Unraveling habitat-driven shifts in alpha, beta, and gamma diversity of hummingbirds and their floral resource (#95322)

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Unraveling habitat-driven shifts in alpha, beta, and gamma diversity of hummingbirds and their floral resource

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Background . Biodiversity, crucial for understanding ecosystems, encompasses species richness, composition, and distribution. Ecological and environmental factors shape species diversity in communities, categorized into alpha (within habitat), beta (between habitats), and gamma (total region) diversity. Hummingbird communities are influenced by habitat, elevation, and seasonality, making them an ideal system for studying these diversities, shedding light on mutualistic community dynamics and conservation strategies.**Methods**. Over a year-long period, monthly surveys were conducted to record hummingbird species and their visited flowering plants across four habitat types (oak forest, juniper forest, pine forest, and xerophytic shrubland) in Tlaxcala, Mexico. Three locations per habitat type were selected based on conservation status and distance from urban areas. True diversity measures were used to assess alpha, beta, and gamma diversity of hummingbirds and their floral resources. Environmental factors such as altitude and bioclimatic variables were explored for their influence on beta diversity.**Results**. Our data reveal high heterogeneity in species abundance among habitats. For flowering plants, gamma diversity encompassed 34 species, with oak forests exhibiting the highest richness, while xerophytic shrublands had the highest alpha diversity. In contrast, for hummingbirds, 11 species comprised the gamma diversity, with xerophytic shrublands having the highest richness and alpha diversity. Notably, certain floral resources like *Loeselia mexicana* and *Bouvardia ternifolia* emerge as key species in multiple habitats, while hummingbirds such as *Basilinna leucotis*, *Selasphorus platycercus*, and *Calothorax lucifer* exhibit varying levels of abundance and habitat preferences. Beta diversity analyses unveil habitat-specific patterns, with species turnover predominantly driving dissimilarity in composition. Moreover, our study delves into the relationships between these diversity components and environmental factors such as altitude and climate variables. Climate variables, in particular, emerge as significant contributors to dissimilarity in floral resource and hummingbird communities, highlighting the influence of environmental conditions on species distribution. **Conclusions**. Our results shed light on the complex dynamics of hummingbird-flower mutualistic communities within diverse habitats and underscore the importance of understanding how habitat-driven shifts impact alpha, beta, and gamma diversity. Such insights are crucial for conservation strategies aimed at preserving the delicate ecological relationships that underpin biodiversity in these communities.

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Abstract

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Methods. Over a year-long period, monthly surveys were conducted to record hummingbird species and their visited flowering plants across four habitat types (oak forest, juniper forest, pine forest, and xerophytic shrubland) in Tlaxcala, Mexico. Three locations per habitat type were selected based on conservation status and distance from urban areas. True diversity measures were used to assess alpha, beta, and gamma diversity of hummingbirds and their floral resources.

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Results. Our data reveal high heterogeneity in species abundance among habitats. For flowering plants, gamma diversity encompassed 34 species, with oak forests exhibiting the highest richness, while xerophytic shrublands had the highest alpha diversity. In contrast, for hummingbirds, 11 species comprised the gamma diversity, with xerophytic shrublands having the highest richness and alpha diversity. Notably, certain floral resources like *Loeselia mexicana* and *Bouvardia ternifolia* emerge as key species in multiple habitats, while hummingbirds such as *Basilinna leucotis*, *Selasphorus platycercus*, and *Calothorax lucifer* exhibit varying levels of abundance and habitat preferences. Beta diversity analyses unveil habitat-specific patterns, with species turnover predominantly driving dissimilarity in composition. Moreover, our study delves into the relationships between these diversity components and environmental factors such as altitude and climate variables. Climate variables, in particular, emerge as significant contributors to dissimilarity in floral resource and hummingbird communities, highlighting the influence of environmental conditions on species distribution.

Conclusions. Our results shed light on the complex dynamics of hummingbird-flower mutualistic communities within diverse habitats and underscore the importance of understanding how habitat-driven shifts impact alpha, beta, and gamma diversity. Such insights are crucial for conservation strategies aimed at preserving the delicate ecological relationships that underpin biodiversity in these communities.

Introduction

The study of biodiversity, the intricate interplay of life forms within ecosystems, serves as a means to depict the structural patterns in communities because it is a key indicator of their complexity, interactions, and stability (*Tilman, Reich & Knops, 2006*; *Campbell, Murphy & Romanuk, 2011*). Its study extends beyond a mere cataloging of species; it involves a comprehensive examination of the richness, composition, and distribution of species, spanning from local to regional scales (*Jost, 2006*). Ecological factors, both biotic (i.e., species interactions) and abiotic (e.g., temperature and precipitation), influence the distribution of species and population density within a community (*Pearson & Dawson, 2003*; *Benton, 2009*). These environmental and biological factors act as filters that determine which species can survive and thrive in a specific area, and their coexistence is contingent upon their specific needs and requirements based on competition for resources (*Wisz et al., 2013*). This way, diversity within communities is primarily shaped by these ecological processes (*Chesson, 2000*).

It is widely recognized that species diversity exhibits spatial heterogeneity. For example, at a regional scale, significant disparities in species richness have been widely documented among habitats (e.g., *MacArthur, 1965*; *Būhning-Gaese, 1997*). These spatial trends have given rise to the concept of three levels of species diversity: alpha (α), beta (β), and gamma diversity (γ) (*Whittaker, 1960*). The partitioning of biodiversity into three components offers a powerful framework to unravel the intricacies of these diversity patterns. Firstly, alpha diversity characterizes species richness and abundance within a single habitat, providing insights into the structure of local communities. Secondly, beta diversity quantifies the turnover of species between habitats, shedding light on the ecological processes driving community assembly and turnover. Lastly, gamma diversity, encompassing total species richness across multiple habitats, reflects broader regional diversity patterns (*Whittaker, 1960*). Fundamental topics in ecological research have revolved around distribution patterns and mechanisms that maintain species diversity across environmental gradients (*Lyons & Willig, 2002*; *McCain, 2009*; *Wang et al., 2017*). Understanding these patterns and mechanisms is crucial for devising strategies and measures aimed at preserving species diversity in the face of environmental changes.

Because of their feeding ecology, hummingbirds (Aves: Trochilidae) are closely tied to their floral resources (*Abrahamczyk & Kessler, 2015*). Their extreme specialization in dependence on nectar consumption has led these tiny birds to often track the availability of nectar sources by following the blooming of flowers, an ability that enables them to survive and thrive in various habitats across the Americas (*Leimberger et al., 2022*). The dynamics shaping hummingbird communities have been explored in numerous studies, revealing an intriguing trend. Hummingbird communities in low-lying habitats (≤ 50 m a. s. l.), encompassing both dry and humid forests, experience an upsurge in both species richness and abundance (*Buzato, Sazima & Sazima, 2000*). In contrast, a different scenario unfolds in habitats surrounded by temperate vegetation at higher and colder elevations (> 2000 m a. s. l. with temperatures around -5°C), such as cloud forests and coniferous forests. In these habitats, there is a tendency for a decrease in the richness and abundance of hummingbird species (*Graham et al., 2009*; *Partida-Lara et al., 2018*). Interestingly, this general pattern doesn't account for the remarkable species richness in the montane region of the Andes, where elevation has instead generated diverse topographical features that have promoted high speciation rates (*Rahbek et al., 2007*).

In addition to the habitat type's impact on the structure of hummingbird communities, seasonality also exerts an effect due to variations in environmental variables that directly influence the floral resources they utilize, such as precipitation. In this regard, it has been demonstrated that in habitats with scarce precipitation, such as tropical dry forests, the peak flowering of plants visited by hummingbirds primarily occurs during the dry season (*Arizmendi & Ornelas, 1990*; *Bustamante-Castillo, Hernández-Baños & Arizmendi, 2018*). Conversely, in temperate environments such as conifeous forests, the flowering peaks of these plants align with the rainy season (*Des Granges, 1979*; *Lara, 2006*). In response to this seasonal effect in the environment, there is typically a positive relationship where a greater number of flowers (i.e., flowering peaks) denotes higher diversity and abundance of hummingbirds at the local level (*Cotton, 2007*). Therefore, the dynamics of this relationship over time can led hummingbird communities to undergo restructuring (*Wolf, Stiles & Hainsworth, 1976*; *Arizmendi & Ornelas, 1990*; *Lara, 2006*).

The interaction between hummingbirds and flowers is an ideal context to explore the three diversity components. The diversity of both these groups may be influenced by factors such as resource availability, and habitat specialization. By dissecting the alpha, beta, and gamma diversity patterns within this context, we aim to uncover the mechanisms driving the assemblages and maintenance of these intricate mutualistic communities. Central Mexico is a hotspot of ecological diversity, characterized by its varied topography, altitude gradients, and climatic variability (*Sánchez-Cordero et al., 2005*). This ecological heterogeneity provides a unique backdrop for exploring biodiversity patterns and underlying ecological processes. Among the states within this region, Tlaxcala, the smallest state in the country (after the capital Mexico City), holds a unique geographical position that facilitated the collection of comprehensive data on the diversity of hummingbirds and their flowers across different vegetation types. This provided insights into the dynamics of these communities within a confined yet ecologically diverse area. The main goal of our research was to unravel the alpha, beta, and gamma diversity patterns within hummingbird-flower communities across the most representative habitats of the region: the oak forest, pine forest, juniper forest, and xerophytic scrubland. These habitats encompass environmental conditions ranging from typically humid and cold to dry and warm and are mainly found covering altitudinal ranges from 2400 to 2700 m a.s.l., although pine forests can be found at elevations as high as 4000 m a.s.l. at the highest point in the region, La Malinche volcano. Considering the variability in our studied habitats, we expected significant variations in alpha, beta, and gamma diversity in hummingbird-flower communities across oak forest, pine forest, juniper forest, and xerophytic scrubland habitats due to their distinct environmental conditions. Additionally, we hypothesized that abiotic factors such as altitude, temperature, humidity, and resource availability would influence species composition between these habitats (beta diversity). Finally, we expected higher alpha diversity in habitats with more varied conditions, while beta diversity will likely correlate with specific environmental factors distinguishing each habitat. Our study holds theoretical significance in elucidating the complexities of alpha, beta, and gamma diversity within mutualistic systems. Moreover, from a practical standpoint, our findings can inform conservation strategies aimed at preserving the delicate ecological relationships that underpin the biodiversity of these communities.

Materials & Methods

Study area

From February 2014 to January 2015, samplings were carried out in four types of vegetation (hereafter referred to as “habitats”) characteristic of the state of Tlaxcala, Mexico: oak forest (OF), juniper forest (JF), pine forest (PF), and xerophytic shrubland (XS). Based on digital land use and vegetation maps at a 1:250,000 scale, as well as information about the vegetation within the state of Tlaxcala (*INEGI, 2009, 2010*; *Acosta, Delgado & Cervantes, 1992*; *Luna, Morrone*

& Espinoza, 2007), three locations were selected for each habitat (Figure 1). For their selection, these locations met the following requirements: (i) belong to conserved areas according to *INEGI* (*2010*), (ii) be distant from areas [illegible] km), and urban (iii) be separated from each other to ensure sampling independence (average distance between locations greater than 13 km). Subsequently, for each habitat and covering the three selected locations, five 500 m transects were placed with 20 m wide bands on each side, and a minimum distance of 100 m between transects. For each transect, its georeference and altitude (m a.s.l.) were obtained using a portable GPS (Garmin Etrex 30). A total of 20 transects were obtained for the four habitat types.

In each location, the transects were established in sites that could encompass the dominant tree species for the habitat type. In OF, species of the *Quercus* genus predominate, such as *Q.* crassipes, *Q. glaucoides*, *Q. laurina*, and *Q. mexicana*. The dominant tree species in JF is *Juniperus deppeana*. In PF, characteristic species include *Pinus montezumae*, *P. hartwegii*, *P. patula*, and *P. leiophylla*. Finally, in XS, dominant species include *Yucca filifera*, *Nolina longifolia*, *Dasylirion acrotriche*, and *Opuntia robusta* (Figure 1).

Sampling of hummingbirds and their flower plants

To identify and quantify the abundance of hummingbirds (H) and the flowering plants they visited (FP), monthly surveys were conducted over a 12-month period at five transects established for each habitat type. Sampling was carried out from 8:00 to 13:00 h. During this period, all the hummingbirds detected within the transect were recorded, whether they were observed foraging on the flowers, perched, or in flight. The observed individuals were identified with the assistance of specialized field guides (*Williamson, 2001*; *Arizmendi & Berlanga, 2014*). Using this information, we obtained the number of individuals per hummingbird species for each survey.

Concurrently, all FP species within a transect (i.e., plants exhibiting tubular flowers, bright colors, and nectar production; *Faegri & van Der Pijl, 1979*) were recorded. Species that did not fit the proposed ornithophilous syndrome were also included in the records if hummingbirds were observed foraging on them. Floral abundance was measured as the number of open flowers per plant species in each transect. The identification of FP species was conducted using dichotomous keys (*Calderón & Rzedowski, 2001*). Assessment of sample completeness (sample coverage) across samples at each habitat type for H and FP species was performed in RStudio, ver. 2023.03.0+386 (*RStudio Team, 2022*) with 'iNext' function in the iNEXT package (*Hsieh, Ma, & Chao, 2016*).

True diversity measures

To assess the structure and differences in H and FP assemblages in the region, we performed an analysis of regional diversity (gamma diversity) by considering all habitats as a unit. Additionally, we conducted a detailed analysis of local diversity within each habitat (alfa diversity), examined how the respective assemblages differ between communities (beta diversity), and explored the origins of differences among habitats, including species turnover and variations in species richness. Furthermore, we assessed the potential role of environmental factors in explaining differences between communities within each habitat. These concepts are pivotal for understanding biological processes across diverse habitats, the structure of biological communities, and the distribution of species at local and regional level. Their practical applications extend to environmental management and conservation of biodiversity.

Each diversity index, H, can be expressed as its true diversity index or equivalent numbers ($^{q}D(H)$), also referred to as Hill numbers (*Jost, 2006*; *Moreno et al., 2017*). Equivalent numbers represent the essential components (i.e., species, communities) that a balanced community with equally common species would possess, assuming that the diversity index of the balanced community matches that of the real community (*Jost, 2006, 2010*; *Pereyra & Moreno, 2013*). Thus, effective numbers depict the structure of the real community in equivalent units, enabling comparisons of the degree of change between communities (*Jost, 2006*; *2007*). Effective numbers ^{q}D derived from the following formula (*Jost, 2007*):

$$^{q}D = (\sum_{i=1}^{S} p_i^q)^{1/1-q}$$

where p_i is the relative frequency of species i, q is the order of true diversity measurement, and S is the number of species. The parameter q has an exponential property that determines the sensitivity of the index to the relative abundance of species (*Jost, 2006*; *2007*). Species richness corresponds to the diversity index of order 0 and is insensitive to the relative frequency of species. The true diversity measure of order 1 is equivalent to the exponential of Shannon's entropy and weights rare and common species proportionally to their abundance. The diversity measures of order 2 are equivalent to Simpson's inverse measures, which favor abundant species while excluding rare ones (*Hill, 1973*; *Jost, 2006*; *2007*).

To measure diversity across the region encompassing the four habitat types, we computed the true gamma diversity index (${}^{q}D\gamma$) using the multiplicative partitioning of regional diversity (${}^{q}D\gamma$) as proposed by *Whittaker* (*1960; 1972*), where ${}^{q}D\gamma = {}^{q}D\alpha * {}^{q}D\beta$, and ${}^{q}D\beta = {}^{q}D\gamma / {}^{q}D\alpha$. The equivalent numbers, expressed as ${}^{0}D\alpha$, denotes the number of species in the communities (${}^{0}D\beta$) required to match the total species count in the region (${}^{0}D\gamma$).

To evaluate diversity at a local level, we calculated alfa diversity (orders q=0,1,2) for the community composition within each habitat (OF, JF, PF, XS) concerning the H and FP species assemblages.

Among communities, changes in species composition are explained by β diversity (*Whitaker, 1960*). The β diversity can arise from two processes: species turnover (B_{-3}) and differences in species richness (B_{rich}); both indexes identify the source of disparities between communities (*Carvalho, Cardoso & Gomes, 2012*). These two components explain β diversity additively ($B_{cc} = B_{-3} + B_{rich}$). To derive β diversity and its components (B_{-3}, B_{rich}), three measures were calculated: a) species common to both sites, b) species exclusive to one site, and c) species exclusive to the other site (see formulas in *Carvalho, Cardoso & Gomes, 2012*). B_{cc} represents a proportion of dissimilarity between two communities, where 0 indicates that communities share all species, and 1 corresponds to communities that do not share any species. Additionally, species turnover (B_{-3}) varies from 0 (when species composition is identical) to 1 (when species composition is entirely different). The values of B_{rich} follow the same scale from 0 to 1 (when species richness is equal or different respectively).

Furthermore, following *Jost* (*2007*), gamma diversity was calculated for orders q = 0 and q = 1, considering the unequal weighting of H and FP communities. Alpha diversity, essential for understanding each community's composition was assessed for orders 0, 1 and 2. Finally, beta diversity and its components across the four habitats for both communities were computed according to *Carvalho, Cardoso & Gomes* (*2012*) and *Carvalho et al.,* (*2013*). All analysis were performed with RStudio, ver. 2023.03.0+386 (*RStudio Team, 2022*), using the vegan package

The relationship between beta diversity and environmental factors

Subsequently, the correlation of β diversity (B_{cc}, B_{-3}, B_{rich}) and environmental factors such as altitude, and 22 bioclimatic variables obtained from the WorldClim website (http://www.worldclim.org), was assessed using Mantel tests (*Sokal & Rohlf, 1995*). For this purpose, the values of each bioclimatic variable were extracted for each transect, and a principal component analysis (PCA) was performed to condense the abiotic variables. Highly correlated variables were removed, as well as those with less contribution to the components explaining >90% of the variance. The selected variables were annual precipitation (Bio12), precipitation of wettest quarter (Bio16), and altitude. Dissimilarity matrices were constructed using the Bray-Curtis method for the selected variables. Simple and partial Mantel tests were conducted with 9,999 permutations. The Mantel tests were computed with RStudio, ver. 2023.03.0+386 (*RStudio Team, 2022*), using the vegan package.

Results

Abundance of flowering plants and hummingbirds

The samplings conducted throughout the study in the four habitat types allowed for the total recording of 34 FP species, which were classified into 22 genera, 17 families, and 11 orders (Supplemental file 1). Of the total quantified flower abundance, 83% was recorded in five FP species: *Loeselia mexicana* (24%), *Bouvardia ternifolia* (14%), *Castilleja tenuiflora* (18%), *Penstemon roseus* (16%), and *Salvia elegans* (11%). The last three FP species belong to the order Lamiales (45% of the total abundance). Likewise, *L. mexicana*, *C. tenuiflora*, and *B. ternifolia* were shared species in all four habitat types, thus being characteristic FP species within the region (Figure 2B). Therefore, the description of the results hereafter will be particularly based on these plant species, as well as in the case of the hummingbird species referred to below.

Regarding the H species, considering all the sampled habitats, a total of 11 species were recorded, classified into 9 genera and one family (Trochilidae). In terms of abundance, three H species comprised 86% of the total abundance. *Basilinna leucotis* was the most abundant hummingbird species in the region (69%), followed in much lower abundance by *Selasphorus platycercus* (11%), *Colibri thalassinus* (6.3%), and *Calothorax lucifer* (5%). The first three H species were recorded in all four habitat types, while *C. lucifer* was only recorded in XS (Supplemental file 1, Figure 2A).

The abundances of the above mentioned FP and H species, exhibited high heterogeneity among the studied habitats. For example, *L. mexicana* was the most abundant FP species (relative to other plant species present) in the sampled sites of JF (69%) and OF (56%), but very scarce in abundance in PF and XS (6%). Likewise, *C. tenuiflora* was particularly abundant in XS (47%). In contrast, it was recorded with low abundance in the other habitats (PF = 12%, OF = 0.1%, JF = 0.2%). Regarding *B. ternifolia*, it was an abundant FP species in JF (27%), XS (21%), and OF (16%), but not in PF (4%). In PF, both *P. roseus* (41%) and *S. elegans* (27%) were abundant species in this habitat. In contrast, in OF, the abundance of both species was low (1.5%), while in JF and XS were not recorded (Supplemental file 1, Figure 3B).

In the case of the H species, their abundances were also highly variable among the sampled habitats. *B. leucotis* was the most abundant species throughout the study in PF (81%), OF (80%), and JF (78%), while in XS was less abundant (15%). Conversely, *S. platycercus* was the most abundant in XS (34%), while in other habitats its abundance was less (JF = 12%, OF = 7%, PF = 1%). In the case of *C. thalassinus*, this specie was one of the most abundant in PF (12%) and showed very low abundances in the remaining habitat types (<4%). Finally, *C. lucifer* was an abundant species found exclusively in the XS habitat (28%) (Supplemental file 1, Figure 3A).

The observed number of FP species and H species in the study seemed to reach an asymptote in relation to our sampling effort across the four sampled habitats (a total of 180 hours of evenly distributed observation efforts for each habitat throughout the study). For FP species, we detected 99.62% for the PF, 99.91% for OF, 99.57% for JF and, 99.95% for XS according to the Chao2 estimator, after conducting 12 samples for each habitat type throughout the study. Likewise, we detected 98.15% of the H species estimated for the PF, 98.40% for OF, 96.25% for JF and 95.16% of those estimated for the XS.

True diversity measures

Richness at regional level of FP was 34 species ($^{0}D\gamma$), with an average local richness ($^{0}\mathrm{D}\alpha$) of 16.5 effective species and 2.06 effective communities ($^{0}D\beta$) necessary to account the regional species richness within the region. This implies that on average, 48.5% ($1/^{0}D\beta$) of the total FP species are present in a single habitat. For H assemblages, the average richness ($^{0}D\alpha$) was 7.3 effective species, representing 66.6% of the total species recorded within the region ($^{0}D\gamma$=11). With a $^{0}D\beta$ of 1.5 effective communities needed to achieve regional richness, it suggests minimal species turnover within the region. Considering the effective communities in H, the species recorded in XS (9 spp.) and OF (2 spp.) contribute to completing the regional richness (Table 1).

In terms of the regional diversity $^{1}D\alpha$ (equiprobable species) in the FP, an average community calculated 4.4 effective species, while 8.7 effective species were observed in the entire region ($^{1}D\gamma$). The communities required to complement $^{1}D\gamma$ are 2 ($^{1}D\beta$), indicating that an average community contained 50% of the equiprobable species in the region. For the H assemblages, an average community displayed 2.5 effective species ($^{1}D\alpha$), an at the region exhibited 3.3 effective species ($^{1}D\gamma$). To complement $^{1}D\gamma$, 1.3 communities were required ($^{1}D\beta$), with an average community encompassing 77% of the equiprobable species in the region (Table 1). Regional diversity ($^{1}D\gamma$) aligned closely with the abundant species recorded within the region (Figure 2).

Regarding alpha diversity (α), the habitat with the highest richness (^{0}D) of FP species was OF (22 species), followed by PF (18 species), and XS (14 species). JF (12 species) had the lowest richness, with the lowest number of effective species of ^{1}D (2.1) and ^{2}D (1.8), particularly recording two dominant species (*L. mexicana* and *B. ternifolia*) (Figure 3B). In contrast, habitats with the highest number of effective species in orders 1 and 2 are PF ($^{1}D = 5.3$, $^{2}D = 3.8$) and XS ($^{1}D = 5.2$, $^{2}D = 3.5$), respectively (Figure 4B; Table 2). Consequently, in terms of order 1 diversity, on average, PF and XS exhibited 2.5 times more diverse than JF and 1.4 times more diverse than OF. PF presented the five most abundant species within the region (*P. roseus*, *S. elegans*, *C. ternuiflora*, *L. mexicana*, *B. ternifolia*) (Figure 2B), while XS shared three species with PF (*C. ternuiflora*, *B. ternifolia*, *L. mexicana*) and had two exclusive abundant species (*Salvia chamaedryoides* and *Salvia melissodora*) (Supplemental file 1).

The habitat with the highest diversity of H species was XS, recording the highest richness ($^{0}D = 9$) and the greatest number of effective species ($^{1}D = 5.2$, $^{2}D = 4.2$). In this habitat, five abundant hummingbird species were found, two of which ranked among the most abundant species in the region, and one was exclusive to XS (*B. leucotis*, *S. platycercus*, *S. rufus*, *A. colubris*, and *C. lucifer*, respectively) (Figure 3A). In contrast, the lowest diversity of H species was observed in OF, PF, and JF, with assemblages having a similar number of effective species. Considering the order 1 diversity measure, XS was, on average, 2.77 times more diverse than OF, JF, and PF (Figure 4A; Table 2).

Beta diversity (β)

The B_{cc} values obtained among the FP communities indicate dissimilarity ranging from 0.52 to 0.77 (where 1 represents maximum dissimilarity). XS is dissimilar compared to the other three habitats (>0.70) (Table 3). The dissimilarity among all communities is primarily attributed to species turnover ($B_{\_3}$), except in OF vs. JF, where dissimilarity is attributed to differences in richness (B_{rich}) (Figure 5B; Table 3). The H communities have dissimilarity ranging from 0.38 to 0.56. Overall, dissimilarity is driven by species turnover (Figure 5A; Table 3). When evaluating the beta diversity between pairs of sites, a very similar trend was found for FP and H. Where, the dissimilarity was mainly due to $B_{\_3}$, with low contribution in B_{rich}. However, the highest values total beta (B_{cc}) occurred between habitats of FP and low values in assemblages of H, showing more similarity in species composition of H between habitats (Figure 5).

The relationship between beta diversity and environmental factors

Mantel's simple and partial tests for FP species, between beta components and selected environmental factors in the study, showed a positive correlation in B_{cc} dissimilarities with climate variables and altitude ranging from $r = 0.34$ to $r = 0.45$. Partial correlations confirm that climate variables contribute more in the relationship. Similar results were obtained for species turnover ($B_{\_3}$), with correlation coefficients ranging from $r = 0.27$ to $r = 0.4$ (Table 4). In summary, we found variation in the species turnover rate for both measured variables (altitude and climate variables). However, environmental conditions had a greater effect on the dissimilarity of FP species assemblages. For H species assemblages, differences in B_{cc} and $B_{\_3}$ are explained by climate variables ($r = 0.45$) and not by altitude (Table 4). Correlations for richness differences ($B_{\_rich}$) were not significant in either case (FP and H).

Discussion

Our study adds a crucial layer of understanding to the intricate ecosystems of our research region by unraveling the complex relationships between flowering plants (FP) and hummingbirds (H). Documenting 34 FP species, spanning 22 genera, 17 families, and 11 orders, underscores the ecological significance of the floral community (*Potts et al., 2010*; *Ollerton, Winfree & Tarrant, 2011*). The implications of this diversity resonate profoundly, encompassing ecosystem stability, pollination dynamics, and overall biodiversity (*Hoehn et al., 2008*).

The prevalence of five key FP species—*Loeselia mexicana*, *Bouvardia ternifolia*, *Castilleja tenuiflora*, *Penstemon roseus*, and *Salvia elegans*—in terms of flower abundance is notable. The flowers of these five FP species are red, which aligns with the fact that 84% of the plants visited by hummingbirds in the Americas are red (*Scogin, 1983*). These species may hold keystone positions in the ecosystem, influencing community composition and structure (*Paine, 1969*). This result is consistent with previous suggestions highlighting that North American bird-pollinated flora is dominated by temperate herbaceous lineages, such as *Castilleja* and *Penstemon* (*Abrahamczyk & Renner, 2015*). Therefore, their prominence serves as an indicator of their vital roles in the ecological web. Furthermore, the presence of characteristic FP species shared across all four habitat types underscores their ecological importance and potential role as indicators of habitat health (*Lechner, Chan & Campos-Arceiz, 2018*).

Within the realm of hummingbird diversity, our study identifies 11 recorded species, categorized into 9 genera within the family Trochilidae. Hummingbirds are highly diverse and abundant in the Americas, particularly in tropical and subtropical regions (*Howell & Webb, 1995*). However, their species richness tends to decrease as we move towards higher latitudes and elevations, such as temperate habitats. Despite this, the hummingbird species richness at our study region is relatively higher compared with other temperate forests of North and South America, where up to 13 species may be present (*Abrahamczyk & Renner, 2015*; *López-Segoviano, Bribiesca & Arizmendi, 2018*). Typically, hummingbird communities are mainly composed of medium-sized species (*Stiles, 1981*), of which resident species tend to be the most abundant (*Arizmendi & Ornelas, 1990*). In our study habitats, seven out of eleven hummingbird species may be considered medium to large-sized (*Arizmendi & Berlanga, 2014*). Among these, the resident *Basilinna leucotis* emerges as the dominant hummingbird species, constituting a substantial 69% of the regional hummingbird population. This dominance extends beyond numerical abundance, potentially influencing plant-hummingbird interactions, with cascading effects on plant reproductive success and community structure (*Stiles, 1981*; *Magrach et al., 2020*). Interestingly, the second most abundant hummingbird species was the long-distance migrant *Selasphorus platycercus*. The presence of this species was recorded throughout most of the year in all four habitat types, suggesting that in this region, both resident and winter migratory populations can be found and may even reproduce in these habitats. Based on these findings, it seems that at least some hummingbird species, such as *B. leucotis* and *S. platycercus*, demonstrate adaptability to multiple habitat types, suggesting a degree of habitat generalism. These species were found in multiple habitats, indicating they can utilize a range of environments for foraging and nesting.

The observed heterogeneity in species abundance across different habitats within our research region offers a captivating glimpse into the tapestry of ecological dynamics. These variations in species abundance likely reflect habitat-specific resource availability, microclimatic conditions, and niche partitioning (*Whittaker, 1960*; *Magurran et al., 2010*). This mosaic of habitats beckons researchers to delve deeper into the ecological processes at play. Our findings hold profound implications for conservation and habitat management, underlining the pressing need to consider habitat preferences and ecological niches (*Margules & Pressey, 2000*). Understanding the intricacies of resource utilization patterns among FP and H species within different habitats guides the strategic prioritization of habitats for protection and conservation, thereby sustaining biodiversity and ecosystem services (*Whittaker, Willis & Field, 2001*; *Krauss et al., 2010*).

The computation of true gamma diversity (${}^{q}D\gamma$) and true beta diversity (${}^{q}D\beta$) provides a quantitative foundation for unraveling the regional biodiversity of FP and H species. These metrics, integral to contemporary ecological research (*Chao et al., 2014*), lay the groundwork for informed regional biodiversity assessments and conservation planning (*Jost et al., 2010*). The revelation of low species turnover for H assemblage suggests some stability in the species composition across the habitats but higher turnover for FP reflects the presence of habitat specialists alongside widespread species. Species turnover is influenced by the availability and variety of resources within each habitat, which determine the communities composition. As a result, the biota undergoes changes based on the specific requirements for food resources and spatial aspects of the species (*Halffter, 1998*). This observation highlights the complexities of ecological dynamics within the region, offering insights into the interconnectedness of species and their environments (*Vellend et al., 2017*; *Chase et al., 2011*). This nuanced understanding of species turnover has far-reaching implications for ecosystem connectivity and resilience. The presence of habitat specialists signals unique ecological roles and dependencies within their respective ecosystems, urging conservationists to consider the holistic preservation of habitats (*Devictor et al., 2007; Cardinale et al., 2012*).

Our exploration of alpha diversity among different habitats unveils intriguing patterns of species richness and evenness. Habitats such as Pine Forest (PF) and Xeric Scrubland (XS) stand out as bastions of high alpha diversity of flowering plants, suggesting the presence of diverse and evenly distributed species assemblages (*Magurran, 1988*; *Grime, 1998*). In contrast, Juniper Forest (JF) exhibits lower diversity, beckoning further investigation into the drivers of this pattern, including resource availability and biotic interactions (*Connell, 1978*; *Tilman, 1982*). XS, on the other hand, shines as a habitat with high diversity for both FP and H species, especially in terms of order 1 (species accounting almost all of the total abundance and proportionately). Understanding the variations in alpha diversity among habitats has profound implications for crafting effective land management and conservation strategies. Our findings underscore the imperative to prioritize the protection and restoration of diverse habitats to maintain biodiversity and enhance ecosystem resilience (*Noss & Cooperrider, 1994*).

The exploration of beta diversity, especially the dissimilarity among FP and H communities, unveils the uniqueness of species assemblages across habitats. The high dissimilarity observed in Xeric Scrubland (XS) points to the existence of distinctive ecological communities, potentially shaped by factors such as dispersal limitation, environmental gradients, or species interactions (*Legendre et al., 2009*). The dissimilarity in species composition is primarily due to species turnover, implying unique ecological roles and contributions of different species to each habitat. These findings emphasize the paramount importance of preserving a variety of habitats to safeguard the diverse assemblages they harbor. By prioritizing conservation efforts across heterogeneous landscapes, we promote ecosystem resilience and augment the capacity of these ecosystems to adapt to changing environmental conditions (*Pressey et al., 2007*; *Hobbs et al., 2014*).

We found a significant relationship between environmental factors (specifically climate variables) and dissimilarities in both FP species and H species assemblages. The positive correlation observed in B_{cc} indicates that as climate variables and altitude vary, the dissimilarity in the composition of FP species increases. Furthermore, the results show that climate variables play a more influential role in this relationship compared to altitude. This suggests that the climatic conditions of a habitat are particularly important in shaping the composition of FP. The variations in species turnover ($B_{\_3}$) also align with this pattern, reinforcing the impact of environmental conditions on the diversity and composition of FP species. In the case of hummingbird species, the dissimilarities in B_{cc} and $B_{\_3}$ are mainly influenced by climate variables, not altitude. This emphasizes the significance of climate in determining the composition and diversity of hummingbird species across different habitats.

However, our study did not find significant correlations for richness differences ($B_{\_rich}$) for both FP and H species. This implies that differences in species richness between habitats were not strongly related to the measured environmental variables and altitude. Thus, the positive correlations detected between beta diversity and climate variables, offer compelling insights into the potential influence of climate change on species composition within our research region (*Bellard et al., 2012*). The ramifications of shifting climate conditions extend to alterations in species distributions, impacting ecological dynamics and the provisioning of ecosystem services (*Parmesan, 2006*).

Previous studies have shown that climate change can be particularly threatening to hummingbirds by affecting the phenology of floral resources on which they depend (*Inouye et al. 2000*; *McKinney et al. 2012*). Even minor changes in blooming dates may be of consequence, as hummingbirds will eventually arrive after flowering begins, which could reduce their nesting success (*Aldridge et al. 2011*; *McKinney et al. 2012*). This disruption in the flowering phenology within and among different habitats can affect both latitudinal and altitudinal migration undertaken by hummingbirds following these floral resources. The established interaction networks between hummingbirds and their floral resources should be incorporated into future studies of geographic distribution models and climate change. Thus, our findings accentuate the central role played by environmental conditions in shaping species assemblages (*Chase et al., 2011*). This knowledge informs the development of effective habitat conservation and restoration strategies that account for the influence of climate and topography on ecosystem structure and function (*Sax et al., 2007*; *Hobbs et al., 2014*).

Conclusions

Our study provides a comprehensive understanding of the abundance, composition, and diversity of flowering plants and hummingbirds across different habitat types. The identified dominant FP and H species play crucial roles in the ecological dynamics of these habitats. Moreover, the analysis of true diversity measures and beta diversity highlights the importance of community species turnover and regional species richness. Habitat variations significantly influence abundance and diversity patterns, emphasizing the need for habitat-specific conservation strategies.

The findings of this research not only deepen our knowledge of ecological interactions but also underscore the necessity of considering environmental factors in biodiversity conservation. Understanding how habitats shape the diversity and composition of these critical ecological components is essential for effective conservation and sustainable management of natural ecosystems. These insights are pivotal for future research and conservation efforts, providing a solid foundation for further investigation into the intricate relationships between hummingbirds, flowering plants, and their habitats. By considering the dynamic interplay of environmental variables and biodiversity, we can develop informed strategies to protect and preserve these invaluable ecological partnerships for future generations.

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Table 1 (on next page)

Multiplicative partition of the gamma diversity (true diversity, modified from Jost, 2007) into its components: Dγ (regional diversity), Dβ (effective communities), and Dα (average alpha).

Dα and Dγ are expressed in the same units of species, while Dβ is expressed in communities. Superscripts correspond to diversity values of orders 0 and 1, based on Hill numbers representing the effective number of species or communities.

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	Gamma diversity			
Dγ diversity and its components	**Hummingbirds**		**Flowering Plants**	
	^{0}D	^{1}D	^{0}D	^{1}D
Dγ	11	3.3	34	8.7
Dβ	1.5	1.3	2.06	2
Dα	7.3	2.5	16.5	4.4

Table 2(on next page)

Alpha diversity (true diversity, modified from *Jost, 2006*) of hummingbirds and their flowering plants in oak forest (OF), juniper forest (JF), pine forest (PF), and xerophytic shrubland (XS).

Superscripts correspond to diversity values of orders 0, 1, and 2, represented by Hill numbers, reflecting the effective number of species.

Table 2. Alpha diversity (true diversity, modified from *Jost, 2006*) of hummingbirds and their flowering plants in oak forest (OF), juniper forest (JF), pine forest (PF), and xerophytic shrubland (XS). Superscripts correspond to diversity values of orders 0, 1, and 2, represented by Hill numbers, reflecting the effective number of species.

	Alpha diversity					
	Hummingbirds			Flowering Plants		
Habitat Type	^0D	^1D	^2D	^0D	^1D	^2D
OF	7	2.2	1.5	22	3.7	2.6
PF	7	2	1.4	18	5.3	3.8
JF	6	2.2	1.5	12	2.1	1.8
XS	9	5.2	4.2	14	5.2	3.5

Table 3(on next page)

Beta diversity based on the partition of total beta diversity (B_{cc}), species replacement [$B_{\_3}$] and species richness differences [β_{rich}]) for hummingbirds and their flowering plants.

This analysis was carried out across four sampled habitat types: oak forest (OF), juniper forest (JF), pine forest (PF), and xerophytic shrubland (XS).

Table 3. Beta diversity based on the partition of total beta diversity (B_{cc}), species replacement [$B_{\_3}$] and species richness differences [β_{rich}]) for hummingbirds and their flowering plants. This analysis was carried out across four sampled habitat types: oak forest (OF), juniper forest (JF), pine forest (PF), and xerophytic shrubland (XS).

Beta diversity						
	Hummingbirds			**Flowering Plants**		
Habitat type	$B_{\_3}$	B_{rich}	B_{cc}	$B_{\_3}$	B_{rich}	B_{cc}
OF vs. JF	0.25	0.13	0.38	0.09	0.43	0.52
OF vs. PF	0.44	0	0.44	0.43	0.14	0.57
OF vs. XS	0.36	0.18	0.55	0.43	0.29	0.71
JF vs. PF	0.44	0.11	0.56	0.50	0.25	0.75
JF vs. XS	0.20	0.30	0.50	0.60	0.10	0.70
PF vs. XS	0.20	0.20	0.40	0.62	0.15	0.77

Table 4(on next page)

Correlation results (Mantel tests) between beta diversity of hummingbirds and their flowering plants, altitude and climatic variables were analyzed for each locality.

Additionally, we conducted Partial Mantel tests to examine the results after eliminate the effects of altitude (Climate Variables-Altitude) and climatic variables (Altitude-Climate Variables).

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	Hummingbirds							
	Altitude		Climate Variables		Climate Variables-Altitude		Altitude-Climate Variables	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Bcc	-0.01	0.50	**0.45**	**<0.01**	**0.45**	**<0.01**	0.03	0.41
B_3	-0.20	0.91	**0.31**	**<0.01**	**0.30**	**<0.01**	-0.19	0.90
Brich	0.22	0.13	0.01	0.41	0.03	0.34	0.22	0.13
	Flowering Plants							
	Altitude		Climate Variables		Climate Variables-Altitude		Altitude-Climate Variables	
	r	*p*	*r*	*p*	*r*	*P*	*r*	*P*
Bcc	**0.34**	**<0,01**	**0.45**	**<0.01**	**0.51**	**<0.01**	**0.42**	**<0.01**
B_3	**0.27**	**0.01**	**0.40**	**<0.01**	**0.44**	**<0.01**	**0.33**	**<0.01**
Brich	-0.05	0.54	-0.12	0.93	-0.12	0.94	-0.06	0.57

Figure 1

Maps showing the monitored habitats and locations in the state of Tlaxcala, Mexico, where the diversity of hummingbirds and their flowering plants was studied.

(A) Geographical location. (B) Monitored locations within each habitat. The colored circles represent the transects established for each habitat type: Oak forest (OF) in green, juniper forest (JF) in purple, pine forest (PF) in yellow, and xerophytic shrubland (XS) in blue. Sources: ESRI, Garmin, INEGI, (2009). Uso del suelo y vegetación, escala 1:250000, serie IV. 2009, and Qgis version 2.18, 2016. QGIS Geographic Information System. QGIS Association. http://www.qgis.org . Photo credit: Hellen Martínez-Roldán.

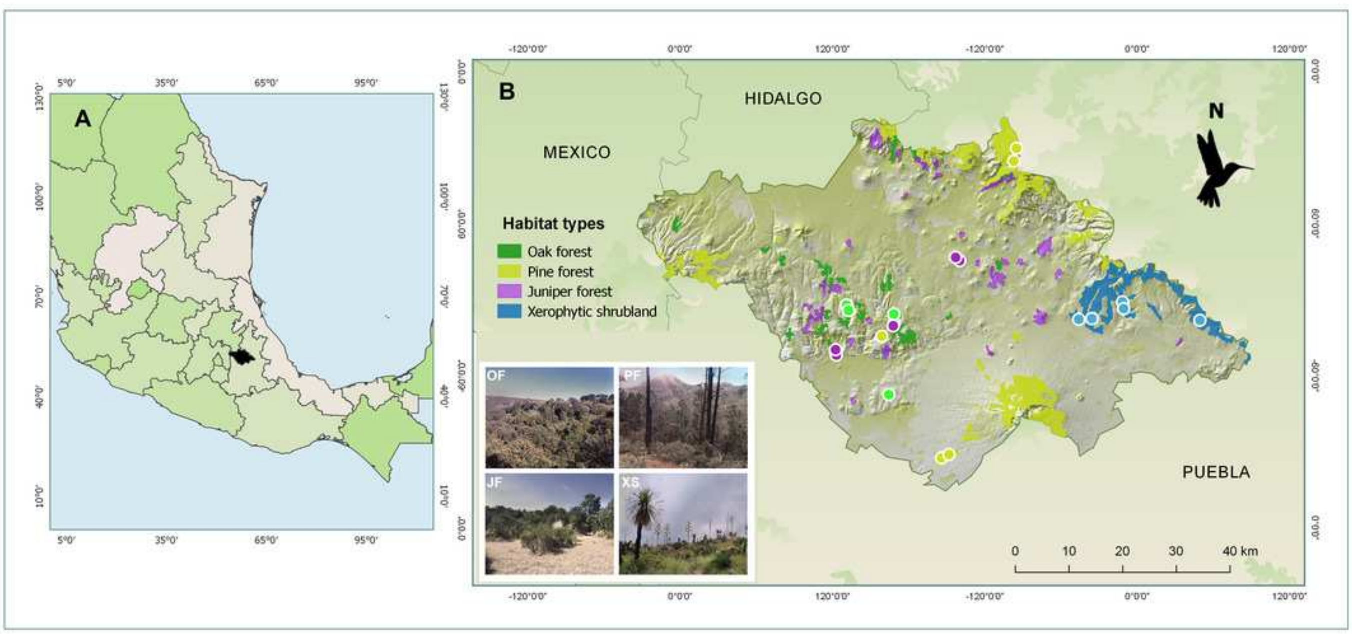

Figure 2

Rank/abundance plots for hummingbirds and their flowering plants species at the regional level in Tlaxcala, Mexico.

Rank/abundance curves show the distribution of hummingbird and plant species from most to least abundant. (A) *Basilinna leucotis* (BASLEU), *Selasphorus platycercus* (SELPLA), and *Colibri thalassinus* (COLTHA) highly dominate in all sampled habitat types, while (B) *Loeselia mexicana* (LOEMEX), *Castilleja tenuiflora* (CASTEN), *Penstemon roseus* (PENROS), *Bouvardia ternifolia* (BOUTER), and *Salvia elegans* (SALELE) were the most abundant plant species within the region. Photo credit: Ubaldo Marquez-Luna, Hellen Martínez-Roldán, Juan Manuel González, María José Pérez-Crespo and Carlos Lara.

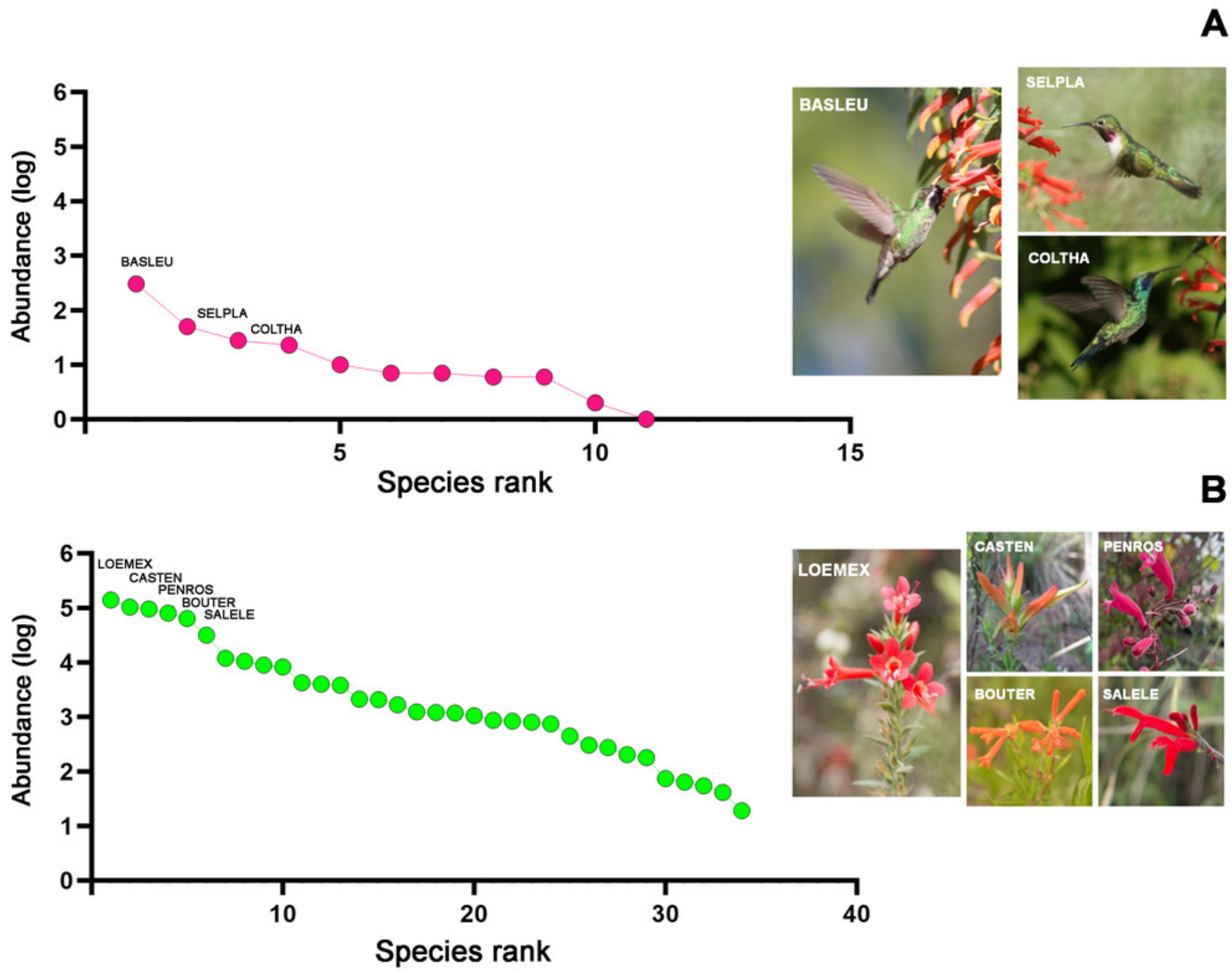
A
Abundance (log)
Species rank
BASLEU
SELPLA
COLTHA
B
LOEMEX
CASTEN
PENROS
BOUTER
SALELE

Figure 3

Rank/abundance plots for the hummingbirds and their flowering plant species by each sampled habitat type.

(A) *Basilinna leucotis* (BASLEU), *Selasphorus platycercus* (SELPLA), *Colibri thalassinus* (COLTHA) and *Calothorax lucifer* (CALLUC) highly dominate in all sampled habitat types, while (B) *Loeselia mexicana* (LOEMEX), *Castilleja tenuiflora* (CASTEN), *Penstemon roseus* (PENROS), *Bouvardia ternifolia* (BOUTER), *Salvia elegans* (SALELE), *S. polystachya* (SALPOL) and *S. melissodora* (SALMEL) were the most abundant plant species in each sampled habitat type: Oak forest (OF), juniper forest (JF), pine forest (PF), and xerophytic shrubland (XS).

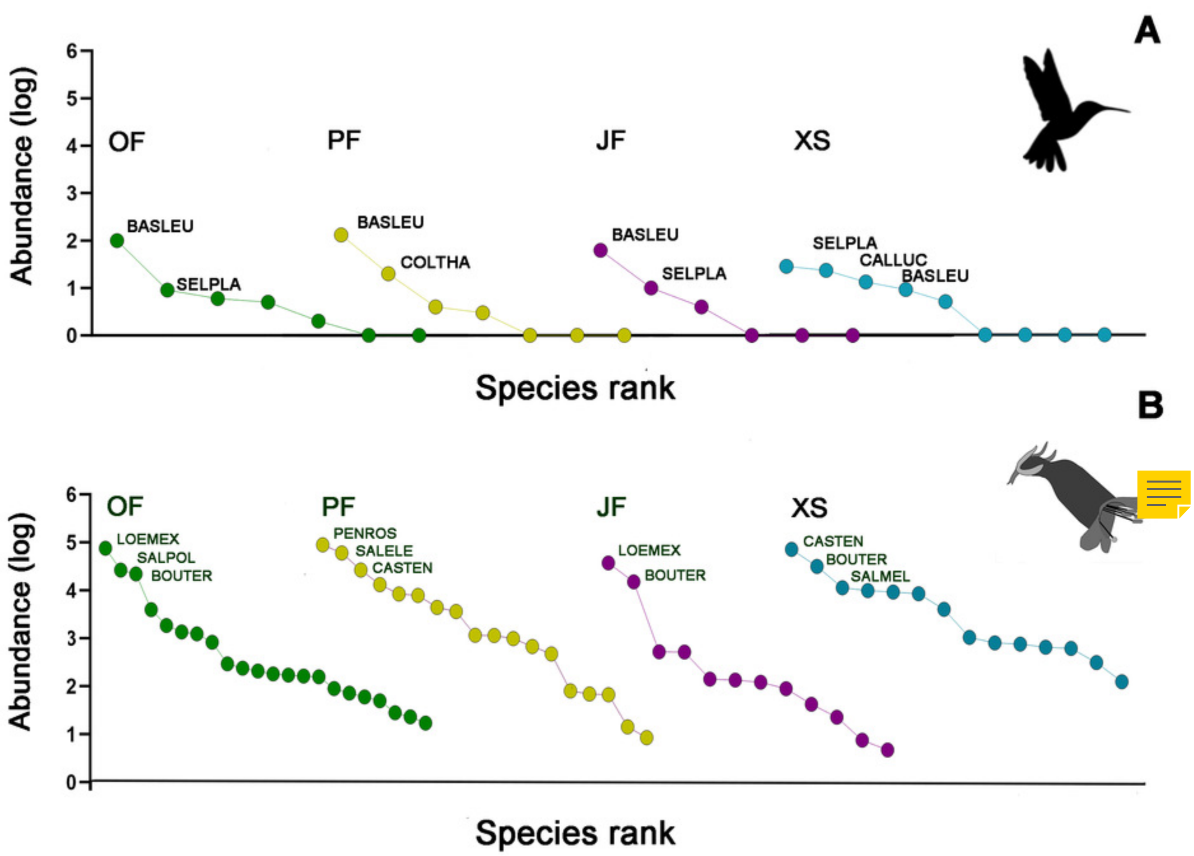

Figure 4

Alpha diversity profiles of hummingbirds and their flowering plant species in the four sampled habitat types.

By following the true diversity concept (*Jost, 2006*), we obtained the diversity profiles for (A) hummingbirds and (B) flowering plants, showing variation in the number of effective species for each sampled habitat type: Oak forest (OF), juniper forest, pine forest (PF), and xerophytic shrubland (XS). Superscripts correspond to diversity values of orders 0, 1, and 2; values for orders 1 and 2 are shown as Hill numbers, representing the effective number of species.

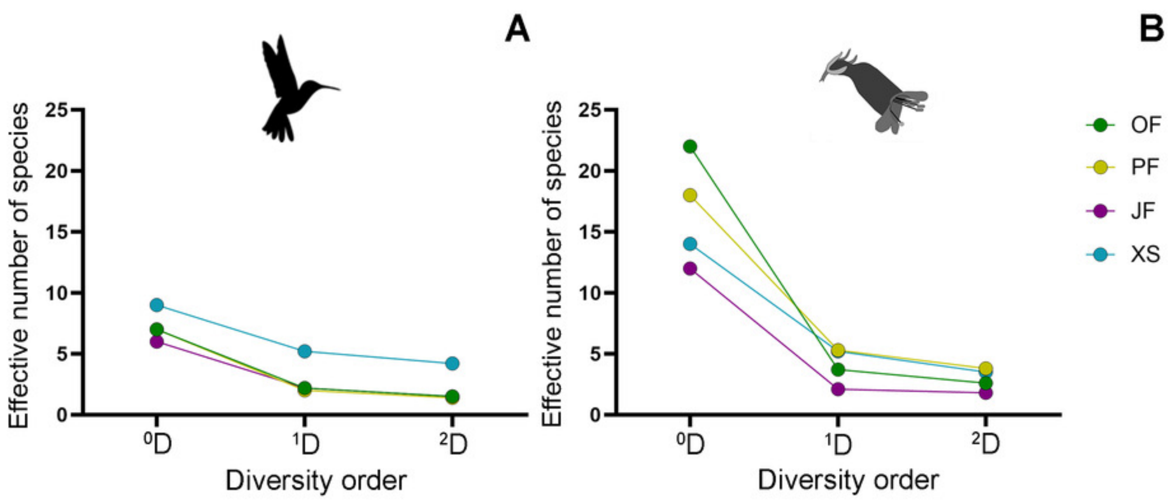

Figure 5

Contribution of species turnover and differences in species richness to beta diversity of hummingbirds and flowering plants.

Plots show beta diversity of (A) hummingbirds and (B) their flowering plant species, where each segment shows the proportion of each component for each habitat pair: Oak forest (OF), juniper forest (JF), pine forest (PF), and xerophytic shrubland (XS).