

A standardized, unbiased protocol for protected areas soundscape monitoring

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Abstract

Standardized, long-term acoustic monitoring protocols for protected areas (PAs) remain scarce, limiting cross-site comparability and biodiversity reporting. Here we describe and test a protocol that combines a spatially balanced passive acoustic monitoring (PAM) design with an analytical workflow based on seven acoustic indices organized into 20 acoustic regions defined by time period and frequency range. Tested with one year of recordings at the Serra do Cipó National Park (Brazil), the protocol identified four acoustic regions showing the greatest seasonal differences: three pre-dawn acoustic regions (AR02, AR03, AR04) most contrasting between spring and autumn, driven by anuran vocalizations and insect activity; and one daytime high-frequency acoustic region (AR12) most contrasting between summer and autumn, driven by different insect assemblages. Rainfall increased in spring but did not alone explain the frequency and time selectivity of the detected patterns, demonstrating that the protocol is sensitive to both biological and geophysical drivers of soundscape change. The protocol is biologically unbiased, modular, and adaptable to PAs of different sizes and ecosystem types. It is currently being expanded across five Brazilian biomes in core areas of Biosphere Reserves. It provides a reproducible basis for generating standardized, comparable baselines to support long-term soundscape monitoring.

Introduction

Protected areas (PAs) are the cornerstone of global biodiversity conservation strategies, providing refugia for species, maintaining ecosystem processes, and contributing to climate regulation^{1,2}. The Kunming-Montreal Global Biodiversity Framework established ambitious targets for PA expansion and effective management, including the 30×30 goal of protecting at least 30% of land and oceans by 2030³. Meeting these targets requires robust, scalable monitoring systems capable of tracking biodiversity states and trends over time and across ecosystems⁴.

Passive acoustic monitoring (PAM) has emerged as a cost-effective approach for large-scale biodiversity monitoring, enabling continuous data collection with minimal wildlife behavioural disturbance⁵. The development of autonomous recording units (ARUs) has allowed researchers to collect hundreds or thousands of hours of audio that capture the acoustic activity of diverse components of the biological community simultaneously. Analysis through soundscape ecology – which examines biological (biophony), geophysical (geophony), and anthropogenic (anthrophony) sounds in a landscape⁶ – offers a biologically inclusive view of ecosystem dynamics without requiring prior species knowledge.

Acoustic indices are mathematical filters that quantify how acoustic energy is distributed across time and frequency^{7,8}. They can be computed per frequency bin, enabling fine-scale spectral resolution that supports detailed soundscape analysis and visualization. The Acoustic Complexity Index (ACI⁹), Temporal Entropy (Ht¹⁰), and Event Count (EVN¹¹) form the basis for long-duration false-colour (LDfC) spectrograms – composite images that summarize days or seasons of acoustic data as colour

patterns¹². Additional indices — Background Noise (BGN), Signal-to-Noise Ratio (SNR), Acoustic Evenness Index (AEI), and Acoustic Diversity Index (ADI) — capture complementary dimensions of the acoustic environment^{11,13,14}.

Despite this analytical potential, the lack of standardized protocols limits cross-site comparability of soundscape data¹⁵. Most existing studies use heterogeneous recording designs and analytical approaches, and many target specific taxa, introducing biological bias which may not be informative when studying ecosystem patterns. Soundscape-based approaches can overcome this by detecting meaningful ecosystem-level patterns without species-specific information — a capacity demonstrated from human-modified tropical forests¹⁶ to global comparative studies across biogeographic realms¹⁷. Biologically unbiased protocols that can detect change from any sound source are particularly valuable for long-term monitoring. Generating standardized baselines reproducibly across multiple PAs is also a prerequisite for robust transnational collaborative monitoring¹⁸.

The acoustic region approach, developed by Campos et al.^{15,19}, addresses several of these challenges by dividing the soundscape into 20 units defined by specific frequency ranges and time periods, enabling independent statistical analysis and interpretation within each acoustic region. This approach has been tested in a Brazilian national park¹⁵ and in New Zealand forests under contrasting pest control regimes¹⁹.

Here we describe and test a standardized soundscape sampling protocol developed within the scope of the Brazilian federal PA monitoring programme (*Programa Monitora*) and registered in the BON in a Box platform³⁸. We demonstrate its application using one year of recordings from the rocky grassland of the Serra do Cipó National Park (Espinhaço Biosphere Reserve, Brazil), focusing on detecting seasonal patterns and identifying the acoustic regions and sound sources responsible for the main seasonal differences. Our aim is to show that the protocol generates robust seasonal soundscape baselines from which long-term trends can be monitored, while remaining unbiased with respect to which biological group drives any detected change.

Methods

Study area

The Serra do Cipó National Park (ParnaSC) is located in the Espinhaço Mountain Range, Minas Gerais, Brazil (19°40'S, 43°30'W; Fig. 1), within the Cerrado biodiversity hotspot. The park is a core area of the Espinhaço Biosphere Reserve, recognized by UNESCO. The eligible sampling area, designated '*Alto Palácio*', is characterized by rupestrian fields (*campos rupestres*) — a rocky grassland ecosystem with an herbaceous-shrub physiognomy growing on quartzitic soils at elevations above 900 m (see Supplementary Fig. S11). These ecosystems show high levels of plant and animal endemism and host a

rich acoustic community of birds, anurans, and insects²⁸. Rupestrian fields represent approximately 85% of the ParnaSC ecosystem cover, making *Alto Palácio* representative of the park main ecosystem.

Sampling design

The sampling design was developed within the scope of the *Programa Monitora* (Chico Mendes Institute for Biodiversity Conservation – ICMBio) to be spatially representative, temporally balanced, modular, and adaptable to PAs of different sizes, shapes, and ecosystem types (Table 1). A random-systematic design was adopted: four quadrants were randomly selected from a grid superimposed on the eligible area, and one recorder was placed at each vertex of each selected quadrant. This combination ensures recorders within a quadrant maintain fixed spatial relationships (systematic component) while the selection of quadrants is left to chance (random component), eliminating systematic placement bias. The number of quadrants can be increased or decreased without changing the analytical approach, giving the protocol its modular character.

A grid with cells of 707 m per side (0.5 km², compatible with the TEAM protocol²⁰) was superimposed on the eligible area, yielding 35 cells. Four cells (13, 16, 20, and 29) were randomly selected. One AudioMoth autonomous recorder was installed at each vertex of each selected cell, yielding 16 sampling stations across four quadrants. To increase battery autonomy and field resistance, the AudioMoths were adapted to run on up to six D-type batteries inside weather-resistant enclosures (L. R. Padovese, personal communication, 2023). At each quadrant, three recorders operated at 48 kHz and one at 384 kHz for bat (ultrasound) monitoring (Fig. 2). All recorders operated at 16-bit resolution, producing uncompressed WAV files, installed at approximately 1.5 m above ground level.

Recorders were programmed to sample one minute every ten minutes (1/10-min schedule), generating 144 one-minute files per recorder per sampling day. One day was recorded every ten days (1/10-day schedule), enabled by a custom firmware provided by Open Acoustic Devices (Alex Rogers, personal communication, 2023), which allows calendar-anchored schedules on AudioMoth recorders. The recording calendar was anchored to 1 January 2023. The dataset spans from June 2024 to May 2025, covering four seasons in recording order: winter (June–August 2024), spring (September–November 2024), summer (December 2024–February 2025), and autumn (March–May 2025). Nine sampling dates per season combined with 16 recorders yielded 144 sampling-days per season (576 sampling days and 82,944 one-minute files in a year).

Table 1

Key properties of the soundscape monitoring protocol and their practical implications for protected area monitoring.

Protocol property	Description
Random-systematic spatial design	Four quadrants are randomly selected from a grid superimposed on the eligible area. Within each quadrant, recorders are placed at fixed relative positions (vertices). Random selection eliminates spatial bias; systematic placement ensures even coverage.
Spatial representativeness	The eligible area represents the dominant PA ecosystem, selected for conservation status and logistical access. Grid and random quadrant selection ensure representative sampling.
Temporally balanced schedule	Recordings taken one minute every ten minutes and one day every ten days, anchored to a fixed calendar date (1 January 2023). Captures shifts in acoustic event timing across the year, relevant for long-term climate change monitoring.
Synchronized multi-PA sampling	The fixed shared calendar allows different PAs to be sampled in different months by different teams while remaining synchronized, enabling national and international seasonal comparisons.
Modularity and adaptability to PA size and shape	The number of quadrants can be increased or decreased without changing the analytical approach. A single quadrant (four recorders) is the minimum deployable unit. In highly fragmented landscapes, natural fragments (allowing installing four recorders at a minimum distance of 707 m from each other) can be pre-defined and randomly selected instead of grid cells.
Cross-PA comparability	The same indices, acoustic regions, and statistical framework are applied regardless of biological community. Soundscapes from PAs with very different communities can be directly compared. No species-level baseline is required.
Multi-scale data aggregation	Data can be aggregated or disaggregated at multiple spatial scales (recorder, quadrant, eligible area, PA, biome) and temporal scales (day, month, season, year), enabling navigation from local gradients to global comparisons.
Contrast comparisons	For assessing acoustic impact of a localized pressure (e.g. mine, road, dam), quadrants can be selected at increasing distances from the source and compared with the protected baseline within the PA.
Complementary species-level analyses	The same recordings can be analysed with species-specific methods (automated classifiers, manual annotation, call libraries) to generate occurrence and abundance data complementary to soundscape outputs. One-quarter of the recorders are set to a sampling rate of 384 kHz to enable bat studies.
Unbiased activity detection	The workflow detects differences from any sound source – biophony (any taxonomic group), geophony, or anthrophony – without a priori knowledge of which taxa are present. The 20 acoustic regions add a frequency-time dimension of scale navigation.

Acoustic indices and seasonal false-colour spectrograms

All audio files were resampled to 22,050 Hz to standardize recordings and reduce computational demands while retaining the frequency range relevant to the selected indices. Seven acoustic indices were computed per frequency bin (bin width ≈ 22 Hz; 512 bins over 0–11,025 Hz) using Python 3.14: ACI⁹, Ht¹⁰, EVN, BGN, SNR¹¹, AEI, and ADI¹³. Index calculations were based on the scikit-maad package (v1.3.10²¹), updated and modified for the per-bin computation required by the acoustic region approach. These indices were selected because they can all be computed per frequency bin and capture complementary acoustic properties with established ecological interpretations^{8,14}.

Three pre-processing transformations were applied: (i) statistical saturation at the 99th percentile to mitigate extreme outliers; (ii) inversion of Ht as $(1 - \text{Ht})$, so higher values reflect greater temporal acoustic activity, consistent with the other indices; and (iii) min-max scaling to standardize all indices to the range 0–1.

For each season, a seasonal LDFC spectrogram was generated by mapping the median ACI (red channel), inverted Ht (green channel), and EVN (blue channel) values across all 144 recorder-days per season. LDFC spectrograms were originally developed to summarize single days of acoustic data as colour images^{12,23}; here we extend this approach to represent entire seasons by computing median index values across all recorder-days within each season — a novel application that is only possible with index-based, species-independent representations of the soundscape. The median was used to reduce the influence of individual recorder locations or atypical events. The analytical workflow followed two stages (Fig. 3): (1) acoustic index calculation forms the basis for the seasonal LDFC spectrogram generation and also for the identification of acoustic regions of interest through data aggregation into acoustic regions, statistical analysis, and the final identification of acoustic regions showing the strongest seasonal differences; (2) visual and aural inspection of a random subsample of audio files from the identified acoustic regions, yielding a description of the sound sources driving the main differences. Full workflow details, including the statistical decision tree, are provided in the supplementary material (see Supplementary Methods S1 and Fig. S2)

Acoustic regions and statistical analysis

The soundscape was divided into 20 acoustic regions (see Supplementary Fig. S3) following Campos et al.^{15,19}, defined by the intersection of five daily time periods (00:00–05:29, 05:30–09:00, 09:01–17:29, 17:30–20:59, and 21:00–23:59) and four frequency bands (0–988 Hz, 988–3,609 Hz, 3,610–7,906 Hz, and 7,907–11,000 Hz). For each acoustic region, index values of all audio files within the corresponding time and frequency window were summed per recorder per day, yielding one value per acoustic region, day, and recorder. The dataset of aggregated index values comprised 11,520 values per index across the four seasons (20 acoustic regions $\times$ 36 sampling days $\times$ 16 stations).

Statistical analysis followed a structured workflow aimed at producing parametric tests where possible (Supplementary Fig. S2). Data for each acoustic region were tested for normality (Shapiro-Wilk). If

normally distributed, a repeated-measures ANOVA was performed; if not, five transformations were applied sequentially (square root, $\log_{10}$, Box-Cox, Yeo-Johnson, log-modulus), and the first transformation achieving normality was followed by repeated-measures ANOVA. If no transformation normalized the data, a Friedman test was applied. Post-hoc comparisons used pairwise t-tests (normal data) or Nemenyi tests (non-normal data). Effect sizes were assessed using Cliff's delta²⁶, calculated as $\delta = (2 \times \text{CLES}) - 1$, and classified as large ($\delta \geq 0.474$), medium (0.330–0.473), small (0.147–0.329), or negligible (< 0.147). Generalized eta-squared (η^2) and Kendall's W were extracted from ANOVA and Friedman tests respectively to enable a unified effect-size ranking. Statistical analyses used the pingouin²⁴ and scikit-posthocs²⁵ Python modules. For each index, the top 10% of absolute Cliff's delta values (12 of 120 comparisons per index: 20 acoustic regions $\times$ 6 season pairs) were identified. Acoustic regions appearing in this top 10% set across any index were counted; those with the highest counts were selected for sonotype annotation.

Sonotype annotation

A random 5% sample of audio files from the acoustic regions of interest and their most contrasting season pairs was selected for manual annotation, with focus on the corresponding frequency range of each acoustic region (AR). For AR02, AR03, and AR04, the most contrasting pair was spring–autumn; for AR12, summer–autumn. The sampling pool comprised 9,216 one-minute audio files for AR02, AR03, and AR04 (pre-dawn period 00:00–05:29, two seasons), from which 443 files were randomly drawn for annotation (approximately 5%; 4.8%). For AR12, the sampling pool comprised 14,112 one-minute audio files (daytime period 09:01–17:29, two seasons), from which 669 files were annotated (approximately 5%; 4.74%). In total, 1,112 audio files were annotated. Annotation was carried out by seven researchers using Raven Pro 1.6²⁷. Each one-minute file was filtered to the frequency range of the corresponding acoustic region, visually and aurally inspected, and each sound event classified into one of 10 sonotype classes: Amphibia, Birds, Insecta, Rain, Wind, Continuous Noise, Artefact, Not Identified, and Silence. Mammalia was initially included as a class but was excluded after yielding no annotations. The relative proportion of each class per acoustic region and season was calculated from total annotated duration.

Results

Seasonal false-colour spectrograms

The seasonal LDFC spectrograms showed visually distinct acoustic signatures for each season, presented in recording order: winter, spring, summer, and autumn (Fig. 4). Autumn showed the lowest index values across all frequency bands and time periods. In spring, high ACI values (red channel) became prominent at low to mid frequencies during the pre-dawn hours. In summer, intense ACI and EVN activity concentrated at mid to high frequencies during central daytime hours (09:00–17:00). Winter presented a pattern similar to autumn, with slightly higher values than autumn. Both seasons showed a

marked reduction in index values relative to spring, particularly in the pre-dawn low-to-high frequency bands and the daytime high-frequency band.

Acoustic regions with the greatest seasonal differences

Of 840 Cliff's delta values calculated (7 indices $\times$ 6 season pairs $\times$ 20 acoustic regions), 378 (45.0%) were negligible, 321 (38.2%) small, 102 (12.1%) medium, and 39 (4.6%) large (Supplementary Table S12). Four acoustic regions appeared most frequently among the top 10% largest effect sizes across all seven indices (Fig. 5): AR03 (3,610–7,906 Hz; 00:00–05:29) and AR04 (7,907–11,000 Hz; 00:00–05:29) tied at the highest frequency ($n = 11$ index-comparison combinations each), followed by AR02 (988–3,609 Hz; 00:00–05:29; $n = 10$) and AR12 (7,907–11,000 Hz; 09:01–17:29; $n = 10$).

For AR02, the largest effect sizes were for the spring–autumn pair (Cliff's delta up to 0.63, ACI; large effect), indicating substantially higher index values in spring. For AR03, the same spring–autumn contrast dominated (Cliff's delta up to 0.56, ACI). For AR04, large effects also appeared for spring–autumn (Cliff's delta up to 0.56, ACI). In AR12, the contrasting pair differed: the largest effects were for the summer–autumn comparison (Cliff's delta up to 0.60, ACI), with index values markedly higher in summer.

Sonotype annotation

A total of 1,112 audio files were annotated by seven researchers, generating 7,597 annotations across the four acoustic regions of interest. The relative duration proportions of each sonotype class by acoustic region and season are shown in Fig. 6.

In AR02, silence occupied 60.4% of annotated duration in autumn and 31.1% in spring. Wind dominated in autumn (17.1%), followed by rain (9.7%). In spring, rain increased markedly (27.0%), as did anuran activity (1.1% autumn vs. 6.5% spring). Bird vocalizations rose from 0% in autumn to 1.5% in spring. In AR03, silence dominated in autumn (73.2%), with rain at 8.2%. In spring, silence fell to 47.8%, rain increased to 27.6%, and anuran activity rose from 1.0% to 3.5%. In AR04, silence was very high in both seasons (84.6% autumn; 65.8% spring). Insect activity was the most notable biophonic signal: 3.7% in autumn but absent in spring (0.0%). Rain increased substantially (5.6% autumn vs. 26.4% spring). In AR12, silence was high in both seasons (86.3% autumn; 81.3% summer). Insect activity showed the opposite pattern from AR04: nearly absent in autumn (0.5%) but substantially higher in summer (4.7%). Rain was higher in summer (6.9%) than in autumn (3.5%).

Discussion

This study demonstrates that a standardized, biologically unbiased soundscape monitoring protocol can successfully detect ecologically meaningful seasonal differences in a protected area located in a species-rich tropical ecosystem. Using a spatially balanced PAM design, seven acoustic indices per

frequency bin, and the acoustic region framework, we identified seasonal patterns and characterized the sound sources responsible for the main differences in the soundscape.

Autumn consistently showed the lowest acoustic activity across all acoustic regions of interest (Fig. 6B). This is consistent with Campos et al.¹⁵, who found autumn as the quietest season in the same park but in a different ecosystem type (Cerrado savanna). Similarly lower autumn activity was reported in New Zealand forests¹⁹. These results suggest that autumn may serve as a useful reference season for long-term monitoring, as its lower biophonic baseline facilitates detection of deviations. The consistency of autumn as a reference for natural soundscapes will be tested as the protocol expands across biomes.

In the low- to mid-frequency pre-dawn acoustic regions AR02 and AR03, anuran vocalizations were the primary biophonic component decreasing from spring to autumn, consistent with anuran reproductive biology in the Cerrado: calling peaks during the rainy season onset in spring²⁹. Rainfall also increased from autumn to spring across AR02, AR03, and AR04. However, rain alone does not account for the selectivity of the detected differences. Rain generates broadband noise and would affect all 20 acoustic regions simultaneously (see Supplementary Information- S6); instead, only four specific acoustic regions stood out, and within those, the most pronounced changes were associated with biological signals at specific frequencies and times. This selectivity demonstrates that acoustic indices applied within defined acoustic regions can report on the contribution of biological and geophysical drivers of soundscape change.

Insect vocalizations dominated the high-frequency acoustic regions AR04 and AR12, which share the same frequency band (7,907–11,000 Hz) but differ in time period: AR04 covers pre-dawn hours and AR12 covers daytime. The seasonal patterns were opposite: insects were more active in autumn in AR04 and in summer in AR12. Two hypotheses to be tested in the future may explain this difference in insect acoustic activity: (i) different taxonomic groups are responsible for the contrasting activity pattern of acoustic regions AR04 and AR12; or (ii) insects change their stridulation behaviour across seasons, being more active during pre-dawn hours in autumn and during daytime in summer (Fig. 7). The acoustic region framework, by treating time periods and frequency bands as independent analytical units, revealed this contrast – which would have been masked in analyses integrating the full diel cycle or full frequency spectrum. This aligns with the growing recognition that acoustic indices at ecologically meaningful time and frequency scales are effective for monitoring insects in tropical environments, where their activity is a sensitive indicator of environmental change^{17,14}.

Artefact and Continuous Noise classes were also recorded during annotation but are not considered part of the soundscape: Artefact events were associated with the acoustic response of the recorder to high-intensity sounds, producing brief wideband signals across the full frequency range of the spectrogram; Continuous Noise was linked to occasional recorder malfunction rather than to environmental sound sources (see Supplementary S4). No anthrophony was detected in any of the annotated files, indicating that the soundscape of the study area reflects exclusively biophony and geophony components (Fig. 6B).

The protocol has several properties supporting adoption at national and global scales (Table 1). The 1/10-min and 1/10-day schedules are sufficient to characterize the soundscape without the need for continuous recording^{30,15}. Seven indices across 20 acoustic regions provide a multi-dimensional representation without requiring prior species knowledge, which is essential in species-rich understudied ecosystems. The workflow is fully implemented in Python, and a software tool is currently under development to provide a complete analytical toolkit for environmental agencies and researchers. These properties address the call for standardized, comparable protocols^{8,17}.

Data collected under this protocol also support contributions to Essential Biodiversity Variables (EBVs) across multiple classes³¹, relevant for the reporting requirements of the Kunming-Montreal Framework monitoring architecture^{32,33,34}. The protocol is currently being expanded to five Brazilian biomes (Amazon, Atlantic Forest, Cerrado, Caatinga, and Pantanal) and internationally, with collaborations in Germany, Namibia, Japan, and New Zealand at different stages of maturity¹⁸. Such globally coordinated baselines are essential for understanding how soundscapes differ naturally across biogeographic realms and for detecting ecosystem-level changes driven by climate change and habitat degradation^{17,4}.

Several limitations should be noted. The dataset covers a single year at a single PA, limiting conclusions about long-term trends. Sonotype annotation covered approximately 5% of files from the four most contrasting acoustic region–season combinations only. Future work should integrate automated species identification of sonotype classes and for target taxa, combine soundscape baselines with species-specific surveys, and evaluate the protocol across multiple years and biomes to test the generality of the seasonal patterns described here.

In conclusion, this protocol provides a feasible, reproducible, and biologically unbiased approach for soundscape monitoring of protected areas. The demonstration in the rupestrian fields of the Serra do Cipó National Park shows that it detects seasonal differences driven by anurans, insects, and geophony across distinct acoustic regions, and generates seasonal soundscape baselines from which long-term trends can be tracked. As the protocol scales to multiple biomes and continents, it has the potential to contribute standardized, globally comparable indicators of ecosystem acoustic activity for PA management and international biodiversity reporting.

Declarations

Data Availability

The acoustic index calculations, examples of audio files with sonotype annotations, the entire dataset of Cliff's delta, table with the complete sonotype results, and other additional information are available at the GitLab public page of this project (<https://gitlab.com/monitoramentoacustico/protocol-article-support-information>). More details on the sampling protocol are available in the BON in a Box platform³⁸. The complete audio datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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Author Contributions

I.B.C. conceived and designed the study, led fieldwork, developed the analytical framework, secured funding, acquired the research permits, and wrote the manuscript. I.B.C. and S.M.A. contributed equally to manuscript writing. T.C.S., S.M.A., Z.A.S.V., L.C.G.G., C.C.P., and M.C.M.A. participated in fieldwork and data collection. T.C.S., I.B.C., S.M.A., and C.C.P. contributed to AudioMoth firmware adaptation. T.C.S., I.B.C., S.M.A., Z.A.S.V., L.C.G.G., and M.T.B.S. contributed to the development and adaptation of the analytical framework. Z.A.S.V. and T.C.S. developed the Python analytical pipeline. I.B.C., T.C.S., L.R.P., T.S.G., and F.H.G.R. contributed to the conceptual design of the study. T.C.S., S.M.A., M.T.B.S., L.C.G.G., S.R.S., I.R.G., and M.M.A.P. performed sonotype annotation. I.B.C., T.C.S., S.M.A., M.T.B.S., and Z.A.S.V. reviewed the manuscript.

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Competing Interests

The authors declare no competing interests.

Supplementary information is available for this paper. Correspondence and requests for materials should be addressed to I.B.C.

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Figures

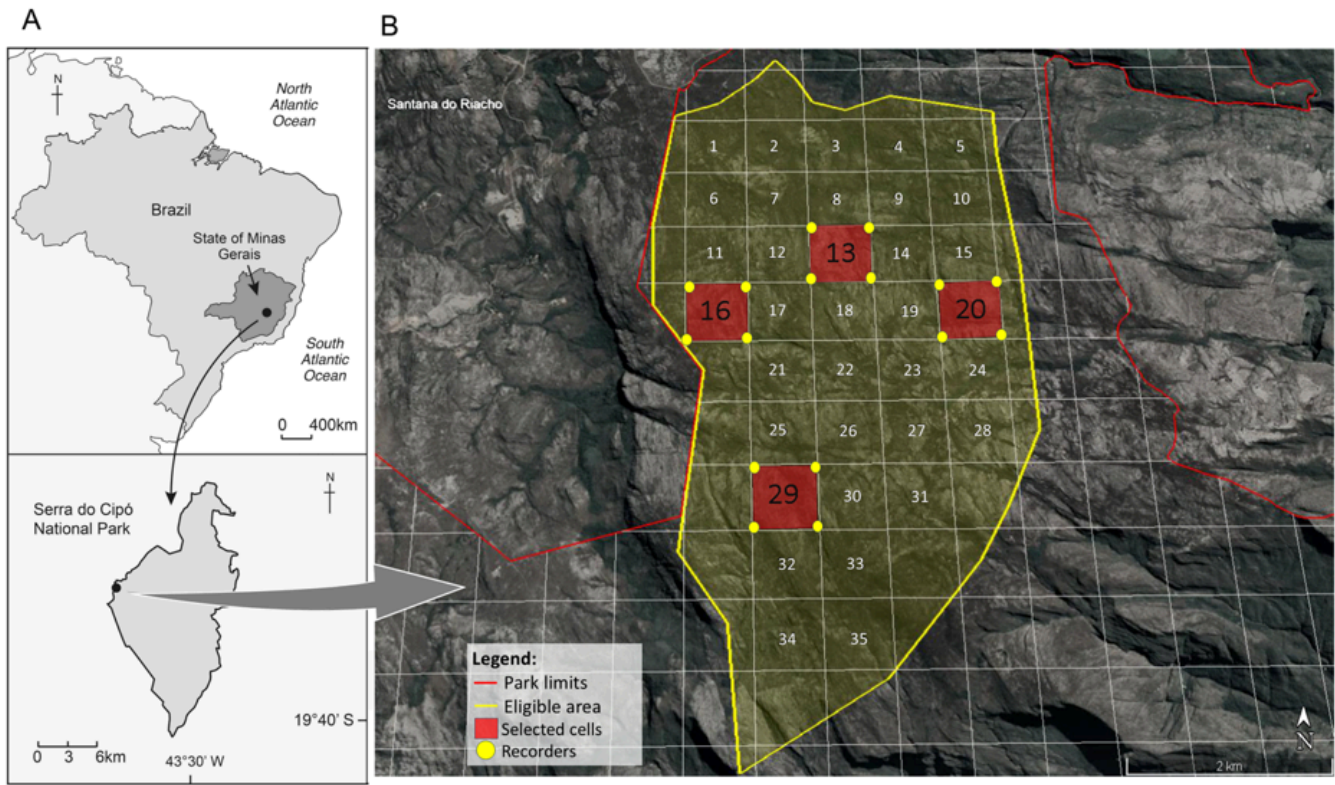


Figure 1

A) Location of the Serra do Cipó National Park within Brazil and B) detail of the eligible sampling area (*Alto Palácio*) showing the grid superimposed on the area and the four randomly selected quadrants (cells 13, 16, 20, and 29, highlighted in red). Yellow dots indicate the 16 AudioMoth recording stations installed at the vertices of the four selected quadrants. The eligible area boundary is shown in yellow and the national park boundary in red (see Supplementary Table S15 and Fig. S16).

Sampling Design

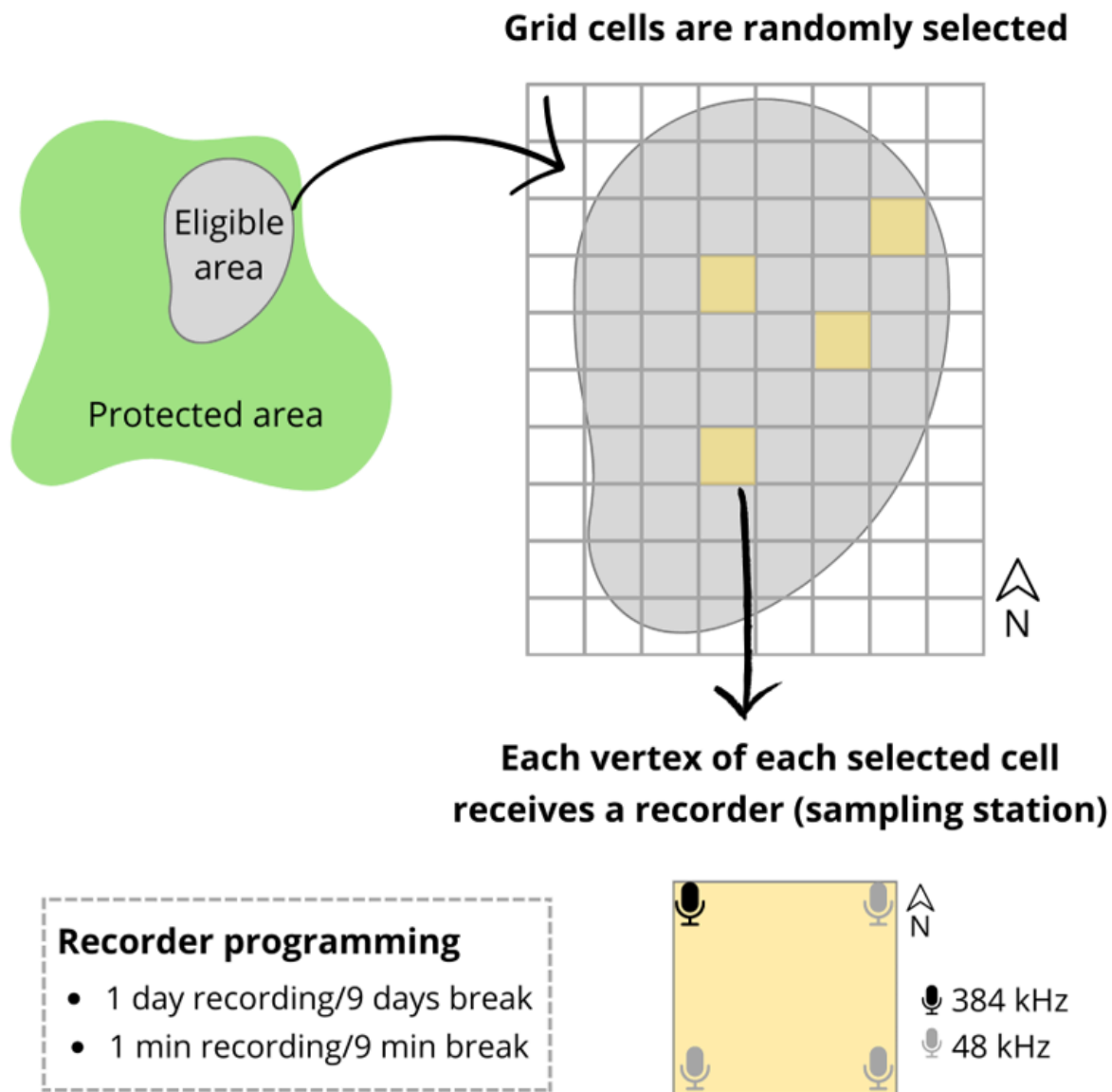


Figure 2

Random-systematic sampling design. Within the eligible area, a grid is superimposed and four cells are randomly selected. One autonomous recorder is installed at each vertex of each selected cell, resulting in 16 sampling stations across four quadrants. Three recorders per quadrant operate at 48 kHz sampling rate; one operates at 384 kHz for ultrasound (bat) sampling. The recording schedule is one minute every ten minutes, one day every ten days. The modular structure allows aggregation of data at multiple temporal and spatial scales, from individual recorders to biome-wide comparisons.

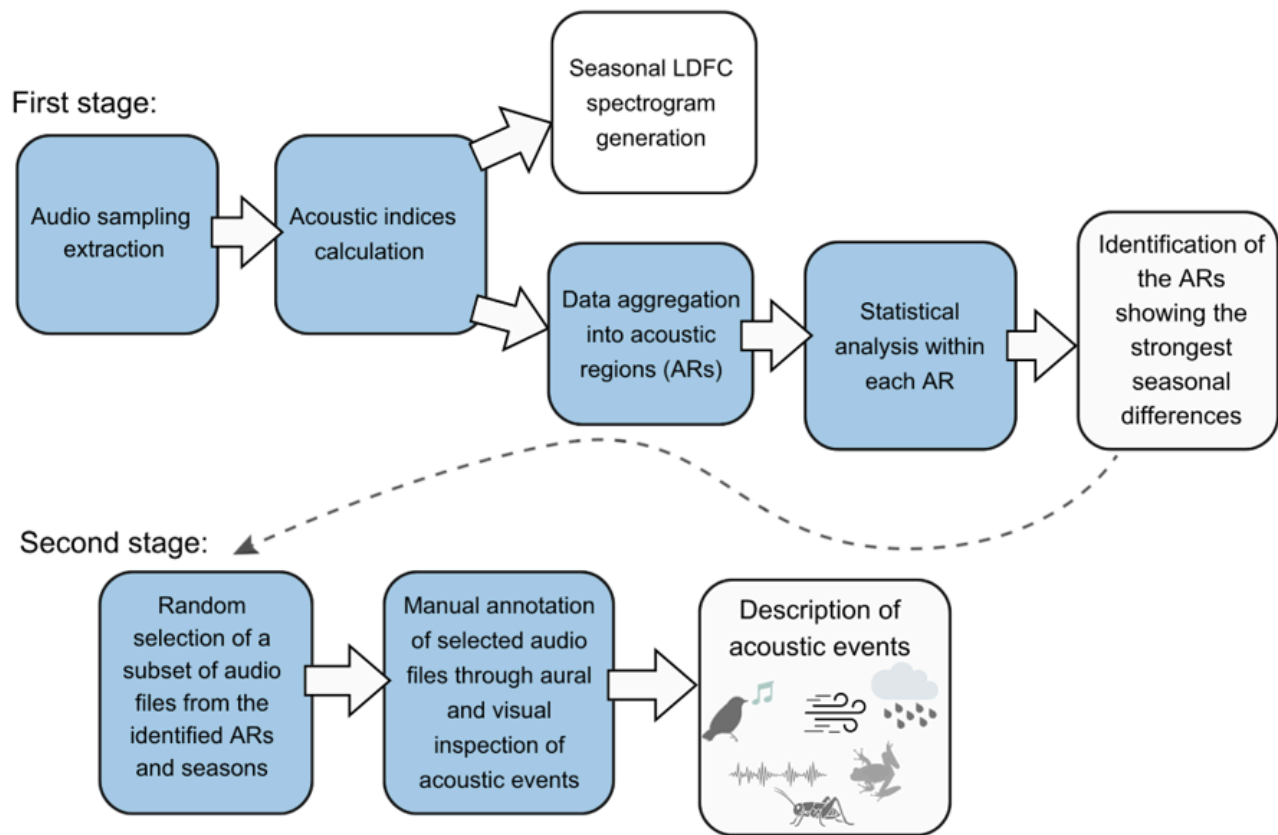


Figure 3

Two-stage analytical workflow for soundscape assessment based on acoustic regions. In Stage 1, after acoustic index calculation, seasonal LDFC spectrograms are generated and index values are aggregated per recorder-day into acoustic regions; statistical tests are then applied, and acoustic regions of interest showing the strongest seasonal differences are identified. In Stage 2, a random subset of audio files from the identified acoustic regions and their most contrasting season pairs is selected for manual sonotype annotation through aural and visual inspection. Adapted from Campos et al.^{15,19}.

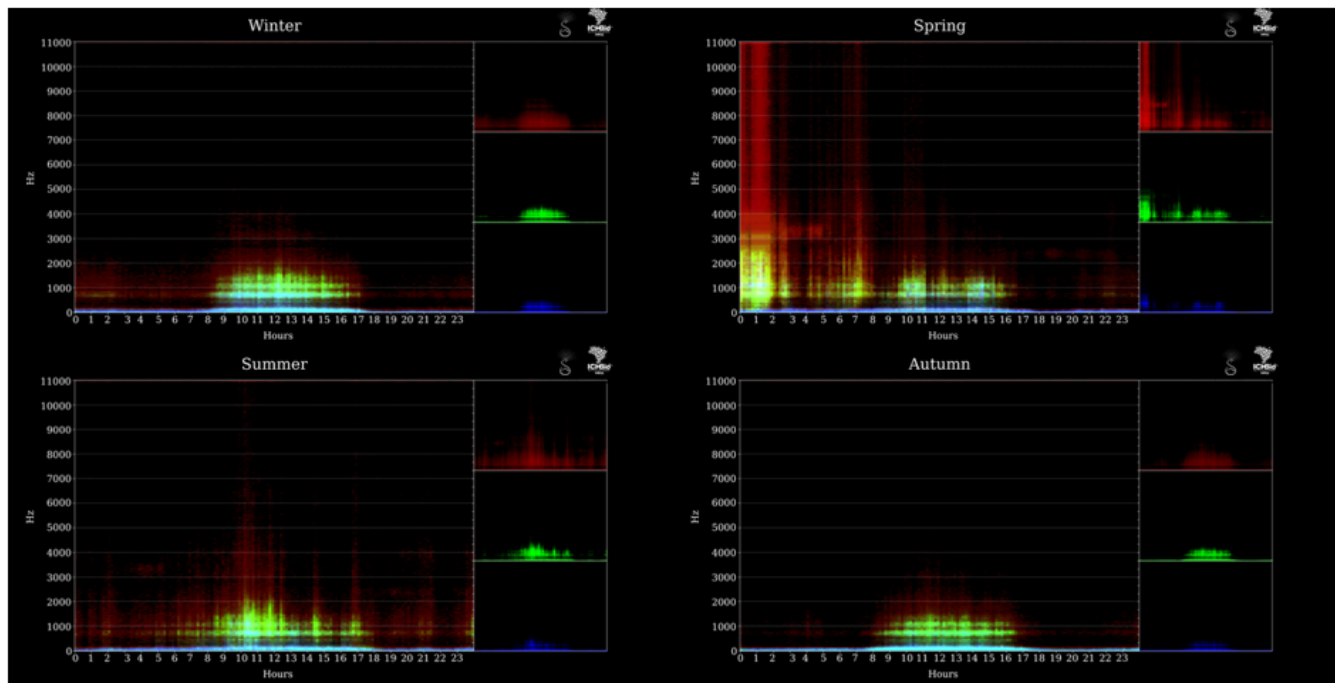


Figure 4

Seasonal long-duration false-colour (LDFC) spectrograms representing the median Acoustic Complexity Index (ACI; red channel), inverted Temporal Entropy (Ht; green channel), and Event Count Index (EVN; blue channel) values per frequency bin and time of day across 144 sampling-days per season: winter (June–August 2024), spring (September–November 2024), summer (December 2024–February 2025), and autumn (March–May 2025). The x-axis shows time of day (00:00–23:59); the y-axis shows frequency (Hz, 0–11,000). Insets to the right of each panel display median values of each index independently. Autumn showed the lowest overall index values. In spring, high ACI values (red) are prominent at low to mid frequencies during the pre-dawn period. Sonotype annotation confirmed elevated anuran and insect activity in acoustic regions AR02 and AR03 during spring. In summer, elevated EVN and ACI values at high frequencies during central daytime hours correspond to insect activity in acoustic region AR12. Autumn consistently shows lower acoustic activity relative to its contrasting season across all four acoustic regions of interest.

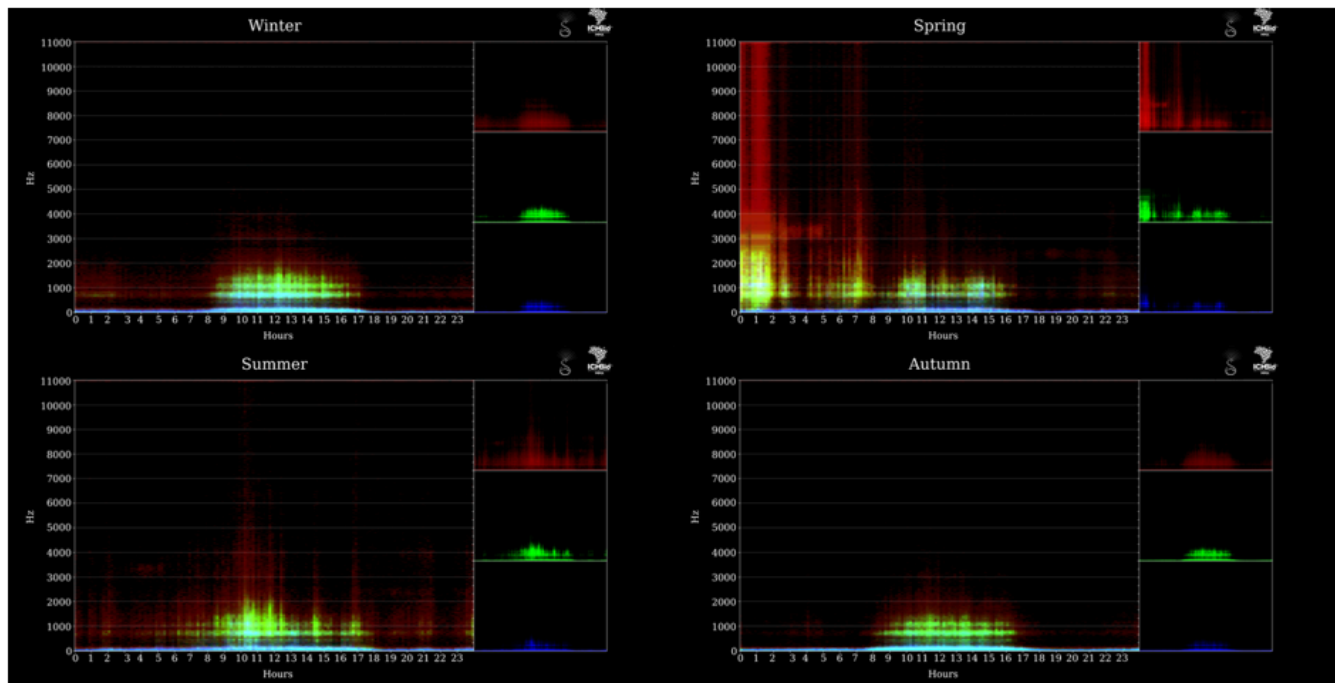


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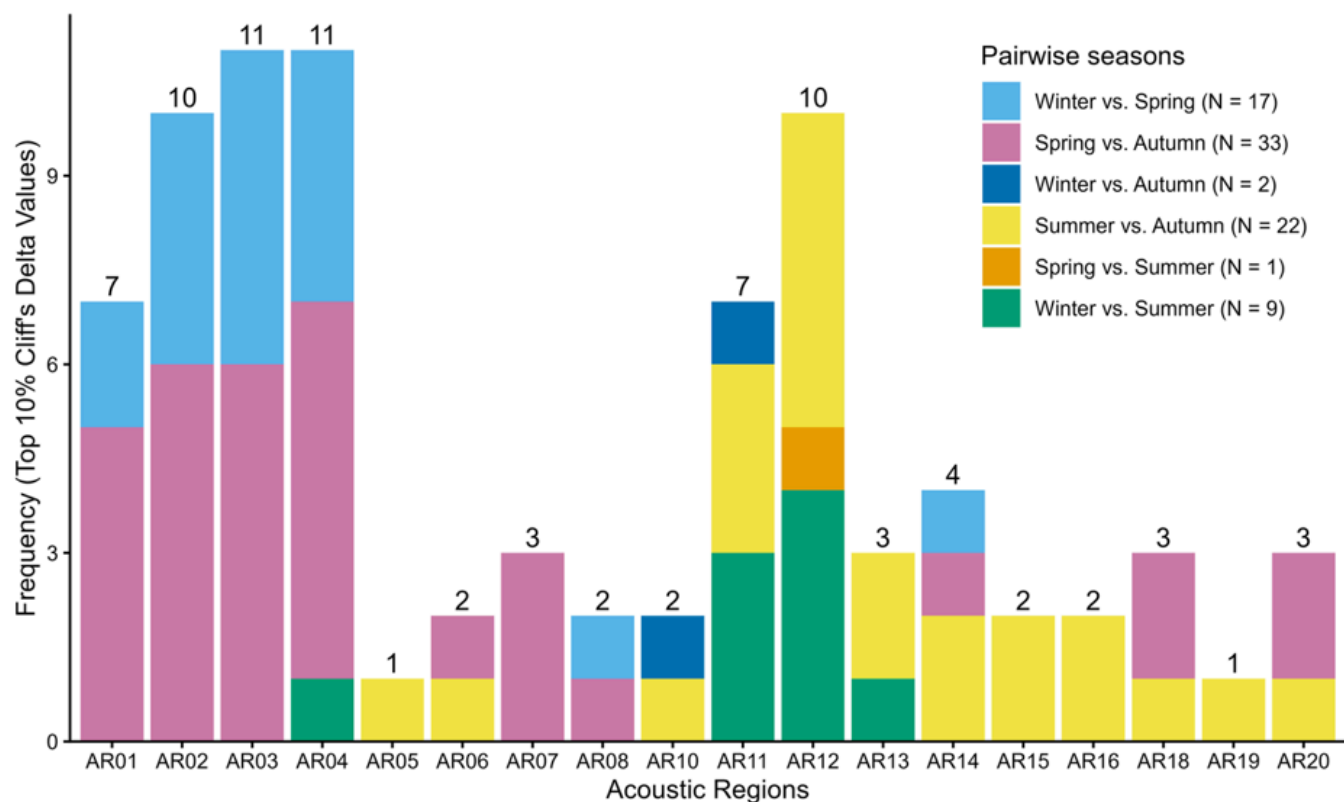


Figure 5

Number of times in which each of the 20 acoustic regions appeared among the top 10% largest absolute Cliff's delta values across all seven indices and six pairwise season comparisons. Regions AR03 and AR04 tied at the highest count ($n = 11$), followed by AR02 and AR12 ($n = 10$ each), and were selected for sonotype annotation. Colours show the number of appearance of each pairwise season comparison among the top 10% largest absolute Cliff's delta values across all acoustic regions and indices. The spring–autumn comparison dominates ($n = 33$), followed by summer–autumn ($n = 22$), consistent with autumn–spring being the most contrasting pair for AR02, AR03, and AR04, and autumn–summer for AR12.

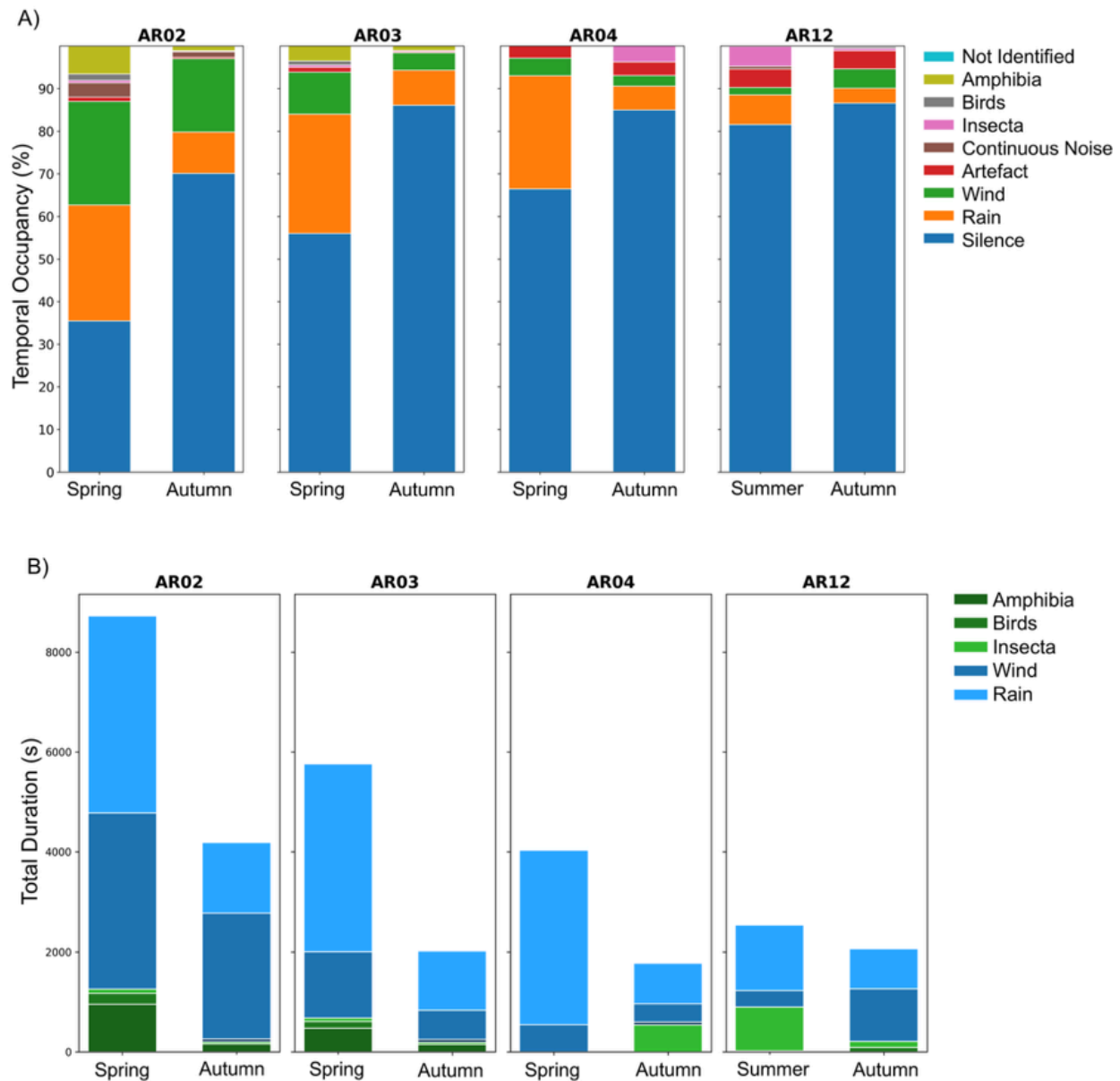


Figure 6

(A) Relative proportion of time per sonotype class (% of total annotated duration) by season and acoustic region for the four acoustic regions of interest (AR02, AR03, AR04, and AR12) and their most contrasting season pairs (spring–autumn for AR02, AR03, and AR04; summer–autumn for AR12). Classes shown: Silence, Rain, Wind, Artefact, Amphibia, Insecta, Birds, Continuous Noise, Not Identified. Note the contrasting insect patterns between AR04 (more active in autumn) and AR12 (more active in summer), and the increased Amphibia activity in spring compared to autumn in AR02 and AR03. (B) Total duration of geophony classes (Wind and Rain – different shades of blue) and biophony classes (Amphibia, Birds, Insecta – different shades of green) per acoustic region and season.

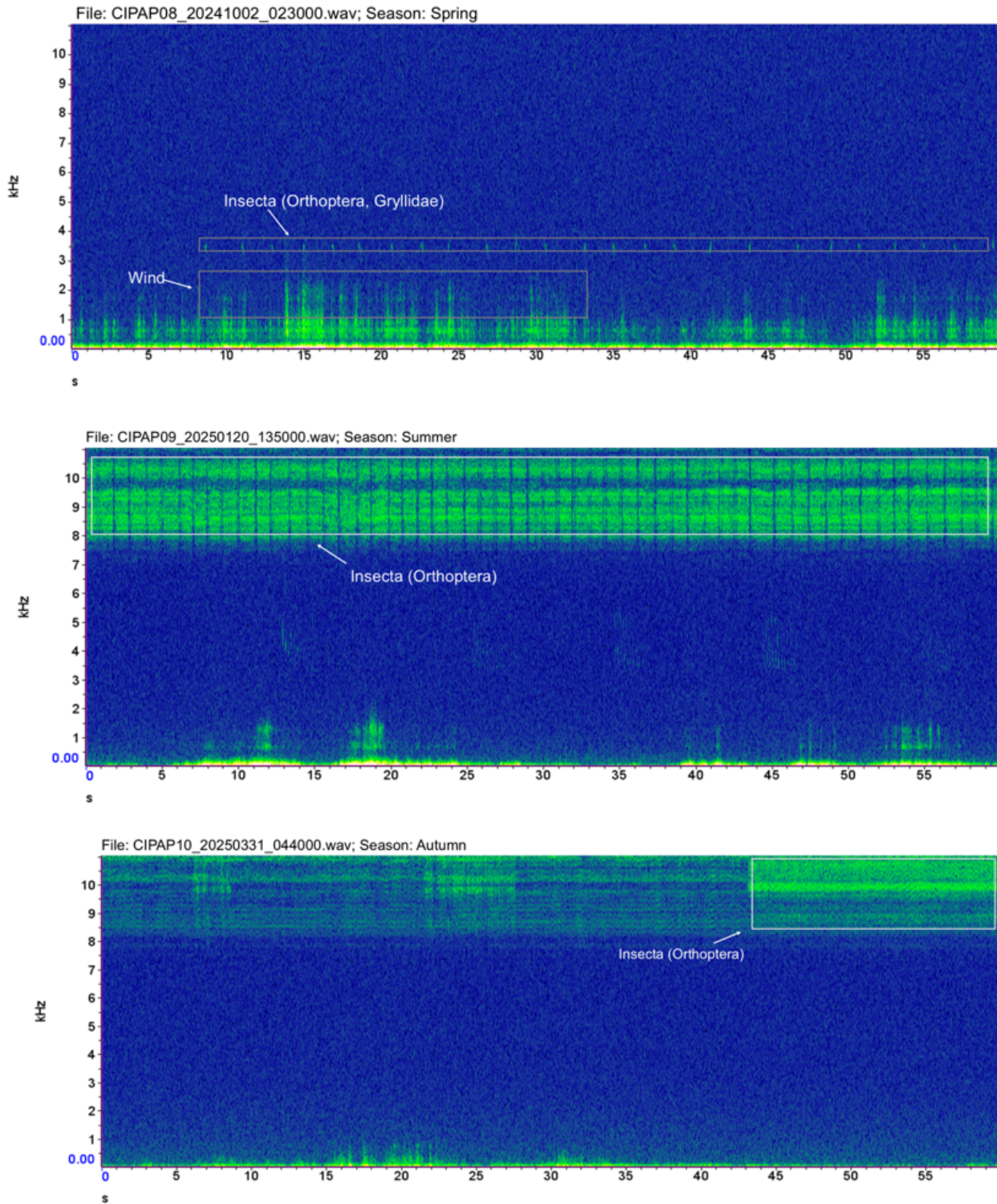


Figure 7

Individual spectrograms illustrating contrasting insect stridulation patterns in the two high-frequency acoustic regions of interest. Top: a 1-min recording from AR04 (7,907–11,000 Hz; pre-dawn) in autumn, showing continuous insect stridulation between approximately 8–9 kHz. Bottom: a 1-min recording from AR12 (7,907–11,000 Hz; daytime) in summer, showing insect stridulation in a different frequency and

temporal pattern. Another insect stridulation pattern is shown in spring for a different acoustic region, suggesting that these acoustic regions contain the activity of different insect taxonomic groups.

Supplementary Files

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- [CamposetalSciReportssupp.pdf](#)