

Supplementary Information

A standardized, unbiased protocol for protected areas soundscape monitoring

Campos et al., Scientific Reports — Bioacoustics Collection (2026)

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Note: Full acoustic index calculations, all Cliff's delta values, sonotype complete annotations, and selected annotated audio files are deposited in the GitLab public repository (<https://gitlab.com/monitoramentoacustico/protocol-article-support-information>).

Supplementary Methods S1 — Sequential description of the statistical analyses performed

Statistical analysis was designed to produce parametric tests where possible, as these are generally more robust than non-parametric alternatives. The workflow was applied independently within each of the 20 acoustic regions and for each of the seven acoustic indices.

Step 1: Pre-filtering.

Data with near-zero variance were excluded from further analysis. Data with fewer than two sampling units ($n < 2$) were also excluded.

Step 2: Normality testing.

The remaining data were tested for normality using the Shapiro-Wilk test ($\alpha = 0.05$).

Step 3: Parametric path.

If data were normally distributed, a repeated-measures ANOVA was performed to test for differences across the four seasons.

Step 4: Transformation path.

If data were not normally distributed, five transformations were applied sequentially: (i) square root; (ii) base 10 logarithm; (iii) Box-Cox; (iv) Yeo-Johnson; (v) log-modulus. The first transformation achieving normality (Shapiro-Wilk $p > 0.05$) was followed by a repeated-measures ANOVA.

Step 5: Non-parametric path.

If no transformation normalized the data, a Friedman test was applied.

Step 6: Post-hoc comparisons.

Following either ANOVA or Friedman test, post-hoc comparisons between all six pairwise season combinations (winter–spring, winter–summer, winter–autumn, spring–summer, spring–autumn, summer–autumn) were conducted. For normally distributed data, pairwise t-tests were used; for non-normally distributed data, Nemenyi tests were applied.

Step 7: Effect sizes.

Effect sizes for all pairwise season comparisons were assessed using Cliff's delta (δ), calculated from the Common Language Effect Size (CLES) as $\delta = (2 \times \text{CLES}) - 1$. Effect sizes were classified as: large ($\delta \geq 0.474$), medium (0.330–0.473), small (0.147–0.329), or negligible (<0.147), following Romano et al. (2006). To enable comparison across parametric and non-parametric paths, generalized eta-squared (η^2) was extracted from ANOVA results and Kendall's W from Friedman results; both range from 0 to 1 and were used to construct a unified effect-size ranking.

Step 8: Selection of acoustic regions of interest.

For each index, the top 10% of absolute Cliff's delta values were identified (12 of 120 comparisons per index: 20 acoustic regions $\times$ 6 season pairs). Each acoustic region appearing in this top 10% set for any index was counted once. Acoustic regions with the highest total counts across all indices were selected for sonotype annotation.

Artefact and Continuous Noise classes.

During sonotype annotation, two non-soundscape classes were identified. Artefact events were associated with the acoustic response of the recorder to high-intensity sounds (typically caused by wind-driven objects striking the recorder support), 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. Both classes were excluded from biophony and geophony analyses. No anthrophony was detected in any annotated file.

Software.

All statistical analyses were implemented in Python 3.14 using the pingouin (Vallat, 2018) and scikit-posthocs (Terpilowski, 2019) modules. The full statistical output, including all 840 Cliff's delta values, is available at the project GitLab repository.

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Supplementary Figure S2

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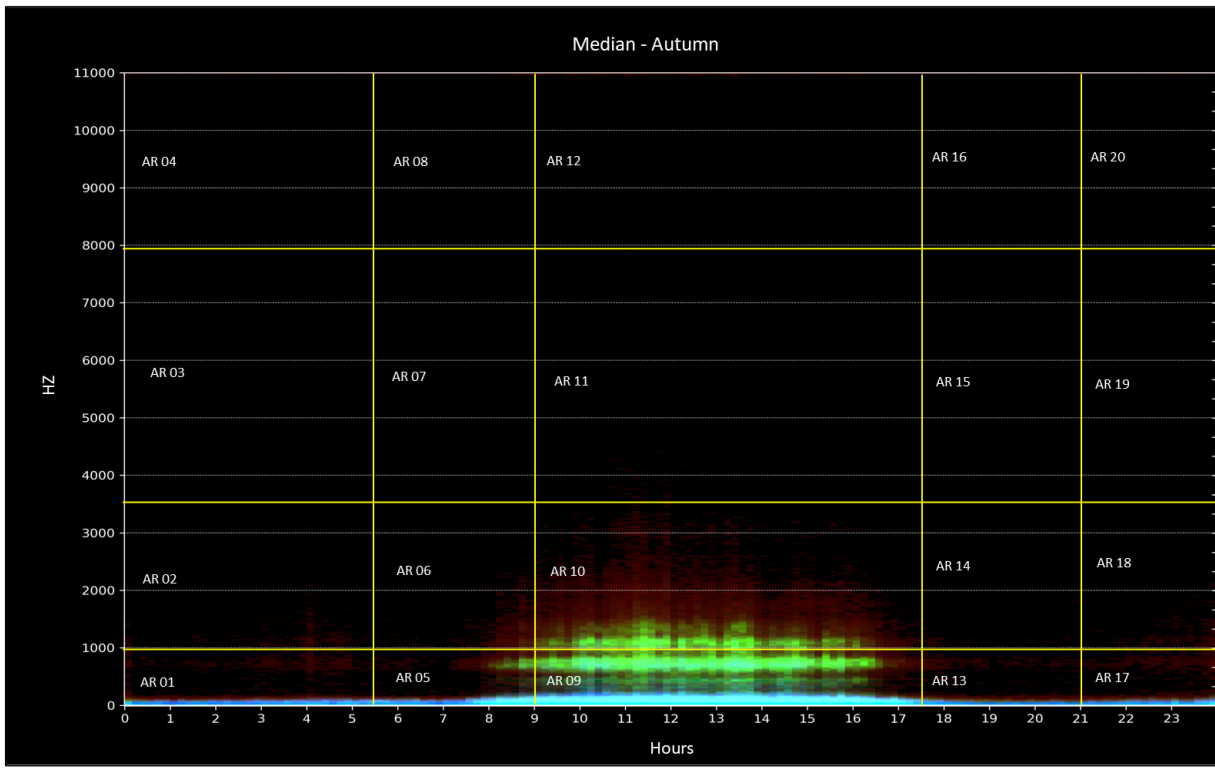
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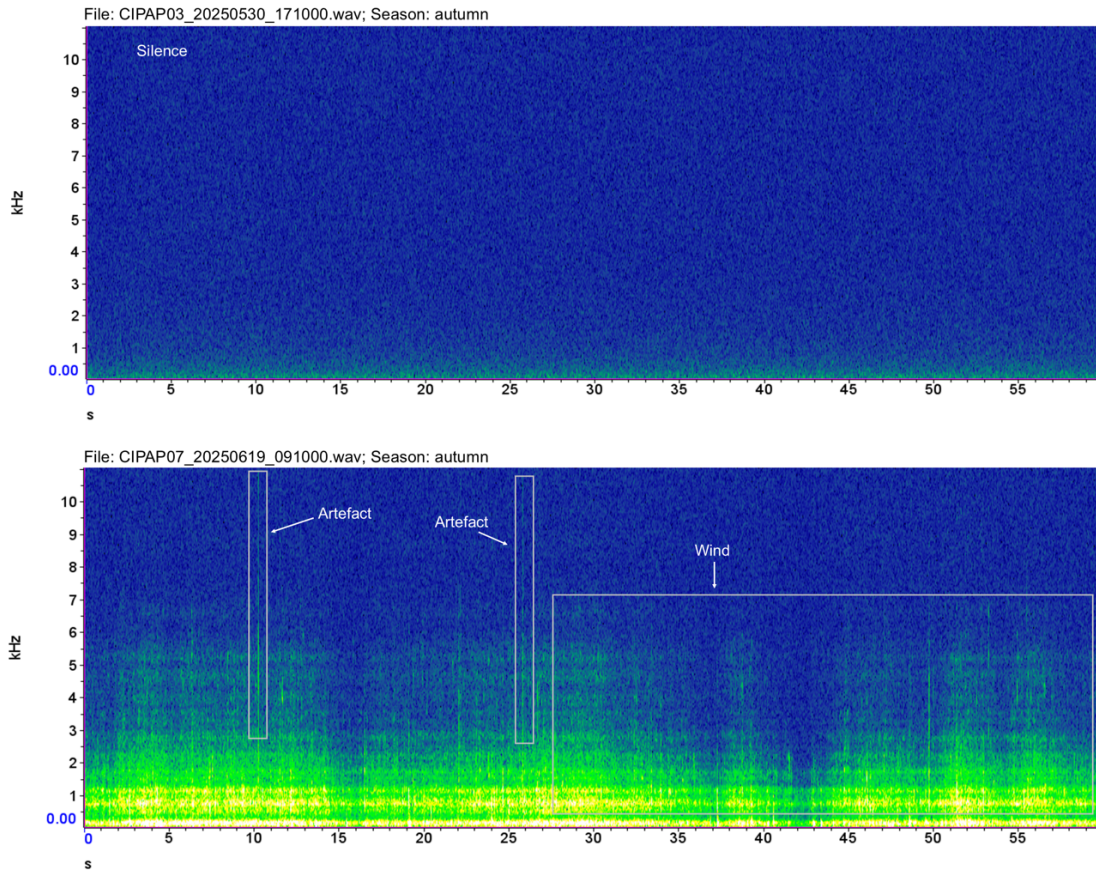
Supplementary Figure S1. Statistical decision tree applied within each acoustic region and for each acoustic index. Starting from the aggregated data per acoustic region, per recorder, per sampling day, data are sequentially filtered for near-zero variance and minimum sample size ($n \geq 2$), tested for normality (Shapiro-Wilk test), and directed to a repeated-measures ANOVA (normally distributed data, or data normalized after transformation) or a Friedman test (non-normally distributed data after all transformations). Post-hoc comparisons are conducted with pairwise t-tests (normal path) or Nemenyi tests (non-normal path). Effect sizes are assessed with Cliff's delta (δ) for all pairwise season comparisons.

Supplementary Figure S3 - Illustration of the 20 acoustic regions



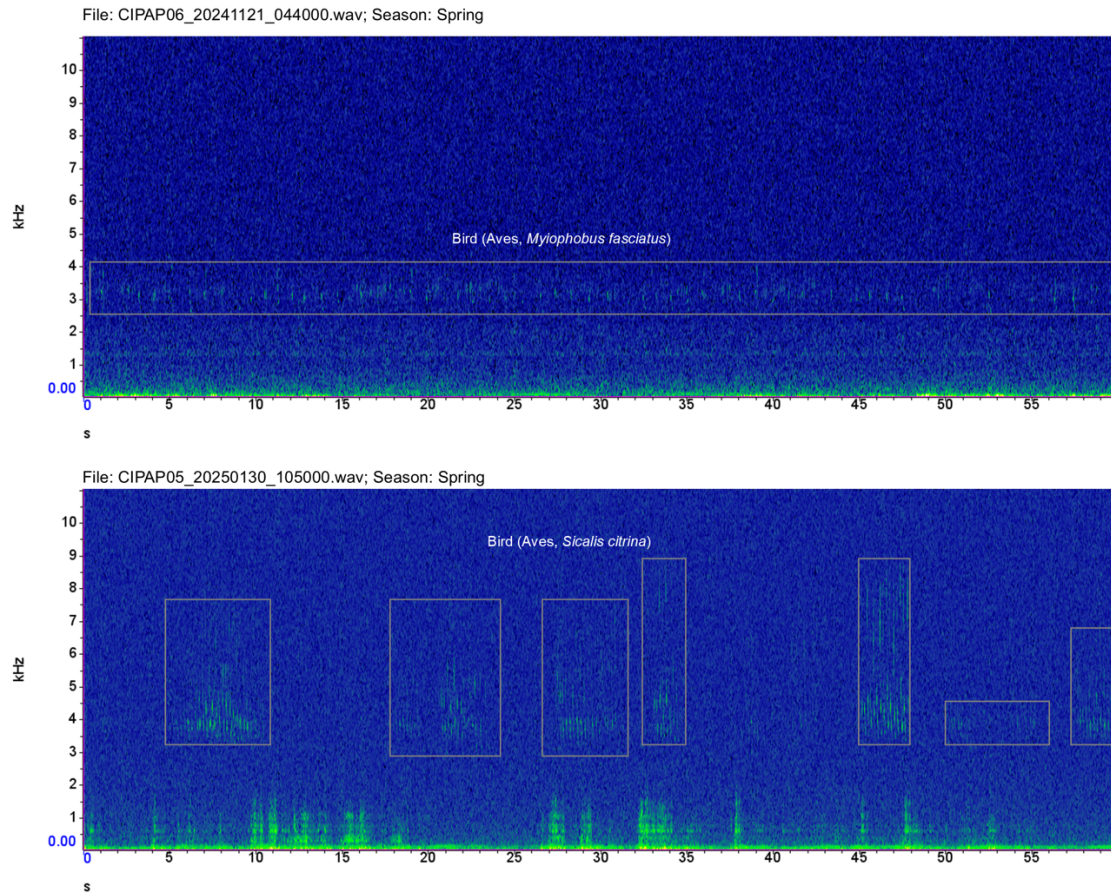
Supplementary Figure S2. Illustration of the 20 acoustic regions defined by the intersection of five daily time periods and four frequency bands (0–988 Hz, 988–3,609 Hz, 3,610–7,906 Hz, and 7,907–11,000 Hz), following Campos et al. (2021, 2022). The autumn seasonal LDFC spectrogram (March–May 2025) is used as background. Yellow lines indicate the temporal and spectral boundaries of the acoustic regions, labelled R01 to R20. Acoustic regions R02, R03, R04, and R12 showed the greatest seasonal differences in the present study. Here low levels of acoustic activity are clear to these four acoustic regions in autumn.

Supplementary Figure S4 — Sonotype example: Silence and Artefact



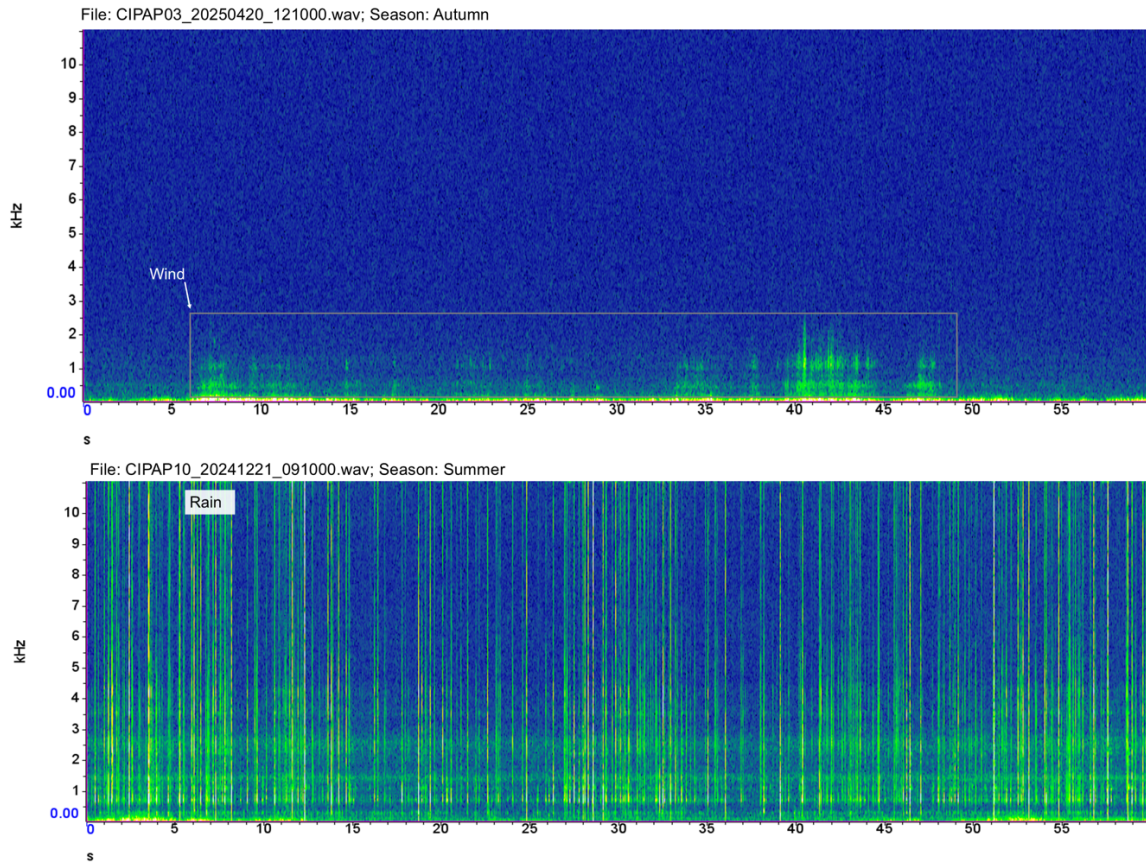
Supplementary Figure S3. Examples of the Silence and Artefact sonotype classes. Both panels show one-minute spectrograms from acoustic region R12 (7,907–11,000 Hz; daytime period 09:01–17:29) in autumn. **Top:** Silence — the absence of any detectable biological or geophysical sound within the frequency range of acoustic region R12. The spectrogram shows a uniform, low-amplitude background across the entire duration. **Bottom:** Artefact — brief wideband events spanning the full frequency range of the spectrogram, produced by the acoustic response of the recorder support to high-intensity mechanical disturbances (e.g. wind-driven vibration or impact). Artefact events are distinguishable from biological or geophysical sounds by their extremely short duration and instantaneous full-frequency coverage. This class is not part of the soundscape and was excluded from all biophony and geophony analyses.

Supplementary Figure S5 — Sonotype example: Birds



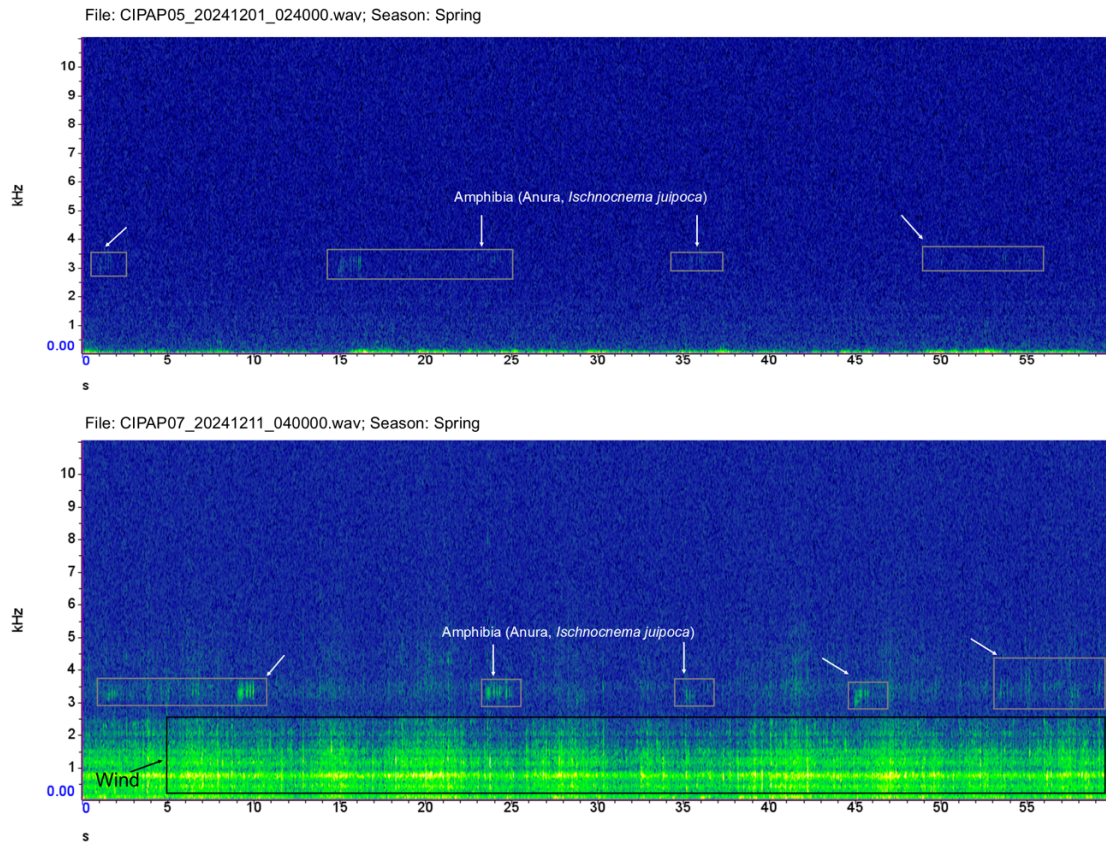
Supplementary Figure S4. Examples of bird vocalizations illustrating two distinct acoustic signatures from the Birds sonotype class. Both panels show one-minute spectrograms from acoustic regions R02 (988–3,609 Hz) and R03 (3,610–7,906 Hz) in spring (pre-dawn period 00:00–05:29). **Top:** Bird vocalizations with a structured tonal pattern consisting of repeated short elements concentrated in the lower frequency bands of R02. **Bottom:** Bird vocalizations with a different acoustic signature, showing broader frequency use extending into R03 and a distinct temporal pattern. The contrasting bird vocal signatures illustrate the diversity of acoustic patterns grouped within the Birds sonotype class.

Supplementary Figure S6 — Sonotype example: Wind and Rain



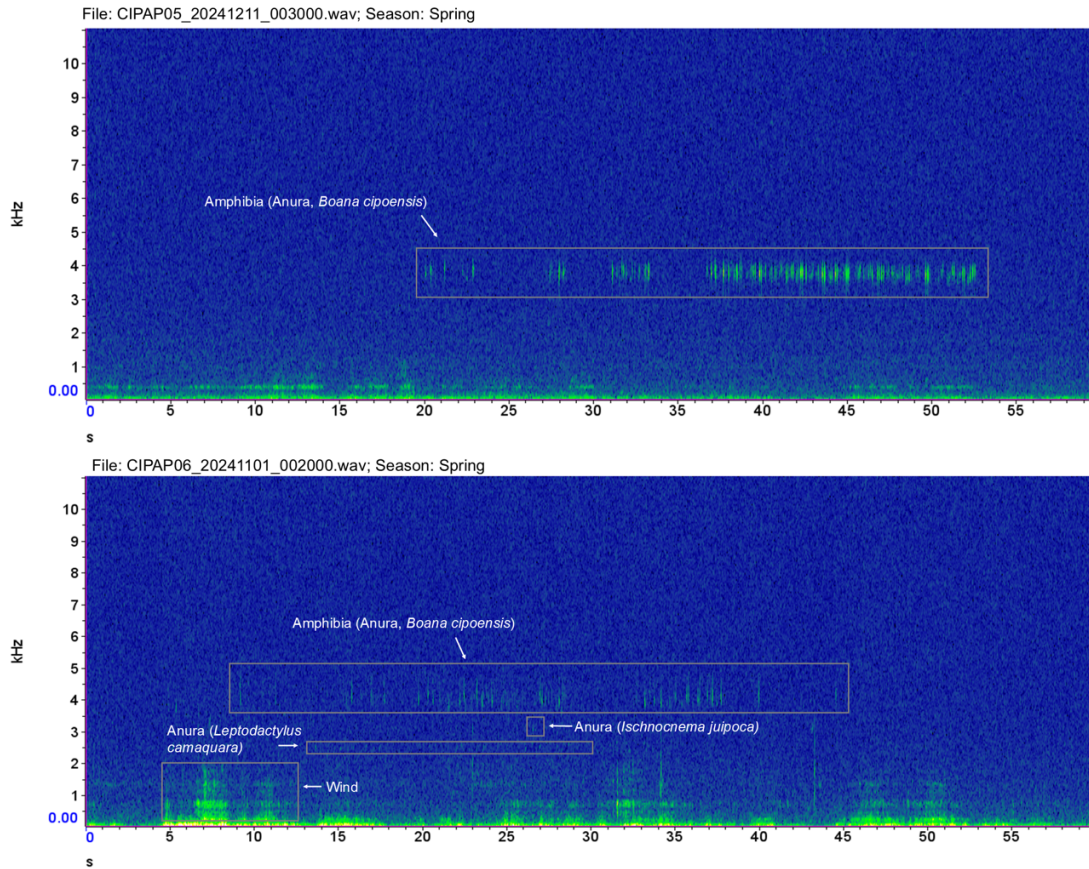
Supplementary Figure S5. Examples of the Wind and Rain geophony sonotype classes. Both panels show one-minute spectrograms sampled at the daytime period (09:01–17:29). **Top:** Wind — characterized by diffuse broadband energy below 3 kHz, with intensity varying over time. Wind events do not produce discrete sound pulses but rather a sustained, fluctuating background energy level. **Bottom:** Rain — characterized by more uniform broadband energy with a distinct pattering texture resulting from the random impact of individual raindrops. Rain produces a higher and more spectrally uniform energy level than wind and occupies the entire frequency range. Both classes are geophony and contribute to the background noise floor.

Supplementary Figure S7 — Sonotype example: Amphibia



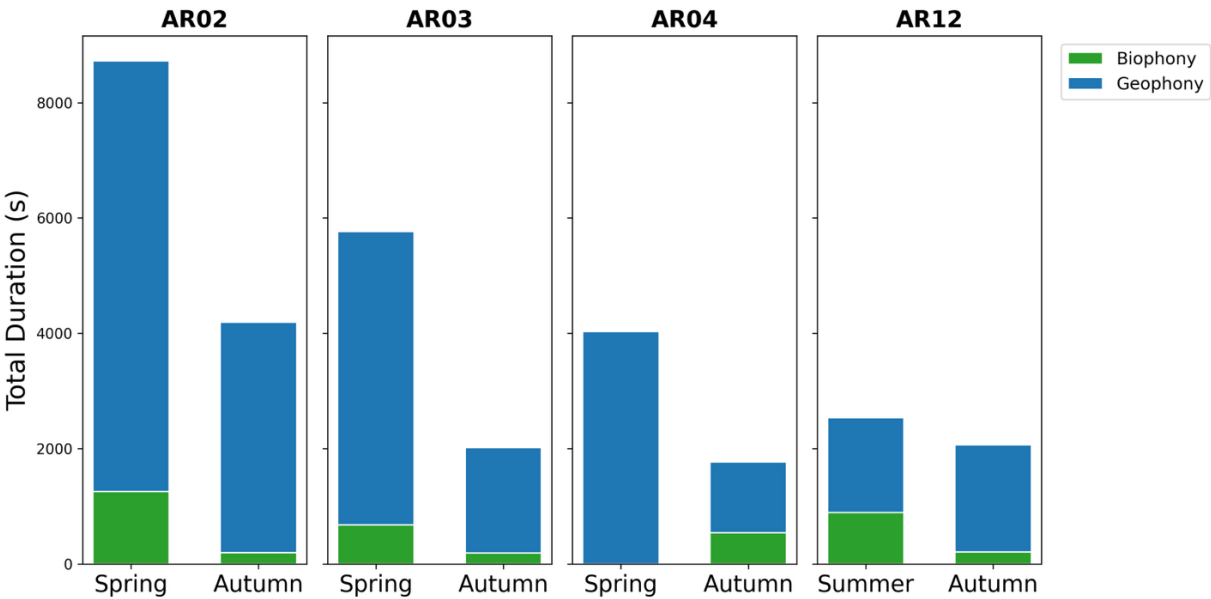
Supplementary Figure S6. Examples of the Amphibia sonotype class in the context of different background conditions. Both panels show one-minute spectrograms from acoustic regions R02 (988–3,609 Hz) and R03 (3,610–7,906 Hz) in spring (pre-dawn period 00:00–05:29). **Top:** Anuran vocalizations visible as repeated calls in a relatively quiet background. The calls show a regular temporal pattern consistent with *Ischnocnema juipoca*. **Bottom:** The same recording period with anuran vocalizations overlapping in time with Wind events visible as spread energy in the lower frequency bands. The co-occurrence of Amphibia and Wind illustrates how multiple sonotype classes can be simultaneously present within the same one-minute file, each annotated independently across the frequency range of the respective acoustic region.

Supplementary Figure S8 — Sonotype example: Amphibia (different species)



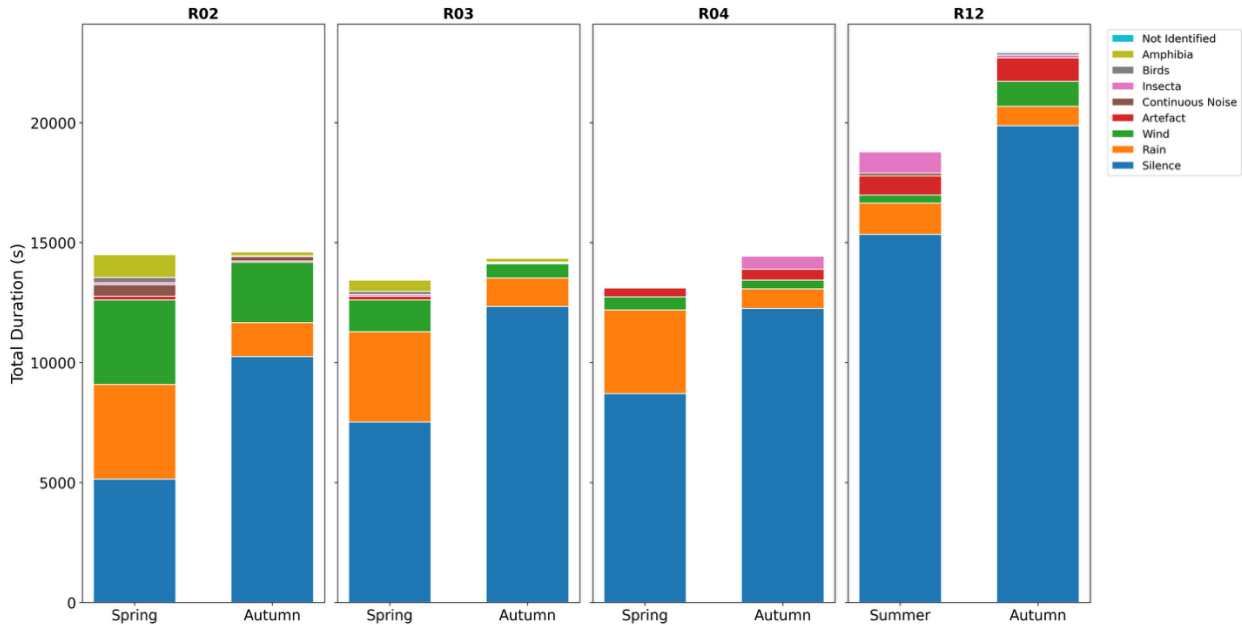
Supplementary Figure S7. Examples illustrating the diversity within the Amphibia sonotype class. Both panels show one-minute spectrograms from acoustic regions R02 (988–3,609 Hz) and R03 (3,610–7,906 Hz) in spring (pre-dawn period 00:00–05:29). **Top:** A single anuran species, visible as a narrow band in R02 sustained throughout most of the recording. **Bottom:** Three anuran species vocalizing simultaneously, visible as overlapping calls at different frequencies within R02 and R03. Wind events appear as diffuse broadband energy between anuran call bouts.

Supplementary Figure S9 — Sonotype analyses: Biophony and Geophony



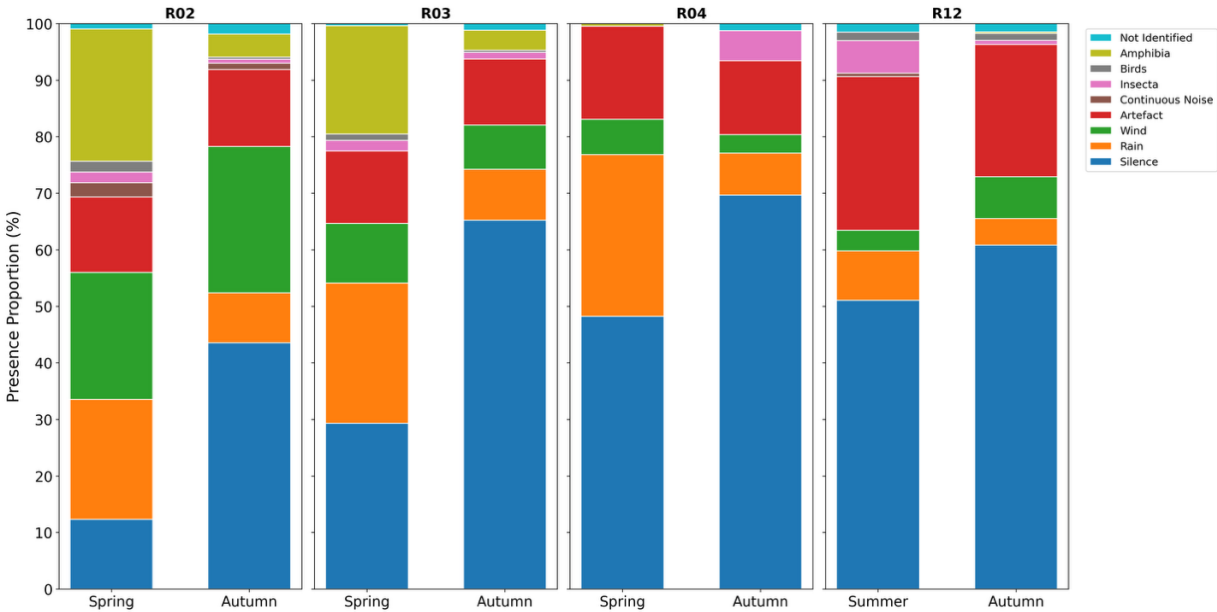
Supplementary Figure S8. Total duration in seconds of Biophony and Geophony by season and acoustic region for the four acoustic regions of interest (R02, R03, R04, and R12) and their most contrasting season pairs (spring–autumn for R02, R03, and R04; summer–autumn for R12). Biophony (encompassing Amphibia, Birds, Insecta) is shown in green; Geophony (encompassing Wind, Rain) in blue. The other classes were excluded from the figure to show clearly that differences were detected for both Biophony and Geophony. In a context of climate change, monitoring the changes of geophony in protected areas is extremely relevant, as is its relation with biophony.

Supplementary Figure S10 — Sonotype analyses: Total duration of each class



Supplementary Figure S9. Total annotated duration (seconds) of each sonotype class by season and acoustic region for the four acoustic regions of interest and their most contrasting season pairs. Unlike proportional analyses, total duration reflects the absolute time occupied by each class within the annotated sample. Note that the total annotated duration is not the same for both seasons within each acoustic region because annotation overlap — where two or more classes are annotated simultaneously — increases the summed duration beyond the actual recording time.

Supplementary Figure S11 — Sonotype analyses: Presence frequency of each class



Supplementary Figure S10. Proportion of annotated one-minute files (%) in which each sonotype class was detected at least once, by season and acoustic region for the four acoustic regions of interest and their most contrasting season pairs. This metric reflects the frequency of occurrence of each class across files, independently of the duration of individual events. Silence was counted only for files in which no other sonotype class was annotated within the acoustic region. Unlike duration-based metrics (Supplementary Figs. S8–S9), presence frequency is not affected by annotation overlap and gives equal weight to brief and sustained acoustic events.

Supplementary Table S12 — Sonotype annotation proportions by acoustic region and season

Supplementary Table S3. Relative proportion of sonotype classes (% of total annotated duration) by season and acoustic region for the four acoustic regions of interest and their most contrasting season pairs (spring–autumn for R02, R03, and R04; summer–autumn for R12). Values calculated from raw annotation duration data (Raven Pro 1.6 selection tables). Dashes (—) indicate classes with < 0.05% annotated duration. Total duration (s) refers to the total annotated time per acoustic region and season (including Silence). Note that summed proportions may exceed 100% due to annotation overlap when multiple classes were annotated simultaneously within the same file. Artefact and Continuous Noise are not part of the soundscape (see Supplementary Methods S1). Cont. Noise: Continuous Noise; Not ID.: Not Identified.

Acoustic region	Season	Silence (%)	Rain (%)	Wind (%)	Amphibia (%)	Birds (%)	Insecta (%)	Artefact (%)	Cont. Noise (%)	Not ID. (%)	Total (s)
R02	Spring	31.1	27.0	24.1	6.5	1.5	0.6	1.0	7.3	0.9	14,620
R02	Autumn	60.4	9.7	17.1	1.1	0.0	0.2	0.4	10.6	0.4	14,674
R03	Spring	47.8	27.6	9.7	3.5	1.0	0.5	1.1	7.5	1.3	13,615
R03	Autumn	73.2	8.2	4.1	1.0	0.0	0.3	0.3	12.5	0.4	14,396
R04	Spring	65.8	26.4	4.1	0.0	—	—	2.8	—	0.9	13,224
R04	Autumn	84.6	5.6	2.5	—	—	3.7	3.2	—	0.4	14,487
R12	Summer	81.3	6.9	1.8	—	0.1	4.7	4.3	0.6	0.3	18,861
R12	Autumn	86.6	3.5	4.6	0.0	0.4	0.5	4.3	—	0.2	22,960

Note: Full acoustic index calculations, all Cliff's delta values, sonotype complete annotations, and selected annotated audio files are deposited in the GitLab public repository (<https://gitlab.com/monitoramentoacustico/protocol-article-support-information>).

Supplementary Table S13 — Top Cliff's delta results

Supplementary Table S1. Selected Cliff's delta effect sizes from the top 10% of absolute delta values across the 20 acoustic regions and seven acoustic indices (n = 840 total comparisons). Only the largest-effect comparisons are shown. The complete table with all 840 Cliff's delta values is available at the GitLab public repository (<https://gitlab.com/ivanbcampos/protected-areas-soundscape-monitoring/>). Effect size classes: large ($|\delta| \geq 0.474$), medium (0.330–0.473), small (0.147–0.329), negligible ($|\delta| < 0.147$). CLES: Common Language Effect Size. Direction indicates the season with higher index values.

Season pair	p-value	Significant	CLES	Cliff's Δ	Cliff's Δ	Magnitude	Direction	Acoustic region	Index
Spring vs Autumn	3.11×10^{-5}	Yes	0.814	0.628	0.628	Large	Spring	R02	ACI
Summer vs Autumn	1.74×10^{-5}	Yes	0.808	0.616	0.616	Large	Summer	R11	ACI
Winter vs Summer	2.55×10^{-4}	Yes	0.193	-0.614	0.614	Large	Summer	R11	ACI
Winter vs Summer	1.03×10^{-3}	Yes	0.198	-0.603	0.603	Large	Summer	R12	ACI
Winter vs Spring	2.05×10^{-3}	Yes	0.201	-0.598	0.598	Large	Spring	R02	ACI
Winter vs Spring	2.05×10^{-3}	Yes	0.201	-0.598	0.598	Large	Spring	R03	ACI
Summer vs Autumn	2.82×10^{-3}	Yes	0.798	0.596	0.596	Large	Summer	R12	ACI
Spring vs Autumn	4.39×10^{-1}	Yes	0.779	0.558	0.558	Large	Spring	R04	ACI
Spring vs Autumn	5.90×10^{-3}	Yes	0.778	0.556	0.556	Large	Spring	R03	ACI
Winter vs Spring	5.11×10^{-1}	Yes	0.223	-0.554	0.554	Large	Spring	R04	ACI
Spring vs Autumn	6.52×10^{-1}	Yes	0.768	0.537	0.537	Large	Spring	R01	ACI
Winter vs Spring	5.18×10^{-1}	Yes	0.215	-0.572	0.572	Large	Spring	R03	EVN

Season pair	p-value	Significant	CLES	Cliff's Δ	Cliff's Δ	Magnitude	Direction	Acoustic region	Index
Winter vs Spring	6.03×10^{-1}	Yes	0.225	-0.551	0.551	Large	Spring	R04	EVN
Spring vs Autumn	4.91×10^{-1}	Yes	0.769	0.539	0.539	Large	Spring	R03	EVN
Spring vs Autumn	6.06×10^{-2}	Yes	0.778	0.556	0.556	Large	Spring	R02	Ht
Summer vs Autumn	7.85×10^{-1}	Yes	0.748	0.496	0.496	Large	Summer	R12	Ht
Spring vs Autumn	3.65×10^{-1}	Yes	0.771	0.541	0.541	Large	Spring	R03	Ht
Spring vs Autumn	6.32×10^{-3}	Yes	0.787	0.573	0.573	Large	Spring	R02	SNR
Summer vs Autumn	8.59×10^{-4}	Yes	0.785	0.571	0.571	Large	Summer	R12	SNR
Winter vs Summer	7.48×10^{-2}	Yes	0.215	-0.569	0.569	Large	Summer	R12	SNR
Spring vs Autumn	9.33×10^{-2}	Yes	0.776	0.552	0.552	Large	Spring	R03	SNR
Spring vs Autumn	5.04×10^{-3}	Yes	0.729	0.459	0.459	Medium	Spring	R02	BGN
Winter vs Spring	1.15×10^{-6}	Yes	0.292	-0.416	0.416	Medium	Spring	R02	BGN
Spring vs Autumn	8.76×10^{-6}	Yes	0.691	0.382	0.382	Medium	Spring	R03	BGN

Supplementary Table S14 — Full protocol properties table

Supplementary Table S2. Full description of the key properties of the standardized soundscape monitoring protocol and their practical implications for protected area monitoring. An abbreviated version of this table is provided as Table 1 in the main article.

Protocol property	Description	Practical implications for monitoring
Random-systematic spatial design	Four quadrants randomly selected from a grid superimposed on the eligible area. Recorders placed at fixed relative positions (vertices) within each quadrant. Random selection eliminates systematic spatial bias; systematic placement ensures even coverage.	Geometrically consistent across PAs of different dimensions. Eliminates placement bias while guaranteeing spatial coverage of the eligible area.
Spatial representativeness	Eligible area defined to represent the dominant PA ecosystem, selected for conservation status and logistical access. Grid and random quadrant selection ensure representative sampling.	Ensures sampling reflects the ecological state of the area without deliberate placement bias.
Temporally balanced schedule	1 min/10 min; 1 day/10 days. Calendar anchored to 1 January 2023. Custom firmware by Alex Rogers (Open Acoustic Devices, pers. comm., 2023) makes this calendar-anchored schedule possible on AudioMoth recorders.	Captures shifts in acoustic event timing across years (e.g. anuran breeding phenology), relevant for long-term climate change monitoring.
Synchronized multi-PA sampling	Fixed shared calendar allows different PAs to be sampled by different teams in different months while remaining synchronized.	Enables national and international comparisons with comparable seasonal coverage across PAs sampled at different times of year.
Modularity and adaptability to PA size and shape	Number of quadrants can be increased or decreased without changing the analytical approach. Minimum: one quadrant (four recorders). Grid cell size adjusted to fit PA dimensions. In fragmented landscapes, natural fragments can be pre-defined and randomly	Adaptable to different resource levels and PA configurations. Maintains methodological consistency across sites of very different size, shape, and accessibility.

Protocol property	Description	Practical implications for monitoring
	selected (min inter-recorder distance 707 m; max 1.5 km).	
Cross-PA comparability	Same indices, acoustic regions, and statistical framework applied regardless of biological community present.	PAs with very different communities and levels of prior ecological knowledge can be directly compared without species-level baselines.
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).	Supports navigation from local gradients to global comparisons, and from short-term variation to long-term trends within a single dataset.
Contrast comparisons	Grid superimposed over zone of influence of a localized pressure (mine, road, dam). Quadrants selected at increasing distances from the source and compared with PA reference baseline.	Allows detection of distance-decay gradients in acoustic disruption; directly relevant for environmental licensing and impact assessment adjacent to PAs.
Complementary species-level analyses	Same recordings analysed with automated classifiers, manual annotation, or call libraries to generate species occurrence and abundance data. One-quarter of the recorders are set to a sampling rate of 384 kHz to facilitate bat studies.	Soundscape and species-level outputs are complementary and derived from the same dataset, maximizing return on fieldwork investment.
Unbiased activity detection	Workflow detects differences from 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.	Applicable in species-rich or poorly characterized ecosystems where species-specific approaches are impractical. No species-level baseline required.

Supplementary Table S15 – Coordinates of Sampling Units (recorders) at the Serra do Cipó National Park

Sampling Units	Latitude	Longitude
CIPAP01	-19.276519	-43.51868
CIPAP02	-19.276417	-43.511592
CIPAP03	-19.28306	-43.5118
CIPAP04	-19.283143	-43.519095
CIPAP05	-19.283231	-43.531507
CIPAP06	-19.283503	-43.525757
CIPAP07	-19.289565	-43.525161
CIPAP08	-19.289601	-43.531831
CIPAP09	-19.283208	-43.5052
CIPAP10	-19.283028	-43.498422
CIPAP11	-19.288714	-43.497611
CIPAP12	-19.289452	-43.504934
CIPAP013	-19.302434	-43.525049
CIPAP014	-19.302307	-43.518344
CIPAP015	-19.308822	-43.518699
CIPAP016	-19.308715	-43.525021

Table S4. Location of the Sampling Units (recorders) at the Serra do Cipó National Park. The standardized naming convention used for recorder locations in the acoustic monitoring protocol consists of: 1) The first three letters identify the protected area (i.e., CIP for Serra do Cipó National Park, PNSC), 2) followed by two letters identifying the eligible area (i.e., AP for *Alto Palácio*). Including the eligible-area code allows multiple eligible areas to be monitored within the same protected area while maintaining clear site identification, and 3) the final two digits (01-16) correspond to the individual recorder locations.

Supplementary Figure S16 - I Photos of recorders and the rupestrian grassland environment in the Serra do Cipó National Park

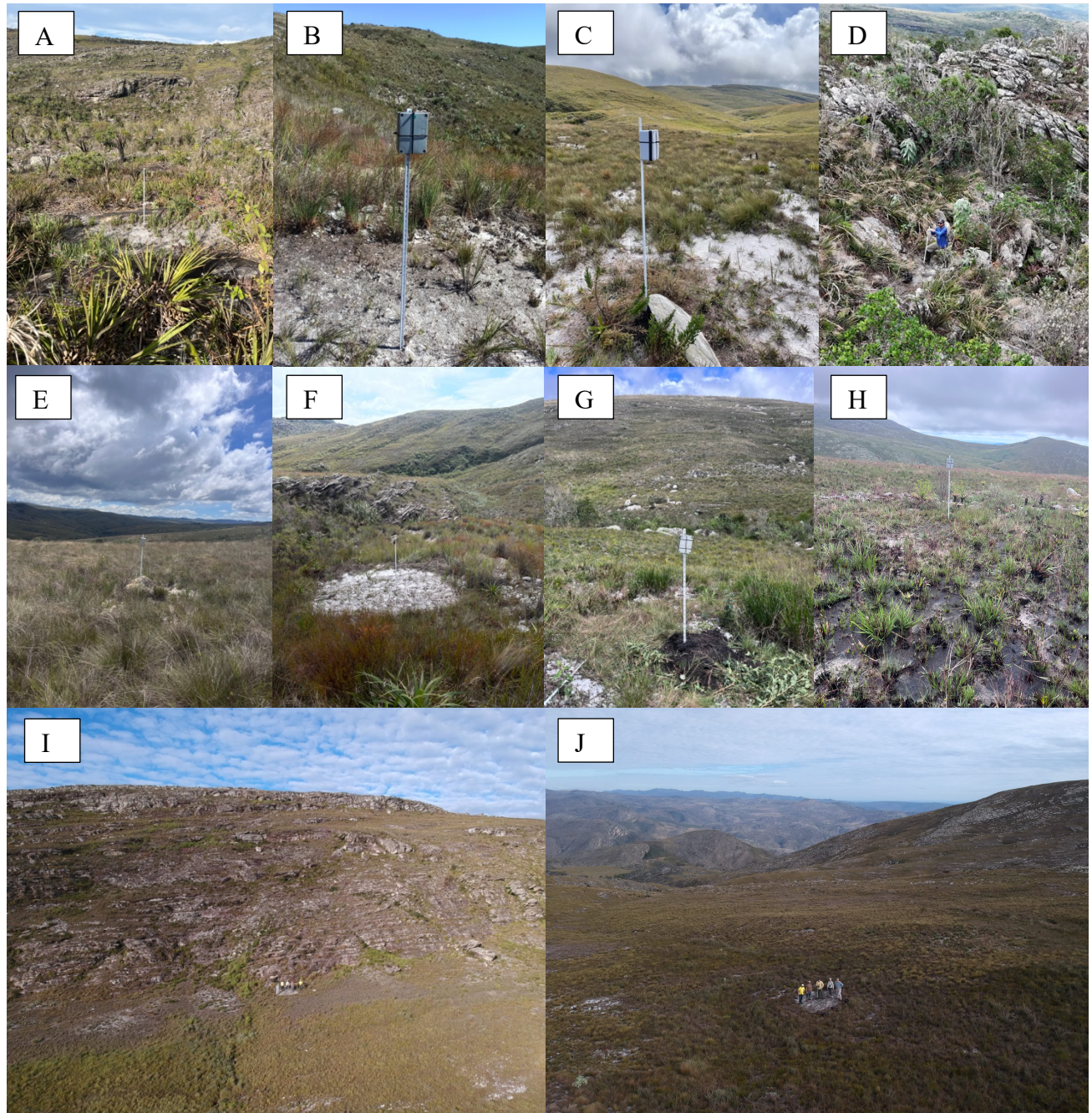


Figure S11. Location of the Sampling Units (recorders): A) CIPAP10, B) CIPAP06, C) CIPAP13, D) CIPAP02, E) CIPAP01, F) CIPAP12, G) CIPAP05, H) CIPAP14, I) CIPAP15, and J) CIPAP16. The naming convention used: 1) The first three letters identify the PA (i.e., CIP for Serra do Cipó National Park), 2) following two letters identifying the eligible area (i.e., AP for *Alto Palácio*), and 3) the final two digits (01-16) correspond to the individual recorder locations.