stored at -20 °C until further processing. To control for potential contamination, a Winkler funnel without any sample but with an ethanol container was included as a negative control (Liu et al., 2019).

The five sites were sampled in each season in both regions, with parallel transects at each site separated by 1 m (two transects per site: one in the rainy season and one in dry season). This design resulted in a total of 204 samples (10 per transect plus four controls), which were later pooled by transect into five samples per area (Huatulco and Chamela) and season (rainy and dry), totaling 20 samples (Table S1).

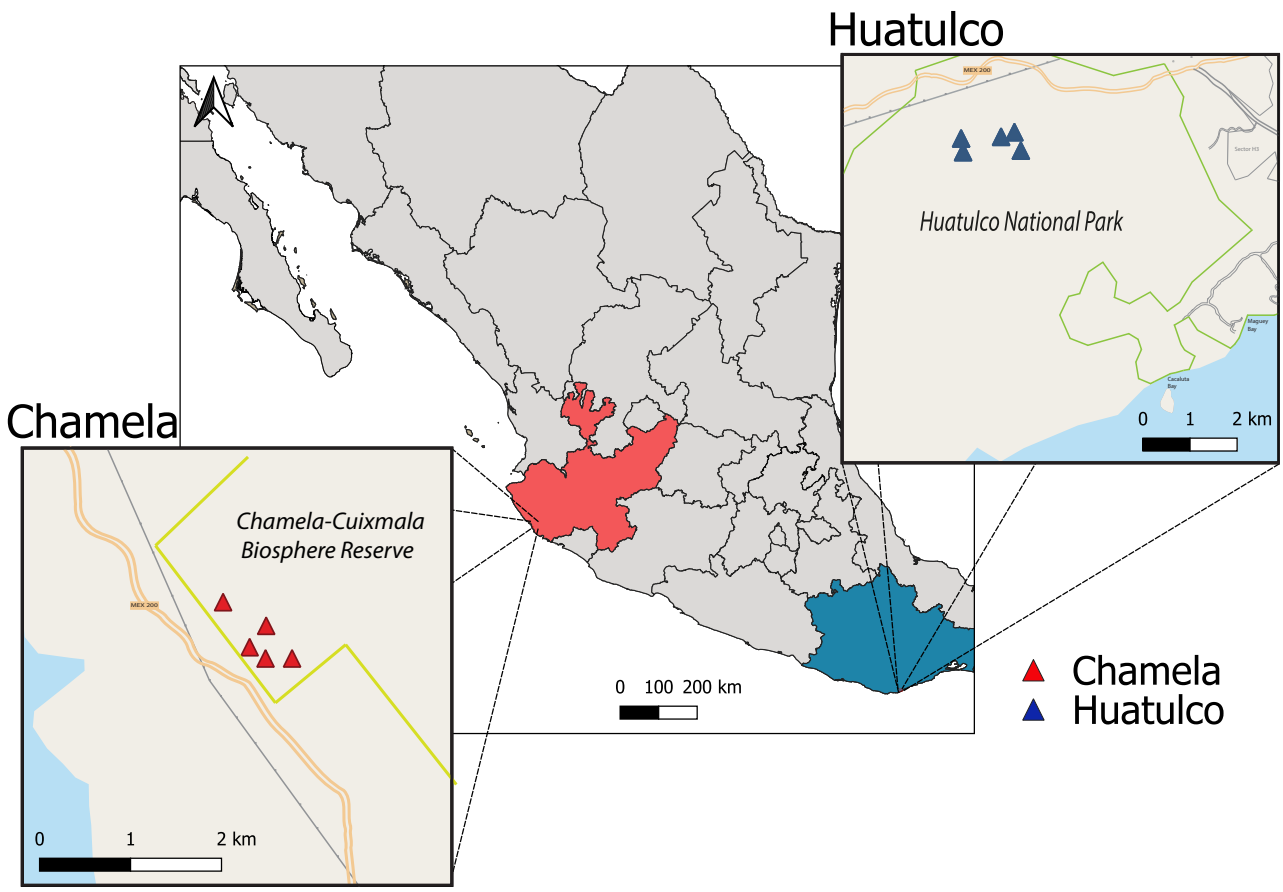

Figure 1: Map of the two tropical dry forest study areas, Chamela (A) and Huatulco (B), showing the location of the sample sites.

Laboratory sample processing

Samples preserved in 95% ethanol were examined under a Zeiss® SteREO Discovery V8 stereoscopic microscope at 10 and 40x to separate arthropod specimens from debris, which could interfere with subsequent DNA extraction and amplification. A total of 200 m^2 of leaf litter and soil were analyzed, corresponding to 20 transects (5 transects per season for both Chamela and Huatulco). Following sorting, samples were drained of ethanol and left to dry overnight. Dried samples were individually ground into a fine powder using an Ultra Turrax Tube Drive grinder (IKA®) with sterile disposables tubes (IKA®) and 10 stainless steel pellets. For each sample, two 25 mg aliquots of fine powder were placed in 1.5 ml tubes as extraction subsamples to favor biodiversity detection. Rigorous controls were implemented through the process to detect, trace and control contamination: an open Eppendorf tube while transferring the powder into the tubes as a negative control (transfer negative control), and a sample with identified species native of other continents: (*Papilio demoleus*, *Speyeria cybele*, *Hamanumida daedalus* and *Hebomoia glaucippe*) as a positive control.

DNA extraction followed Ivanova et al. (2006), with lysis carried out overnight with incubation at 56°C, with 180 µl of lysis buffer and 20 µl of proteinase K. Supernatant was placed in silica columns with binding mix, and DNA subsequently eluted in 180 µl of wash buffer. The final products were quantified using a Qubit® 2.0 fluorometer (Invitrogen) and the eluted subsamples were diluted to 2 ng/ µl. PCR amplification targeted a 418 bp of the animal barcode locus using the arthropod BF3 and BR2 primers (Elbrecht et al. 2019), with a two-step PCR protocol: first PCR (PCR1) amplified the targeted BF3/BR2 fragment, and second PCR (PCR2) added P5/P7 Illumina adapters with individual identification barcodes (6 to 8 bp) attached to them to maximize multiplexing. PCR1 had an initial denaturation of 95 °C for 5 min, followed by 30 cycles of 94°C for 30 s, 46°C for 30 s, and 72°C for 50 s, with a final extension at 72°C for 5 min. PCR2 had an initial denaturation of 2 min, followed by 15 cycles of 94°C for

40 sec, 51°C for 1 min, and 72°C for 1 min, with a final extension of 72°C for 5 min.

Each PCR reaction had a total volume of 25 µl, composed of 2.5 µL of buffer (10X), 0.5 µL of dNTPs (10 mM), 1 µL of each forward and reverse primers (10 mM), 0.2 µL of mi-Taq (5 U/µL), 16.8 µL of extra pure molecular grade water, and 2 µL of DNA or 1x diluted PCR1 template. A negative control containing reaction mix without DNA (PCR control) was included every 96 subsamples. Each subsample was PCR-amplified three times, with each product tagged with a unique barcode to allow independent tracking and contamination detection. PCR products were purified using magnetic beads (ratio 0.8:1 µL beads/product), ligated to platform adapters, and paired-end sequenced using the Illumina MiSeq instrument (250 bp) at the Canadian Center for DNA Barcoding (CCDB).

Bioinformatic processing and taxonomic assignment

Raw sequencing reads were first processed to remove adapters and filtered for quality using AdapterRemoval v.2.3.0 (Schubert et al., 2016) and Sickle v.1.33 (Joshi & Fass, 2011). Sequencing errors were subsequently corrected using Bayes-Hammer via SPAdes 3.12.0 (Nurk et al., 2013) and paired-end reads were merged with PANDAseq v.2.11 (Masella et al., 2012). Reads were demultiplex into samples using DAMe v.1.0 (Zepeda-Mendoza et al., 2016), and PCR technical replicates per sample were filtered using *filter.py* from DAMe, retaining only reads present in all three PCRs with abundance ≥10 reads in the final output. Chimeras were identified and removed through VSEARCH (Rognes et al., 2016), and reads were clustered into Molecular Operational Taxonomic Units (MOTUs) with SUMACLUST v.1.0.36 (Mercier et al., 2013) at a conservative species-level similarity threshold of 97 %. The resulting MOTUs were further error corrected using the LULU package (Frøslev et al., 2017) in R v.3.6.1 (R Core Team, 2017), to discriminate between rare MOTUs and low frequency MOTUs resulting of sequencing errors, based on co-occurrence across samples.

After quality filtering and MOTUs clustering, subsamples were merged into 20 biological samples for the study, in addition to one Negative (NC) and one Positive control (PC). The positive control was used to detect contamination (*e.g*. tag-jumping) during sequencing and to adjust filtering parameters in DAMe, whereas the negative control served to detect any potential contamination during laboratory processing.

Taxonomic assignment was performed by sequence similarity, comparing MOTU nucleotide sequences against the BOLD Systems database using the BOLD package v.1.3.0 (Chamberlain 2020) in R v.3.6.1 (R Core Team, 2017). A conservative approach was adopted: assignments were retained at the species, genus, and order/family levels only when MOTUs showed sequence similarities of ≥ 97%, ≥ 95%, and ≥ 85%, respectively. For MOTUs exhibiting similarity between 80–85% we retained only higher-rank information (i. e. class and phylum), and assignments with similarity lower than 80% were discarded to control for chimeras and non-target PCR artefact error sequences. Each MOTU was retained only at the lowest taxonomic level with unambiguous identification. When a MOTU matched multiple entries at a particular taxonomic level, the immediate higher taxonomic rank level with a unique identification was selected.

Statistical Analyses

All biodiversity and community assembly statistical analyses were conducted in R v. 3.6.1 (R Core Team, 2017). Read abundances were converted to incidence data, recording the presence (1) or absence (0) of MOTUs in each sample, regardless of read counts. MOTUs diversity, which served as a proxy for species diversity, was represented by accumulation curves generated with the iNEXT package v.3.0.0 (Hsieh et al. 2020) using incidence-based frequencies and Hill's number q set to 0. These curves plotted the total number of MOTUs against the number of sample units to evaluate sampling effort. Additionally, biodiversity estimators Chao and first-order Jackknifing were calculated using the vegan v.2.6-4 package (Oksanen et al. 2022) to further assess species richness and completeness of sampling.

Differences in richness between regions, between seasons, and their interaction were tested using a two-way ANOVA. Prior to ANOVA, assumptions of normality and homogeneity of variance were verified using Shapiro-Wilk and Levene´s test, respectively. A Bonferroni correction (Armstrong, 2024) was applied to account for multiple comparisons, setting $\alpha = 0.05/3 \approx 0.017$ as significance threshold for each individual test.

Arthropod community composition of each tropical deciduous forest region and season was visualized using non-metric multidimensional scaling (NMDS) analyses in vegan v.2.6-4 R package (Oksanen et al. 2022). For MOTU-level analysis, NMDS was based on Jaccard dissimilarity matrix (presence/absence), with differences in community structure and diversity between these two regions and between seasons were statistically assessed using a multi-factorial permutation-based ANOSIM (Clarke, 1993) in vegan v.2.6-4 (Oksanen et al. 2022). Correlation plots for these MOTU-level patterns were generated using corrplot package v.0.95 (Wei et al., 2017). Community

composition was also evaluated at family level using MOTU abundances per family, with NMDS based on Bray-Curtis distances, and family-level community structure tested using a multi-factorial Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2001). Together, this approach allowed for a robust evaluation of spatial and temporal variation in arthropod assemblages across different taxonomic resolutions.

The prevalence of the seven most species-rich arthropod groups across regions and seasons was assessed using a Fourth Corner Model (Brown et al. 2014) with the "traitglm" function from mvabund v.4.2.1 (Wang et al. 2020). This GLM-based analysis identified significant associations between MOTU assignment and season in each region, with three explanatory variables: season (dry/rainy), MOTU assignment to the seven most species-rich arthropod groups, and the interaction between season and MOTU assignment (i.e. the "fourth-corner matrix"; Brown et al. 2014).

Compositional changes in species between and within regions, and between seasons were evaluated using Sørensen indices for multiple samples (Baselga, 2010), calculated from incidence-based MOTU data. This approach partitions total beta diversity into turnover (species replacement) and nestedness (species gain or loss) components using betapart v.1.6 (Baselga and Orme, 2012). Within this framework, pairwise total β-diversity (ranging from 0 to 1) quantifies dissimilarity between assemblages, with values near 1 indicating completely distinct communities and values near 0 indicating identical communities. Turnover values close to 1 indicate that dissimilarity is primarily driven by species replacement, whereas nestedness values near 1 indicate that dissimilarity is driven mainly by differences in richness (Baselga, 2010). This partitioning provides insights into the ecological processes underlying compositional change.

To complement pairwise beta diversity, zeta diversity analysis (Hui & McGeoch 2014) was applied using zetadiv v.1.2.1 (Latombe et al. 2020) to evaluate multisite compositional change and species turnover across increasingly spatial scales. Zeta diversity measures the number of species shared across multiple sites, sequentially calculating shared species across 2, 3, 4… n sites. This approach allows assessment of the contributions of both rare (shared by few sites) and common (shared by many sites) species to overall turnover. Analyses were conducted for all sites and seasons combined (Huatulco + Chamela), as well as for each region separately. Two models were compared: a non-geographic model including all possible site combinations, and a Nearest-Neighbour model accounting for spatial distance. Model selection was based on the Akaike Information Criterion (AIC), with the lowest value and $\Delta AIC \geq 10$ indicating the best fit. The selected model was then fitted to exponential and power law functions to infer whether turnover was driven by stochastic processes or ecological niche differentiation (Hui & McGeoch et al. 2014; McGeoch et al. 2019). A power law fit suggests niche differentiation, where rare species drive compositional changes, while an exponential fit indicates stochastic assembly. Zeta decline (number of species shared by increasing number of sites) and zeta-ratio curves (probability of encountering a species with additional samples), were plotted to analyze species turnover across increasing numbers of sites, integrating the contributions of common species at higher orders.

Together, Baselga's pairwise beta diversity and zeta diversity approaches provide complementary insights into biodiversity patterns and compositional change in leaf litter arthropods communities. While pairwise beta diversity captures localized dynamics mainly driven by rare species, zeta diversity evaluates multisite patterns considering both rare (low- order) and common (high-order) species. Combined, these approaches provide a comprehensive understanding of species turnover rates and compositional patterns. All analyses were performed for each region and season, considering MOTUs as well as the seven most species-rich arthropod groups: Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Araneae, and Acari.

Results

Arthropod species diversity

High-throughput sequencing generated 23,201,852 raw 250 bp reads (R1 and R2), and sequencing was successful in all subsamples. All negative control samples showed no detectable reads. After quality-filtering, adapter removal, error correction, and merging of R1 and R2, a total of 18,885,578 high quality fragments with a length of 418 bp were obtained. Clustering at 97% resulted in 1238 MOTUs (4892 ± 472.6 reads per MOTU) assigned to Arthropoda. Diversity estimations using Chao1 and first-order Jackknife indices indicated that approximately 66% of the total expected biodiversity was recovered (Chao's MOTUs: 1947.3 ± 77.6; Jackknife's MOTUs: 1818.5 ± 161.3). Rarefaction and extrapolation curves suggested that more than 20 additional samples would be needed to approach complete biodiversity sampling and stabilize the curve, with current sample coverage close to 0.8 (Figure 2). Similar results were observed across all arthropod groups except Diptera, which exhibited a steeper rarefaction slope than the rest, indicating lower sampling completeness for this order (Supplementary Figure S1).

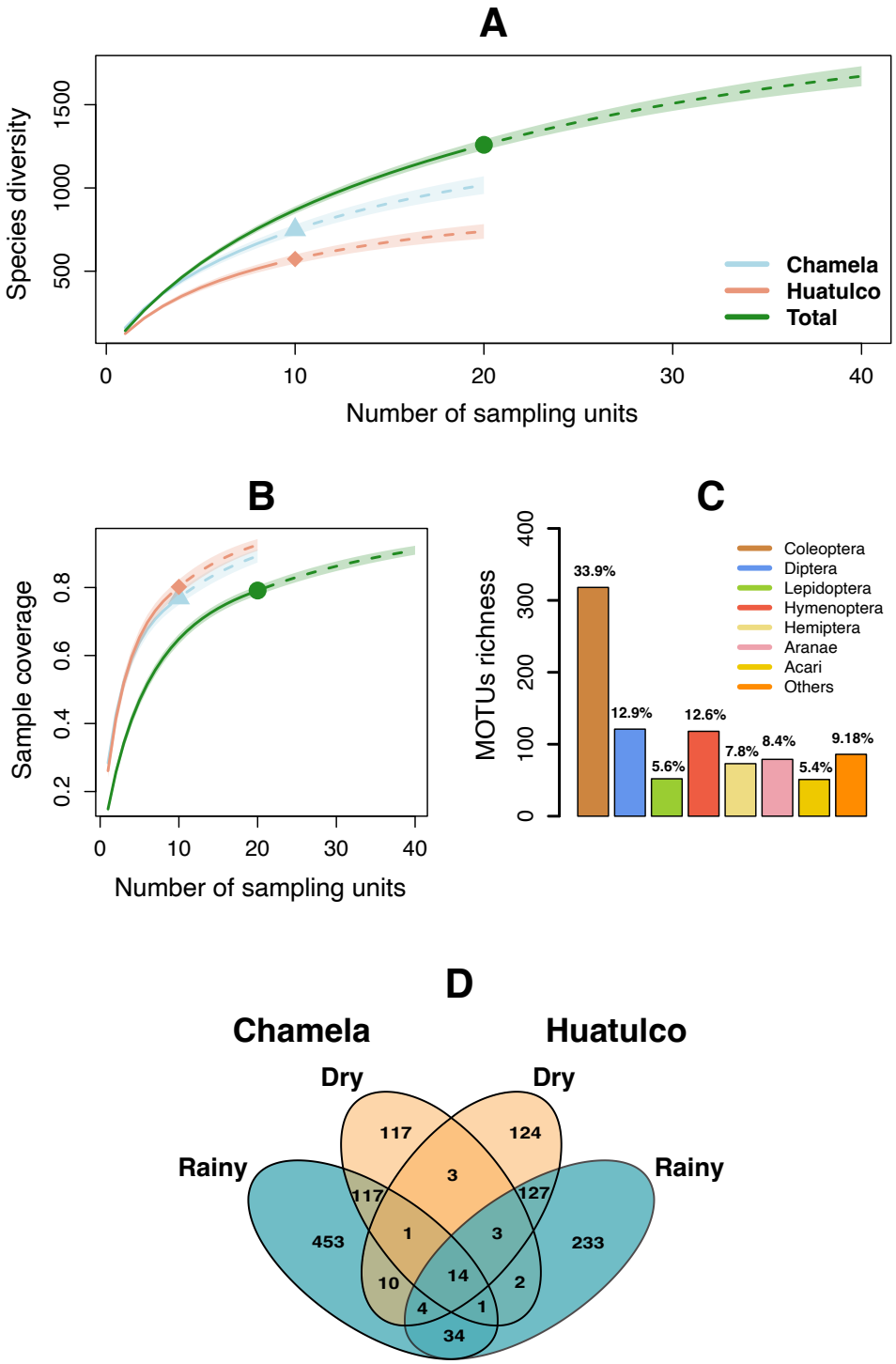

Figure 2: Species accumulation curves for leaf litter mini-Winkler extraction samples generated by iNext for Chamela and Huatulco (20 sites). **A)** Incidence frequency-based species richness (MOTUs) per sampling unit (solid line) and extrapolation (dashed line) over the total sampling period, for Chamela and Huatulco, respectively, and for the total sampling (Chamela + Huatulco). Shaded area represents 95% confidence interval. **B)** Sample coverage (sampling efficiency of sampling units to recover the expected biodiversity) per number of sampling units, for the total sampling and for Chamela and Huatulco. Accumulation curves indicated that sampling allowed nearly 80% sampling coverage of the given area and sampling period and allowed us to recover over 50% of the total leaf litter diversity, in each area. **C)** Barplot with taxonomic affiliations of MOTUs in the seven principal groups, and the rest of the lesser represented groups recovered grouped in the 'others' category, and their relative abundance, for Chamela + Huatulco, Coleoptera, Diptera, and Hymenoptera represent almost 60% of the MOTUs diversity. **D)** Venn diagram of number species MOTUs across dry (orange ellipses) and rainy (blue ellipses) season for Chamela (left ellipses) and Huatulco (right ellipses). Number of MOTUs shared between season and areas are shown in the diagram.

TABLE 1: ANOVA´s results for comparisons for All Arthropods, Coleoptera, Diptera, Hymenoptera, Hemiptera, Lepidoptera, Araneae, and Acari, between areas (Chamela and Huatulco) and between seasons (Dry and Rainy). All are parametric ANOVA but Lepidoptera, where a Kruskal-Wallis´ test was applied. *= Significantly different ($p \leq 0.017$).

	All Arthropods		Coleoptera		Diptera		Hymenoptera		Hemiptera		Lepidoptera[#]		Araneae		Acari	
Area	F	*P*	F	*p*	F	*p*	F	*p*	F	*p*	X^2	*p*	F	*p*	F	*p*
Chamela *vs* Huatulco	19.71	**<0.001***	16.86	**<0.001***	11.48	**<0.005***	47.88	**<0.001***	5.01	0.04	7.53	0.02	9.69	**<0.01***	0.02	0.89
Season	48.9	**<0.001***	66	**<0.001***	52	**<0.001***	117	**<0.001***	42.6	**<0.001***	3.6	0.08	0.7	0.42	2	0.17
Area x Season	13.84	**<0.001***	15.03	**<0.001***	23.68	**<0.001***	19.97	**<0.001***	6.82	0.02	2.94	0.11	0.1	0.76	8.2	**<0.01***
Chamela – Dry *vs* Rainy		**<0.001***		**<0.001***		**<0.001***		**<0.001***		**<0.001***		0.09		0.98		0.04
Huatulco – Dry *vs* Rainy		0.14		0.04		0.38		**<0.005***		0.06		1		0.85		0.75
Dry – Chamela *vs* Huatulco		0.96		1		0.73		0.34		0.99		0.88		0.24		0.19
Rainy – Chamela *vs* Huatulco		**<0.001***		**<0.001***		**<0.001***		**<0.001***		0.02		0.03		0.11		0.26

[#]Kruskal-Wallis test X^2

Taxonomic assignment using the BOLD database allowed MOTUs to be identified at various taxonomic levels: all to phylum, 99.4% to class, 72,8% to order, 62.8% to family, 6.3% to genus, and 1.9% to species (Supplementary Table S2). MOTUs were distributed among 8 classes, 27 orders, and 184 families. Insecta was the most species-rich class (851 MOTUs; ~70% of the total), comprising 11 orders and 123 families, with Coleoptera being the most species-rich order (318 MOTUs), followed by Diptera (121), Hymenoptera (118), Hemiptera (73), and Lepidoptera (50). Arachnida was represented by 282 MOTUs (22.8%), with Araneae (79 MOTUs) and Acari (51 MOTUs) as its most species-rich groups (Figure 2). Collembola, Diplopoda, and Chilopoda contributed smaller proportions, with 61 (5 %), 15 (1.12%), and 16 (1.3 %) MOTUs, respectively, whereas Diplura, Malacostraca, and Symphyla each contributed less than 1% of the MOTUs (Supplementary Table S2). Among the most species-rich arthropod families identified, Formicidae was the most diverse (Hymenoptera; 82 MOTUs), followed by Cecidomyiidae (Diptera; 71 MOTUs), Chrysomelidae (Coleoptera; 57 MOTUs), Curculionidae (Coleoptera; 41 MOTUs) and Staphylinidae (Coleoptera; 37 MOTUs). The subclass Acari included 20 and three families from superorders Acariformes and Parasitiformes, respectively, whereas the order Araneae was represented by 21 families.

ANOVA analyses revealed significant differences in MOTU richness between the two study areas for all arthropods combined (All Arthropods), as well as for Coleoptera, Diptera, Hymenoptera, Hemiptera, and Araneae (Table 1; Supplementary Figure S2). In both regions, species richness tended to be higher during the rainy season. In Chamela, species richness significantly differed between the rainy and dry seasons ($F= 13.84$, $p < 0.001$) for all groups except for Lepidoptera, Araneae, and Acari, whereas in Huatulco only Hymenoptera showed seasonal variation (Table 1). During the dry season, richness did not differ between Chamela and Huatulco, but in the rainy season Chamela generally supported significantly higher diversity for All Arthropods, Coleoptera, Diptera, and Hymenoptera (Table 1).

Community composition

NMDS of community composition in the two tropical deciduous forest regions revealed a clear separation in the ordination space (stress = 0.069), indicating that arthropod communities in Chamela and Huatulco are compositionally distinct (Figure 3; Figure S3). Community composition also differed considerably between seasons within each region. Although dispersion between the two areas was not significant (dispersion test's function *betadisper*: $F= 1.3$, $p = 0.32$), greater overlap between seasons was observed in Huatulco compared to Chamela (Figure 3). ANOSIM results confirmed significant differences between Chamela and Huatulco communities for all arthropods combined ($R= 0.99$, $p < 0.001$), as well as for each group analyzed

separately (Table 2). In Chamela, communities differed between seasons for all arthropods combined and each group separately, whereas in Huatulco this pattern was found for all groups except Lepidoptera and Acari. Between regions, insect communities were significantly different for all groups during the rainy season, and for all groups except Diptera during the dry season (Table 2).

Family-level analyses of community structure showed similar patterns to those observed at the MOTU level (NMDS Figure S4; PERMANOVA results Table S3), with seasonality as a dominant driver of community variability ($R^2 = 0.43$, $p < 0.001$), explaining 82% of community variance in Chamela and 46% in Huatulco ($p < 0.01$). Regardless of season, area explained 47-48% of the variance in both dry and rainy periods ($p < 0.01$; Table S3). These results reflect true shifts in community composition rather than differences in dispersion among areas and seasons (*betadisper*'s $p > 0.1$ in all cases). Specific families were identified as drivers of these patterns (envfit; Table S4), with rainy season assemblages characterized primarily by Coleopteran families: Staphylinidae ($r^2 = 0.72$, $p = 0.001$), Cantharidae ($r^2 = 0.66$, $p < 0.005$) and Lycidae ($r^2 = 0.60$, $p < 0.005$), among others. Conversely, dry season communities showed a significant contribution from the arachnid family Salticidae ($r^2 = 0.56$, $p < 0.005$) and the dipteran family Stratiomyidae ($r^2 = 0.44$, $p < 0.01$).

TraitGLM revealed distinct seasonal patterns in arthropod group prevalence. Non-insect taxa, particularly Araneae, were positively associated with the dry season in both regions, with Acari additionally associated with the dry season in Huatulco. In contrast, the rainy season in Chamela favored insect groups, specifically Hymenoptera and Diptera. No arthropod taxa were strongly associated with the rainy season in Huatulco. Overall, no arthropod group prevalence was negatively associated with any region or season (Figure 4).

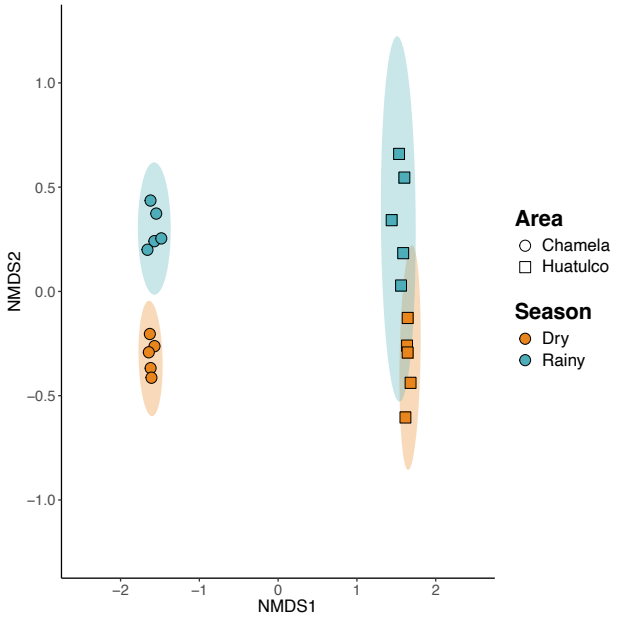

Figure 3: NMDS of community composition based on MOTUs in samples. Each point represents a sample, and distances in the NMDS space (NMDS1 vs NMDS2) reflect differences in MOTU composition; communities (sites) with more similar MOTU assemblages plot closer together. Symbols indicate locality (circles = Chamela, squares = Huatulco), and colours indicate season (blue = rainy season, orange = dry season). Group centroids and 95% confidence ellipses are shown for each site–season combination.

Beta and Zeta diversity patterns

Beta diversity index based on the Sørensen index showed high species dissimilarity between Chamela and Huatulco, with species turnover rather than nestedness

TABLE 2: Community composition comparison (ANOSIM with 9999 permutations) for Chamela and Huatulco, for All Arthropods, Coleoptera, Diptera, Hymenoptera, Hemiptera, Lepidoptera, Araneae, and Acari, between areas (Chamela and Huatulco) and between seasons (Dry and Rainy). *= Significantly different.

	All Arthropods		Coleoptera		Diptera		Hymenoptera		Hemiptera		Lepidoptera		Araneae		Acari	
Area	R	*p*	R	*p*	R	*p*	R	*p*	R	*p*	R	*p*	R	*p*	R	*p*
Chamela *vs* Huatulco	0.99	**<0.001***	0.93	**<0.001***	0.18	**<0.05***	0.92	**<0.001***	0.27	**<0.01***	0.62	**<0.001***	0.88	**<0.001***	0.54	**<0.001***
Season	0.21	**<0.05***	0.29	**<0.01***	0.22	**<0.005***	0.12	0.09	0.35	**<0.001***	0.15	0.04	0.88	**<0.001***	0.66	**<0.001***
Chamela – Dry *vs* Rainy	1	**<0.01***	1	**<0.005***	0.9	**<0.05***	0.99	**<0.01***	0.65	**<0.01***	0.5	**<0.05***	0.36	**<0.01***	0.7	**<0.01***
Huatulco – Dry *vs* Rainy	0.74	**<0.01***	0.83	**<0.01***	0.35	**<0.05***	0.53	**<0.01***	0.75	**<0.01***	0.24	0.06	0.23	**<0.05***	0.18	0.15
Dry – Chamela *vs* Huatulco	1	**<0.01***	0.97	**<0.01***	0.27	0.14	1	**<0.01***	0.45	**<0.01***	0.75	**<0.01***	1	**<0.01***	0.6	**<0.01***
Rainy – Chamela *vs* Huatulco	1	**<0.01***	0.97	**<0.01***	0.61	**<0.05***	1	**<0.01***	0.93	**<0.01***	0.62	**<0.05***	0.73	**<0.01***	0.9	**<0.01***

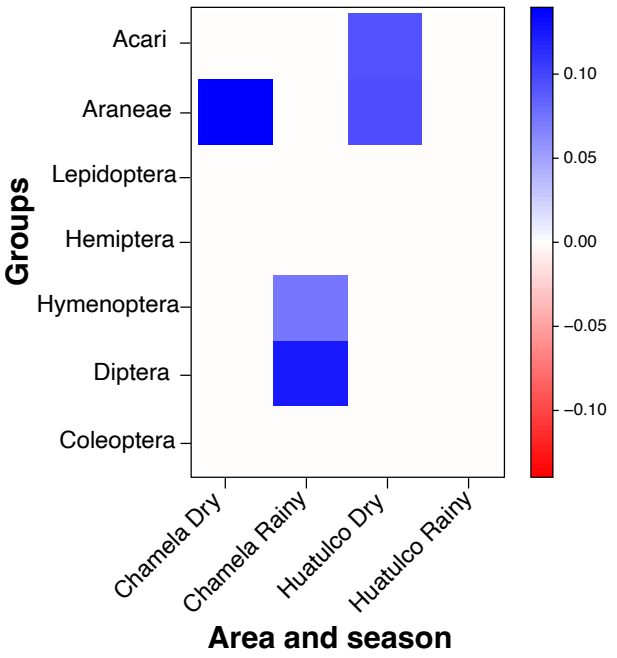

Figure 4: Heatmap of the prevalence of arthropod groups across areas and seasons, based on traitGLM analyses with LASSO penalty. The color gradient represents the strength and direction of the associations between the seven most represented arthropod groups and the combined effects of area and season, with blue indicating negative relationships and red indicating positive ones.

shaping community differences (Table 3). High turnover was observed both between and within regions and across seasons in each area (Table 3), indicating that species replacement rather than simple species loss or gain is the main driver of species change.

Zeta diversity model selection based on AIC showed contrasting patterns depending on spatial scale: between Chamela and Huatulco, species change across sites was best explained by a Nearest-Neighbour model, suggesting spatially structured and abrupt turnover between regions (ΔAIC > 30). In contrast, within each region, a non-geographic model best explained the turnover pattern (ΔAIC > 10 for both Huatulco and Chamela). In all cases, zeta diversity analysis revealed a sharp decline in species shared across multiple samples, with almost no species shared by four or more samples, whether considering both regions together (Chamela + Huatulco) or each separately (Figure 5). Most arthropod groups exhibited rapid declines with increasing number of compared sites, with Coleoptera, Hymenoptera, and Lepidoptera present across all sites in Chamela, and only Hymenoptera present in Huatulco (Figure 5). When both regions were analyzed together (Chamela + Huatulco), all groups exhibited steep declines in species retention, with no species shared across more than 10 sites (Figure 5). In all cases (Huatulco + Chamela, and Chamela and Huatulco separately) zeta diversity analysis fitted a power law better than an exponential model (ΔAIC > 10; Figure S5).

TABLE 3: Species β-diversity measured with Sørensen index, between areas (Chamela and Huatulco), within areas, and between seasons (dry and rainy seasons). Total β-diversity (left, beta.SOR) was partitioned into turnover (beta.SIM) and nestedness (beta.SNE). β-diversity (0→1) near 1 means that insect assemblages have different MOTUs, near 0 means that they have the same MOTUs. Turnover (0→1) close to 1 means that dissimilarity is driven only by replacement of MOTUs (i.e., without changes in richness). Nestedness close to 1 means that dissimilarity is driven only by differences in species richness.

		Turnover (beta.SIM)	Nestedness (beta.SNE)	Dissimilarity (beta.SOR)
Chamela vs Huatulco		0.912	0.024	0.936
Chamela		0.796	0.066	0.861
Huatulco		0.824	0.039	0.863
Season				
Chamela	Cham_1	0.618	0.124	0.742
	Cham_2	0.762	0.094	0.856
	Cham_3	0.534	0.228	0.762
	Cham_4	0.565	0.220	0.784
	Cham_5	0.562	0.177	0.739
Huatulco	Huat_1	0.645	0.123	0.767
	Huat_2	0.714	0.086	0.800
	Huat_3	0.814	0.057	0.871
	Huat_4	0.689	0.020	0.709
	Huat_5	0.532	0.125	0.657

Discussion

Our study provides one of the most comprehensive assessments to date of leaf litter arthropod diversity and community structure in Mexican tropical deciduous forests, creating an important taxonomic and molecular baseline database for this group of organisms across the two studied regions. We detected 1238 arthropod MOTUs across the two protected areas, with diversity estimates suggesting that the actual richness likely exceeds 1,800–1,900 MOTUs. This highlights the extraordinary diversity harbored in this biome and the effectiveness of metabarcoding for capturing more than half of this hidden biodiversity, particularly among small-bodied and cryptic taxa that are often overlooked by traditional methods (Taberlet et al., 2012).

The leaf litter arthropod assemblages showed high diversity coupled with marked spatial and temporal

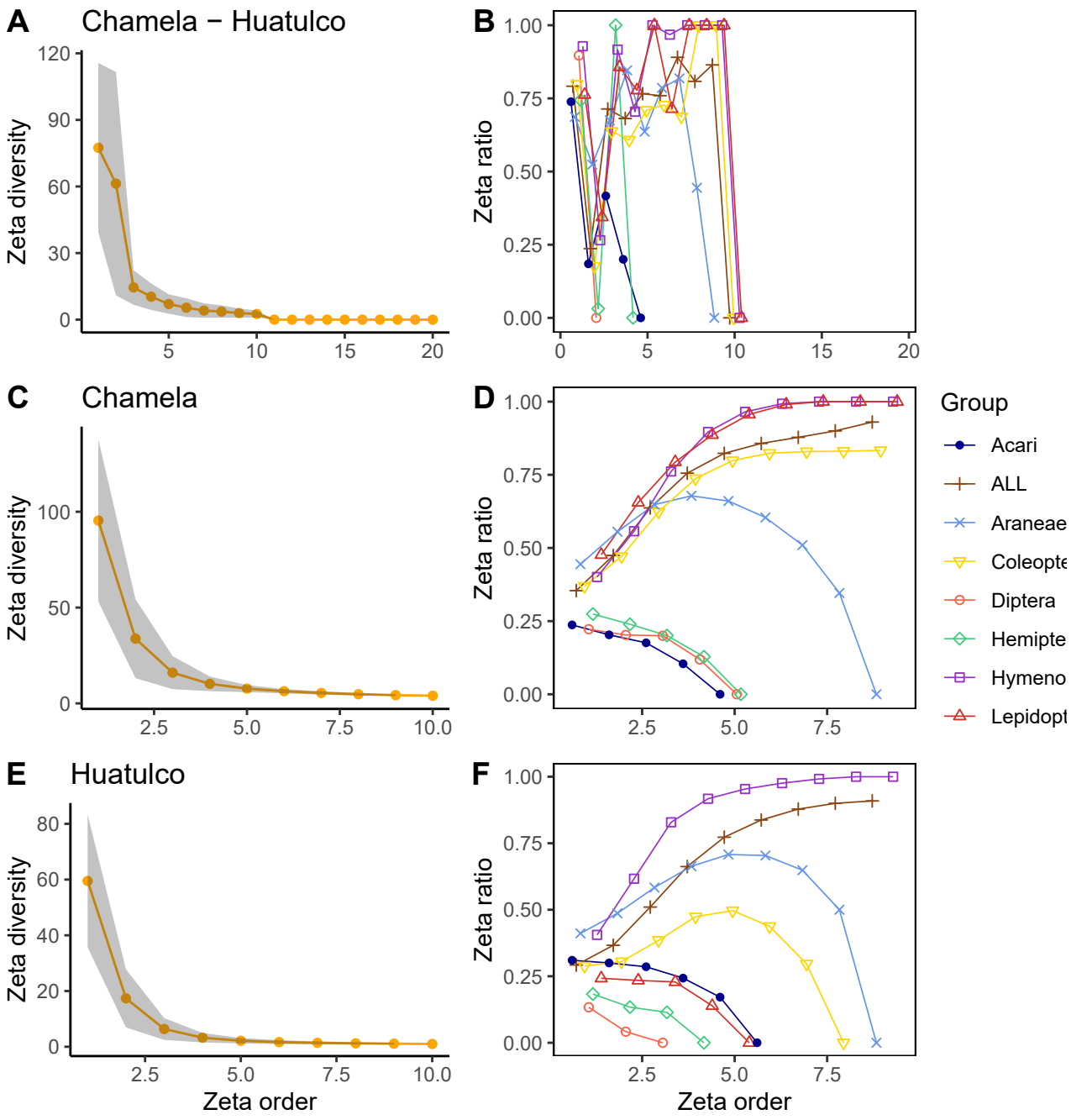

Figure 5: Zeta-diversity decline and 0–1 scaled zeta ratio (i.e., the retention probability of common species in the community) for species shared across sites in Chamela and Huatulco. Panels A–B show Chamela + Huatulco combined, C–D Chamela only, and E–F Huatulco only, for all MOTUs and for the seven main arthropod groups. Zeta-diversity decline curves (**A, C, E**) indicate strong compositional turnover across sites, with the number of shared species dropping sharply beyond 2–3 sites, few species shared among four sites, and virtually none shared when more than four sites are considered. The zeta ratio (**B, D, F**) highlights the high prevalence of the small subset of species likely to be sampled across sites: Hymenoptera and Lepidoptera in Chamela, and Hymenoptera in Huatulco, retain shared species across all sites, whereas no species are shared across all sites when Chamela and Huatulco are analyzed together.

turnover and distinct community compositions between areas and seasons. The dominant representation of the insect orders Coleoptera, Diptera, and Hymenoptera–defined by the high proportion of MOTUs assigned to these orders–is consistent with previous studies conducted in tropical forests (Wardle et al., 2006; Vasconcelos et al., 2005; Cole et al., 2016; Beng et al. 2018; Cole et al. 2019). The high richness of Formicidae (ants), Cecidomyiidae (gall midges), and several beetle families emphasizes the ecological importance of these groups in leaf litter processes such as decomposition, nutrient cycling, predation, as well as the above- and below- ground communities mediated by fallen galls and fungal associations. Arachnids, particularly spiders (Araneae) and mites (Acari), also contributed substantially to the community composition, reflecting their roles as predators and detritivores. Below, we discuss in detail the importance of the arthropod taxonomic and molecular baseline database generated for Neotropical tropical deciduous forests, the spatial and temporal patterns observed, and the ecological and conservation implications derived from our results.

Diversity and composition of leaf litter arthropods in tropical deciduous forests

Consistent with previous studies in these forests using different collecting techniques, we found that most arthropod MOTUs were collected during the rainy season (Cole et al., 2019). Arthropod species richness is strongly influenced by moisture availability, as many species tend to move into deeper soil layers in response to changes in humidity, resulting in a higher species richness during the rainy season than in the dry season (Gómez-Anaya & Palacios-Vargas 2004; Palacios-Vargas et al., 2007). Our results, however, provide more detailed information on the specific higher arthropod taxa occur across seasons, offering valuable insights that can guide future taxonomic surveys and the discovery of new species.

While information on arthropod diversity in leaf litter from tropical forests is generally available (Cole et al., 2016, Villaseñor-Amador et al., 2024, Ahuatzin et al., 2019, Basset et al., 2015), much less is known specifically about Mexican tropical deciduous forests (Palacios-Vargas et al., 2007). The few studies conducted in these forests have identified Acari and Collembola as the dominant arthropod groups. For instance, Palacios-Vargas et al. (2007) found Prostigmata (Acari), Cryptostigmata (Acari), Collembola, and Mesostigmata (Acari) to be the most abundant orders based on individual counts without distinguishing species or morphospecies. In contrast, our metabarcoding-based analysis, which reflects taxonomic diversity rather than abundance, revealed a predominance of insects, high diversity of Acari, and a poor representation of Collembola. The differences between both studies are likely explained by methodological factors: Palacios-Vargas et al. (2007) sampled mainly soil (11 cm in diameter and 5 cm in depth), whereas we collected a larger volume of leaf litter, which favors the detection of more vagile litter-dwelling organisms and a more complete representation of their diversity.

Comparisons with other studies in Mexican tropical deciduous forests further underscore the efficiency of metabarcoding for assessing arthropod diversity. For instance, Cole et al. (2016), using pitfall traps over two weeks in both the dry and rainy seasons, recorded 25 orders, 52 families, and 302 species or morphospecies, whereas our metabarcoding approach recovered more than 1200 species in a considerably shorter period. Similarly, Pérez-Toledo et al. (2020) documented 145 ant species along an elevational gradient, while we identified 82 species (52%) at sea level. Likewise, Villaseñor-Amador et al. (2024) found 55 morphospecies of weevils in leaf litter along an elevational gradient in El Cielo in northern Mexico, whereas our study alone detected 44 species. Altogether, these comparisons illustrate the remarkable ability of metabarcoding to reveal hidden arthropod diversity, even from limited temporal sampling.

While nearly all MOTUs were identified to order and most to family, only 6.3% reached the genus and 1.9% the species level. Our ability to assign MOTUs to lower taxonomic ranks was constrained by gaps in the BOLD reference database, accentuating the prevalence of "dark taxa" in these forests and emphasizing the urgent need to expand and improve DNA barcode libraries for Neotropical arthropods. Similarly, in a recent comprehensive metabarcoding survey of insects along an elevational gradient in subtropical Mexico (Villaseñor-Amador et al. 2025), only 22.6% of MOTUs matched any DNA vouchered specimens in BOLD or GenBank, and only 6.4% MOTUs matched sequences identified to species level in these databases. Strengthening these databases is crucial not only to enhance biodiversity assessments but also to support ecological and conservation research aimed at elucidating the factors that drive arthropod diversity and turnover in these forests (Hebert et al., 2016).

Spatial and seasonal variation in arthropod community structure

Our results reveal significant differences in arthropod richness and community composition between the tropical deciduous forests of the Chamela and Huatulco reserves,

as well as between rainy and dry seasons. These findings are consistent with previous results obtained for these areas using Malaise-sampled insects (Benites et al. 2025). Such patterns likely reflect both regional environmental variation and the pronounced seasonality characteristic of tropical deciduous forests. Seasonal changes in temperature, moisture, and resource availability can strongly influence arthropod activity, abundance, and community turnover (Bestelmeyer et al., 2000). The observed differences among key groups–such as Coleoptera, Diptera, Hymenoptera, and Araneae–suggest that both spatial and temporal heterogeneity are important drivers of diversity in the leaf litter arthropod community of these forests (Palacios-Vargas et al., 2007; Basset et al., 2015).

The intensity of the seasonal effect was particularly evident in Chamela at the family level, where it accounted for 82% of the community variance, suggesting a near-complete taxonomic turnover considerably more pronounced than the 46% observed in Huatulco. While both areas are seasonally deciduous, Huatulco lies at the "wetter end" of the Mexican tropical deciduous forest gradient, with substantially higher mean annual precipitation than Chamela (Alvarado and Terrazas, 2023; Takano-Rojas et al., 2023). Therefore, arthropod communities in Chamela may be more sensitive to or experience harsher hydric stress conditions. This pattern, where seasonal compositional change appears more pronounced in Chamela than in Huatulco, was also reported for flying insects in these areas (Benites et al., 2025).

Seasonality affected species composition more strongly than species richness in Huatulco, implying that intra-annual variability resulted primarily in species replacements rather than net losses and gains within the different arthropod groups. Moreover, Hymenoptera were positively associated with the rainy season in both areas. Leaf-litter ants, as the most abundant family within Hymenoptera, have been shown to respond strongly to both local and regional climatic variation, particularly to precipitation and temperature along tropical elevational and environmental gradients (Longino & Colwell 2011; Longino & Branstetter 2019; Pérez-Toledo et al., 2020). These findings support the view that both spatial heterogeneity and strong seasonal fluctuations play key roles in structuring arthropod communities in tropical deciduous forests. Seasonality also affected arthropod prevalence differently: insect groups were significantly associated with the rainy season, whereas spiders and mites were positively associated with the dry season. Higher density of Acari has been reported in dry season, a pattern associated with litterfall seasonality and organic matter decomposition (Palacios-Vargas et al., 2007). Our family-level environmental fitting identified the arachnid family Salticidae as a primary driver of dry season community assemblages, while rainy season was defined by a coleopteran explosion, with predatory and fungivorous families driving these assemblages. This is not surprising, as Coleoptera was the most species-rich order; however, it also suggests that this insect explosion is tightly coupled with the onset of the rains, while arachnids may be better adapted to the dry season (Hernández and Caballero, 2016; Martínez-Hernández et al., 2022; Mavasa et al., 2022).

Patterns of beta and zeta diversity further supported these spatial and seasonal trends. Both indices revealed high species turnover between localities and seasons, indicating strong spatial structuring and temporal replacement of taxa. The steep decline in zeta diversity across sample orders reflects the predominance of rare and site-restricted MOTUs, suggesting that most species occur in few samples and that communities are largely composed of locally unique taxa. This high compositional turnover underscores the strong influence of environmental heterogeneity and seasonal dynamics on arthropod community assembly in tropical deciduous forests (Hui & McGeoch, 2014). Significant community turnover was observed even between proximate sites (approximately 650 m), suggesting a critical role of micro habitat environment in shaping leaf-litter arthropods communities. Local variables that can vary significantly over short distances, such as canopy openness, leaf-litter depth, soil moisture retention, and vegetative composition, can influence the physical and chemical properties of leaf litter, creating distinct microhabitats. The fact that a non-geographic model best explained the turnover pattern as well as the turnover pattern fitting a power law, supports that niche differentiation, rather than purely stochastic processes, drives species turnover and distribution patterns in these forests (McGeoch et al. 2019).

A total of 36.5% of the MOTUs recovered in leaf litter were also recorded in a six-month Malaise trap metabarcoding survey conducted previously in the same tropical deciduous forest regions (Benites et al., 2025). Specifically, between 30% and 40% of the MOTUs belonging to Araneae, Coleoptera, Diptera, and Hymenoptera were shared between both sampling methods, whereas only about 5% of Acari species detected here were captured by Malaise traps. Notably, approximately 75% of the leaf litter lepidopteran MOTUs were also recorded in the Malaise sampling, with nearly

90% of the Lepidoptera species present in both datasets corresponding to predominantly nocturnal taxa whose larvae typically move and forage within the leaf litter (Dugdale, 1996; Hohn & Wagner, 2000; Legal, 2022). The number of species shared between leaf litter and Malaise sampling indicates that above- and belowground arthropod communities are closely connected, showcasing the importance of leaf litter as both a refuge and a foraging resource in seasonally harsh environments. The proportion of shared MOTUs was higher in Chamela (40%) than in Huatulco (29%), which may reflect greater ecological stability and a stronger coupling between ground and aerial arthropod assemblages in the former site. However, this pattern may be partly influenced by the higher number of Malaise traps used in Chamela, which likely increased sampling completeness (Benites et al. 2025).

Together, our results reveal the interplay between spatial and seasonal factors in shaping arthropod community structure in Neotropical tropical deciduous forests and underscore the complementary nature of ground and above-ground sampling, as well as the key role of leaf litter in sustaining arthropod diversity (Bardgett & Wardle, 2010; Basset et al., 2015). The consistency of our findings at both MOTU and family level reinforces the robustness of the observed spatio-temporal patterns. While species-level turnover was remarkably high, significant differences in composition at family level indicates that factors shaping species distribution also act at broader taxonomic resolution.

Sampling completeness and methodological considerations

Although we recovered a substantial proportion of the estimated total diversity (~66%), rarefaction and extrapolation curves indicate that additional sampling would be required to approach an asymptote, particularly for highly diverse or under-sampled groups such as Diptera. The use of mini-Winkler extractors combined with bulk-sample metabarcoding proved highly effective for capturing a broad spectrum of leaf litter arthropods. Nevertheless, future studies could benefit from integrating complementary collecting techniques–such as pitfall traps–or alternative high-throughput strategies like megabarcoding (Meier et al., 2024; Meier et al., 2025; Souto-Vilarós et al., 2025). Recent multi-protocol metabarcoding surveys in tropical forests have shown that combining multiple trap types (e.g. Winkler, pitfall, Malaise, light traps) substantially increases taxonomic coverage and sampling efficiency (Souto-Vilarós et al., 2025), which would ultimately improve the completeness of reference databases and biodiversity assessments in tropical deciduous forests arthropod communities.

Methodological limitations should also be considered when interpreting our results. Beng et al. (2018) found that single sampling events can capture less than 25% of the arthropod diversity collected over a 12-month period in leaf litter, with substantial variation depending on the sampling month and taxonomic group. This limited temporal coverage can influence biodiversity estimates and community composition patterns. Additionally, climatic phenomena affecting the Pacific coast, such as El Niño Southern Oscillation and La Niña, considerably influence the intensity and duration of dry and rainy seasons and can therefore shape long-term arthropods community assemblages in these forests (Beng et al. 2018). Single-year surveys, such as the one presented in here, may underestimate the underlying complexity of multi-year biodiversity dynamics. Our design would therefore benefit from extending the sampling period to capture a more representative fraction of the annual arthropod diversity, as suggested by our coverage estimate exceeding 60%, as well as year-to-year sampling and comparison.

Despite the low percentage of MOTU assigned to species, the consistency of our findings at family level highlights its utility when studying highly diverse communities as the tropical deciduous forests. The fact that seasonal and geographical signal persists, and it is even amplified, at the family level suggests that biodiversity monitoring in these megadiverse biomes can remain considerably informative even when species-level identification is limited.

Ecological and conservation remarks

The high diversity and distinct community composition observed between sites and seasons emphasize the ecological complexity and conservation value of Mexican tropical deciduous forests. These forests face ongoing threats from land-use change, fragmentation, and climate variability (Miles et al., 2006). Our findings underscore the need to protect multiple reserves and to consider temporal dynamics in management plans, as both spatial and seasonal variation contribute to overall biodiversity. Furthermore, the predominance of rare and site-specific MOTUs, as suggested by beta and zeta diversity analyses, indicates that local extinctions or habitat loss could result in disproportionate losses of unique taxa.

Disturbances such as fire, logging, or conversion to agriculture reduce litter depth and alter microhabitat conditions, negatively impacting arthropod communities (Cole et al. 2016; Mbenoun Masse & Yede, 2017). Recovery of arthropod diversity after disturbance is typically slow, even when litter dynamics (*e.g.,* litterfall

and decomposition rates) recover more rapidly (Cole et al., 2019). Conservation and restoration strategies that promote habitat heterogeneity and maintain or enhance litter layers are therefore more effective in supporting arthropod abundance and functional diversity (Meloni & Varanda 2015; Cole et al. 2016). Management plans should therefore prioritize both habitat heterogeneity and connectivity to sustain both local and regional leaf litter diversity.

Leaf litter offers arthropods microclimatic buffering against climatic extremes and provides essential food resources under harsh conditions, particularly in regions with high seasonality or temperature variability such as tropical deciduous forest (Decaëns et al., 2006; Hamilton, 2015). The importance of these refugia is expected to increase under ongoing climate change, as they help mitigate the effects of drought and temperature fluctuations on arthropod persistence. Maintaining litter layers and habitat heterogeneity is thus crucial for conserving arthropod communities and the ecological processes they support.

Finally, our results emphasize the importance of generating comprehensive metabarcoding reference libraries and baseline datasets for Neotropical arthropods. Such resources are critical for improving taxonomic resolution in biodiversity assessments and serve as long-term benchmarks for monitoring community change and evaluating the effectiveness of conservation actions in tropical dry forests. Yet, we are only exploring the tip of the biodiversity iceberg, as the vast majority of tropical arthropod species remain undescribed (Stork, 2018; Chimeno et al, 2023). Addressing the current Linnaean shortfall is therefore essential to fully realize the potential of metabarcoding approaches in documenting and conserving tropical arthropod diversity.

Author Contributions

A.Z.R. and P.B. conceived the research; P.B., A.Z.R., K.P.A., F.E.Z.H., N.B.B., A.G.B., and M. J. participated in fieldwork; P.B., K.P.A., A.G.B., and A.M.P.D. contributed to sample preparation for DNA extraction and sequencing; P.B. and K.P.A. conducted data curation and data analysis; P.B. and A.Z.R. wrote the manuscript. All authors reviewed and approved the final version
of the manuscript.

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Data Availability

Raw sequence data generated in this study have been deposited in the National Center for Biotechnology Information (NCBI) Sequence read Archive (SRA) under BioProject ID: PRJNA1436763 (study accession number: SRP683543; BioSample accessions: SAMN56483305, SAMN56483306)."

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