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Neotropical insectivorous bats adjust echolocation calls in response to vegetation structure

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Echolocation is a crucial process in bat ecology, but it is influenced by different biotic and abiotic factors. The structural complexity of the vegetation can filter which species can be part of an assemblage because bats have to modify the spectral and temporal parameters of their echolocation calls. We evaluated how insectivorous bats modulate their echolocation calls among sites that vary in vegetation structural complexity in the Llanos of Colombia in three habitats. We used canonical correspondence analysis (CCA) to correlate vegetation variables per plot with the spectral and temporal variables of the echolocation signals from bats using those habitats. We found that delta frequency and delta time had the greatest variability in relation to the percentage of tree cover and diameter at breast height. Our results indicate that pulses, in terms of frequency and time, tend to fluctuate more in cluttered habitats. We then selected the two species with the highest number of recordings (*Saccopteryx bilineata* and *Neoeptesicus* cf. *furinalis*) and compared spectral and temporal variables among sites. For *Neoeptesicus* cf. *furinalis* showed a predictable behavior: pulses were shorter, and more frequency modulated in highly cluttered habitats. However, and contrary to our expectations, the pulses of *Saccopteryx bilineata* tended to be longer and with a smaller frequency modulated component in highly cluttered habitats. These results demonstrate that bats modulate their echolocation calls in different ways according to the structure of vegetation. Modulation of echolocation has important implications for sensory ecology and bat composition in tropical landscapes.

Key words: Audiomoth, functional diversity, habitat filtering, plant structural complexity, savannas.

La ecolocalización es un proceso crucial en la ecología de los murciélagos, pero está influenciada por diferentes factores bióticos y abióticos. La complejidad estructural de la vegetación puede determinar qué especies pueden formar parte de un ensamblaje, ya que los murciélagos deben modificar los parámetros espectrales y temporales de sus llamadas de ecolocalización. Evaluamos cómo los murciélagos insectívoros modulan sus llamadas de ecolocalización en lugares con diferentes niveles de complejidad estructural de la vegetación en los Llanos de Colombia en tres hábitats. Aplicamos un análisis de correspondencia canónica (CCA) para correlacionar las variables de vegetación por parcela con las variables espectrales y temporales de las señales de ecolocalización de los murciélagos que habitan esos lugares. Descubrimos que la delta de frecuencia y la delta de tiempo presentaron una mayor variabilidad en relación con el porcentaje de cobertura arbórea y el diámetro a la altura del pecho. Nuestros resultados indican que los pulsos tienden a fluctuar más en términos de frecuencia y tiempo en hábitats densamente estructurados. Posteriormente, seleccionamos las dos especies con mayor número de grabaciones (*Saccopteryx bilineata* y *Neoeptesicus* cf. *furinalis*) y comparamos sus variables espectrales y temporales en los distintos lugares. En *Neoeptesicus* cf. *furinalis* mostró un comportamiento predecible: los pulsos eran más cortos y presentaban una mayor modulación de frecuencia en hábitats muy densos. Sin embargo, y contrario a nuestras expectativas, los pulsos de *Saccopteryx bilineata* tendieron a ser más largos y con un componente de modulación de frecuencia menor en hábitats muy densos. Estos resultados demuestran que los murciélagos modulan sus llamadas de ecolocalización de diferentes maneras de acuerdo con la estructura de la vegetación. La modulación de la ecolocalización tiene implicaciones importantes para la ecología sensorial y la composición de murciélagos en paisajes tropicales.

Palabras clave: Audiomoth, complejidad estructural de la vegetación, diversidad funcional, hábitat como un filtro, sabanas.

Functional diversity plays a crucial role in regulating community structure, determining ecosystem functioning (Tilman *et al.* 1997; Naeem and Wright 2003), and influencing niche partitioning, guild membership and habitat selection in animals (García-Morales *et al.* 2016; Pigot *et al.* 2020). Functional diversity refers to the range of morphological or physiological traits among species, with functional traits being the key attributes that enable organisms to cope successfully with their environment (Hooper *et al.* 2005; Villéger *et al.* 2010). Therefore, functional diversity helps us understand how habitats can filter which species are found in a given area (Petchey and Gaston 2006). For insectivorous bats, the functional diversity of echolocation signals can help determine how vegetation structure at small spatial scales may filter species based on their ability to discriminate prey echoes from background clutter or emit signals that optimize detection in open areas (Schnitzler and Kalko 2001).

Echolocation is a sensory system that operates along a continuum, rather than being strictly divided into open-space and clutter-adapted signals. The spectral and temporal characteristics of echolocation calls allow bats to detect, classify and locate prey in different levels of vegetation clutter (Schnitzler and Kalko 2001). In densely vegetated environments, bats require broadband, short-duration pulses to navigate efficiently around obstacles

(Broders *et al.* 2004; Barataud *et al.* 2013). In contrast, in open areas, bats emit echolocation calls with narrower bandwidths and longer durations, which enhance long-range prey detection (Kalko and Schnitzler 1993). However, some species show a high degree of plasticity, modulating their echolocation parameters to different vegetation structures and foraging conditions (Neuweiler 2003).

Bats can also modify the timing and rate of their echolocation pulses depending on environmental constraints (Fenton *et al.* 2016). For instance, during critical hunting moments, bats decouple their call emissions from wingbeats to enhance sensory flow, rapidly increasing their call rate when they are close to their prey (Stidsholt *et al.* 2021). However, this behavior comes at an energetic cost, so when not actively hunting, bats synchronize their calls with wingbeats to optimize energy efficiency (Stidsholt *et al.* 2021). Similarly, when navigating through highly cluttered environments, bats tend to increase their call emission rates to improve spatial resolution (Falk *et al.*, 2014). Yet, in the presence of conspecifics, they reduce emission rates and even omit pulses to minimize interference, which can ultimately impact their navigation and collision avoidance (Adams *et al.* 2017).

Vegetation complexity, which serves as a proxy for clutter, can act as a strong selective force, shaping echolocation structures and filtering community composition (Yovel *et al.* 2009). Greater habitat heterogeneity provides more ecological niches, which promotes species diversity (Langridge *et al.* 2019). Conversely, a reduction in structural complexity negatively affects bat diversity (Meyer *et al.* 2016) and poses a threat to several taxonomic groups (Meyer and Kalko 2008; Jones *et al.* 2009; Estrada-Villegas *et al.* 2010; Cruz *et al.* 2016). For example, Díaz-B *et al.* (2023) demonstrated that environmental conditions in Colombian dry forests significantly influence functional traits of bats, including echolocation pulse structure, diet, vertical foraging behavior and trophic level. However, despite increasing research on the drivers of functional diversity in Neotropical bats, few studies have examined how vegetation complexity modulates the functional diversity of echolocation signals in insectivorous bat ensembles (Cisneros *et al.* 2015; Núñez *et al.* 2019). This knowledge gap is particularly relevant in naturally heterogeneous landscapes such as savannas. In the Colombian Llanos, savannas are often flanked by gallery forests, and sometimes there are soft forest edges with sparce tree cover in the transition between gallery forests and savannas (Romero-Ruíz *et al.* 2004).

To better understand how vegetation structure influences echolocation in Neotropical bats, we assessed the effect of vegetation complexity on the functional diversity of echolocation pulses in an ensemble of insectivorous bats in the savannas of Colombia. Based on Denzinger *et al.* (2016), we predicted that bats in cluttered habitats would echolocate at higher frequencies and shorter durations, whereas bats in open areas would emit lower frequency, longer duration calls. We also expected the acoustic parameters of the bat ensemble to vary with habitat complexity. Specifically, we predicted that species should modulate their vocalizations to the structural characteristics of their environment.

Previous studies have shown that species such as *Saccopteryx bilineata* and members of the genus *Eptesicus* modify their echolocation signals according to vegetation complexity: in cluttered habitats they emit highly modulated frequency pulses, whereas in open areas they reduce modulation (Ratcliffe *et al.* 2011; Jakobsen *et al.* 2012). In this study, we first describe the acoustic parameters of the insectivorous bat ensemble and then test our predictions by correlating vegetation clutter with the variability of spectral and temporal parameters of echolocation calls for all species in the ensemble, as well as for *Saccopteryx bilineata* and *Neoeptesicus* cf. *furinalis* separately.

Materials and Methods

Study area and vegetation coverage. We conducted our study in El Caduceo Natural Reserve, municipality of San Martin, department of Meta, Colombia (3.6711° N, -73.6585° W, Elevation: 377.8 MASL). The reserve is located at the piedmont of the Eastern Cordillera and the onset of the Llanos (Basto-González 2009) (Supplementary material, Figure 1). The Colombian Llanos are mainly composed of natural savannas and gallery forests (Lasso *et al.* 2011). Savannas are mostly covered with native grasses and are mainly used for cattle grazing, and the forest boundaries between gallery forests and savannas have not shifted due to stable land use patterns and the lack of significant encroachment or deforestation (Romero-Ruíz *et al.* 2004).

In El Caduceo, 70 hectares are covered with gallery forests while the remaining 103 hectares are savannas used for cattle farming and agriculture in small parcels (Casallas-Pabón *et al.* 2017). The area experiences a monomodal rainfall regime, with a dry season from December to March (Correa *et al.* 2006). We selected three habitats: open (S), cluttered (BT), and highly cluttered (BM) environments. Open areas (S) consisted mainly of pastures used for cattle grazing and agricultural crops, such as pineapples. Cluttered sites (BT) were in the transitional area between open areas and highly cluttered environments, and were composed of scattered, thin, and tall pioneer trees characteristic of early successional stages, interspersed with open savanna patches. Highly cluttered sites (BM) were located within gallery forests, which exhibited greater structural complexity, including a diverse assemblage of tall trees, palms, dense understory vegetation, as well as numerous saplings.

We measured diameter at breast height (DBH), canopy width and percent cover in 18 plots, six per habitat. We also measured the percentage of vegetation coverage with a concave spherical densiometer (Rivera-Gallego and Pinzón-Florián 2022) in each plot. Vegetation structure data were collected on the same sites where we conducted

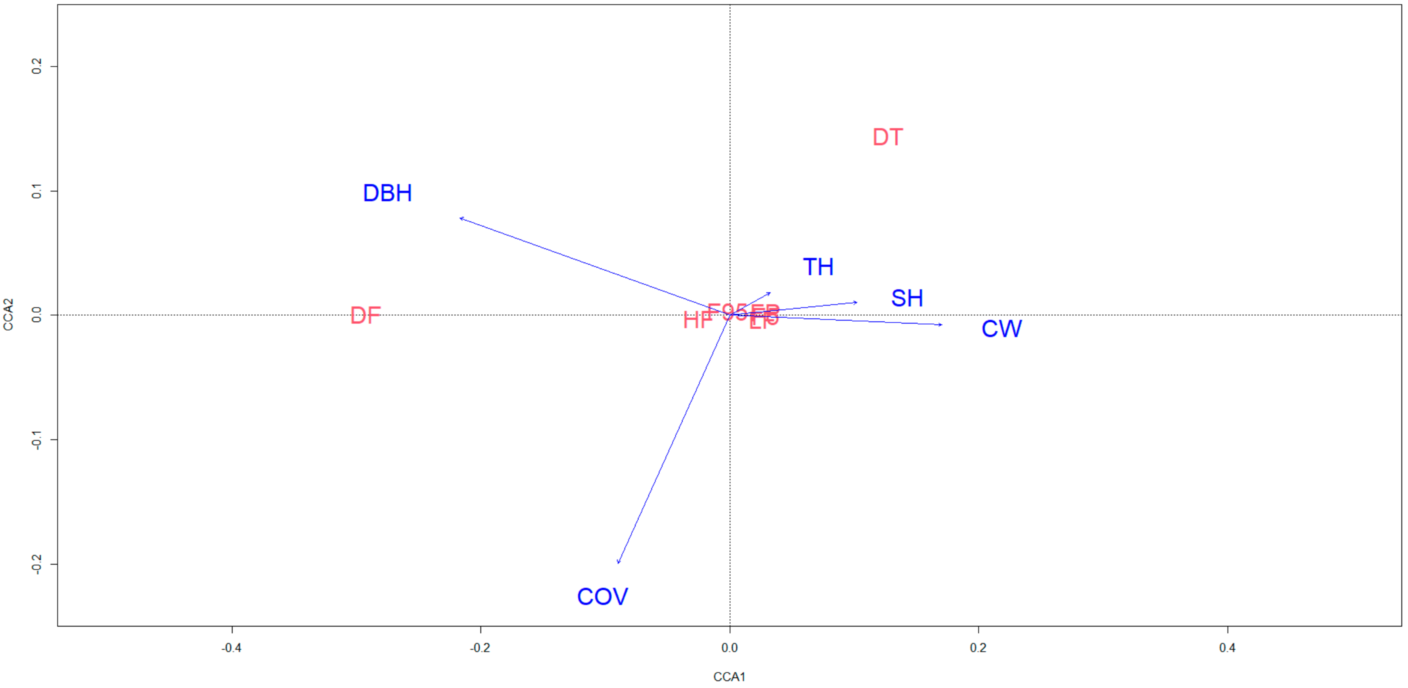

Figure 1. Canonical correspondence analysis (CCA) showing that spectral and temporal parameters vary according to the vegetation variables. The parameters showing the most significant changes were Δ Frequency (DF) and Δ Time (DT). The eigenvalues for the first and second axes were 0.3790 and 0.0474, respectively. Spectral and temporal parameters in red. LF: Low frequency, High frequency: HF, Δ Time: DT, Frequency 5%: F5, Frequency 95%: F95, Δ Frequency: DF, Peak frequency: FP. Vegetation variables in blue. Total height: TH, Stem height: SH, Canopy width: CW, DBH, % Coverage: COV.

acoustic monitoring to ensure comparability between environmental variables and acoustic parameters across sites (please see below). Since vegetation structure remains stable over short time periods (months or years), data collection of vegetation variables did not require the same sampling effort as bat monitoring (Willcox *et al.*, 2017).

Bat capture and reference calls. We captured and recorded bats from February 11th to March 9th, 2023. Four 6 × 2.5 m nets were used on each habitat and deployed from 17:30 to 23:30 for 18 nights. We relocated nets every night. Captured bats were placed in cloth bags, measured, and sexed (Supplementary material, Figure S2). We followed the Guidelines of the American Society of Mammalogists for proper handling protocols (Sikes *et al.* 2011).

We used taxonomic keys to identify all the captured bats (Díaz *et al.* 2016 ; Díaz *et al.* 2021). All bats from the family Phyllostomidae bats were released immediately. Although some phyllostomid bats are insectivorous, we did not include any species from this family because their echolocation calls are unidirectional (all other families are omnidirectional) and are not adequately sampled with our sound recorders (Jakobsen *et al.* 2013). For reference calls, we used a Pettersson M-500 USB microphone, with Pettersson-BatMicRecorder software at a sampling rate of 386 kHz and a resolution of 16 bits. We started recording when the individual was released until it was no longer in sight. The recording methodology involved releasing bats at the site where they were found on the night of capture. Our study is in compliance with Permit No. 2467 (25 October 2023) issued by the Colombian Environmental Licensing Authority for collecting and manipulating wildlife for non-commercial scientific research purposes.

Echolocation calls were recorded and analyzed using Raven Pro 1.6. (Cornell Lab of Ornithology, Cornell Univeristy, USA). Both reference and passive acoustic recordings (see next section) were processed in Raven Pro 1.6. Spectrograms were generated using a Hamming window with a 512-point FFT, 100% frame size, and 80% overlap. A sonotype can be defined as a distinct acoustic category that groups echolocation calls sharing similar structural characteristics, regardless of their signal frequency (Roemer *et al.* 2021). Based on this definition, we labeled each call and measured the following spectral-temporal parameters: start time (s); end time (s); low frequency (kHz); high frequency (kHz); Δ Time delta (s); 5% frequency (kHz); 95% frequency (kHz); Δ Frequency delta (kHz); peak frequency (kHz). We also labeled each recording with sonotype; taxon; sex; recording type; and quality. The audio quality was evaluated using the signal-to-noise ratio (SNR), which quantifies the quality of a signal in the presence of noise and is expressed in decibels (dB). The selection of these parameters was based on Martínez-Medina *et al.* (2021).

Passive acoustic sampling. We used nine Audiomoth recorders using the following parameters: 250 kHz sampling rate, medium gain, 1740 seconds of inactive time, and 60 seconds of active time. We sampled from 17:30 to 5:30 daily for three weeks. In total, we had nine recording sites, three in each habitat. Each recorder was placed four meters above ground level in all three habitats and at least 150 meters apart to avoid pseudoreplication (Pryde and Greene

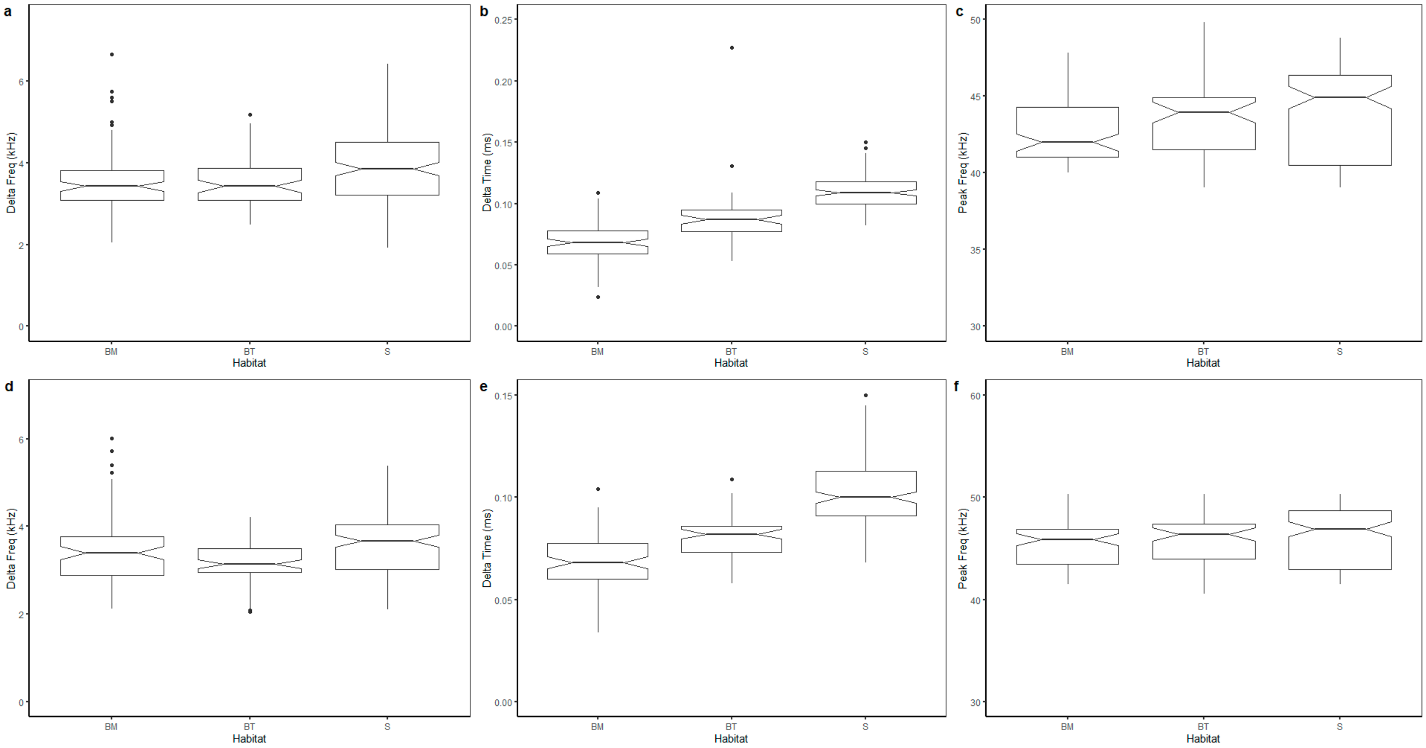

Figure 2. Spectral and temporal parameters of the two *Saccopteryx bilineata* pulses vary between the different sites, Open (S), Clutter (BT) and High Clutter (BD), as indicated by the lack of overlap in the notches of the box plots. **a.** Δ Frequency of the low pulse **b.** Δ Time of the low pulse **c.** Peak frequency of the low pulse **d.** Δ Frequency of the high pulse **e.** Δ Time of the high pulse **f.** Peak frequency of the high pulse.

2016). Recording sites were relocated weekly to avoid nights without recordings. Spectral-temporal parameters from search calls were analyzed and labeled in Raven Pro1.6. (Cornell Lab of Ornithology, Cornell University, USA), including start and end times, low and high frequencies, Δ Time delta, bats presence, sonotype, taxon, habitat classification, feeding behavior, and recording quality (Martínez-Medina *et al.* 2021). Sonotype identifications were informed by previous studies (Jung *et al.* 2007; Jung *et al.* 2014; Arias-Aguilar *et al.* 2018).

Data Analysis. We used a Principal Component Analysis (PCA) to identify patterns in vegetation structure, using the following R libraries: FactoMineR (Lê *et al.* 2008), factoextra (Kassambara and Mundt 2020), REdaS (Maier 2022) and ggplot2 (Wickham 2016). Then we used canonical correspondence analysis (CCA) to determine whether the spectral and temporal parameters of the insectivorous bat ensemble were modulated given the complexity of the vegetation. To do this, we first averaged the data obtained for each vegetation variable and each spectral and temporal variable per plot across the three habitats (18 plots in total). Then we created two matrices with the same number of rows, one with the vegetation data per plot, and another with the spectral and temporal variables per plot. Then, we used the function "scale" to normalize the data because all variables were in different units and then calculated the CCA using the library Vegan (Oksanen *et al.* 2022).

To determine differences in spectral and temporal parameters of echolocation signals among habitats, we selected species recorded at least 10 times in each habitat to ensure sample representativeness. Only two species met our criteria: *Saccopteryx bilineata* (Number of recordings per habitat: BT= 33, BM= 28, S= 49) and *Neoeptesicus* cf. *furinalis* (Number of recordings per habitat: BT= 15, BM= 10, S= 49).

We calculated boxplots for the following parameters: Bandwidth (Δ Frequency, kHz), Pulse Duration (Δ Time, s), and Peak Frequency, as these parameters best characterize echolocation pulses (Jung *et al.* 2007). Subsequently, we used a Kruskal-Wallis test to compare the means of each parameter across habitats, and used a Dunn-Bonferroni test for multiple comparisons (Supplementary material, Table S1, S2). For the aforementioned tests, we used the following R libraries: PMCMRplus (Pohlert 2021), ggplot2 (Wickham 2016), and ggpubr (Kassambara and Mundt 2020), o and the FSA library (Ogle *et al.* 2025). Given that vegetation variables were not used in these analyzes, our comparisons between habitats for *Saccopteryx bilineata* and *Neoeptesicus* cf. *furinalis* are based on our whole sampling (three weeks).

Results

We found 19 species, being *Saccopteryx bilineata* and *Neoeptesicus* cf. *furinalis* the most relatively abundant across all three habitats (Supplementary Material, Table S3 and Figure S4 to S6). The first PCA axis of vegetation variables explained 38.3% of the data, whereas the second axis explained 19.9%. The variables that explained most of the variance in Axis 1 and 2 were DBH, and Total Height, respectively (Supplementary material, Table S4). The PCA plot indicated that BM and BT are clustered together. Since there were no vegetation variables in habitat S, all plots from S are aggregated into a single point (Supplementary material, Figure S3).

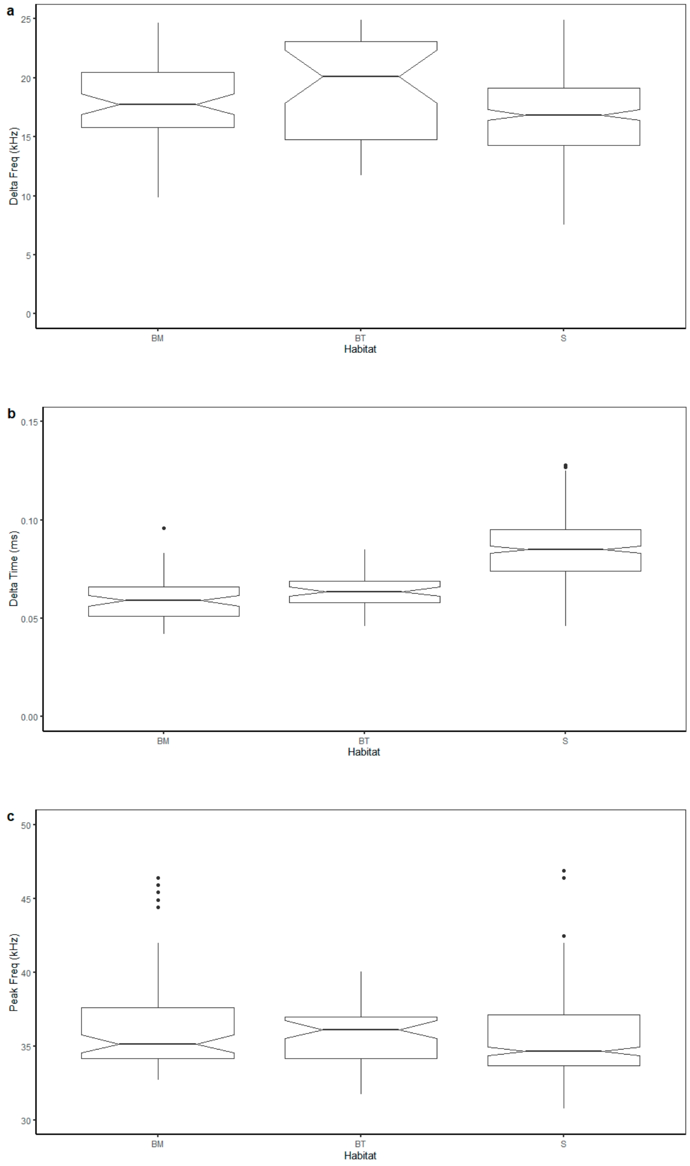

Figure 3. Spectral and temporal parameters of the *Neoeptesicus* cf. *furinalis* pulses vary between the different sites, Open (S), Clutter (BT) and High Clutter (BD), as indicated by the lack of overlap in the notches of the box plots. **a.** Δ Frequency (kHz) **b.** Δ Time (s) **c**. Peak frequency (kHz).

Relationship between vegetation variables and acoustic parameters. The first axis of the CCA indicates that DBH had the strongest influence on the spectral and temporal parameters (-0.67), while percentage cover (COV) was the most influential variable on the second axis (-0.62). Among the spectral-temporal variables, Δ Frequency (kHz) was most influenced by the first axis (-0.29), while Δ Time (s) was mainly influenced by the second axis (0.14) (Figure 1).

Spectral and temporal differences in two species present in the three habitats. We found that the Δ Frequency (kHz) (*P*= 1.987e-05), Δ Time (ms) (*P*= 2.2e-16), and peak frequency (kHz) (*P*= 4.89e-12) of the low pulse of *Saccopteryx bilineata* (Figure 2) were significantly different among habitats. Multiple comparisons for peak frequency only showed a significant difference between BM-S (*P*= 0.002). For Δ Frequency (kHz), we observed significant differences between BM-S (*P*= 0.002) and BT-S (*P*= 0.004). For Δ Time (ms), we found significant differences between BM-BT (*P*= 1.32e-04), between BM-S (*P*= 9.34e-40), and between BT-S (*P*= 3.23e-13).

Moreover, we also found significant differences among habitats for the high pulse of *Saccopteryx bilineata* in Δ Frequency (kHz) (*P*= 0.0001), Δ Time (ms) (*P*= 1.72e-05), and peak frequency (kHz) (*P*= 4.89e-12). The multiple comparisons tests only showed a significant difference between BM-S (*P*= 0.031). For Δ Frequency (kHz), we only found significant difference between BT-S (*P*= 0.0001). However, for the Δ Time (s), there is a significant difference between BM-BT (*P*= 1.40e-03), between BM-S (*P*= 2.3e-34), and between BT-S (*P*= 1.84e-13). These results indicate that environmental factors significantly influence the echolocation calls of *Saccopteryx bilineata* (Supplementary material, Figure S7). The significant differences in Δ Frequency and peak frequency between habitats suggest that this species is modifying their vocalizations, probably to optimize foraging efficiency and sound detection in response to the specific characteristics of their environment.

For *Neoeptesicus* cf. *furinalis* (Figure 3), there were significant differences among habitats in the Δ Frequency (kHz) (*P*= 0.004), Δ Time (s) (*P*= 2.20e-16), and peak frequency (kHz) (*P*= 1.39e-05). Regarding Δ Frequency (kHz), there was a significant difference among all three habitats: BT-S (*P*= 2.44e-06), BM-S (*P*= 5.99e-02), and BM-BT (*P*= 4.70e-02). For the Δ Time (s), there is a significant difference between BM-S (*P*= 1.13e-30), between BT-S (*P*= 4.15e-17), and finally between BT-BM (*P*= 6.81e-01) (Supplementary material, Figure S8).

Discussion

Our results confirm that bats can modify their echolocation calls according to the vegetation structure of their surroundings. These results suggest that vegetation structure, particularly tree size (*i.e.*, DBH), plays a key role in the variation of bat call frequency, whereas canopy cover affects the temporal aspects of calls, such as pulse duration. Our results indicate that different aspects of habitat complexity influence different acoustic parameters, reflecting how bats modify their echolocation to navigate and forage in environments with varying levels of clutter.

The most variable parameters were Δ Frequency (kHz) and Δ Time (s) for the whole ensemble. For *Saccopteryx bilineata*, we found rather unusual results that contradicted our predictions (Fenton *et al.* 2016). Initially, we assumed that in a more cluttered environment, pulses would be shorter and with a wider Δ Frequency delta to better avoid environmental obstacles (Moss *et al.* 2011). However, our results suggest that *S. bilineata* does the opposite: we recorded a shift to larger frequencies and a shorter in open spaces compared to cluttered environments. In comparison, and as we expected, *Neoeptesicus* cf. *furinalis* had longer duration calls with narrower Δ Frequency delta in open spaces, and short pulses with a higher Δ Frequency delta in highly cluttered areas (Moss *et al.* 2011).

Bats adjust their spectral and temporal parameters according to vegetation structure. To understand how the temporal and spectral parameters of echolocation pulses vary in bats, it is key to first determine how vegetation structure varies. The cluttered area (BT) is a transitional forest that has been restored for about 15 years. In this area, tall trees predominate, but with a lower DBH and with a smaller canopy width. In comparison, the highly cluttered space (BM) is a mature forest with very little disturbance in the last 50 years, according to landowners. Trees present in the highly cluttered area have different heights and diameters, with an average height of 25 m, and an average DBH higher than BT. Therefore, the highly cluttered space has a thick intertwined canopy whereas the cluttered space did not.

Bats adjusted the spectral and temporal parameters of their echolocation calls according to the complexity of vegetation structure. The type of acoustic environment that bats need to detect, classify and avoid is directly related to structural variables such as tree height, canopy density, and DBH because these parameters determine the number and type of obstacles and need to be sorted out (Schnitzler and Kalko 2001). For instance, Δ Frequency increased in sites with higher DBH, while Δ Time increased in areas with lower percentage cover. These results indicate that echolocation pulses become more modulated in more cluttered environments, supporting our predictions.

Larger trees generally contribute to greater clutter because of their sheer size and because they promote a denser and taller understory (Chazdon *et al.* 2010). Moreover, pulse interval became longer in less cluttered spaces, also supporting our prediction. Sites with lower percentage of canopy coverage, by definition, are much more open and less obstructed. In these types of conditions, bats are not forced to increase their rate of sound emission to update their perception of their environment (Fenton *et al.* 2016). We do not exclude other factors that may influence how bats modulate their echolocation signals. For instance, bat size (López-Cuamatzi *et al.* 2020) and feeding behavior (Fenton *et al.* 2016) also play a role in structuring echolocation calls. Yet, our results support our prediction that bats will echolocate at higher frequencies for shorter durations in cluttered areas (Denzinger *et al.* 2016).

Differences in spectral-temporal parameters in two species across three habitats. Saccopteryx bilineata is known for its vocal learning abilities (Knörnschild *et al.* 2006) and exhibits complex acoustic social interactions (Davidson and Wilkinson 2004). As shown by Ratcliffe *et al.* (2011), we observed that *S. bilineata* can alter its echolocation signals as needed, especially in relation to the type of habitat where it is foraging. For instance, Jakobsen *et al.* (2012) showed that *S. bilineata* significantly reduced the intensity and duration of its calls when flying in a flight cage, a proxy for a closed space environment. This aligns with our findings; Δ Time was higher in open environments (S) compared to sites with some obstacles (BT) or to highly cluttered environments (BM). In other words, pulses were longer in open spaces than in cluttered spaces. Our results support the idea that bats reduce the duration of their echolocation calls in cluttered environments. In cluttered habitats, the speed of receiving and processing environmental information is crucial, and this modification in call duration could facilitate such processing (Denzinger *et al.* 2016).

On the other hand, Δ Frequency and peak frequency showed a surprising behavior. We expected that Δ Frequency should be highest in cluttered spaces as bats increase their frequency modulation so they can effectively differentiate prey from vegetation (Denzinger *et al.* 2016). However, and contrary to our predictions, we observed that Δ Frequency and peak frequency were significantly higher in open spaces compared to cluttered spaces. Both results contradict existing literature. We think there are three different, not mutually exclusive hypotheses that can help us explain these unforeseen results.

First, call structure could change due to prey size (Aldridge and Rautenbach 1987). It is possible that smaller insects are found in greater numbers in the savanna (open spaces), so increasing both peak frequency and the range of frequency modulation of the echolocation signal would be advantageous and facilitate the detection of smaller prey at closed distances. Further studies assessing prey size across habitats would help us evaluate this hypothesis.

Second, *S. bilineata* might be increasing the frequency of their calls in open spaces to increase the directionality of their sonar beam. Jakobsen *et al.* (2012) found that *S. billineata* emits at higher frequencies to achieve more directional sonar beams, which increases the resolution and level of detail in their acoustic field of view. Seems contradictory to increase frequency and frequency modulation in open spaces because a reduction in call intensity also reduces the chances that a bat will detect an echo from a distant prey (Jakobsen *et al.* 2012). However, a narrower sound beam produces more intense sounds at the center of the beam, increasing the range at which bats can receive echoes, thus increasing the chances of detecting prey (Jakobsen *et al.* 2013). It is commonly assumed that bats use more energy when emitting echolocation calls at higher frequencies.

Initial studies by Speakman *et al.* (1989) showed that bats expend more energy at rest as vocal frequency increases. However, later studies showed the energy demand of echolocation was negligible compared to the high energy demand of flight (Speakman and Racey 1991; Voigt and Lewanzik 2012). Currie *et al.* (2020) found that increased signal intensity, but not frequency, increased energy expenditure even during flight. Therefore, more detailed studies in echolocation physiology will help us determine whether *S. billineata*, and maybe other species, increase their frequency range of emission to produce narrower beams in open spaces.

A third option as to why *S. bilineata* increased its frequency and decreased its pulse duration in open areas could be to avoid acoustic interference. *Saccopteryx leptura*, *Peropteryx macrotis*, and *Molossops temminckii* forage in similar areas as *S. bilineata*, and there could be an overlap with the echolocation calls of these species and *S. bilineata*. These three other species produce calls in similar frequency ranges (Supplementary material, Table 5), which could lead to acoustic interference.

Acoustic interference is related to acoustic niche partitioning, where coexisting species adjust their acoustic signals to minimize masking and enhance foraging efficiency (Siemers and Schnitzler 2004; Ey and Fischer 2009; Roemer *et al.* 2019). However, we believe this hypothesis seems unlikely because the abundance of these three species in the sampled habitats was low, reducing the likelihood of significant acoustic interference with *S. bilineata*.

Alternatively, the shift in *S. bilineata*'s call structure might not be solely related to acoustic overlap but could also be a strategy to reduce direct competition with other insectivorous bats in cluttered environments (Beilke *et al.* 2021). In cluttered habitats, where prey availability and species overlap may be higher, *S. bilineata* might exploit open spaces to reduce competition with species that hunt similar prey sizes. In these cluttered environments, where dense vegetation makes prey capture more difficult (Rainho *et al.* 2010), *S. bilineata* often shows a proportional aggregative response to increased prey abundance, whereas clutter-adapted species may remain unaffected by such variations (Müller *et al.* 2012). These findings support the hypothesis that *S. bilineata* may shift to open spaces as a strategy to access prey more efficiently and reduce competitive interactions.

Identifying the specific Molossidae species present in each habitat would help clarify whether this behavioral shift is driven by competition avoidance or by other adaptive factors. In sum, our results suggest that *S. bilineata* may have greater plasticity in its echolocation structure than previously thought. Further studies on prey size, sonar beam adjustments, and call overlap avoidance would be needed to better understand the potential costs of this plasticity.

Contrary to our results for *S. bilineata*, the spectral and temporal parameters of *N.* cf. *furinalis*, were consistent with theory (Barataud *et al.* 2013). Δ Frequency was higher in cluttered spaces compared to open spaces, suggesting that this species indeed benefits from having a broader frequency range in cluttered environments to efficiently navigate obstacles. Moreover, *N.* cf. *furinalis* showed shorter pulses in cluttered environments and longer pulses with lower frequency modulation in open spaces. The peak frequency for this species did not show significant differences between habitats.

Limitations. This study had two major limitations. First, our sampling period is short. We only sampled for one month and during the peak of the dry season. We do not know whether our results would be different in other times of the year where insect population abundance would be different across habitats, or if tree deciduousness would alter the acoustic space between habitats that bats must sort. It would be advisable that other studies integrate different climatic seasons to get a more complete picture of how vegetation complexity affects the echolocation structure of the insectivorous bat ensemble. The second limitation was the lack of data on insect size present in the different habitats, and the prey size that *S. bilineata* prefers among habitats. More information on the foraging ecology of this species would help us understand the unusual behavior we found.

Conclusions

Vegetation complexity significantly influences temporal and spectral parameters of bat echolocation calls in an insectivorous bat ensemble in the Llanos of Colombia. The structure of echolocation calls were significantly affected by diameter at breast height (DBH) and percentage of vegetation coverage (COV). We found unexpected call behavior in *Saccopteryx bilineata*, potentially influenced by various factors such a s prey sizes, sonar beam adjustments, and overlap avoidance, including direct competition with other species of similar size, even in the absence of acoustic overlap. Sorting among these hypotheses would help elucidate the potential costs of echolocation plasticity. Contrary to *S. bilineata*, the echolocation behavior of *N.* cf. *furinalis* followed the expected pattern according to theory. Understanding how bats change their spectral and temporal parameters according to different levels of vegetation complexity can provide insights into how species manage, or fail to manage, naturally open areas, such as the Colombian Llanos, or open areas resulting from anthropogenic activities.

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Supplementary material

This article contains Supplementary Material or Data Files, which can be downloaded from the journal website.