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PAISAGENS ACÚSTICAS E USO DE MONITORAMENTO ACÚSTICO PASSIVO EM BIOMAS BRASILEIROS

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PAISAGENS ACÚSTICAS E O USO DE MONITORAMENTO ACÚSTICO PASSIVO EM BIOMAS BRASILEIROS

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RESUMO

O estudo de paisagens acústicas vem ganhando atenção da comunidade científica nos últimos anos, mostrando-se como uma importante ferramenta na avaliação tanto de saúde ambiental como dos efeitos de mudança no uso da terra e mudanças no clima em várias escalas temporais e espaciais. Em ambientes tropicais, a alta biodiversidade, associada ao conhecimento taxonômico mais restrito para muitos grupos (como insetos) e menor investimento financeiro em pesquisas, faz com que estudos de ecologia acústica sejam mais desafiadores que em outras regiões. Por serem também as áreas de grande importância para conservação, o uso de monitoramento acústico mostra-se ainda mais relevante na medida em que pode facilitar estudos de longo prazo a um custo mais baixo que tradicionais estudos ecológicos. Diante de um panorama de constantes inovações tecnológicas e métodos de análise de dados na área de ecologia acústica, essa tese teve como objetivo geral avaliar o uso do monitoramento acústico passivo em biomas brasileiros, fornecendo protocolos de análise e contribuindo com o avanço da ecologia acústica em áreas tropicais. A tese está dividida em três capítulos e um apêndice com outras publicações não relacionadas diretamente à tese. No primeiro capítulo descrevemos padrões de utilização do espaço acústico em uma área de Caatinga, através do agrupamento em clusters de múltiplos índices acústicos. Na estação seca, o som do vento foi predominante nas gravações, enquanto na estação chuvosa, observamos alta presença de biofonia. Variações diárias incluíram aves e vento ocupando o espaço acústico durante o dia, enquanto insetos predominaram no período noturno. No segundo capítulo, também em uma área de Caatinga, nós buscamos compreender como um parque eólico modifica a paisagem acústica ao seu redor. Encontramos que a atividade biofônica aumenta com a distância dos aerogeradores, sugerindo que aves e insetos tem seus padrões de atividade acústica modificados em resposta à presença das turbinas. No terceiro capítulo utilizamos dados de Caatinga, Cerrado e Mata Atlântica, para testar a eficiência de índices acústicos em predizer a riqueza de espécies de aves e a discriminação de composição de comunidades entre os biomas. A performance dos índices acústicos variou entre os habitats, e características das comundiades de aves como predominância de espécies e faixas de frequência utilizadas por elas podem estar afetando os resultados dos índices. Mesmo assim, uma combinação de índices acústicos foi capaz de diferenciar as comunidades dos três biomas.

Palavras-chave: Índices Acústicos; Paisagens Acústicas; Ecologia Acústica; Floresta sazonalmente seca; Caatinga; Cerrado; Mata Atlântica

ABSTRACT

Soundscape studies has becoming more popular nowadays as an important tool with the potential to evaluate environmental health, changes in land use, climate change, in several temporal and spatial scales. In tropical environments, the high biodiversity associated with a restricted taxonomic knowledge for many taxa and the lack of financial resources make the acoustic ecology studies more challenging than in other regions. At the same time, tropical areas are important in terms of biodiversity conservation, and the use of acoustic monitoring is even more relevant once it can facilitate long-term studies at a lower cost than traditional ecological surveys. Facing a reality of rapid technological innovations and data analysis methods in the acoustic ecology field, this thesis has the main goal of evaluate the use of passive acoustic monitoring in Brazilian biomes, providing analysis protocols and contributing to advance of acoustic ecology in tropical regions. The thesis is divided in three chapters and an appendix with other production non-related to the thesis. In the first chapter we describe patterns in use of acoustic space in a Caatinga area by clustering multiple acoustic indices. During dry season, wind was predominant in the recordings, while in rainy season, was biophony. Daily variations included the birds and wind occupying the acoustic space during the day, while insects predominated at night. In the second chapter, also in a Caatinga area, we aimed to understand how a wind farm facility modifies the soundscape around it. We've found that biophonic activity decreases as we move closer to the turbines, suggesting that both birds and insects have their acoustic activity patterns modified in response to wind turbines presence. In the third chapter, we used data from Caatinga, Cerrado and Atlantic Forest, to test whether single indices are able to predict bird species richness and if a composition of several indices allow discrimination of different species' composition. We've found that single indices performance is variable among habitats, and features of birds' communities as predominance of species and frequency bands used may be affecting the indices results. Even so, a combination of indices was able to distinguish among biomes.

Keywords: Acoustic Indices; Soundscape; Acoustic Ecology; Seasonally Dry Tropical Forest; Caatinga; Cerrado; Atlantic Forest

INTRODUÇÃO GERAL

Essa seção tem como objetivo apresentar as bases teóricas que deram origem a esse trabalho. O texto apresenta uma explicação de conceitos e abordagens mais comuns utilizadas na área de ecologia acústica. Embora escrito em português, apresentamos a tradução dos termos-chave em inglês entre parêntesis, uma vez que é o idioma utilizado nos três capítulos. Para tanto, dividimos em quatro subseções: (1) Conceitos fundamentais no estudo de paisagens acústicas, (2) Uso de monitoramento acústico passivo em estudos ecológicos, (3) Conservação de ambientes acústicos saudáveis, (4) Abordagens metodológicas e desafios práticos.

1. Conceitos fundamentais no estudo de paisagens acústicas

Os sons são utilizados por diversos grupos animais como principal forma de comunicação e, portanto, são foco de estudo de uma área de conhecimento conhecida como bioacústica. A bioacústica investiga a função dos sinais acústicos na comunicação animal, e é historicamente uma área mais voltada às interações entre indivíduos. O comitê técnico de Bioacústica Animal da Acoustical Society of America (https://tcabasa.org/) inclui que em escalas mais amplas, o som não é apenas uma troca de energia e informação, mas é capaz de mediar (e até mesmo simbolizar) relações entre emissores, receptores e seus ambientes, refletindo um sistema dinâmico de comportamentos característico de uma abordagem ecológica.

O termo paisagem acústica (soundscape) foi proposto pela primeira vez em um contexto urbano, definido como propriedades acústicas das cidades que ajudam as pessoas a se localizarem em certos espaços (Southworth, 1969). Ainda hoje, é um conceito utilizado no planejamento de cidades e manifestações artísticas (Raimbault & Dubois, 2005). A partir de 1977 começou a ser usado também para abordar questões relacionadas a ambientes naturais. Schafer (1977) define uma paisagem acústica como sendo “as características acústicas de uma área que refletem seus processos naturais” (uma retrospectiva histórica mais detalhada pode ser encontrada em Zorzetto, 2019).

Os componentes de uma paisagem acústica (Figura 1) incluem emissões sonoras de origem biológica – biofonia (biophony), de origem em processos geofísicos – geofonia (geophony) e antropogênica – antropofonia (antropophony). Biofonia inclui todos os sons emitidos por animais, também chamados zoofonia (zoophony) (Ferreira et al., 2018). Geofonia inclui sons como chuva, vento, e trovões. Antropofonia engloba os sons advindos de atividades urbanas e industriais (Krause, 1987; Pijanowski et al., 2011). Esses três componentes ocupam o mesmo espaço acústico, e assim, fenômenos acústicos oriundos de geofonia ou antropofonia interagem, modificam e tem o potencial de impactar a biofonia.

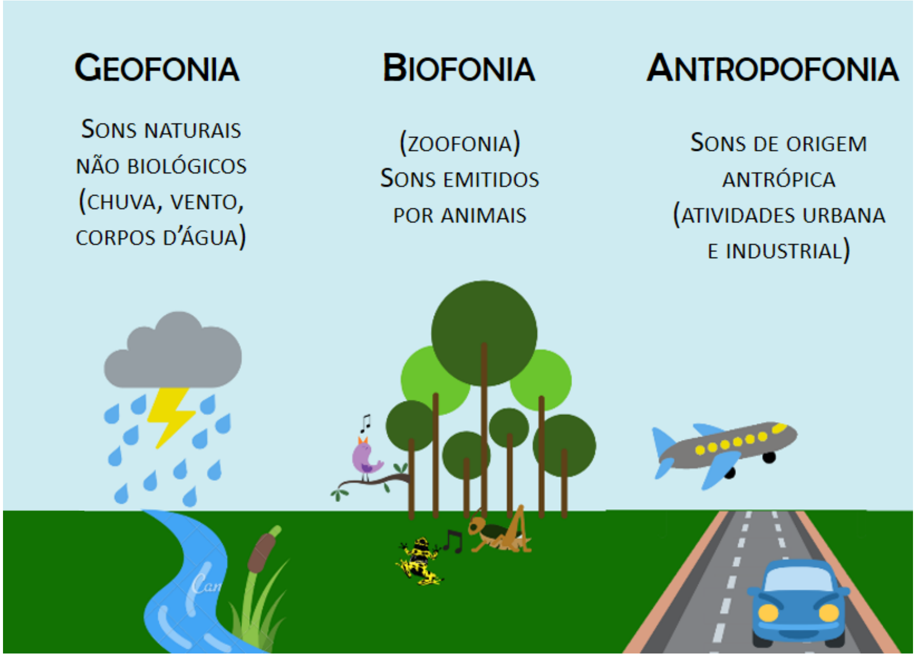

Figura 1 – Fontes de emissão sonora que compõe uma paisagem acústica.

Considerando essa interação entre os três componentes da paisagem acústica, três hipóteses diferentes e não mutuamente exclusivas foram propostas para explicar como as características vocais de diferentes espécies animais variam e se adaptam ao ambiente no qual evoluíram: Hipótese de Adaptação Morfológica, de Adaptação Acústica e de Nicho Acústico.

A Hipótese de Adaptação Morfológica (Morphological Adaptation Hypothesis – MAH) refere-se às limitações morfológicas e fisiológicas de determinado organismo, que restringem as características das suas emissões acústicas (Wallschlager, 1980). As características vocais das espécies também são moldadas e selecionadas ao longo do tempo por fatores ambientais como cobertura vegetal, compactação do solo, meio de transmissão. Esse fenômeno é conhecido como Hipótese da Adaptação Acústica (Acoustic Adaptation Hypothesis – AAH) (Ey & Fischer, 2009; Morton, 1975).

Essas duas hipóteses explicam em parte as pressões que levam as espécies a terem distintos padrões de emissão acústica. Uma terceira pressão seria a de minimizar sobreposições no tempo e espaço entre sinais sonoros, maximizando a transmissão de informação ao receptor. A essa hipótese damos o nome de Hipótese de Nicho Acústico (Acoustic Niche Hypothesis) (Krause, 1993). Segundo ela, cada espécie teria seu próprio nicho acústico no espectro de tempo-frequência e, ambientes mais antigos, mais diversos e menos perturbados possuiriam nichos acústicos amplamente ocupados. Modificações nessa ocupação seriam então possivelmente um sinal de perturbações ambientais (Pijanowski et al., 2011) ou alterações em processos ecológicos.

2. Uso de Monitoramento acústico passivo em estudos ecológicos

O monitoramento acústico passivo (MAP) consiste na gravação de arquivos de áudio sem que haja a necessidade da presença do pesquisador operando o gravador. Esses sistemas de gravação automáticos permitem que amostragens sejam feitas em uma larga escala espaço-temporal, reduzindo os esforços e gastos com trabalhos de campo, além de diminuir potenciais impactos que os observadores possam ter sobre o comportamento natural dos animais. MAP envolve a gravação de sons utilizando uma variedade de sensores acústicos, a depender do estudo, e uma posterior extração de dados relevantes a partir dos áudios (Fig. 2). Até o momento, a maior parte dos trabalhos de MAP em ambientes terrestres tem um foco voltado para estudos com quirópteros, em regiões temperadas, com gravações feitas no período noturno e incluem uma análise manual dos arquivos (Sugai et al., 2019).

Um produto das gravações de áudio é a amostragem de espécies que podem ser identificadas a partir de dados acústicos. Nesse sentido, Shonfield & Bayne (2017) avaliaram a eficácia desse método comparado a pontos de escuta (um método de amostragem tradicionalmente utilizado) chegando à conclusão de que, de um modo geral, a análise de dados provenientes de MAP é bastante eficiente e permite que diversos pontos sejam amostrados ao mesmo tempo e que os dados sejam armazenados e avaliados por mais de um especialista. Uma limitação dessa metodologia é para grupos de organismos que não apresentam suas emissões sonoras identificadas e catalogadas, como é o caso dos ortópteros (Riede, 2017).

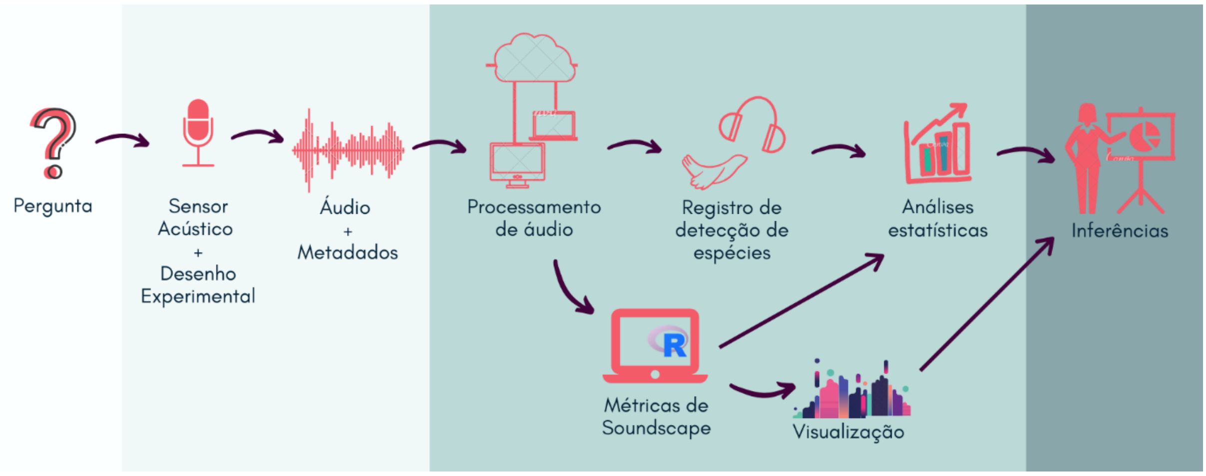

Figura 2 – Workflow de estudos de monitoramento acústico passivo (modificado de Gibb et al., 2019)

3. Conservação de ambientes acústicos saudáveis

As mudanças antropogênicas no ambiente acústico, especialmente desde a revolução industrial, vêm aumentando substancialmente, de forma que dificilmente é possível encontrar um local no mundo, seja na superfície da terra ou no fundo dos oceanos, que tenha suas características sonoras naturais totalmente preservadas. Os impactos acústicos do crescente aumento de ruído no ambiente são bem estudados em humanos e seus efeitos incluem alterações no ritmo de sono, aumento do risco de doenças cardíacas e prejuízos auditivos e cognitivos (Fritschi et al., 2011). O efeito da poluição sonora sobre outras espécies de animais é mais bem estudado em ambientes aquáticos, mas vêm ganhando atenção em ambientes terrestres mais recentemente (Shannon et al., 2015).

De acordo com a literatura, a vida selvagem apresenta repostas ao ruído a níveis sonoros acima de 40dB, mas quantificar os efeitos de antropofonia em animais é um desafio, já que a sensibilidade auditiva varia de acordo com os taxa, contextos e histórias de vida (Shannon et al., 2015). Essas fontes de alteração da paisagem sonora também não podem ser desconectadas de outras mudanças, como alteração do habitat e distúrbio visual, o que dificulta uma interpretação precisa das respostas biológicas ao ruído (Summers, Cunnington, & Fahrig, 2011).

O uso de soundscapes em estudos relacionados a conservação vêm crescendo nos últimos anos, uma vez que a comparação das paisagens sonoras em áreas sob pressões antrópicas diferentes pode nos fornecer um entendimento rápido da intensidade dos impacto causados (Astaras, Linder, Wrege, Orume, & Macdonald, 2017; Burivalova et al., 2017) e fornece uma ferramenta eficiente e relativamente barata para monitorar tendências. Burivalova et al. (2019) aponta o potencial do uso de monitoramento acústico associado a imagens de satélite para avaliação do sucesso de medidas de conservação em áreas tropicais. O uso dessas ferramentas é também recomendado em sistemas de produção agrícola que, embora não naturais, terão um papel cada vez mais importante na manutenção da biodiversidade (Doohan et al., 2019). Além disso, gravações acústicas constituem um banco de dados extenso e que pode ser utilizado por um longo período de tempo, analisado sob diferentes óticas e múltiplos pesquisadores e, portanto, vêm sendo consideradas como “cápsulas de tempo”, e serão criticamente importantes para comparações nas próximas décadas (Sugai & Llusia, 2019).

4. Abordagens metodológicas e desafios práticos

Uma vez que estudos de ecologia acústica possuem focos diferentes, encontramos na literatura uma ampla variação em equipamentos utilizados, frequências amostrais e esquemas temporais de amostragem. De acordo com Phillips (2018), a partir de uma revisão bibliográfica de trabalhos de paisagem acústica, a maioria dos trabalhos utiliza uma frequência de amostragem de 44.1 ou 48 kHz, o que significa que emissões sonoras de até 22 ou 24 kHz, respectivamente, serão amostradas com qualidade (é preciso um mínimo de duas amostras por ciclo – Teorema de Nyquist, Nyquist, 1928).

O esquema temporal de amostragem pode variar de gravações contínuas (Phillips et al., 2018) a até alguns minutos por dia (Machado et al., 2017; Sugai et al., 2019). A exploração de resultados provenientes de diferentes esquemas de amostragem (Pieretti et al., 2015) são importantes nessa etapa de desenvolvimento da ecologia acústica e visam auxiliar inclusive na redução de gastos relacionados a alimentação de equipamentos de gravação, armazenamento de dados e subsequentes processamento e análise.

Nos últimos anos observamos uma significativa redução de custos associados a equipamentos e tecnologia de armazenagem e análise. Enquanto há poucos anos apenas uma empresa fornecia a maior parte de gravadores autômatos utilizados em estudos de ecologia acústica, hoje open-source hardwares como Audiomoth (Hill et al., 2018) permitem que estudos de monitoramento acústico sejam feitos a um custo mais baixo. Essa facilidade permite também que grandes bancos de dados sejam criados e, consequentemente, novos desafios para a sua análise surjam.

A análise de grandes bancos de dados acústicos não pode ser feita da mesma forma que estudos de bioacústica com foco em poucas espécies ou em restritos espaços de tempo. Para solucionar esse impasse no processamento dos dados, começaram a ser desenvolvidos índices acústicos, métricas que resumem determinadas características presentes nas gravações e que podem refletir processos ecológicos presentes nelas (Sueur et al., 2014). A avaliação da eficiência dessas métricas ainda é incipiente (Ferreira et al., 2018; Gasc et al., 2015; Gasc et al., 2013). A eficiência de certos índices em refletir diversidade de aves por exemplo, parece variar de acordo com a região (Eldridge et al., 2018). A utilização de índices para monitorar e diferenciar taxa distintos dentro das mesmas gravações representa um desafio a mais no estudo de soundscapes, especialmente em áreas com alta biodiversidade (Ferreira et al., 2018). A combinação de índices acústicos está sendo usada mais recentemente para auxiliar nessa complexa caracterização das paisagens acústicas (Phillips et al., 2018).

Índices acústicos também são utilizados para auxiliar na visualização de grandes bancos de dados. Em trabalhos de bioacústica, utiliza-se espectrogramas, representações visuais do som em três dimensões: frequência, tempo e amplitude. Em gravações de longa duração, entretanto, a utilização de simples espectrogramas torna-se inviável. Índices acústicos associados à visualização em espectrogramas de falsa cor (False Colour Spectrograms - FCS) têm fornecido resultados interessantes em grandes bancos de dados e facilitado a interpretação (Towsey et al., 2014). Os FCS estão sendo usados para identificar padrões em áudios de longa duração e, mais recentemente, pesquisadores vêm analisando sua eficácia para identificação de grupos taxonômicos ou espécies (Towsey et al., 2018).

OBJETIVO GERAL E ORGANIZAÇÃO DA TESE

Diante desse panorama de constantes inovações tecnológicas e métodos de análise de dados, essa tese teve como objetivo geral avaliar o uso do monitoramento acústico passivo em biomas brasileiros, fornecendo protocolos de análise e contribuindo com o avanço da ecologia acústica em áreas tropicais.

A tese está dividida em três capítulos e contém três apêndices: um texto de divulgação científica publicado na revista Ciência Hoje Crianças, e duas publicações não relacionadas diretamente com a tese. No primeiro capítulo descrevemos padrões de utilização do espaço acústico em uma área de Caatinga, analisando variações sazonais e padrões diários da biofonia. No segundo capítulo, também em uma área de Caatinga, buscamos responder como um parque eólico modifica a paisagem acústica do seu entorno. No terceiro capítulo utilizamos dados de Caatinga, Cerrado e Mata Atlântica para responder como índices acústicos refletem diferenças em riqueza e composição de comunidade de aves nos três biomas.

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CAPÍTULO 1

THE CAATINGA ORCHESTRA: CLUSTERING MULTIPLE ACOUSTIC INDICES TRACK TEMPORAL CHANGES IN A SEASONALLY DRY TROPICAL FOREST

Eliziane G. Oliveira, Milton Cezar Ribeiro, Paul Roe,
Renata S. Sousa-Lima

The Caatinga Orchestra: Clustering Multiple Acoustic Indices track Temporal Changes in a Seasonally Dry Tropical Forest

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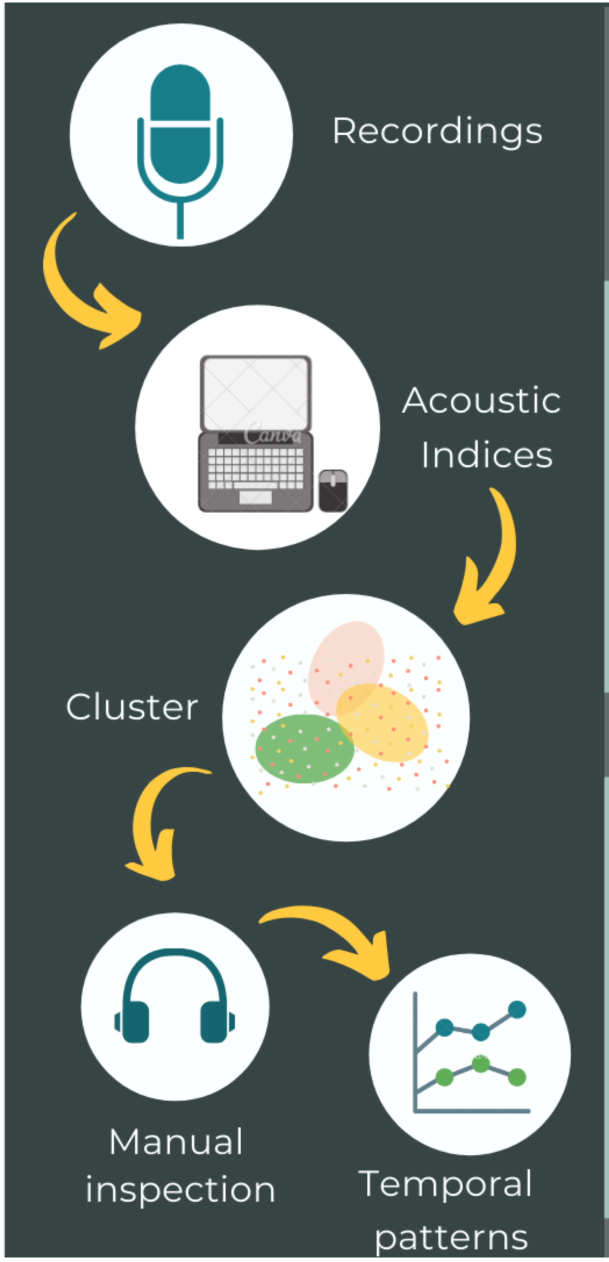
Highlights

- Clustering of eleven acoustic indices were used to discriminate the main sound sources present in the Caatinga soundscape
- The diurnal period was dominated by sounds of birds and wind, while at night insect acoustic activity was prevalent
- Acoustic indices revealed seasonal differences in animal activity
- Biophony (insects and birds) was more prevalent during the rainy season while in the dry season, when vegetation is deprived of leaves, wind (geophony) dominated
- Entropy of the Spectral Peaks (EPS) was influenced by birds and orthopterans. Orthopteran activity also drove the diel patterns of Entropy of Average Spectrum (EAS) and High Frequency Cover (HFC) indices.

IDENTIFYING TEMPORAL PATTERNS OF CAATINGA SOUNDSCAPES USING ACOUSTIC INDICES



Clustering of eleven acoustic indices discriminate the sound sources in the **Caatinga** soundscape, revealing their daily and seasonal patterns

Diurnal period dominated by sounds of **birds and wind**

At night, **insect** acoustic activity was prevalent

Biophony was predominant in the rainy season

In dry season, **geophony** (wind) predominated

Abstract

Advances in technologies for data acquisition, storage and analysis have boosted Acoustic Ecology studies, but there is still a lack of protocols and it is unknown which methodologies can be applied to answer ecological questions in different environments with varying temporal and spatial dynamics. Tropical forests are generally more complex than temperate ones, in terms of use of acoustic space and species diversity. The seasonally dry tropical forest in Brazil is a threatened biome, with two marked seasons that shape the vegetation and animal activity patterns. In this study, we investigate the applicability of passive acoustics in monitoring seasonally dry tropical forests (SDTF), describing the soundscape and tracking diel patterns and seasonal changes. Combining multiple indices, visualization through false colour spectrograms and clustering we describe the acoustic activity of the main faunal groups that compose the biophonic orchestra in a SDTF area in Northeast Brazil. Distinct patterns were found between day – when birds and wind were the main sound sources – and night – with Orthopterans occupying a large frequency band. Other sound sources in the SDTF soundscape included cicada, rain, and anthropogenic interference such as domestic animals, cars and gunshots. Clustering of eleven acoustic indices was useful to distinguish sound patterns from several sources, especially in the dry season. Further investigation within each cluster showed specific relationships among selected indices and different sound sources. Birds were associated with Entropy of the Spectral Peaks (EPS) and Orthopteran also had a relationship with EPS, as well as with Entropy of Average Spectrum (EAS) and High Frequency Cover (HFC). Variation in diel values of these selected indices, as well as the number of samples included in each cluster category, were successfully used to describe the acoustic activity of Birds and Orthopteran and to track changes between rainy and dry seasons. A better understanding of the soundscape dynamics in a highly seasonal tropical environment was achieved by applying cheap and reliable novel methodologies to study biodiversity in geopolitical regions where funding for conservation initiatives is limited.

Keywords: Acoustic Indices, Soundscape, Biophony, Geophony, Acoustic Ecology

1. Introduction

The use of acoustic data to study ecological processes relies on the fact that sounds play a fundamental role for many species and ecosystems, being used by vertebrates and invertebrates in a variety of functions and contexts (Bradbury & Vehrencamp, 1998). Employment of acoustic tools is non-invasive and provides data about variation on temporal and spatial patterns of species richness (Depraetere et al., 2012; Duarte et al., 2015) and changes in behaviour (Parks et al., 2011). Passive acoustic monitoring (PAM) has become popular due to recent advances in technologies for data acquisition, storage and analyses (Burivalova et al., 2019; Gibb et al., 2019; Merchant et al., 2015). Major caveats to these methods include that they only work for vocal species and it is difficult to identify individuals calling animals for most taxa (Gibb et al., 2019).

The popularization of autonomous passive acoustic monitoring was followed by the exponential growth of soundscape ecology (Pijanowski et al., 2011). The term "soundscape" appeared for the first time in an urban context (Southworth, 1969) and describes the acoustic characteristics of a given area that reflects their processes (Schafer, 1977) and, in natural environments, creates dynamic acoustic patterns (Pijanowski et al., 2011). We can recognize three different sound sources in a soundscape (Krause, 1987): biophony, arguably zoophony (Ferreira et al. 2018) (sounds produced by animals, usually related to communication), geophony (sounds from physical processes, like wind, rain, thunder), and anthropophony (sounds from human made activities or machines). The study of soundscapes has applications in several temporal and spatial scales to address questions about environmental health, changes in land use, and climate change, to name a few (Blumstein et al., 2011; Burivalova et al., 2019; Buxton et al., 2018; Farina, 2014; Krause & Farina, 2016; Pijanowski et al., 2011; Tucker et al., 2014).

As a relatively new research field, with a potential to generate an enormous amount of data, researchers are now dealing with data management and wish to find fast and reliable ways to visualize and interpret acoustic big data (Phillips et al., 2018; Sueur et al., 2014; Towsey et al., 2014). The use of acoustic indices to summarize the information in audio-recordings has been explored. An acoustic index is an statistic that summarizes some aspects of structure and distribution of acoustic energy, and potentially reflects ecological processes (Towsey et al., 2014). The relationship between acoustic indices and community diversity are still poorly understood (Burivalova et al., 2019; Ferreira et al., 2018; Gibb et al., 2018) and, besides the great potential to be used in a more broad context (Aide et al., 2017), most literature still focus on avian community (Machado et al., 2017; Pieretti et al., 2015; Zhang et al., 2016). Given the diversity of acoustic patterns within a recording, it is not expected that a single index could precisely describe the soundscape (Buxton et al., 2018; Ferreira et al., 2018). Therefore, recent studies are proposing the combination of several non-redundant indices to achieve best results (Phillips et al., 2018). More visual approaches in characterizing soundscapes rely on the idea that is impossible to identify patterns in long duration recordings by only listening to them (Sankupellay et al., 2015). To address this caveat, multiple acoustic indices' visualization has been proposed using two techniques: false colour spectrograms (Sankupellay et al., 2015) and data ordination (Phillips et al., 2018; Sankupellay et al., 2015).

Here we propose the utilization of multiple visualization tools to validate the applicability of passive acoustic monitoring in seasonally dry tropical forest (SDTF) characterization, aiming to point out weaknesses and strengths of this methodology in such unique environments. The

SDTF in Brazil, called Caatinga, has two distinct seasons: dry and rainy. The vegetation cover among seasons changes drastically, as well as the animal activity. The reproductive period of birds, anurans, mammals and insects in the area is highly dependent on the water supply (Santos et al., 2011).

The objective of this study was to describe the Brazilian SDTF soundscape dynamics and track diel patterns and seasonal changes. Specifically, we aimed to answer: (1) Which indices can better describe SDTF acoustic activity, considering multiple taxonomic groups? (2) Are there diel patterns? (3) Which animal groups contribute to changes in the observed diel patterns? (4) Do acoustic patterns change between rainy and dry seasons?

We hypothesize that a single index will not be able to reflect all acoustic activity, given the diversity of signals emitted by animals. We expect that in such tropical environments, birds will dominate the diurnal period and insects will fill in the nightscape. Anurans are not expected to be present once the recording sites were not close to water bodies (information about the sampling sites are in Appendices, Table A.1). We also expected less contribution of avian fauna and a shorter dawn and dusk choruses during the dry season, as a response to climate. Regarding insects, we do not expect changes in the daily activity period as seasons change, but we do expect that the number of individuals, and even species, will reflect more intense use of the acoustic space during the wet season, when most species are exploiting the abundance of water as a resource for adults and early stages of insect's life cycles.

2. Methodology

2.1. Study area

Seasonally Dry Tropical Forests represents 20% of world tropical forests (Hansen et al., 2010), are distributed within Americas, Africa, Eurasia and Australasia (Miles et al., 2006) and share some similarities such as a significant numbers of endemic species which are susceptible to desertification processes, and are often absent from priority conservation actions (Miles et al., 2006; Santos et al., 2011). Within Brazil, the SDTF is distributed over nine states in an area of 836,001 km² (MapBiomas, 2019). Annual rainfall ranges from 200-800 mm and the rainy season lasts for 3 to 5 months (Olmos et al., 2005).

The study area is located within Serra do Feiticeiro (Figure 1) which is the most pristine Caatinga area in Rio Grande do Norte State (Cordero-Schmidt et al., 2018; Marinho et al., 2018; Vargas-Mena et al., 2018). Besides the conservation importance, there is no legal preserve in or around the area and a massive windfarm has been planned to be constructed in the next few years. Therefore, this paper also aims to contribute rare prior baseline data to monitor how the windfarm construction will influence the soundscape in the future (Dahl et al., 2012).

2.2. Data acquisition

In each of the five sampling points, we installed one autonomous recorder (Song Meter SM3®, Wildlife Acoustics, Inc., Concord, Massachusetts), with an omnidirectional waterproof microphone, attached to a tree at 1.5 m high. The sensors were programmed to record one minute every 15 minutes at a sample rate of 44.1 kHz and 16 bits, in WAVE format. Data were collected in 2017 over 26 consecutive days each season simultaneously in each of the five sites (Figure 1). Additionally, two SM2 with the same specifications were used during the 2018 rainy season, also recording for 26 days, in two other sites simultaneously (Figure 1). Due to equipment malfunction, data from one of the recorders from the 2017 rainy season was removed from analyses. The recorders were distributed so that our sample was representative of the landscape in the region. Sites varied in vegetation structure, distance from roads, terrain inclination. Distance between sampling points varied from 200 to 2,000 meters (Figure 1). Additional information about sampling sites are presented in Appendices (Table A.1 and Figure A.1).

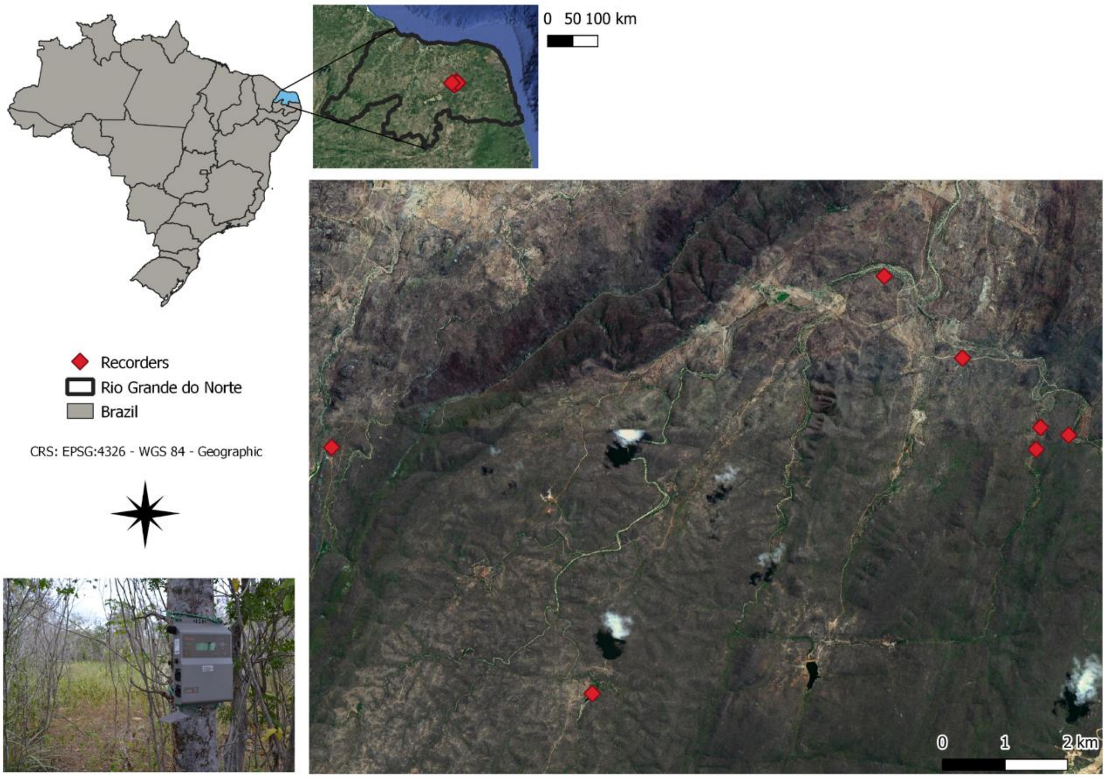


Figure 1 - Location of sites within an area of Caatinga sampled using song meters distributed in the municipality of Lajes, Rio Grande do Norte State, Brazil and a close-up photograph of one SongMeter 3 installed in the area.

2.3. Data processing

To better understand the biological content of the data, we manually counted sonotypes in a subsample of the recordings (1-minute every 30 minutes on files from 3 consecutive days, for each season, N= 290). We define “sonotype” as a note or series of notes with a distinct pattern that may represent a species vocalization (Aide et al., 2017; Ferreira et al., 2018). The use of this approach has some caveats, although for insects and amphibians one sonotype equals one species, in birds we have a different scenario. The same species can have a wide vocal repertoire, including mimetic signals from other species, which may lead us to biases in the number of species resulting from this approach. Nonetheless, it is valid since the limitations to identify sounds to the species-level is well understood (Aide et al., 2017) and our aim was not identifying species. Insects are currently the most problematic taxa in terms of species identification. The challenge and weakness of this methodology for soniferous insect species is that their vocal parameters have a strong association with body size and environmental conditions such as humidity and temperature (Robinson & Hall, 2002). Thus, a single insect species comprised by individuals with size variation could be accounted for two or more times.

Sonotypes were detected using manual inspection of files for their spectral features in Raven Pro 1.5® (Cornell Lab of Ornithology, Ithaca, NY). Each sonotype was placed in one of four categories: birds, insects, anurans, or mammals. We also registered the presence of geophony (e.g. wind, rain) and antropophony (e.g. vehicles, gunshots).

Once the recorders are exposed to adverse conditions in the field, it is common that some files are damaged (Sankupellay et al., 2015). Although it is also a common practice to manually remove files with too much wind, rain or anthropogenic noise. Instead here we use all files since we consider that those sound sources are also part of the soundscape and should be accounted for.

2.4. Acoustic Indices

The acoustic indices calculations and visualization were generated using Analysis Program (Towsey et al., 2016). In total, we use 14 Summary and six Spectral Indices (see section “Data visualization” below) (Table 1). The summary indices represent a single statistic calculated across a broad frequency range to measure different aspects of the acoustic energy within that time-period, in our case, 30 seconds. Meanwhile, the spectral indices are vectors calculated within predefined frequency bands and time periods, but also summarise several aspects of the acoustic energy distribution (Towsey, 2017).

Table 1. Indices calculated using the Analysis Program. For details about calculations, see references. Note that ACI, ENT, EVN, BGN, PMN, R3D were calculated at both spectral and summary versions. The spectral calculations were used to build False Colour Spectrograms.

ACI	Acoustic Complexity Index	Originally developed to reflect bird activity, excluding constant and low frequency sounds (like human generated noise). Very sensible to other sound sources, like rain. In this study, based on bird species observed, we chose to use the frequency band of 0.5 to 11 kHz.	Pieretti, Farina, & Morri, 2011; Towsey, 2017
ENT	Temporal Entropy	Measurement of acoustic energy concentrated in each frequency bin (Spectral Temporal Entropy), across the wave envelope.	Towsey, 2017
EVN	Events per second	Average number of times the decibel envelope crosses a BGN +3 dB threshold, per second.	Towsey, 2017
BGN	Background noise	Noise profile calculated from the decibel waveform. Note that this index is used to calculate others (like EVN).	Towsey, 2017
SNR	Signal to noise Ratio	Difference between the BGN value and the maximum value in the decibel envelope.	Towsey, 2017
ACT	Activity	Fraction of values in the 30 seconds decibel envelope that exceed 3 decibels above the BGN value. Constant sounds with high signal to noise ratio (like cicada chorus) are reflected in high ACT values.	Towsey, 2017
HFC	High Frequency Cover	Fraction of the noise-reduced decibel spectrogram in the high frequency band (11-22.050 kHz) that exceed 3 dB above BGN.	Towsey, 2017
MFC	Mid Frequency Cover	Fraction of the noise-reduced decibel spectrogram in the mid frequency band (0.5-11 kHz) that exceed 3 dB above BGN.	Towsey, 2017
LFC	Low Frequency Cover	Fraction of the noise-reduced decibel spectrogram in the low frequency band (below 0.5 kHz) that exceed 3 dB above BGN.	Towsey, 2017
EAS	Entropy of Average Spectrum	Concentration of mean energy in the mid-band of the mean energy spectrum.	Towsey, 2017

EPS	Entropy of the Spectral Peaks	Concentration of spectral maxima values in the mid frequency band (0.5 – 11 kHz).	Towsey, 2017
ECV	Entropy of Coefficient of Variation	Similar calculation to EAS, but in ECV the mid-band spectrum is derived from the variance divided by the mean of the energy values in each frequency bin.	Towsey, 2017
CLC	Cluster Count	Measure of the degree of internal acoustic structure, or spectral diversity, within the mid-band. Therefore, it is an index that can reflect bird diversity.	Towsey, 2017
SPD	Spectral Peak Density	A measure of the number of cells in the mid-frequency band that are identified as being local maxima. Not normalized to be independent of frame size and frame overlap.	Towsey, 2017
R3D	Three Ridge Indices	Combination of maximum values of three ridge indices (horizontal, upward slope, downward slope) which attempts to detect harmonic structures in the mid-band.	Towsey, 2017

2.5. Data visualization

Sound visualization is an important tool considering large datasets and it is based on the assumption that the human pattern perception is better at integrating long visual representations than long aural stimuli (Sankupellay et al., 2015). In this sense, the use of False Colour Spectrograms (FCS) capitalizes on the human capability to discriminate red, green, and blue and is generated based on a combination of acoustic indices. Three different and non-redundant indices are assigned to one of three colours (red-green-blue, RGB). The pixel size depends on the sampling rate (vertical axis) and temporal window (horizontal axis). Six acoustic indices were used in two different visualizations: ACI-ENT-EVN and BGN-PMN-R3D.

2.6. Clustering the dataset

The k-means is one of the most popular and relatively simple clustering algorithms and requires that a fixed number of clusters is determined prior to the analysis. The algorithm used here was a variation of the k-means, known as k-means++, that results in more accurate centroids and minimizes the distance between points within each cluster (Campbell et al., 2006). To determine the ideal number of clusters we used a smaller dataset with high biophonic activity. Days with rain, strong winds and recorder malfunction were discarded by inspecting the False Colour Spectrograms. This smaller dataset consisted in eight days, two for point 02 and two on point 03, in the rainy and in the dry season, respectively. The indices were normalised between 2 and 98 percentile bounds and scaled between 0 and 1. We performed a correlation matrix to remove highly correlated summary indices (Pearson's correlation > 0.75) (Gage et al., 2017). In total, we used 11 of the 14 summary indices (Appendices, Table A.2).

We tested two indices that are metrics for evaluating clustering algorithms: Silhouette Index (Rousseeuw, 1977; R Package 'cluster') and Dunn Index (Dunn, 1974; R Package 'clValid'). As observed by Phillips et al. (2018), although these are the most used indices for this purpose, they do not always work. The Silhouette Index achieved best values for our sample with 5 clusters, which is a low number to represent complex acoustic communities (Phillips et al., 2018), and the Dunn Index showed incongruent results, with best values in 15 and 50 clusters. Considering the caveats of such validation metrics, we decided to manually browse 376 files and labelled sonotypes as: Orthopteran, Cicada, Birds, Wind, Quiet, Clipping, Frogs, Donkey, Anthropophony. Within Orthopteran, Cicada and Birds we also created a "Low activity" subcategory which was used when the acoustic signals were faint (low signal to noise ratio) or appeared only sporadically in the file. When k=50, the number of clusters assigned to each manually labelled class reached a plateau, except for Orthopteran, that kept increasing. An explanation for this increase may be the great number of files containing Orthopteran vocalization, and not necessarily an effect of the number of clusters. Therefore we used the optimal number of clusters as 50 which was coherent with one of the Dunn Index best values.

The complete dataset comprises more than 24,000 one-minute files from all seven different sampling points. These files were further divided into 30-seconds segments to achieve a better resolution and avoid losing data due to clipping. Files were removed from further analyses by excluding those with Clipping Index values >500. The Clipping Index is a measure of the proportion of audio segment that presented clipping, which compromises the information, indices calculation and, thus, would affect the clustering. We normalized the rest of the data and excluded the correlated variables (as explained above), then we performed a k-means++ clustering, using k=50, maximum number of iterations =500 (Campbell et al., 2006; R Package 'LICORS').

We validated the content of clusters by manually verifying 10 files within each cluster, in a total of 500 files. Each file was labelled with one to four classes (birds, orthopterans, cicadas, wind, rain, quiet) which were further ranked by importance (i.e., the amount of occupation of the acoustic space). Differences in cluster categories frequencies among seasons were verified using a Chi-Squared test.

2.7. Clusters visualization

We chose the Uniform Manifold Approximation and Projection for Dimension Reduction (UMAP) for data visualization (Figure 5). UMAP is a new technique for data reduction, and presents some advantages compared to other popular data reduction algorithms, such as PCA and SammonMap (McInnes et al., 2018). The UMAP analyses were generated in R (package 'umap') and the graphs were created using package 'ggplot2'.

2.8. Relationship between clusters and acoustic indices

To understand the weight of acoustic indices on each cluster we use radar plots analysis (R package ‘fmsb’), for each one of the 25 clusters associated with a single label (Figure 6). Besides using the normalized values, we also reduced the noise by using a medoid value for each variable (Phillips et al., 2018). This way, we could easily visualize which variables have the most influence on each cluster.

2.9. Diel and seasonal patterns

To understand how the diel and seasonal patterns could be identified using the clusters, we chose 5 days per season in each sample point and looked for the relationship between the time of the day (24hs) and the number of files associated to each sound label. We also used the most important acoustic indices from the radar plots as a measure of the contribution of each one of our main biophonic labels.

3. Results

3.1. Visualization using False Colour Spectrograms (FCS)

We compared the spectrograms (examples can be found on Appendices, Figure A.2) with the FCS to verify which sound sources were contributing more to the patterns observed (Figure 2). In a 24h resolution, it is possible to visualize the nocturnal insects at the grey scale spectrogram, but not the diurnal biophonic activity, which are easily observed in the FCS. This is especially evident for short duration signals, the case for most bird calls, and an example can be seen in the dawn chorus (indicated with red arrows in Figure 2).

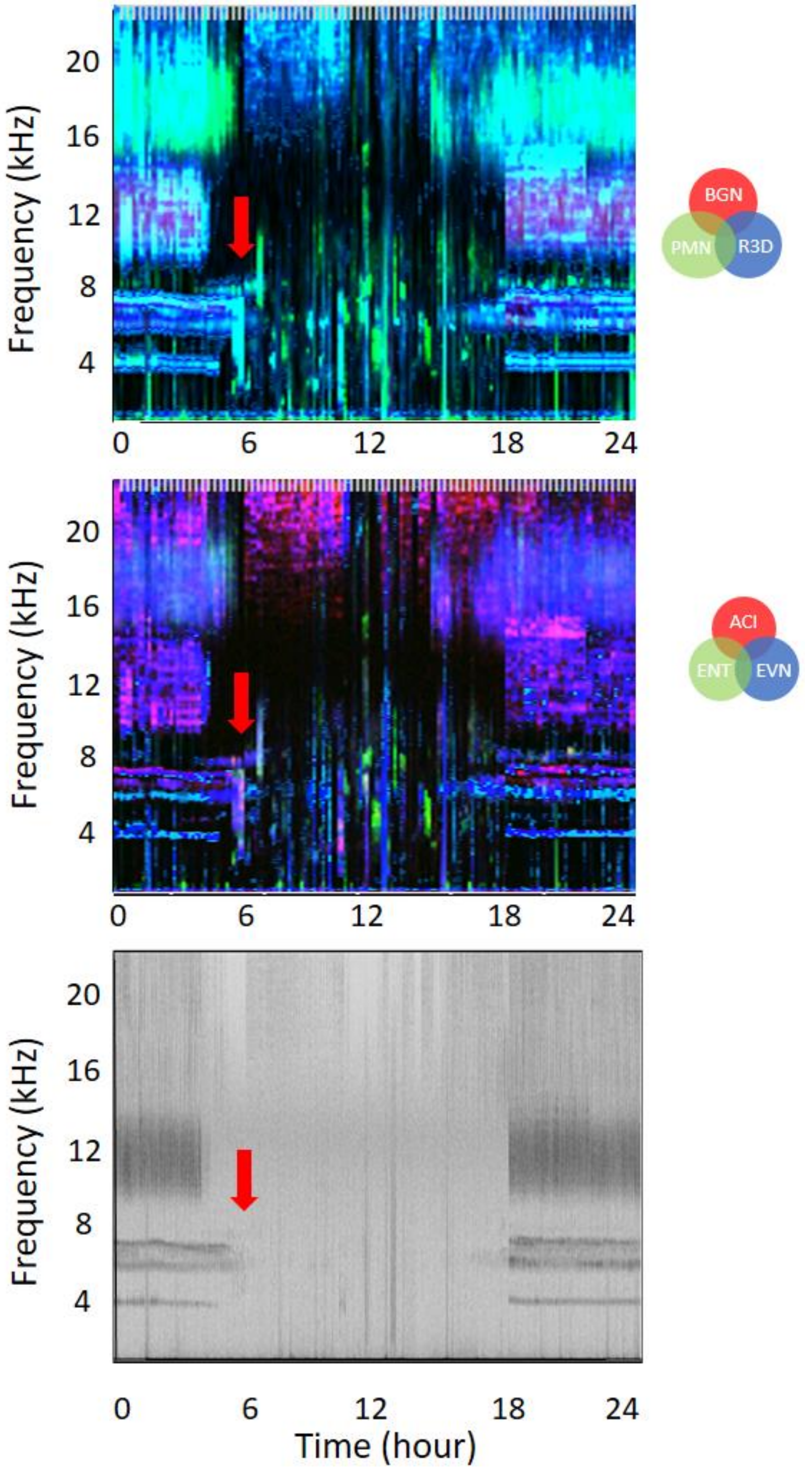


Figure 2 – 24-hour visualization, using one-minute samples every 15 minutes. A total of 96 files were used to compose these images, no gaps between them. On top, two False Colour Spectrograms, using three different indices to compose each of the two Red-Green-Blue visualizations. The indices and

colours used in each FCS are indicated beside each image. In the bottom, a greyscale spectrogram of the same period, FFT=1024, 50% overlap. Red arrows indicate the dawn chorus.

Although the bird activity is visible during twilight and daylight (from 04h30 to 17h30 approximately), the characteristics of the sounds produced by this group are not easily visualized and individualized with the FCS technique in our recording scheme (Figure 3). On the other hand, insects presented clear patterns of occupation of acoustic space and were better visualized in the FCS, since their vocalizations occupy narrower frequency bands with long durations. In a first look, it is even possible to estimate the number of sonotypes by counting different bands. The activity at night was dominated by Orthopterans, but it is also possible to identify Cicada's calls as horizontal bands during the day between 5 and 7 kHz in the rainy season (Figure 3).

The background noise representing the low intensity wind can be observed as the reddish colours (Figure 3, bottom FCS). Strong winds directly hitting the microphone as seen with a distinct pattern of green and were a limiting factor to the use of FCS during the dry season (vertical stripes on the FCS, Figure 4). On the other hand, this visual analysis makes it possible to detect a decrease in animals´ acoustic activity in the dry season compared to the rainy season.

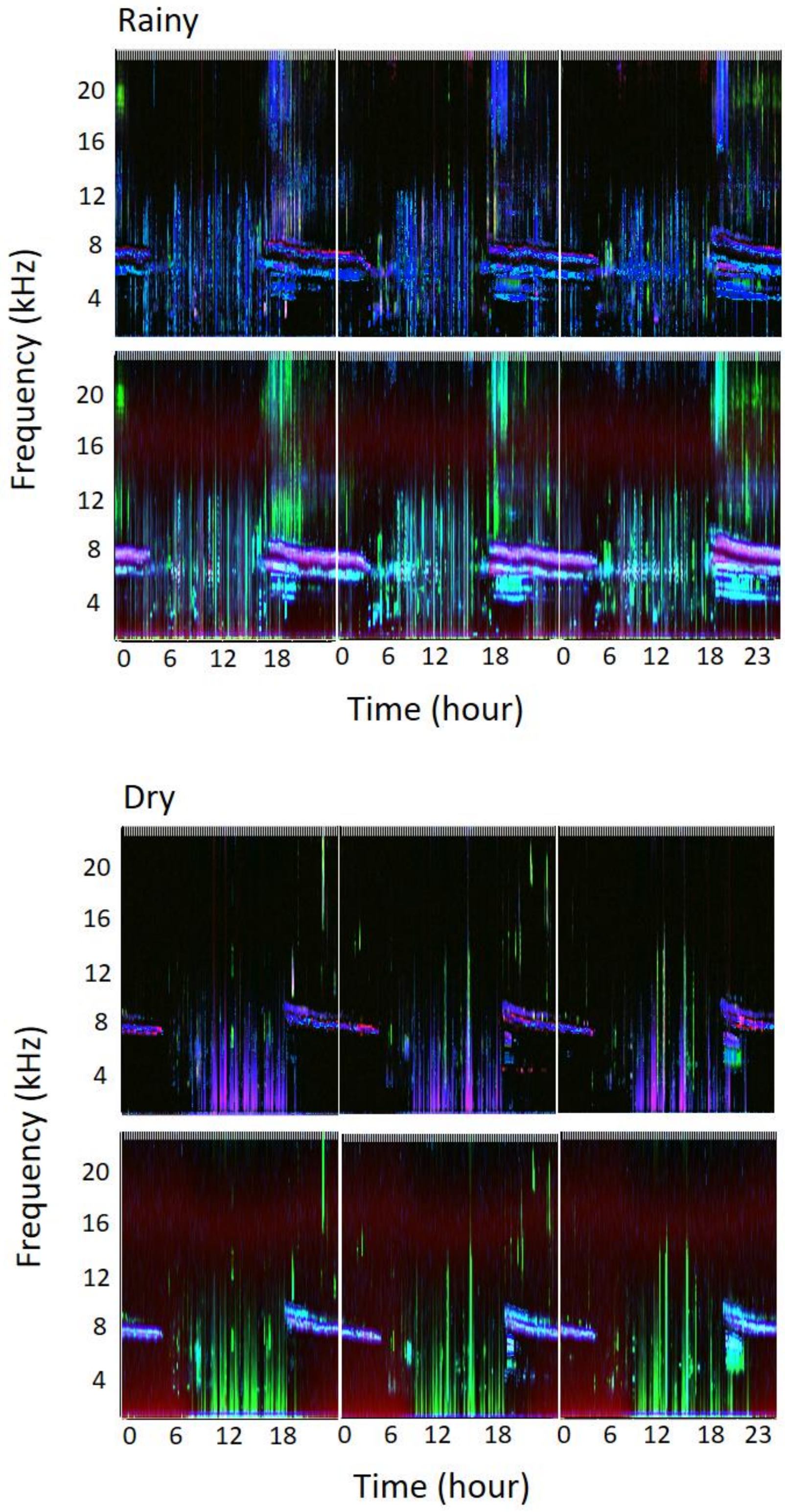


Figure 3 - Examples of 24-hour soundscape visualizations of a seasonally dry tropical forest at the Municipality of Lajes, Rio Grande do Norte State, Brazil using False Colour Spectrograms. Three days in the rainy season (top) and three days in the dry season (bottom). Top images (for both seasons) were made using the indices ACI-ENT-EVN. Bottom images (for both seasons) were made using the indices BGN-PMN-R3D.

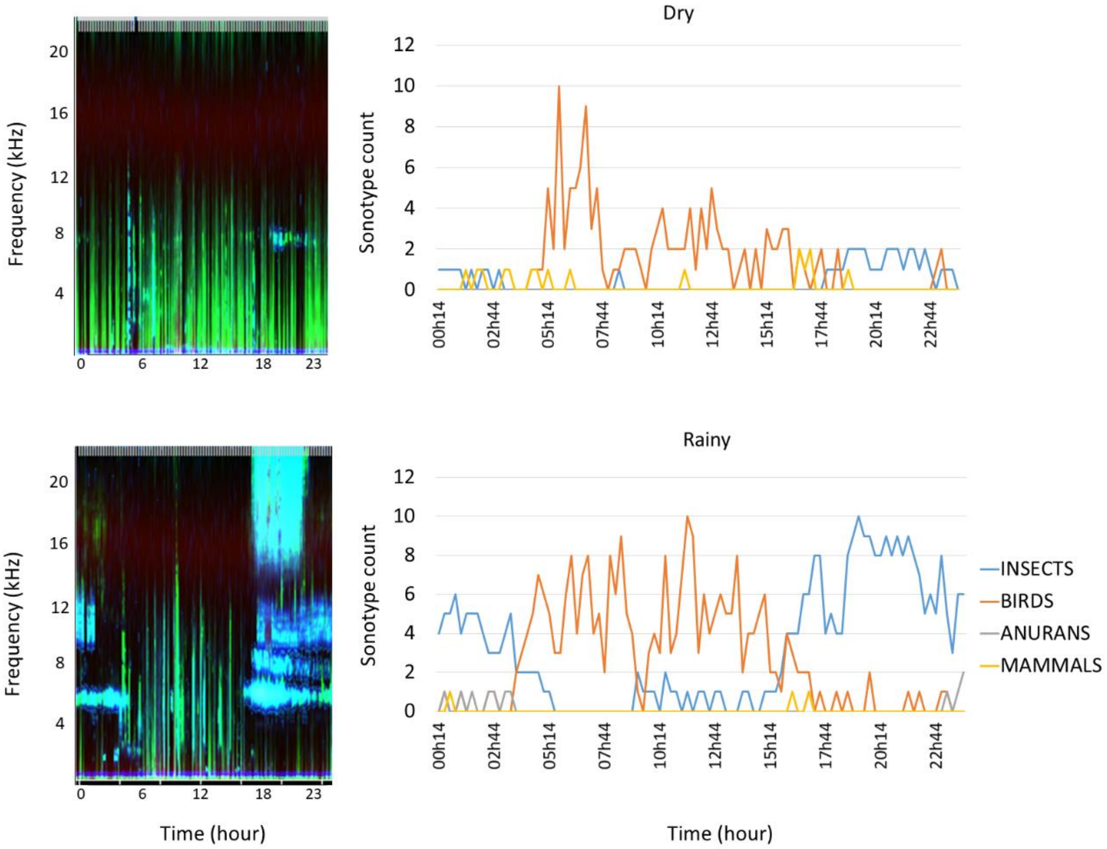


Figure 4 – Comparison between False Colour Spectrograms and graphs with the number of sonotypes manually identified for each animal category. Note that during the dry season the wind dominates the FCS visualizations and impairs inferences on acoustic activity diel fluctuations. FCS were built with the combination of BGN-PMN-R3D.

3.2. Data Clustering

The number of clusters labelled as each of the categories (Birds, Cicadas, Orthopterans, Quiet and Wind) can be seen on Table 2. The first part of the table lists only the clusters assigned to a single label. During the dry season, more than 70% of the clusters fit into this single label-category, but during the rainy season, the percentage drops to 50%. In both seasons, the clustering was more successful in grouping files with Wind, Orthopterans and with very low acoustic activity (Here referred as Quiet). The frequency of all categories, except Birds+Quiet, were significantly different between seasons.

Table 2 – Number of clusters and files assigned to each label. The clusters are divided in those assigned to only one label, to two labels and, three or more/inconsistent labels. Percentages are shown within parenthesis. Significant differences between seasons are indicated as *(p<0.001).

Label	Number of clusters	Files assigned (%)	
		Dry	Rainy
Birds *	1	0	637 (2.56)
Cicadas *	1	204 (0.82)	5 (0.03)
Orthopteran *	10	1793 (12.72)	6155 (24.8)
Quiet *	5	3691 (26.18)	2301 (9.27)
Wind *	8	4545 (32.24)	3358 (13.52)
Total	25	10233 (71.96)	12456 (50.6)
Birds+Orthopteran *	4	246 (1.74)	2246 (9.04)
Birds+Wind *	3	1749 (12.4)	1434 (5.77)
Birds+Quiet	1	317 (2.24)	455 (1.83)
Orthopteran +Cicadas *	2	225 (1.6)	770 (3.1)
Orthopteran + Quiet *	1	214 (1.51)	513 (2.06)
Orthopteran + Wind *	3	97 (0.68)	1970 (7.93)
Birds + Rain *	1	66 (0.46)	206 (0.84)
Wind + Rain *	1	0	831 (3.34)
Total	16	5828 (20.63)	8425 (34.22)
Birds+Orthopteran+Wind *	4	292 (2.07)	2043 (8.29)
Inconsistent *	5	856 (6.07)	1694 (6.82)
Total	9	1148 (8.1)	3737 (13.7)

The distribution of the clusters is shown in Figure 5. We chose to show only the files assigned to a single label, once the visualization of clusters assigned to multiple labels jeopardizes interpretation of the results due to the great number of different colours used. The clusters distribution grouped together the categories Wind and Quiet, as expected. Also, we labelled as Wind, files with different intensities of wind. Thus, files with presence of low wind could have been confounded with those labelled as Quiet.

Clusters labelled as Orthopterans varied in activity level, which resulted in the presence of this label in different groups of clusters. The group located in the upper right corresponded to the low activity level, mainly in the dry season, but also in late night hours in the rainy season (an UMAP with files coloured as dry and rainy season is shown in Figure A.3). Files labelled as Birds were segregated from most of the other files but presented some similarities with part of the files labelled as Orthopteran during the rainy season.

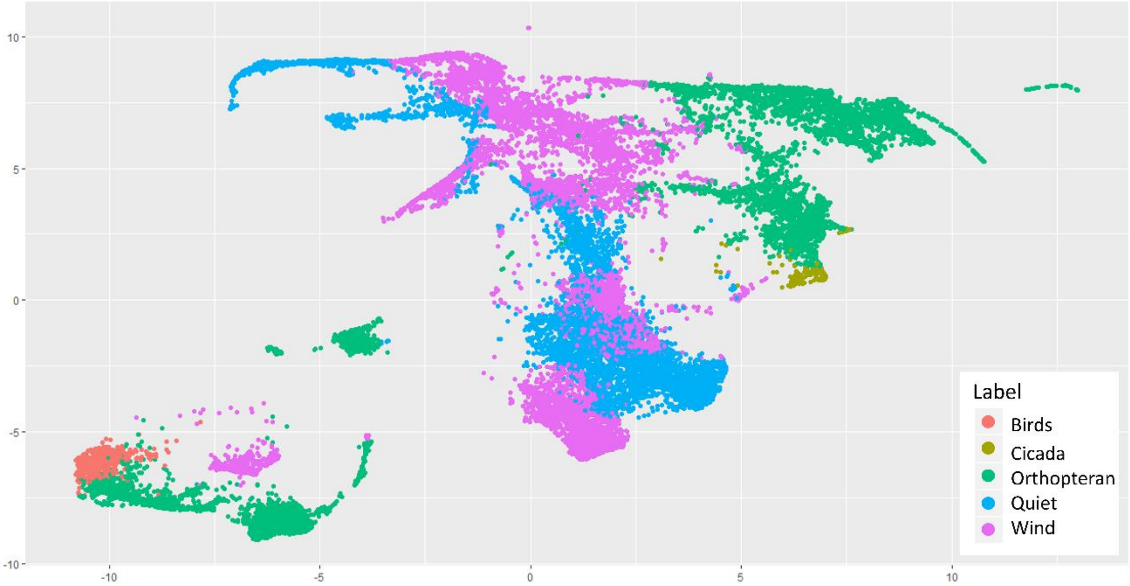


Figure 5 – Uniform Manifold Approximation and Projection for Dimension Reduction (UMAP) Clusters visualization. The colours represent single-label files.

3.3. Relationship between clusters and acoustic indices

The weight of indices in each cluster was explored using radar plots (Figure 6). Orthopteran clusters presented two different patterns, one linked to EPS (Entropy of Peaks Spectrum) and EAS (Entropy of Average Spectrum) and other highly influenced by HFC (High Frequency Cover). This last one reflects acoustic patterns of the Tettigoniidae family, which produce high frequency sounds (Montealegre-Z and Mason, 2005). The clusters labelled as Quiet, Wind, and Cicada presented high values of Background Noise (BGN). EPS, although important to describe Orthopteran activity, showed the highest values in the clusters labelled as Birds.

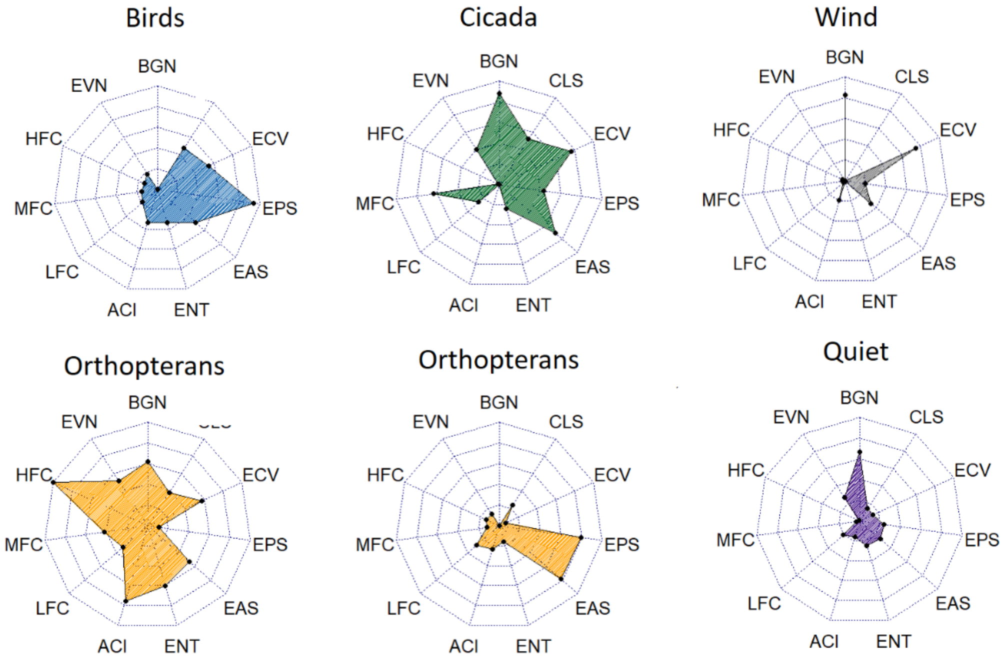


Figure 6 - Radar plots showing the indices weights in clusters associated with a single label. Here, the medoid values were used to achieve a better visualization. BGN (Background Noise), CLS (Cluster Count), ECV (Entropy of Coefficient of Variation), EPS (Entropy of Peaks Spectrum), EAS (Entropy of Average Spectrum), ENT (Temporal Entropy), ACI (Acoustic Complexity), LFC (Low Frequency Cover), MFC (Medium Frequency Cover), HFC (High Frequency Cover), EVN (Events per Second). More details about the indices are presented in Table 1.

3.4. Diel and Seasonal Patterns

Finally, we used two methods to investigate the diel patterns of our main biophonic sources contributing to the soundscapes (Figure 7) and how these patterns vary between seasons. In graphs A to D we used the number of files labelled to each animal taxa according to the clustering presented previously. Figure 7 shows similar general patterns of biophonic activity as those generated by manual identification (Figure 4). Figure 7 also shows that acoustic activity from insects and birds are almost mutually exclusive, showing little temporal overlap, markedly in the dry season. Figure 8 shows the temporal dynamics of three indices that are most representative in the clusters labelled as Birds (EPS), mid-frequency band Orthopteran (EAS) and high-frequency band Orthopteran (HFC). It is interesting to observe that Orthopterans belonging to the Tettigoniidae family (HFC) had a distinct temporal pattern compared to other Orthopterans, being active only at dusk in the rainy season. EPS has a strong association with both Birds and Orthopteran clusters. Therefore, the diel pattern of EPS is different between seasons, following the acoustic activity of Birds in the dry season, but also influenced by Orthopteran activity in the rainy season.

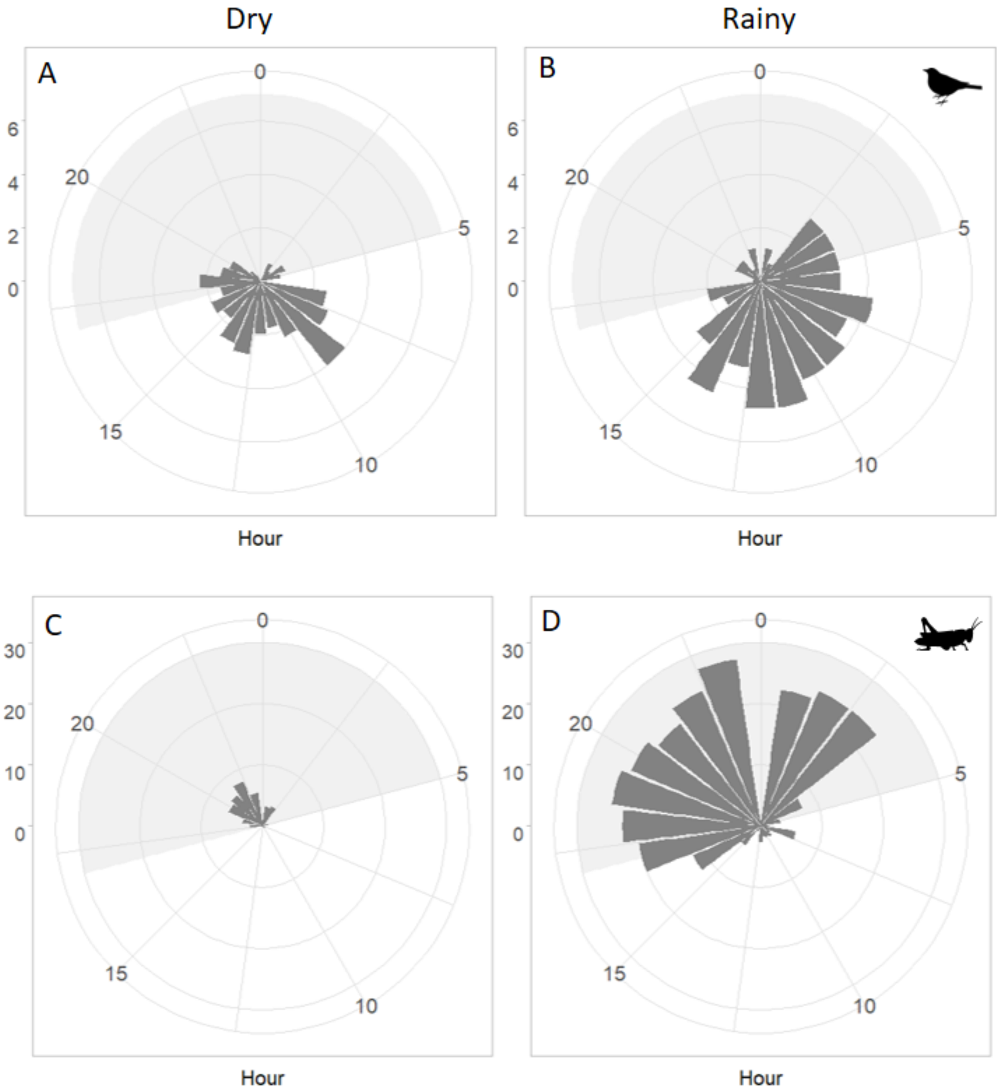


Figure 7 - Diel patterns (shaded area represents nigh time) of acoustic activity presence of the most representative groups (Orthopteran and Birds) during Dry and Rainy seasons. A

and B refer to files labelled as Birds, and C and D to the ones labelled as Orthopteran throughout 24 hours, using the average number of files of a subsample of five consecutive days from each of the four recorders.

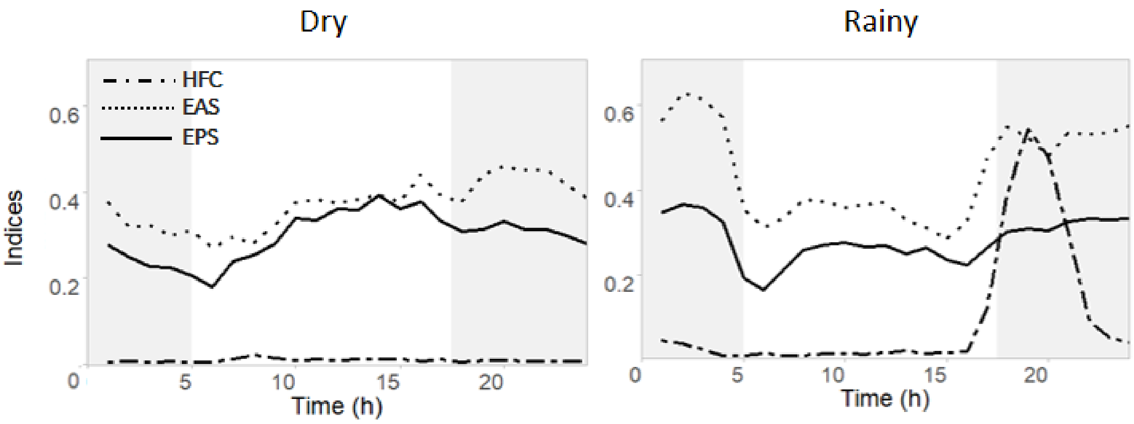


Figure 8 – Fluctuations in the mean value of three different indices along the 24 hours, each one associated to one biophonic group: EPS (Entropy of Spectral Peaks, associated to Birds), HFC (High frequency Cover, associated to Tettigoniidae orthopterans), and EAS (Entropy of Average Spectrum, associated to other Orthopteran families). We used the average of subsample of five days from four recorders.

4. Discussion

4.1. Acoustic Indices

Here, the use of multiple acoustic indices achieves good results in tracking the daily and seasonal activity patterns in the soundscape. Previous works in similar biomes using single acoustic indices were able to use them to find variation in the soundscapes and acoustic activity between seasons, forest cover (Rankin & Axel, 2018), impact from open mining (Duarte et al., 2015), and distance from roads (focusing on birds, Machado et al., 2017). With a focus on multiple taxa, four acoustic indices (Acoustic Diversity, Evenness, Entropy, and Normalized Difference Soundscape) were correlated with insects' richness, but the relationship with bird acoustic activity wasn't clear (Ferreira et al., 2018). Our approach uses a combination of indices to produce more reliable characterization of acoustic signals, allowing us to differentiate sound sources on the environment more precisely. The use of multiple indices has been suggested to increase the reliability of these metrics in environmental monitoring (Eldridge et al., 2018; Towsey et al., 2014).

4.2. Visualization through FCS

The use of False Colour Spectrograms has some advantages based on the human capabilities of rapidly processing visual information. FCS provide a quick and reliable look at the data in its entirety and show daily acoustic patterns, also enabling the identification of the main factors responsible for the Caatinga acoustic fingerprint and the variation between its two defined seasons: dry and rainy.

Visible acoustic activity patterns were different between seasons, especially when comparing the two main sources of biophony - birds and insects. These animals showed higher acoustic activity in the rainy season, as expected, considering the reproductive period of these groups is linked to the food and water availability during the wet season (Cavalcanti et al., 2016; Wolda, 1988). The observed temporal segregation in acoustic activity between these two faunal groups suggest temporal partition of the soundscape but we can also speculate about acoustic crypsis of insects during birds´ activity period, or acoustic avoidance (as coined by Curio, 1976). Acoustic avoidance may denounce a possible ecological prey-predator relationship and/or evolutionary processes of predation avoidance at play. Bird guilds pure-insectivorous, insectivorous-frugivorous, and insectivorous-granivorous account for 78% of all bird guilds in the region (Cavalcanti et al., 2016), which make this speculation plausible.

The wind was the main factor affecting data integrity and signal detectability, which was also expected. Wind is constant at the Serra do Feiticeiro, which also attracts Windfarms Companies to the region, where one of the biggest windmill complexes is expected to be built in the next few years. The wind (and resulting overload and clipping in the recordings) was more pronounced in the Dry season when vegetation structure - leafless trees and no herbs - allows wind to directly hit the microphones. Strong winds might also act as a masking agent of acoustic communication and can influence negatively in the decisions to call during windy periods, which would in turn affect the dynamics of the soundscape. Removing all those files was an option, but we believe that it would not realistically depict the sampled soundscapes. Additionally, we wanted to take on the challenge of investigating soundscape sampling in areas with open vegetation, as dry forests and savannas.

The potential to identify groups or even species using FCS was explored by Towsey et al. (2018) and Indraswari et al. (2018). Acoustic space partitioning among insect species is clearly observed in FCS of SDTF at a much greater temporal scale that usually done (Schmidt & Balakrishnan, 2014). While vertical stratification of insects assemblages is often observed in areas with high canopy (Jain & Balakrishnan, 2011), our area is dominated by shrubs and small trees. Therefore, we suggest that the main strategy for acoustic masking avoidance among insects is frequency partitioning. In this sense, the FCS can allow studies of acoustic niche partitioning at a broader scale.

To the best of our knowledge, this was the first study that employed FCS in non-continuous recordings and produced very satisfactory visualizations. The use of non-continuous recordings schemes has logistic advantages (i.e., reduction of costs related to data acquisition and storage, longer duration of passive acoustic monitoring efforts, Sousa-Lima et al., 2013). Disadvantages include the reduction in the sampling resolution and loss of opportunities to detect target species that are rare or less vocal. However, to make more general inferences and comparisons across soundscapes and their main sound sources, this methodology deemed successful.

4.3. Clustering evaluation

We evaluated how effective was clustering of the summary indices by listening to a sample of each cluster, examining the False Colour Spectrograms, UMAP visualization and investigation of daily patterns. Additionally, we determined how the main environmental sound sources affected the indices, inferring which index best predicts the occupation of acoustic space by each sound source.

Entropy of Spectral Peaks (EPS) is a measurement of concentration of spectral maxima in the mid-band (Towsey, 2017), in our case from 0.5 to 11 kHz, a frequency band occupied by Birds, but with influence from insect stridulation as well. The index presented higher values in clusters classified as containing acoustic activity from Birds but was also present in Orthopteran clusters. The 24h variation observed in Figure 8 is a reflex of this finding. In the dry season, when Orthopteran activity is low, the EPS values increase during the day, in a pattern that resembles the Birds Activity pattern. On the Rainy season, when Orthopteran are highly active, they appear as the main driver to both EPS and EAS daily variation.

Entropy of Average Spectrum (EAS) was highly correlated to Orthopteran acoustic activity. The index is a measure of the concentration of mean energy within the mid-band of the mean-energy spectrum. These insects were predominant within the mid-band, particularly at night, sometimes filling almost the entire mid-band frequency. Nonetheless, other clusters with high Orthopteran acoustic activity showed a different pattern, with high values of High Frequency Cover (HFC). This index is a measure of the energy concentration on high frequency bands (here, from 11-22 kHz), assumed to be indicating acoustic activity from Tettigoniidae. The Background Noise (BGN) was highly correlated with two of our cluster categories, Quiet and Wind. This index is calculated from the energy distribution in the waveform envelope and was expected to be high on files with low (or no) biophony.

Within the same taxa some similarities in the sound characteristics are expected, reflex of physiological constraints, such as sound production mechanisms. For example, all around the world, most orthopterans will produce sound by stridulating (Montealegre-Z & Mason, 2005; Riede, 1998), resulting in similar sound patterns. On the other hand, acoustic communities are highly dependent on their species composition and other environmental factors that may lead to a change in the use of acoustic space. Sound sources identified in our work didn't affect the summary indices the same way that the ones identified by Phillips et al. (2018) in two Australian forests.

Compared to Phillips et al. (2018), insect clusters also presented high values of EAS, but the authors did not find the HFC related to these animals. Besides the differences in insect assembly, this can also be a result of the sampling frequency used and the values applied to be considered as Low, Medium and High frequency in calculations. The HFC was more related to cicada activity. The bird clusters also presented differences, being more related to MFC, LFC and even ACI, while in our work, was more related to EPS.

4.4. Insects

The disproportional occupation of acoustic space by insects in our data brings to our attention the importance of considering this group in ecoacoustics studies. Especially in semi-arid environments, insects represent an important food source for other animals and, although there is a consensus that they are good bioindicators (Brown, 1991), there is limited evidence to support this assumption.

In humid tropical environments, there is almost no variation between the insect abundance through the seasons, but a reduction can be observed in tropical environments with a severe dry season, as the Brazilian SDTF. This reduction can be caused by several factors as stress related to food decline, which can lead to adaptations that include dormancy, diapause or migration (Pinheiro et al., 2002).

The use of acoustic monitoring has the potential to become a key tool in the study of insects communities (Lehmann et al., 2014), even accelerating the discovery of new species. Besides that, there is a lack of consistent and reliable acoustic libraries for the Insect group. For example, there are at least ten thousand Orthoptera species that produce sound as a form of communication, but only 10% of those are included in digital acoustic libraries (Riede, 2017).

The temporal vocalization patterns in Orthoptera are variable and dependent on environmental features - especially humidity and temperature. These patterns can be continuous through the day, have peaks on dusk , peaks on dawn, only during the night, only during the daylight (Fischer et al., 1996). In tropical regions, it is common to have a nocturnal pattern, as observed in our Caatinga data. Predator avoidance is probably one of the major factors leading to this acoustic pattern. Orthopteran species living in open vegetation (as the SDTF) are easily visualized by birds, thus are more nocturnal, in comparison to species living in dense vegetation (Robinson & Hall, 2002). Changes in frequency features related to temperature changes through the night were also visible in our FCS. This phenomenon was observed by Phillips et al. (2018). The temperature does not affect the frequency directly, but the wave sound speed in the medium (Endler et al., 2000).

The limitation in the use of passive acoustic monitoring to study insects relies on the fact that information degradation in these animals can occur at short distances. The interval between pulses in orthopterans calls is an important information which could be lost in distances as short as 2 meters, once the echoes derived from the signals fill the spaces between them (Simmons, 1988). When we explore the greyscale spectrograms of our data, we observe that only part of the orthopteran signals presents resolution enough to make posterior comparisons with databases to identify species.

4.5. General conclusions about the use of PAM in SDTF

The use of Passive Acoustic Monitoring is a relatively new technique applied to terrestrial environments and there are important open questions to be solved. The relative distance between sampled sites depends on the recording system sensitivity, but also on the signal recorded. Here we include constraints as amplitude, frequency range, position of emitter, vegetation, topography, temperature, and humidity (Farcas et al., 2016). An effective sampling relies, thus, on the coverage of different species, their vocal characteristics, and should also vary according to time and space (Gibb et al., 2018).

The development of the acoustic ecology field with improved processing and analyses of time series can provide much information about the environment to managers, ecologists and conservationists (Burivalova et al., 2019; Almo Farina & Gage, 2018). We believe that such efforts are necessary in regions with low scientific knowledge and low financial investment in research and conservation, as the Brazilian Caatinga (Santos et al., 2011) and other tropical dry forests.

Our main challenges working with acoustic data in a dry forest was the constant presence of wind, especially in the dry season, when vegetation loses foliage. Also, the strong presence of insects associated with the relatively low abundance of birds compared to other tropical regions (again, more evident on the dry season) steers our attention to this group, that is still left aside in most ecoacoustics studies (Aide et al., 2017). Some acoustic indices are dependent on the frequency band selected for inclusion in the calculations (i.e., the frequency range is adjustable) which can bias the interpretation of the soundscape. We highlight the importance of considering the insects in both experimental design and analyses. Despite these challenges, our approach was able to track seasonal changes and patterns of animal activity, reflected in the use of acoustic space.

This paper represents a contribution to a better understanding of the soundscape dynamic in a highly seasonal tropical environment. Soundscape ecology and its translation into acoustic indices to study ecological processes in tropical environments is still very incipient. Seasonally dry tropical forests (SDTF) are globally threatened and receive less economic intake in conservation initiatives when compared to other tropical environments (Dirzo et al., 1995; Santos et al., 2011), which makes even more important that fast and cheap methodologies to study biodiversity are explored.

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6. Appendices

Figure A.1 – Examples of areas sampled. Note the differences in top photos, taken at the same place during rainy and dry seasons.

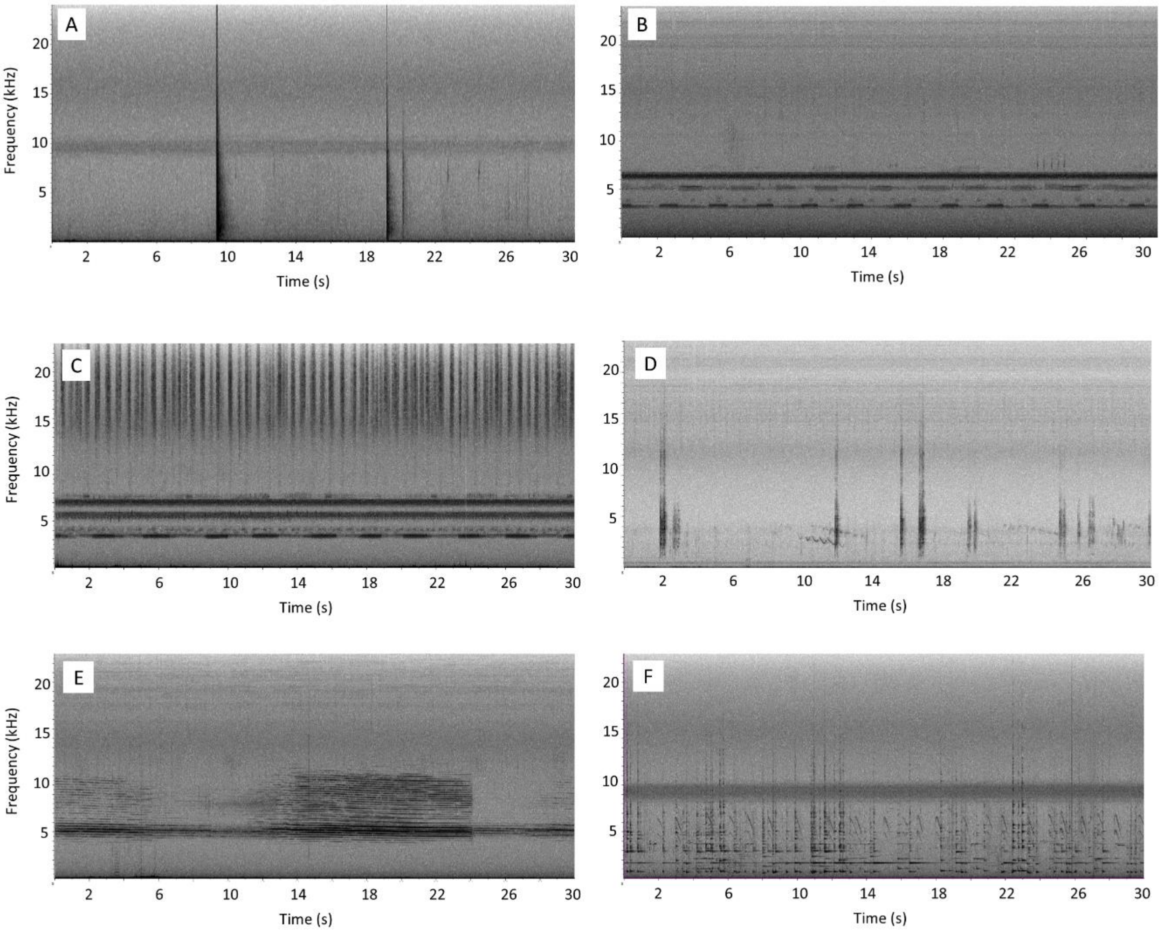

Figure A.2 – Grey scale spectrograms of six distinct acoustic patterns: Gunshots (A), Orthoptera (B), Orthoptera with high frequency band stridulation (C), Birds (D), Cicada (E) and Bell (F).

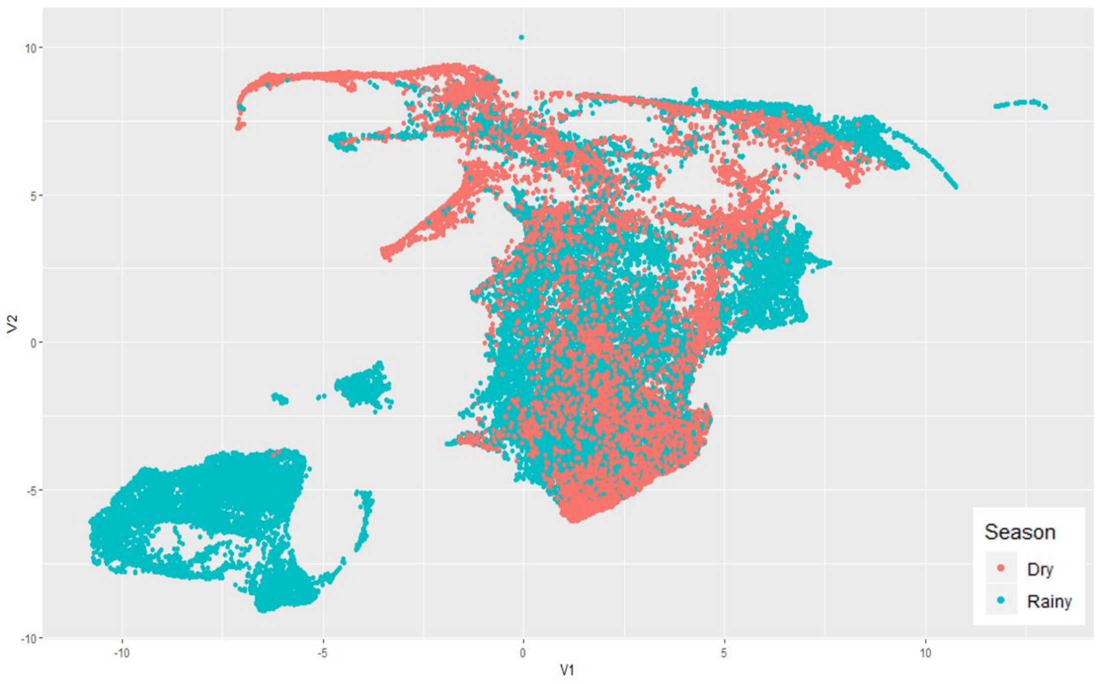

Figure A.3 – Uniform Manifold Approximation and Projection for Dimension Reduction (UMAP) Clusters visualization. The colours represent files recorded in dry an rainy season.

Table A.1. General description of sampling sites used in the study. *Please note the region has an unpredictable rain regime and intermittent rivers that may flow once in every five years. To measure the nearest water body, we considered only the ones that have water most part of the year (in most cases, human-made reservoirs).

Point	Coordinates	Habitat	Elevation (m)	Nearest road (m)	Nearest water body (m)*
1	5°47'2.1"S 36°8'58.1"O	Rocky ground, vegetation composed mostly by shrubs and cactus.	224	600	2900
2	5°47'42.1"S 36° 8'3.0"O	Terrain inclination around 30°, SM installed in a hill, exposed to wind guts. Vegetation include trees and shrubs.	268	650	2100
3	5°47'49.5"S 36°8'19.9"O	Close to a dry water course, Terrain inclination around 30°. Vegetation composed of trees, bromeliads, and cactus. Presence of large rocks, used as shelter by bats, small mammals and lizards.	257	600	2300
4	5°46'19.6"S 36°9'38.7"O	Open area, used as cotton plantation in the past, in regeneration. Vegetation dominated by shrubs, herbs and cactus.	223	320	1230
5	5°49'56.20"S 36°12'10.3"O	Human made reservoir. As one of the largest in the region, it contains water most part of the year. Poaching is common during the dry season in the area.	303	130	50
6	5°47'38.1"S 36° 8'17.6"O	Area dominated by herbs and sparse trees.	243	30	2200
7	5°47'48.4"S 36°14'25.6"O	SM positioned on top of a cave, used by bat colonies and other small mammal species and lizards. Vegetation is mostly composed by shrubs and small trees.	325	100	2200

Table A.2. Correlation Matrix of fourteen indices for the complete dataset. Pearson's Correlation > 0.75 are in bold. The eleven indices used after removal of the high correlated ones are presented in bold.

	BGN	SNR	ACT	**EVN**	**HFC**	**MFC**	**LFC**	**ACI**	**ENT**	**EAS**	**EPS**	**ECV**	**CLS**	SPD
BGN	1	0.420	0.254	0.205	0.342	0.313	0.313	0.120	0.370	0.071	0.523	0.137	0.386	0.398
SNR	0.420	1	0.792	0.733	0.154	0.431	0.810	0.181	0.895	0.220	0.186	0.116	0.558	0.314
ACT	0.254	**0.792**	1	0.828	0.172	0.449	0.866	0.140	0.618	0.177	0.200	0.121	0.456	0.330
EVN	0.205	0.733	**0.828**	1	0.099	0.256	0.747	0.077	0.634	0.125	0.091	0.119	0.389	0.188
HFC	0.342	0.154	0.172	0.099	1	0.520	0.196	0.319	0.107	0.040	0.136	0.029	0.423	0.878
MFC	0.313	0.431	0.449	0.256	0.520	1	0.526	0.398	0.375	0.017	0.248	0.043	0.611	0.837
LFC	0.313	**0.810**	**0.866**	0.747	0.196	0.526	1	0.175	0.708	0.153	0.239	0.100	0.496	0.388
ACI	0.120	0.181	0.140	0.077	0.319	0.398	0.175	1	0.173	0.044	0.041	0.050	0.231	0.365
ENT	0.370	**0.895**	0.618	0.634	0.107	0.375	0.708	0.173	1	0.202	0.131	0.113	0.498	0.256
EAS	0.071	0.220	0.177	0.125	0.040	0.017	0.153	0.044	0.202	1	0.411	0.524	0.130	0.033
EPS	0.523	0.186	0.200	0.091	0.136	0.248	0.239	0.041	0.131	0.411	1	0.095	0.247	0.261
ECV	0.137	0.116	0.121	0.119	0.029	0.043	0.100	0.050	0.113	0.524	0.095	1	0.035	0.045
CLS	0.386	0.558	0.456	0.389	0.423	0.611	0.496	0.231	0.498	0.130	0.247	0.035	1	0.639
SPD	0.398	0.314	0.330	0.188	**0.878**	**0.837**	0.388	0.365	0.256	0.033	0.261	0.045	0.639	1

CAPÍTULO 2

WIND FARM INFLUENCE ON THE SOUNDSCAPE OF A SEASONALLY DRY TROPICAL FOREST

Eliziane G. Oliveira, Milton Cezar Ribeiro, Paul Roe,
Renata S. Sousa-Lima

Wind farm influence on the soundscape of a seasonally dry tropical forest

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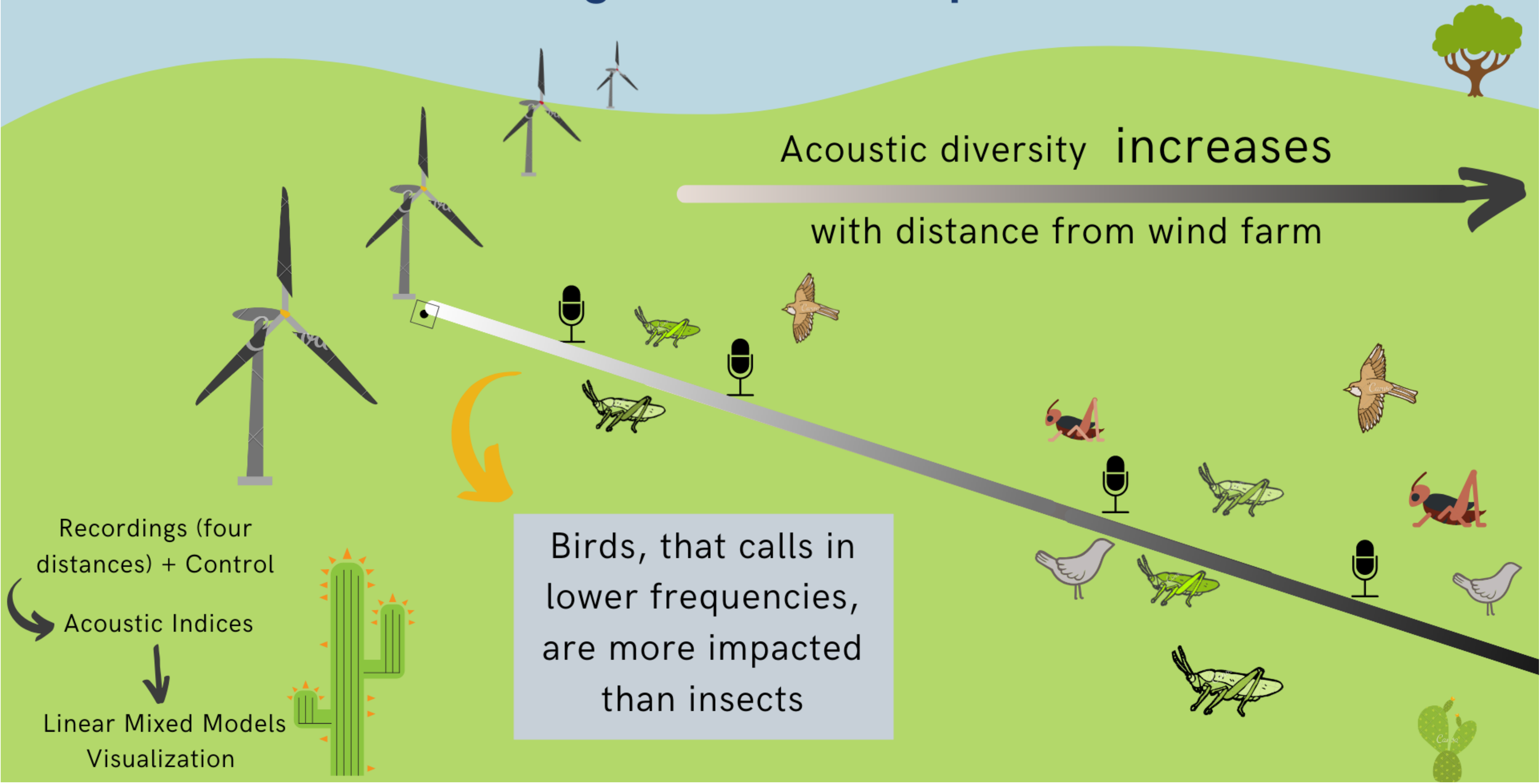
Planning to submit to "Science of the Total Environment"

How do wind farms change the soundscape?



Acoustic diversity increases

with distance from wind farm

Birds, that calls in lower frequencies, are more impacted than insects

Recordings (four distances) + Control

Acoustic Indices

Linear Mixed Models Visualization

Abstract

Passive acoustic monitoring of terrestrial environments can detect soundscape changes in temporal and spatial scales, but in tropical environments, its applicability is still limited due to the complexity of these natural systems. Most tropical systems are currently under anthropogenic influence. Here we addressed wind farm effects on a seasonally dry tropical forest in Northeastern Brazil. We aimed to understand how the sound energy distribution and acoustic diversity vary as a function of distance from wind farm turbines. We sampled seven points, four in the wind farm area, and three in a control area. Soundscape composition was described in terms of temporal and spectral patterns, using False Colour Spectrograms (FCS) and Spectral Probability Density (SPD) visualization, respectively. Two acoustic indices (Temporal Entropy and Signal to Noise Ratio) were used as response variables in Linear Mixed Models to verify if the changes in the use of acoustic space were related to the distance from the turbines. Soundscape samples were chosen to capture the activity of birds (daytime) and insects (nighttime). As we move closer to the turbines, the biophonic activity decreases, especially in the lower frequencies. Distance from turbines affected the variation in acoustic indices' values both in the morning and at night. Our findings suggest that both birds and insects have their acoustic activity patterns modified in response to wind turbines presence, denouncing that the effect of wind farming on natural soundscapes is evident at small spatial scales.

Keywords: Acoustic impact, Ecoacoustics, Wind Energy, Acoustic Indices, Acoustic monitoring

1. Introduction

Acoustic signals are plastic and can be adjusted and modified in short periods of time according to environmental conditions (Parks et al., 2011). As a result, changes in land use can rapidly be detected acoustically by identifying loss in species richness or changes in vocalization patterns (Farina, 2014; Zwart et al., 2016). Hence, variations of temporal and spatial patterns on animal diversity can be captured non-invasively with passive acoustic monitoring (Depraetere et al., 2012; Duarte et al., 2015).

Bioacoustic and soundscape analyses are powerful tools to monitor biodiversity in tropical forests (Fuller et al., 2015; Tucker et al., 2014) since such complex systems require more sampling effort using traditional methods. Soundscape analyses have been proposed as a mean to inform decision making in environmental policies (Doser et al., 2019), but few studies address its applicability and evaluate to which extent it can be used, especially in tropical environments (Burivalova et al., 2019). This acoustic approach can detect several taxonomic groups at variable distances depending on the signal and the environmental conditions (Darras et al., 2016).

Eolic energy farming is a growing industry because it harvests a renewable energy source that is assumed to have low environmental impact. Environmental impact assessments indicate that the most affected taxa in terrestrial wind farm facilities are birds and bats (Anderson et al., 1999; Kunz et al., 2007). Nonetheless, most studies focus on only a single group or species and are biased towards direct mortality caused by collision with the turbines (de Lucas et al., 2012; Drake et al., 2015; Smallwood et al., 2009).

One additional problem associated with wind farming documented in the literature is the acoustic impact (Rabin et al., 2006; Zwart et al., 2016). Anthropogenic sound pollution is usually concentrated in lower frequency bands and can contaminate natural areas, even in the most remote places that are rarely monitored (Reed et al., 2012; Wrightson, 1999). Noise pollution can overlap with animal calls, affecting sound perception by individuals, impairing signal detection and causing behavioural alterations in receivers and senders, such as potentially costly vocal adjustments (Patricelli & Blickley, 2006; Reed et al., 2012; Slabbekoorn & den Boer-Visser, 2006). When exposure to noise is chronic, effects go beyond acoustic communication, and can induce changes in spatial distribution or reproductive traits in several species (Barber et al., 2010; Kunc & Schmidt, 2019). Although noise pollution in terrestrial habitats has been reported mainly for vertebrates (Shannon et al., 2016), there is an increasing interest in the study of its impact on insects (Duarte et al., 2019), especially in tropical habitats, where insects are most representative (Aide et al., 2017).

This work was motivated by the necessity of better understanding the relationship between wind farm facilities and the acoustic community around it. Specifically, we aimed to answer the following questions: (1) How does the sound energy distribution vary along a distance gradient from turbines? (2) Do acoustic indices capture the variation observed along this gradient? (3) How can we use these measurements to infer impacts on animals living around the turbines?

We hypothesize that (1) sound energy (amplitude) would have high values distributed in more frequency bands in the most distant points from the noise source (turbines); (2) Signal to Noise Ratio (SNR) and Temporal Entropy Index (ENT) will capture the changes in soundscape along a distance (SNR in the amount of acoustic energy proportional to the amplitude of noise and ENT will be able to capture changes in the amplitude of short duration signals, e.g. bird calls); (3) animals with sound signals concentrated in lower frequencies in the spectrum would be more impacted, due to overlap of calls and noise in the same frequencies. To the best of our knowledge, this is the first study applying acoustic ecology to address wind farming effects and testing the application of acoustic monitoring for evaluating wind farm ecological effects in tropical environments.

2. Methodology

2.1. Study sites

The wind farm facility sampled in this study is located in Jandaíra, Rio Grande do Norte State, Northeast Brazil (05° 18' 30.1" S, 36° 02' 31.4" W). The majority of this area is characterized by abandoned pastures, in varying stages of natural regeneration. Recorders were installed in a fragment where vegetation structure and plant species composition still resemble the original vegetation in the area.

The area used as control is a preserved area located in the municipality of Lajes, Rio Grande do Norte State (05° 47' 42.1" S, 36° 08' 03.0" W). The vegetation structure and topography of the area are different from the wind farm, but previous inspection of the recordings revealed a similarity in the use of acoustic space and general acoustic activity patterns (see False Colour Spectrograms section for details).

2.2. Recordings

Data collection started at the end of the rainy season in June 2017. We used five SM3 (Song Meter SM3®, Wildlife Acoustics, Inc., Concord, Massachusetts), recording for 26 days at a sampling rate of 48 kHz. One of the recorders failed and the data was not used for further analyses. We placed the SM3 recorders along a 1 km line perpendicular from the turbines, spaced 200 meters between them to ensure the independence of sampling points (Pieretti et al., 2015) (Figure 1). As a control, two recorders were placed in a more preserved area, with six km of distance between them (Figure 1).

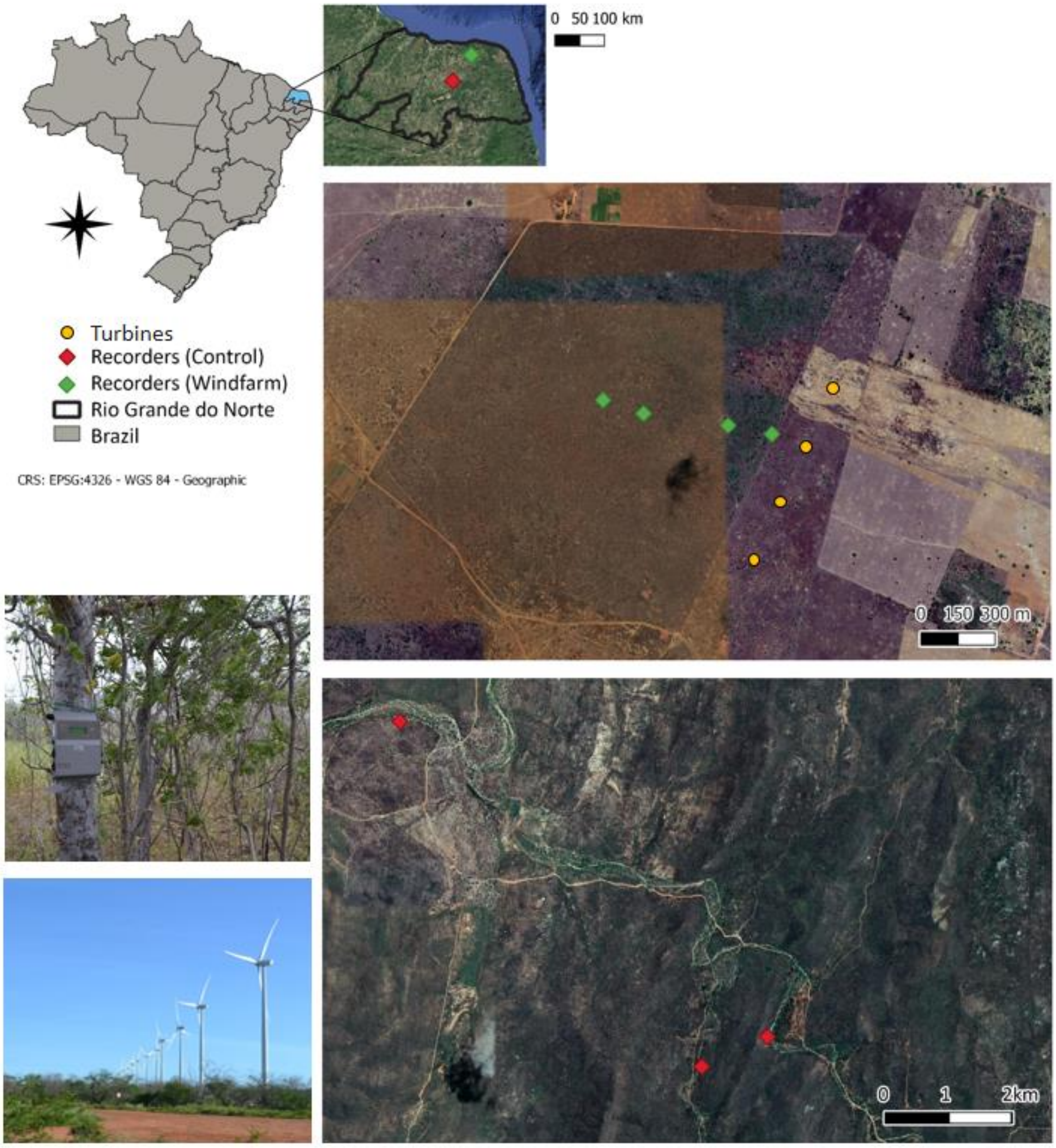


Figure 1 – Location of recorders in the two study areas. On top, the wind farm area, within the municipality of Jandaíra, Rio Grande do Norte State (RN, Brazil), and on the bottom, the control area, within the municipality of Lajes, RN, Brazil. In site photos on the left are from wind farm area and show a SongMeter 3 recorder and the wind turbines.

2.3. Characterization of species and sonotypes

The number of bird and insect sonotypes was measured in a subsample of 300 minutes, randomly extracted from morning (05h00 to 07h00) and evening periods (18h00 to 20h00) at the most distant point in the wind farm area. A sonotype can be described as a distinct note or series of notes that represents one type of vocalization of a species (Aide et al., 2017; Ferreira et al., 2018). We chose to use sonotypes because it is difficult to identify insects to the level of species. Two ornithologists identified the bird species. From this sound database, we extracted the values of dominant frequency of all acoustic signals. A minimum of 10 samples for each species/sonotypes were used.

2.4. Sound level distribution

We generated sound level distributions over the frequency spectrum using Spectral Probability Density (SPD) analyses (Merchant et al., 2013). Though there are several averaging methods for ambient noise spectra, the most common and the one we used here, is the RMS level, where the mean is computed before it is converted to dB. We used the PAMGuide function for MatLab R2018, following Merchant et al. (2015). The parameters were set to default, except Linear scale, Gain of 24 dB, zero-to-peak voltage of the analogue-to-digital converter (vADC) of 1.414.

2.5. Acoustic Indices

To inspect the differences along the distance gradient from the turbines, we used two subsets of data. One, focusing on bird species, with data from 05h00 to 08h00. Other, focusing on insects, with data from 18h00 to 20h00. In both of them, minutes were sampled in a scheme of 1 minute every 15 minutes (Pieretti et al., 2015). Here, we used 15 days of recordings, the same days were sampled in both control and wind farm areas.

We use the open software QUT Ecoacoustics Analysis Program (Towsey et al., 2016) to calculate the acoustic indices. Six spectral indices were used to produce the False Colour Spectrograms (ACI, ENT, EVN, BGN, PMN, R3D see Table 1). Based on inspection of the values distribution of all acoustic indices, we decided to use Temporal Entropy (ENT) and Signal to Noise Ratio (SNR) to verify the differences between recording points (see Statistical Analysis for details). The ENT was used in both spectral and summary calculations. The difference between them is that, for the spectral indices, values are assigned to each frequency band. In the summary calculations, indices' values were calculated to produce a single value for each 1-minute sample.

Table 1. Description of acoustic indices used. For details about calculations, see references. The faunal groups (Birds or Insects) to which the indices are more correlated are indicated between parentheses.

BGN	Background Noise (Towsey, 2017): Noise profile calculated from the decibel waveform.
ENT	Temporal Entropy (Towsey, 2017): Measurement of acoustic energy concentrated in each frequency bin (Spectral Temporal Entropy), across the wave envelope. It is a good measure of signals that conentrate energy in short periods, such as birds.
EVN	Events per Second (Towsey, 2017): Average number of times the decibel envelope crosses a BGN +3 dB threshold, per second.
ACI	Acoustic Complexity Index (Pieretti et al., 2011): Originally developed to reflect bird activity, excluding constant and low frequency sounds (like human generated noise). Very sensible to other sound sources, like rain.
PMN	Power minus Noise (Towsey, 2017): The maximum decibel value in each frequency bin of the noise- reduced decibel spectrogram.
R3D	Three Ridge Indices (Towsey, 2017): Combination of three other Ridge Indices (Horizontal, Vertical, Downward Slope). The ridge indices attempt to detect the harmonic structure presented in calls.
SNR	Signal to Noise ratio (Towsey, 2017): Difference between the BGN value and the maximum value in the decibel envelope.

We used the acoustic indices to build False Colour Spectrograms (FCS) (Towsey et al., 2014), in order to visualize the acoustic space occupation and daily patterns of animal groups. The FCS images are the result of a combination of three indices, represented by three colours (red-green-blue). Each of these three indices chosen detects part of the information within the recording. We used the following indices to generate two different FCS: ACI-ENT-EVN and BGN-PMN-R3D.

2.6. Statistical Analyses

We used R (R Core Team, 2018) and *lm4* (Bates, Mächler, Bolker, & Walker, 2015) to perform a linear mixed effects analysis of the relationship between acoustic index and distance from turbines. Distance from turbines was entered as fixed effect. As random effects, we included area (windfarm and control) and time (day per sampling point). Visual inspection of residual plots did not reveal deviations from normality or homoscedasticity. P-values were obtained by likelihood ratio tests of the full model with distance against the null model.

3. Results

3.1. Signals' characterization

We obtained a total of 630 hours of recordings, including control and wind farm areas, and the sampling of continuous one-hour recordings during dawn (5h30 to 06h30) and dusk (17h30 to 18h30) periods. Continuous recordings were used to build False Colour Spectrograms and to identify bird species and insects sonotypes (in subsamples – 300 minutes total). In the wind farm area, we identified a total of 29 bird species (presented in Supplementary Material, Table S1). Bird vocalizations presented dominant frequency mean value of 3246.8 Hz (varying from 515 to 7448.9 Hz) and insect sounds presented a mean value of 7938 Hz (varying from 3188 to 18797 Hz) (Figure 2).

Figure 2 – Boxplot showing distribution of dominant frequencies values for birds and insects, detected in recordings at dawn and dusk hours close to a wind farm facility, municipality of Jandaíra, Rio Grande do Norte state, Brazil.

3.2. Sound level distribution

To visualize the energy distribution along the frequency bands, we generated a Spectral Probability Density (SPD) plot (Figure 3). The SPD plots show the empirical probability density of sound levels in each frequency band.

The general pattern of the SPD for the wind farm area presents two peaks around 5 kHz e 10 kHz (Figure 3). At the closest point to the turbines, activity around 5 kHz is less evident than in the other sample points. This is the frequency band most used by birds but is also occupied by insects (Figure 2). The frequencies around 10 kHz, on the other hand, are used almost exclusively by insects, and mainly of the signals come from one Orthoptera group – the katidids (Figure S1). Around 5 kHz we can visualize another difference among sample sites: while in the point at 100 meters there is only one peak in the RMS values (pink line, examples are pointed with red arrows), the others shown two or even three peaks, suggesting a higher diversity in biophonic signals (probably due to diverse insect activity).

The control area, represented in Figure 3, shows a different pattern, with no prominent peak between 10 and 14 kHz. To compare, we inspected the spectrograms (Figure S1) and detected the constant presence of one Orthopteran sonotype in the wind farm areas at night occupying these higher frequency bands. It is also interesting to note the differences in RMS peaks between 2 and 7 kHz between wind farm and control areas. The control area presented several peaks, and one likely explanation for this is higher diversity of sonotypes occupying those frequency bins (Figure S1).

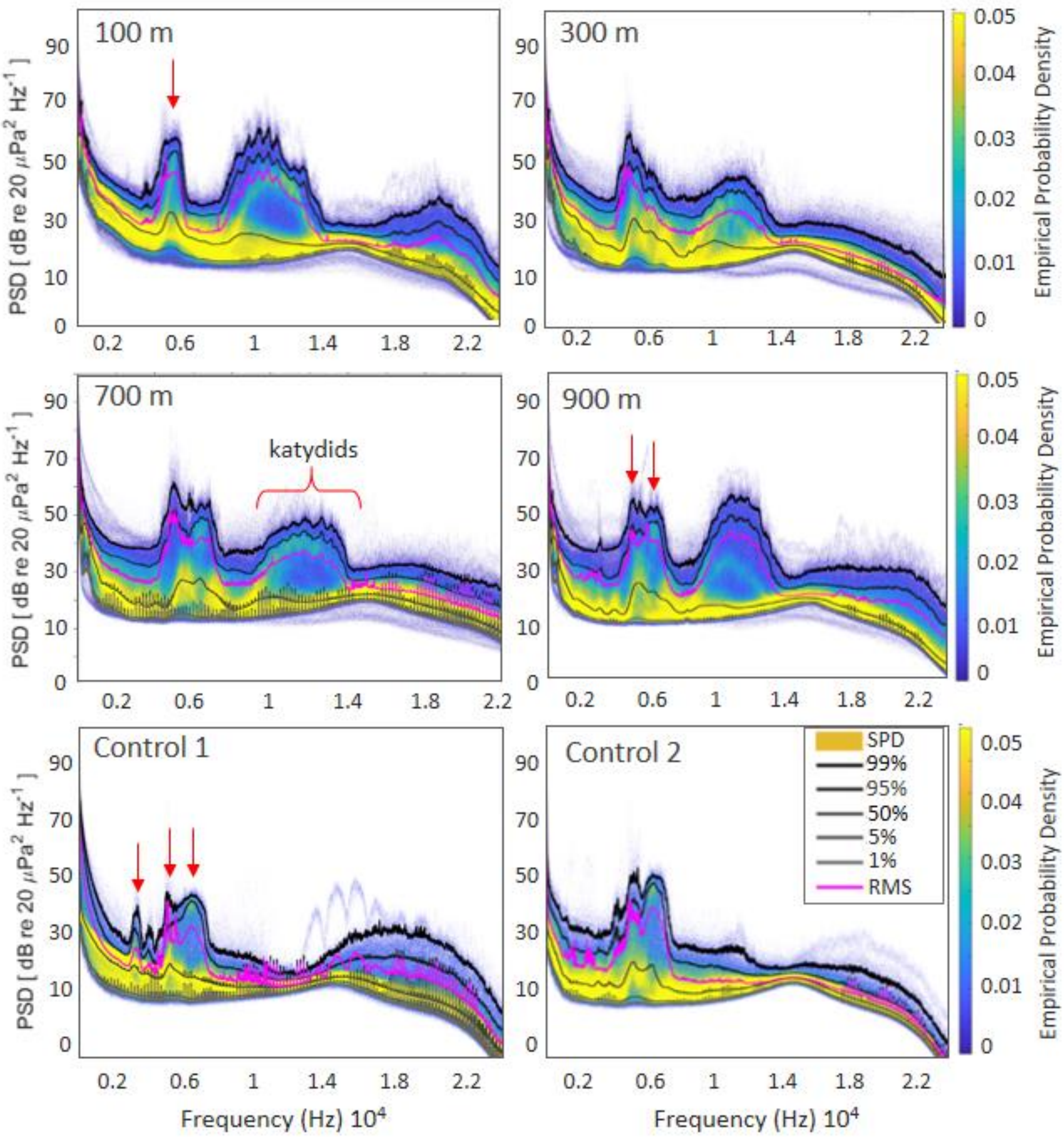


Figure 3 - RMS (Root Mean Square) level of the PSD (Power Spectrum Density), percentiles and SPD (spectral probability density) of four points in the wind farm area (distances from the turbines as it shows). The control area is represented by Control 1 and 2. Red arrows indicate examples of peaks and katydid's activity.

3.3. False colour spectrograms

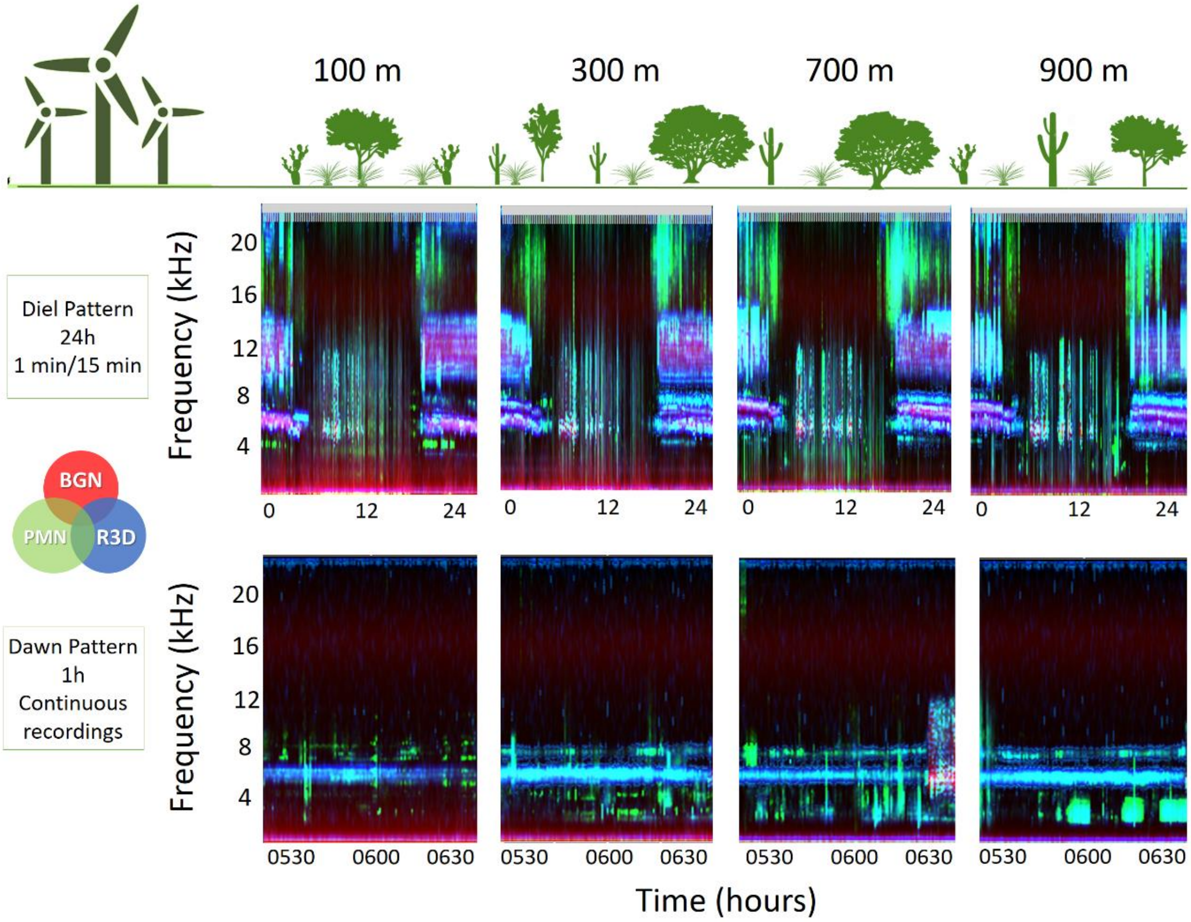


Figure 4 – Example of False Colour Spectrograms of the same day in the four points recorded. Indices used in this visualization were BGN (red), PMN (green), R3D (blue), calculated at a resolution of 30 seconds. FCS on the top represent the diel patterns, with 24h at a resolution of one minute every 15 minutes. At the bottom, a zoom in the morning built with one hour of continuous recordings. Distance between points 1 and 2, and 3 and 4 is 200 meters. Between 2 and 3 is 400 meters.

The FCS shows (in red along the bottom of the plots) the amount of background noise registered through the gradient distance from the turbines (Figure 4). In the first point (100 meters from the turbines) this noise band is evident up to 4 kHz, but 900 m away from the turbines it is reduced to the first kHz. The insect activity appears dominating the night period in all four sample points, with apparently no evident differences. When we look at the bird activity, however, there are some clear differences between the points, as shown in the bottom FCS in Figure 4. Those differences can be seen in green and blue colours, in short duration signals between 2 and 5 kHz. The long duration signals, represented as horizontal bands during the entire one-hour period, reflect Orthopteran activity.

3.4. Acoustic Indices

The two indices chosen to inspect the variation in acoustic activity in a distance gradient presented similar patterns, with values increasing with distance, both in the morning (Figure 5; SNR: Chi-squared (1) = 13.65, p<0.001; ENT: Chi-squared (1) = 7.48, p<0.01) and in night periods (Figure 6; SNR: Chi-squared (1) = 22.14, p<0.001; ENT: Chi-squared (1) = 11.74, p<0.001). Results from model selections are presented in Table 2.

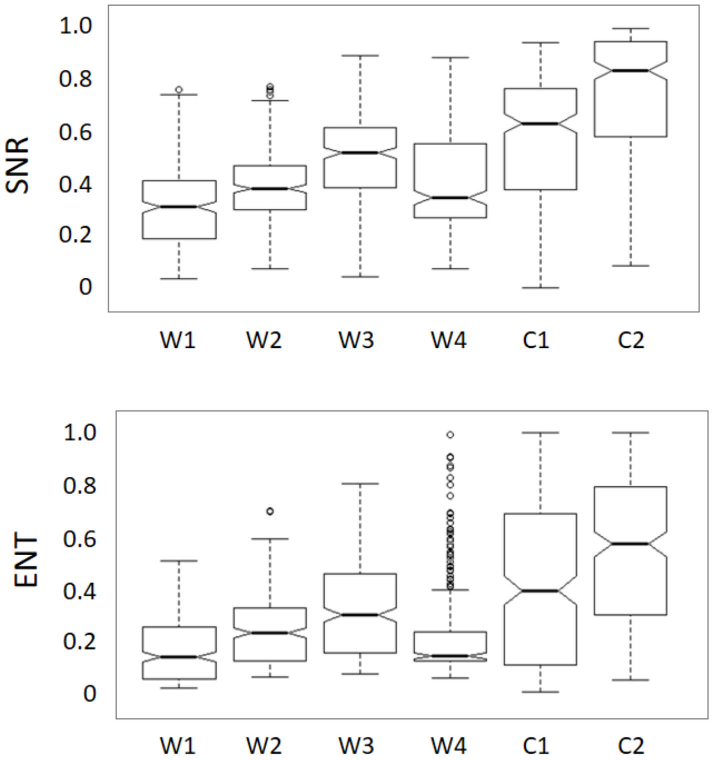


Figure 5 – Acoustic indices variation during the morning period (from 0500 to 0800). On top, boxplots showing the distribution of Indices values, according to distance. W1 to W4 refers to the wind farm area, from the closest to the most distant point from the turbines. C1 and C2 refers to Control points, sampled in a different area.

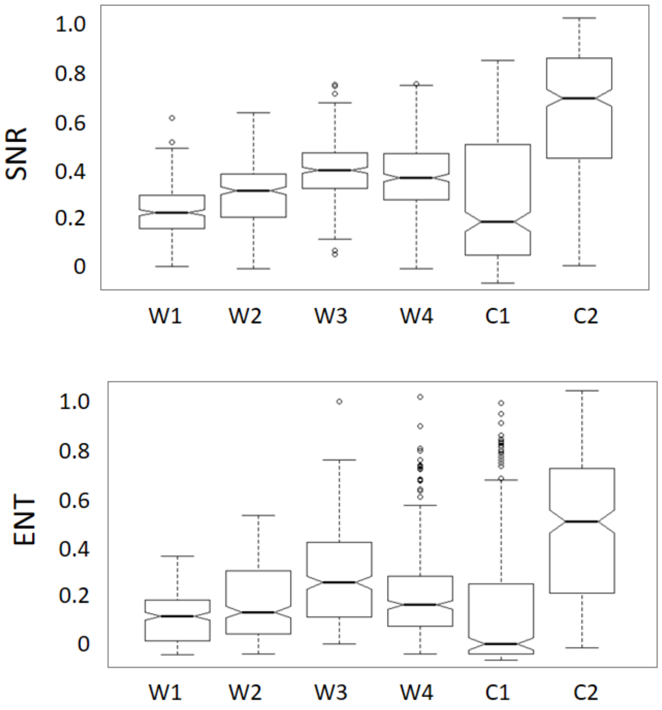


Figure 6 – Acoustic indices variation during night periods recordings (from 1700 to 2000). On top, boxplot showing the distribution of Indices values, according to distance. W1 to W4 refers to the wind farm area, from the closest to most distant point from turbines. C1 and C2 refers to Control points.

Table 2 – Results of mixed models selected from comparison with null models (ANOVA). All models include as random factors area (treatment/control) and time (hour and day, per sampling point). All models were checked for normality and homoscedasticity.

Model		**Coefficients**			
Period	*Index*	*Random variables (SD)*		*Fixed variable (SE)*	
Day	SNR	Area: Time:	0.022 (0.15) 0.034 (0.184)	Distance:	0.130 (0.042)
Day	ENT	Area: Time:	0.008 (0.09) 0.033 (0.182)	Distance:	0.067 (0.035)
Night	SNR	Area: Time:	<0.001 0.032 (0.179)	Distance:	0.049 (0.007)
Night	ENT	Area: Time:	<0.001 <0.001	Distance:	0.027 (0.007)

4. Discussion

We addressed the impacts of wind farms using three methods (FCS, SPD and two Acoustic Indices) to explore how the soundscapes dynamics are being affected by proximity to wind turbines. The frequency bins used by most birds overlapped with the noise emitted by the turbines, while only a few insects suffer this acoustic masking. Our results suggest that both birds and insects have different activity patterns associated with wind farm turbines, denouncing that the effect of wind farming on natural soundscapes is noticeable at small spatial scales.

4.1. Soundscape characterization

Most calls from birds and insects (Figure 2) did not overlap in their dominant frequencies, although they do in time, with some insects also calling during the day. The Orthopterans dominated the night soundscape, with temporal partitioning in relation to other insect groups frequent in tropical regions - the day time active cicadas (Diwakar & Balakrishnan, 2007). The frequency range used by insects in our study (dominant frequencies between 3.1 kHz and 18.9 kHz) is similar to the one found in previous studies (Ferreira et al., 2018; Gasc et al., 2013; Penone et al., 2013; Phillips, 2018; Pijanowski et al., 2011), and the calling patterns we observed for Orthopterans differ between groups, with katydids using a broad frequency range and crickets calling with pure tone songs (Figure S1). Inasmuch, crickets seem to separate their signals better in the frequency spectrum (Schmidt & Balakrishnan, 2014) and this partitioning is also related to known patterns of highly diverse communities (Kostarakos et al., 2008; Schmidt et al., 2013).

Birds were vocally active during the day, especially in the first hours of the morning – the dawn chorus. This phenomenon is widely studied, and theories about the reasons why it occurs include optimization of foraging time and favourable time for sound transmission in the atmosphere (Berg et al., 2006; Hutchinson, 2002). Dominant frequency in bird calls varied from 515 Hz to 7.5 kHz, a range also comparable to other studies (Ferreira et al., 2018; Wiley & Richards, 1982) and potentially more affected by anthropogenic noise (Slabbekoorn & Ripmeester, 2008).

The potential noise masking effect on the lower frequency bands (up to 4 kHz) could be visualized on False Colour Spectrograms (FCS), as a drastic reduction of bioacoustic signals on lower frequency bands dominated by noise (reddish colour, bottom left panel, Figure 4). Other possible explanation for the lack of signals in those frequency bands could be the displacement of animals that use those bands for reasons other than acoustic masking, such as avoidance of the physical structure of the wind farm. It is also important to remember that, even if the individuals are not directly affected by acoustic masking of their signals, they can still be impacted by the noise depending on the frequency range sensibility of auditory system (Robert & Beason, 2004).

Concerning the community of animals in such affected areas, the acoustic niche hypothesis predicts that animals will compete for the acoustic space, occupying separate frequency bands (Krause, 1993). Hence, an environment richer in acoustic signals can also be considered more biodiverse. The Spectral Probability Density (SPD) plot summarizes the information about spectral values and shows that the sample points with more peaks in Root-mean-square values can be considered richer acoustically (more diversity in signal types), or having more complex acoustic communities. RMS of the raw audio signal was successfully used to track temporal and spatial dynamics both in terrestrial (Eldridge et al., 2018; Rodriguez et al., 2014) and marine environments (Bertucci et al., 2016).

4.2. Acoustic Indices

Our results shown that acoustic activity increases with distance from the turbines, both during the morning and at night, suggesting an impact on bird and insect communities. These results are in contrast with a recent study in a temperate region (Florentin et al., 2015) that investigated soundscape dynamics in the vicinity of a single wind turbine. The authors found no impact on bird community based on ACI and Spectral Entropy indices. However, they also highlight the difficulty of making general assumptions due to limitations of the study such as low sampling rate, short time period and limited sampling (Florentin et al., 2015). We believe that our results were different because the area sampled by Florentin et al. (2015) only have one wind turbine and it is placed in a region with low bird diversity. Another study using acoustic indices to measure anthropic impacts on tropical areas showed results similar to ours. Duarte et al. (2015) showed significant differences in ACI (Acoustic Complexity Index) in an area with iron mining activity and a control area within the Brazilian Atlantic Forest, with higher biophony values away from the mining area.

Most studies addressing anthropic impact on acoustic communities are focused on birds (Buxton et al., 2018), because they are good models on terrestrial environments: they are widespread, have their taxonomy and call identification well established, and are cost-efficient to monitor (Venier et al., 2012). Based on the spectral distribution of bird song (Figure 2), we found that the acoustic impact from turbine noise affects more the frequency bands used by birds, as documented in the literature (Slabbekoorn & Ripmeester, 2008). In fact, birds are considered the most impacted faunal group around wind farm facilities, according to previous studies (e.g. Dahl et al., 2012; Percival, 2005; Tellería, 2009). Here, even selecting only the first hours in the morning to sample specifically the avian dawn chorus, we could still detect the presence of insects, but it is much less evident than at night. This represents an extra challenge in the use of acoustic indices to measure the specific activity of birds and could explain why some acoustic indices traditionally used for birds do not work (e.g. ACI, Pieretti et al., 2011). The abandonment of generalization of acoustic indices is an issue recently addressed by researchers. Particular indices may not be suitable for different regions, considering how variable the acoustic communities and the data sampling are (Bradfer-Lawrence et al., 2019; Sugai et al., 2019).

We observed diminished Orthopteran calling activity close to the wind turbines, an effect that could be explained by acoustic masking. Insects are an interesting group to be used in environmental monitoring (Riede, 1998), although it is under investigated, mainly due to lack of baseline studies. But it is known that strategies to avoid acoustic masking also reported for vertebrates can occur in invertebrates as well. This includes increasing the amplitude of the calls (Duarte et al., 2019; Nemeth & Brumm, 2010), spatially moving away from noise source (Bunkley et al., 2017), or increase the intensity of masking frequencies (Lampe et al., 2012). Other strategies common in vertebrates, such as changing calling time are not common (Raboin & Elias, 2019) but can occur in invertebrates (Duarte et al., 2019). Further explorations could focus on species identification and measurement of call parameters to better understand the phenomenon behind the decrease observed.

5. Conclusions

The eolic energy industry is an interesting disturbance model, as the facilities can be placed in areas with less interference in the vegetation than other energy plants, such as hydroelectric. Thus, apart from direct collision (Bispo et al., 2013; Grodsky et al., 2013), other changes in animal community are related to the acoustic impact from the turbines noise in these areas (Rabin et al., 2006; Zwart et al., 2016, this study). Recently the use of acoustic monitoring as an environmental assessment tool has been supported and encouraged. In addition to other traditional methods (Burivalova et al., 2019), the use of acoustic indices can measure and quantify human-driven changes in the environment (Doser et al., 2019). Here, we found that: (1) the acoustic diversity, represented by occupation of frequency bands, increases with distance from the turbines; (2) both ENT and SNR indices capture the acoustic variation along a distance gradient, during morning and evening periods; (3) birds that call in lower frequencies suffer more from acoustic overlap than insects.

We suggest that further studies should employ multiple acoustic indices on modelling the anthropic disturbance on soundscapes, once the combination of acoustic indices can provide a better understanding of soundscape patterns (Eldridge et al., 2018). We also recommend that communities of soniferous insects are studied, filling the gap of information that exists for tropical insects, and exploring the use of this group in environmental health measurements (Duarte et al., 2019; Raboin & Elias, 2019).

The use of soundscape-based analyses to inform and guide environmental policies is still incipient. In the case of wind farms, we recommend the inclusion of noise propagation modelling taking into account also the vocal characteristics of the animals in the area, as well as the spatial configuration of the wind farms. The size of the wind farms and the proximity of turbines to each other may have unexpected local effects on the range of area impacted by their noise. As shown here, species can be impacted in a different way. Therefore, the use of soundscape-based analyses in environmental monitoring should be employed with caution, since impacts will depend on local propagation, spatial and temporal configuration of human activities and the features of local animal communities.

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Supplementary material

Table S1 – Bird species identified from a 300 minutes sample in the wind farm area.

Taxon	**English Name**
Tinamidae	
Crypturellus parvirostris	Small-billed Tinamou
Accipitridae	
Rupornis magnirostris	Roadside Hawk
Charadriidae	
Vanellus chilensis	Southern Lapwing
Columbidae	
Columbina picui	Picui Ground-Dove
Strigidae	
Glaucidium brasilianum	Ferruginous Pygmy-Owl
Bucconidae	
Nystalus maculatus	Spot-backed Puffbird
Picidae	
Veniliornis passerinus	Little Woodpecker
Cariamidae	
Cariama cristata	Red-legged Seriema
Falconidae	
Caracara plancus	Southern Caracara
Thamnophilidae	
Myrmorchilus strigilatus	Stripe-backed Antbird
Formicivora melanogaster	Black-bellied Antwren
Dendrocolaptidae	
Lepidocolaptes angustirostris	Narrow-billed Woodcreeper
Rhynchocyclidae	
Tolmomyias flaviventris	Yellow-breasted Flycatcher
Todirostrum cinereum	Common Tody-Flycatcher
Hemitriccus margaritaceiventer	Pearly-vented Tody-tyrant
Tyrannidae	
Euscarthmus meloryphus	Tawny-crowned Pygmy-Tyrant
Phaeomyias murina	Mouse-colored Tyrannulet
Myiarchus tyrannulus	Brown-crested Flycatcher
Pitangus sulphuratus	Great Kiskadee
Empidonomus varius	Variegated Flycatcher
Vireonidae	
Cyclarhis gujanensis	Rufous-browed Peppershrike
Troglodytidae	
Troglodytes musculus	Southern House Wren
Cantorchilus longirostris	Long-billed Wren
Polioptilidae	

Polioptila plumbea	Tropical Gnatcatcher
Icteridae	
Icterus jamacaii	Campo Troupial
Thraupidae	
Coryphospingus pileatus	Pileated Finch
Coereba flaveola	Bananaquit

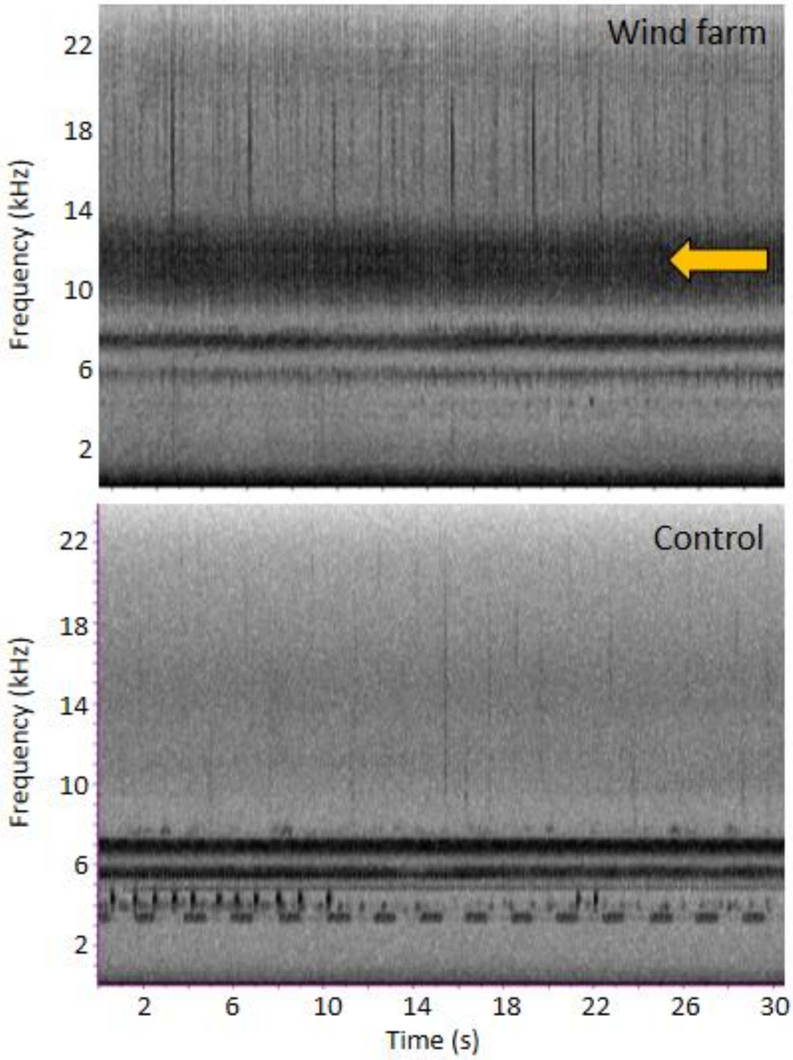

Figure S1 – Spectrograms (FFT=512) of 30 seconds of sounds recorded at 19h15 in two different areas in Rio Grande do Norte state in Brazil. Above the wind farm area and below the control area. Patterns observed (dark banding) represent Orthopteran acoustic activity. On wind farm area, the arrow points at katidids' acoustic activity.

CAPÍTULO 3

ACOUSTIC INDICES PREDICT BIRD SPECIES RICHNESS AND COMMUNITY COMPOSITION IN TROPICAL BIOMES

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Acoustic Indices predict bird species richness and community composition in tropical biomes

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Can acoustic indices predict bird species richness?

NDSI and AR were the best predictors, but varied between biomes

A combination of acoustic indices was able to discriminate the bird communities in the biomes

Dominance of some bird species and concentration of their acoustic signals in a few frequency bands can bias acoustic indices results

Abstract

Passive acoustic monitoring is considered an important tool to quantify biodiversity and track changes in animal acoustic activity. To analyse the large volume of recordings, acoustic indices have been developed to summarize the information in the recordings. However, the relationship between indices developed to quantify animal activity and the traditional estimates of biodiversity is still controversial. Here we tested whether single indices can predict bird species richness and if a composition of several indices allow discrimination of different species' composition in three biomes in Brazil: Cerrado (Savanna), Caatinga (Seasonally Dry Forest), and Atlantic Forest (Tropical Moist Broadleaf Forest). We identified a total of 132 bird species: 25 in the Cerrado, 53 in the Caatinga, and 69 in the Atlantic Forest. We found that for all biomes pooled together, NDSI was correlated to the number of bird species identified in each recording. Other indices were correlated when considering only Cerrado (NDSI) and Atlantic Forest (negative correlation with AR). In Caatinga no single index was correlated with bird diversity. The reasons why those indices perform differently are discussed based on the spectral characteristics of the birds' calls and species' predominance. The combination of six acoustic indices allowed discrimination of these three biomes. Our results indicate that the use of acoustic indices as a proxy of avian diversity in tropical environments can vary, according to characteristics of the avian community and phytophysignomies. Future studies should better explore how those local peculiarities can be considered in calculations and interpretation of results.

Keywords: Ecoacoustic indices, Soundscape, Biodiversity Monitoring, Avian Community

1. Introduction

Passive acoustic monitoring has become popular in the last decade as an important tool to measure ecological processes, temporal and spatial changes in animal activity patterns (Blumstein et al., 2011; Campos-Cerqueira & Aide, 2016; Lellouch et al., 2014; Machado et al., 2017), and to identify the richness of determined group (Shonfield & Bayne, 2017; Wimmer et al., 2013; Zhang et al., 2016). Birds are one of the most studied group in terrestrial passive acoustic monitoring studies (Sugai et al., 2019), which enables sampling distinct areas simultaneously for long periods of time, with no interruption. It can help, for example, the detection of rare species which would go unnoticed in a bird survey using traditional methodology, such as fixed-radius sampling points and mist nests (Holmes et al., 2014). Recent studies showed that the acoustic monitoring for counting species is not only a complimentary methodology, but can be used alone, as the results are similar to other traditional methodologies (Alquezar & Machado, 2015), or can even outperform direct observations by humans (Darras et al., 2019). The major challenge in using acoustic monitoring is the large amount of data generated, which makes it difficult for experts to hear all the files (Towsey, Wimmer, Williamson, & Roe, 2014). For this reason, several recording and analysis schemes have been tested, so that the manual effort of the specialists can be optimized. One efficient scheme to sample diurnal birds is the analysis of dawn chorus, when animals are more active (Wimmer et al., 2013).

With the premise that richer communities are expected to have richer acoustic environments, ecological indices that unveil this acoustic diversity can be used as a proxy of biodiversity (Sueur et al., 2014). In the past years computational tools were developed in order to improve the use of increasingly larger acoustic datasets in environmental monitoring, behavioral and ecological studies, especially through acoustic indices (Burivalova et al., 2017;

Towsey et al., 2018; Zhang et al., 2016). Acoustic indices were introduced in the field of acoustic ecology to summarize information within acoustic recording, allowing researchers inferring processes occurring in a landscape in an automated way (Sueur et al., 2014).

Previous work comparing acoustic indices with other methodologies of estimating bird diversity and richness were carried on a variety of environments, and sampling designs and the findings about which index (or indices combinations) better summarize soundscape information are variable (Table 1). Factors that may be causing this variation are related to birds' vocal features, such as the shape of sound units and their amplitude, and also to the frequency of occurrence of each species within communities (Zhao et al., 2019). Vocal features can be related to the vegetation where the species vocalize. Areas with a dense vegetation will attenuate higher frequency sounds, while in areas with open vegetation, the sound tends to propagate more freely allowing all frequencies to be detected at distance (Bradbury & Vehrencamp, 1998). Such peculiarities may hinder the generalization of findings so studies testing the applicability of using sounds to characterize and monitor biodiversity should consider the effect that different habitats have on the results.

Table 1 – Summary of studies that investigated the relationship between acoustic indices and animal species' richness.

Reference	Spatial design	Area	Sampling design	Species richness estimative	Acoustic Indices	Main results
Depraetere et al., 2012	Three recorders, 300 m apart. Gradient of tree density	Forest-cropland France	74 days, 3 hrs dawn, 3 hrs dusk, 150s every 15 min. Total= 222 hrs of recordings at 44.1 kHz	Manual inspection of subsample	Acoustic Richness, Dissimilarity Index	Acoustic richness in agreement with traditional aural identification. Higher values in young forest. Peak at dawn chorus. Dissimilarity Index indicated differences between areas.
Mammides et al., 2017	97 recorders, 250m apart. Two different areas	Rubber plantations, tropical and subtropical forest; China.	Recordings made at the same time the count surveys, for the same duration (15 min) at 44.1 kHz.	Point Count surveys	Acoustic Richness, Acoustic Complexity, Biodiversity Index, NDSI, Acoustic Diversity, Acoustic Evenness	No index with strong correlation. Total Entropy, Acoustic diversity, Acoustic evenness moderate correlations. Environmental dissimilarity didn't affect indices performance
Towsey et al., 2014	One recorder	Open forest, with Eucalyptus. Australia	5 days, continuous recordings at 22.05 kHz	Manual inspection files	Average Signal Amplitude, Background Noise, Signal to Noise Ratio, Acoustic activity, Count of acoustic events, Average duration of acoustic events, Entropy of the signal envelope, Acoustic Complexity, Mid-band activity, Entropy of the average spectrum, Entropy of spectral maxima, Entropy of spectral variance, Spectral diversity, Spectral persistence.	Spectral diversity*, Spectral and Temporal Entropy, Acoustic complexity were the best indicators. *best index in single performance

Reference	Spatial design	Area	Sampling design	Species richness estimative	Acoustic Indices	Main results
Izaguirre, et al., 2018	60 points (5 recorders in each of 12 areas) 200 m apart	Dry Tropical Forest. Costa Rica	Continuous recordings (2 hr) during dawn and dusk, 10 min every hour for the rest of the day. 2 consecutive days. Sampling rate of 44.1 kHz.	Bird point counts. Bird abundance, richness, diversity and evenness	Acoustic evenness, Acoustic diversity, Acoustic complexity, Biodiversity index, NDSI, Total Entropy, Median Amplitude Envelope, Number of peaks	Acoustic complexity correlated to bird abundance, Number of Peaks with bird diversity.
Machado et al., 2017	30 Points	Savannah. Brazil	15 minutes in each point. Sampling rate of 48 kHz.	Manual inspection of the files	Acoustic diversity Index, NDSI	Acoustic Diversity Index associated with species richness, values of NDSI lower in areas close to highways
Moreno-Gómez et al., 2019	3 areas (600 m apart)	Valdivian Forest. Chile	1 min per hour, 24h/day. Sampling rate of 44.1 kHz.	Manual inspection of the files	Spectral Entropy, Temporal Entropy, Total Entropy, Acoustic Complexity Index, Acoustic Diversity Index, Acoustic Evenness Index, Bioacoustic Index	Temporal Entropy, Acoustic Diversity Index and Acoustic evenness Index associated with bird richness
Jorge et al., 2018	12 points	Atlantic Forest. Brazil	10 minutes recording/10 minutes paused. Sampling rate of 48 kHz.	Point-count survey	Total Entropy, Acoustic Complexity Index, Acoustic Diversity Index, Acoustic Evenness Index, Bioacoustic Index, NDSI	Moderate correlation with Acoustic Evenness Index. Other correlations were considered biased by the observer during the data collection.

Here, we used data from three tropical biomes in Brazil. A Seasonally Dry Tropical Forest (SDTF) in the Northeast referred as Caatinga, a Savanna in central Brazil, known as Cerrado, and the Tropical Moist Broadleaf Forest, the Brazilian Atlantic Forest on the Southeast region of the country. Our main goal was to understand how acoustic indices can be used to infer information about richness and species composition of bird communities, aiming to answer: (1) Can acoustic indices be used as a proxy of species richness considering different environments? (2) Can the features of birds' calls influence the relationship between species richness and acoustic indices? And, (3) Can the differences in avian community composition among areas be predicted by acoustic indices?

We hypothesize that (1) Indices can respond in two different ways to bird diversity: i) Indices that are based on occupation of frequency bands (e.g. Acoustic Diversity Index) will be correlated to species richness and ii) Indices based on measurement of activity (e.g. Total Entropy) will be related to the amount of acoustic activity, which may or may not have a relationship with richness and can indicate other processes occurring in the area, such as effects of human activities; (2) Indices will be affected by the frequency bandwidth occupied by birds' calls. In areas where species present more even distribution of energy in frequency bands, indices based on occupation of those bands will present higher values; (3) Combination of acoustic indices will account for different features of the acoustic space, enabling the identification of differences among areas.

2. Methodology

2.1. Acoustic recordings

In all areas, we installed recorders during the rainy season, and the sampling minutes were sorted within a one-month period. In the selected month, we sampled one hour during the morning chorus, the period of time when most bird species are vocalizing (Wimmer et al., 2013). We used Song Meter Digital Field Recorders (SM2, in Cerrado. SM3 in Atlantic Forest and Caatinga) (Wildlife Acoustics, Inc., Massachusetts), positioned 1.5 m high, with at least 400 meters between them to avoid superposition (Figure 1). Sampling rate varied from 44.1 kHz to 96 kHz, 16 bit WAV format, but for the calculation of acoustic indices (see below) files were downsampled to 24 kHz. Other details about data collection are presented below for each site.

2.2. Study sites

Caatinga is a Seasonally Dry Tropical Forest in northeastern Brazil. It is a semi-arid region, with precipitation varying around 200-800 mm annually, and a rainy season of 3 to 5 months (Olmos et al., 2005). Our study area, the Serra do Feiticeiro is considered one of the most important areas of Caatinga within the state of Rio Grande do Norte (Marinho et al., 2018). The area is not part of a legal reserve, and human occupation in small farms is present in the region. The area is composed of an open arbustive and arboreal vegetation, and most tree species do not pass 5m of height. We recorded at the end of rainy season, from June to July 2017.

The Brazilian Savanna, known as Cerrado, is considered a biodiversity hotspot (Strassburg et al., 2017), with phytophysiognomies that range from open grassland to closed canopy forests and gallery forests adjacent water courses (Oliveira-Filho & Ratter, 2002). The climate is seasonal tropical, and the rainy season ranges from October to March (Queirolo & Motta-Junior, 2007). We collected data from November to December 2017, in the Serra da

Canastra National Park, Minas Gerais State. Vegetation in the areas was mainly composed by shrubs, small trees and open grassland.

The Atlantic Forest is also considered a biodiversity hotspot (Myers et al., 2000) and covers areas along the coastline from southern to northeastern Brazil. The weather is usually warm and wet year round, but some phytophysiognomies experience a dry season from April to September (Morellato & Haddad, 2016). We recorded during November 2016, in a fragmented landscape area, and recorders were placed in areas at least 60m away from the border.

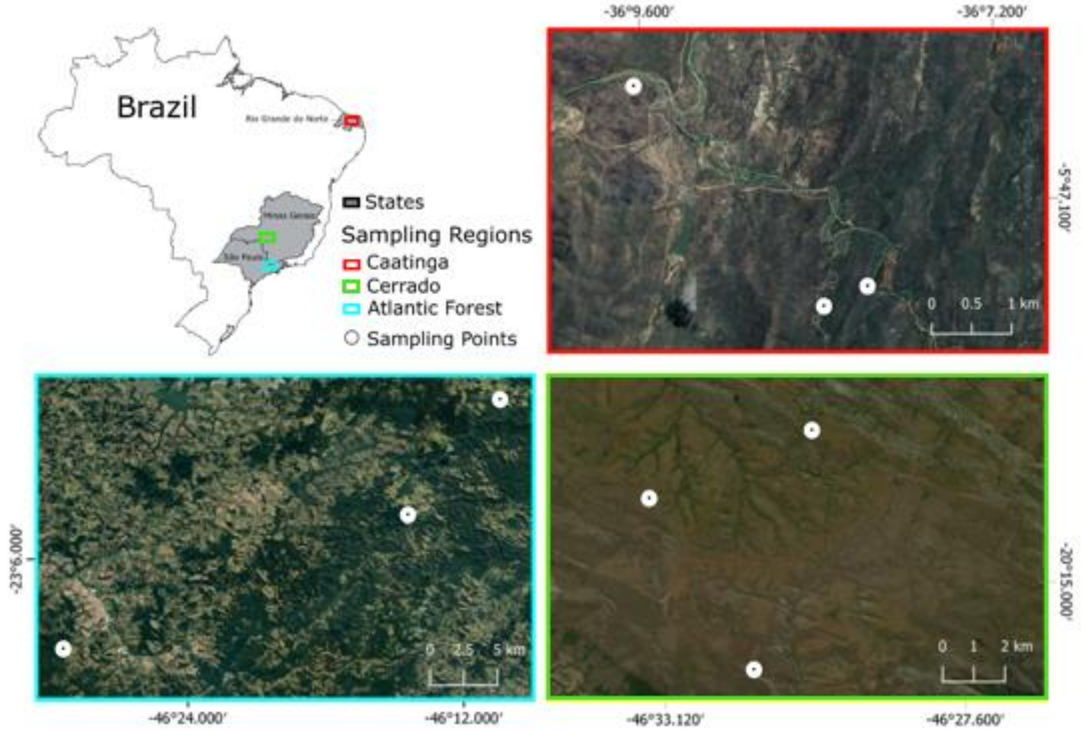

Figure 1 – Nine recording sites distributed equally in three Tropical Biomes. Brazilian Savanna (Cerrado), Seasonally Dry Tropical Forest (Caatinga) and Tropical Broadleaf Forest (Atlantic Forest) were used to sample the soundscapes using autonomous recorders. Note the difference in spatial scale among the areas sampled.

2.3. Data analysis

For species identification, we subsampled our data by randomly sorting 60 second audio clips. For each biome we used 300 minutes, 100 in each of three points. Those were analysed by two ornithologists in each biome (AG and JD in Caatinga, VT and LG in Atlantic Forest, EO and CG in Cerrado), that identified the species aurally and visually inspecting the spectrograms, using the software Raven Pro 1.5. Annotations generated in the software were translated to a presence/absence matrix in R platform (Supplementary Material 1). From this data base we also investigated the ten species that were most identified in each biome, and also inspected the frequency range used by them.

2.4. Acoustic Indices

To calculate the acoustic indices, we used data from one hour per day, for 25 days, for each of the nine points. In total, approximately 16200 one-minute files were processed. We chose six ecological indices available at R software (R Core Team, 2018) packages ‘soundecology’ (Villanueva-Rivera & Pijanowski, 2016) and ‘seewave’: Acoustic Diversity Index (ADI), Total Entropy (H), Acoustic Complexity Index (ACI), Bioacoustic Index (BI), Acoustic Richness (AR), Normalized Difference Soundscape Index (NDSI) (Table 2). Indices were calculated at a resolution of 60 seconds and maximum frequency values were set to 12 kHz, ensuring that bird calls were captured, but other sound sources occupying higher frequency were left out.

Table 2 – Description of six Acoustic Indices used. Details about index calculation can be found on references.

Index	Description	Reference
ADI	Acoustic Diversity Index. Is the result of applying the Shannon Diversity Index to each frequency bin (the default is 1kHz). Also, by default, there is a cutoff value of -50 dBFS, to remove the faint sounds. According to this index, greater values will represent greater number of	(Villanueva-Rivera *et al.*, 2011)

	active bands and, therefore, more diverse acoustic communities.	
H	Total Entropy. Is the product of both temporal and spectral entropies. Values are close to 0 for a single pure tone and increases up to 1 with the occupation of more frequency bands and amplitude modulations.	(Sueur *et al.*, 2008)
ACI	The Acoustic Complexity Index was designed to measure biophony activity (mainly birds), considering the variability in the amplitude values (greater than 3db) of close time units in each frequency bin. By using this calculation, it assumes that human-made sounds, usually constant and concentrated in lower frequency bins, are excluded	(Pieretti, Farina, & Morri, 2011)
BIO	Bioacoustic Index was proposed to measure relative avian song abundance by calculating the area under the normalized power spectrum that includes all frequency bands and is greater than the minimum intensity of each curve. Minimum frequency was set to 500 Hz.	(Boelman *et al.*, 2007)
NDSI	Normalized Difference Soundscape Index, estimates the proportion of anthropogenic acoustic activity compared to biophonic in a soundscape. The anthropogenic frequency bin was defined from 0 to 500 Hz.	(Kasten *et al.*, 2012)
AR	Acoustic Richness. Is a ranked index based on the rank of two other indices: M (Median of the amplitude envelope) and Ht (Temporal Entropy).	(Depraetere *et al.*, 2012)

2.5. Statistical Analysis

Aiming to compare only the days for which we had species identified, we've decided to use the average of the number of bird species identified in each one-minute recording by day, and did the same to calculate Acoustic Indices values. To test the correlation among the number of species identified per minute and acoustic indices, we performed Spearman's correlation in two different subsets: using data from all biomes and separated by biome.

We've calculated similarity between points based on species composition and results of acoustic indices to understand if the combination of acoustic indices can predict the species composition. For species composition we used the Jaccard index followed by a hierarchical cluster analysis. For the Acoustic Indices, we first ran a kmeans clustering (k=40), followed by a calculation of a matrix of distance and then a hierarchical clustering. We used the packages 'vegan' (Oksanen et al., 2019) and 'cluster' (Maechler et al., 2015) on R statistical program (R Core Team, 2018).

3. Results

3.1. Species richness

We identified bird species based on listening and viewing spectrograms, which lead us to a total of 132 bird species in the nine sampling locations. The Atlantic Forest was the biome with most species, 69, while in Caatinga we identified 53 bird species. Cerrado was the biome with less species identified, 25 (Supplementary Material − Table S1). The average number of species identified per file was also higher in Atlantic Forest (4.42 species), followed by Caatinga (2.8 species) and Cerrado (2.7 species).

We also investigated the occurrence of species, and the ten most common species in each biome are presented in Table 3. Cerrado and Atlantic Forest showed few species that were registered in more than 44% of the files, while in Caatinga we observed a more equal distribution of species among files (all less than 30%). The spectral characteristics of birds' calls was also investigated (Table 3, Figure S2), and the frequency bands used by the species revealed that in Atlantic Forest most species use frequency bands from 3-6 kHz, but few calls in higher frequency bands. In Cerrado, we observed a similar pattern, but with more species using higher frequency bands. Caatinga species presented another pattern, with a more homogeneous occupation of frequency bands.

Table 3 – Fist ten most common species identified in each biome, the respective percentage of files in which they appear and their call frequency range.

Biomes	**Species**	**% of files**	**Frequency range**
Caatinga			
	Tolmomyias flaviventris	28.7	5-12 kHz
	Nystalus maculatus	20.7	1-2 kHz
	Troglodytes musculus	19.0	1-8 kHz
	Eupsittula cactorum	16.7	1-12 kHz
	Columbina picui	13.3	0.3-0.8 kHz
	Todirostrum cinereum	12.0	3-12 kHz
	Myiarchus tyrannulus	10.7	1-5 kHz
	Myrmorchilus strigilatus	10.7	2-5 kHz
	Columbina minuta	9.3	0.3-0.8 kHz
	Coereba flaveola	8.7	4-11 kHz
Cerrado			
	Sicalis citrina	44.0	2-8 kHz
	Rhynchotus rufescens	43.0	1-3 kHz
	Ammodramus hummeralis	36.7	2-10 kHz
	Anthus nattereri	35.7	2-8 kHz
	Nothura maculosa	20.3	1-3 kHz
	Anumbius annumbi	11.7	2-5 kHz
	Synallaxis albescens	3.3	1-11 kHz
	Cariama cristata	2.3	0.5-5 kHz
	Embernagra platensis	2.0	2-10 kHz
	Neothraupis fasciata	1.6	5-12 kHz
Atlantic Forest			
	Vireo chivi	51.0	2-5 kHz
	Myiothlypis leocublephara	42.3	3-10 kHz
	Mionectes rufiventris	42.0	0.5-8 kHz
	Basileuterus culicivorus	37.0	3-10 kHz
	Conopophaga lineata	26.0	2-4 kHz
	Dysithamnus mentalis	20.7	0.5-4 kHz
	Lathrotriccus euleri	20.3	1-5 kHz
	Corythopis delalandi	19.3	2-4 kHz
	Pachyramphus polychopterus	10.7	1-8 kHz
	Thamnophilus caerulescens	9.3	1-3 kHz

3.2. Acoustic Indices predicting bird species richness

The distribution of the acoustic indices showed different patterns among the biomes and sampling points (Figure 2). Acoustic Complexity Index presented higher mean values and more evenly distributed in the Atlantic Forest. While in ACI and BIO results, Caatinga presented lower values than Atlantic Forest, and similar to Cerrado. Total Entropy (H) is higher in Caatinga, especially in point “a”, which also appear to increase the values of AR for this point. We found no overlap among sampling points (labelled as “g”, “h” and “i” in Fig. 1) considering AR values in the Atlantic Forest. Significant differences were found for all indices among biomes when sample sites were pooled together (Supplementary Material, Table S3).

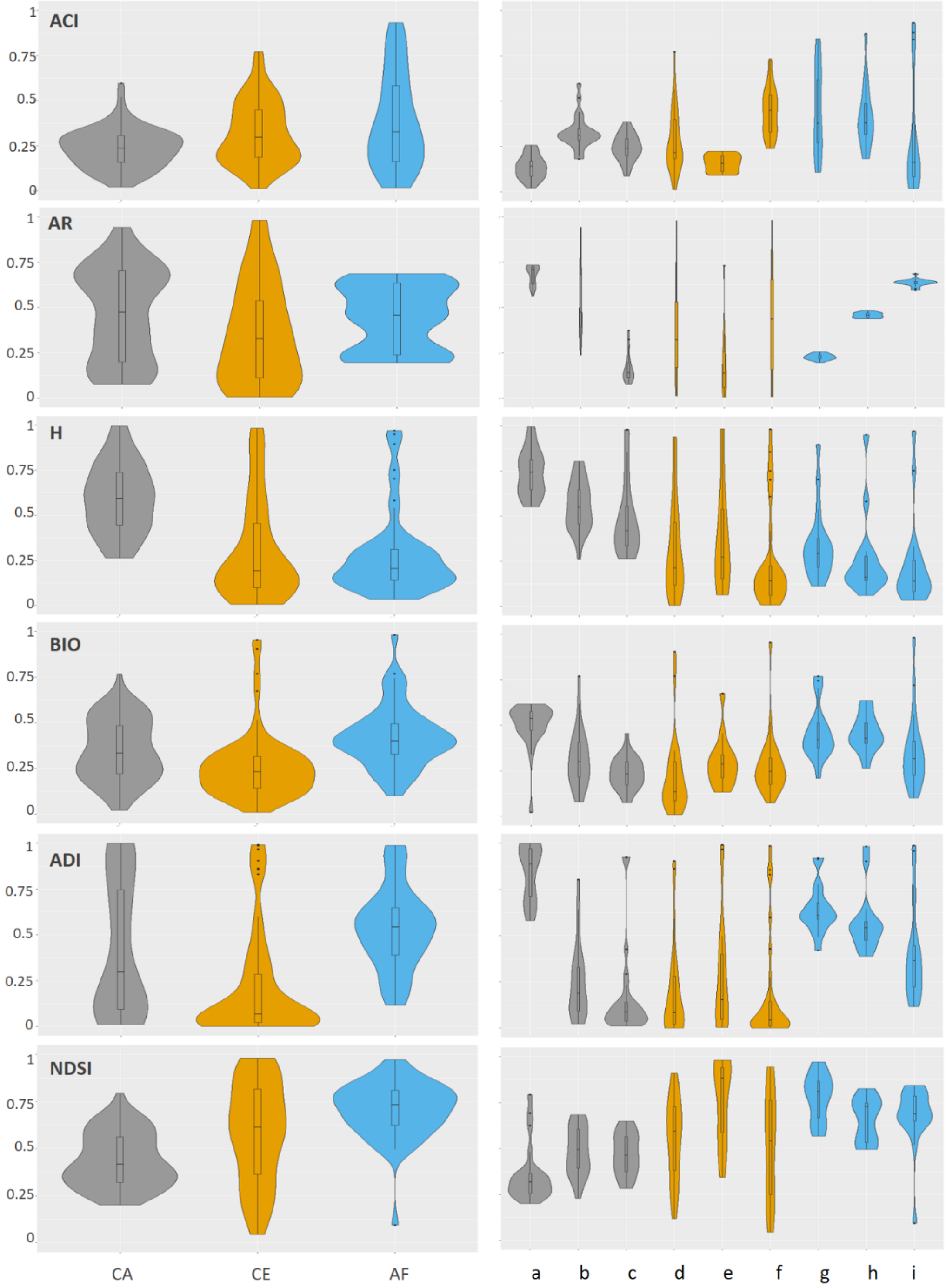

Figure 2 – Distribution of acoustic indices values among biomes and separated into sampling points. Y axis represents normalized values of Acoustic Complexity (ACI), Acoustic Richness (AR), Total Entropy (H), Bioacoustic Index (BIO), Acoustic Diversity (ADI), and Normalized Difference Soundscape Index (NDSI), averaged by daily values. Letters “a” to “i” identify the sampling points. Significant differences were found for all indices among biomes when sample sites were pooled together (CA, CE and AF).

The correlation between number of species and acoustic indices was significant for NDSI when we considered all biomes pooled together, and also within Cerrado (Table 4 and Figure 3). The Atlantic Forest showed a negative correlation between AR and species richness (rho = -0.42). In the Caatinga no index was correlated to richness.

Table 4 - Spearman rho correlation coefficients between daily average of number of species and acoustic indices. Results are shown for all biomes combined and for each three biomes (Caatinga, Cerrado and Atlantic Forest). *p < 0.05 after a multiple comparison test (False Discovery Rate).

	NDSI	ACI	ADI	BIO	H	AR
All Biomes	0.31*	0.08	0.15	0.01	-0.12	-0.11
Caatinga	0.1	0.21	0.05	-0.06	0.13	0.004
Cerrado	0.35*	-0.16	0.18	-0.22	0.23	-0.25
Atlantic Forest	-0.003	0.05	-0.01	0.05	-0.11	-0.42*

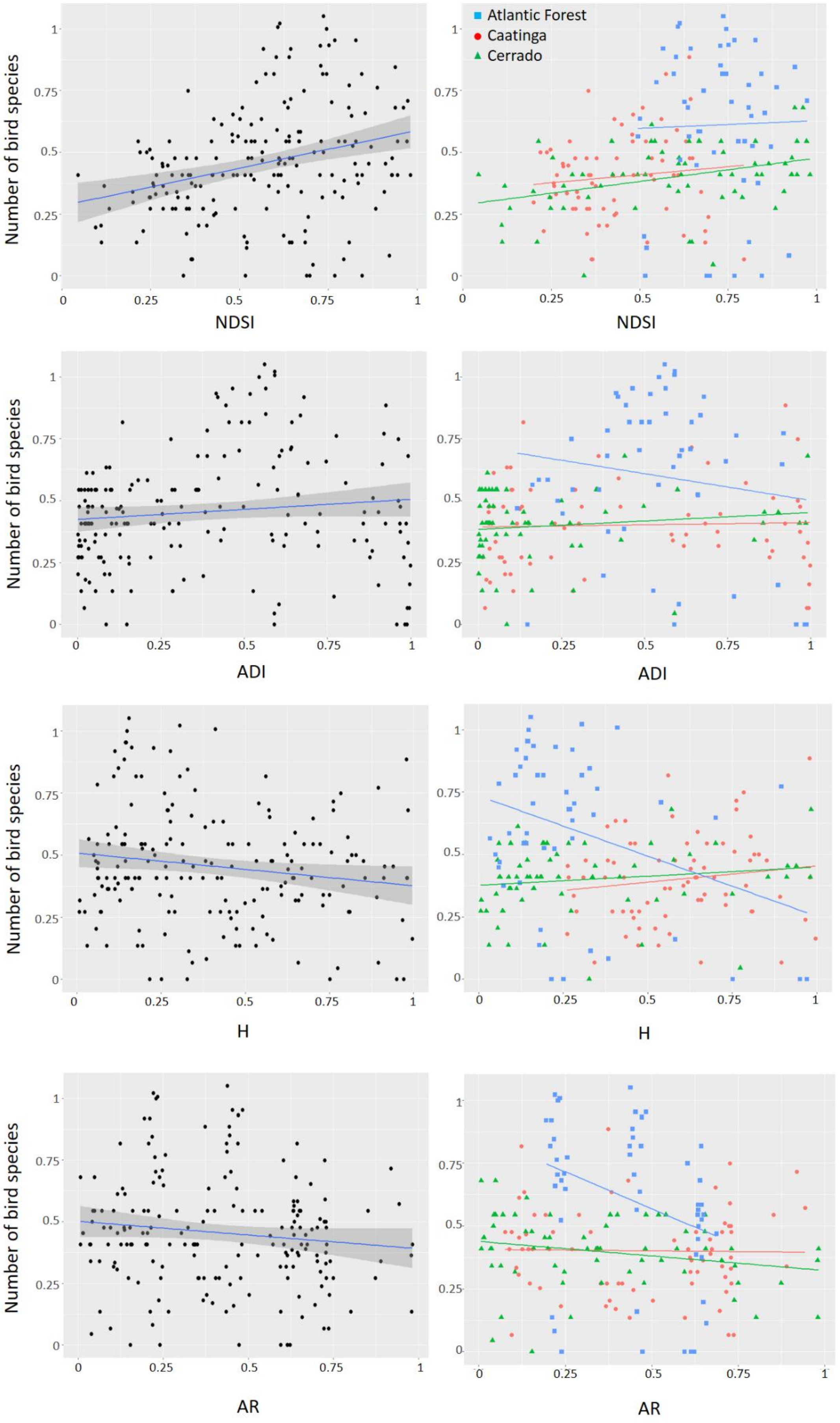
Number of bird species
Atlantic Forest
Caatinga
Cerrado
0
0.25
0.5
0.75
1
NDSI
ADI
H
AR

Figure 3 – Relationship between daily averaged values of acoustic indices and number of bird species identified (normalized values). Graphs on the left were made with data from all points, shaded area represent the standard error. On the right, separated by biomes: Atlantic Forest (Blue squares), Caatinga (Red dots), Cerrado (green triangles).

3.3. Acoustic Indices predicting bird species composition

Our dendrogram of similarity of bird species composition revealed a separation between the biomes, with points within the same biome being grouped together (Figure 4). The dendrogram built with Acoustic Indices values presented a similar pattern (Figure 5), with only one point grouped a different biome. Looking at the distribution of acoustic indices (Figure 1) we've found that this point presented higher values of H, BIO, AR and ADI compared to the other sampling points in Caatinga. The mean number of species identified per recording at point "a" is even smaller than the others in Caatinga (a= 0.36. b= 0.37. c= 0.46), so the cause could be a higher predominance of wind in the recordings.

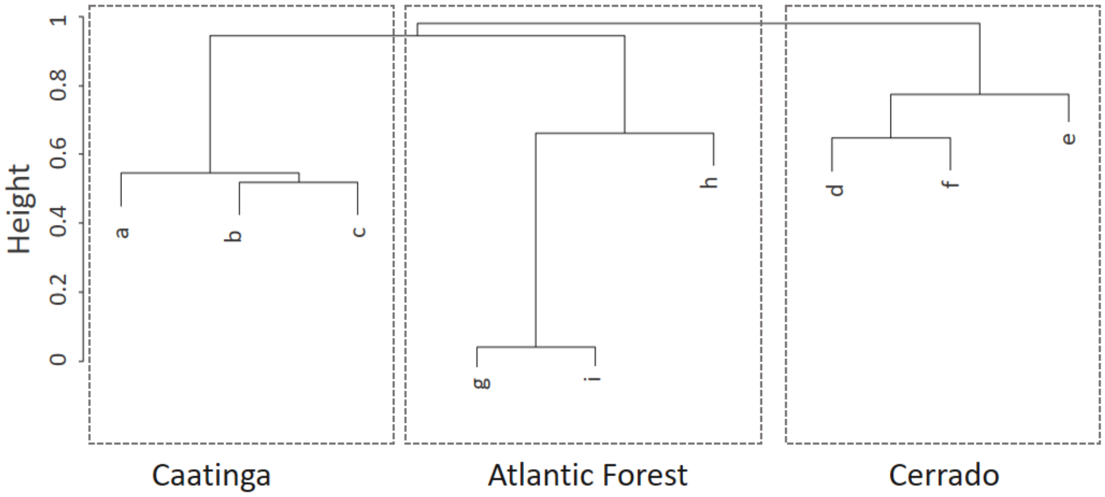

Figure 4 – Dendrogram of the similarity (calculated by Jaccard Index) of the sampling points, accordingly to the bird species composition for the Caatinga, Cerrado and Atlantic Forest.

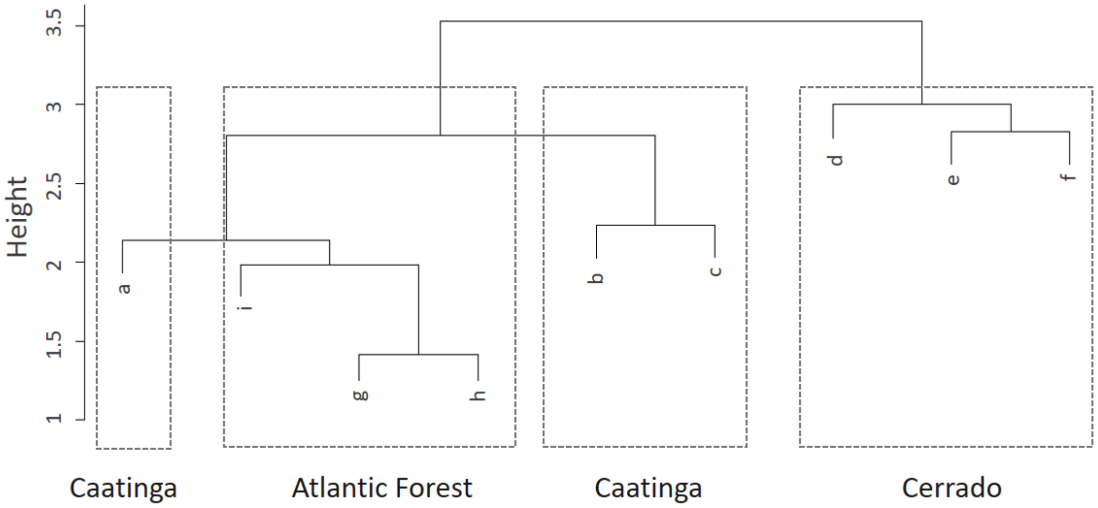

Figure 5 – Dendrogram of the similarity of the sampling points, accordingly to the values of six Acoustic Indices for the Caatinga, Cerrado and Atlantic Forest.

4. Discussion

We identified more bird species in the Atlantic Forest, followed by Caatinga and Cerrado. Nonetheless, despite the overall number of species was lower in Cerrado, the number of species identified in each recording was similar to Caatinga (average of Cerrado= 0.39 and Caatinga= 0.40). This result suggests predominance of fewer species in Cerrado, which was corroborated by the ranking of the most common species (Table 3), and by the pattern of low diversity with high activity indicating a strong acoustic competition and species territoriality (Izaguirre et al., 2018). The frequency bands used by birds are in agreement with the theory that states vegetation plays a role in selecting the species' calls (Morton, 1975). According to the Acoustic Adaptation Hypothesis (AAH) (Ey & Fischer, 2009), forest areas will have species with calls concentrated in lower frequency bands, as we observed in Atlantic Forest (Figure S1). In open environments, where transmission is more efficient and soundwaves are less scattered by the vegetation, species will have calls with wider frequency range, as we observed in Caatinga (Figure S1).

The results from correlations between acoustic indices and species richness varied when we considered the biomes individually or pooled together. NDSI was correlated to bird richness from all biomes, and in sites within the Cerrado. This corroborates with the findings of a previous study that considered only Cerrado (Machado et al., 2017). NDSI is an index that represents more the influence of anthropic sounds on the soundscape than the number of species itself. Our results indicate that we have more human-made sounds in the Caatinga area (lower values of NDSI), which is supported by the fact that the Caatinga area sampled isn't within a legal reserve and suffers acoustic impact from roads and other human activities nearby.

Acoustic Diversity Index wasn't correlated to species richness here, although we found a tendency when all biomes were pooled together. ADI was a good predictor of bird richness in other studies (Mammides et al., 2017; Moreno-Gómez et al., 2019). This index is assumed to capture the diversity of signals, but a concentration of signals in some frequency bands may cause incongruences in ADI values, as observed in our Atlantic Forest data with a tendency to a negative correlation with richness (Figures S1 and 3).

Total Entropy (H) was considered a good indicator of bird diversity by Zhao et al. (2019), but their results were dependent on the sound unit feature, which may explain why we did not find a significant correlation in our study. H is a product of Temporal Entropy (Ht) and Spectral Entropy (Hf), which separately, were significantly correlated to richness in previous studies (Moreno-Gómez et al., 2019; Towsey et al., 2014). Ht is used in the calculation of another index used in our study, the Acoustic Richness (AR). The Acoustic Richness was developed to capture information in noisy and open environments, with low signal to noise ratio and high presence of wind and rain (Depraetere et al., 2012). Here, we found a complete separation of values of three sampling points in Atlantic Forest (Figure 2), the environment in which the use of the index was, in theory, less appropriate. AR was negatively correlated to the number of species in Atlantic Forest, and we found the same negative tendency within Cerrado.

Looking at how AR is calculated (see Table 2), we verified that instead of several species, one or a few highly active bird species were predominant in the Cerrado and Atlantic Forest data (Table 3) and might have inflated the values of this index (Zhao et al., 2019). The characteristics of acoustic communities are probably the reason why the our results diverge from those from a French forest (Depraetere et al., 2012).

Overall, indices that consider information of distribution of energy in frequency bands are more effective in predicting species richness than indices that quantify more acoustic activity, when we consider distinct environments. The later ones can be biased by the concentration of several species in fewer frequency bands and by the amplitude values of dominant species' calls (Gasc et al., 2015; Zhao et al., 2019). Here, Caatinga was the biome with a more even distribution of calls among frequency bands and with less predominance of species. Even so, we couldn't find a correlation between richness and any of the indices tested, probably because our recordings were obtained by the end of the rainy season, when calling activity might be diminished. For this reason, we recommend that further investigations also include other features of the communities such as territoriality of species (that can bias the indices by calling close to microphone for a long time) and the time of the year in which the recordings are made, as calling behaviour associated with mating and territoriality increases during the reproductive period (Bradbury & Vehrencamp, 1998).

Our results shown that acoustic indices could also be used to differentiate acoustic communities, with only one point being grouped in the wrong biome (point "a - Caatinga" grouped with Atlantic Forest, Figure 5). AR, BIO, ADI and H indices' values were higher in this odd sampling point than in others (Figure 2), which should indicate higher animal activity, but also might be caused by other sound sources (natural abiotic and anthropogenic). Inspecting the files, we detected that the constant presence of wind could be the cause of this odd result. Wind was already pointed as a source of inaccurate acoustic indices values, as well as anthropogenic noises, both present in the area (Depraetere et al., 2012; Fairbrass et al., 2017).

5. Conclusions

This was the first study carried in different tropical biomes that aimed to test the efficacy of using acoustic indices to predict species richness, and it was interesting to compare the same methodology among different environments. The premise of an acoustic index is to be able of summarize some information in a given area, and thus they are supposed to work in a wide variety of environments. Ours and all previous studies cited here found a correlation between species richness and some of the acoustic indices used, but not always the same ones. Why some indices perform better on some biomes than in others? Some of the acoustic indices assume that we have a relationship between frequency bins occupation and biodiversity, based on the Acoustic Niche Hypothesis (Krause, 1987). Other indices are based on the measurement of overall amount of acoustic energy. These assumptions may be too general since we here show great variability among and within habitats. A careful look at the characteristics of the acoustic communities can give some guidance when making such assumptions (Zhao et al., 2019). Information about the vegetation and the likely features of the local acoustic community may also aid in generalizations about which indices should be explored.

The assumption that acoustic indices can be used in rapid biodiversity assessments and conservation policies itself (Burivalova et al., 2019) still need further validation since the concept of biodiversity itself that considers only species richness may be faulty, and nowadays the functional and phylogenetic diversity are also been considered (Isaac et al., 2007). Integrate those concepts to sound analyses is another challenge, since the relationship between the phylogenetic distance, dissimilarity of acoustic features, and the role of the environment in temperate (Gasc et al., 2013) and tropical habitats (Trigg, 2015) is not yet generalizable enough to be the basis for ecological assumption when it comes to soundscape analyses.

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Supplementary Material

Table S1 – Bird species identified in three biomes, CA=Caatinga, CE=Cerrado, AF=Atlantic Forest.

Species	Common name	CA	CE	AF
Tinamidae				
Crypturellus obsoletus	Brown Tinamou			X
Crypturellus tataupa	Tataupa Tinamou			X
Rhynchotus rufescens	Red-winged Tinamou		X	
Nothura maculosa	Spotted Nothura		X	
Accipitridae				
Elanus leucurus	White-tailed Kite		X	
Rupornis magnirostris	Roadside Hawk	X	X	
Columbidae				
Columbina squammata	Scaled Dove	X		
Patagioenas picazuro	Picazuro Pigeon			X
Patagioenas plumbea	Plumbeous Pigeon			X
Leptotila verreauxi	White-tipped Dove	X		X
Leptotila rufaxilla	Gray-fronted Dove			X
Strigidae				
Glaucidium brasilianum	Ferruginous Pygmy-Owl	X		
Apodidae				
Panyptila cayennensis	Lesser Swallow-tailed Swift			X
Trogonidae				
Trogon surrucura	Surucua Trogon			X
Bucconidae				
Nystalus maculatus	Spot-backed Puffbird	X		
Picidae				
Picumnus temminckii	Ochre-collared Piculet			X
Veniliornis passerinus	Little Woodpecker	X		
Cariamidae				
Cariama cristata	Red-legged Seriema		X	X
Falconidae				
Herpetotheres cachinnans	Laughing Falcon	X		
Falco femoralis	Aplomado Falcon		X	
Psittacidae				
Eupsittula aurea	Peach-fronted Parakeet		X	
Eupsittula cactorum	Cactus Parakeet	X		
Forpus xanthopterygius	Blue-winged Parrotlet	X		
Brotogeris tirica	Plain Parakeet			X
Pionus maximiliani	Scaly-headed Parrot			X
Triclaria malachitacea	Blue-bellied Parrot			X
Thamnophilidae				
Myrmorchilus strigilatus	Stripe-backed Antbird	X		
Dysithamnus mentalis	Plain Antvireo			X

Herpsilochmus rufimarginatus	Rufous-winged Antwren			X
Thamnophilus caerulescens	Variable Antshrike			X
Hypoedaleus guttatus	Spot-backed Antshrike			X
Batara cinerea	Giant Antshrike			X
Pyriglena leucoptera	White-shouldered Fire-eye			X
Drymophila ochropyga	Ochre-rumped Antbird			X
Conopophagidae				
Conopophaga lineata	Rufous Gnateater			X
Grallariidae				
Grallaria varia	Variegated Antpitta			X
Formicariidae				
Chamaeza campanisona	Short-tailed Antthrush			X
Scleruridae				
Sclerurus scansor	Rufous-breasted Leaftosser			X
Dendrocolaptidae				
Lepidocolaptes angustirostris	Narrow-billed Woodcreeper	X		
Xenopidae				
Xenops rutilans	Streaked Xenops			X
Furnariidae				
Furnarius rufus	Rufous Hornero		X	
Lochmias nematura	Sharp-tailed Streamcreeper			X
Automolus leucophthalmus	White-eyed Foliage-gleaner			X
Anabazenops fuscus	White-collared Foliage-gleaner			X
Anumbius annumbi	Firewood-Gatherer		X	
Synallaxis albescens	Pale-breasted Spinetail		X	
Cranioleuca pallida	Pallid Spinetail			X
Pipridae				
Chiroxiphia caudata	Swallow-tailed Manakin			X
Tityridae				
Pachyramphus polychopterus	White-winged Becard			X
Pachyramphus validus	Crested Becard			X
Platyrinchidae				
Platyrinchus mystaceus	White-throated Spadebill			X
Rhynchocyclidae				
Mionectes rufiventris	Gray-hooded Flycatcher			X
Leptopogon amaurocephalus	Sepia-capped Flycatcher			X
Corythopis delalandi	Southern Antpipit			X
Tolmomyias sulphurescens	Yellow-olive Flycatcher			X
Tolmomyias flaviventris	Yellow-breasted Flycatcher	X		
Todirostrum cinereum	Common Tody-Flycatcher	X		
Poecilotriccus plumbeiceps	Ochre-faced Tody-Flycatcher			X
Hemitriccus margaritaceiventer	Pearly-vented Tody-tyrant	X		
Tyrannidae				
Euscarthmus meloryphus	Tawny-crowned Pygmy-Tyrant	X		

Phaeomyias murina	Mouse-colored Tyrannulet	X		
Pitangus sulphuratus	Great Kiskadee		X	
Myiodynastes maculatus	Streaked Flycatcher			X
Megarynchus pitangua	Boat-billed Flycatcher			X
Tyrannus melancholicus	Tropical Kingbird			X
Empidonomus varius	Variegated Flycatcher	X		
Sublegatus modestus	Southern Scrub-Flycatcher	X		
Lathrotriccus euleri	Euler's Flycatcher			X
Vireonidae				
Cyclarhis gujanensis	Rufous-browed Peppershrike			X
Vireo chivi	Chivi Vireo			X
Corvidae				
Cyanocorax cyanopogon	White-naped Jay	X		
Hirundinidae				
Stelgidopteryx ruficollis	Southern Rough-winged Swallow		X	
Troglodytidae				
Troglodytes musculus	Southern House Wren	X		
Cantorchilus longirostris	Long-billed Wren	X		
Polioptilidae				
Polioptila plumbea	Tropical Gnatcatcher	X		
Turdidae				
Turdus leucomelas	Pale-breasted Thrush			X
Turdus rufiventris	Rufous-bellied Thrush			X
Turdus albicollis	White-necked Thrush			X
Motacillidae				
Anthus nattereri	Ochre-breasted Pipit		X	
Passerellidae				
Zonotrichia capensis	Rufous-collared Sparrow		X	
Ammodramus humeralis	Grassland Sparrow		X	
Parulidae				
Setophaga pitiayumi	Tropical Parula			X
Basileuterus culicivorus	Golden-crowned Warbler			X
Myiothlypis flaveola	Flavescent Warbler			X
Icteridae				
Pseudoleistes guirahuro	Yellow-rumped Marshbird		X	
Thraupidae				
Neothraupis fasciata	White-banded Tanager		X	
Schistochlamys ruficapillus	Cinnamon Tanager		X	
Tangara desmaresti	Brassy-breasted Tanager			X
Tangara cayana	Burnished-buff Tanager			X
Sicalis citrina	Stripe-tailed Yellow-Finch		X	
Sicalis luteola	Grassland Yellow-Finch		X	
Coryphospingus pileatus	Pileated Finch	X		
Dacnis cayana	Blue Dacnis			X
Coereba flaveola	Bananaquit	X		X
Embernagra platensis	Great Pampa-Finch		X	

Emberizoides herbicola	Wedge-tailed Grass-Finch		X	
Saltator similis	Green-winged Saltator			X
Saltator fuliginosus	Black-throated Grosbeak			X
Cardinalidae				
Habia rubica	Red-crowned Ant-Tanager			X
Cyanoloxia brissonii	Ultramarine Grosbeak	X		
Fringillidae				
Spinus magellanicus	Hooded Siskin		X	
Euphonia chlorotica	Purple-throated Euphonia			X

Table S2 – Descriptive statistics for seven Acoustic Indices. Data is presented as mean (standard deviation).

	Caatinga	Cerrado	Atlantic Forest
NDSI	0.439 (sd= 0.234)	0.604 (sd= 0.304)	0.714 (sd= 0.225)
ACI	0.242 (sd= 0.198)	0.307 (sd= 0.227)	0.392 (sd= 0.292)
ADI	0.425 (sd= 0.409)	0.222 (sd= 0.316)	0.537 (sd= 0.280)
AEI	0.688 (sd= 0.370)	0.875 (sd= 0.265)	0.653 (sd= 0.274)
BIO	0.357 (sd= 0.235)	0.263 (sd= 0.222)	0.429 (sd= 0.235)
H	0.600 (sd= 0.254)	0.317 (sd= 0.298)	0.272 (sd= 0.247)
AR	0.470 (sd= 0.312)	0.335 (sd= 0.308)	0.445 (sd= 0.169)

Table S3 – Results of Kruskal-Wallis and Wilcox pairwise comparison for each Acoustic Indices, between the three biomes.

	Chi-Squared	df	p-value	Wilcox Test
NDSI	1762.7	2	<0.01	<0.01 for all pair comparison
ACI	448.5	2	<0.01	<0.01 for all pair comparison
ADI	1624.7	2	<0.01	<0.01 for all pair comparison
AEI	2026.9	2	<0.01	<0.01 for all pair comparison
BIO	1055.1	2	<0.01	<0.01 for all pair comparison
H	2696.3	2	<0.01	<0.01 for all pair comparison
AR	673.4	2	<0.01	<0.01 for all pair comparison

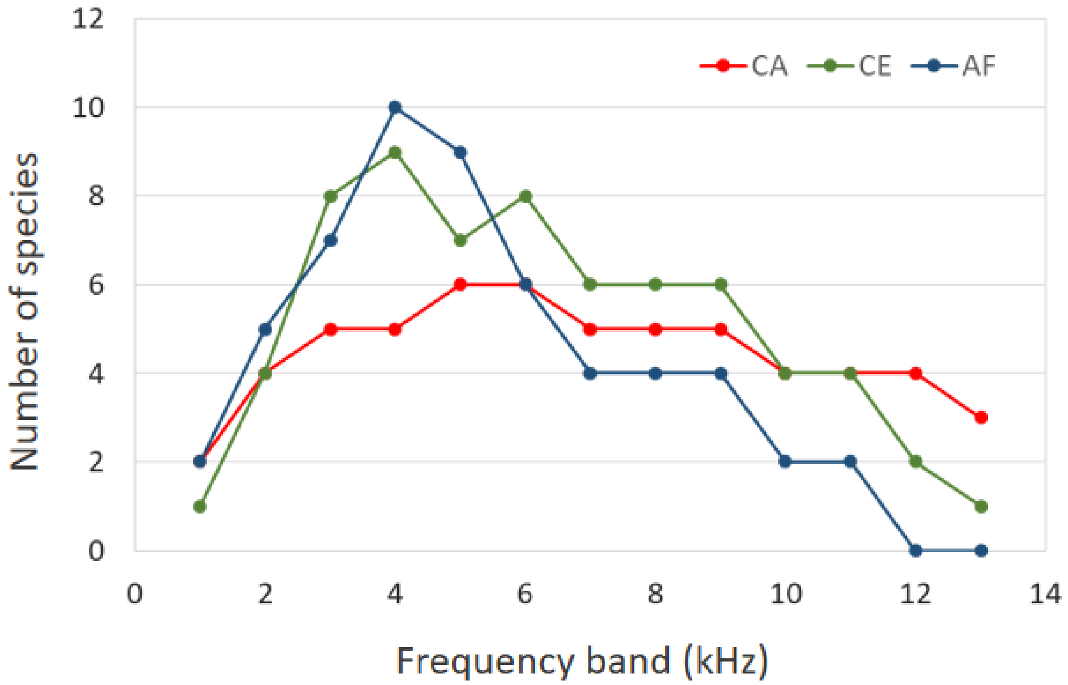

Figure S1 – Frequency bands used by the ten most commonly identified bird species in each biome.

CONCLUSÕES FINAIS

Algumas das questões que a ecologia acústica busca responder são ligadas às métricas usadas como um proxy da biodiversidade de uma determinada área, da sua saúde ambiental e de como alterações decorrentes de processos temporais, espaciais, ou de mudanças no habitat podem ser identificados nas gravações de áudio. Essas questões foram abordadas pelos três capítulos desta tese.

No primeiro capítulo, utilizamos múltiplos índices acústicos descritivos para mapear a distribuição temporal de sons provenientes de biofonia e geofonia, diferenciando os dois grupos animais que compuseram a sinfonia da Caatinga, aves e insetos. Outros elementos mais esporádicos nas gravacoes, como anuros, tiros, carros e animais domésticos, foram identificados em inspeções manuais, mas não constituíram fontes significativas de energia acústica a ponto de serem identificados pela metodologia empregada. A Caatinga é um ambiente que sofre forte pressão antrópica. Portanto, fontes de emissão sonora associadas a ocupação da terra, como espécies invasoras e caça, podem ser objeto de estudos futuros que se utilizem de detectores automáticos, para investigar especificamente estas fontes de som e quantificar sua presença no ambiente.

O Capítulo 2 tratou de uma das alterações humanas na paisagem acústica da Caatinga. Parques eólicos são uma fonte de mudança na paisagem caatingueira crescente nos últimos anos, e a influência acústica desta atividade foi investigada. Encontramos uma diminuição da atividade de aves e insetos ligada à proximidade com os aerogeradores, fontes de emissão de ruído e que representam um disturbio tambem fisico na paisagem natural deste bioma. Espécies com vocalizações de frequências mais baixas que não estão sendo detectadas perto das turbinas possivelmente tem sua comunicação comprometida ao redor das turbinas. Esse fenômeno de mudança da composição, abandono de área, ou silenciamento de espécies ao redor das turbinas deve ser compreendido em maior escala por estudos futuros. Em regiões com alta densidade de parques eólicos, onde a influência de uma linha de aerogeradores pode se sobrepor ao de uma linha vizinha, os efeitos sobre a fauna podem ser sinérgicos e mais ainda mais fortes.

Nos dois primeiros capítulos, os índices acústicos foram usados com sucesso para diferenciar fontes de emissão sonora e quantificar atividade acústica, mas uma das principais premissas dessas métricas é a de ser capaz de serem usadas como um proxy de riqueza de espécies. Assim, utilizamos dados de Caatinga, Mata Atlântica e Cerrado para tentar entender, no capítulo 3, de que forma os índices se relacionam com o número de espécies de aves presentes nas áreas. Os resultados de diferentes índices variaram entre as áreas e também para todas as áreas consideradas juntas. Características da comunidade de aves, como composição de espécies e bandas de frequências mais utilizadas são fatores que podem explicar essa variação. Características da vegetação das áreas, que agem como um filtro evolutivo e sonoro para as faixas de frequência utilizadas pelas aves, podem ser um indicativo de quais índices podem ser mais adequados em diferentes ambientes.

Na Caatinga, principal bioma explorado nessa tese, observamos uma limitação no uso de índices acústicos como proxy de riqueza de espécies (Cap 3). O emprego de múltiplas métricas (Caps 1 e 2), por outro lado, foi capaz de caracterizar padrões temporais, mensurar atividade e construir visualizações da comunidade sonora neste bioma. No entanto, isso demonstra uma fragilidade na generalização das metodologias de estudo de paisagens acústicas e como é importante que a validação e inspeção manuais sejam feitas. Por ter forte contato com as ciências da computação, quanto maiores são os bancos de dados analisados, maior o distanciamento da realidade do ambiente. Assim, o trabalho do ecólogo é fundamental na interpretação desses resultados e na validação e escolha dos métodos a serem empregados. Essa tese deu um passo à frente nessa etapa em que se encontra a área de ecologia acústica, de indicar padrões gerais que podem ser incorporados desde a escolha das análises. No nosso caso, embora a Caatinga seja um ambiente rico em diversidade, as características do ambiente como vegetação aberta e consequente presença forte de vento são um limitante em estudos de paisagem sonora. A sazonalidade marcada e a redução da atividade dos animais devido ao clima também reduz as chances de detecções das espécies com o uso de monitoramento acústico passivo em boa parte do ano. Esse conhecimento da dinâmica das paisagens acústicas pode ser utilizado em outras áreas que apresentem características ambientais semelhantes, auxiliando na escolha de métodos na coleta e análise de dados.

Por ser uma nova área de estudo, que acumula menos de uma década de contribuições substanciais à ciência e que é fortemente influenciada pelos avanços da área tecnológica, nesses quatro últimos anos vimos um aumento considerável de metodologias sendo criadas e novas perguntas a serem respondidas. Novas regiões são estudadas e, da mesma forma que a vegetação e comunidades animais, as paisagens acústicas possuem peculiaridades que ainda estão sendo exploradas. Boa parte das métricas foram desenvolvidas em regiões temperadas, onde a diversidade de animais e seus sons não é tão rica quanto nos trópicos. Esse trabalho também cobre um vazio de conhecimento da área de ecologia acústica em regiões tropicais, demonstra algumas limitações dos métodos e ainda deixa muitas perguntas em aberto para serem desenvolvidas em trabalhos futuros.

APÊNDICES

1. Ferreira, L. M**., Oliveira, E. G**., Lopes, L. C., Brito, M. R., Baumgarten, J., Rodrigues, F. H., & Sousa-Lima, R. S. (2018). What do insects, anurans, birds, and mammals have to say about soundscape indices in a tropical savanna*. Journal of Ecoacoustics*, 2(March), PVH6YZ. https://doi.org/10.22261/JEA.PVH6YZ

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

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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

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

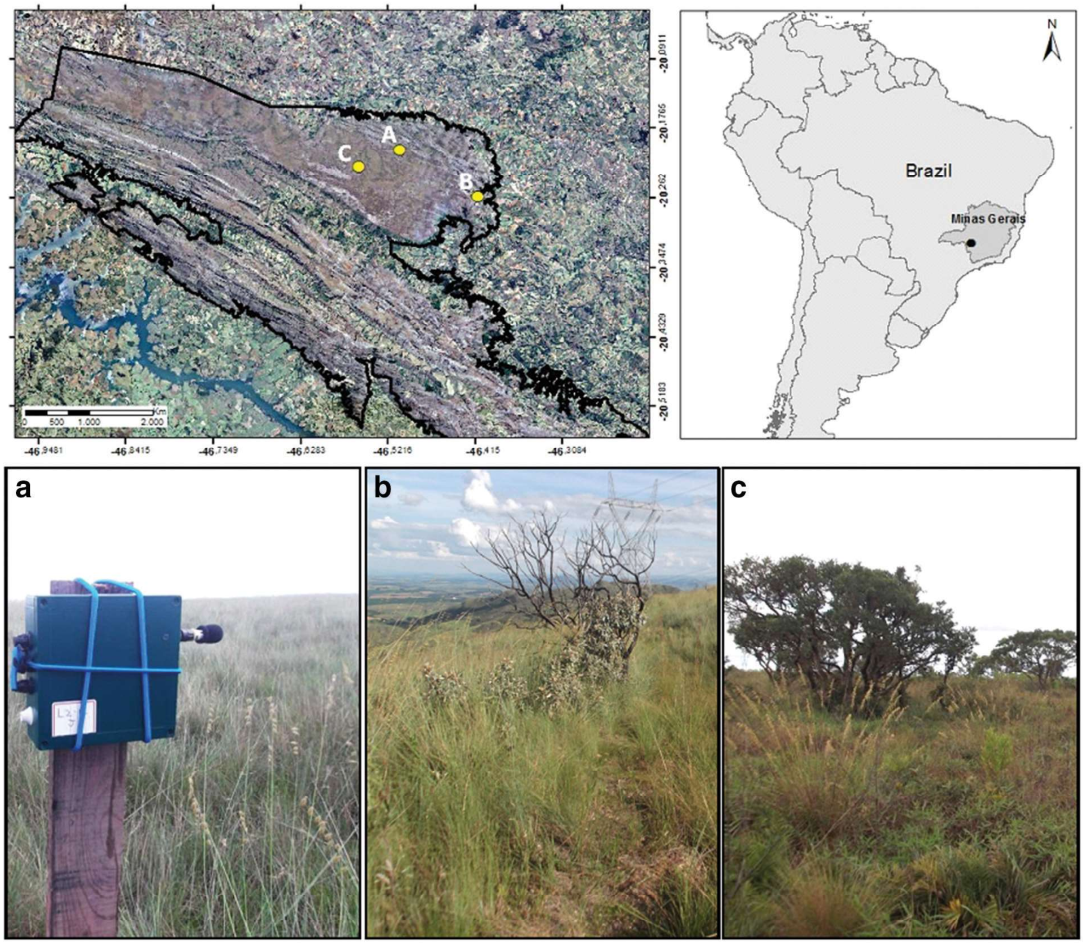

Figure 1. The three studied sites at Serra da Canastra National Park, Minas Gerais state, Brazil.

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 ± 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). 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 analyst 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 (α) with the spectral power area of a predefined anthropophony bin (β) 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

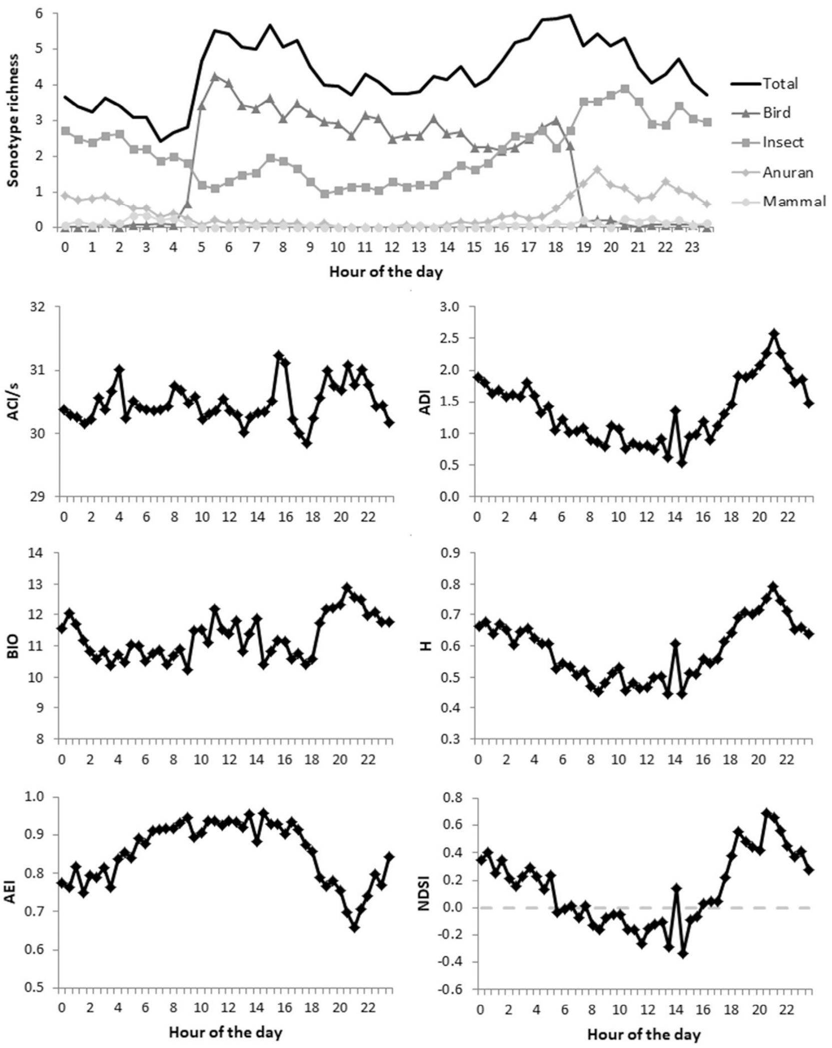

Figure 2. Temporal distribution of the seven day average of sonotype richness (*top*) and acoustic indices extracted from recordings in three different sites at Serra da Canastra National Park, Minas Gerais state, Brazil.

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. $\text{rho}_{\text{NDSIxAEI}} = -0.854$, max. $\text{rho}_{\text{NDSIxH}} = 0.959$, 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 ($\text{rho}_{\text{BIOxH}} = -0.218$, $p < 0.01$).

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*	**0.520***
Insect	0.167	−0.053	**0.673***	**0.697***	**0.673***	**−0.590***
Anuran	0.053	−0.165	**0.773***	**0.824***	**0.819***	**−0.731***
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***	**−0.709***	**−0.717***	**0.501***
Insect	−0.273	0.026	0.104	0.274	0.267	0.107
Anuran	−0.384*	**−0.544***	**0.605***	**0.725***	**0.755***	−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	**0.630***	**−0.715***	**−0.731***	**−0.639***	**0.756***
Insect	0.356	−0.285	0.439*	0.413*	0.460*	−0.429*
Anuran	−0.004	−0.398*	**0.683***	**0.684***	**0.652***	**−0.774***
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***	**0.713***	**−0.541***
Bird	−0.459*	**−0.695***	**−0.575***	**−0.557***	**−0.511***	**0.596***
Insect	0.447*	**0.780***	**0.855***	**0.890***	**0.875***	**−0.792***
Anuran	**0.604***	**0.838***	**0.879***	**0.893***	**0.885***	**−0.822***
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).

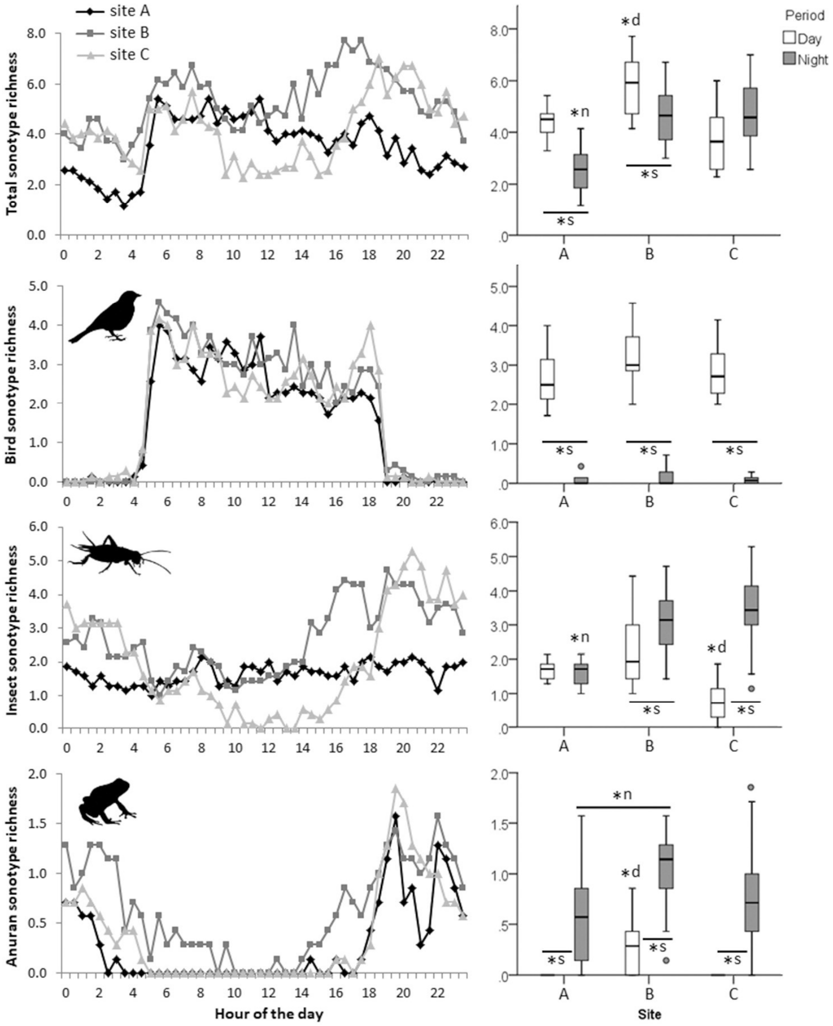

Figure 3. Sonotype richness in three different sites at Serra da Canastra Brazilian National Park, Minas Gerais state, Brazil.

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, $\{N_{day} = 26; N_{night} = 22\} \times 3$ 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

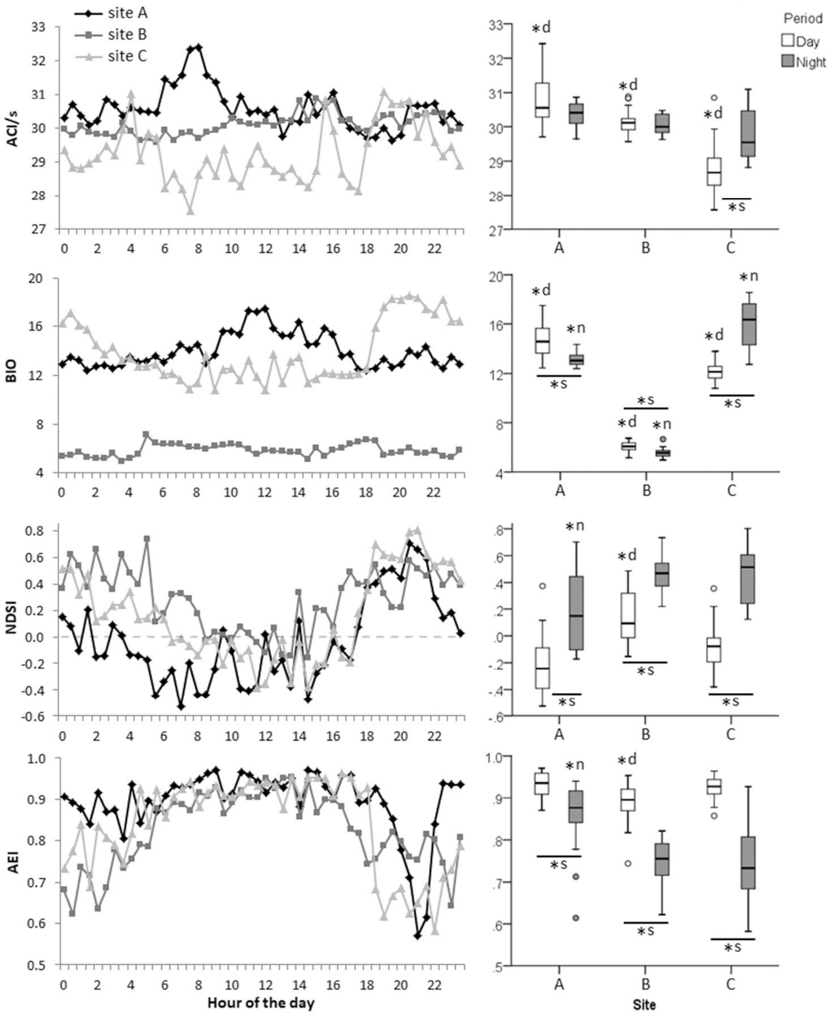

Figure 4. Acoustic indices in three different sites at Serra da Canastra Brazilian National Park, Minas Gerais state, Brazil.

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)

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Competing interests

All authors declare that they have no conflict of interest.

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ZUM ZUM
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COACH
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O som da natureza

Concentre-se para responder: você ouve o barulho das águas de córregos e cachoeiras, das folhas das árvores quando o vento bate, das aves cantando e de outros animais que se escondem na vegetação – onde estamos? Na floresta, claro! Agora você ouve barulhos de sirenes, buzinas, máquinas furando o chão, vozes de muita gente ao mesmo tempo – onde estamos? Na cidade? Acertou! A noção de saber onde estamos somente por meio dos sons é criada por um dos nossos sentidos: a audição. O som tem importantes funções não apenas para nós, humanos, mas também para os outros animais, que se comunicam por meio dos sons que emitem. Portanto, ouvir é importante para bicho e para gente!

Nós, humanos, criamos sons para nos comunicar: pronunciamos palavras, frases, conversamos, cantamos, e fazemos isso em um ou mais idiomas até! Para alguns pesquisadores, essa linguagem tão elaborada, isto é, essa capacidade tão especial de falar (emitir som), ouvir e responder foi o que fez a nossa sociedade evoluir tanto.

Mas, e quando falamos de outros animais – como pássaros, macacos, baleias, sapos, abelhas... –, o que eles querem dizer com os sons que emitem? Essa é exatamente a pergunta que os biólogos que trabalham na área de bioacústica tentam responder. Nem sempre é uma tarefa fácil!

O que é bioacústica?

A bioacústica estuda os sons emitidos pelos animais para se comunicar. Esses sons podem ser muito variados e com as mais diversas funções,

Ilustrações Jaca

dependendo do animal. Aves, anfíbios e insetos, por exemplo, emitem sons para encontrar seus parceiros e defender seus territórios. Macacos podem gritar para se comunicar dentro do grupo, informar onde podem encontrar água ou comida. Cada espécie tem a capacidade de ouvir melhor o que os membros de sua própria espécie estão dizendo, como se estivem sintonizados.

Mas sabia que os sons emitidos por determinados animais nem sempre são percebidos pela audição humana? Morcegos e golfinhos, por exemplo, se comunicam de uma forma que permite um localizar o outro – é o que chamamos ecolocalização.

A ecolocalização não é percebida por nós, seres humanos, porque esses ultrassons são mais agudos do que o som mais agudo que conseguimos escutar. Por outro lado, os elefantes emitem um outro tipo de som, o infrassom, que é muito grave mesmo, mais grave do que o mais greve dos sons que podemos ouvir e, por isso, não conseguimos escutar também.

Paisagens que falam

Além de utilizar o som para estudar a comunicação entre os animais, recentemente surgiu uma área de estudo chamada ecologia de paisagens acústicas. Opa, paisagem? Como assim? Vamos relembrar o começo desse texto: a gente consegue reconhecer uma área apenas ouvindo os sons ao redor, não consegue? Pois é, quando estudam paisagens acústicas, os pesquisadores não analisam cada som dos animais separadamente, eles estão mais interessados em entender como os sons de diferentes origens se combinam para formar a identidade acústica de uma área.

Complicado? Que nada! Presta atenção: a identidade acústica de um lugar é como a impressão digital de uma pessoa. Assim como cada pessoa tem uma impressão digital que não é igual a de ninguém, cada local tem sua identidade acústica própria! Na identidade acústica, além dos sons dos animais, são incluídos também outros barulhos da natureza, como os de trovões, ventos, chuvas...

Como os sons da natureza variam de acordo com a época do ano, um mesmo ambiente pode ter sons diferentes ao longo do ano, certo? Assim, os cientistas se perguntam: que animais fazem mais barulho na primavera? E nas outras estações? Essas são perguntas que a ecologia de paisagens acústicas tenta responder.

1, 2, 3, gravando!

Agora, como é que os cientistas tentam encontrar respostas? Se você pensou que eles gravam sons de diferentes locais em diferentes períodos, acertou! Mas ninguém precisa ficar segurando um gravador no meio de uma floresta, por exemplo, por meses ou até anos. Já existem gravadores automáticos, que podem ser deixados no local que se deseja estudar por longos períodos de tempo.

Com essas gravações, é possível verificar como os sons se modificam ao longo do tempo, comparar sons de diferentes áreas, e até mesmo ter uma rápida ideia de que área possui mais espécies de animais.

Som que vira imagem

Mas quem é que ouve todas essas gravações? Bem, por mais que seja interessante ouvir os sons da natureza para estudá-los, os cientistas precisam de formas mais rápidas para entender o que está acontecendo nas gravações. É por isso que costumam usar programas de computador que transformam o som em imagem!

Hora de gritar “UAU!” e perguntar: por que transformar sons em imagens? Ora, porque é muito mais rápido olhar para as gravações, do que escutá-las.

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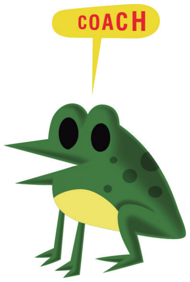
Som natural

Muitos cientistas estão disponibilizando essas gravações para que todo mundo possa escutar e aprender mais sobre os sons da natureza. Quer ouvir?
https://www.wikiaves.com.br/
http://www.legrandorchestredesanimaux.com/en



Fotos Wikipédia

A volunteer cleans oil from rocks on a beach in northeastern Brazil.

PHOTO: ANTONELLO VENERI/AFP/GETTY IMAGES

Edited by **Jennifer Sills**

Retraction

After publication of the Report "Site-selective enzymatic C—H amidation for synthesis of diverse lactams" (*1*), efforts to reproduce the work showed that the enzymes do not catalyze the reactions with the activities and selectivities claimed. Careful examination of the first author's lab notebook then revealed missing contemporaneous entries and raw data for key experiments. The authors are therefore retracting the paper.

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Brazil oil spill response: Time for coordination

In his News In Depth story "Mystery oil spill threatens marine sanctuary in Brazil" (8 November 2019, p. 672), H. Escobar discusses a dense crude oil spill that arrived at Brazil's northeastern tropical coast in late August 2019. Given its extent (more than 3000 km) (*1*) and the recorded impacts (*2*), this spill is considered the most severe environmental disaster ever recorded in tropical coastal regions. More than 40 marine protected areas and a unique set of poorly explored coastal ecosystems (*3*) that include intertidal rocky shores, rhodolith beds, sandy beaches, mangroves, estuarine systems, seagrass beds, and coral reefs have been affected. Exacerbating the ecological, social, and economic impacts, Brazil's government action has been inadequate.

The Brazilian federal government has shown poor coordination with the nongovernmental organizations, military, civil society, states, and Brazilian municipalities to address the oil spill's effects (*4*). The lack of coordination and proper transparent guidelines made a rapid response nearly impossible. The federal government disbanded the executive and support committees responsible for oil-spill accidents (Contingency Plan for Oil Pollution team) in early 2019 (*4*). The resulting lack of leadership delayed the governmental response to the oil spill (*5*). Moreover, the recent budget cuts to science (*6*, *7*) and unraveling of environmental policies (*8*, *9*) undermine the capacity of Brazilian institutions to understand and solve the impacts of this uncontrolled environmental disaster.

Shallow and deep oil extraction is a delicate matter. The inadequate response to this disaster highlights the importance of establishing science-based solutions to prevent extensive and long-term impacts of coastal and offshore oil extraction. Governments must execute a coordinated response so as not to aggravate the problems.

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Brazil oil spill response: Government inaction

In his News In Depth story "Mystery oil spill threatens marine sanctuary in Brazil" (8 November 2019, p. 672), H. Escobar describes the contamination of 2500 km of Brazil's northeast coast caused by oil from an offshore oil spill, which is threatening marine biodiversity, livelihoods, and human health in one of the country's most iconic and touristic places. The spill has already affected 15 marine protected areas (*1*) and had incalculable impacts on wildlife and

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ecological services, which could last for decades (*2*, *3*). Escobar also mentioned the Brazilian government's delayed action and disinformation campaign in response to the spill, but he does not sufficiently describe the government's malfeasance.

Brazil's federal government has been profoundly lax in the face of this environmental catastrophe. On 17 October 2019, the Federal Prosecution Service, responsible for ensuring social and individual rights in matters of public interest, denounced the government's inaction (*4*). The government responded that the Contingency Plan for Oil Pollution Incidents (*5*) had already been activated, with "necessary adaptations," but never clarified what those adaptations were (*4*). This Contingency Plan was improperly implemented: It should have contained a comprehensive set of guidelines to organize an integrated action plan that mitigated further contamination from the spill and alleviated its impacts (*5*).

While the government neglected its responsibilities, volunteers from civil society risked their lives to help remove more than 5000 tons of oiled residue from 980 areas (*6*), including beaches and mangroves, often without support or personal protective equipment (*7*). State and local governments have collaborated as best as they can, but they depend on federal agencies' direction and resources. The oil is no longer reaching the beaches (*6*), but environmental and human health monitoring will be necessary for several years (*8*).

In less than a year, Brazil has experienced multiple environmental tragedies, including a mudslide (*9*), uncontrolled fires in the Amazon (*10*), and now an oil spill. Despite these threats, the Bolsonaro government has dismantled environmental policy (*10*). Brazilian biodiversity is crucial for ecological services and climate regulation (*11*). Civil society, researchers, nongovernmental organizations, and international markets should pressure the Brazilian government to reverse its destructive environmental agenda.

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Brazil oil spill response: Protect rhodolith beds

In his News In Depth story "Mystery oil spill threatens marine sanctuary in Brazil" (8 November 2019, p. 672), H. Escobar highlights important ecosystems that have been affected by the spill. However, he did not mention the Brazilian rhodolith beds—the most extensive, abundant, and diverse biogenic carbonate habitats in the South Atlantic (*1*). The oil spill severely threatens these ecosystems, which comprise a staggering 2 x 10^{11} tons of carbonatic bank (*2*), stretch from 5°N to 27°S along the Brazilian coast, and cover a seabed potential area of 229,000 km^2 (*1*).

Brazil's rhodolith beds are recognized as an oasis of diversity (*3*). Although they harbor species of great economic and ecological value, they remain unprotected. The oil pollution will likely cause major socio-environmental and economic losses, similar to those caused by the Deepwater Horizon incident in the Gulf of Mexico (*4*). The contamination will compromise the region's food security as well as biodiversity conservation and efficient management. Moreover, the ongoing oil spill could have global consequences given the potential biogeochemical role of rhodolith beds in the oceanic carbon balance (*1*, *5*). Thus, this event must not be downplayed or concealed, as has been attempted in the case of Brazilian mining accidents (*6*) and Amazon deforestation and fires (*7*). We advocate urgent action to evaluate and mitigate the oil spill and to remediate and restore areas on the oil slick route. Brazil must follow in the footsteps of Australia and Europe (*8*) and prioritize rhodolith bed conservation.

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COMPETING INTERESTS

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