

# What do insects, anurans, birds, and mammals have to say about soundscape indices in a tropical savanna

## Original paper

### Article history:

Received: 5 October 2017

Accepted: 3 January 2018

Published: 27 February 2018



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### Peer review:

Double blind

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### Keywords:

Acoustic Indices; zoophony; tropical savanna; sonotype richness; diel patterns; insects

### Citation:

Ferreira L. M., Oliveira E. G., Lopes L. C., Brito M. R., Baumgarten J., et al. (2018). What do insects, anurans, birds, and mammals have to say about soundscape indices in a tropical savanna. *Journal of Ecoacoustics*. 2: #PVH6YZ.  
<https://doi.org/10.22261/JEA.PVH6YZ>

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## Abstract

The application of acoustic indices is incipient and still needs validation before it can reliably characterize soundscapes and monitor rapidly disappearing hot-spot areas as the Brazilian tropical savanna (Cerrado). Here we investigate which of six acoustic indices better correlate with the 24 h zoophony richness of insects, anurans, birds, and mammals. We sampled one minute every 30 minutes for seven days on three sites in Serra da Canastra National Park (Minas Gerais state, Brazil) and extracted the sonotype richness and six indices based on recordings with a bandwidth of up to 48 kHz. The Acoustic Diversity, Evenness, Entropy, and Normalized Difference Soundscape indices followed the temporal trends of the sonotype richness of insects and anurans. The Acoustic Complexity (ACI) and Bioacoustic (BIO) indices did not correlated with sonotype richness. ACI and BIO were influenced by sonic abundance and geophony. We emphasize the need to include insects and anurans on soundscape and acoustic ecology analyses and to avoid bias on avian fauna alone. We also suggest that future studies explore measures of sonic abundance and acoustic niche occupation of sonotypes to complement measures of zoophony richness and better understand what each faunal group is telling us about indices.

## Introduction

A soundscape can be described as the sonic structure of a given landscape, accounting for signal composition, diversity, intensities and temporal patterns (Schafer, 1977; Pijanowski et al., 2011b). The soundscape is comprised of various sound sources, including biotic and abiotic components. The biotic component of the soundscape has been defined as Biophony (arguably Zoophony): the collective sounds produced by living organisms in nature. Geophony is comprised by sounds produced by geophysical elements, and Anthropophony comprises sounds of anthropogenic origins (Pijanowski et al., 2011a,b). Soundscape ecology aims to understand the structure and dynamics of ecological processes that are mediated or can be described by sounds. On the other hand, Ecoacoustics has been recently described as the discipline that includes soundscape ecology and other research that incorporate both sound and ecology (Sueur and Farina, 2015).

The application of acoustic methods to study ecology at the population and the community levels is increasing. Such methods deploy non-invasive technologies capable of generating large amounts of data on varying temporal and spatial scales (Van Parijs et al., 2009; Duarte et al., 2015; Stepanian et al., 2016; Leach et al., 2016). The use of autonomous recorders brings an additional advantage, since it is independent of the observer presence and enables long-term recordings, allowing for sampling in large temporal scales and at multiple simultaneous locations (Bardeli et al., 2010; Holmes et al., 2014). As such, acoustic variances can be compared among sites, accounting for landscape structure and heterogeneity.

Data generated through automatic recordings can be used to answer multiple questions but the large amount of data collected implies challenges for its management and analysis. In this context, different acoustic indices have been developed to aid the interpretation of these large data sets. The extraction of acoustic indices would be a good solution to those challenges, since they would be capable of summarizing the structure and distribution of the acoustic energy in a recording, in order to reflect contents of ecological interest (Towsey et al., 2014). Some of the existing indices are calculated in order to estimate the amplitude, equitability, richness, heterogeneity, and temporal patterns of a community or soundscape (Sueur et al., 2014).

However, the development of acoustic indices is still incipient and may suffer various biases (such as interference from different levels of background anthropic noise; Gasc et al., 2015). Further studies are needed to increase the reliability of acoustic indices as robust analytical tools for ecology applications (Sueur et al., 2014). *In situ* physical measurements and manual analyses of recordings are still frequently needed to verify and validate the information provided by them and to help improve the way they are extracted. Ecologically important phenomena, such as the impact of climate change on biodiversity, are occurring quickly and reliable indices capable of describing changes in landscapes due to these kinds of phenomena with an efficient processing time are in high demand (Krause and Farina, 2016). Biodiversity hot-spot areas are also disappearing fast and would benefit from a methodology capable of quickly assessing their large scale characteristics and trends to advise conservation actions while there is still time to yield positive results (Machado et al., 2017).

The relationship between acoustic indices and species richness has been studied in the Cerrado (Pieretti et al., 2015; Machado et al., 2017). However, all inferences were restricted to birds (Machado et al., 2017) or emphasized the avian contribution to the zoophony using a single index (Pieretti et al., 2015). These examples highlight the fact that soundscape ecology has been dominated by research focused on ornithology (Boelman et al., 2007; Farina et al., 2011; Depraetere et al., 2012; Pieretti et al., 2011; Kasten et al., 2012; Towsey et al., 2014; Tucker et al., 2014; Fuller et al., 2015; Gasc et al., 2017; Machado et al., 2017; Mammides et al., 2017). This bias is to be expected in the early steps of this emerging methodology, since birds are conspicuous, diverse, relatively easy to identify at the species level, diurnal and concentrated temporally during the dawn chorus, which makes visual validation of the recordings much simpler (Wimmer et al., 2013; Alquezar and Machado, 2015).

The need for integrating anuran, insect, and mammalian data to complement avian data into soundscape analyses, including the nocturnal biodiversity, has already been pointed out by some authors (Fuller et al., 2015). Broadening the range of sonic fauna in soundscape research would help

explain cases of poor association between some acoustic indices and bird diversity (Mammides et al., 2017) as well as drive the soundscape ecology field towards a more comprehensive characterization of the zoophony.

Here we aim to describe the 24 h temporal acoustic richness pattern of four different animal groups contributing energy to the soundscape (anurans, birds, insects and mammals) at three sites within a Cerrado protected area and to investigate which of six selected acoustic indices better corresponds to the zoophony patterns found.

We predict that the acoustic entropy (H; Sueur et al., 2008b) and the Acoustic Diversity Index (ADI; Villanueva-Rivera et al., 2011) will not be specific to any faunal group and correlate more strongly with total zoophony richness. We also predict that the Acoustic Evenness Index (AEI; Villanueva-Rivera et al., 2011) will be different in each of the three recording sites even if they have similar zoophony richness values, as the species composition may result in different acoustic niche partitioning. Signals are under selective pressure to be received, which can drive acoustic niche partitioning to avoid masking and competition by other sonic entities (Krause, 1987; Pijanowski et al., 2011b). The Normalized Difference Soundscape Index (NDSI; Kasten et al., 2012) should vary according to the anthropophony but some studies have found a relationship of this index with biophony (Fuller et al., 2015). Inasmuch, we have included this index predicting that NDSI will be sensitive to diurnal and nocturnal zoophony richness as found by Fuller et al. (2015). Finally, we predict that the Acoustic Complexity Index (ACI; Pieretti et al., 2011) and the Bioacoustic index (BIO; Boelman et al., 2007), which measure biophonic activity, will correlate with zoophony richness of the predominant groups.

## Materials and methods

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### Study area and recording sites

The Cerrado is a vast tropical savanna ecoregion in Brazil, and the only tropical savanna region acknowledged by Myers et al. (2000) as a biodiversity hot-spot. Cropland expansion is the main threat for this habitat, which has lost more than half of its original area (Françoso et al., 2015) since less than 2.2% of the area is protected (Klink and Machado, 2005). The Cerrado has over 12,000 species of vascular plants, of which 4,252 are endemic (Silva and Bates, 2002; Zappi et al., 2015). Phytophysiognomies in the Cerrado are predominantly savannas, but it also comprises habitats that range from open grassland to closed canopy forests, as well as gallery forests adjacent to perennial water courses (Oliveira-Filho and Ratter, 2002; Silva and Bates, 2002).

The recordings were made at Serra da Canastra National Park, located southwest of Minas Gerais state, Brazil (Figure 1). The climate is seasonal tropical with a cold, dry season from April to September and a hot, rainy season from October to March (Queirolo and Motta-Junior, 2007). Annual mean temperature is 22°C with 1,500 mm precipitation. Altitudes vary from 750 m to 1,490 m (MMA/IBAMA, 2005). Recording sites were situated between 1,230 m and 1,400 m of altitude.

Recordings were done from 22 to 28 November 2016. The park is open to public from 08:00 a.m. to 06:00 p.m., but received very few visitors on the recording days due to the beginning of the rainy season (MMA/IBAMA, 2005; personal observation of the absence of vehicle sounds on the recordings). Site geographical coordinates were: A -20.203790 -46.507400, B -20.261250 -46.411920, and C -20.224520 -46.557210 (decimal degrees, WGS 84 datum).

Site A (Figure 1) was situated within an open grassland area (*campo limpo*; Ribeiro and Walter, 1998) located 11.97 km from the park entrance. This site is not accessible to park visitors and is located 3 km away from the closest road. This was the site with the least anthropic influence. Site B was located 45 m from the main park road (dirt road) and 193 m from the park entrance, where the vegetation is comprised of tall grass, shrub and a few low trees (*campo sujo*; Ribeiro and Walter, 1998). This was the site with the highest anthropic influence, as vehicle traffic is common during the touristic season and urban and rural areas are closer than to the other sites. Additionally, there is a small stream alongside the road at site B. Site C was located 15.90 km from the park entrance and 45 m from a secondary dirt

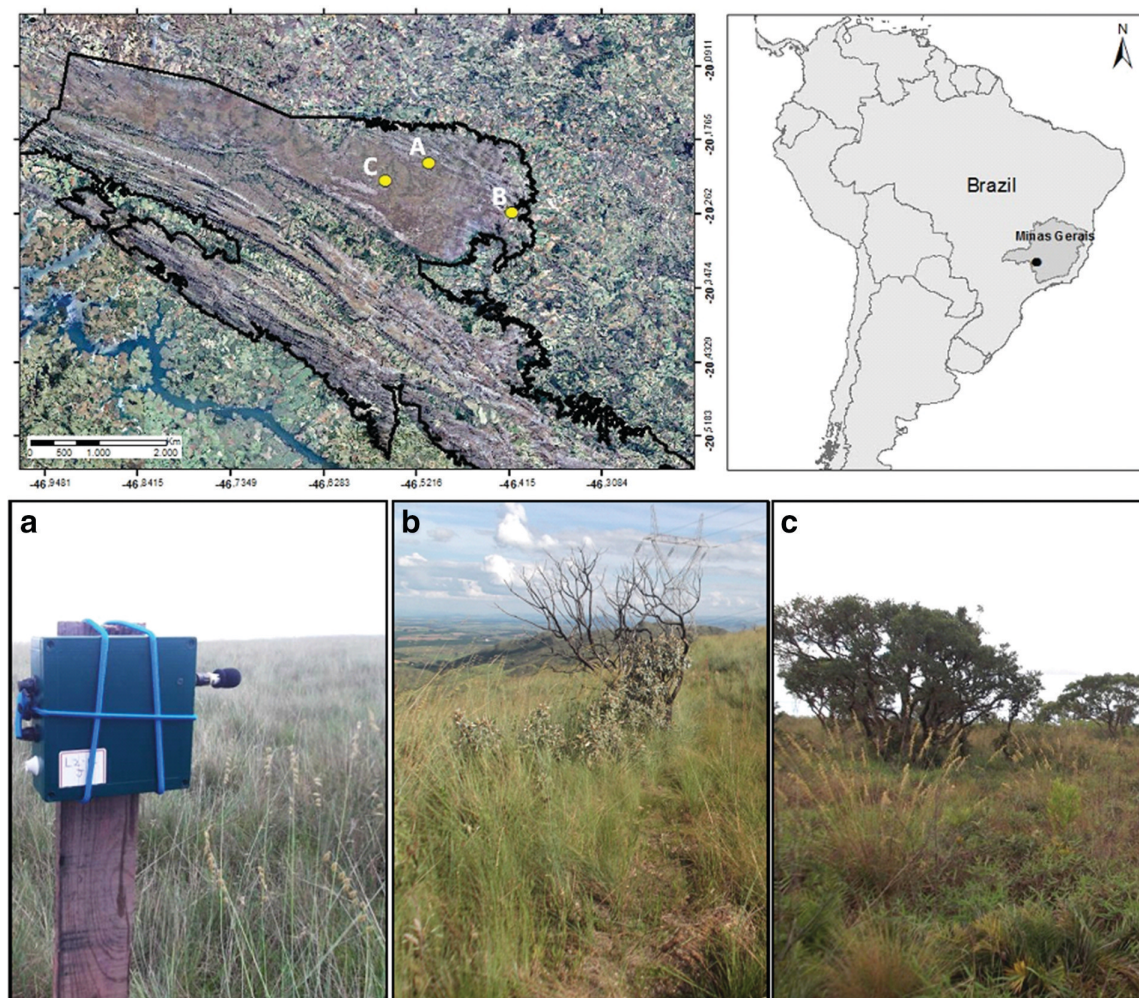


Figure 1. The three studied sites at Serra da Canastra National Park, Minas Gerais state, Brazil.

Site A photograph shows the autonomous recorder SongMeter SM2+ (Wildlife Acoustic, Inc., Concord, Massachusetts).

road, where vegetation was limited to shrubs and short trees (*cerrado ralo*; Ribeiro and Walter, 1998). This site is on a sightseeing touristic route but only some visitors go that far while visiting the park.

## Recordings

On each site (Figure 1) a single autonomous recorder (Song Meter SM2+; Wildlife Acoustics, Inc., Concord, Massachusetts) with an omnidirectional weatherproof microphone (SMX-II; Wildlife Acoustics, Inc.;  $-36 \pm 4$  dB sensitivity [0 dB = 1V/pa@1 kHz]; 20 Hz–20 kHz flat frequency response) was attached to 1.4 m high wooden stake. We used the Song Meter SM2+ Configuration Utility software version 3.2.4 (Wildlife Acoustics, Inc.) to program the equipment to record one minute each 30 minutes for seven days at a sampling rate of 96 kHz and 16 bit WAV coding form. This configuration yielded 336 one-minute files for each site. All 1,008 files were used in the analyses.

## Sonotype counting

We conducted a manual account of the zoophony by counting sonotypes, *i.e.*, the equivalent to “acoustic morphospecies” used by Aide et al. (2017). Those authors define an acoustic morphospecies (or sonotype) as a note or series of notes uniquely distinct that represents one type of vocalization of a species. If a series of different notes is uttered together, the entire set was classified as a single sonotype.

As noted by Aide et al. (2017), while each sonotype probably represents a single species of insects and anurans, in birds a one-to-one match is less likely since many species have multiple vocalizations or may mimic the calls of another species.

We emphasize that sonotype counting here is a measure of richness, not an abundance estimate: each sonotype was counted only once per minute file as a potential species, independent of the number of times it appears in each one-minute file. Nonetheless, one sonotype can, and normally do, reappear on the same file and in other files. Different from Aide et al. (2017) who measured sonotype richness per area, here we were interested in the 24h variation patterns and measured sonotype richness per minute file, i.e., sonotypes were counted again if they reappear in another file.

Sonotypes were identified according to the analysts auditory perception and spectrogram characteristics on Raven Pro 1.5 software (Cornell Lab of Ornithology, Ithaca, NY; [www.birds.cornell.edu/raven](http://www.birds.cornell.edu/raven)). This methodology was an adaptation of those from Riede (1996) and Gasc et al. (2013), being equivalent to that of Aide et al. (2017). Each one-minute file was viewed as a spectrogram which was generated in a range of window FFT sizes varying from 512 to 4,096 points (Hann window type) so that the resulting visual representation was most fitted to each type of sound. Each different sound was counted and classified into four categories: insect, anuran, bird or mammal (See Figure S1 and Table S2 on the supplementary material).

Manual sonotype counting was divided among four different analysts who each browsed and processed part of the data. Three analysts were responsible for files from five non consecutive days (240 files) and one was responsible for files from six non consecutive days (288 files). Each day was composed of 48 sequential one-minute files (one every half hour) in each of the three sites (a total of 21 sampled days). Distribution among analysts was done ensuring that no single person was responsible for an entire site or the same day at different sites to avoid observer bias. All analysts had experience in bioacoustics and in the use of Raven software but were not specialists in any of the particular animal groups cited. When analysts did not reach a consensus on which category sonotypes belonged to, those sonotypes were sent to specialists for identification.

## Acoustic indices

Six acoustic indices (ADI, AEI, H, NDSI, ACI and BIO) were calculated on R statistical program (R Development Core Team, 2014) with the function “multiple\_sounds” from the packages “soundecology package” (Villanueva-Rivera and Pijanowski, 2016) and “seewave-R” (Sueur et al., 2008a). Those indices were selected to compare our results with other papers that investigate the diel patterns of acoustic indices (Villanueva-Rivera et al., 2011; Fuller et al., 2015).

ADI calculates the Shannon diversity index considering each frequency band as a different category, i.e., species, and the proportion of an occurring sound in each band as the frequency, i.e., abundance (Villanueva-Rivera et al., 2011). The greater the number of active bands and the more even the proportion of their activity, the more diverse the acoustic community. AEI also uses the amount of activity in each frequency bin but instead, applies the Gini coefficient to measure how even the occupancy distribution is (Villanueva-Rivera et al., 2011). The closer the index is to 1 the less even the acoustic community is. H has a similar concept to that of the ADI, also using Shannon's index in a more complex calculation that results from the product of a time diversity index and a frequency diversity index based on amplitude envelopes (Sueur et al., 2008b). NDSI was designed to estimate the proportion of anthropogenic disturbance in a soundscape (Kasten et al., 2012). It does so by comparing the greater biophony bin spectral power area ( $\alpha$ ) with the spectral power area of a predefined anthropophony bin ( $\beta$ ) using the formula  $(\alpha - \beta)/(\alpha + \beta)$ . Positive values correspond to the prevalence of biophony whereas negative values result from high anthropophony levels. ACI was designed to measure biophony activity independent of anthropophony based on the assumption that biotic sounds vary in intensities while human related noise show constant intensities (Pieretti et al., 2011). BIO is also an abundance index originally proposed to measure relative avian song abundance by calculating the area under the normalized power spectrum that is greater than the minimum intensity of the curve (Boelman et al., 2007).

For ADI and AEI, the maximum frequency was set to 48,000 Hz and the freq\_step was set to 1,000 Hz. The ACI and BIO were calculated excluding the first 300 Hz, which contains mainly wind noise (Geophony). For the NDSI we considered the 100–300 Hz band as containing anthropophony and the 300–43,000 Hz band containing biophony (zoophony). The inferior threshold for ACI, BIO and NDSI is lower than used in most papers. Nonetheless, we chose this threshold to avoid excluding from the biophony mammal and anuran sounds previously observed in this frequency band during manual sonotype counting. All other settings were left as the default values (Villanueva-Rivera and Pijanowski, 2016).

Due to equipment failure some of files are less than 60 seconds long (min. = 47 s). The ACI is a temporal cumulative index (Pieretti et al., 2011) and thus would be influenced by different durations of samples. To balance this and make ACI indices comparable, we divided the ACI values by the duration (in seconds) of the files, as recommended in the R “soundecology package” manual (Villanueva-Rivera and Pijanowski, 2016). The balanced values may be very different from other studies and are referred here as ACI/s.

## Statistical analyses

Sonotype richness variation along the 24 hours was plotted using the mean value (N=7) calculated from counts of different sonotypes within each half hour (48 points) of 7 different days. The same was done for the acoustic indices, resulting in a 24 h temporal series including all data. Additionally, a 24 h series was calculated for each of the three sites separately. We chose to work with 24 h means instead of a continuous temporal series from the 7 consecutive days of recording to reveal diel zoophonic patterns for sonotype richness and acoustic indices. Those patterns better represent site characteristics than isolated days and can be an effective way of describing local soundscape structure and discriminating among sites (Villanueva-Rivera et al., 2011; Gasc et al., 2013; Fuller et al., 2015).

Kolmogorov-Smirnov tests indicated that our sample did not have a normal distribution. The 24 h mean series of indices and manual sonotype counts were compared with Spearman’s correlation and a regression analysis was conducted for ADI. To test differences among sites we averaged the variables in two subsets according to local sunrise and sunset (day: 05:30 a.m. to 06:00 p.m.; night: 06:30 p.m. to 05:00 a.m.). Manual sonotype counts and acoustic indices were then compared with a Kruskal-Wallis test and subsequent Mann-Whitney post hoc Bonferroni corrected tests (15 comparisons; final significance level 0.0033). All statistical analyses were conducted in SPSS Statistic 17.0 (SPSS, Inc. 2011. Chicago, IL, USA).

Minimum distance between sites was 5 km (Site A to C). This distance was considered sufficient for sites to be independent in the time window used (seven days). Some mammals, as maned wolves, may travel such distances daily, but we registered this species only once in this study. Accounts of other mammals included only bats. Besides, overall mammal count was low enough to have negligible effects on site independency.

## Additional tests

We compared our results controlling for some potential sources of variation by comparing datasets. To test the influence of equipment interference noise (all frequencies, 1–4 s; see Figure S1), we excluded the file parts containing noise events with Raven Pro 1.5 in site C, and recalculated the indices for this site. To test the influence of the rain we excluded the first two days (in which there was rain almost all the time, especially on site C) and recalculated the 24 h mean series and correlations.

Additionally, we calculated NDSI using 1–2 kHz as anthropophony and 2–10 kHz as biophony to compare our results with those from published studies, since we used very different frequency bins that better suited our data for the calculation of NDSI.

## Results

### Diel patterns

Sonotype counting (Figure 2) revealed a marked diel pattern of higher diurnal acoustic richness of birds and inversely, a higher nocturnal acoustic richness of insects and anurans. Insect and anuran sonotype richness was higher in the first half of the night. Mammals had a very low sonotype count and were also concentrated at night. When observing the total sonotype richness pattern for the Cerrado, a dawn and dusk chorus is clearly perceptible, although the dusk chorus is more influenced by a rise in the number of insect rather than bird sonotypes. Maximum sonotype value in a single file was 15, minimum 0 and average 4.35. Insects had the higher values (mean 2.11/file; min. 0; max. 12), followed by birds (mean 1.74/file; min. 0; max. 10), anurans (mean 0.432/file; min. 0; max. 5) and then mammals (mean 0.07/file; min. 0; max. 3). A list of all sonotypes can be found in the Table S2.

ACI/s and BIO (Figure 2) reached higher values in the first half of the night, indicating an increase in activity in this period, but otherwise had a very heterogeneous pattern. ADI, H, and NDSI showed

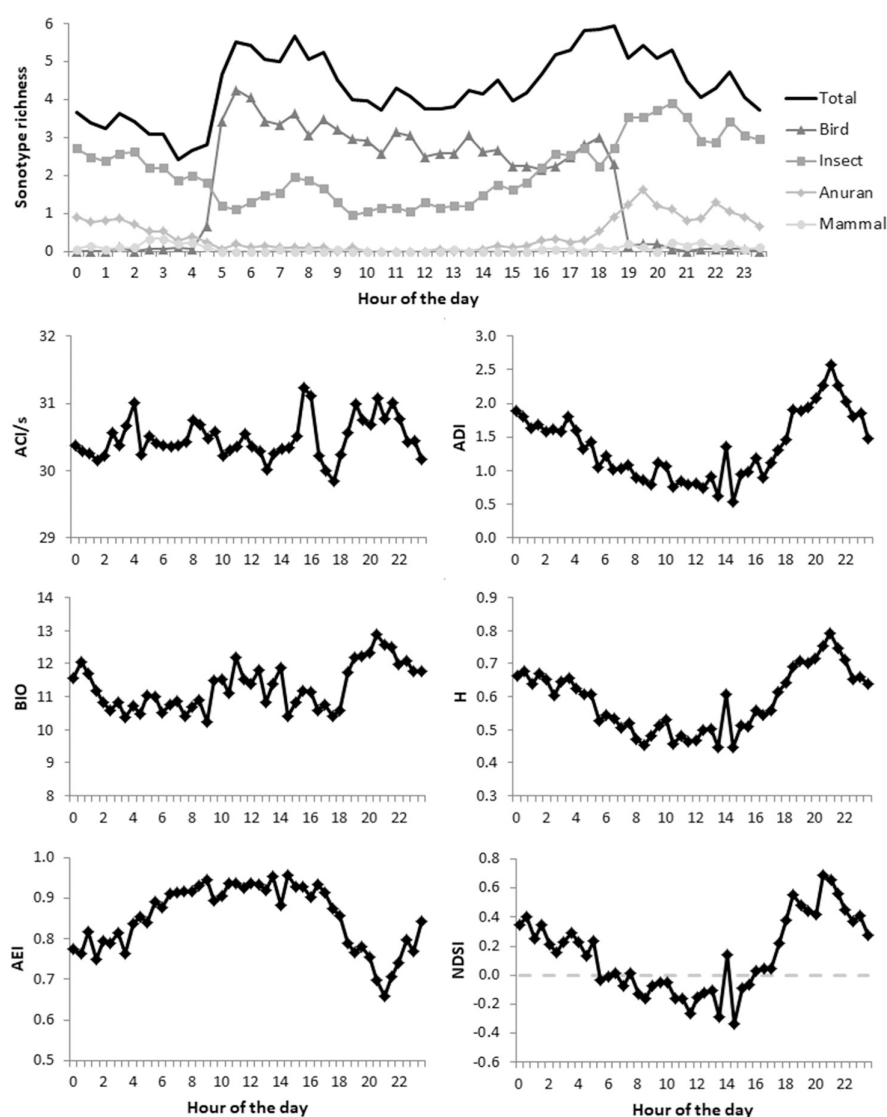


Figure 2. Temporal distribution of the seven day average of sonotype richness (top) and acoustic indices extracted from recordings in three different sites at Serra da Canastra National Park, Minas Gerais state, Brazil.

very similar patterns, a “U” curve with low diurnal diversity and high nocturnal diversity. AEI showed an inverse pattern from those three, indicating high heterogeneity during the day and a higher evenness at night.

### Correlation among sonotype richness and indices

Spearman’s rho correlation coefficients (Table 1) among sonotype richness and acoustic indices for all sites were highly variable (from |0.057| to |0.824|) and the best correlated index for each animal group also varied. We considered correlation coefficients lower than 0.5 to be weak, between 0.5 and 0.7 moderate, and higher than 0.7 as strong.

None of the indices explored here achieved a high correlation coefficient with the total sonotype count which included all three sites (max. = 0.309 for ADI). Anurans had the higher absolute mean correlation coefficient across indices (|0.561|), followed by insects (|0.476|), while total sonotype richness had the lowest (|0.209|). NDSI had the highest absolute mean correlation coefficient across groups (|0.530|), while ACI/s and BIO values did not correlate with sonotype richness of any animal group ( $p > 0.01$ ).

Contrary to our predictions, ACI and BIO did not correlate well with avian sonotype richness nor with any other group. Also contrary to our predictions, H and ADI correlated more strongly to specific animal groups than with the total sonotype richness. H and ADI correlated strongly with anurans, moderately with insects and weakly with mammals and birds. Nocturnal groups (anurans, insects and mammals) correlated positively with H and ADI while birds correlated negatively. NDSI had a similar pattern and correlated with the nocturnal vocally active groups as we predicted. AEI presented an inverted pattern (almost the mirror image of H and ADI), correlating positively with birds and negatively with nocturnal groups of animals.

Correlations among ACI, H, NDSI and AEI were very high (Table S1; min.  $\rho_{\text{NDSI} \times \text{AEI}} = -0.854$ , max.  $\rho_{\text{NDSI} \times \text{H}} = 0.959$ , absolute mean = |0.917|). ACI/s and BIO did not correlate with each other nor with the other indices ( $p > 0.01$ ) with one exception: H ( $\rho_{\text{BIO} \times \text{H}} = -0.218$ ,  $p < 0.01$ ).

### Differences among sites

When comparing correlations between sonotype richness and indices among the different sites separately (Table 1), a few sonotype groups correlated with ACI/s and BIO, but the results were inconsistent, the same index having positive coefficients in one site and negative in another for the same animal group (for example, birds).

Correlations had in general higher coefficients values on site C than in the other sites (Figure 3). In site C, BIO correlated strongly with insect and anuran sonotype richness ( $\rho = 0.780$  and  $0.838$  respectively) and H showed the overall highest correlation with anurans ( $\rho = 0.893$ ).

Total sonotype richness from site A correlated negatively (weak/moderate) with ADI, H, and NDSI, but in site C the correlation was positive (moderate/strong). In site B, total sonotype richness did not correlate with those indices. Bird sonotype richness correlated negatively (mean  $\rho = -0.640$ ) and anuran sonotype richness positively (mean  $\rho = 0.751$ ) with ADI, H, and NDSI in all three sites. Insect sonotype richness in site A did not correlate with ADI, H and NDSI ( $p > 0.01$ ). Correlation of these later indices with insect sonotype richness in site B was weak (mean  $\rho = 0.437$ ;  $p < 0.01$ ), and strong in site C (mean  $\rho = 0.873$ ;  $p < 0.01$ ). Site C was the only site that ADI, H and NDSI indices did not correlate with mammal sonotype richness ( $p > 0.01$ ). AEI always followed an inverse correlation direction than those of ADI, H, and NDSI.

To further investigate the relationship of indices and sonotype diversity, we chose the ADI to perform model selection for regression analyses (Figure 4; see Figure S2 on the supplemental Material). The linear model had the best fit to our data. Considering all sites, ADI was the index that best explained anuran sonotype variance ( $R^2 = 0.550$ ). Additionally, ADI explained over 84% of insect sonotype variance in site C ( $R^2 = 0.843$ ).

Table 1. Spearman rho correlation coefficients showing the relationship among the seven day average of sonotype richness and acoustic indices.

| All sites |         |                      |                       |                       |                       |                       |
|-----------|---------|----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
|           | ACI/s   | BIO                  | ADI                   | H                     | NDSI                  | AEI                   |
| Total     | 0.081   | -0.169               | 0.309*                | 0.267*                | 0.265*                | -0.165                |
| Bird      | -0.107  | -0.129               | -0.442*               | -0.449*               | -0.488*               | <b>0.520*</b>         |
| Insect    | 0.167   | -0.053               | <b>0.673*</b>         | <b>0.697*</b>         | <b>0.673*</b>         | -0.590*               |
| Anuran    | 0.053   | -0.165               | <u><b>0.773*</b></u>  | <u><b>0.824*</b></u>  | <u><b>0.819*</b></u>  | <u><b>-0.731*</b></u> |
| Mammal    | -0.057  | -0.080               | 0.365*                | 0.379*                | 0.407*                | -0.397*               |
| Site A    |         |                      |                       |                       |                       |                       |
|           | ACI/s   | BIO                  | ADI                   | H                     | NDSI                  | AEI                   |
| Total     | 0.217   | 0.433*               | -0.448*               | -0.499*               | -0.500*               | 0.478*                |
| Bird      | 0.348   | 0.492*               | -0.610*               | <u><b>-0.709*</b></u> | <u><b>-0.717*</b></u> | <b>0.501*</b>         |
| Insect    | -0.273  | 0.026                | 0.104                 | 0.274                 | 0.267                 | 0.107                 |
| Anuran    | -0.384* | -0.544*              | 0.605*                | <u><b>0.725*</b></u>  | <u><b>0.755*</b></u>  | -0.446*               |
| Mammal    | -0.066  | -0.347               | 0.583*                | 0.560*                | 0.625*                | -0.496*               |
| Site B    |         |                      |                       |                       |                       |                       |
|           | ACI/s   | BIO                  | ADI                   | H                     | NDSI                  | AEI                   |
| Total     | 0.178   | 0.488*               | -0.283                | -0.348                | -0.181                | 0.340                 |
| Bird      | -0.146  | <b>0.630*</b>        | <u><b>-0.715*</b></u> | <u><b>-0.731*</b></u> | -0.639*               | <u><b>0.756*</b></u>  |
| Insect    | 0.356   | -0.285               | 0.439*                | 0.413*                | 0.460*                | -0.429*               |
| Anuran    | -0.004  | -0.398*              | 0.683*                | 0.684*                | 0.652*                | <u><b>-0.774*</b></u> |
| Mammal    | -0.173  | -0.467*              | 0.574*                | 0.603*                | 0.510*                | -0.587*               |
| Site C    |         |                      |                       |                       |                       |                       |
|           | ACI/s   | BIO                  | ADI                   | H                     | NDSI                  | AEI                   |
| Total     | 0.228   | 0.465*               | 0.639*                | 0.678*                | <u><b>0.713*</b></u>  | -0.541*               |
| Bird      | -0.459* | -0.695*              | -0.575*               | -0.557*               | -0.511*               | 0.596*                |
| Insect    | 0.447*  | <u><b>0.780*</b></u> | <u><b>0.855*</b></u>  | <u><b>0.890*</b></u>  | <u><b>0.875*</b></u>  | <u><b>-0.792*</b></u> |
| Anuran    | 0.604*  | <u><b>0.838*</b></u> | <u><b>0.879*</b></u>  | <u><b>0.893*</b></u>  | <u><b>0.885*</b></u>  | <u><b>-0.822*</b></u> |
| Mammal    | 0.105   | 0.085                | 0.232                 | 0.225                 | 0.231                 | -0.205                |

Coefficients are shown for all sites combined (top) and for each three individual site at Serra da Canastra Brazilian National Park, Minas Gerais state, Brazil. \* Indicates significant correlations ( $p < 0.01$ ). Correlations in which coefficients are in bold are considered moderate ( $> 0.5$ ) and those with underlined coefficient values are considered strong ( $> 0.7$ ).

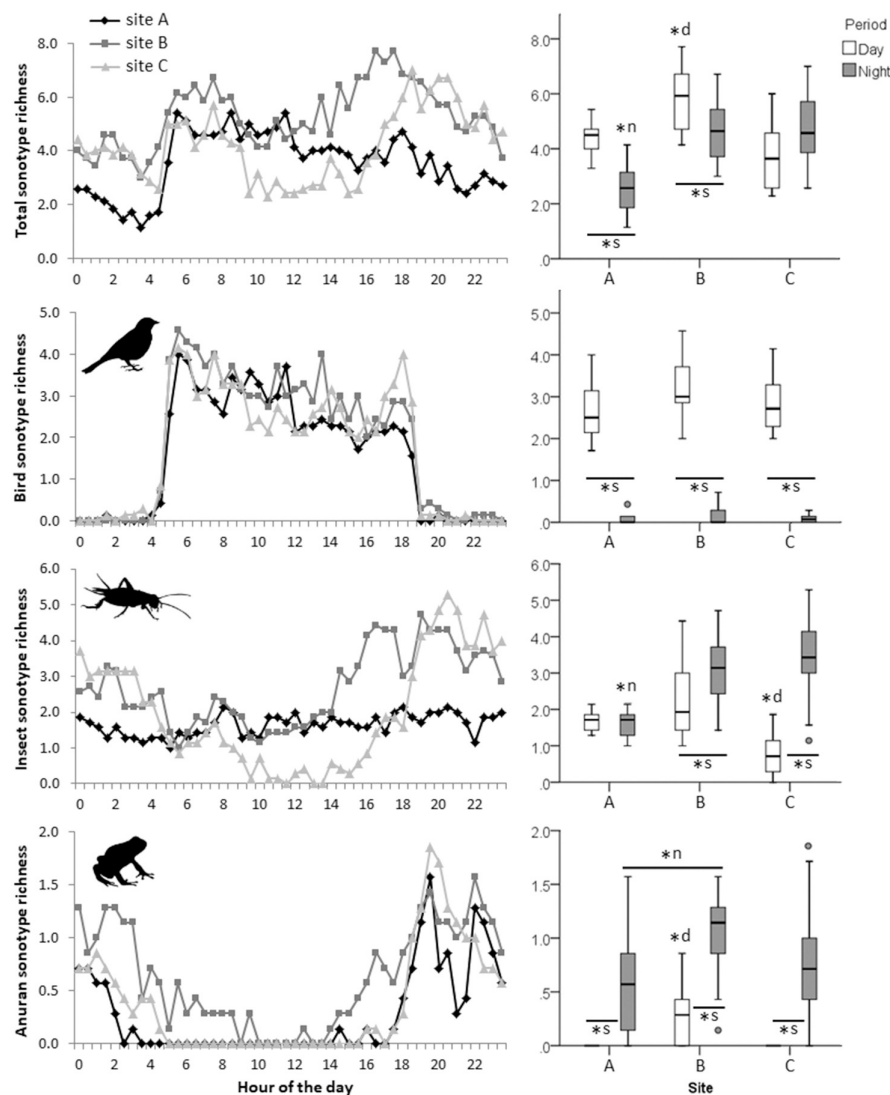
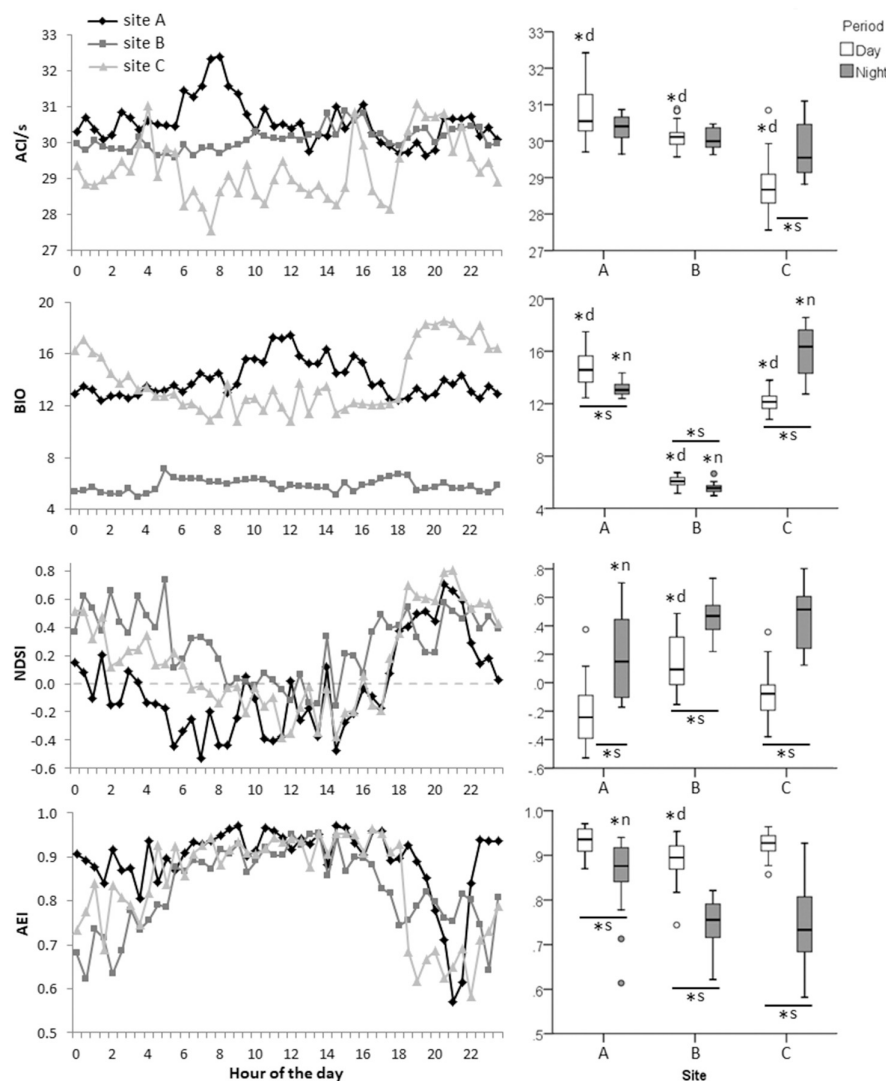


Figure 3. Sonotype richness in three different sites at Serra da Canastra Brazilian National Park, Minas Gerais state, Brazil.

Graphs on the left show the temporal distribution of the seven day average of sonotype richness. Boxplots on right show comparisons among sites and between day and night (first and third quartiles and median values are shown). Vertical bars are 95% confidence intervals. Circles are outliers. \*s indicates a significant difference between day and night in the same site. \*d indicates significant differences among sites during the day. \*n indicates significant differences among sites at night.

Kruskal-Wallis tests revealed significant differences in sonotype richness and indices among recording sites, both day and night (all tests resulted in  $p < 0.001$ ,  $df = 5$ ,  $\{N_{\text{day}} = 26; N_{\text{night}} = 22\} \times 3$  sites; see tests details in supplementary Table S3). Total sonotype richness (Figures 3 and S3) during the day was higher in site B than in the other two sites and during the night lower in site A than in the other two sites. For sites A and B, diurnal sonotype richness was higher than at night. This last result can be explained by a more pronounced drop in the diel pattern of sonotype richness in the middle of the day in site C than in the other two sites. Site C showed a more intense bird dusk chorus when compared to the other two sites. However, day and night bird sonotype means were equal for all sites.

Indices variation among sites is shown in Figure 4. ACI/s had an increase around 8:00 AM in site A, while in site B, ACI/s showed relatively constant values across the 24 h, and in site C, ACI/s had overall lower values during the day. Diurnal mean values followed a gradient from highest in site A to lowest in site C, while nocturnal mean values were equal across sites. BIO index for site B was higher in



**Figure 4.** Acoustic indices in three different sites at Serra da Canastra Brazilian National Park, Minas Gerais state, Brazil.

Graphs on the left show the temporal distribution of the seven day average of acoustic indices. Boxplots on right show comparisons among sites and between day and night (first and third quartiles and median values are shown). Vertical bars are 95% confidence intervals. Circles are outliers. \*s indicates a significant difference between day and night in the same site. \*d indicates significant differences among sites during the day. \*n indicates significant differences among sites at night.

the day than at night, but both were lower compared to the other two sites. Site A had higher day values while site C had higher night values. The ADI, H, NDSI and AEI (inversely) all followed almost identical patterns (Figure 4 shows only NDSI and AEI, see Figure S3 for other indices), being lower during the day and higher at night in all sites.

### Additional tests

The exclusion of noise interference had a very small impact on indices and no impact at all in 24 h patterns (data not shown). The biggest changes occurred in calculations of BIO (0.75 change in value) and ADI (0.2 change in value), but even those changes were considered small enough not to impact our results. Therefore, here we only show the results from the analyses of the original files, noise included.

Exclusion of the rainy days smoothed the diel patterns of ACI/s in site C, which made the variation in its values clearer: a marked increase between 20 h and 21 h 30 and a decrease during the day (data not shown). ACI/s and BIO correlation coefficients in site C increased for bird and total sonotype richness and in the other two sites increased for insect and total sonotype richness (data not shown). As significant differences occurred only in the abundance indices and as we did not account for abundance, comparisons were not possible, therefore we choose to leave those results in the original form as well.

NDSI calculated with commonly used frequency bins (1–2 kHz anthropophony/2–10 kHz biophony) still resulted in negative diurnal values and positive nocturnal values, with even greater differences between day and night (data not shown). The values were more similar between sites, with only site B presenting higher diurnal values than site C. Similarly to the other additional test we decided to consider the analyses bins that best fit our data.

## Discussion

As noted before, we aimed to describe variation in 24 h zoophony patterns and therefore, measured sonotype richness per minute file (*i.e.*, the same sonotype would be counted more than once along the day if it reappeared in different one-minute files). Aide et al. (2017) had a different approach because those authors were interested in comparing locations and measured total sonotype richness of each area (*i.e.*, the same sonotype was counted only once per location independent of the number of files it appeared).

Considering the data we used we did not find support for our prediction that ADI and H would follow the total zoophony richness trend (which included all four animal groups). Instead, ADI and H followed the temporal trends of the sonotype richness of insects and anurans, which was also the case for the other diversity indices (NDSI and AEI). This emphasizes the need to include anurans and insects on soundscape analyses and avoid to bias on avian fauna alone. NDSI was not sensitive to diurnal zoophony nor did AEI point differences between sites or periods with the same zoophony richness (Figures 3 and 4).

Also contrary to our predictions, in general, the indices of biophonic activity (ACI/s and BIO) did not correlate with the diel sonotype richness pattern of the most prevalent groups (birds and insects). In fact, ACI/s and BIO did not follow any animal group temporal trend, nor the total sonotype richness which included sonotype counts of all groups combined. Those indices (especially BIO) discriminated sites well because they take into account the abundance of biophony, which also resulted in differences of ACI/s and BIO during periods with the same richness (Figure 4).

The diel pattern of the diversity indices tested here (ADI, H and NDSI) correlated with the sonotype richness of specific taxonomic groups, positively for nocturnal sonic groups, and negatively for diurnal sonic groups (inversely for AEI). The correlations were stronger for anurans followed by insects, reinforcing the notion that these are the animal groups that have more to say about soundscape indices!

The sampled period coincides with the insect abundance period for the Cerrado (Silva et al., 2011), which might have biased our results. Insect stridulation had a greater influence on indices than anuran calls because they were more pervasive in all files. Anurans appeared in only 30.3% of files, their chorus rarely formed a continuous band on spectrograms, and anuran sonotypes occupied a narrow band (all below 3.8 kHz, see Table S2). By contrast, insects were present in 82.5% of files, their chorus had a high duty cycle and formed continuous bands at a large temporal scale. Insect sonotypes were found up to 48 kHz (although most concentrated between 2.5–7 kHz). A recent study counting sonotypes (acoustic morphospecies) also found that insect richness was the main driver of acoustic space use (Aide et al., 2017).

Higher correlation coefficients for anurans than for insects may have arisen due to the nature of our data. We measured richness and not diversity, the latter also takes into consideration the abundance of sounds. Diversity indices use both occupation of frequencies and time. Occupation in time is sometimes viewed as a correlate of species abundance (Villanueva-Rivera et al., 2011). That could

mean that the 24 h diversity pattern indicated by the diversity indices (low-day and high-night values) was a result of differences in the distribution of values between anurans and insects. The anurans were virtually absent during the day as opposed to the insects that stridulate during the day but more (or more individuals stridulating) at night. This results in a similar value for richness and abundance for anurans, and a discrepancy in those values for insects. Future investigation on the sonic abundance of those taxonomic groups is needed to clarify this issue.

We fail to find relationships between acoustic indices and bird sonotype richness. The broad frequency bandwidth (0/0.3 to 48 kHz) used in the analyses may have reduced the relative impact of birds on the indices as they occupy a more restricted frequency band. Alternatively, even when using a much more limited bandwidth (0–10 kHz) acoustic indices have been correlated negatively with bird richness and/or presented high values at night (Villanueva-Rivera et al., 2011; Fuller et al., 2015; Mammides et al., 2017). Therefore, analyses bandwidth might not have such strong leverage on the relative importance of animals groups to acoustic indices. Insects and anurans might be influencing those indices more than birds, even in lower frequency bands. In fact, most insect and all anuran sonotypes occupied frequencies below 7 kHz (see Table S2).

Detection of mammal sonotypes were rare (6.6% of files) and comprised almost in its entirety on bats (except for a single file with maned wolf calls — *Chrysocyon brachyurus*). It should be noted that many bats vocalize at higher frequencies than we recorded (Kunz and Brock, 1975; Russo and Jones, 2003) which probably biased our sonotype counts towards the lower frequency vocalizing species. All the above indicates the methodology used here is not efficient to adequately account for mammal richness. Recording at appropriate frequencies, continuously, for longer periods, followed by automated detection (Rocha et al., 2015) would be a better approach.

Although we identified airplane noise on our data, they are very rare and there were no vehicle traffic at all nor other human associated sounds in our sample. Overall, NDSI did not perform better than the other indices tested here. For instance, different from the work of Fuller et al. (2015), this index was only sensitive to nocturnal biophony instead of presenting an additional diurnal pattern related to birds. Hence, we do not recommend the use of NDSI for soundscape analyses of sites with similar low anthropophony.

AEI did not differ between sites or periods with the same richness among sites. As we did not measured differences in sonotypes across sites or differences in sonotype acoustic niche occupation (both could be an acoustic correlate of beta diversity) we cannot be sure if the studied sites really have similar acoustic niche occupation — or even same species composition and abundance — or if the AEI was not sensitive to site differences. Villanueva-Rivera et al. (2011), who proposed the ADI and AEI, also showed similar results for both indices.

Biophony activity indices (ACI and BIO) did not correlated with the predominant groups (birds and insects) or with total sonotype richness unless rain is excluded (see “additional tests” in the Results session). Even when we excluded the rain they fail to show the bird dawn and dusk choruses (except for BIO in site B), which was detectable in the manual inspection of files. These periods of highest zoophonic activity are clearly visible in the sonotype richness pattern of all three sites as well as in other studies with ACI and BIO (Fuller et al., 2015; Gasc et al., 2017). The overlapping bird songs of many species and individuals might have resulted in a relatively constant intensity band, which at least for the ACI/s, would impair biophony detection (Pieretti et al., 2011; Gasc et al., 2015). Mammides et al. (2017) reported low performance of the ACI in a region of high bird richness. Even removing the two days with more rain our data may still contain occasional rain noise and a large amount of wind noise derived from setting a lower frequency filter during data acquisition than in most bird focused studies (300 Hz instead of 2,000 Hz). ACI and BIO appear to be very sensitive to geophony (and probably some anthropophony types; Pieretti et al., 2011) requiring substantial data filtering to yield robust correspondence to biological events (Boelman et al., 2007; Depraetere et al., 2012; Gasc et al., 2015).

We conclude that ADI and H were the indices that better corresponded to the tropical savanna zoophony richness and that they were mainly influenced by insect and anuran sounds. Regarding the others indices, future studies on sonic abundance and acoustic niche occupation are needed to

complement our inferences. Future effort should focus on how insect sonic abundance influences indices in comparison to their acoustic richness and if AEI, ACI/s, and BIO are reflecting sonic abundance and niche occupation among sites and periods with the same calculated richness.

Although bird sonic data alone are used as proxies for soundscape structure, overall species richness and habitat health (Pieretti et al., 2011; Kasten et al., 2012; Towsey et al., 2014; Tucker et al., 2014; Gasc et al., 2017; Mammides et al., 2017), acoustic indices based only on avian sounds may not be sufficient to account for overall richness. Here, avian sonic richness did not vary across sites, but local differences in zoophony were better captured by insects. In fact, no single animal group would provide a complete scenario about acoustic ecology. Therefore, we suggest including other taxonomic groups when investigating zoophony richness. Insects and anurans are highly acoustically active, being important contributors to the sonic world and not accounting for them will generate an incomplete description and understanding of soundscapes and underlying acoustic ecological processes. Insects, in particular, seem to be important determinants of the acoustic space use (Aide et al., 2017) and therefore central to the partitioning of acoustic niches (Krause, 1987; Pijanowski et al., 2011b). Proper investigation of terrestrial soundscapes should at least include insects, anurans and birds on the analyses.

We should reinforce that in this study we used a limited number of sites resulting in a lack of site replicates. Also, sonotype counting involves a degree of subjectivity that will always be a limitation to this approach. We believe this methodology is very promising and performed well despite the small number of sites.

In order to validate and better understand indices, we suggest exploring measures of sonic abundance and acoustic niche occupation of sonotypes besides richness. Also, it would be ideal to measure geophony and anthropophony to understand their influence on indices and on soundscapes. Finally, we suggest further refinement of acoustic methodologies so that one may be able to discriminate between taxonomic groups and even allowing for ranking their contribution to the overall zoophony. By doing so, one could potentially detect ecological interactions among animal communities, *e.g.*, prey-predator interactions, which, in the end, is the acoustic ecology we are so fascinated with.

## Supporting material

**Figure S1.** Example of spectrogram showing sonotype richness. Analysts identified sonotypes in this spectrogram and classified them as 5 birds, 3 insects, 1 anuran and 1 mammal. There are also two types of noise: a large equipment interference (1) and wind noise (2; 0–300 Hz). Spectrogram generated with Raven Pro 1.5, 4,096 FFT size, Hann window, 50% overlap, brightness and contrast, from a 1 minute, 96 kHz sample rate, 16 bit wav file. (PNG)

**Figure S2.** Sonotype richness and Acoustic Diversity Index (ADI) regression analyses. Each site has 48 points that correspond to 1 minute samples each half hour averaged from 7 days of recordings in three different sites at Serra da Canastra Brazilian National Park, Minas Gerais state, Brazil. (PNG)

**Figure S3.** Mammal sonotype richness (top) and acoustic indices in three different sites at Serra da Canastra Brazilian National Park, Minas Gerais state, Brazil. Graphs on the left show the temporal distribution of the seven day average. Boxplots on right show comparisons among sites and between day and night (first and third quartiles and median values are shown). Vertical bars are 95% confidence intervals. Circles are outliers. \*s indicates a significant difference between day and night in the same site. \*d indicates significant differences among sites during the day. \*n indicates significant differences among sites at night. (PNG)

**Table S1.** Spearman rho correlation coefficients showing the relationship between acoustic indices for all sites combined (Serra da Canastra Brazilian National Park, Minas Gerais state, Brazil). \* Indicates significant correlations ( $p < 0.01$ ). Correlations in which coefficients are in bold are considered moderate ( $> 0.5$ ) and those with underlined coefficient values are considered strong ( $> 0.7$ ). (DOCX)

**Table S2.** Sonotype list. ([XLSX](#))**Table S3.** Kruskal-Wallis and Mann-Whitney results for the comparisons among sites and between day and night. ([DOCX](#))

## Acknowledgements

We thank Elaine Cunha for producing the map. For equipment preparation and field assistance, we are very grateful to V. Sábato, J. Santos and M. Montagno do Valle, and also to P. Dodonov for logistic assistance.

## Funding sources

We are thankful to several institutions that supported this work. The Coordination for the Improvement of Higher Education Personnel in Brazil (CAPES) granted scholarships to LMSF, EGO, LCL, and MRMB. The Brazilian National Council for Scientific and Technological Development (CNPq) granted to FHGR support from the Universal Call (grant number 474000/2010-9) and from the Productivity in Research (grant number 301665/2011-7). The Brazilian Foundation for the Support of Minas Gerais State Research (FAPEMIG) awarded FHGR two research grants (grants PPM-00429-08 and PPM-00299-11). We also thank for the support from Idea Wild, the Graduate Program in Psychobiology (UFRN) and the Graduate Program in Ecology (UFRN).

## Competing interests

All authors declare that they have no conflict of interest.

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