Amazonian soundscapes: unravelling the secrets of insect acoustic niches in diverse habitats

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Abstract

Insects are the most diverse animal taxon on Earth and play a key role in ecosystem functioning. However, they are often neglected from biodiversity surveys due to the difficulties of monitoring this small and hyper-diverse taxon. With technological advancements in biomonitoring and analytical methods, these shortcomings may finally be addressed. Here, we performed passive acoustic monitoring at 141 sites (eight habitats) in the Amazon to advance insect surveys using acoustic methods. We first describe the frequency range occupied by three soniferous insect groups (cicadas, crickets, and katydids) to calculate the Acoustic Evenness Index (AEI). Then, we assess the temporal and spatial variations of AEI among the insect categories, and finally we investigate the relationship among vegetation structure variables and AEI for each category. Overall, crickets occupied lower and narrower frequency bands than cicadas and katydids. AEI values varied among insect categories and across space and time. There was higher acoustic activity before sunrise and lower acoustic activity was recorded at pastures. Canopy cover was positively associated with crickets' acoustic activity but not with katydids. Our findings contribute to a better understanding of the role of time, habitat, and vegetation structure in shaping insect activity within the diverse Amazonian ecosystem.

1. Introduction

Global diversity has declined worldwide in recent decades in what is often considered the sixth major extinction event (Cowie et al. 2022). However, the biodiversity crisis is not homogeneous and varies among areas and taxonomic groups. For example, biodiversity loss is greater in tropical forests, where deforestation is causing extinctions at unprecedented rates (Brooks et al. 1999) and is also greater for insects than for other taxa (van Klink et al. 2020). Insects are the most diverse group of animals, comprising around 80% of all described animal life (Stork 2018), which together with their small size and our limited knowledge about their ecology (c. 80% of insect species remains undescribed, Cardoso et al. 2011), have made them challenging to study. However, technological advances during the last decades have provided new scalable, automated, and non-invasive methods for monitoring insects at large spatial and temporal scales (van Klink et al. 2022, Van Klink et al. 2024).

Among the recently developed automated and non-invasive techniques for biomonitoring is passive acoustic monitoring (PAM, Sugai et al. 2019). PAM requires the placement of programmed autonomous sound recorders in the field and followed by recording interpretation (manually or using automated software). This technique has proven to be a suitable method for monitoring wildlife across many regions and taxa (Sugai et al. 2019). However, invertebrates are, by far, the biological group least monitored using this technique (<5% of the studies, Sugai et al. 2019). Likewise, most research has been concentrated in the northern temperate zone (65% of the studies, Sugai et al. 2019), even though one of the main advantages of using PAM relies on its ability to monitor wildlife in ecosystems difficult to reach for ecologists, such as tropical forests (Deichman et al. 2018, Pérez-Granados and Schuchmann 2020, Do Nascimento et al. 2021).

A diverse range of insects produce sounds and therefore PAM enables a scalable and standardized methodology for monitoring soniferous insects, which dominate nocturnal soundscapes in the tropical region (Aide et al. 2017, Müller et al. 2023). Indeed, insects can be acoustically detected far away from the recorders (e.g., up to 100 m), which is a much longer distance than insect detection range using pitfalls or nets (Jeliazkov et al. 2016). Moreover, PAM surveys can be coupled with automated species recognition, thus allowing for species-specific monitoring with less effort than traditional survey methods (van Klink et al. 2022, Symes et al. 2024). However, this approach is limited by the lack of available training data for the algorithms, and this is especially problematic for insects (ter Hofstede et al. 2020, Symes et al. 2022).

PAM-based surveys collect soundscape recordings, which contain available information about the state of biological assemblages. A soundscape can be described as the sonic structure of a given location, accounting for signal composition, diversity, intensity and temporal patterns (Schafer 1977). Soundscapes are usually divided into biotic and abiotic components according to main sound sources, with the biotic component, usually defined as biophony, composed of the collective sounds produced by living organisms (Pijanowski et al. 2011). Several acoustic indices have been developed to aid in the interpretation of soundscapes and have often proven their ability to assess species richness, composition, or whether acoustic activity varies within different ecosystems (Buxton et al. 2018, Bradfer-Lawrence et al. 2020, Do Nascimento et al. 2020, Allen-Ankins et al. 2023, Müller et al. 2023; but see Alcocer et al. 2022 for limitations). Several groups of insects produce sounds at very specific (e.g., higher than birds) and narrow frequencies (Metcalf et al. 2021), and therefore acoustic indices could be an effective alternative for monitoring the relationship between insect acoustic activity and other variables of interest. Indeed, some of the existing indices already have been used as a surrogate of insect richness or composition (Aide et al. 2017; Müller et al. 2023) and to describe temporal patterns of insect communities (Ferreira et al. 2018, Burivalova et al. 2022). However, our current knowledge about the use of acoustic indices for monitoring insects is still limited and their effectiveness is expected to vary among regions owing to different background noise and species compositions (Müller et al. 2023, Scarpelli et al. 2023).

Here, we draw upon the acoustic adaptation hypothesis (Morton 1975) to test the effects of habitat type and vegetation structure on the acoustic signals produced by insect communities. We recorded soundscapes and collected vegetation data at 141 sites across eight habitat types (from natural to anthropogenic) representing dominant habitat types found in the Amazon basin. Our approach comprises three main goals: (1) to describe manually the frequency range occupied by three soniferous insects groups (cicadas, crickets, and katydids) in our study area, and to calculate acoustic indices targeting these groups to semi-automatically study their ecology (Burivalova et al. 2022, Metcalf et al. 2021), (2) to assess the temporal (hourly) and spatial (habitat type) variations of acoustic indices among insect groups, and (3) to investigate the relationship between vegetation structure variables and acoustic indices on targeted insect groups to better understand relationships between insect activity and habitat structure. We predict that: (a) insect acoustic activity will vary among habitat types because habitat type influences insect community composition as well as their acoustic communication and behaviour (Römer and Lewalds 1992, Schmidt and Balakrishnan 2015). More specifically, we expect forested habitats (and those with higher vegetation complexity) to have more diverse insect communities and thus a more diverse acoustic environment, while open (more homogeneous) habitat types would have more homogeneous insect communities and thus less diverse acoustic environments, due to more favourable microenvironment conditions in forested habitats that may sustain greater insect richness and abundance. We also predict that (b) insect acoustic activity will vary among recording hours because several environmental factors (e.g., moonlight, temperature) may influence their activity and sound production throughout the night in tropical forests (Oliveira et al. 2021, Gomez-Morales and Acevedo-Charry 2022, Scarpelli et al. 2023, Symes et al. 2024). Our findings will contribute to a better understanding of the role of time, habitat and vegetation structure in shaping insect acoustic activity within the diverse Amazonian ecosystem and might be useful to guide future research on other tropical regions.

2. Methods

(a) Study sites

We conducted this research in and around Viruá National Park (VNP), Roraima, Brazil, in the north of the Brazilian Amazon (Figure 1). VNP was established in 1998 and is 240,000 ha (ICMBio, 2014). The climate in VNP is warm and wet with mean annual temperature of 26°C and mean annual precipitation of ~2,000 mm (ICMBio, 2014). Rainfall is mostly concentrated from May to September (ICMBio, 2014). VNP is regulated by floods that create a vegetation mosaic ranging from dense forests to grasslands and has most major habitats found across the Amazon biome (ICMBio, 2014). These habitats share common species, but also harbour unique fauna and flora. The study was carried out in eight different habitat types, separated according to their vegetation characteristics and flooding regime (Figure 1). The eight habitats surveyed can be grouped into three main categories: i) open habitats (Burned campina, Campina, and Pastures), ii) flooded forests (Igapó, Islands, and Várzea), and iii) non-flooded forests

(Campinarana and Terra-firme). In general, open habitats have lower vertebrate species richness than forested habitats, and Campiranana, Igapó, and Island forests are not as diverse as Terra-firme and Várzea forests, yet it is unclear if these patterns hold for invertebrates (see Do Nascimento et al. 2020 for additional information on habitats).

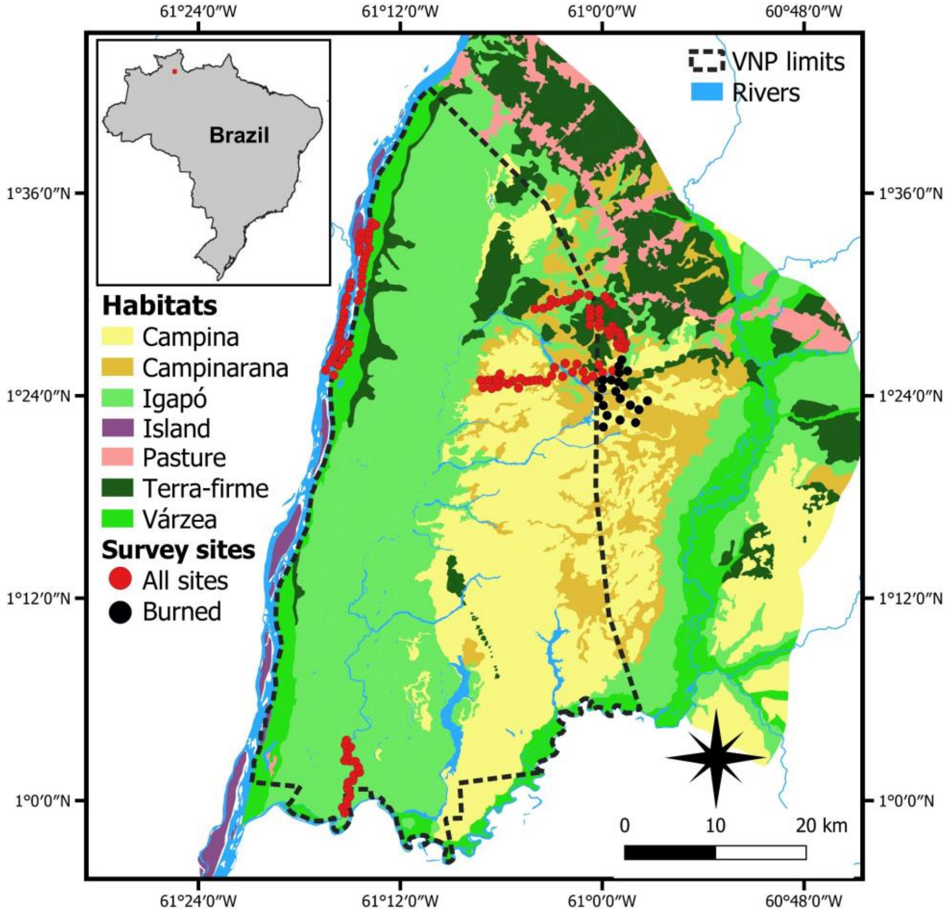

Figure 1. Location of the surveyed sites (141) across the eight habitat types studied in the Viruá National Park, Roraima, Brazil. The inset shows the location of the study area in northern Brazil.

(b) Passive acoustic monitoring

We used Arbimon acoustic recorders (Campos-Cerqueira & Aide, 2016) to collect acoustic data during the dry season, from November 2016 to February 2017, in the eight habitat types. We deployed recorders at 20 replicate sites in each habitat, except for Pastures (six replicates because they comprised a small area in the VNP); and Campinarana and Terra-firme

(18 and 17 replicates, respectively, because of recorder malfunctions). Therefore, the final acoustic dataset was collected in 141 sites. Recorders were separated by at least 500 m to minimize the risk of overlapping detections across recorders (vocalizations of birds and frogs recorded with the employed recorder can be detected up to ~100 m, Campos-Cerqueira et al. 2020). We attached recorders to trees or fixed poles at the height of 1.5 m. Acoustic devices were programmed to record 1 min of audio every 10 min (144 1-min recordings per day) for six days in each sampling site (sample rate = 44.1 kHz; resolution = 16 bit; format = WAV). After six days, the 20 recorders were moved to a different habitat type and the method repeated. We inspected recordings for file corruption, heavy rain, and wind and removed those files when any of these circumstances occurred (details in Do Nascimento et al. 2020). The acoustic dataset we used for subsequent analysis had 95,141 one-minute recordings (1,585 hours).

(c) Vegetation structure survey

Vegetation structure data were collected within a 20-m radius plot from each acoustic recorder location (141 sites) after the devices were moved to a different habitat type to avoid interference with the recordings. For each site, we measured the percent canopy cover, canopy height, litter depth, number of small trees (Diameter at Breast Height – DBH > 1 cm to < 10 cm), number of large trees (DBH > 10 cm), and shrub cover. For each vegetation structure variable, we used the mean value per site for subsequent analysis. Details about the vegetation structure survey methodology can be found in Do Nascimento et al. 2020.

(d) Spectral frequencies occupied by insects

To characterize the spectral frequencies occupied by insects two experienced researchers (LAN and JBRA) with insect sounds and the study area manually reviewed 1,152 one-minute audio files (19.2 hours) from one randomly chosen day/site (24 hours) per habitat type. The amount of time manually reviewed was similar or higher to that considered in most prior research using acoustic indices and sonotypes (e.g., 228 minutes in Gasc et al. 2018, 672 minutes in Duarte et al. 2021, 63 minutes in Burivalova et al. 2022, and 1,342 minutes in Gomez-Morales and Acevedo-Charry 2022).

For each file, we annotated the minimum and maximum frequencies of three main sound-producing insect groups in the study area using a sonotype approach (details in Aide et al. 2017): Cicadidae (Cicada), Grylloidea (Crickets), and Tettigoniidae (Katydids). We used Audacity (Audacity Software 3.3.3) to visually inspect the spectrograms and annotated all selected files using selection boxes around each sound (like Burivalova et al. 2022). When in doubt about the identification of a sonotype, we would consult each other or other specialists to reach consensus. In total, we annotated 1,714 sonotypes across insect groups. We also annotated if any other animal or sound would overlap in the frequency range occupied by the insects because this would allow us to focus our analysis on periods of little to no overlapping sounds (163 sonotypes were annotated). We also registered the beginning and ending time of insect choruses (i.e., two or more insects producing a very characteristic and continuous acoustic signature that may last for hours, Figure S1) for each site and habitat reviewed. Additionally, we estimated the chorus length for each habitat type as the time difference between the ending and beginning time of insect choruses.

Our analysis revealed the cicada sounds were not present across all habitats in our manually reviewed dataset, therefore this group was excluded from subsequent analysis.

Nonetheless, we have opted for including in the results the spectral frequencies occupied by cicadas, which might be useful to guide future studies in the Amazon.

(e) Acoustic index calculation

We used the Python package “scikit-maad” (Ulloa et. al 2021) and Anaconda (Anaconda Software Distribution, 2016) with Python 3.9.17 to calculate the Acoustic Evenness Index (AEI, see description in Villanueva-Rivera et al. 2011) on three average frequency ranges mainly occupied by Crickets (5,000 Hz to 7,500 Hz), Katydids (8,000 Hz to 17,000 Hz), and what we called “All insects” category - encompassing both crickets and katydids (5,000 Hz to 17,000 Hz) using the default settings provided in the package (frequency step = 500 Hz). The AEI measures unevenness among frequency band steps returning a value close to one when sound intensity is restricted to few frequencies and a value close to zero when soundscapes are saturated with sounds (Bradfer-Lawrence et al. 2023, Do Nascimento et al., 2020). Therefore, recordings with lower values of AEI can be interpreted as recordings with higher sound activity, while recordings with higher AEI values can be interpreted as recordings with limited sound activity (Figures S1–S8). The AEI was chosen to describe insect acoustic activity based on prior research in the study area, which showed that the AEI was the acoustic index that better explained ($R^2 = 81\%$) the relationship between soundscapes and vegetation structure outperforming several other acoustic indices on this task (Do Nascimento et. al 2020). We focused the index calculation on the nocturnal period because during this period there was minimal frequency overlap of crickets and katydids sounds with other animals or sound sources at our manually annotated dataset. In fact, only three out of 1,152 files reviewed had birds overlapping with crickets which reassures that the AEI values estimated during the nocturnal period will reflect the acoustic activity of insects.

We considered the nocturnal period to be the time elapsed between evening and morning astronomical twilight (Foote et al. 2017, Pérez-Granados and Schuchmann 2021), as extracted from www.timeanddate.com. To standardize the number of recordings analysed per site and due to the low variation in twilight timing during the study period, we considered as nocturnal those recordings made between 1900 h to 0500 h, which was always between the evening and morning twilight times.

(f) Statistics

First, to assess variations of the AEI among hours and habitat type, we fitted generalized linear mixed models (GLMM, Gaussian family) using the values (log transformed) of the AEI targeting each insect category (“All insects”, Crickets, and Katydids) as the response variable, hour of recording (“ten levels”) and habitat type (“eight levels”) as fixed effects and recording station as a random effect to control for variations within habitats owing to site. One independent GLMM was fitted for each insect category (“All insects”, Crickets, and Katydids). The Gaussian family was selected after testing different distribution families and choosing the most appropriate according to the Akaike information criteria (AIC; Burnham and Anderson, 2004) and visually inspection of the residuals. Model performance was evaluated by plotting standardized residuals versus fixed variables, normal Q-Q plots and histograms of residuals. No concrete pattern was found in any case. When a fixed effect was found to be significant, a Tukey’s post hoc test was performed to test for differences among levels.

Second, to determine the influence of vegetation structure variables on the AEI, we fitted linear mixed models (LMM) using the mean values (log transformed) per site of the AEI targeting each insect category as the response variables (one independent LMM per insect category), six vegetation structure variables (canopy cover, canopy height, litter depth, number of large trees, number of small trees, and shrub cover) as fixed effects, and habitat type as a random effect to control for variations owing to habitat groups. Fixed effects were scaled to make their parameter estimates comparable within models. We performed model selection using the AICc. We selected the top four performing models based on ΔAICc and considered models to be similar if $\Delta AICc < 2$ (Burnham and Anderson, 2004). We checked for multicollinearity of predictors and removed canopy height from the analysis. Residuals of the models were checked for linearity, homoscedasticity, independence, and normality. Finally, we calculated the marginal and conditional R^2 values (Nakagawa and Schielzeth, 2013) to estimate the proportion of variance explained by fixed and random effects.

All statistical analyses were performed with R 3.6.2 (R Development Core Team 2021). We used the packages “lme4” (Bates et al. 2015) for the GLMM construction, “lmerTest” (Kuznetsova et al. 2014) to calculate the significance of fixed effects, “multcomp” (Hothorn 2008) for post hoc comparison tests, “nlme” (Pinheiro et al. 2019) for the LMM construction, “car” (Fox and Weisberg, 2019) and “SjPlot” (Lüdecke, 2020) for checking model assumptions, and “R2glmm” (Jaeger, 2017) to extract R^2 values. The level of significance adopted was $p < 0.05$.

3. Results

(a) Mean spectral frequencies occupied by insects and chorus temporal patterns

Cicadas were only found in Burned Campinas, Campinaranas, and Terra-firme sites. In Campinaranas, the mean maximum frequency of Cicadas was much higher (16.21 kHz) than at

Terra Firme and Burned Campinas (8.64 and 8.95 kHz, respectively), but just one call was registered in Campinaranas. Crickets were found across all eight habitats surveyed, however, the variation among habitats between minimum and maximum frequencies was small in magnitude compared to the other insect groups (range between 5.13-7.73 kHz in all habitats). Katydids were also found across all habitats surveyed, and higher variations between minimum and maximum frequencies were identified across habitats (Table 1). For example, mean minimum and maximum frequencies of Katydids in Burned Campina were of 7.71 and 8.77 kHz, respectively, while in Island the mean minimum and maximum frequencies were of 12.11 kHz and 15.54 kHz (Table 1).

Table 1. Minimum and maximum frequencies (kHz, mean ± SD) manually measured for sonotypes (N) of three insect groups across eight Amazon habitat types.

Insect group	Habitat type	N	Minimum frequency	Maximum frequency
Cicadas	Burned campina	3	3.28 ± 0.35	8.95 ± 1.88
	Campinarana	1	3.06	16.21
	Terra-firme	77	3.19 ± 1.43	8.64 ± 2.84
Crickets	Burned campina	118	5.46 ± 1.09	6.48 ± 1.27
	Campina	118	5.27 ± 1.1	5.62 ± 1.28
	Campinarana	173	6.75 ± 1.81	7.73 ± 1.76
	Igapó	236	5.68 ± 1.56	7.17 ± 1.71
	Island	206	5.13 ± 1.4	7.13 ± 1.61

	Pasture	147	5.37 ± 1.09	6.41 ± 1.36
	Terra-firme	330	6.15 ± 2.61	7.26 ± 2.53
	Várzea	96	6.13 ± 0.83	7.02 ± 1.06
Katydids	Burned campina	4	7.71 ± 1.15	8.77 ± 1.35
	Campina	12	9.34 ± 0.46	12.63 ± 0.47
	Campinarana	2	11.51 ± 2.98	16.00 ± 3.81
	Igapó	15	9.02 ± 2.7	17.30 ± 2.14
	Island	56	12.11 ± 0.64	15.54 ± 1.87
	Pasture	35	8.73 ± 1.25	11.75 ± 2.48
	Terra-firme	73	9.29 ± 1.58	17.29 ± 2.15
	Várzea	12	12.83 ± 0.8	14.74 ± 0.26

The onset and end of the insect chorus greatly varied across habitats, lasting the longest (18 hours) on Islands and the shortest (7 hours) on Várzea (Table 2).

Table 2. Temporal patterns of insect chorus activity across Amazonian habitats based on the analysis of one randomly chosen replicate site for each habitat type during a 24-hour period.

Habitat	**Beginning time**	**Ending time**	**Chorus length**
Burned campina	1710 h	0540 h	12 h 30 m
Campina	1840 h	0310 h	8 h 30 m
Campinarana	1510 h	0810 h	17 h 0 m

Igapó	1740 h	0700 h	13 h 20 m
Island	1600 h	1000 h	18 h 0 m
Pasture	1730 h	0620 h	13 h 20 m
Terra-firme	1240 h	0600 h	17 h 20 m
Várzea	1800 h	0100 h	7 h 0 m

(b) The influence of the hour and habitat type on the AEI targeting insect sounds

According to GLMMs, the AEI values of "All insects" (5,000 Hz to 17,000 Hz), Crickets (5,000 to 7,500 Hz), and Katydids (8,000 Hz to 17,000 Hz) significantly varied across recording hours and habitat types (Table 3). Overall, there was a significant decreasing pattern of AEI values from sunset to sunrise, which was very consistent for the three insect categories (Figure 2). According to post-hoc tests, the higher AEI values were obtained from the recordings made at 1900 h and 2000 h for all tree insect categories, while the lowest AEI values were observed at 0200 h for Crickets (Figure 2B), and between 0200 h to 0400 h for "All insects" and Katydids (Figure 2A, 2C).

Table 3. Summary table of type-III partitioning of variances testing the effects of recording hour and habitat type on the variations of the Acoustic Evenness Index per insect category. Recordings were collected at eight habitat types and during ten different recording hours. Detailed results of the GLMMs per insect category can be found at Table S1–S3 in the online supplementary material.

Insect Category	**Fixed effect**	**Sum Sq**	**Mean Sq**	**DF**	**F**	***p***
All	Recording hour	1090.49	17	416	203.31	**<0.001**
	Habitat type	11.98	1	33	2.87	**0.008**
Crickets	Recording hour	73.91	1	401	19.68	**<0.001**
	Habitat type	28.54	8	33	9.77	**<0.001**
Katydids	Recording hour	1272.89	43	415	270.71	**<0.001**
	Habitat type	18.43	3	33	5.04	**<0.001**

At the habitat level, there were more subtle differences across habitat types compared to the differences found at hourly scale (Figure 2). AEI values for all three insect categories were significantly higher for Pasture than the other habitats (Figure 2), although similar AEI values (non-significant differences according to post-hoc tests) were observed at Pastures and Burned campina for Crickets (Figure 2E), and similar values (non-significant) at Pasture and Islands for Katydids (Figure 2F). Lower AEI values were observed in Campina and Várzea for “All insects” category, in Várzea and Island for Crickets, and in Burned campina and Campina for Katydids.

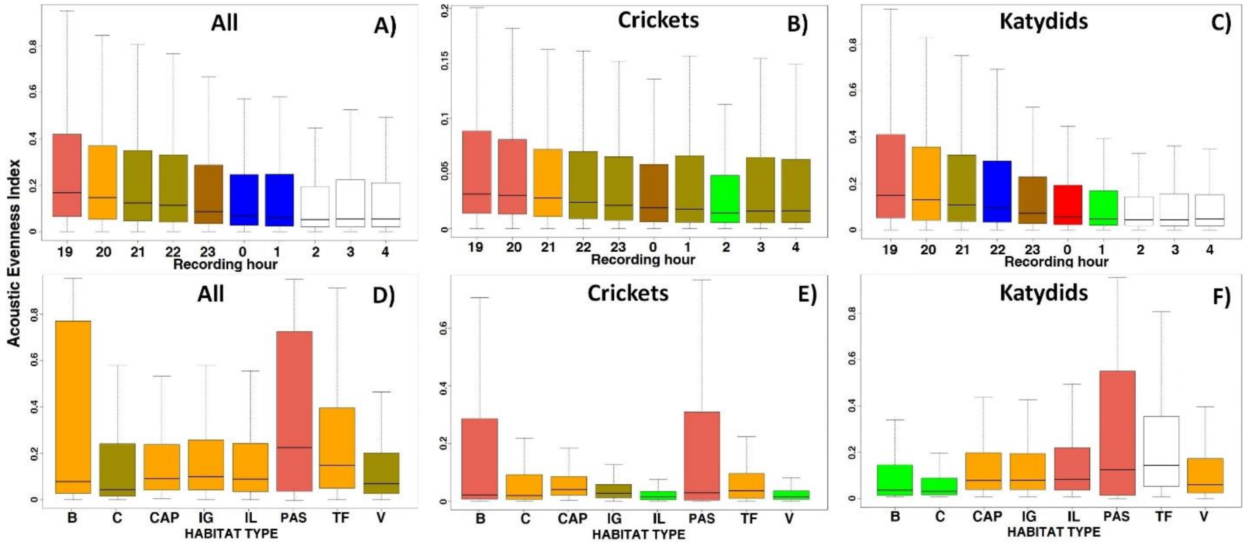

Figure 2. Boxplots showing the variation of AEI values as a function of recording nocturnal hours (A-C) (19:00 to 04:00) and (D-F) habitat type. Results are shown separately for each insect category. Different colours show significant differences in AEI values between recording hours or habitat type from Tukey's post hoc test. B=Burned campina, C=Campina, CAP=Campinarana, IG=Igapó, IL=Island, PAS=Pasture, TF=Terra-firme, and V=Várzea.

(c) The influence of vegetation structure on the AEI targeting insect sounds

Across the top performing models (Table S4), only the AEI calculated in the frequency range dominated by Crickets was significantly and positively associated with canopy cover and negatively associated with shrub cover (Figure 3). The top performing models for AEI targeting Katydids and "All insects" category included null models, therefore we did not consider these groups to be significantly related to any of the vegetation structure variables, although canopy cover and the number of small trees was marginally associated with the "All insects" category. Conditional R^2 of the Cricket top perform model was 41% while the marginal R^2 of canopy cover and shrub cover were, respectively, 41% and 5%.

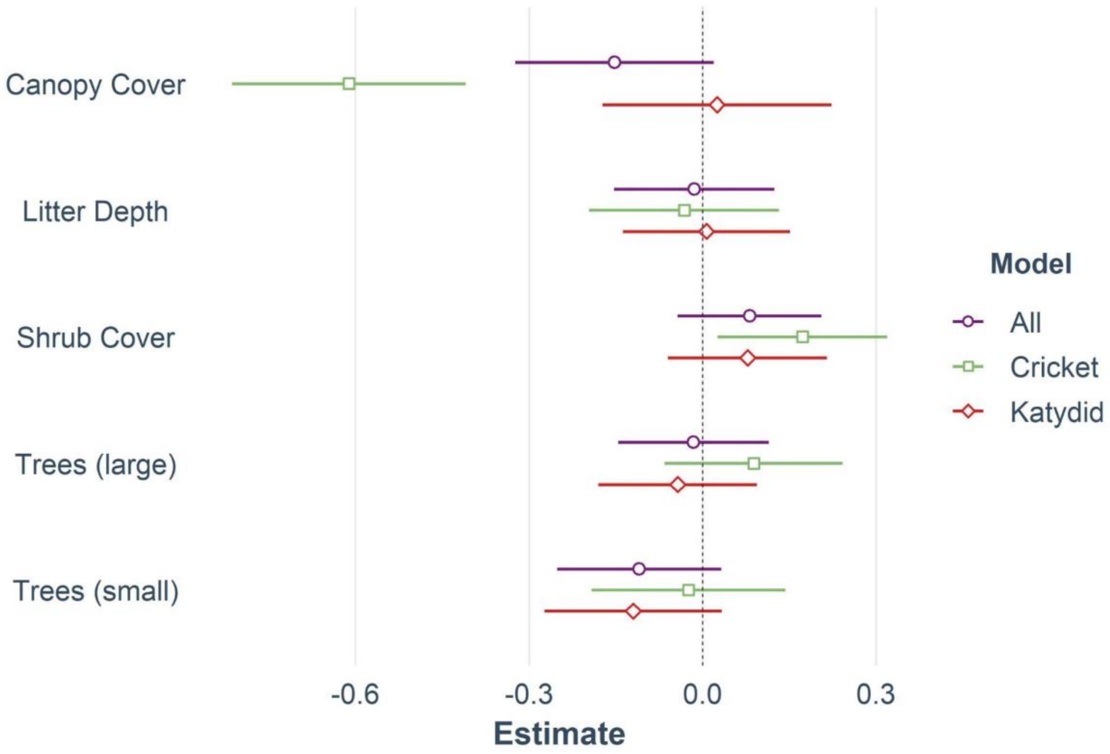

Figure 3. Results of linear mixed models for three insect categories ("All insects", Crickets, and Katydids) showing the effects of five vegetation structure variables on the AEI values calculated for each insect category. Symbols are the normalized coefficients' estimate values and horizontal lines represent the 95% confidence intervals. Estimates were normalized by subtracting raw values by the mean and dividing by the standard deviation. Detailed results of the LMMs can be found at Table S5 in the online supplementary material.

4. Discussion

Acoustic indices are useful tools to analyse large quantities of audio data and investigate ecological patterns at broad spatial and temporal scales. Their usefulness is particularly high when coupled with expert ecological knowledge about the studied region and with other analytical methods or available datasets that may help to interpret the underlying ecological patterns. Here, we demonstrate how manual annotation of a subset of recordings was useful in calculating the Acoustic Evenness Index (AEI) for a time and frequency range sensitive to different insect groups that dominate nocturnal soundscapes in the Neotropics. Overall, we found that crickets occupy lower and narrower frequency bands than cicadas and katydids. We also found that AEI values varied among insect categories across space and time, with a higher acoustic activity (i.e., lower AEI values) during the hours before sunrise and lower acoustic activity (higher AEI values) in Pastures across all insect categories analysed. Canopy cover was positively associated with Cricket acoustic activity but not with Katydids. Our findings contribute to our understanding of the role of time, habitat, and vegetation structure in shaping insect acoustic activity within the hyper-diverse habitats of the Amazon using a non-invasive and semi-automated approach based on soundscapes that is scalable (Müller et al. 2023).

The manual annotation process allowed us to verify the absence of other (non-target) vocally-active taxa during the night, and therefore reduce their influence when assessing changes in AEI values. Indeed, birds were only detected in three of the 1,152 files reviewed, while anurans appeared in few recordings, but their vocalizations were always made at frequency bands lower than the ones used by insects (e.g., Figure S1). The frequency measurements provided in our study might be useful as a baseline for future research aiming to work with these groups of insects in the Amazon. However, prior research has demonstrated the existence of geographical variation in insects' frequencies, partly driven by habitat type, and even of dialects within the same species, and so we encourage researchers to develop their own group-specific frequency bands (Claridge and Morgan 1993, Chen et al. 2019, Metcalf et al. 2021).

We found a clear pattern of increasing acoustic activity (lower AEI values) from sunset to sunrise across all three insect categories analysed (Figure 2), in agreement with our prediction that insect acoustic activity will significantly vary throughout the night in Amazonian habitats.

Our results agree with prior research in tropical forests from Panama (Symes et al. 2022) and Colombia (Gomez-Morales and Acevedo-Charry 2022), where acoustic activity of crickets and katydids also significantly varied throughout the night. However, the results were species-dependent. For example, in Colombia two cricket sonotypes were more often detected around sunset while five katydids sonotypes were detected more homogeneously throughout the night (Gomez-Morales and Acevedo-Charry 2022). The authors attributed some of this variation among groups to cricket acoustic activity being influenced by rainfall, while katydids were more influenced by moon phase. In Panama, Symes et al. (2022) found that 24 katydid species were detected throughout the night, but some species were reported to call more often right after sunset and right before sunrise, with no moonlight effect (Symes et al. 2024). It is important to note that we focused our study during the nocturnal period and excluded the sunset and sunrise choruses from our analysis to avoid frequency and time overlap with other soniferous species, if we could easily filter out non-target sounds (e.g. birds) to calculate the AEI (see advances in source separation in Tolkova 2023), perhaps we would also detect more insect activity right after sunset and right before sunrise as reported in prior research (Symes et al. 2022, Gomez-Morales and Acevedo-Charry 2022). Despite this limitation about the use of acoustic indices, a soundscape analysis approach is likely capturing the activity of much more insect species than species-specific approach and thus reflecting broader patterns of calling behaviour (Müller et al. 2023, Scarpelli et al. 2023).

When analysing the effect of habitat type on AEI values targeting three insect categories, we did not find any clear pattern emerging from the results (Figure 2). Nonetheless, the variation across habitats was significant in all insect categories. Pastures displayed the lowest acoustic activity overall (higher AEI values) while forests (e.g., Várzea and Islands) had more acoustic activity for Crickets (lower AEI values). This result was expected as pastures are human modified habitats likely hosting lower insect diversity than forests (Do Nascimento 2020). But only by using fine scale habitat vegetation measurements the patterns in the data became clearer, unravelling how canopy cover has a positive effect ($R^2 = 41\%$) on Crickets calling activity but not on Katydids or the broad “All insects” category (Figure 3). Therefore, partially confirming our prediction that more complex habitats (e.g., Várzea forests) will have higher insect acoustic activity than less complex habitats (e.g., Campina grasslands). Interestingly, crickets seem to call more often from sites with less shrub cover (Figure 3), which are more often found in flooded forests, such as Várzea and Islands, than non-flooded forests, such as Terra-firme. This shrub cover variation among forests could also be the result of flood pulses that wash out these ecosystems seasonally (Householder et al. 2021) but in turn may also bring nutrients and other resources important for cricket survival. Further research could monitor Várzea and Islands during the flooding period to test if acoustic activity of insects is higher when the herbaceous vegetation is submerged. To the best of our knowledge, this is the first study to test how major habitat formations from the Amazon basin may structure the calling activity of different insect groups using a semi-automated approach based on acoustic indices. The effect of habitat type and vegetation structure on insect calling activity have been discussed for a long time (Couldridge and van Staaden 2004) but has rarely been tested on a scale that is relevant for decision making and conservation (Schmidt and Balakrishnan 2015, Jeliazkov et al. 2016). Soundscapes and acoustic indices could enable these tests on an unprecedented scale (Roe et al., 2021).

In this study, we employed acoustic indices to assess the temporal and spatial variations of insect acoustic niches. However, the use of acoustic indices for monitoring insects can extend far beyond and might be useful to answer traditional (and new) ecological questions regarding insect activity. Currently, our knowledge about how and which drivers shape insect acoustic activity is very limited, and the use of passive acoustics and indices could clearly contribute to better understanding this topic. For example, the methodology applied might be useful to assess the relationship between climate conditions and insect acoustic activity (Scarpelli et al. 2023), as well as to assess seasonal variations on such relationships (e.g., rainfall is not expected to have the same impact during the wet and dry season in tropical forests). Likewise, it could also help to understand how other drivers, such as astronomical (e.g., moonlight), human noise, or predation pressure (e.g., birds, bats) may have an impact on the acoustic activity of insects (Scarpelli et al. 2023, Belwood and Morris 1987). We are aware that acoustic indices need to be used with caution (Bradfer-Lawrence et al. 2023), but our results suggest that acoustic indices can greatly advance insect automated monitoring after carefully selecting the optimal frequency bands for certain insect groups and with a validation of low interferences in the selected frequencies (Metcalf et al. 2021).

5. Conclusions

We have demonstrated that the use of acoustic indices can be a useful tool to accurately describe the temporal and spatial variation of insect acoustic activity at scale in the Amazon. We hope that our assessment will encourage researchers and managers to judiciously use this readily and user-friendly tool. The estimation of acoustic indices is an automated process, which can be implemented in R (or through friendly interfaces, such as Kaleidoscope Pro) with little informatic knowledge, and as demonstrated here (after being validated through manual inspection of the sonograms) holds the potential to shed light into the nocturnal ecology of insects, an aspect of their life cycle difficult to ascertain using traditional surveys methods. Further research should evaluate the utility of this technique to monitor a wider range of insects, including those that call in the diurnal period, a time of the day under which the utility of acoustic indices for monitoring insects may be decreased owing to frequency overlap with other vocally-active taxa (Scarpelli et al. 2023). Future studies could also focus on applying the techniques described here to monitor insects calling in the ultrasonic spectrum, which also presents many challenges (Madhusudhana et al. 2024).

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