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Soundscapes of the Yungas Andean forest: Identifying the acoustic footprint of an anuran assemblage

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ABSTRACT

Soundscapes are composed by the conjunction of sound patterns in each space and time. The biophony produced by the advertisement calls of anurans are one of the most outstanding features of a soundscape, particularly in subtropical montane areas such as the Yungas Andean forests of NW Argentina. We recorded soundscapes with automated recording units along an altitudinal gradient and throughout a whole year in the Calilegua National Park. Using a novel combination of visual analysis and acoustic indices we have been able to identify the anuran acoustic fingerprint in the soundscapes of a seasonal subtropical montane forest. In addition, we detected a decrement in using the acoustic communication channel along the altitudinal gradient by anuran assemblages, in agreement with a decrement in species richness. Our results showed a seasonal effect of the vocalization patterns, splitting the group of sounds recorded in two main periods along the year. In this study we presented the first description of Argentina's altitudinal strata of the Yungas Andean forests. This information provides a solid basis for monitoring programs of direct and indirect anthropogenic impacts. Additionally, we highlight the importance of understanding how soundscapes are composed in a naturally protected area and, posit them as a novel ecosystem service that could benefit human health. We emphasise that Natural Soundscapes should be protected and must be included as intangible heritage in the management plans of protected areas in the country. Likewise, the methods proposed in this study encourage settling down the basis for implementing long-term acoustic monitoring of anuran species and other acoustic communities in the Yungas ecoregion.

1. Introduction

Animal acoustic communities are individuals that vocalise, exchanging information in each time and space (Farina and James, 2016). Anuran choruses, formed by a diverse number of species that vocalise simultaneously, constitute a component that stands out within the acoustic community mainly because of their markedly seasonal patterns (Ulloa et al., 2019; Duarte et al., 2019) and form an outstanding portion of the biophony of a soundscape (Ryan, 2001; Gerhardt and Huber, 2002; Wells and Schwartz, 2007). To minimise interspecific competition for the acoustic communication channel, anuran species of a community tend to avoid the spectral and temporal overlap of their calls (Drewry and Rand, 1983; Krause, 1987; Narins, 1995; Schwartz and Wells, 1983; Schwartz and Bee, 2013). The sum of these spectro-temporal interactions makes an acoustic community of anurans to have a particular and extended fingerprint within a soundscape (Farina and James, 2016).

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From an ecoacoustical approach, it is possible to study the temporal patterns of the calling activity of an anuran community along environmental gradients (Weiperth et al., 2016) and through long-term monitoring programs (Duarte et al., 2019). The frequency bands used by an anuran acoustic community have a well-defined spectral signature (Farina and James, 2016). In recent years, useful tools have been developed for the analysis and precise description of acoustic communities, through the combination of acoustic indices and visualisation tools employing false colour spectrograms (Towsey, 2017; Sankupellay et al., 2015). The implementation of acoustic indices is becoming more frequent in the field of ecoacoustics (Farina and Gage, 2017). In addition, the visualisation of the extended time-lapses allows obtaining qualitative and quantitative representations of the acoustic spectrum in a soundscape (Towsey et al., 2014). Both tools can identify daily patterns and seasonal changes of the acoustic communities in environments with a marked climatic seasonality (Garcia Oliveira et al., 2021).

In the specific case of the subtropical montane of North-wetern Argentina (Southern Andean Yungas ecoregion), they are characterised by high environmental heterogeneity, where the marked elevational gradients are accompanied by a physiognomic and floristic gradient (Grau and Brown, 2000). Likewise, this ecoregion is influenced by subtropical climate with a monsoon regime forming forests with contrasting characteristics according to the dry or wet season of the year (Grau, 2005). The elevation gradient of the Yungas conditions the species richness of the anuran assemblages that inhabit different phytogeographic strata of these montane cloud forests (Vaira, 2002; Boullhesen et al., 2021). In addition to this altitudinal variation in species richness, it is a marked seasonality in their reproductive activity where most of the anuran species that inhabit the Yungas of Argentina are vocally active during the aestival season (Vaira, 2002). The anuran acoustic communities in the Calilegua National Park, can occupy a wide range of sound frequencies, ranging from very low frequencies, such as 250 Hz in the case of the advertisement call of *Leptodactylus macrosternum* (Heyer and Giaretta, 2009), to 4600 Hz as in the case of *Dendropsophus nanus* (Teixeira et al., 2016).

The Southern Andean Yungas ecoregion (Fig. 1 A) is the southernmost extension of the range of the megadiverse tropical Andes region (Myers et al., 2000), and is also one of the most biodiverse ecoregions in Argentina (Burkart et al., 1999). Its forests represent less than 1% of the continental territory of Argentina. They have undergone dramatic land-use change, with almost complete loss of premontane forest and extensive alterations of the upper altitudinal floors of the Yungas (Brown et al., 2006; 2009). This mountainous forest faces constant pressure from the human population, which is advancing with agricultural expansion and the introduction of livestock, drastically reducing its original extent (Brown et al., 2006). These changes have led to forest remnants, severely threatened by human influence (Brown et al., 2006). The Parque Nacional Calilegua (PNC) is one of the most representative protected areas of the Yungas Andean forests (Malizia et al., 2010), and there is vast information about its biodiversity (Vaira, 2002; Grau et al., 2016; Ganem et al., 2013). Its diversity decreases along the altitudinal gradient, and it has been documented a high complementarity in the assemblage's composition (Vaira, 2002; 2002; Boullhesen et al., 2021). The remarkable diversity of anurans and a high proportion of endemisms make this protected area of great importance for amphibian conservation in Argentina (Vaira, 2002; Vaira et al., 2017). Until now, to our knowledge there is no record of how the Calilegua soundscapes are formed nor its acoustic communities and how this species turnover correlates with the acoustic communication channel.

The aim of this work was to identify and characterise the acoustic fingerprint of the anuran assemblages of the PNC. In addition, we compared the use of the acoustic communication channel of the anuran community along their altitudinal stratum and its seasonal

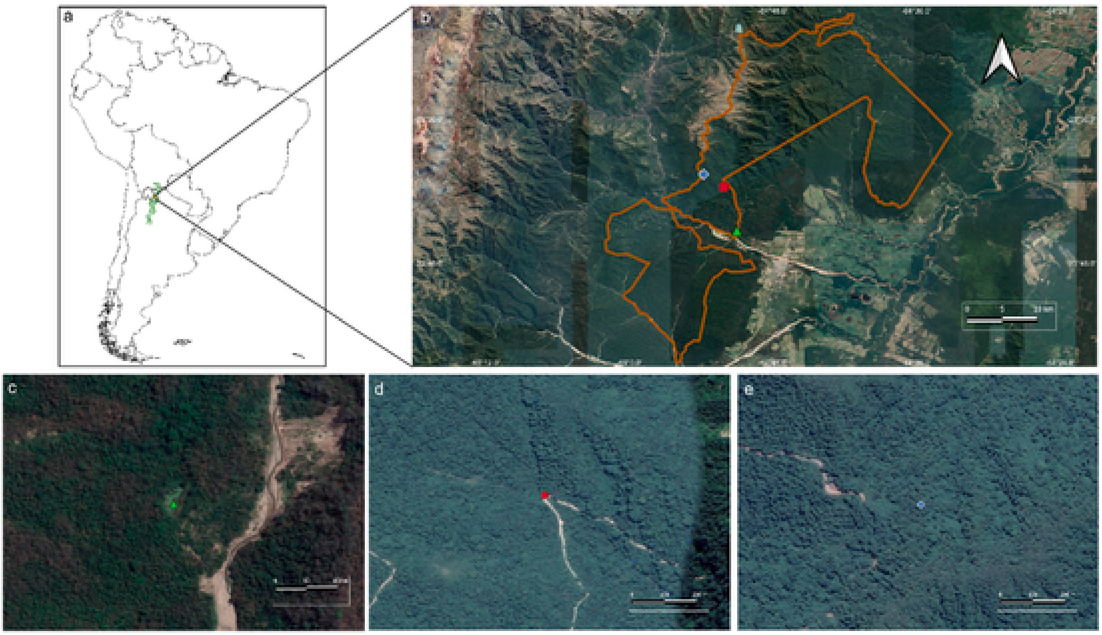

Fig. 1. a) South America showing the Yungas Ecoregion in green; b) Calilegua National Park; c) PF = Premontane Forest; d) LMF = Lowe Montane Forest; e) UMF = Upper Montane Forest. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

variation. In this study, we propose using of available visualisation and analytics tools such as acoustic indices to describe different soundscapes and identify anuran acoustic fingerprint along an altitudinal gradient in north-western Yungas.

2. Methods

2.1. Study area

The study was developed in the Parque Nacional Calilegua (PNC), in the province of Jujuy, NW Argentina (Fig. 1 b). This protected area covering76,320 ha of Yungas Andean forests (Malizia et al., 2010).

We selected three sites with representative reproductive habitats, commonly used by the entire anuran assemblage of the PNC along the altitudinal gradient of the three phytogeographic strata occurring in this protected area (Boullhesen et al., 2021). We proposed that there will be a decrease in the complexity of the acoustic communication channel from the richest to the lowest species assemblages. Also, we suggested that given the marked seasonality reflected in the reproductive activity of the anuran assemblage of the PNC (Vaira, 2002), an increase in the use of the acoustic communication channel of the anuran assemblage will be expected from the autumn-winter season to the spring-summer season.

The lowest site corresponds to the Premontane Forest stratum (PF) (Fig. 1 c), located at 23°45′16.84″ S; 64°50′59.35″ W, at an altitude of 650 m asl. It includes a permanent lentic pond with an approximate area of 1114 m^2, surrounded by deciduous trees such as the "Cebil Rojo" (*Anadenanthera colubrina*) and the "Sauce criollo" (*Salix humboldtiana*). The intermediate site belongs to the Lower Montane Forest stratum (LMF) (Fig. 1 d), located at 23°41′36.84″ S; 64°52′5.04″ O, at an altitude of 1125 m asl., includes a permanent stream surrounded by an evergreen forest of "Nogal criollo" (*Juglans australis*), "Cedros" (*Cedrela balansae*) and "Pacará" *(Enterolobium contortisiliquum*). The highest site belongs to the Upper Montane Forest (UMF) (Fig. 1 e), located at 23° 40 ′28.56″ S; 64°53′44.15″ W, at an altitude of 1650 m asl. This site is a primary woodland dominated by trees of the family Myrtaceae.

2.2. Passive acoustic monitoring

Three Song Meter SM4 (Wildlife Acoustics, Inc., Concord, MA, USA) automated recording units (hereafter ARUs) were installed at 1.5 m above ground level. The ARUs were active from September 2017 to September 2018 and programmed to record three consecutive minutes per hour (72 min/day) on the MONO channel (following Shirose et al., 1997; Márquez et al., 2014). The recordings were stored in digital files in uncompressed format (.WAV) in 16-bit resolution with a frequency range of 16 kHz. The recordings were analyzed in the laboratory using Raven Pro © 1.5 software (Center for Conservation Bioacoustics, 2014). To identify the species of anurans, we used a spectrogram visualisation tool programmed with Hanning-type windows, a resolution of 512 bands and a bandwidth of 100%. Only vocalising adult males could be recorded with this survey method (Boullhesen et al., 2021). From the complete data set, we analyse a total of 2589 recordings corresponding to one day per month at each sample site.

2.3. Long-false colour spectrograms

To identify the acoustic fingerprint of the anuran assemblages along the study sites, false colour spectrograms were constructed using the QUT Ecoacoustics Audio Analysis Software v.17.06.000.34 (Towsey et al., 2018). This analysis was recommended as an effective visualisation tool to identify anuran species in long recordings (Brodie et al., 2022). The analysis was conducted in two steps.

Step 1) Six acoustic indices were calculated following the methodology proposed by Towsey (2017) with the combination of 6 acoustic indices: ACI – ENT – ENV and BGN – PMN – BPE for a long recording (corresponding to 1 day recorded per month in each site = 15:30 h). The Acoustic Complexity Index (ACI) was calculated from the amplitude of the spectrogram. For each frequency link during a 60-s recording, this index computes the absolute average of the frequency amplitude ratio in successive sound spectra. The Acoustic Entropy Index (ENT) calculates the temporal entropy of all values in each frequency link of the amplitude spectrogram. The Events Per Second rate (EVN) is a vector that counts the acoustic events in each frequency link. The Background Noise index (BGN) estimates the background noise calculated for each recording. The Power Minus Noise (PMN) rating calculates the difference between the maximum decibel value on each frequency link and the decibel value of the BGN rating for the corresponding frequency links. Spectral Peak Search index (SPS) is a measure that detects the presence of frequency peaks in a spectrogram.

Step 2) Using the output of the previous step (the result of the combination of the six acoustic indices), long-false colour spectrograms were plotted (Sankupellay et al., 2015). The long-false colour spectrograms result from the information obtained from the recordings of the combination of the six acoustic indices calculated. This analysis was performed using the software AnalysisPrograms available on GitHub (Towsey et al., 2018). MB visually inspected the long-false colour spectrograms for de identification of anurans acoustic fingerprints.

2.4. Soundscape analysis

Nine acoustic indices were calculated to describe the soundscapes along the three sites and the two main periods of the years recorded at the PNC. The description of the soundscapes by calculating the acoustic indices was focused on the frequency range occupied by the species of anurans recorded in the altitudinal gradient of the different phytogeographic strata in the PNC (Boullhesen et al., 2021). The set of acoustic indices was defined to characterise the acoustic fingerprint of the anuran assemblage at each site recorded, comparing them, and evaluating their acoustic patterns throughout the study period. Two types of acoustic indices were calculated following the definitions proposed by Sueur et al. (2014): 1) indices that reflect the acoustic complexity of a soundscape = Bioacoustic Index (Bio) (Boelman et al., 2007); Acoustic Entropy Index (H) (Sueur et al., 2008); Acoustic Richness Index

(AR), Acoustic Amplitude Index (M) and the Temporal Entropy Index (H_t) (Depraetere et al., 2012); Acoustic Diversity Index (ADI) and Acoustic Equity Index (AEI) (Villanueva-Rivera et al., 2011); Acoustic Complexity Index (ACI) (Pieretti et al., 2011) and 2) an index that reflects the relative contribution of biophony and anthrophony to a given soundscape, the NDSI (Normalised Differential Soundscape Index), calculated using a simple formula: NDSI = b – a/b + a where b is biophony (sounds between 2 and 11 kHz) and a corresponds to anthrophony (sounds between 1 and 2 kHz) (Kasten et al., 2012). The NDSI ranges from −1 (low-frequency domain = anthrophony) to 1 (high-frequency domain = biophony). All indices were calculated using the *soundecology* and *seewave* packages in R software version 4.0.2 (R Core Team, 2021).

2.5. Statistical analysis

To evaluate the dissimilarity of the recorded soundscapes we split the year recorded in the two main periods: spring-summer = September 21 th to March 20 th; and autumn-winter = March 21 th to August 31 th). Violin-box plots were constructed with monthly values (one day per month = 2589 recordings files) of each one of the computed indices along the sites and the main periods recorded using the *ggstatsplot* package version 0.8.0 (Patil, 2021). These graphs were fitted accompanied by a non-parametric statistic to evaluate the existence of differences in the acoustic indices between the two main seasons of the year (Mann-Whitney test) and between the sites recorded (Kruskal-Wallis test) (Patil, 2021).

The acoustic indices values were then standardised using a range from 0 to 1 to make them comparable among the three recorded sites (Schielzeth, 2010) apart from the NDSI index that has a range between −1 and 1 and was transformed as: NDSI* = (NDSI + 1)/2 (Fairbrass et al., 2017). To explore the differences in the soundscapes between the recorded sites, a multivariate analysis of the homogeneity of the variance dispersion group was performed, using the "*betadisper*" function of the *vegan* package version 2.5–6 (Oksanen et al., 2019). This analysis fits a distance-based dissimilarity matrix used as input to calculate and visualise the variability between beta diversity distributions between data sets (Anderson et al., 2006). The matrix of the nine calculated acoustic indices was used as input data for the analysis (section 2.1). To perform this analysis, the average of the distances of the group members to the centroid of each data group was used. To evaluate the contrast of means calculated from the matrix, a Tukey post-hoc analysis was performed, creating a set of confidence intervals between the distances to the centroid. To plot this dissimilarity matrix, a Principal Coordinates Analysis (PCoA) was performed with model 1, where: the principal coordinates correspond to the sites (factor = "Site") and with model 2, where: the principal coordinates correspond to the main periods of the year (factor = "Seasons").

3. Results

The year-round false-colour spectrogram made it possible to visualise and identify the acoustic fingerprint used by anuran assemblages recorded in each location in the altitudinal gradient of the PNC and to identify a clear seasonal activity pattern of frog's assemblages (Fig. 2). The acoustic fingerprint of the entire anuran community of the PNC was between 200 and 5000 Hz. A marked decrease in the acoustic range was recorded from the anuran community of Premontane Forest to the Upper Montane Forest (Fig. 2A–C). In addition, an increase in the background noise gradient (Fig. 3A–C) was recorded from the PF (lower elevation site) to the MF site (higher elevation).

Several indices of acoustic complexity of the soundscape (H, ADI, AEI, Bio and NDSI) showed significant differences between the two main periods of the year 1) and between the three recorded study sites. The Acoustic Complexity Index (ACI) showed significant differences between the study sites but was not different between the periods of the year. Likewise, the Acoustic Amplitude index (M) showed significant differences between the study sites and no significant differences were detected between the periods of the year (Fig. 4A–B).

The beta diversity analysis of the model fitted with all the acoustic indices calculated showed significant differences between sites ($n = 2589$; ANOVA $p < 0.001$) and between the two main periods of the year (ANOVA $p < 0.001$). The analysis of the homogeneity of variances (Tukey test) of the model showed significant differences between the UMF and PF soundscapes ($p < 0.05$). The Principal Coordinate Analysis of Model 1 (factor = "Site") did not show a clear dissimilarity of the acoustic information provided by the indices among the three recorded sites, where the first two axes explained 62.88% of the data variability (Fig. 5A). However, it stands out that PF and UMF sites presented a greater dissimilarity of the acoustic information recorded concerning to the LMF site (Fig. 5 A). Model 2 (factor = "Period") showed a higher dissimilarity among the acoustic information recorded in the two seasons of the year with the first two axes, which explains 62.88% of the variability of the data (Fig. 5 B).

4. Discussion

With this study, we provide a standpoint to understand how soundscapes are conformed in a diversity hotspot natural protected area of the Southern Andean Yungas ecoregion in Argentina (Myers et al., 2000), as well as characterise the acoustic fingerprint of the PNC' anuran assemblage for the first time. Witch, proved to be an area of relatively high amphibian diversity compared to other montane forest areas surveyed in Argentina (Vaira, 2002).

4.1. Anuran acoustic fingerprint in the PNC

By implementing acoustic indices calculations and their visualisation with year-round long false colour spectrograms (Figs. 2 and 3), we were able to identify the acoustic niche of the anurans within the soundscape of the complex orography of the PNC. Which corresponds to the most representative protected area of the Yungas ecoregion in the province (Malizia et al., 2010). The acoustic community of anurans occupied a wide range of frequencies along the three study locations in the PNC. The calls made by a community of anurans can be very diverse according to their spectral features, the time spent calling and the diversity of species that inhabit an en-

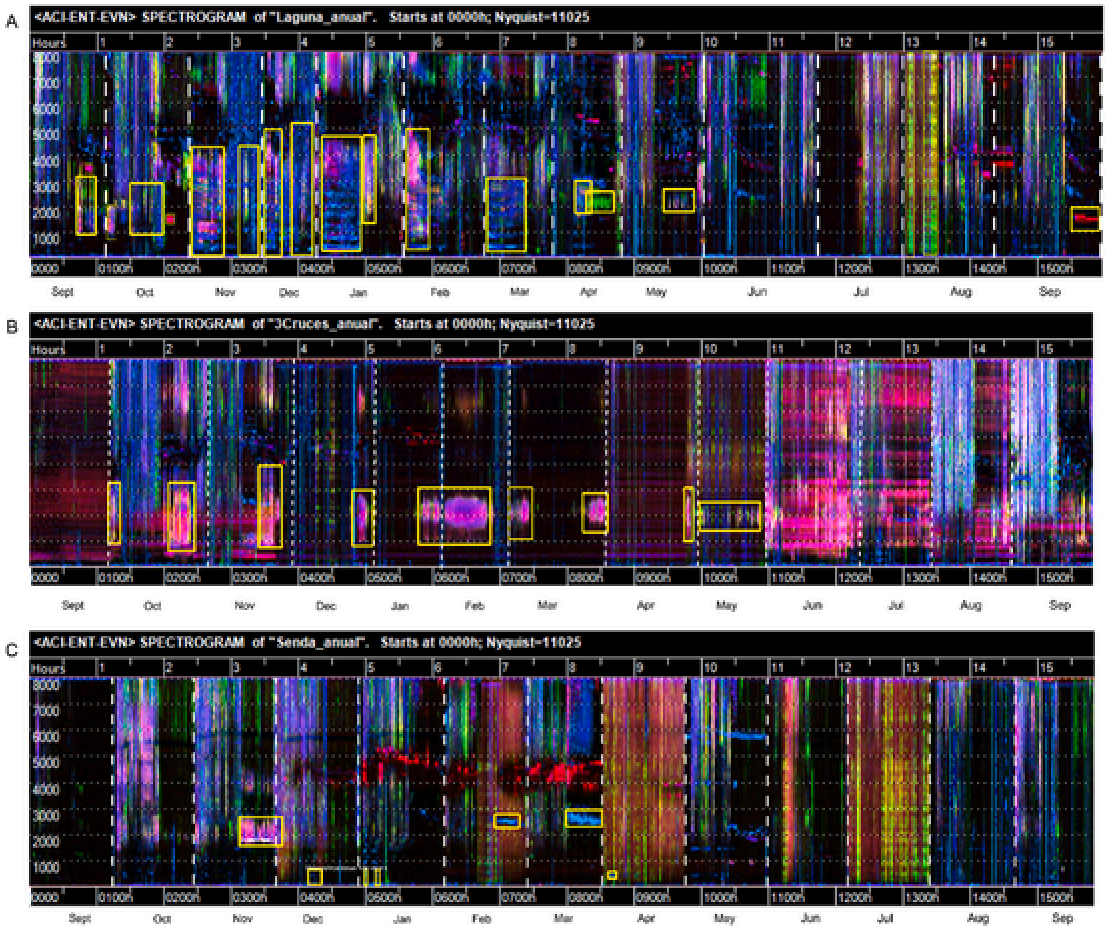

Fig. 2. Long-False colour spectrogram at the three sites monitored with the combination of ACI-ENT-EVN indices. A = Premontane Forest; B = Lower Montane Forest; C = Upper Montane Forest; Yellow boxes = anuran calls. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

vironment (Duarte et al., 2019; Sinsch et al., 2012). In addition, the choruses made by anurans make up a sound fingerprint that stands out in the biophony of a soundscape (Ferreira et al., 2018), mainly in neotropical regions where the events of the chorus may reflect relationships of this reproductive activity and are triggered by climatic factors (Ulloa et al., 2019).

4.2. The Yungas Andean forests soundscapes

A marked difference was recorded between the soundscapes within the three monitored sites at the PNC. These acoustic differences could be linked to the anuran assemblage composition and species turnover along the altitudinal gradient monitored (Boullhesen et al., 2021). In addition, differences between the soundscapes recorded could be reflected by the high level of heterogeneity identified for the Yungas Andean forests (Grau and Brown, 2000), considering the characteristics of the habitat conditions and their relation to sound patterns (Pijanowski et al., 2011; Fuller et al., 2015). We detected a significant difference between seasonal soundscapes in the wet and dry seasons throughout the year. This could be associated with the marked seasonality characterised by the monsoon regime in the area (Grau, 2005). The coincidence of the rainy and warm seasons may also condition the activity patterns of the living organisms that inhabit this forested area, being more evident in ectothermic animals such as anurans. The soundscapes described in this work allow us to highlight the marked climatic seasonality of the Yungas Andean Forest of north-western Argentina (Brown and Malizia, 2004), which in turn plays a determinant role in the reproductive cycles of the anuran assemblage of the PNC (Vaira, 2002). In this way, the biophony of the soundscape will be conditioned by the climatic factors where they live (Pijanowski et al., 2011), fluctuating in regions where the climate is markedly seasonal.

The altitudinal decrement of anuran richness in the Yungas Andean forests was clearly reflected in the use of the acoustic communication channel from the site belonging to the lowest and most diverse stratum (PF), where the acoustic fingerprint covered a wide spectral range from 250 to 4600 Hz (Heyer and Giaretta, 2009; Teixeira et al., 2016), to the highest site of the study where the acoustic spectrum was more limited. This could be associated with the calling ranges of the two species recorded at the upper site, vocalising in a narrower range of frequencies between 1700 and 2000 Hz (Ferrari and Vaira, 2008; Akmentins et al., 2021). This is a clear contrast with the 17 species registered vocalising in the Premontane Forests area (Boullhesen, 2022). The marked difference in

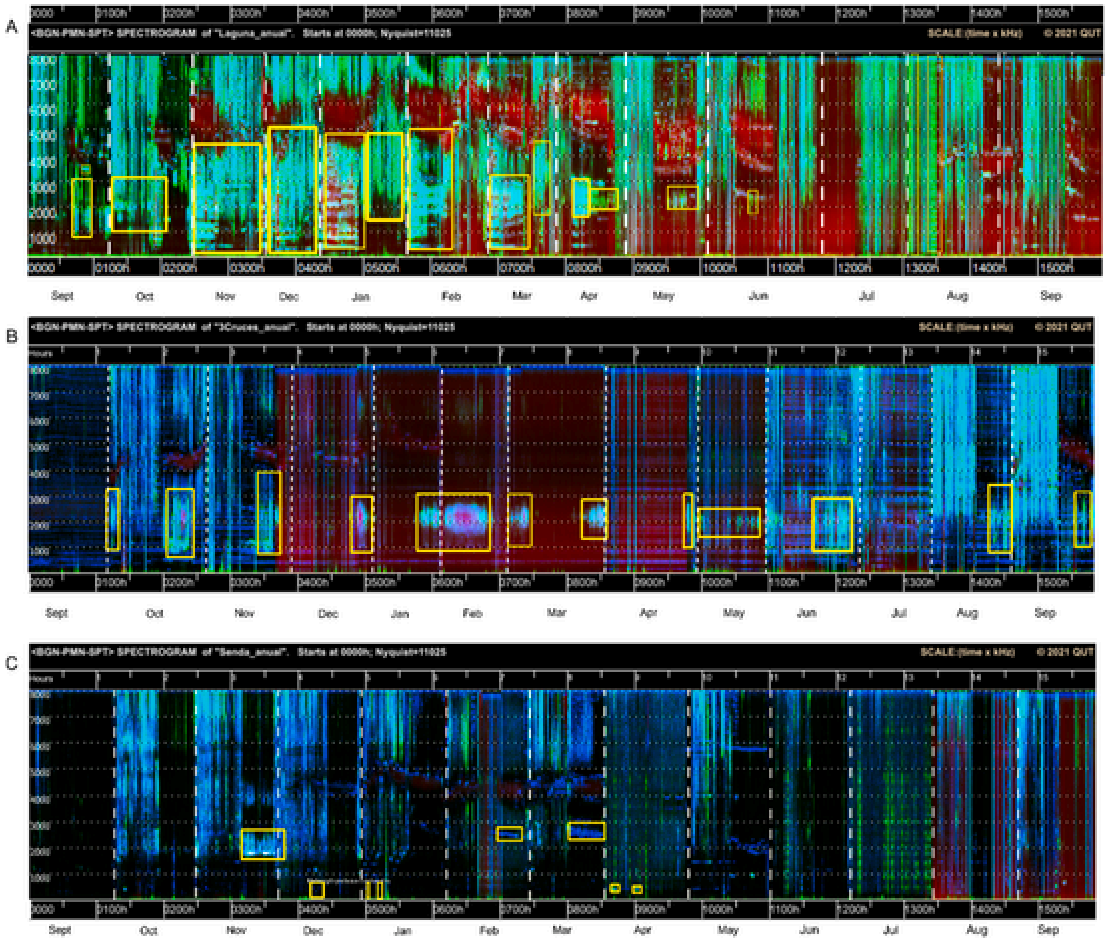

Fig. 3. Long-False colour spectrogram with the combination of BGN-PMN-SPT indices. A = Premontane Forest; B = Lower Montane Forest; C = Upper Montane Forest; Yellow boxes = anuran calls. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the acoustic spectrum, coupled with the recorded differences in soundscapes, seems to be a good proxy of the diversity of anurans detected in the natural protected area.

4.3. Use of acoustic indices for soundscape's description

The Acoustic Complexity Index (ACI) values reflected a higher acoustic complexity for the LMF site, compared to the other sites recorded. This could be explained by the intense geophony of the permanent stream river recorded throughout the year that may be influencing the index values (Depraetere et al., 2012). High values of ACI were also recorded in stream environments in an Atlantic Forest landscape (Scarpelli et al., 2020). In addition, this site is close to an unpaved road frequently travelled by motor vehicles, which contributes to high levels of anthrophony at the study site, which also may increment the values of the index. This was also reflected in the Normalised Differential Soundscape Index (NDSI) spring-summer period values, which are higher than the autumn-winter period, probably due to the increased tourist activities during this period of the year. Usually, sound patterns fluctuate according to landscape configuration, regional climate, human activities, and the acoustic communities that inhabit them (Pijanowski et al., 2011; Fuller et al., 2015).

Bioacoustic index values reflected a clear difference between the upper and lower monitored sites, being higher in the lower and intermediate sites than the upper site, in agreement with previous reports of the biophony in these montane forest areas (Boullhesen et al., 2021). However, in contrast to our hypothesis, the spring-summer period registered lower Bio values than the autumn-winter period. This index was originally developed to reflect sound pressure levels across the band spectrum elaborated by birds (Boelman et al., 2007). However, further investigations have shown mixed results with both positive and negative correlations to bird species richness (Fuller et al., 2015; Mammides et al., 2017) in areas with numerous vocalising species. In addition, many of these indices are sensitive to background noise, as intense rains, and windy days, are likely to occur during the summer season in the Yungas ecoregion (Grau, 2005).

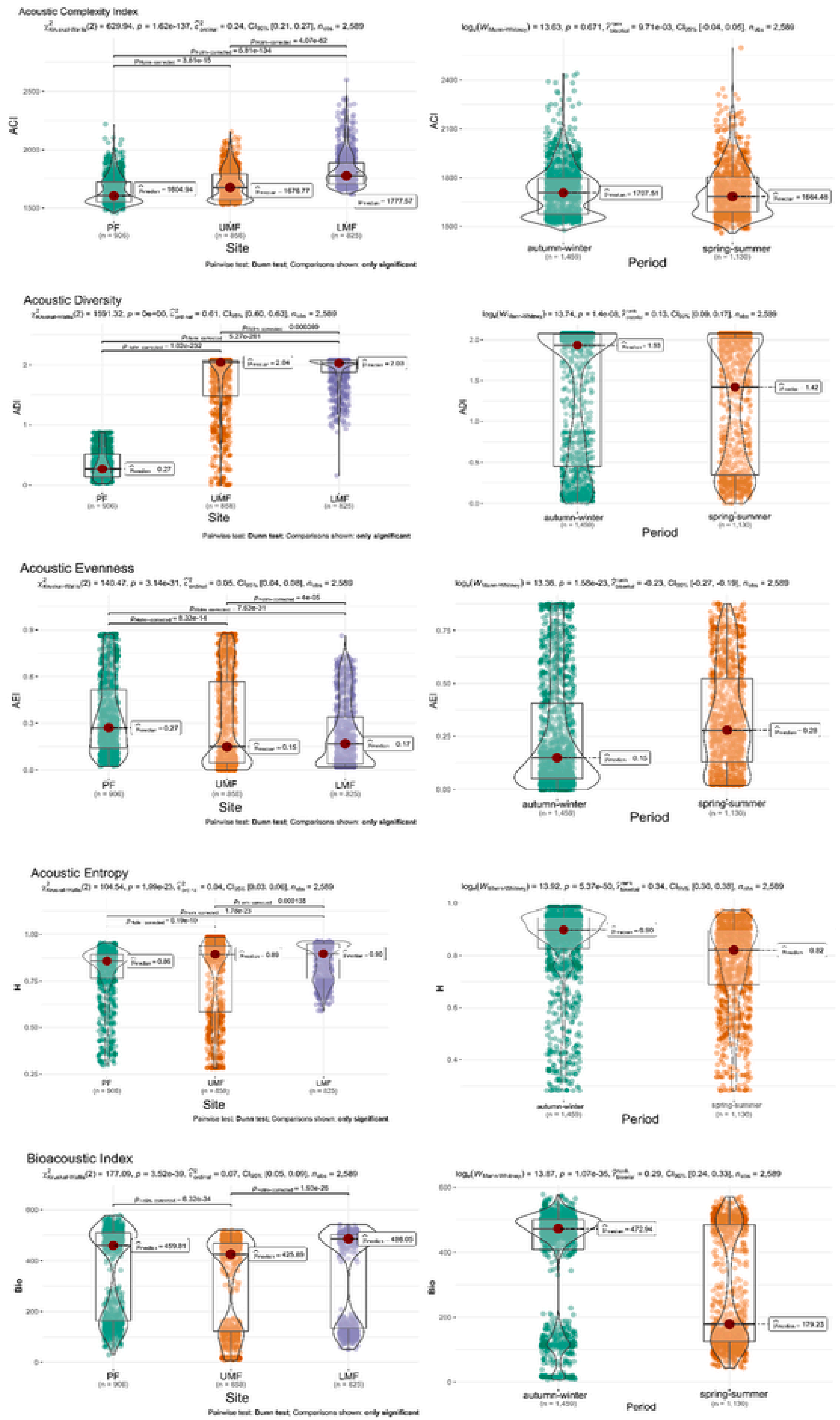
Acoustic Complexity Index
ACI
Acoustic Diversity
ADI
Acoustic Evenness
AEI
Acoustic Entropy
H
Bioacoustic Index
Bio
PF
UMF
LMF
Site
autumn-winter
spring-summer
Period
Pairwise test: Dunn test; Comparisons shown: only significant

Fig. 4. Violin-box plots of the monthly averages of each acoustic index in the study sites (A) and during the two main periods of the year (B). Red dot = mean of each data group. N = number of recordings. p = significance value; CI = confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

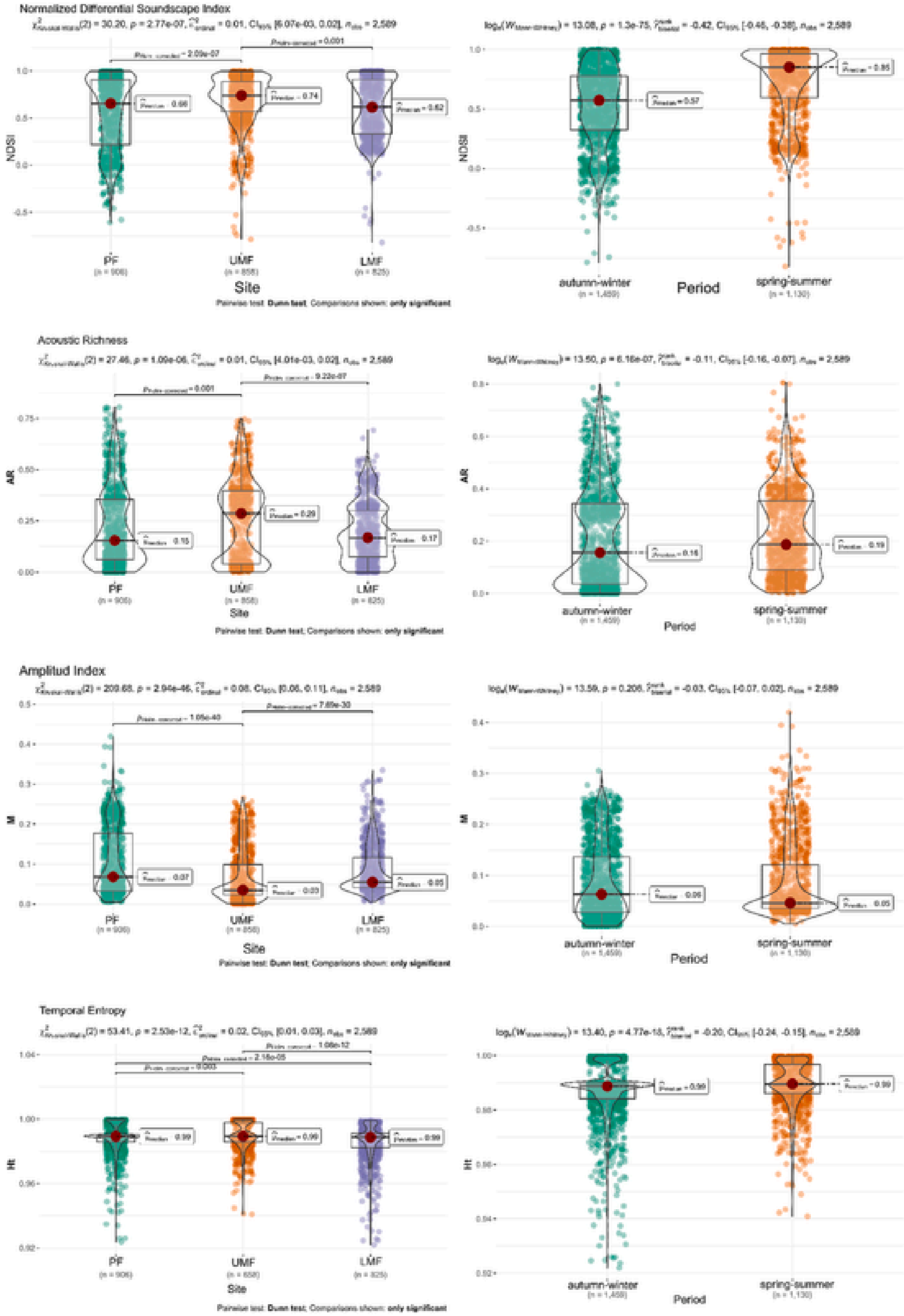

Fig. 4. (*continued*)

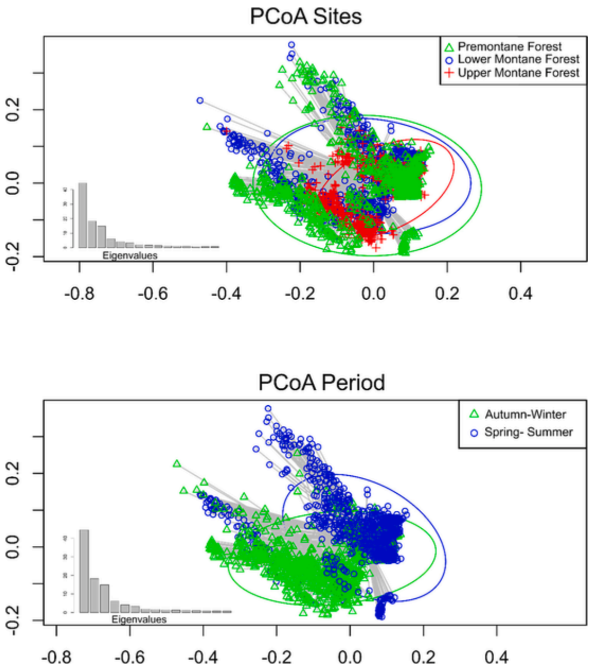

Fig. 5. A y B. Multidimensional analysis of the acoustic information obtained with the dissimilarity matrix of the calculated acoustic indices in three sites along an elevational gradient of Parque Nacional Calilegua. Bar graphs = eigenvalues magnitudes. A = Projection of axes 1 and 2 with the "Site" factor as an explanatory variable. B = Projection of axes 1 and 2 with the "Period" factor as an explanatory variable. Ellipses = 95% confidence intervals.

5. Conclusions

This work constitutes the first description of the soundscapes in different altitudinal strata in a protected area of the Yungas Andean forests of Argentina. Considered as one of the most biodiverse ecoregions in the country (Malizia et al., 2010). Especially, the PNC which harbours endemic and threatened anuran species (Duellman, 2015). Descriptions of natural soundscapes by applying different acoustic indices are increasing in the region (Scarpelli et al., 2020). Herein, we outline the first description of the soundscapes preserved by the PNC along an altitudinal gradient and through different seasons. The natural sounds sheltered in protected areas, such as national parks, were evidenced as a component that favours human well-being (Buxton et al., 2021). In this way, we suggested as crucial to include the soundscapes of protected areas in the Yungas of Argentina, as part of management plans to monitor and preserve the intangible sound heritage (Levenhagen et al., 2020). In addition, we encourage future studies in soundscapes employing the combination of analytical tools such as: long-false spectrograms visualisations with a group of acoustic indices calculations for better understanding of sound patterns.

We highlight the importance of further describing other acoustic communities in this ecoregion using PAM and the analytical framework tested here. Furthermore, the implementation of PAM and soundscapes analytics aiming to the identification of acoustic fingerprints in other ecoregions will provide comparable results with our study. The recorded results here become important mainly due to the climatic crisis and direct human-induced impacts that this diverse ecoregion is facing (Myers et al., 2000; Brown and Pacheco, 2006; Lavilla and Heatwole, 2010).

Author contribution statement

MB and MSA conceived the idea; MB, MSA, MV, and RMB designed the methodology: MB and MSA undertook the PAM; MB analyzed the data; MB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Uncited references

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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