

Relationships between species assemblage richness and acoustic indices

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Abstract. *Biodiversity assessment is increasingly reliant on acoustic indices derived from bioacoustic recordings. This study evaluates the performance of five acoustic indices—Acoustic Richness Index (H_s), Von Neumann Entropy (H_v), Acoustic Diversity Index (ADI), Bioacoustic Index (BIO), and Acoustic Evenness Index (AEI)—in controlled environments. We generated simulated assemblages by randomly mixing vocalizations from distinct species and specimens, creating 24,000 bird assemblages and 2,000 anuran assemblages. Each index was calculated for these datasets to assess its relationship with species richness. The results revealed that these indices exhibited a positive correlation with species richness in the assemblages. However, background noise significantly distorted these correlations. Our findings underscore the need for further research to clarify the specific conditions under which acoustic indices can reliably quantify biodiversity.*

1. Introduction

The term “biodiversity” refers to the variety of life on Earth, encompassing both species richness and ecosystem diversity. Biodiversity can be inferred from acoustic signals produced by organisms during communication, movement, and interactions with their environment [Sueur et al. 2008]. These bioacoustic signatures enable researchers to assess biodiversity non-invasively by analyzing natural soundscapes, often through passive acoustic monitoring (PAM) systems [Sueur et al. 2014]. However, developing robust methods to monitor and quantify ecological changes in these complex acoustic communities remains an ongoing challenge.

The analysis of soundscapes provides a powerful framework for qualifying and quantifying environmental dynamics. This framework enables monitoring ecosystem changes and assessing animal diversity across spatial and temporal scales. Bioacoustic indices, which are mathematical tools derived from soundscape recordings, serve as unsupervised methods for quantifying species diversity. These indices rely on specific features of audio signals, such as frequency, amplitude, and temporal patterns, to infer ecological information [Farina and Gage 2017].

Recent studies have explored bioacoustic indices to quantify biodiversity [Pieretti et al. 2011, Towsey et al. 2014, Zhao et al. 2019, Sánchez-Giraldo et al. 2021]. While these studies have shown promising results, the extent to which these indices can serve as reliable proxies for measuring diversity remains unclear [Mammides et al. 2017, Bicudo et al. 2023, Sethi et al. 2023,

Llusia 2024, Mammides et al. 2025]. Several factors may influence the accuracy of these estimates, including background noise, rainfall, overlapping sounds, the presence of multiple species vocalizations, and the distance to the microphone, among others [Bradfer-Lawrence et al. 2024].

Given the discrepancy between authors who support the use of bioacoustic indices and those who critique their reliability, we aimed to study and compare how five different bioacoustic indices respond to variations in species number and background noise. To achieve this, synthetic soundscapes were generated to simulate 24,000 bird assemblages and 2,000 anuran assemblages, each five seconds long. These soundscapes were created using real forest audio data, forming random assemblages ranging from one to five species to simulate an increase in species richness. All our experiments and code are available in the repository: https://github.com/Yunevda/synthetic_assemblages.git

2. Related works

[Pieretti et al. 2011] evaluated the practical utility of the Acoustic Complexity Index (ACI) through correlation analyses. Their findings revealed a significant correlation between ACI values and the number of bird vocalizations, but only weak associations with acoustic frequency and intensity ranges. The authors attributed this limitation to interference from geophonic (e.g., wind, rain) and anthropophonic (e.g., human activity) noise within soundscapes. Despite these challenges, they concluded that the ACI remains a valuable tool for monitoring songbird activity and detecting environmental disturbances. Similarly, [Towsey et al. 2014] assessed 14 acoustic indices for quantifying avian diversity and found that such indices enhance species identification accuracy compared to traditional field methods, particularly in automated biodiversity monitoring.

In contrast, [Eldridge et al. 2018] reported mixed results across ecosystems: acoustic indices exhibited strong correlations with bird species richness in temperate habitats but weaker correlations in tropical regions. The latter, they argued, reflects the heightened biodiversity complexity of tropical soundscapes, which include vocalizations from non-avian taxa. Expanding on this, [Alcocer et al. 2022] identified a moderate positive correlation between acoustic indices and species diversity metrics but cautioned against treating indices as direct biodiversity proxies. Their work emphasized the need to account for methodological limitations when interpreting index-derived data.

Similarly, [Bicudo et al. 2023] highlighted the poor predictive power of acoustic indices in hyper-diverse tropical ecosystems, drawing on field recordings from the Amazon's Balbina hydroelectric reservoir. While their results supported a positive correlation between indices and biodiversity, the authors stressed that acoustic heterogeneity in tropical soundscapes complicates accurate diversity estimation. However, their conclusions are constrained by the lack of publicly available datasets, necessitating further experimental validation.

The efficacy of acoustic indices for biodiversity assessment remains contentious. Recent studies propose that combining multiple indices may yield modest improvements in accuracy, though such approaches remain limited by inherent biases [Alcocer et al. 2022, Sethi et al. 2023, Bicudo et al. 2023, Mammides et al. 2025]. For instance, [Bradfer-Lawrence et al. 2024] argued that the compression of complex acous-

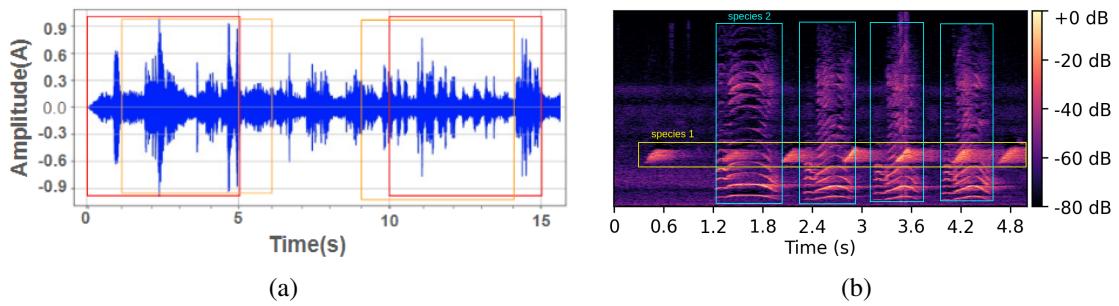


Figure 1. (a) An example of segmentation applied to the audio file XC175797.ogg, divided into five-second segments with a one-second overlap. **(b)** The spectrogram of Segment_7643.wav which consists of two species: *White-rumped Munia* and *Indian Peafowl*.

tic data into simplified index values inherently obscures ecological nuance. They further noted that most indices rely on unsupervised information quantifiers, underscoring the importance of identifying and mitigating biases in their application.

To address these challenges, the present study employs a novel approach: simulating controlled acoustic ensembles of bird and amphibian vocalizations and background noise, to systematically assess the behavior of the bioacoustic index both in isolation (e.g., vocalizations or noise alone) and under combined interference conditions.

3. Materials and Methods

The data used to generate realistic avian vocalization ensembles were sourced from the BirdClef 2024 competition [Klinck et al. 2024], which focuses on biodiversity monitoring in the sky islands of the Western Ghats, a UNESCO World Heritage Site in southern India. This dataset comprises 23,528 audio files (.ogg format) spanning 182 bird species, with each recording lasting a minimum of five seconds and sampled at $f_s = 32$ kHz. To broaden taxonomic representation, we incorporated 2,000 anuran vocalizations from nine distinct species, sourced from field recordings in the Amazon rainforest [Colonna et al. 2012]. The assemblage creation procedure follows three stages:

- 1. Segmentation:** Audio files are partitioned into segments to isolate vocalizations of interest while minimizing silent intervals (Figure 1(a));
- 2. Mixing:** Segments are systematically combined to simulate natural vocalization overlap. An example of two mixed species can be observed in the spectrogram shown in Figure 1(b); and
- 3. Noise Isolation:** Background noise profiles are extracted from silent or low-activity portions of recordings to enable controlled noise-introduction experiments (Figure 2(b)).

In this stage, the amplitude of each audio file is first normalized to the range $[-1, 1]$. Only recordings lasting at least five seconds are segmented using a sliding window approach with a one-second overlap, as illustrated in Figure 1(a). The root mean square (RMS) value is then computed for each segment. The segment with the highest RMS value is selected, as it is expected to contain the clearest and highest-amplitude syllable of the vocalization.

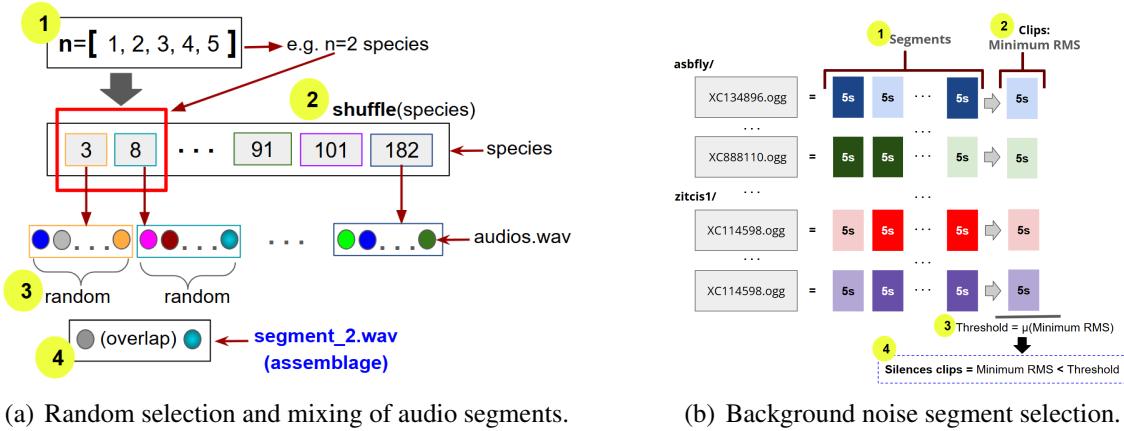


Figure 2. Procedure for creating assemblages.

3.1. Audio mixing

The audio mixing process, illustrated in Figure 2(a), consists of four steps designed to create audio segments containing one to five different species. Step 1, the number of species n in each assemblage is randomly sampled from a uniform distribution, $n \sim \mathcal{U}(1, 5)$. Step 2, the species records are shuffled, ensuring that segments from the same species remain grouped to prevent species repetition within a single audio mix. Step 3, one segment is randomly selected from each species using a uniform distribution to form the ensemble. Finally, at Step 4, a new audio segment is generated. To minimize potential biases, every step of the process is randomized. As a result of this procedure, two files are generated: (a) a new audio file (the assemblage with n species), and (b) a .CSV file containing metadata such as the experiment run identifier, the number of mixed species, the species included in the mix, the names of the original audio files, and the filename of the newly created segment, as illustrated in Figure 1.

3.2. Background noise segment selection

As illustrated in Figure 2(b), the process begins with Step 1, which involves segmenting each species' audio files into 5-second clips. In Step 2, the RMS value of each five-second segment is calculated, with the segment exhibiting the lowest RMS value being extracted. Step 3 then computes the silence threshold by determining the mean (μ) of all RMS values obtained from these segments. Subsequently, Step 4 constructs a silence dataset comprising all 5-second clips from Step 2 that fall below the mean RMS value established in Step 3. Finally, as part of Step 4's completion, all silence clips are shuffled, enabling the extraction of the first n segments as required for analysis. It is essential to emphasize that, a background noise segment is defined as an audio segment with a minimal root mean square (RMS) value compared to its peers, in which bird or anuran vocalizations are nearly imperceptible or entirely absent.

3.3. Acoustic Indices

In physics, sound is treated as a time series x_t , where the amplitude of the sound wave corresponds to the pressure variations detected by a microphone. In bioacoustics, a sound is conceptualized as a medium of animal communication, with applications in

population monitoring, acoustic ecology studies, and the assessment of noise impacts on species. These acoustic phenomena are measured and quantified through acoustic indices [Towsey et al. 2014].

Acoustic indices are mathematical tools used to characterize digital audio recordings of soundscapes. All indices were computed using the `scikit-maad` library¹ in Python. Among the indices examined in this research, the following have been implemented:

- Acoustic Richness Index: $H_s = H_t \times H_f$, where $H_t = -1/\log(n) \sum_{t=1}^n A(t) \log(A(t))$ is the temporal entropy, $A(t)$ the amplitude envelope, $H_f = -1/\log(n) \sum_{f=1}^n S(f) \log(S(f))$ is the spectral entropy, and $S(f)$ the spectrum [Sueur et al. 2008].
- Normalized Von Neumann Entropy: $H_v = -1/\log(\tau) \sum_{i=1}^{\tau} \lambda_i \log \lambda_i$, where λ_i are the normalized eigenvalues of the autocorrelation matrix R_{xx} and τ is the maximum time lag [Colonna et al. 2020].
- Acoustic Diversity Index: $ADI = -\sum_{i=1}^S p_i \log p_i$, where p_i is the fraction of sound in each i -th frequency band in S number of frequency bands [Villanueva-Rivera et al. 2011].
- Bioacoustic Index: $BIO = \sum_{f_{\min}}^{f_{\max}} \text{FFT}(x_t)$, where $f_{\min} = 2 \text{ kHz}$, $f_{\max} = 8 \text{ kHz}$, x represents the acoustic signal and FFT is the Fast Fourier Transform [Boelman et al. 2007, Rajan et al. 2019].
- Acoustic Evenness Index: the AEI is obtained by dividing the spectrogram into bins and taking the proportion of spectrogram's pixels above the threshold (-50dBFS) in each bin, and then the Gini index is calculated from these bins [Villanueva-Rivera et al. 2011].

4. Results and Discussions

All segmentation, selection, and mixing procedures described in Section 3 were applied to generate a new set of samples, effectively creating a new dataset. We then computed all indices described in Section 3.3 for this dataset. The X-axis of Figure 3 indicates the number of different species mixed in each sample. Here, 0 represents background noise, while values from 1 to 5 correspond to the number of randomly mixed species. Each subplot shows how each index varies with an increasing number of species (birds or anurans). The results show that the H_s , H_v , ADI, and BIO index values increase proportionally with the number of species, as shown in Figures 3(a), 3(b), 3(c) and 3(d). In the case of AEI, the index value tends to consistently decrease as the number of species increases, as shown in Figure 3(e).

According to the values obtained in Figure 3(a) for the bird assemblages, the H_s value for background noise (0.640) represents a moderately complex acoustic environment, with a spectral entropy suggesting the presence of multiple acoustic sources. However, this value is lower than that of the four-species assemblage (0.657), suggesting that despite its spectral complexity, the noise is less diverse and less dynamic than the acoustic environment provided by multiple species. The five-species assemblage (0.681) is observed to have the highest H_s value, indicating greater spectral occupancy and temporal variation, with the introduction of new frequencies and vocalization patterns that contribute to greater complexity in the sound environment.

¹<https://scikit-maad.github.io/>

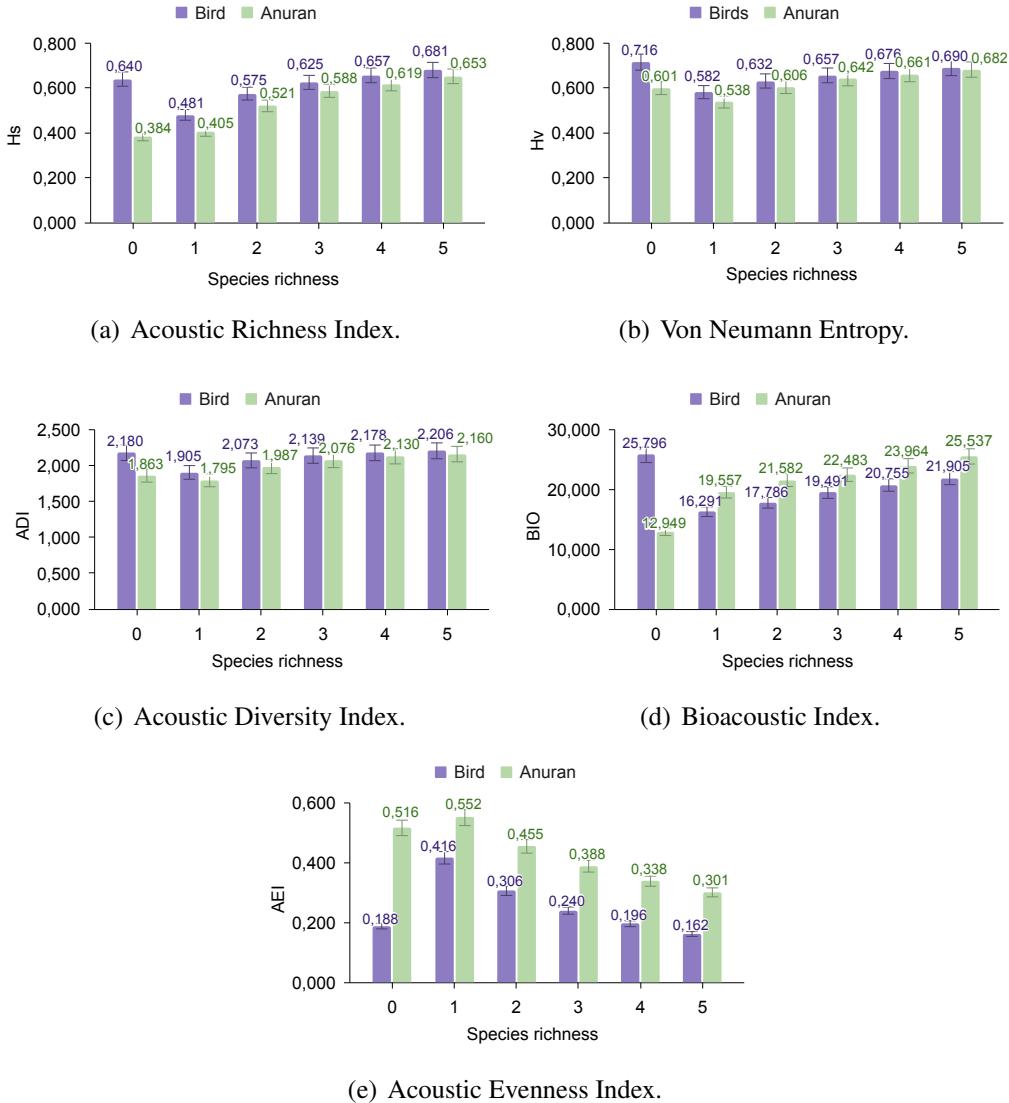


Figure 3. Acoustic Indices and Species Richness (0 denotes the background noise, while values from 1 to 5 correspond to the number of randomly mixed species).

In the anuran assemblage, as illustrated in Figure 3(a), the H_s value for background noise (0.384) indicates a sound environment characterized by minimal acoustic complexity. Consequently, these signals are characterized by a limited frequency range with an equally limited temporal variability. Thereby, a single anuran species ($H_s = 0.405$) exhibits augmented temporal and spectral intricacy relative to the background noise. Increasing the number of species in the assemblage leads to a more diverse sound environment, as evidenced by higher H_s values (e.g., a five-species assemblage has an H_s of 0.653). This increase in H_s indicates a more efficient distribution of frequencies and greater temporal variation in vocalizations. Therefore, it can be inferred that the presence of multiple anuran species results in a more dynamic and diverse sound environment. Conversely, the presence of background noise provides a more simplistic and repetitive acoustic environment.

When evaluating the Von Neumann entropy in single-species assemblages, 0.582 (birds) and 0.538 (anurans), it is evident that they predominantly contain a single signal, which may exhibit periodicity or low spectral diversity. This indicates that the signal follows certain repetitive patterns. Consequently, the eigenvalues of the R_{xx} matrix remain concentrated or follow a predictable distribution, reducing entropy (uncertainty). However, as the number of vocalizing species increases, the signal becomes more complex, and the eigenvalues of the R_{xx} matrix begin to distribute uniformly, increasing uncertainty. This leads to a rise in H_v . As shown in Figure 3(b), entropy values increase as the number of species per assemblage grows.

Background noise in bird assemblages presents a high H_v value (0.716), indicating the absence of a discernible organization in the sound, suggesting a loosely defined structure, possibly composed of multiple mixed frequencies. In this case, the eigenvalues of the R_{xx} matrix are highly uniformly dispersed, which increases uncertainty and, consequently, results in a higher Von Neumann entropy value. For anurans, the background noise has a value of 0.601, indicating that it is not entirely unstructured and may contain some underlying organization, such as small temporal correlations (a certain degree of predictability in the sound) or the predominance of specific frequency bands. However, the level of uncertainty and randomness remains high.

In the results from Figure 3(c), it is observed that the background noise in the bird assemblage presents a relatively high ADI value (2.180), slightly higher than that of the assemblage of 4 species (2.178). This result can be interpreted as a scenario where the energy of the assemblage is occupying multiple frequency bands, and in each frequency band, the proportion of occupied cells is more or less balanced. Furthermore, the proportion of energy calculated in n -th frequency band is similar across all bands. Therefore, it is inferred that the noise assemblage exhibits high complexity, with a large diversity of sound sources, where many different species are present simultaneously, something typical of biodiversity-rich environments. Thus, the more balanced the energy distribution and the more occupied cells, the higher the ADI will be, reflecting greater acoustic diversity.

For anurans, the background noise exhibits a moderate ADI value (1.863), indicating uneven energy distribution across frequency bands—likely due to dominant low-frequency components (e.g., wind or rain). When only one species is present, the ADI decreases slightly (1.795) compared to the noise scenario. This reduction occurs because single-species vocalizations occupy a more limited spectral range specific to that species' acoustic signature.

The BIO (Biodiversity Index) is calculated based on the sound intensity (in dB) of animal vocalizations distributed across different frequency ranges in the acoustic spectrum. In Figure 3(d), the bird assemblage shows that the background noise value (25.796) is the highest compared to assemblages with 1 to 5 species. This suggests that the background noise contains a significant amount of natural sounds (biophony) within the spectrum, indicating a wide variety of acoustic events between $f_{\min} = 2$ kHz, $f_{\max} = 8$ kHz. With one species, the BIO drops to 16.291, decreasing relative to the noise scenario. However, the index increases with the number of species, a trend that aligns with the H_s , H_v , and ADI indices, all of which exhibit a positive correlation with species richness.

For the anurans, the background noise (as shown in Figure 3(d)) is lower than in the other species assemblages (1 to 5 species). This suggests a smaller area under the spectrum, likely due to fewer natural sounds (biophony) within the frequency bands $f_{\min} = 2 \text{ kHz}$, $f_{\max} = 8 \text{ kHz}$. As the number of species increases (from 1 to 5), the BIO value also rises, presumably due to greater frequency diversity expanding the spectral area. Notably, the BIO and H_s indices are the only two metrics with lower values for background noise in anuran calls compared to multispecies assemblages—a desirable property for distinguishing biophony from noise.

The AEI index exhibits a negative correlation with species richness—a counter-intuitive pattern where more species correspond to lower AEI values. This trend is consistent across both bird and anuran assemblages. For birds (Figure 3(e)), the background noise yields a low AEI (0.188), reflecting moderately high evenness due to acoustic energy being evenly distributed across frequency bands. This is supported by the high proportion of frequency cells containing signals above the threshold, resulting in low spectral inequality. In contrast, single-species assemblages show a higher AEI (0.416), indicating reduced uniformity in energy distribution. This suggests the vocalizations are concentrated in fewer frequency bands, with unequal cell proportions amplifying spectral disparity.

In theory, a greater number of species should produce more uneven soundscapes, leading to higher AEI values, since the index is based on the Gini coefficient (where greater inequality yields higher values). However, we observe the opposite pattern: richer assemblages exhibit lower AEI values. This occurs because multi-species soundscapes distribute energy more uniformly across frequency bands, resulting in a smaller Gini coefficient and thus reduced AEI. With the anuran assemblage, the behavior of the AEI value is similar to that of the birds; for example, with 5 species, the AEI value (0.301) is lower than the AEI of 1 species (0.552). Regarding the background noise in anurans, it is high (0.516), indicating that the distribution of sound energy across the frequency bands is not uniform, meaning there is a certain concentration of energy in some bands more than in others.

5. Conclusions

This study aimed to simulate a controlled scenario where the number of vocalizing species in audio recordings (.wav files) was known, enabling evaluation of five acoustic indices' performance in relation to species richness and background noise. We simulated bird and anuran assemblages to (1) test the limits of these indices and (2) observe their responses under controlled conditions. Our findings provide empirical evidence for applying these indices in controlled environments. However, future studies incorporating real soundscapes would yield more robust results.

Four of the five evaluated indices showed a positive correlation with species richness. Specifically, the H_s , H_v , ADI, and BIO indices all increased with greater species numbers, while the AEI index exhibited an inverse relationship, decreasing as species richness increased. These indices yielded consistent results for both background noise and species assemblages (birds and anurans), providing relevant information about spectral distribution of sound patterns, temporal variation in sound intensity, and other acoustic features.

However, all five indices exhibit significant limitations when evaluated in scenarios containing only background forest noise. This occurs because natural background noise can be highly diverse, encompassing biophony (e.g., distant species vocalizations), geophony (wind or rain), and other acoustic components. In such cases, the resulting flat frequency spectrum and temporally even energy distribution (visible in spectrograms) may produce values similar to species-rich assemblages. These similarities can compromise ecological interpretation by: (1) obscuring accurate species richness estimates and (2) potentially mischaracterizing ecosystem status.

This limitation has been previously identified in related studies contributing to this debate [Bicudo et al. 2023, Llusia 2024]. These findings underscore the critical need to account for acoustic environmental context when applying these indices for ecological monitoring. However, determining a priori whether an audio segment originates from noise or species assemblages remains a challenge. Therefore, future studies aiming to measure the acoustic complexity of soundscapes should consider using a set of multiple acoustic indices simultaneously, enabling a more comprehensive characterization of biodiversity.

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