

Research Article

Determining temporal sampling schemes for passive acoustic studies in different tropical ecosystems

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Abstract

Among different approaches to exploring and describing the ecological complexity of natural environments, soundscape analyses have recently provided useful proxies for understanding and interpreting dynamic patterns and processes in a landscape. Nevertheless, the study of soundscapes remains a new field with no internationally accepted protocols. This work provides the first guidelines for monitoring soundscapes in three different tropical areas, specifically located in the Atlantic Forest, Rupestrian fields, and the Cerrado (Brazil). Each area was investigated using three autonomous devices recording for six entire days during a period of 15 days in both the wet and dry seasons. The recordings were processed via a specific acoustic index and successively subsampled in different ways to determine the degree of information loss when reducing the number of minutes of recording used in the analyses. We describe for the first time the temporal and spectral soundscape features of three tropical environments. We test diverse programming routines to describe the costs and the benefits of different sampling designs, considering the pressing issue of storing and analyzing extensive data sets generated by passive acoustic monitoring. Schedule 5 (recording one minute of every five) appeared to retain most of the information contained in the continuous recordings from all the study areas. Less dense recording schedules produced a similar level of information only in specific portions of the day. Substantial sampling protocols such as those presented here will be useful to researchers and wildlife managers, as they will reduce time- and resource-consuming analyses, whilst still achieving reliable results.

Keywords: environmental monitoring, animal conservation, tropical environments, soundscape ecology, sampling protocols

Resumo

Entre as diferentes abordagens para explorar e descrever a complexidade ecológica de ambientes naturais, a análise de paisagem acústica tem fornecido recentemente ferramentas úteis para o entendimento e interpretação da dinâmica de padrões e processos de uma paisagem. Apesar disso, o estudo de paisagens acústicas é uma nova linha de pesquisa que ainda não possui protocolos e métricas aceitas internacionalmente. Este estudo tem como objetivo fornecer as primeiras diretrizes para monitorar paisagens acústicas em três diferentes áreas tropicais localizadas especificamente na Mata Atlântica, no Campo Rupestre e no Cerrado. Cada área foi investigada usando três equipamentos autônomos gravando por 6 dias inteiros durante um período de 15 dias nas estações seca e chuvosa. As gravações resultantes foram processadas utilizando um índice acústico específico e foram sucessivamente subamostradas para determinar o grau de informação perdido quando reduzido o número de minutos de gravações usadas nas análises. Nós descrevemos pela primeira vez, as medidas temporais e espectrais de três ambientes tropicais e testamos rotinas de programação diversas para descrever os custos e benefícios de diferentes desenhos de amostragem, considerando questões de armazenamento e análise de bancos de dados extensos gerados por monitoramento acústico passivo. A programação 5 (gravação de um minuto a cada 5 minutos) manteve o maior número de informações contidas nos registros contínuos em todas as áreas de estudo. Programações de gravação menos intensas produziram um nível similar de informação apenas em porções específicas do dia. Protocolos de amostragens tais como os apresentados aqui são úteis para pesquisadores e gestores de meio ambiente, uma vez que eles podem reduzir tempo e recurso a ser consumido durante análise de dados e ainda fornecer resultados confiáveis.

Palavras-chave: monitoramento ambiental, conservação animal, ambientes tropicais, ecologia de paisagens acústicas, protocolos de amostragem.

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Introduction

Nowadays, passive acoustic monitoring (PAM) is considered to be an invaluable tool for both research and management. PAM collects acoustic data over large spatial and temporal scales and provides detailed, long-term information on animal distribution and variations in community dynamics. This wide-scale data collection inevitably leads to animal populations being better understood and managed more effectively [1]. However, to avoid time- and resource-consuming analyses, acoustic surveys need general guidelines to ensure efficient sampling with experience-based protocols.

Animals produce sounds for diverse biological functions (e.g. communication, mating, building territories, foraging) [2, 3], which can serve as proxies for estimating species fitness and individual behavior, especially in environments that are difficult to access or monitor using conventional methods [4, 5]. In the early 1990s, idiosyncrasies in the study of marine mammal behavior led researchers to develop autonomous acoustic devices for detecting sounds underwater [6]. Subsequently, acoustic recordings of the natural environment became gradually an important technique for monitoring all ecosystems. Passive acoustic monitoring has only recently been proposed for terrestrial environments [7], and the study of the soundscape (the aggregation of sounds from physical, biological and human-made sources, also known as 'soundscape ecology') has rapidly gained attention as a potential tool to evaluate both ecosystem health [8] and the effects of changes in land use and climate at various temporal and spatial scales [9–11].

Advances in technology over the last decade have revolutionized the potential of acoustic surveys. Fixed, programmable acoustic recording sensors can sample continuously for 24 hours a day for prolonged periods of time, allowing for the non-invasive assessment of changes in the distribution and acoustic behavior of entire animal communities throughout a variety of habitats simultaneously. Moreover, all of the recordings can be permanently stored and serve as an everlasting memory of the habitat sounds [1, 5, 12].

This temporal and spatial wide-scale application of soundscape ecology inevitably produces an overwhelming amount of information, with associated difficulties in data management and analysis [13]. Problems include an ever-growing requirement for storage space and the need for time-consuming processing, expensive power supply systems, and field personnel to periodically download data and reinstall the equipment. Common standards and baseline data collection models could be useful to limit unnecessary recordings and trips to the field while ensuring targeted data are collected.

Selecting specific portions of active recording times (ON) and leaving the device off for the rest of the time (OFF) is essential to conserving basic resources and staff-time, especially when constrained by limited funding. On the other hand, reducing ON periods increases the probability of losing important information and may result in a distorted description of the target community. Identifying the appropriate sampling period for a study is therefore essential for using soundscape surveys appropriately, and a good understanding of the daily and frequency patterns of the recorded community is required.

Several acoustic surveys have been conducted in recent years to investigate animal community dynamics and structure [14, 15], species richness and distribution [16–18], relationships with vegetational parameters [19, 20], and human or noise impact [21–23]. However, explicit evaluations of the survey effort required to characterize the acoustic dynamics of different landscapes are generally lacking. Knowledge about temporal variations in such acoustic dynamics could improve the design of future soundscape studies, making soundscape ecology more efficient and applicable for different categories of users (academics and other stakeholders).

Our goal was to describe the type and extent of soundscape information lost with different recording schedules in areas located in three tropical ecoregions (Atlantic Forest, Rupestrian fields and the Cerrado). These environments were chosen because they are priority conservation areas, threatened environments, and contain biodiversity hotspots with high endemism [24–26]. Additionally, studies of tropical soundscapes are limited [18].

On the basis of our results, we tried both to identify a cost-effective scheme for surveying such areas and to suggest the minimum sampling effort required to characterize the soundscape features. This was achieved by identifying when the recording schedule loses acoustic information that is essential for correctly describing the dynamics of the sound activity of that community and its circadian rhythms.

Methods

Study area

The study was conducted in three threatened environments in Minas Gerais, in the southeastern region of Brazil: Atlantic Forest, Rupestrian ferruginous fields and Cerrado *sensu stricto* (Fig. 1).

Atlantic Forest – Environmental station of Peti – The Atlantic Forest is a world biodiversity hotspot with high species richness and high levels of endemism, which are threatened by the rapid loss of native land-cover [25]. We collected data in this biome at the environmental station of Peti in the municipalities of São Gonçalo do Rio Abaixo and Santa Bárbara (19°53'57"S and 43°22'07"W). The reserve is approximately 605 hectares in size and is located in the upper Rio Doce Basin (altitude range: 630-806m). The area harbors 29 anuran species [27], 231 bird species [28] and 46 mammal species [29]. A large part of the reserve is covered by secondary arboreal vegetation, with large trees and a continuous canopy [30].

Rupestrian fields – State Park of Rola Moça - The ecosystems found in ferruginous outcrops known as 'Rupestrian ferruginous fields' or 'Canga' are among the least studied and most endangered areas of Brazil due to restricted geographical distribution and the presence of the country's main iron ore deposits [26]. Rupestrian fields have a relatively continuous herbaceous stratum of sclerophyllous plants, which are small evergreen shrubs located between rocky outcrops that occur at altitudes between 800 and 2,000m. This ecosystem is highly diversified, with more than 4,000 plant species along the Espinhaço Range, and has one of the highest levels of endemism in Brazil [31]. We collected data in the Rupestrian fields at the State Park

of Rola Moça, which is located in the northwest of 'Quadrilátero Ferrífero' (20°03'60"S, 44°02'00"W) at an altitude of approximately 1,450m.

The Cerrado *sensu stricto* – National Park of Serra do Cipó - The Cerrado is a biodiversity hotspot and a highly threatened environment [25]. The Cerrado *sensu stricto* (intended as a sub-category of the Cerrado *sensu lato* that includes a variety of physiognomies) is characterized by the presence of small trees with thick and twisted trunks and branches, while grasses characterize the understory [32]. We collected data in an isolated area of the Cerrado *sensu stricto* at the core of the national park of Serra do Cipó, which is approximately 34,000 hectares in size and is situated at 19°12'19"S and 43°30'43"W. This area provides habitat for 226 bird species [33] and 26 medium-large mammalian species [34].



Fig. 1. Location map of the study areas: Atlantic Forest (AF), Rupestrian fields (RF), and Cerrado *sensu stricto* (CE). The photographs represent the typical surroundings of the three habitats where the acoustic measurements were taken.

Acoustic recordings and data analyses

The climate of southeastern Brazil can be divided into two macro-climatic seasons: a hot wet season, running from October to March, and a cooler dry season from April to September [35]. The soundscape of the three study areas was collected by recording for six non-consecutive days during a period of 15 days during the dry season (Cerrado: 9-23 September 2012; Rupestrian fields: 17-30 April 2013; Atlantic Forest: 4-19 June 2013) and wet season (Cerrado: 15-30 March 2013; Rupestrian fields: 6-21 October 2012; Atlantic Forest: 17 October-1 November 2012). In each sampling area, three SongMeter Digital Field Recorders (SM2) (Wildlife Acoustics, Inc., Massachusetts) were set to record from 00:00 to 23:59h continuously. Accordingly, each area was recorded for 432 hours (24h * six days * three recorders) each season, making 2,592 hours in total. We considered 06:00 and 18:00 to be the approximate times of dawn and dusk, which varied slightly among the different months. One of the three recorders stopped recording after three days during the wet season in the Rupestrian fields, while another recorder in the Atlantic Forest stopped working after 17h on the last recording day during the dry season.

The recorders were placed at a distance of approximately 300m from each other to avoid double sampling the same sounds and to ensure that each recorder was an independent sampler per area. We mounted each recorder on a tree at approximately 1.5m from the ground, and ensured that nearby vegetation would not interfere with recordings. The SM2s recorded at a sampling rate of 44,100Hz, set at 16 bits.

The Kaleidoscope converter utility (Wildlife Acoustics, Inc., Massachusetts) was used to split the collected data into files of one minute in length, which were further processed via the Wavesurfer software [36] powered by the SoundscapeMeter plug-in [37]. One minute resolution was chosen, since most of the recent literature used this time lapse for sound assessments [18, 19, 38, 39], to allow comparisons with previous research.

Among the variety of the available acoustic indices to directly summarize the information in a recording (i.e. [16, 17, 22, 38, 40]), the Acoustic Complexity Index (ACI) [11, 41] was selected for this study, since it is an algorithm designed to measure the spectral complexity of soundscapes, which was recently used to track the dynamics of animal acoustic communities [15] or for comparison with vegetational parameters [19]. In the recent study of Towsey et al. [38], the ACI was found to be one of the best indicators of the biodiversity of a bird community among a list of 14 different acoustic indices, with weaknesses due to the sensitivity to wind gusts.

To analyze the collected acoustic data, a Fast Fourier Transform (FFT) of 512 points was applied, obtaining from every recorded minute a matrix made by 256 frequency bins of 86.13Hz and 5,167 temporal intervals of 0.012s. This matrix was used to calculate the Acoustic Complexity Index, with the following formula:

$$ACI = \frac{\sum_{k=1}^n |I_k - I_{k+1}|}{\sum_{k=1}^n I_k}$$

where $|I_k - I_{k+1}|$ is the absolute difference between two adjacent values of amplitude along a frequency bin, n represents the total number of temporal steps (k) contained in every interval of time in which the calculation is made (in this study, 1s). The sum of the results for all of the frequency bins and temporal intervals is then calculated. To avoid bias due to background ambient noise that is inevitably present in every recording (even if soft), we set an *a priori* filter on the power spectral density (SoundscapeMeter settings: Noise filter =3000 $\mu V^2/Hz$) operating on all the frequency bins, so that the ACI didn't apply to values under the selected threshold. This filter was appositely verified for the type of recording used in order to filter only background noise but not biophonies, and to increase the signal to noise ratio.

Five different recording schedules were then chosen to be simulated: (i) Schedule 5: recording one minute every five minutes; (ii) Schedule 10: one minute every 10 minutes; (iii) Schedule 20: one minute every 20 minutes; (iv) Schedule 30: one minute every 30 minutes; and (v) Schedule 60: one minute every 60 minutes.

These schedules were obtained by selecting the corresponding minutes of each simulated configuration from the continuous recording, thus simulating a recording routine whereby the recorder was not running continuously, but intermittently, at respectively one minute every five, 10, 20, 30 and 60 minutes. A mean of the ACI values was then calculated for each recording hour for both the continuous recordings and the simulated samplings in order to compare the different schedules with the original and complete sampling. These comparisons were conducted for both the temporal and spectral dimensions.

Rain and wind were found to be recognizable in abnormal ACI results [15, 38], especially at lower frequencies. Consequently, when the ACI values highlighted discrepancies with the normal acoustic behavior of the local community, the sound files were aurally checked to verify if the anomaly was due to some atypical biophony or anthrophony (such as insect buzzes on the microphone or transits of airplanes) or to the influence of stormy weather. This allowed us to generate a table of the adverse weather conditions during the recording days, which was filtered from the analyses in selected statistical tests.

Statistics

All of the statistical tests were performed using Statistica v.8.0. We used a non-parametric approach, since the variables did not present a normal distribution pattern, even after transformation of the data values. Non-parametric correlation analyses (Spearman's rho, $p < 0.01$) were conducted to investigate the relationship between the continuous data set and the simulated recording schedules.

To quantify the relative non-conformity of the sampling schedules with the real distribution of the ACI levels along the different hours of the day and the different frequency bins, the percent deviation [42] was calculated using the following formula:

$$(1) \quad \% \text{ deviation} = (\text{actual value} - \text{expected value}) / \text{expected value} \times 100$$

in which the 'actual value' was the ACI value calculated for a simulated configuration (expressed as an hourly mean) and the 'expected value' is the ACI resulting from the continuous recordings. Subsequently, the percent deviation was grouped by temporal slots (hours of the day) and frequency bins (1kHz-wide) to determine specifically where results from the simulated schedules differed from those of the continuous data set.

For both the correlation and percent deviation tests, only frequencies above 500Hz were processed, since under that threshold, the ACI could not well filter the background noise from the environment, which, if included, could have affected the final results. Because it was windy all year round at the Rupestrian fields, the cutoff frequency for the temporal analyses was 1,500Hz to avoid the eventual inclusion of soft wind noise.

Because rain and wind produce sounds and add complexity to soundscape analyses, we decided to treat the temporal and the frequency analyses differently in order to address the consequences of adverse weather and focus on the acoustic behavior of the animal community. The entire data set was used for the temporal analyses in order to include realistic limitations caused by weather conditions. On the other hand, the hours affected by rain and strong wind were left out of the data set when considering the differences in the spectral distribution of sounds (the frequency footprint, *sensu* [41], enabling us to reliably track the acoustic community dynamics and identify which frequencies were most affected when the sampling was less intense.

Results

The singing community

The ACI values varied greatly from the wet to the dry season in all our study areas, with a pronounced change between daytime and nighttime recordings. Figures 2, 3, and 4 show, respectively, the seasonal, temporal, and spectral acoustic complexity variations of the investigated environments based on the complete data set. A summary of their main soundscape features resulting from the ACI is set out in Table 1.

Table 1. Summary of the principal soundscape features of the three environments.

	Wet season (peaks of activity)		Dry season (peaks of activity)		Higher acoustic activity
	(kHz)	(hours)	(kHz)	(hours)	
Atlantic forest	4-6 kHz	18:00-01:00	1 kHz, 4 kHz	18:00-19:00	Wet season
	15-16 kHz	07:00-08:00	15-16 kHz		
Rupestrian fields	3-5 kHz	19:00-20:00	2-4 kHz	12:00-16:00	Wet season
	9-13 kHz		5-7 kHz		
Cerrado	5-6 kHz	18:00-03:00	3 kHz	07:00-18:00	Wet season
	10-17 kHz	12:00	5-6 kHz		
		15:00-17:00	10-14 kHz		

The acoustic complexity of Peti (Atlantic Forest) was especially pronounced during the wet season, with two peaks at approximately 4-6kHz and 15-16kHz. The ACI was especially high from 18:00 to 01:00, mainly due to insects and bats (aural check, NP's personal observation). An additional peak was observed at 07:00, probably due to bird choruses that gradually diminished over the course of the day. In the dry season, lower ACI levels occurred with peaks during dusk, around 18:00, and at 1, 4, and 15-16kHz.

The soundscape recorded in the Rupestrian fields of Rola Moça presented the lower values of ACI, with high variations over the course of the day and more constant levels during the hours of darkness (again, an aural check showed that this was due to insect activity). The wet season had a slightly higher acoustic complexity than the dry season (Fig. 2). Strong winds were present during the entire recording period, but by filtering out the windy hours from the data set, it was possible to register narrow peaks from 3 to 5kHz and from 9 to 13kHz in the wet season, which switched into peaks from 2 to 4kHz and 5 to 7kHz in the dry season.

The sampled areas in Cipó (the Cerrado) had the highest ACI values, especially in the wet season. In the wet season, most of the acoustic complexity was registered above 10kHz, with a narrow peak from 5 to 6kHz; the ACI presented high values preferentially during the night hours (18:00 to 03:00). In the dry season, a higher acoustic complexity was registered during daylight hours (07:00 to 18:00), mostly between the 10 and 14kHz frequency bands. Others peaks of ACI were found from 3 to 6kHz.

Statistical analyses of the sampling schemes

All of the correlations between the ACI values from the scheduled and continuous recordings were significant and positive (Appendix 1). An expected inverse relationship between time OFF and the value of the correlation was found for both the frequency and time analyses. The correlation coefficients were very high ($r > 0.90$; $p < 0.01$) for the more intense sampling period (Schedules 5 and 10) and fell with increasing OFF minutes, especially for Schedule 60. The Rupestrian field correlations generally had the lowest values. The frequency correlations were always found to be higher than the temporal correlations.

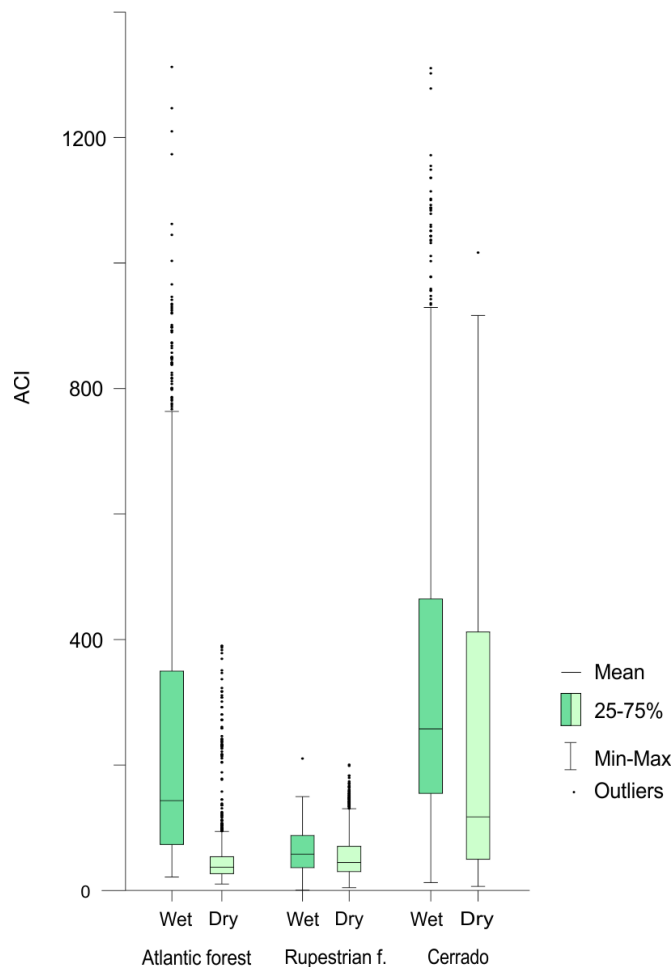


Fig. 2. Box-Whisker plot of the hourly means of the ACI values in the three environments.

The percent deviations were low for the intense sampling schedules and tended to increase when enlarging the OFF period (Appendix 2). As for the correlations, the temporal analyses tended to diverge away from the continuous recordings more strongly than the spectral analyses (Appendix 2). When categorized by hour of the day or 1kHz-frequency bands, interesting trends on the possible major losses of information of the subsampled recording schedules became clearly visible (Appendix 3 and 4). In particular, Schedule 5 assumed values that deviated by a maximum of 10%. Schedule 60 registered substantial deviations of 90% and 80% at specific hours of the day (Cerrado, wet and dry seasons, respectively), and deviations over 30% in the frequency analyses (Rupestrian fields and Cerrado, wet season).

In the wet season, both the Atlantic Forest and the Rupestrian fields seemed to experience a greater loss of information during daylight hours, while in the dry season the deviations were more evenly distributed. In the Cerrado, we found peaks in the deviations at 17-18:00 (both seasons) and 01:00 (wet season). The highest frequency bands registered null deviations in the Atlantic Forest (dry season) and Rupestrian fields (wet season). In the Cerrado during the dry season, low variations were found in the frequencies around 11-13kHz.

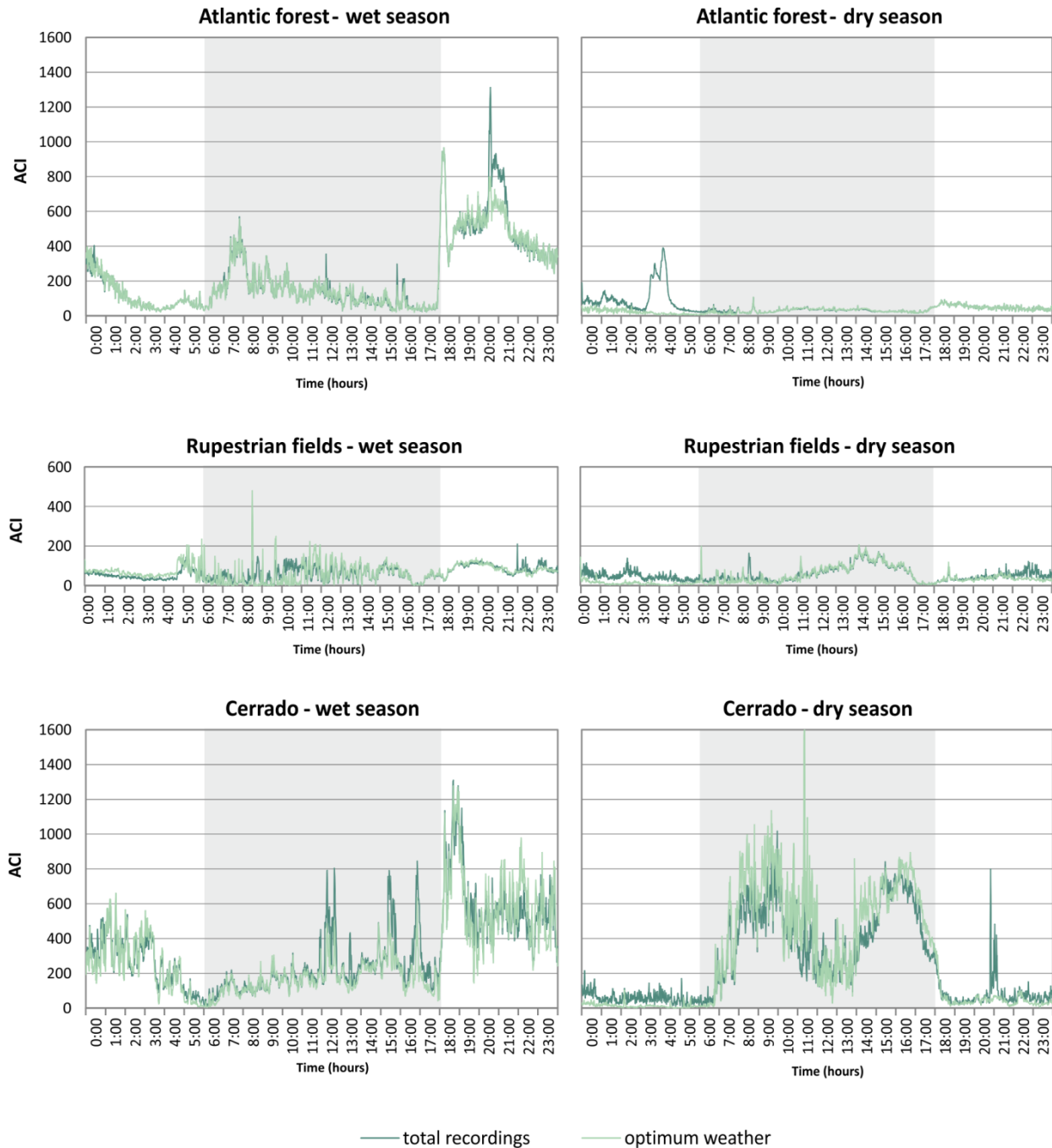


Fig. 3. Temporal trends of the acoustic complexity recorded in the three environments. Each graph represents the mean pattern resulting from sampling on six days at three recording points. The dark lines show the ACI trends when not deleting the files with adverse weather conditions; this highlights discrepancies in the hours of the day in which rain and wind mainly occurred. The grey highlight shows the period of the day comprised between the approximate times of dawn and dusk.

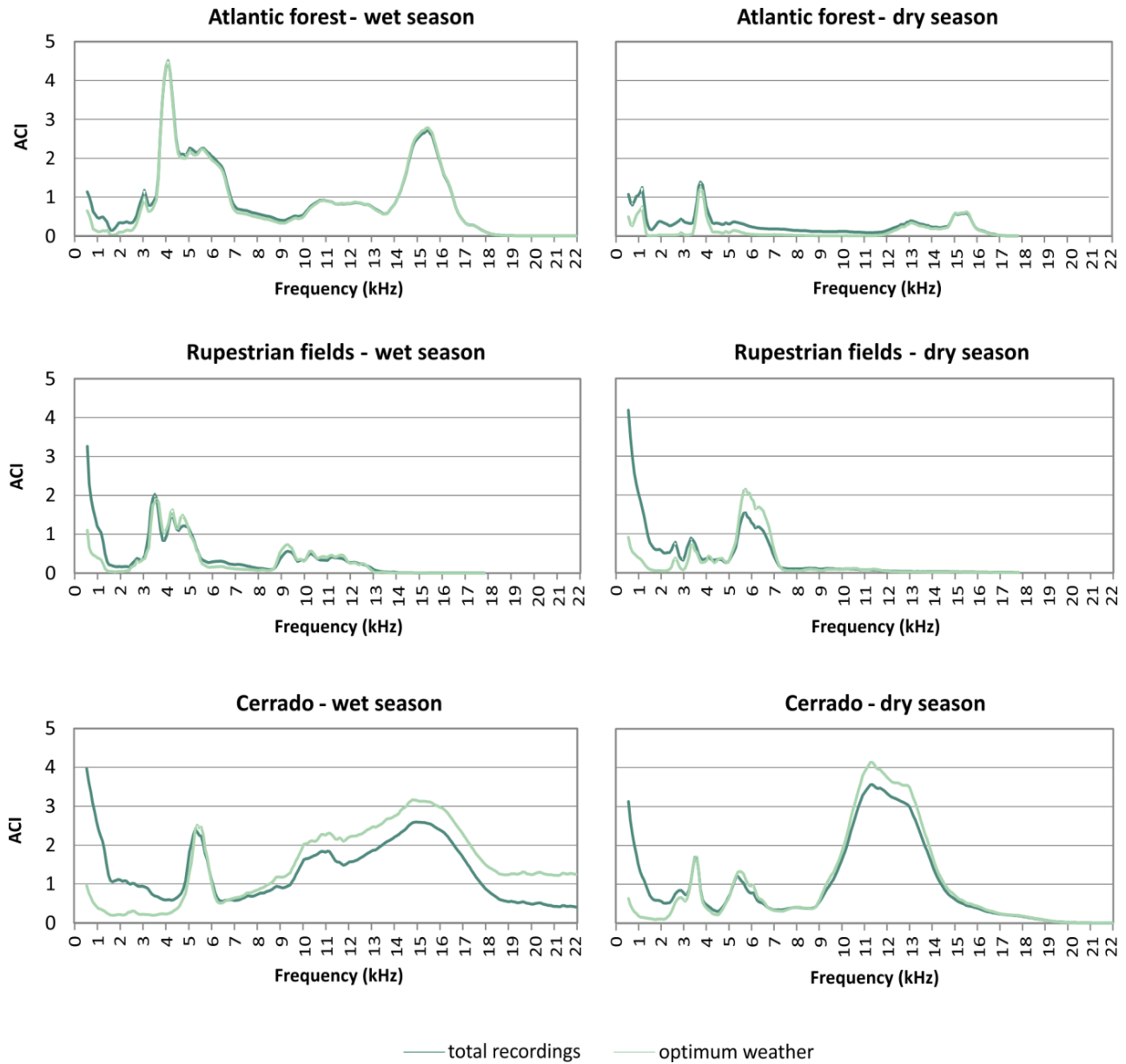


Fig. 4. Frequency distribution of the ACI in the three environments. The dark lines show the ACI trends when not deleting the files that present adverse weather conditions; this highlights discrepancies, especially at the lower frequencies in which the energy of the sounds produced by rain and wind are mainly comprised.

Discussion

Soundscape studies can be particularly useful for exploring fragile and endangered ecosystems that need special attention for their conservation [18]. Our results from simulating recording schedules provided information useful for suggesting the first guidelines for soundscape studies in three tropical areas considered as threatened environments. These suggestions are based upon our overall description of the main dynamics recorded in the three study areas, evaluation of the different sampling schedules as representative of the real acoustic dynamics, and the percentage of information lost when reducing the recording time.

Soundscape characterization

Temporal and spectral characteristics of the soundscape for each study area were unique and largely specific to the climate season. Generally we found a comparatively higher ACI in the wet season, which in Brazil coincides with the breeding season for most species [33, 43, 44], when anurans, birds, and insects produce sounds to achieve mating success. Acoustic complexity differences were clearly noticeable from the diverse trend across the temporal domain (Fig. 3), and by delineating habitat- and season-specific frequency footprints (Fig. 4) (*sensu* [41]) depending on the singing behavior of the emitters acting in each season and environment. Evidence of habitat type acoustic signatures was also found in temperate environments in four forest and two grassland habitat types in Northern Greece [45].

The lower acoustic complexity of the Rupestrian fields is probably related to their high altitude, which directly influences floristic structure and composition (fewer trees, open areas and strong winds). This leads to lower species diversity and, consequently, lower acoustic diversity (Figure 2).

General considerations on the different sampling schedules

All study areas were characterized by falling Spearman Rank correlation coefficients with increasing OFF minutes, showing that there was a gradual loss of correspondence with the real soundscape. This means that in these environments, it is difficult to provide a perfect picture of the acoustic dynamics of the community if the sampling becomes too sparse. It is therefore likely that there will be a loss of important data that could be essential for conservation issues.

This decreasing trend is shown by both temporal and spectral correlations, even if the correlation coefficients are always very high in the latter. We hypothesize that this is probably because the frequency bins have a lower degree of freedom than the temporal analyses, since the spectral emissions were strictly linked to the acoustical organs of indigenous species. Accordingly, animals cannot vary the spectral properties of their emissions, which over the entire day are likely to be registered by less intense sampling, but they can vary the moment and the length of a singing period. In other words, the presented temporal analyses depend on what was singing across all of the spectrum at a certain temporal interval, while the frequency analyses depend on what was singing in the 24 hours of one day in a fixed frequency band. The frequency footprint is thus less variable than the temporal trend across time.

The Rupestrian fields were the most critical environment, since the lowering of the sampled files corresponded to very low correlations with the continuous recordings. The main reason probably is the lower acoustic activity in the area (Fig. 2), which has a higher risk of not being recorded and therefore needs a greater sampling effort to be captured and measured.

The percent deviation analyses provide more detailed evidence about the loss of acoustic information. In general, lower percent temporal deviations were found where the sound emissions were more constant and prolonged in time, such as during the night in the wet season in the Atlantic Forest and Rupestrian fields. At these times, insects are the main protagonists of the acoustic performances, and tend to produce a longer sonic performance than other taxa (mammals, birds). This makes them more easily detectable in all of the sampling schedules, thus minimizing the percent deviations. In contrast, during the day, birds also sang abundantly, but were less constant in their acoustic emissions and more variable over time than the insects, meaning that they may or may not be detected by less intense sampling. This suggests the need for more cautionary sampling during specific hours of the day and a less intense effort at other times, when a greater constancy of sounds occurs.

The percent spectral deviation was found to be at a minimum where the frequency bins were unoccupied (or rarely occupied) by some species, such as in the Atlantic Forest (dry season) and Rupestrian fields (wet season). The narrow peaks visible on the lower frequencies all referred to insects, most likely crickets, while cicadas presented a broader frequency band. In the Cerrado (dry season), the reduction in variation from 10 to 15kHz is related to the continuous and abundant sound emissions of cicadas.

Which sampling routine is better?

The choice of the type of sampling will always depend on the focus of the investigation. Our results may help researchers to opt for the best sampling protocol according to their principal goals.

Our findings show that there are preferential recording schedules for each of the three investigated ecosystems. When the mean soundscape of the community over the six recording days shows a high and continuous presence of sounds, it may be preferable to use less dense recording schedules, since the acoustic information is going to be captured anyway and will be representative of the community. On the other hand, when the acoustic emissions are occasional or intermittent and impossible to predict, the sampling should be more intense to ensure a reliable representation of the soundscape.

Schedule 5 seemed to most reliably depict the soundscapes captured by continuous recordings in all of our study areas. This schedule, which is the most conservative, results in an 80% storage space and battery power reduction compared to the continuous sampling. Schedule 10 seems often to represent a good compromise, which will correspond to a 90% reduction from the continuous sampling, and to a 50% reduction of the energy and storage space occupied by Schedule 5.

Therefore, to create an effective reproduction of the soundscapes, it could be possible to design robust sampling for the Atlantic Forest from 04:00 to 17:00, such as Schedule 5 for the wet season, while a less dense sampling routine could be used from 18:00 to 03:00 without a major loss of information (Schedule 30). In the dry season, it could be enough to record one minute in every 10, or even every 20, although this risks losing some sounds at 7kHz and around 08:00.

For the Rupestrian fields in the wet season, a schedule similar to that for the Atlantic Forest wet season should be applied, the exception being the early morning hours when it is necessary to record one minute in every 20. In the dry season, Schedule 10 should be adopted, which would be a good compromise in both the temporal and spectral domains.

In the Cerrado, schedules 5 or 10 will provide reliable insights into the acoustic diversity of the community, both for the dry and wet seasons. Schedules 60 and 30 should be avoided, especially when recording at 18:00 (dusk).

Additional insights

Soundscape information can sometimes be misleading and interpreted incorrectly. Where the weather intervenes significantly in the soundscape of the environment, as in the Rupestrian fields or the Cerrado (wet season), sounds produced by the rain and wind mask and interrupt the soundscape of the community, meaning that weather condition is an additional variable to take into account, with all of its unpredictability. Moreover, Towsey et al. [38] found that ACI was responsive to wind gusts, and that it was not accurately representing the biological community in adverse meteorological conditions. Certainly, before conducting brief acoustic surveys, which rely on recording for only a few days, it is advisable to select the days that may be less demanding from this point of view. Nevertheless, in the case of long-term investigations (as in the case of fixed stations' detection of acoustic dynamics), a precautionary schedule should be adopted. In the Atlantic Forest in both the dry and wet seasons, we found a lower degree of bias due to sounds from adverse weather conditions. Moderate or strong wind was not noticed in this location, and the rain was easily detectable because of its natural broadband and dominance signal across the spectrogram. In contrast, the Rupestrian fields were always very windy, consequently having an influence on the distribution of sounds through time.

Despite this limitation in the present methodology and the relatively small number of studied days per season, we believe that our results can provide useful insights in how to approach the problem of choosing the correct sampling of the sounds of an ecosystem. Moreover, we trust that the three recording points randomly chosen in each area were so spaced to be independent and to represent the variability of those selected environments.

Nevertheless, we emphasize that it is not possible to apply the results of this study to all locations in all weather conditions. Soundscape dynamics vary enormously from one ecosystem to another, and they even tend to differ between two recording points with the same macroscopic vegetation features on the base of the therein established animal community. Thus, the analyses here proposed can be representative of just the three localities taken under investigation and cannot necessarily be applied to all ecosystems.

Clearly more work could be done with other acoustic indices besides the ACI. Adding further elaborations, including a number of other indices, could certainly improve our results and add more information to enhance understanding of the impact of a particular sampling strategy. This could lead to establishment of an internationally accepted sampling methodology.

As a final consideration, in the present study we tested different sampling schedules keeping the ON duration fixed to one minute, and varying the OFF period from four to 59 minutes. It would also be interesting to test whether the one minute resolution is the best setting for soundscape investigations, or whether a shorter/longer recording interval could be more cost-effective.

Implications for conservation

Sounds provide a valuable, indirect source of information with which to survey animal dynamics and diversity in particular regions of interest [3, 7]. The assessment of acoustic temporal and spectral changes offers a new way to interpret the dynamics of animal communities and understand spatio-temporal variations in community structure across space and time [8, 11, 22]. Given the urgency of climate change and the loss of

habitats, understanding normal levels of variation in acoustic complexity could be fundamental for conservation efforts, enabling managers to decide whether changes in acoustic dynamics warrant further investigation.

Herein, we have produced a starting point for research-guidelines to improve the efficiency of acoustic surveys using analytical methods, by suggesting the sampling effort needed for biologically robust investigations of animal communities in three tropical environments. This could be especially useful for wildlife managers whose choices are limited by economic and staff constrictions. If non-optimal sampling schedules were to be adopted, our results will help to identify the most critical points, both temporal and spectral, when the risk of the loss of information is highest.

Future research may focus on the sampling efforts required in temperate areas or in different tropical ecosystems. Additional insights could be provided by the use of other indices besides the ACI, or by testing variations in length of the ON period (here kept constant to one minute). Suggesting the ideal number of days needed to represent the acoustic community reliably in different seasons throughout the year would also be another important step toward the best protocol design for soundscape investigation.

These kinds of studies are particularly important at this early stage of soundscape ecology research, since this discipline is demonstrating its suitability to both interpret the state of health of environments and to monitor the anthropogenic challenges facing natural environments today.

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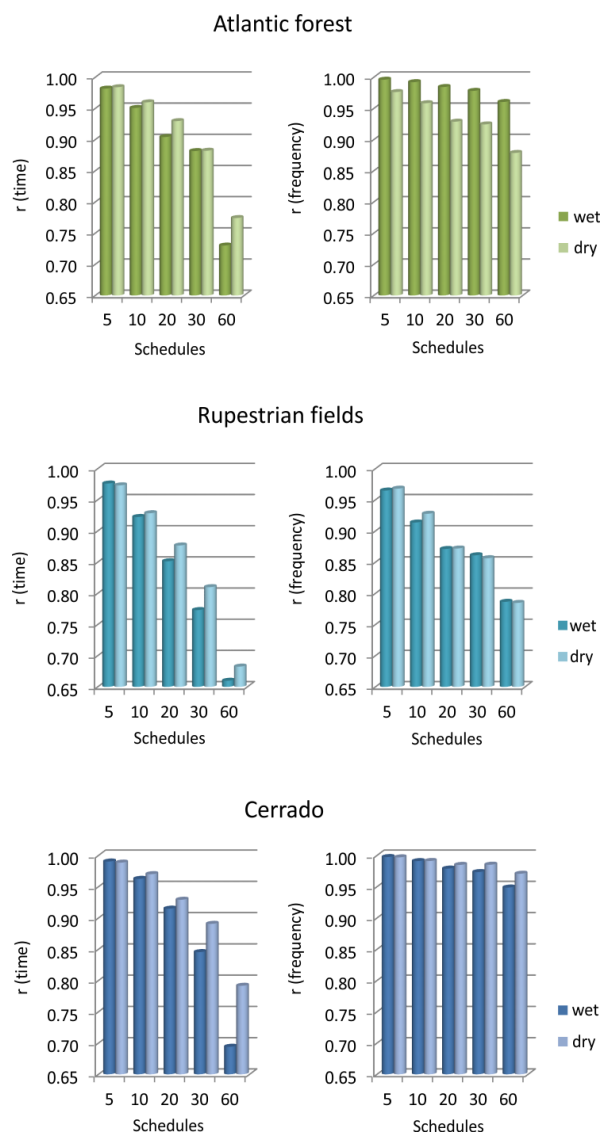
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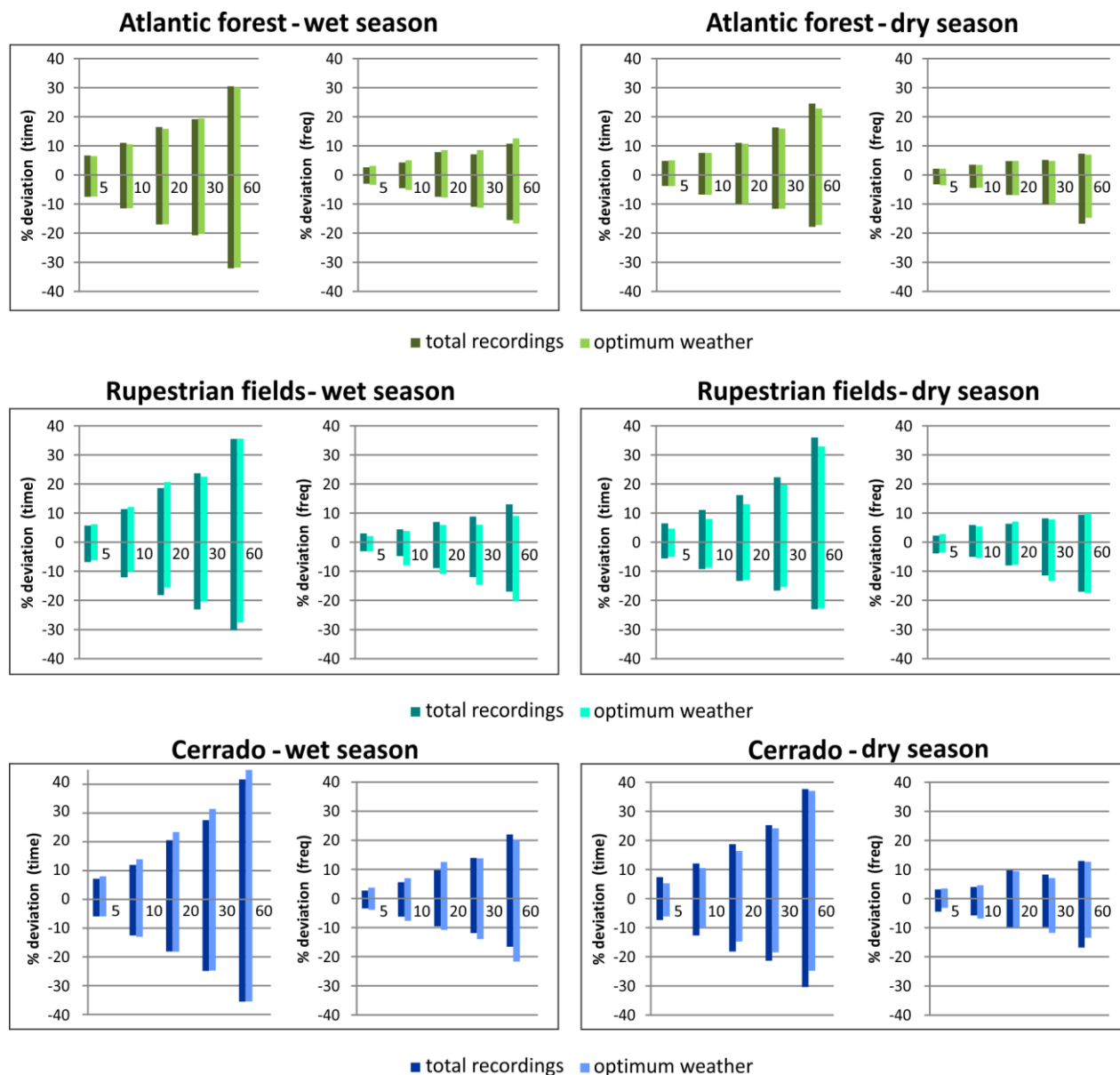
APPENDIX 1

Spearman Rank correlations of the ACI results according to the temporal and spectral comparisons of the different schedules ($p < 0.01$). The ACI results were grouped by hour, comparing the mean value registered each hour by the different recording schedules, or by the frequency bin (1kHz), comparing the mean value registered for every frequency band by the different recording schedules.



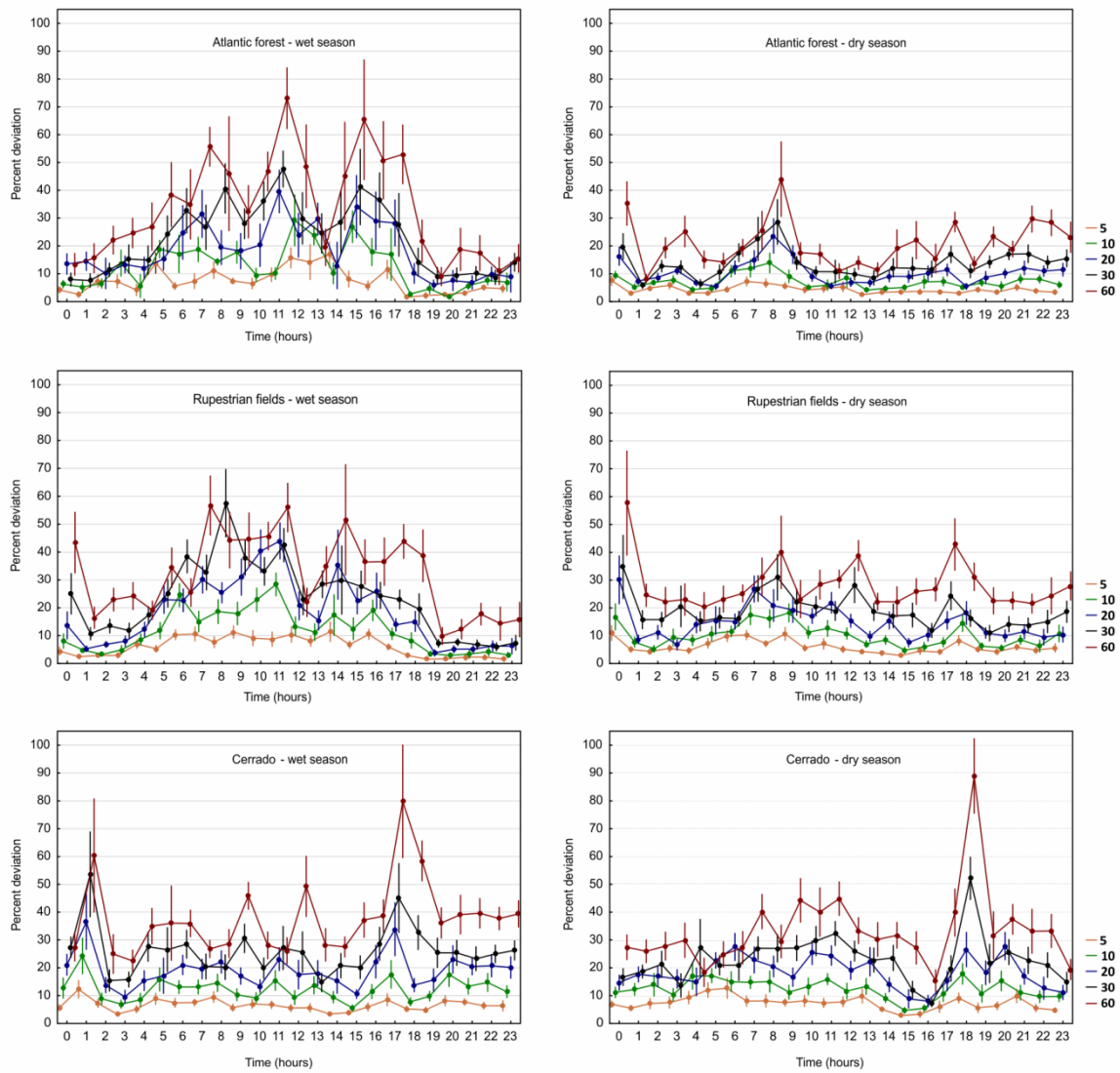
APPENDIX 2

Percent deviations of each subsampling category with respect to the continuous recording. The unfiltered data set and the recordings with the weather perturbations removed (i.e. optimum weather) are shown.



APPENDIX 3

Percent deviations of the five recording routines from continuous recordings aggregated by the time of the day (hours).



APPENDIX 4

Percent deviations of the five recording routines from continuous recordings aggregated by frequency (1kHz).

