Acoustic complexity indices reveal the acoustic communities of the old-growth Mediterranean forest of Sasso Fratino Integral Natural Reserve (Central Italy)

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ABSTRACT

The Sasso Fratino Integral Natural Reserve (Central Italy), a rare example of climax Mediterranean forest, provides an extraordinary opportunity to create an important soundscape reference of old-growth forest. In this study, we describe the soundscape of three localities (Lama, Sasso 950, Sasso 1400) representative of a gradient of variety and complexity of habitats, recorded during the period 10 May to 9 June 2017. Our results reveal temporal partitioning into acoustically homogeneous periods across 24 h suggesting that soniferous species (mainly birds) adopt ecological routines in which their acoustic activity is organized according to specific transient physiological needs. We processed multi-temporal aggregates of 1, 5, 10, and 15 s recordings and calculated the Acoustic Signature (*AS*) with four new indices: Ecoacoustic Events (*EE*), Acoustic Signature Dissimilarity (*ASD*), and their fractal dimensions (D_{EE} and D_{ASD}), derived from the Acoustic Complexity Index (*ACI*). The use of the *EE* and *ASD* greatly improved the *AS* interpretation, adding further details such as the emergence of a clear sequence of patterns consistent with the daily evolution of the overall soundscape. D_{EE} and D_{ASD} confirm the patterns observed using the *AS*, but provide more clarity and detail about the great acoustic complexity that exists across temporal scales in this old-growth forest. The temporal turnover of different acoustic communities occurs as a result of a gradual shift of different homogenous acoustic properties. We conclude that soniferous species use distinct, species-specific temporal resolutions according to their physiological and ecological needs and that the fractal approach used here provides a novel tool to overcome the difficulties associated with describing multi-temporal acoustic patterns.

1. Introduction

Human impacts on our environment are causing a dramatic reduction in ecosystem functionality resulting in a growing silencing of biophony (Carson, 1962; Krause and Farina, 2016; Sueur et al., 2019). Many studies have shown that the impoverishment of taxonomic, phylogenetic, genetic, and functional diversity as a consequence of human impact (Naeem et al., 2012) can be effectively detected using ecoacoustic indices (Laiolo, 2010; Depraetere et al., 2012; Gage and Axel, 2014; Sueur et al., 2014; Fuller et al., 2015; Harris et al., 2016). In particular, human disturbance in forest landscapes of temperate regions is such a common and recurrent process that all forests, even those perceived as well preserved, exhibit some signs of anthropogenic influence on species composition and structure (Caviedes and Ibarra, 2017).

Studies have shown that the structure and composition of forests influences animal communities, their richness, abundance and diversity (MacArthur and MacArthur, 1961; Davidowitz and Rosenzweig, 1998; Tews et al., 2004), daily movement paths (Wells et al., 2006; Fahrig, 2007) and seasonal migratory rhythms (Vickery et al., 2014). In forest ecosystems, soniferous species may not be the most prevalent taxa, however they play an important role in the food web as predators and/or secondary consumers (Sekercioglu, 2006; Van Bael et al., 2008; Kasso and Balakrishnan, 2013), and their presence indicates non-soniferous biodiversity that is part of the trophic web (e.g. animal prey, fruit and seed). In secondary or degraded forests, and under conditions of climatic stress, there is evidence of reduced complexity in acoustic communities (Krause and Farina, 2016) that can be associated with a

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reduction in ecosystem structural complexity (Markham, 1996).

For this reason, the analysis of acoustic patterns using dedicated metrics (e.g. Acoustic Entropy (Sueur et al., 2008), Normalized Difference Soundscape Index (Kasten et al., 2012), Acoustic Complexity Indices (Pieretti et al., 2011) or Ecoacoustic Events (Farina et al., 2016, 2018)) provides an efficient tool to evaluate ecosystem complexity and ecosystem changes under local (habitat degradation and pollution) or global (climate change) pressures (Sueur et al., 2014; Sueur and Farina, 2015). An understanding of soundscape structure and dynamics is also required to evaluate ecosystem resilience, to address management actions, and to plan possible environmental restoration (Dumyahn and Pijanowski, 2011; Pavan, 2017). Moreover, anthrophony and/or technophony (e.g. peak traffic hours, tourism-related traffic patterns, urban noise) can overwhelm natural soundscape patterns through the alteration of how animals vocalize (Brumm and Slabbekoorn, 2005; Parris et al., 2009; Slabbekoorn, 2013; Gil and Brumm, 2014; Zhang et al., 2015), and the masking of daily and seasonal routines and communication (Naguib, 2013; Gil et al., 2015).

While a few studies have been undertaken in old-growth forests (Luther, 2009; Pilcher et al., 2009; Burivalova et al., 2017; Burivalova et al., 2019; Monacchi and Farina, 2019), the majority of theories (Krause, 1993, 2012) and empirical ecoacoustic studies have been inspired by or come from secondary succession forests (de Camargo et al., 2019), or rural (Matsinos et al., 2008) and urban landscapes (Dein and Rüdisser, 2020). Rural and urban landscapes offer little to our understanding natural acoustic conditions or their natural patterns in the absence of human-generated disturbances (Pavan, 2017). Furthermore, the inherent spatial heterogeneity of secondary forests at a landscape scale due to human impact (e.g. deforestation, plantations of exotic taxa) has a strong influence on the structure of animal communities (Tews et al., 2004), and such spatial patterns are evidenced in the heterogeneous biophonic components or soundtopes (Farina, 2014; Fuller et al., 2015).

Evidence suggests that well-preserved (intact) forests support a more complex, interacting community of soniferous and non-soniferous species than disturbed woodlands (Watson et al., 2018). Moreover, in long time intact environments, individual species adapt to the overall community sound spectrum partitioning (Hall and Magrath, 2007; Malavasi and Farina, 2013). In recently modified environments such partitioning is less evident, and the occurrence of frequency and time overlap between species can reduce the efficiency of intra and interspecific communication (Malavasi and Farina, 2013). In addition, in intact forests we expect that acoustic information will change gradually, effectively flowing throughout the day and season, reflecting the phenology of individual species. Regular oscillations are expected from systems in which the mechanisms of resource partitioning are the result of a stable interspecific adaptation (Vandermeer, 2006).

The methodological question remains, however, what temporal resolution should be used to detect the emergence of distinct acoustic patterns and regularities? There is evidence that soundscape patterns represent processes that occur at different temporal and spatial scales (Borker et al., 2019). However, current ecoacoustic methodologies are limited by the fact that the temporal resolution for acoustic data processing is usually arbitrarily selected, regardless of any intrinsic periodicity. Fractal analysis offers a promising alternative and avoids the necessity of selecting an arbitrary temporal scale at which to scan a soundscape. Fractal mathematics can be used to evaluate variation in the temporal or spatial resolution at which the investigation is carried out, producing a dimension (*D*) that grows according to the internal complexity of the system (Parrott, 2010). In this way, a single dimensional system (e.g. a straight line) has a *D* of 1, a two-dimensional system (e.g. a convoluted line that fills a surface) has a *D* of 2, and so on (Mandelbrot, 1983; Barnseley, 1993). When applied to the temporal domain, *D* can provide a good proxy of the temporal complexity of a system. In this study, our objectives were to (1) investigate and compare the daily patterns of complexity in the acoustic communities

within an intact old-growth Mediterranean forest and (2) evaluate the descriptive power of a suite of new generation ecoacoustic indices, including two fractal indices, for examining acoustic communities across multi-temporal resolutions.

2. Methods

2.1. Study area

To further our understanding of complex, natural soundscapes, we have selected to study the forest of Sasso Fratino Integral Natural Reserve (SFINR) (National Park of the Casentinesi Forests, Central Italy) because it is characterized by an intact climax community with minimal human foot-print that is rare in Mediterranean Europe. This forest wilderness of 800 ha is characterised by complete prohibition of human access and of any other anthropic action and is an ideal location for the collection of high-quality acoustic recordings, providing an important reference database for comparing acoustic communities across ecological gradients. The area can be reached by walk only, with permission of the Authorities (Reparto Carabinieri Biodiversità).

The SFINR, located at latitude 43°11′ N; longitude 11°47′ E, was established in 1959 as the first Italian Integral Nature Reserve. With other adjacent Nature Reserves, it belongs to the Natura 2000 site SCI-SPA IT4080001 (http://ec. europa.eu/environment/nature/ natura2000).

Since 1985, the SFINR has been awarded the European Diploma for Protected Areas for its importance and uniqueness as a well-protected pristine ecosystem. In 2017, it was included by UNESCO in the list of "Ancient and Primeval Beech Forests of the Carpathians and Other Regions of Europe World Heritage" (https://whc.unesco.org).

Due to the presence of steep slopes (> 65%) and the extremely irregular landform morphology of the site, this land is not suitable for human land transformation resulting in a negligible anthropogenic impact throughout the last centuries. The intactness of SFINR for so many years has allowed the spontaneous succession of an old-growth forest characterised by high biomass (above 1000 mc/ha) and a rich biodiversity (Bianchi et al., 2011). The vegetation type is characterised as "mountain belt" *Fagus* and *Fagus-Abies series* (Ozenda, 1985). Forest cover is dominated by beech (*Fagus sylvatica*) and white firs (*Abies alba*) covering the slopes up to 1250 m.

The forest mosaic of this old-growth forest shows specific characteristics if compared with managed forests, the most important being the concurrent presence of all of the phases of the forest cycle, with a high component of dead matter. The forest structure appears to be even-aged and single-layered at higher altitudes and uneven-aged and multi-layered in the lower section (Nardi-Berti, 1972; Bianchi et al., 2011).

In the area, atmospheric moisture is high throughout the year and foggy days are quite frequent during the growing season; this allows a hyper humid habitat with rich soil hosting huge biodiversity of invertebrates that drive a complex food network.

Above the altitude of 1250 m, the forest is composed only of beech (Padula, 1983; Bianchi et al., 2011). The climate can be defined as temperate humid with mild winters, without dry seasons, and a relatively hot summer, belonging to the category of Moist Subtropical Mid-Latitude Climates(C) sub type marine (fb) (Köppen, 1936).

2.2. Site selection

Three recording sites were selected inside the SCI-SPA IT4080001 area: one site outside the SFINR, functioning as control, and two sites with a different vegetation composition in the core of the SFINR. La Lama (hereafter "Lama"), at 694 m above sea level, was located in a Biogenetic Reserve adjacent to the SFNIR (Fig. 1a). It is characterized by a flat plain originating from a progressive burial of a barrier lake formed by a landslide which fell from Poggio di Fonte Murata (Olivari,
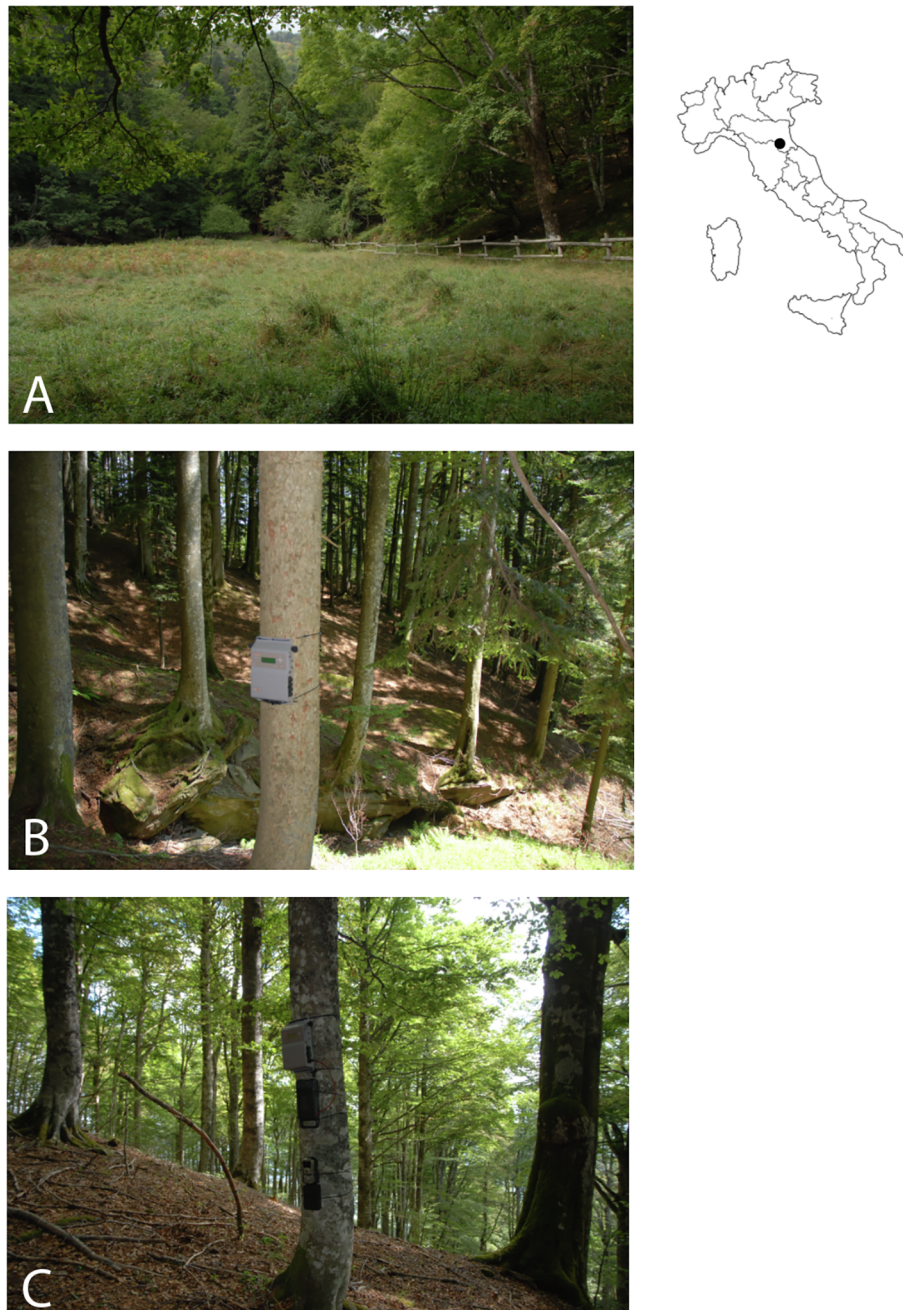
Fig. 1. The study area is represented by three sampling plots: a) Lama is characterized by a flat surface that has resulted from a paleo landslide, and is crossed by a permanent stream. b) Sasso 950 is characterized by a steep slope and a mixed broadleaf forest. c) Sasso 1400 is characterized by a steep slope and a pure beech (*Fagus sylvatica*) forest.

2018) and consists of a small clearing surrounded by a variety of tree species, largely planted (at different times, until 1960) and crossed by a small permanent stream. The recorder position is on the SW side of the clearing; a small building used by the forestry service personnel and by researchers is at the opposite side of the clearing, about 260 meters away. The area represents an anthropogenic ecotone seasonally managed by slashing in the summer; it is crossed by a trekking trail and can be reached by a 20 km long forest service road, closed to public access. The forest around the recorder is mixed, composed mainly by sycamore maple (*Acer pseudoplatanus*), Italian maple (*Acer opalus*), European ash (*Fraxinus excelsior*), manna ash (*Fraxinus ornus*), small-leaved lime (*Tilia cordata*), and large-leaved lime (*Tilia platyphyllos*).

Sasso Fratino 950 (hereafter "Sasso 950") was located inside the SFINR at 950 m above sea level (Fig. 1b) on a steep slope covered by

beech (*Fagus sylvatica*) and fir trees (*Abies alba*). Sasso Fratino 1400 (hereafter "Sasso 1400") was also located inside the SFINR at 1400 m above sea level (Fig. 1c) on a steep slope covered by a pure beech forest.

2.3. Acoustic sampling

As described by Righini and Pavan (2019), acoustic recordings were collected from 10 May 2017 to 9 June 2017 at Lama and Sasso 950, and from 10 May to 7 June 2017 for Sasso 1400 using three Song Meter SM3 autonomous recorders (Wildlife Acoustics, Inc., Maynard, Massachusetts, USA). Recorders were programmed using the Song Meter SM3 Configuration Utility software (Wildlife Acoustics, Inc., Massachusetts, USA) to operate at a sampling rate of 48 kHz with 220 Hz HP filter. The recorders were activated for 10 min every 30 min, producing 48 daily

sessions, ranging from 00:00 to 23:30, totaling across all localities 43,680 min of recording. A sampling schedule of 10 min was used as it represents a tradeoff between ensuring a robust acoustic survey and providing a manageable quantity of data for processing, although a standardized methodology is not yet available (e.g. Pieretti et al., 2015; Thomisch et al., 2015; Elise et al., 2019). Audio files were saved in stereo 16 bit uncompressed *wav* format on 128 GB SDXC cards. Acoustic data were transferred into the sound repository of the Interdisciplinary Centre for Bioacoustics and Environmental Research (CIBRA) of the University of Pavia, Italy (http://www.unipv.it/cibra). Further details about the recording methodology are described in Righini and Pavan (2019).

2.4. Acoustic analysis

The modified standalone software package (Sfractal®, Salutari and Farina, 2019) that represents an updated and expanded version of the EEDI package (Farina et al., 2016, 2018) was used to compute the acoustic metrics. Sfractal requires mono-channel wave files and therefore the left channel of the original stereo acoustic data was extracted using Adobe Audition CC2017.0.2 and then processed using a sliding Short-Term Fourier Transform (STFT) of 1024 samples and a Hanning window with same size without overlap between frames. This resulted in series of power spectrum composed by 512 output spectral lines with a frequency resolution band of 46.875 Hz. Acoustic Complexity Indices (*ACI*) were calculated after applying an empirically selected background filter of amplitude 4 to the power spectrums, to exclude low-energy microphone and analog/digital conversion noise (Pieretti et al., 2011; Farina and Salutari, 2016; Farina et al., 2016). To eliminate residual noise at very low frequency, the first four spectral frequency bands (0–187.5 Hz) were excluded from the computation of *ACI* metrics. The sequence of data processing output produced is illustrated in Fig. 2 and the list of acronyms used is given in Table 1. The inherent

Table 1
Short description of the acronyms utilized.

ACI_{ft}	Acoustic Complexity Index calculated along time
$ACI_{ft\ evenness}$	Evenness of Acoustic Complexity Index calculated along time
ACI_{tf}	Acoustic Complexity Index calculated along frequencies
$ACI_{tf\ evenness}$	Evenness of Acoustic Complexity Index calculated along frequencies
AS	Acoustic Signature
ASD	Acoustic Signature Dissimilarity
AS_d	Acoustic Signature aggregated across days
AS_h	Acoustic Signature aggregated across half-hour interval
AS_k	Acoustic Signature aggregated in 1-kHz interval
$AS_{k,h}$	Acoustic Signature aggregated across half-hour × 1-kHz interval
CV	Coefficient of Variation
D	Fractal Dmension
DASD	Fractal Dimension of Acoustic Signature Dissimilarity
DEE	Fractal Dimension of Ecoacoustic Events
EE	Ecoacoustics Events
EE_d	Ecoacoustics Events aggregated across days
EE_h	Ecoacoustics Events aggregated across half-hour interval
$EE_{h,g}$	Ecoacoustics Events aggregated across half-hour interval × group
EE_g	Ecoacoustics Events from 000 to 999, aggregated in 10 groups of 100 codes each
H'_{EE}	Diversity of Ecoacoustic Events
SFINR	Sasso Fratino Integral Natural Reserve

complexity of the soundscape in each 10-minute recording was investigated at five different temporal aggregations: 1, 5, 10, 15 and 20 s. To better interpret the acoustic metrics, aural and visual inspection of randomly selected wave files was carried out.

2.5. Acoustic Complexity Indices (Appendix A; Eqs. (1) and (2))

ACI is a group of two indices (ACI_f and ACI_t) which are used to calculate the amount of acoustic information/complexity present in an acoustic file (Pieretti et al., 2011; Farina et al., 2016, 2018). ACI_f
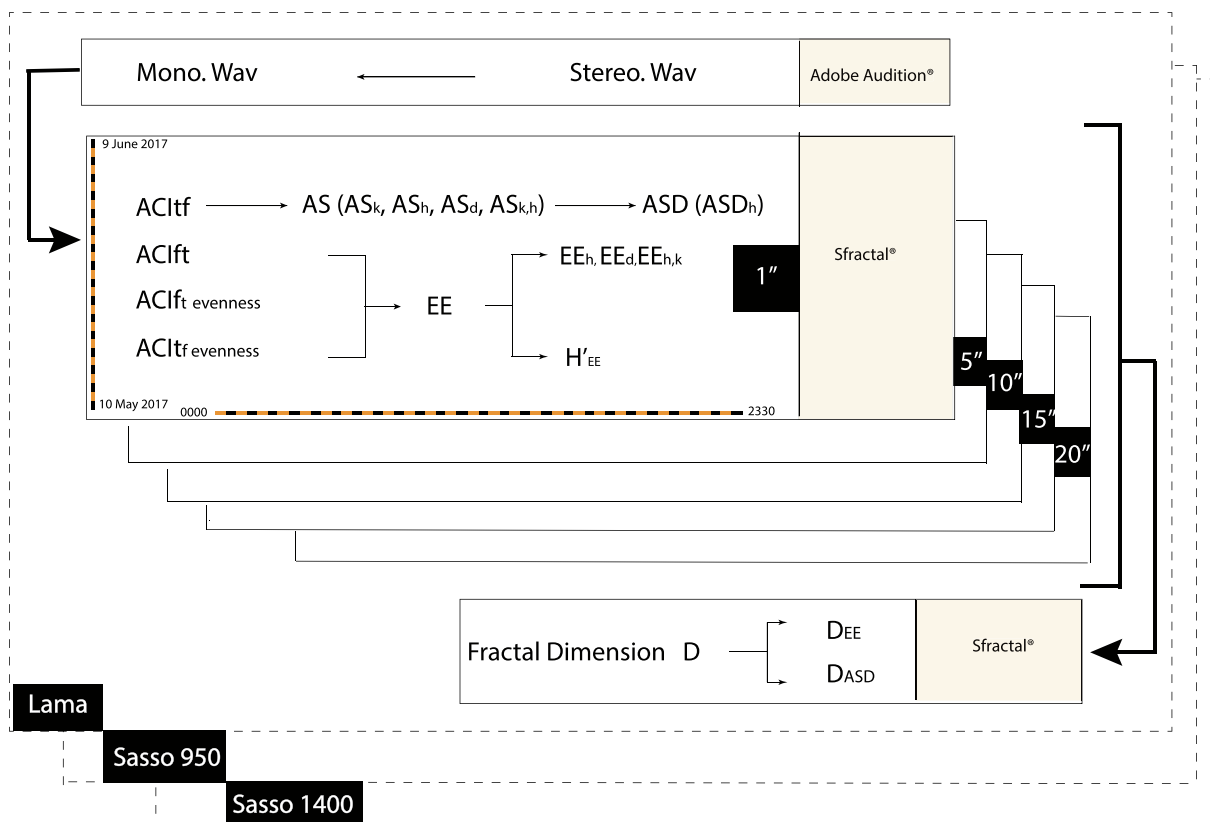

Fig. 2. Graphical representation of the different steps used in the data processing for the three localities (Lama, Sasso 950, Sasso 1400) examined in this study.

measures the acoustic information in each frequency band across a temporal aggregation interval and $ACIf_t$ measures the acoustic information in each temporal frame across the frequency bands. The $ACIt_f$ values obtained along each frequency band f have been aggregated in 1 kHz classes, obtaining 24 1-kHz classes of frequency for each temporal interval.

2.5.1. Acoustic Signature AS

The distribution of $ACIt_f$ values along all the frequencies in selected temporal intervals is termed Acoustic Signature (*AS*). The acoustic signature is a synonym for acoustic footprint or sonotope (Farina, 2014) and is obtained when all the sources of sound at a locality are considered. Every locality has a specific acoustic signature.

In order to facilitate the presentation of results, the *AS* was aggregated in different frequency and time resolutions represented by the following acronyms:

1) AS_k, when aggregated in 24 classes of 1-kHz each;
2) AS_h, when aggregated in 48 daily intervals of half hour each reflecting the sampling design (10 min every half hour);
3) AS_d, when aggregated across days, throughout the study period;
4) $AS_{k,h}$, when the aggregation 1 and 2 are combined.

2.6. $ACIt_{fevenness}$ and $ACIf_{tevenness}$ (Appendix A, Eqs. (3)–(6))

These two indices measure the level of equitability (hereafter "evenness") of acoustic information across frequencies and time, and were computed using Levins evenness *B* algorithm (Levins, 1968; Hurlbert, 1978; Farina et al., 2016, 2018). $ACIt_f$ evenness was calculated across the 512 frequency bands. $ACIf_t$ evenness was calculated for each temporal aggregation.

2.7. Ecoacoustic Events EE (Appendix A; Eq. (7))

The Ecoacoustic Events method is an innovative approach that aims to assign, at each temporal resolution, a specific code that represents variation in three *ACI* metrics ($ACIf_t$, $ACIf_t$ evenness and $ACIt_f$ evenness). These three metrics are not mathematically calculated into a single numeric value, but rather are assigned to a three digit numeral expression that creates an arbitrary code (*sensu* Barbieri, 2003). *EE* is a model that *de facto* assumes that the combination of the three *ACI* metrics represents different aspects of the soundscape through time, according to the sonotope model of ecoacoustics theory (Farina, 2014, p. 17-18). *EE* can also be considered as a cognitive/semiotic template used by species to interpret the acoustic complexity at different temporal resolutions. As a result, every *EE* can be interpreted as an acoustic eco-field used to intercept resources (Farina, 2006; Farina and Belgrano, 2004, 2006). Specifically, by adopting a temporal resolution of 1, 5, 10, 15, and 20 s we have simulated the function-specific perception of a soundscape by a hypothetical species. In reality, these temporal resolutions are an attempt to explore in depth the complexity of a soundscape.

EE index is a conventional numeral expression used to convert any spatial or temporal unit in which a soundscape is segmented according to the sonotope model (Farina, 2014), into a three digit numeral that results from the combination of $ACIf_t$, $ACIf_t$ evenness and $ACIt_f$ evenness (Farina et al., 2016, 2018). This sequence assigns the major importance of the numeral expression to $ACIf_t$, modulated by its evenness and then in the less significant position by $ACIt_f$ evenness. According to this "semiotic" numeral expression, a soundscape is not simply a sequence of specific signs inter-dispersed into a neutral background of acoustic signals, but rather a sequence of ecoacoustic events that operates like an alphabetical sequence of "acoustic text". Consequently, acoustic events are not only a description of some distinct emerging characteristics of a soundscape that can be isolated and categorized from an indistinct acoustic background (Ma et al., 2006), but we postulate that they are functional units actively used by organisms to cognitively fulfill specific functions, consistent with the semiotic model of the (acoustic) eco-field (Farina and Belgrano, 2004, 2006; Mullet et al., 2017).

The analysis of the *EE* was carried out at five temporal resolutions of 1, 5, 10, 15, 20 s and the results were aggregated based on:

1) 48 half-hour steps a day (EE_h);
2) study period (EE_d) (31 days Lama, Sasso 950, and 29 days Sasso 1400);
3) 10 groups of EE_g according to EE code (000–999): (000–100, 101–200 .... 900–999); EE grouping was requested to a synthetic view of EE distribution;
4) and as combination of 1 and 3 $EE_{h,g}$.

2.8. Diversity of Ecoacoustic Events H'$_{EE}$ (Appendix A; Eq. (8))

The diversity of *EE* was calculated using the Shannon diversity index (Shannon and Weaver, 1949) and applied to the entire collection of *EE* (Farina et al., 2018).

2.9. Acoustic Signature Dissimilarity ASD (Appendix A; Eqs. (9) and (10))

The *Acoustic Signature Dissimilarity* index is based on the premise that the distance between one *AS* and another is a proxy for variability and/or diversity of acoustic information, irrespective of the origin of the sound. It is reasonable to assume that biophony will increase dissimilarity because soniferous species will vary their acoustic communication across time. However, much evidence exists that when birds sing actively (from dawn chorus to late morning), the same syllables or song type are repeated several times in sequence, depending on the level of intra and interspecific frequency (Wiley, 1994; Brumm and Slater, 2006; Díaz et al., 2011; and Luther and Gentry, 2013 for a review), and this fact may decrease the *ASD*. To calculate the *ASD*, the Chord Distance Algorithm (Orloci, 1967; Legendre and Gallagher, 2001) was applied to the values of $ACIt_f$ (Farina et al., 2018).

2.10. Fractal Dimension D (Appendix A; Eq. (11))

The complexity of a system can be measured by its fractal dimension and it is a useful tool for describing a specific phenomenon independent of scale. In nature, many systems have fractal-like structures, such as marine coastlines or a dendritic river. Fractals are objects that have scale-invariant patterns (self-similarity, fractional dimension, non-differentiable properties; Mandelbrot, 1983) and have been used in several scientific disciplines (see Frontier, 1987; Feder, 1988 for reviews), including physical geometry (for a review Kusak, 2014) and ecology (Sugihara and May, 1990; Hastings and Sugihara, 1993; Li, 2000; Brown et al., 2002; Halley et al., 2004). Although applications in the field of acoustics are rare (Lyamshev and Adreev, 1997; Makabe and Muto, 2014) or limited to studies of music (Bigerelle and Iost, 2000), a fractal mathematical approach may be more appropriate to explore the complexity of acoustic communities than currently used Euclidean geometry methods, including acoustic indices. Recently Monacchi and Farina (2019) have described the fractal dimension of two tropical acoustic communities. In this study, we assumed that a soundscape is fractal and that this property can be interpreted using *EE* and *ASD* analysis tools. In other words, according to our model natural soundscapes have an inherent complexity created by soniferous species with temporal scaling of their soniferous activity and acoustic perception (passive and active) of their surroundings. The fractal dimension *D,* was calculated according to the box-counting method (Mandelbrot, 1983; Feder, 1988; Li et al., 2009) and applied to *EE* and *ASD* (Monacchi and Farina, 2019).

2.11. Statistical analysis

As the data were not normally distributed, non-parametric Wilcoxon Matched Pairs and the Friedman ANOVA Chi Square were used to test statistical significance at α = 0.05. A cluster analysis was performed, applying the Ward's method and Euclidean distance, and clusters where discriminated with a threshold of 10. All statistical calculations were conducted in Statistica 12® (www.stasoft.com). 3D images of Fig. 5 were produced using Surfer ® (13.5.83 2016). 3D images of Fig. 7 were obtained using MATLAB (Ver R2020a, Natick, MA, USA). The Coefficient of Variation (CV) or relative standard deviation defined as the ratio of the standard deviation to the mean, describes the level of variability within a sample independently by the absolute values of observations (Sokal and Rohlf, 1995), and was used to evaluate the variability of $AS_{k,d}$.

3. Results

3.1. Acoustic Signature AS

3.1.1. AS_k (aggregation 24 1-kHz)

AS_k was similar between Sasso 950 and Sasso 1400 (Wilcoxon Matched Pairs Test: N = 24, T = 108, Z = 1.2, P = 0.23), but significantly different between Lama and Sasso 950/1400 (Wilcoxon Matched Pairs Test: N = 24, T = 0,00, Z = 4.3, P = 0.00) (Fig. 3).

3.1.2. AS_h (aggregation 48 half-hour steps)

AS_h was similar between Sasso 950 and Sasso 1400 (Wilcoxon Matched Pairs Test: N = 48, T = 418.0, Z = 1.7, P = 0.08) but was significantly different between Lama, Sasso 950 and Sasso 1400 (Wilcoxon Matched Pairs Test: N = 48, T = 0.00, Z = 6.0, P = 0.00). Acoustic information first peaked between 04:00 to 08:00 in the morning, and a second lower peak occurred around 20:00 at all three sites (Fig. 4).

3.1.3. $AS_{k,h}$ (aggregation 24 1-kHz × 48 half-hour steps)

The distribution of $AS_{k,h}$ showed distinct patterns throughout the day (Fig. 5). The 1-kHz frequency class varied less throughout the day and frequency groups > 19 kHz were so rare that it was not possible to assign realistic patterns. The 2-kHz and 3-kHz classes have visible peaks between 04:00 and 06:00 in the morning and between 20:00 to 21:00 in the evening coincident with the dawn and dusk chorus. The dusk chorus was less evident at Lama. Cluster analysis applied to the 1-kHz classes revealed a distinct difference between the first 8 or 9-kHz classes and the 10 to 24-kHz classes which showed high similarity. The 1-kHz class was more distant from the other classes at Sasso 950 and Sasso 1400 than at Lama.

The $AS_{k,h}$ showed similar patterns in the three recording locations when a cluster analysis was applied to the half-hourly data, revealing that the daily soundscape was aggregated in homogeneous groups. At Lama, seven groups were distinguished: two groups (1 and 2) for the nocturnal period, group 6 and 7 for the dawn chorus period, two groups (3 and 4) for the morning period, and group 5 for the entire afternoon (Fig. 1a Supplementary material). At Sasso 950 a similar pattern to Lama was found. Group 1 and 2 were associated with night time, group 3 separated the dawn chorus from group 4 and group 5 that represented early and late morning, respectively. Group 6 consisted of the hours of midday, while group 7 included early and late afternoon (Fig. 1b Supplementary material). At Sasso 1400 the nocturnal hours were aggregated into a unique group. Dawn chorus (group 6) was represented only by 05:30 h. Close to this group was group 7, representing the early morning hours. Groups 2, 3, 4 and 5 were heterogeneous and few temporal sequences could be discerned (Fig. 1c Supplementary material).

3.1.4. $AS_{k,d}$ (aggregation 24 1-kHz × days)

The acoustic signature during the period 10 May − 9 June revealed higher variability at Lama, with a decrease from Sasso 950 to Sasso 1400 (Fig. 2 Supplementary material). Variability associated with the seasonal phenology of the soniferous community was particularly
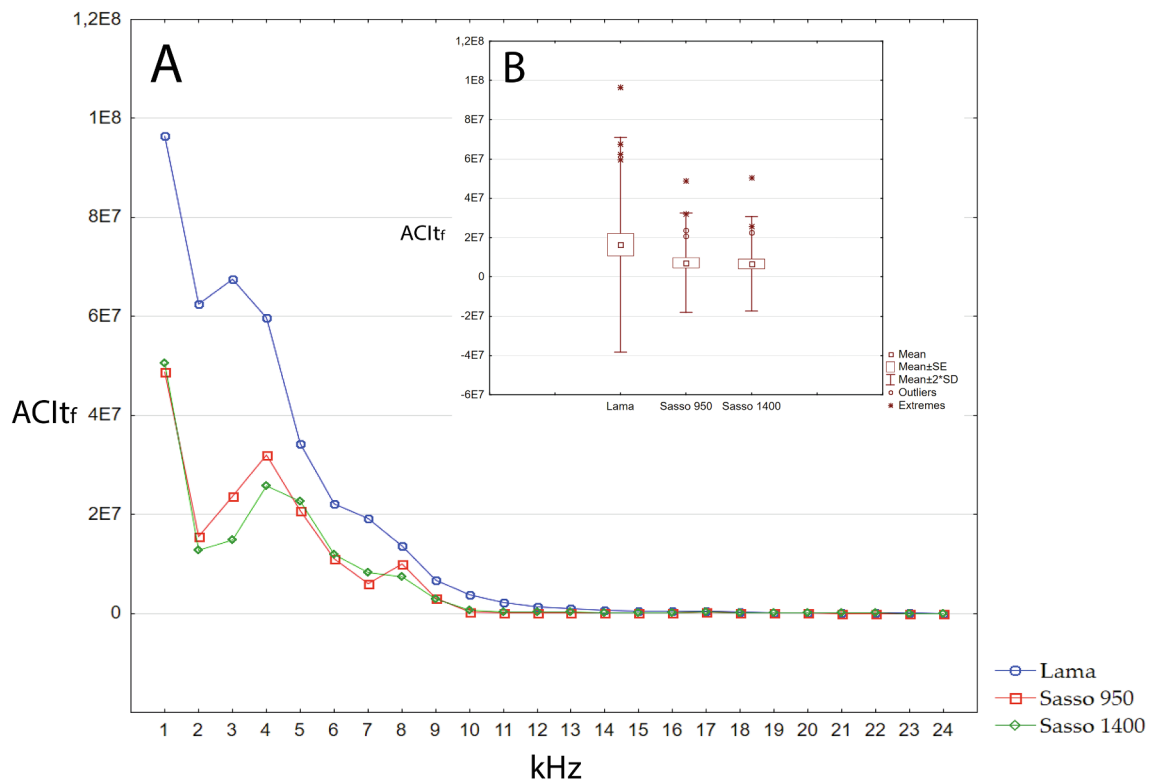

Fig. 3. (A) Distribution of $ACIt_f$ when aggregated according to 1-kHz frequency classes and (B) descriptive statistics for Lama, Sasso 950 and Sasso 1400.
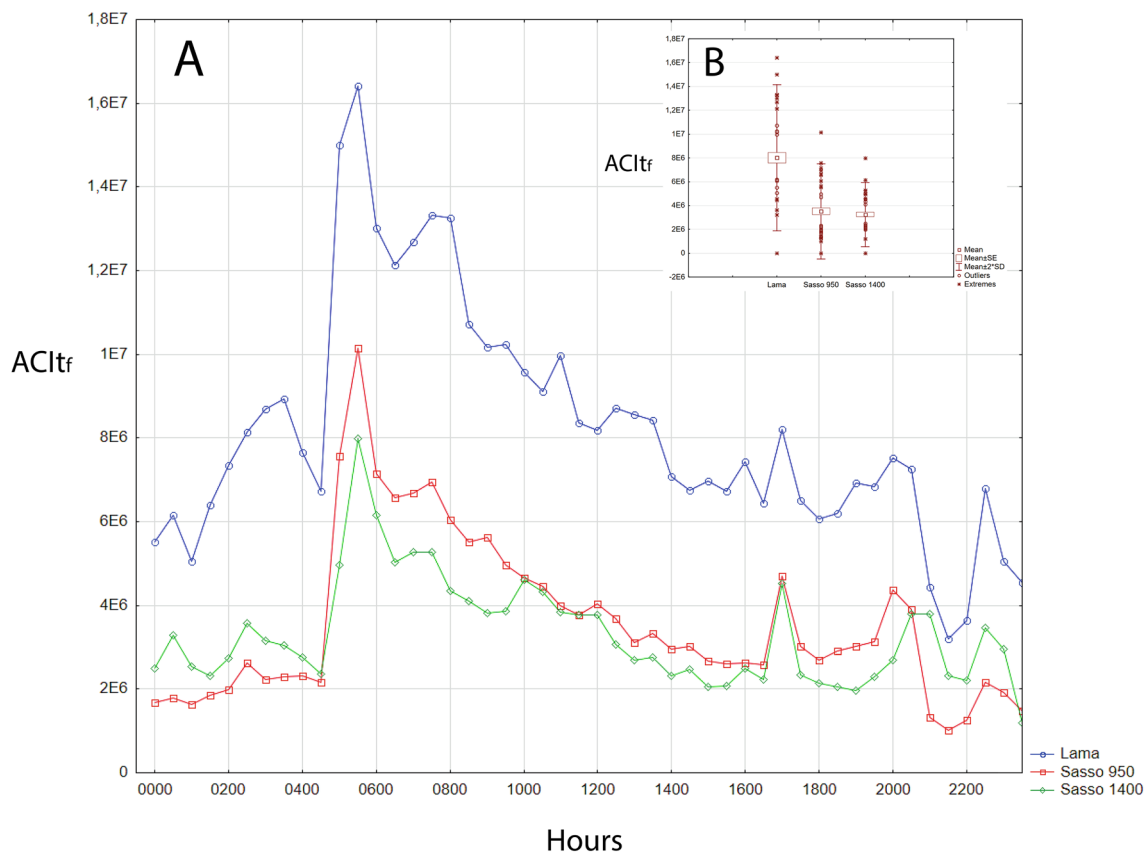
Fig. 4. (A) Distribution of $ACIt_f$ throughout the day and (B) descriptive statistics for Lama, Sasso 950 and Sasso 1400.

evident in the first 1-kHz frequency class in all three sites (Fig. 2 Supplementary material). When the coefficient of variation (CV) was applied to 1-kHz frequency aggregates, variability in $AS_{k,d}$ was highest between 10 and 18 May, then remained more constant until 31 May, followed by an increase in variability until the end of the study period (Friedman ANOVA: N = 24, df = 2, Chi Sqr = 14.6, P = 0.0007) (Fig. 6). Lama exhibited higher variability in the CV of $AS_{k,d}$ and was significantly different when compared with Sasso1400 (Wilcoxon Matched Pairs Test: N = 28, T = 79, Z = 2.82, P = 0.0048), but not with Sasso 950 (Wilcoxon Matched Pairs Test: N = 31, T = 209, Z = 0.76, P = 0.4447). No differences were found between Sasso 950 and Sasso 1400 (Wilcoxon Matched Pairs Test: N = 28, T = 128, Z = 1.71, P = 0.0877).

3.2. Ecoacoustic Events EE

The total number of *EE* and their diversity and evenness are presented in Table 1 of the Supplementary material for each temporal resolution. The richness of EE_d for the five temporal resolutions was significantly different for all sites (Lama: Friedman ANOVA (N = 31, df = 4 Chi Sqr = 88.75, P < 0.0001); Sasso 950: Friedman ANOVA (N = 31, df = 4 Chi Sqr = 76.25, P < 0.0001); Sasso 1400: Friedman ANOVA (N = 28, df = 4 Chi Sqr = 64.33, P < 0.0001). The distribution of $EE_{h,g}$ for each temporal resolution is reported in Fig. 7 for each recording site (Tables 2 and 3 supplementary materials). The second group of *EE* codes (101–200) and the third group of *EE* codes (201–300) revealed a different temporal distribution between Lama and the two Sasso Fratino sites. This temporal distribution was characterized by a strong decrease in the number of *EE* between 04:00 and 06:00 for the group 101–200, and a peak at the same time for the group 201–300. A peak in *EE* was more pronounced at Lama in the 201–300 group, compared with a similar but smaller peak at Sasso 950 and Sasso 1400.

At Sasso 1400, the ten groups of *EE* showed a similar distribution throughout the half-hour sampling for the groups 101–200, 201–300 and 301–400, while the other *EE* groups differ significantly across temporal resolutions (Table 4 Supplementary material). Group 301–400 did not vary at Sasso 950, but did vary significantly for all other groups among temporal resolutions (Table 4 Supplementary material). At Lama, groups 101–200, 201–300 were not significantly different across temporal resolutions, but all the other groups were significantly different (Table 4 Supplementary material).

Based on the cluster analysis, the distribution of *EE* followed a pattern of daily temporal intervals with distinct homogenous time periods (Fig. 3 Supplementary material). This analysis revealed that the distribution of *EE* follows a sequence of temporal daily partitioning that separates the day time into distinct homogeneous periods (sonotopes). For Lama, the number of temporal daily partitions was according to five temporal resolutions, (1s: 6), (5s: 6), (10s: 9), (15s: 9) and (20s: 11). The number of temporal partitions was higher in Sasso 950 (1s: 10, 5s: 9, 10 s: 11, 15s: 12, 20s: 12) and in Sasso 1400 (1s: 17, 5s: 22, 10s: 22, 15s: 25, 20s: 31), and was higher during the nocturnal compared with the diurnal period for all sites (Fig. 8). The 05:00 and 05:30 half-hour intervals were always distinct at every resolution for all three localities. The number of clusters increased with increasing temporal resolution. Sasso 1400 had the highest number of clusters (31) relative to 12 and 9 for Sasso 950 and Lama, respectively, at the temporal resolution of 20 s.

3.3. Acoustic Signature Dissimilarity ASD

When aggregated per hour, ASD_h showed a similar pattern across all three sites, independent of the temporal resolution, with an increase during the day time and an abrupt reduction at night (Fig. 9a). There was no clear trend when examined across days of sampling. An increase in *ASD* at Lama and Sasso 950 was evident from 10 May – June with little between-day variation, while Sasso 1400 exhibited a greater
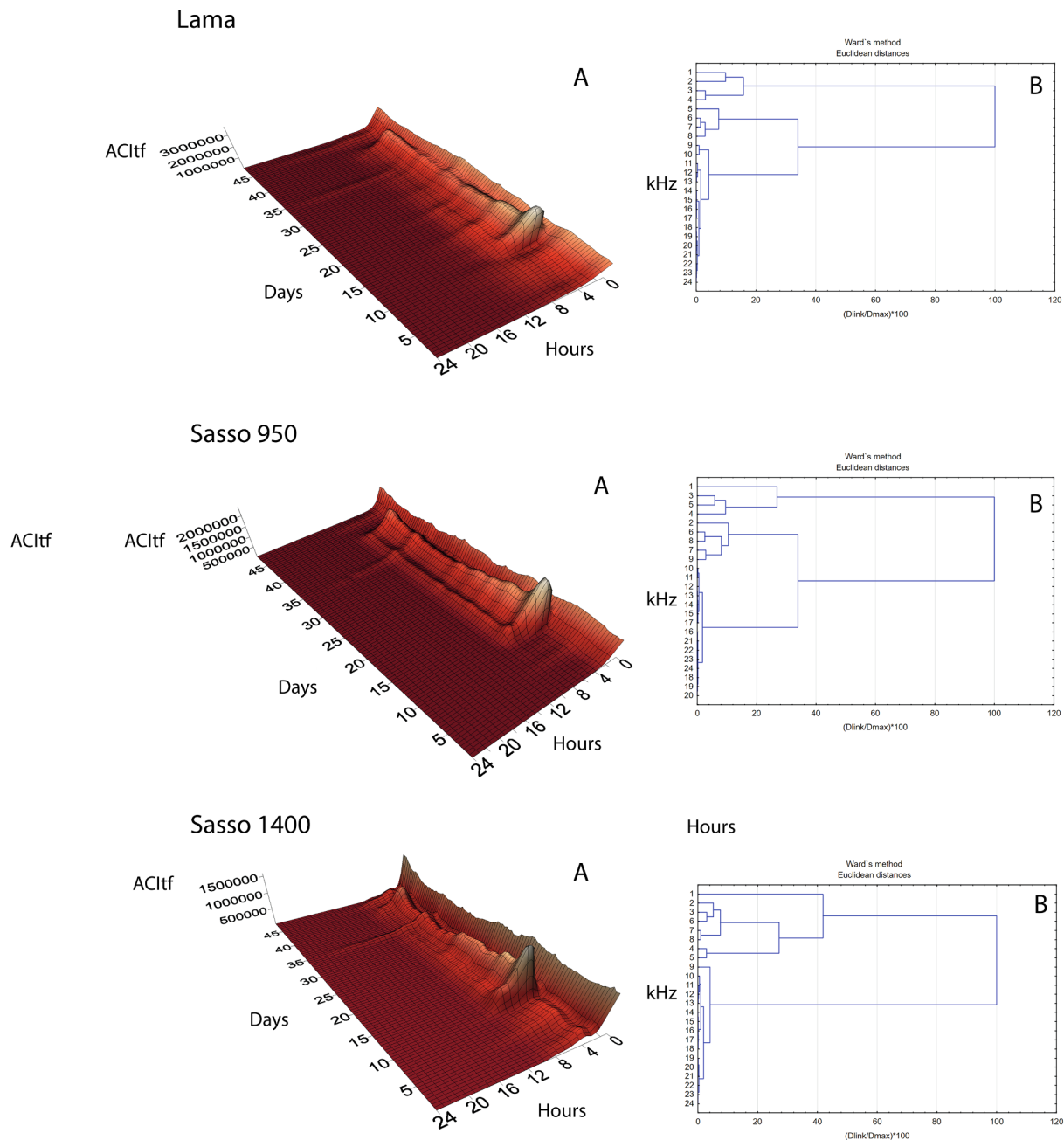
Fig. 5. (A) Distribution across time of day of *ACIt_f* for each of the three study sites, aggregated according to 1-kHz of frequency classes and (B) ordination by cluster analysis of each 1-kHz frequency class according to daily hour distribution.

variability between days than the other two sites (Fig. 9b) (Table 5 Supplementary material).

3.4. Fractal Dimension D

3.4.1. The Fractal Dimension of Ecoacoustic Events, D_{EE}

When D_{EE} was examined across daily half-hour intervals, lowest values were found during the night time, while two peaks occurred at sunrise and sunset, with lower values during the middle of the day (Fig. 10a and Table 6 supplementary material). Significant differences in daily half-hour intervals were found between Lama and Sasso 1400 (Wilcoxon Test: N = 48, T = 232, Z = 3.65, P = 0.0003), and between Sasso 950 and Sasso 1400 (Wilcoxon Test: N = 48, T = 231, Z = 3.66, P = 0.0003). The differences between sites were due to an increase in D_{EE} during the middle of the day between 11:00 and 16:00 at Sasso

1400 (Fig. 10a). There was no significant difference between Lama and Sasso 950 (Wilcoxon Test: N = 48, T = 576, Z = 0.12, P = 0.9020). Fig. 10b shows a distinct decrease in D_{EE} at all sites on 14 May, 1 June and 7 June, with uniform increases on 15 May, 22 May, 28 May, and 3 June at all sites.

D_{EE} throughout the study period was not significantly different between the three sites (Lama × Sasso 950: Wilcoxon Test (N = 31, T = 229, Z = 0.37, P = 0.7096); Lama × Sasso 1400: Wilcoxon Test (N = 29, T = 151, Z = 1.44, P = 0.1505); Sasso 950 × Sasso 1400: Wilcoxon Test (N = 29, T = 144, Z = 1.59, p = 0.1120)).

3.4.2. The Fractal Dimension of the Acoustic Signature Dissimilarity, D_{ASD}

The trend of D_{ASD} across daily half-hour intervals revealed lowest values during day time and highest values during night time (Fig. 11a and Table 5 Supplementary material). There were significant
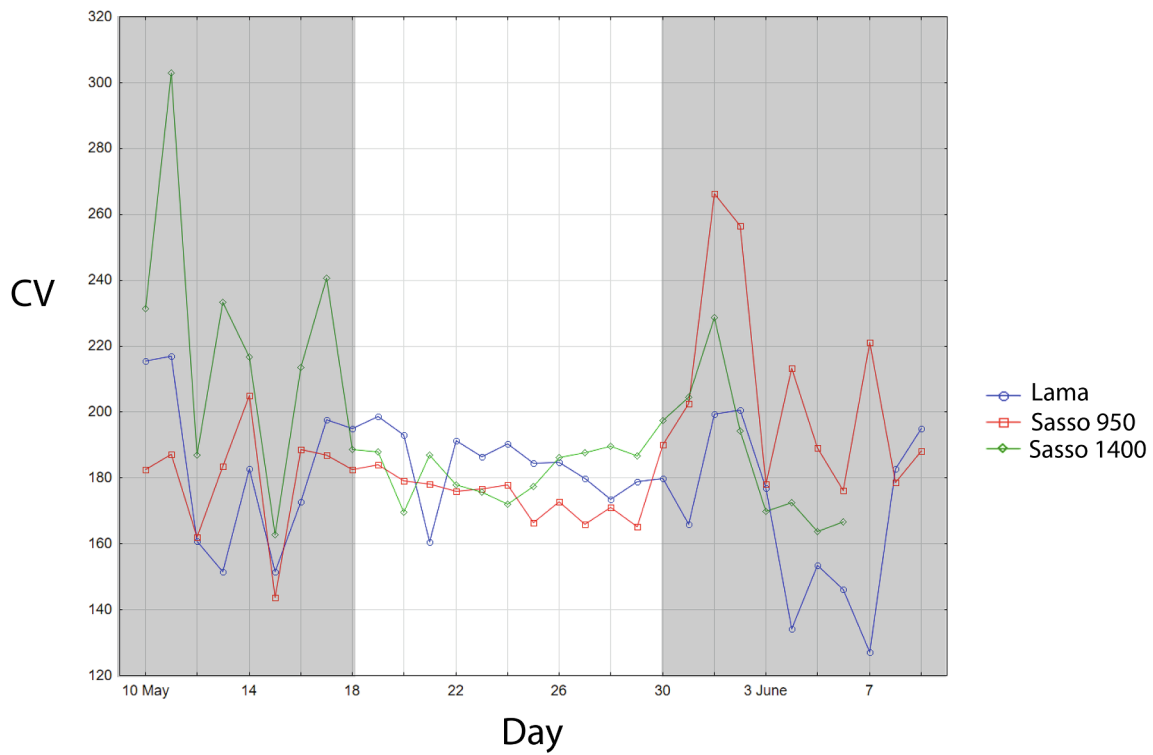
Fig. 6. Trends in the Coefficient of variation (CV) of AS calculated across the 1 kHz frequency classes throughout the study period The two temporal windows in which the CV has a significantly higher value are shaded in grey color.
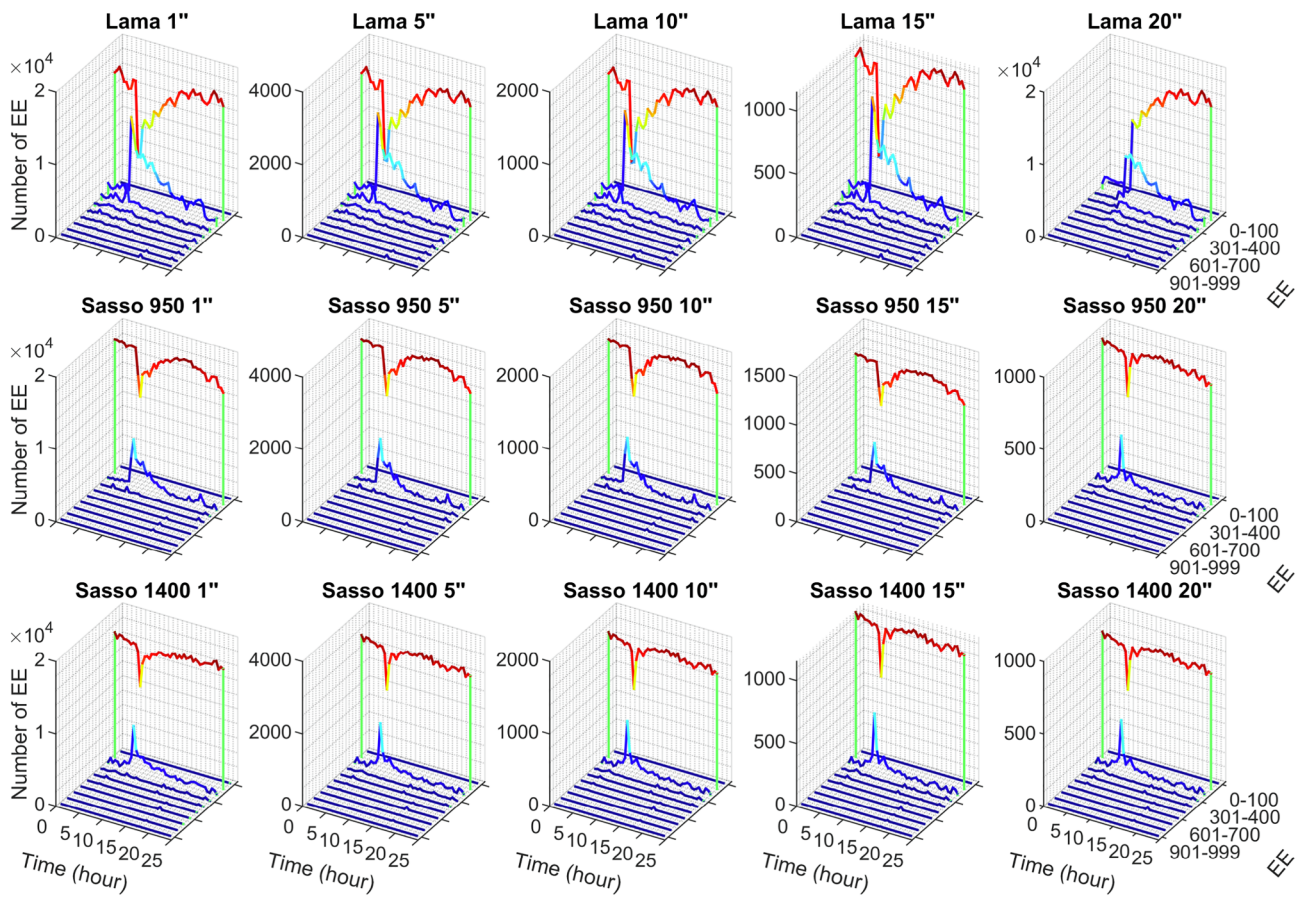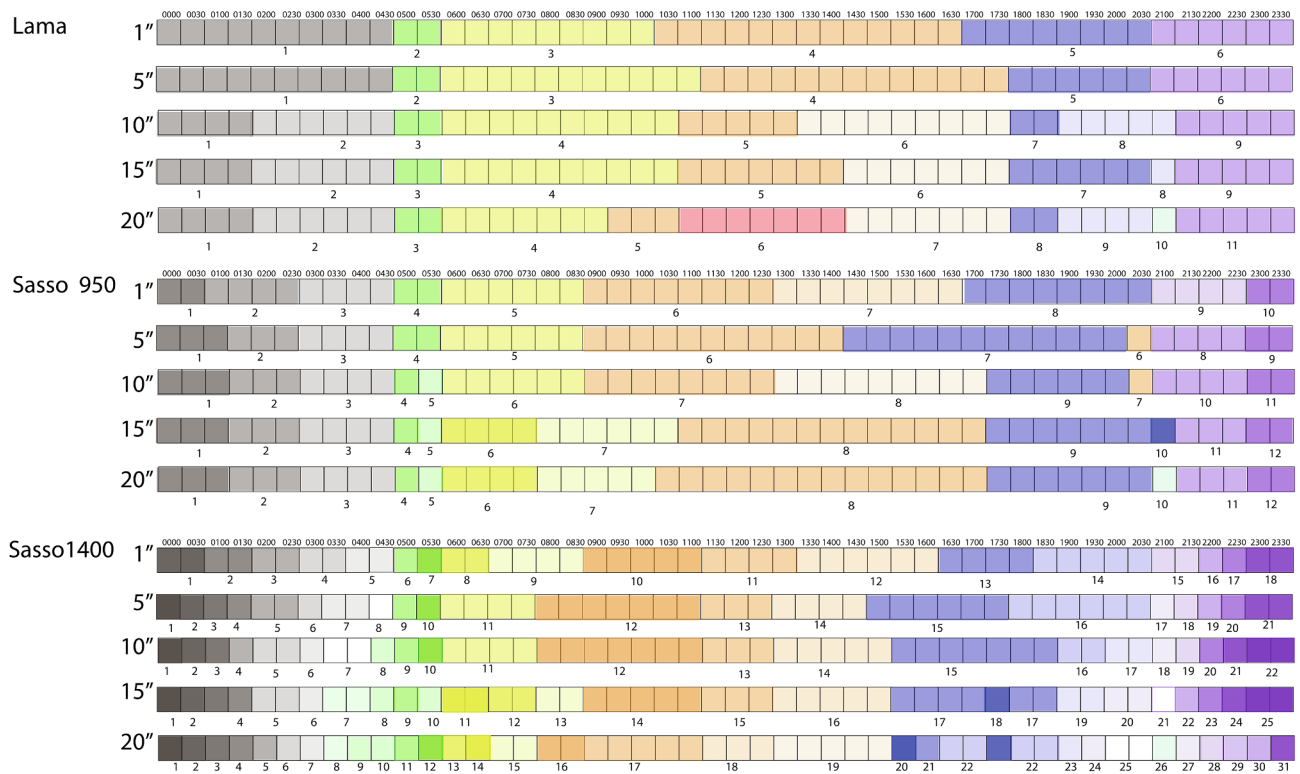

Fig. 7. Distribution of *EEs* across the daily half-hour intervals, aggregated in 10 code classes at five temporal resolutions for each recording locality.

Fig. 8. Aggregation across the 48 half-hour sampling intervals of the EE_h per day. The colors are indicative of the temporal belts. The aggregation resulted from the cluster analysis (see Fig. 1 in the Supplementary material). This aggregation has been repeated for each of the five temporal resolutions and for each recording site. The number refers to the number of clusters recognized throughout the day.

differences in D_{ASD} across daily half-hour intervals between Lama and Sasso 950 (Wilcoxon Test N = 48, T = 39, Z = 5.63, P < 0.0001) and Lama and Sasso 1400 (Wilcoxon Test N = 48, T = 51, Z = 5.51, P < 0.0001). There was no significant difference between Sasso 950 and Sasso 1400 (Wilcoxon Test N = 48, T = 512, Z = 0.78, P = 0.4357). The trend of D_{ASD} across days of the study period revealed lowest values at the end of May (Fig. 11b). D_{ASD} was significantly different across days between Lama and Sasso 950 (Wilcoxon Test N = 31, T = 115 Z = 2.61, P = 0.0092) and Lama and Sasso 1400 (Wilcoxon Test N = 29, T = 95, Z = 2.65, P = 0.0081). There were no significant differences across days between Sasso 950 and Sasso 1400 (Wilcoxon Test N = 29, T = 195, Z = 0.49, P = 0.6266).

4. Discussion

We explored the temporal dynamics of acoustic communities within an intact old-growth forest in Mediterranean Europe. These forests persist in a relatively pristine condition rarely found in this region and therefore represent an important reference system for future research on the impacts of landscape transformation and forest management.

4.1. Acoustic Signature AS

The acoustic signature (AS_h and AS_k) of Lama was significantly different from the other two sample sites in Sasso Fratino. The higher value of AS_h and AS_k probably results from an edge effect where the ecotonal character and unique soil and vegetation structure of Lama has likely resulted in the different acoustic signature found at this site. When we consider the trend of AS_h, all three sites presented similar patterns. For instance, a common peak was evident at 05:30 due to the dawn chorus. From this time onwards, AS_h decreased throughout the day and then increased to a second peak between 16:30 and 17:30 corresponding to the dusk chorus.

The cluster analysis of AS_k revealed three distinct groups: low

frequencies, intermediate frequencies, and high frequencies. We could assign geophonies (water flowing in streams, breezes) at low frequencies (< 1000 Hz), birds at intermediate frequencies (1000–8000 Hz), and insects at high frequencies (> 8000 Hz). AS_k displayed no discernable pattern throughout the day at frequencies < 100 Hz, probably due to stream noise which is the main component of geophony that occurs constantly throughout the day.

While the period examined in this investigation might be considered too short to grasp the entire complex phenomenology of the soundscape, it does in fact provide a good representation of the reproductive cycle of several songbirds found in this old-growth forest. As the study area was located in a montane region, a natural delay in the reproductive phenology of organisms may be expected due to the unpredictability of the montane climatic regime and the onset of spring weather. A concentrated and short phenological cycle of montane organisms can be expected to influence the phenology and rhythms of the acoustic communities (Saracco et al., 2018; Cayan et al., 2001). In the current study, Lama exhibited higher AS_d variability than the other higher elevation sites, but similar trends were also found, although to a lesser extent, in the other two localities up until 15 May and after 30 May. The low variability between 18 and 30 May, common to all three sites, could be associated with the hatching and feeding period for the majority of songbirds that dominate the acoustic community (Righini and Pavan, 2019). These results confirm that our ecoacoustics methodology can describe biological dynamics accurately. The high variability of AS_d during the rest of the study period indicates that the behavior of acoustic communities varies throughout the day and from one day to the next.

While annual cycles are well documented in many organisms, especially in a seasonal context (McNamara and Houston, 2008), studies of short-term cycles allow the discovery of critical behavior and provides great insights into fundamental ecological dynamics (Bru et al., 2011). We found that acoustic signatures were similar throughout the day across all three sites. Furthermore, we found that acoustic
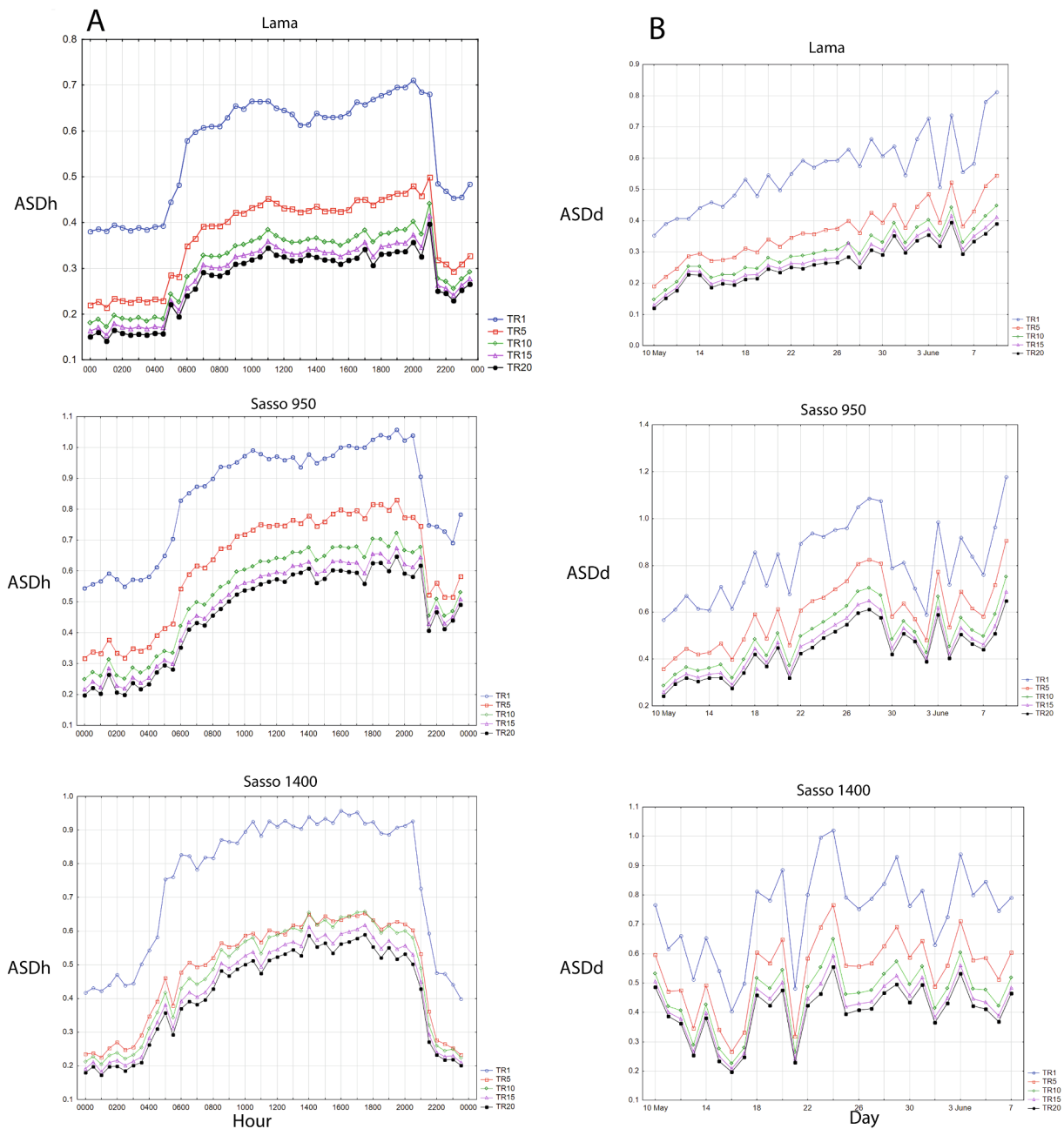
Fig. 9. The Acoustic Signature Dissimilarity every half-hour ASD_h (A), and throughout the study period (ASD_d) (B) for the three localities.

signatures can be grouped according to specific hourly intervals which indicates the occurrence of regular rhythmic acoustic patterns. Such phenomena may be favored, in part, by the absence of human interference, including noise. In fact, there is often conflict between human and animal behaviors and patterns which can have a severe consequence on the acoustic habitat (Mullet et al., 2017), producing modifications of the acoustic cyclical routines of soniferous species such as birds (e.g. Gil et al., 2015). In our study area, the absence of human disturbance has probably allowed acoustic communities to evolve acoustic signatures possessing patterns that have been lost in other disturbed habitats. For example, we observed regularity in the acoustic signature across hours, but alternation between distinct aggregations of hours. Temporal sonotopes (Farina, 2014, p. 17-18) align with sunlight variation (night, sunrise, early morning, late morning, early afternoon, late afternoon), indicating that the daily routine of acoustic communities is the result of factors regulated by species-specific biological needs. Our data suggest that these routines are a rhythmic sequence of

seemingly scheduled animal behaviors that are maintained due to the absence of human activity and landscape change.

4.2. Ecoacoustic Events EE

We found that the number of *EE* significantly varied between the three study sites, for all temporal resolutions. These results indicate that the soundscapes of each site were complex, however, the different patterns were more easily interpreted when the *EE* were aggregated in groups of 100 codes each ($EE_{h,g}$). The first group of $EE_{h,g}$ (0–100) had a minimum value at sunrise in all three study sites. The opposite trend was observed for the second (101–200) and the third (201–300) groups which had maximum values at sunrise. The distribution of $EE_{h,g}$ throughout the day revealed significant differences for groups 101–200, 201–300, 401–500 at Lama, group 201–300 at Sasso 950, and groups 101–200, 201–300, 301–400 at Sasso 1400. These groups were characterized by high amounts of biophony and confirm our previous
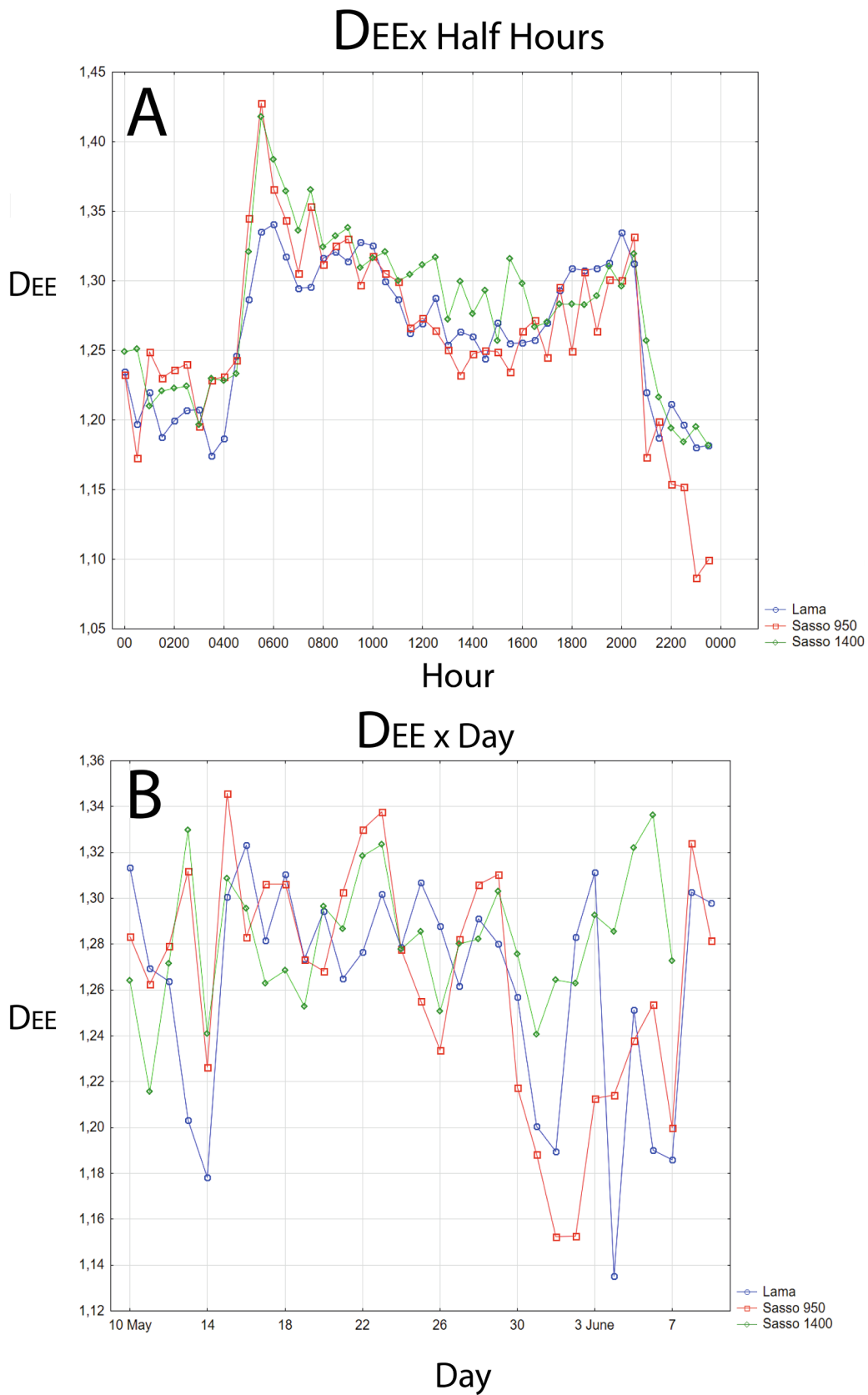
Fig. 10. Aggregation across hours and days of the D_{EE} for the three localities.
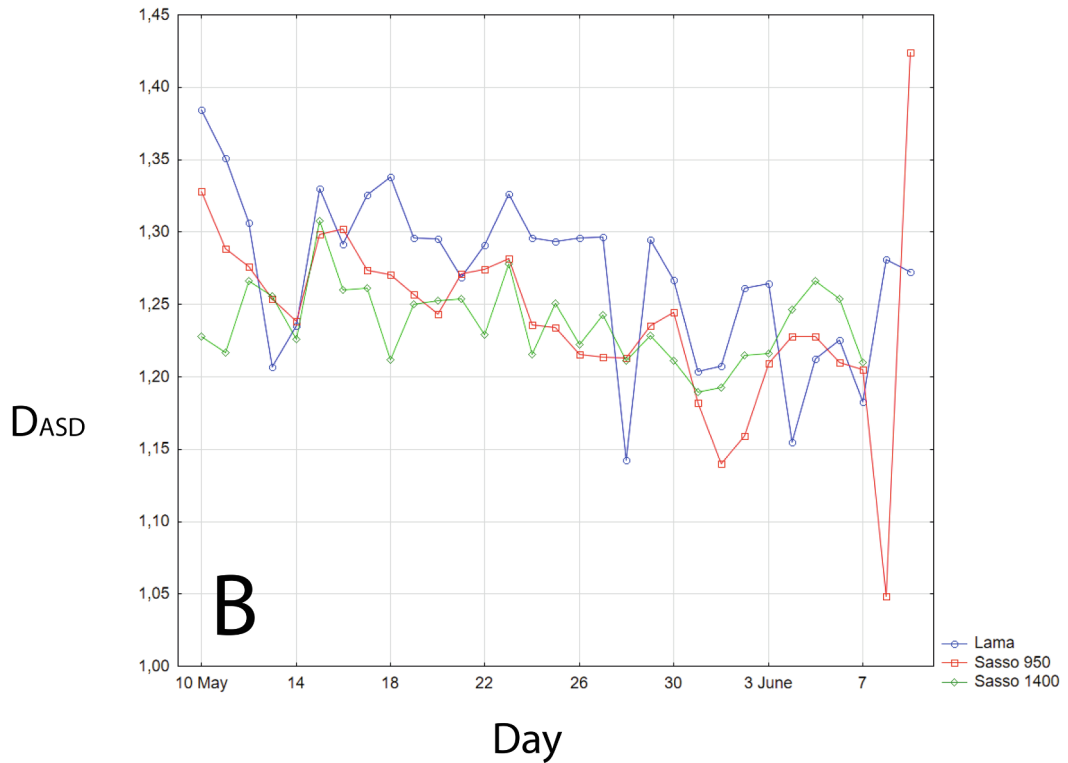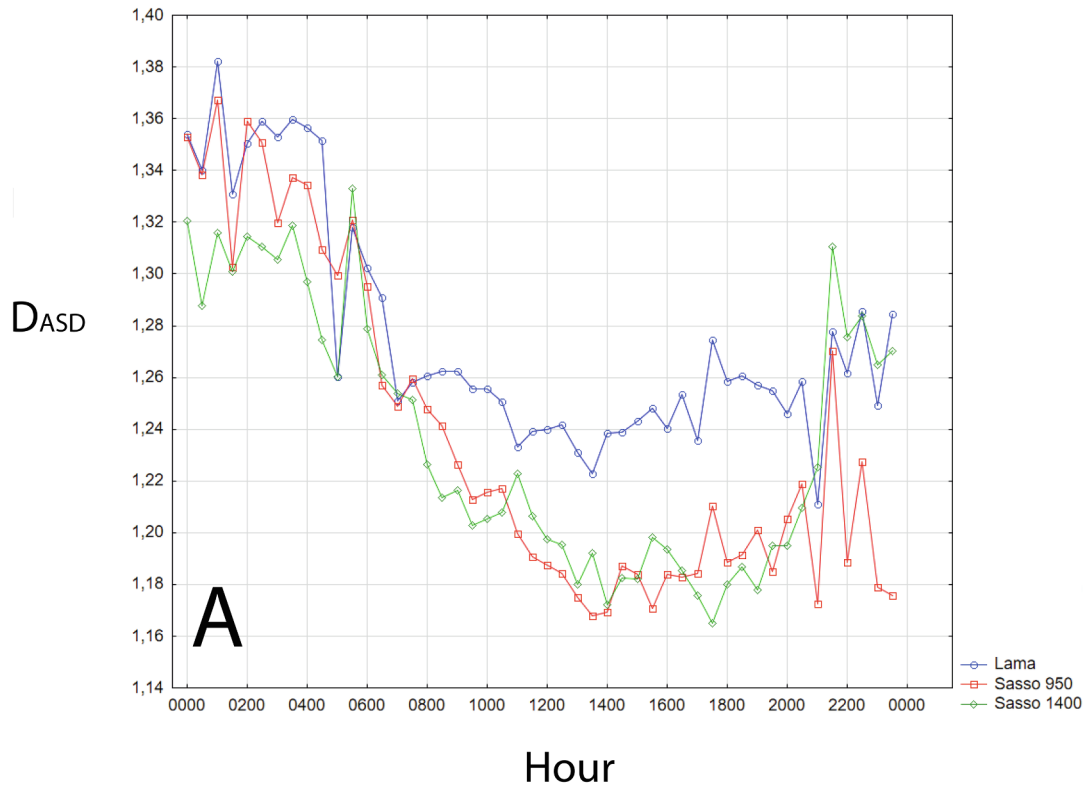
Fig. 11. Aggregation across hours and days of the D_{ADS} for the three localities.

finding that acoustic communities are self-organized and exhibit regular patterns. When the distribution of EE_h was examined across successive half-hour intervals throughout the day, the length of homogeneous periods decreased with an increase in the temporal resolution allowing further elucidation of patterns.

4.3. Acoustic Signature Dissimilarity ASD

The higher ASD_h and ASD_d found for Sasso 950 could be due to the more complex structure of the forest at this site where a mix of broad leaf trees and conifers can impart more canopy and shrubland complexity compared with the other two sites. There was no apparent pattern in ASD_d across the sampling period and this may be attributed to the short reproductive season in mountain forests. However, there was a daily oscillation of ASD_d which may be an effect of microclimatic variability at higher elevations influencing the acoustic activity of birds.

4.4. The Fractal Dimension D of Ecoacoustic Events D_{EE} and of Acoustic Signature Dissimilarity D_{ASD}

Our fractal analysis revealed that D_{EE} was higher during the day than at night, with peaks in early morning and late afternoon. These D_{EE} results confirm the patterns observed using the *AS*, but the fractal approach provides more clarity and detail. Diurnal complexity was largely due to biophony, while at night geophony was the prevailing source of sounds characterized by an intrinsic lower fractal dimension. Throughout the study period, D_{EE} did not show any clear patterns confirming the inter-daily variability of the soundscape. This may be due to variable weather conditions (cloud cover, air temperature and humidity, wind and rain regime) that characterize montane regions and can depress or enhance the emission and broadcasting of sounds. D_{ASD} decreased throughout the day in all three study sites due to differences in the spectral distribution of frequencies confirming a reduction of acoustic complexity from dawn onward. The different patterns shown in the D_{ASD} and the D_{EE} analyses were not contradictory, but rather indicate that *EE* and *ASD* describe different phenomena. *EE* is a proxy of the distribution of acoustic energy, while *ASD* describes how this energy is modulated across frequencies.

5. Conclusions

The development of a new suite of *ACI* indices (*EE, ASD, D_{EE}* and D_{ASD}) has allowed in-depth examination of the structure and dynamics of the acoustic communities of the Sasso Fratino Integral Natural Reserve, providing unprecedented novel insights into the acoustic distinctiveness of this old-growth forest soundscape. Significant differences in the different indices were found between localities and consisted of a higher number of diurnal temporal partitions (sonotopes) in Sasso 950 and Sasso 1400 compared with Lama. In particular, the *EE* analysis revealed a more "organized" dynamic in the two intact, remote

old growth Sasso Fratino sites. This corresponds with an increasing elevation gradient and distance from disturbance. Lama was lower elevation and relatively less natural than these two sites. The daily distribution of *EE* complements the information obtained by the *AS* and confirms the emergence of a clear sequence of patterns consistent with the daily evolution of the overall soundscape including distinct dawn and dusk choruses, punctuated by periods of geophony and night biophonic silence. The distribution of the daily soundscape into homogeneous temporal periods suggests that there are acoustic habits adopted by soniferous species according to specific and seasonally transient physiological needs. It seems probable that soniferous species use distinct, species-specific temporal resolutions according to their physiological and ecological needs, to perceive or to interact with distinct acoustic eco-fields. For this reason, identifying the appropriate temporal resolutions will remain a difficult task when applied at an entire acoustic community and will likely require *a priori* identification of acoustic guilds within acoustic communities. However, our choice of six temporal resolutions and the application of fractal mathematics to *EE* and *ASD*, has produced encouraging results. In fact, the new fractal indices used here (D_{EE} and D_{ASD}) have allowed the description of new patterns that reinforce the information obtained using *AS*, *EE* and *ASD*. Further investigations in other ecosystems where natural conditions remain intact and free from human disturbance, using different temporal resolutions and across a longer, annual sampling period is required to validate these findings.

CREDIT authorship contribution statement

A. Farina: Supervision. **R. Righini:** Investigation, data collection. **S. Fuller:** . **P. Li:** Data curation. **G. Pavan:** Conceptualization, Data collection, Supervision.

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.

Appendix A

Acoustic Complexity Indices (*Pieretti et al., 2011; Farina et al., 2016, 2018*)

$$ACIt_f = \sum_{t=1}^{n_t-1} \left(\frac{|a_{t,f} - a_{t+1,f}|}{a_{t,f} + a_{t+1,f}} \right) \tag{1}$$

$$ACIf_t = \sum_{f=1}^{n_f-1} \left(\frac{|a_{t,f} - a_{t,f+1}|}{a_{t,f} + a_{t,f+1}} \right) \tag{2}$$

where $a_{t,f}$ is the amplitude of each pulse along the spectral line *f* at the time step *t*; n_t is the total number of temporal frames that depends on the temporal aggregation parameter used (e.g., for temporal aggregation 1 second, $n_t = \lfloor 48,000/1024 \rfloor = 46$); and n_f is the number of spectral lines which is 512 in the context of this work.

ACIt$_{fevenness}$ *and* **ACIf$_{tevenness}$** *(Levins, 1968; Hurlbert, 1978; Farina et al., 2016, 2018)*

$$B_{ACIt_f} = \frac{1}{\sum_{f=1}^{n_f} p_f^2} \tag{3}$$

$$B_{ACIf_t} = \frac{1}{\sum_{t=1}^{n_t} p_t^2} \tag{4}$$

where p_f is the relative importance of $ACIt_f$ along the fth spectral line (i.e., $p_f = ACIt_f / \sum_{f=1}^{n_f} ACIt_f$) and p_t is the relative importance of $ACIf_t$ along the tth temporal frame (i.e., $p_t = ACIf_t / \sum_{t=1}^{n_t} ACIf_t$).

The standardized measure is:

$$ACIt_{fevenness} = \frac{B-1}{n_f - 1} \tag{5}$$

$$ACIf_{tevenness} = \frac{B-1}{n_t - 1} \tag{6}$$

Ecoacoustic Event EE *(Farina et al., 2016, 2018)*

$$EE = \langle ACIf_t^{'0,9} | ACIf_{tevenness}^{0,9} | ACIt_{fevenness}^{0,9} \rangle \tag{7}$$

where $ACIf_t'$ results from the normalization of $\sum_{t=1}^{n_t} (ACIf_t)$ for the entire set of data and then aggregated into nine classes of abundance from 0 to 9. $ACIf_t$ *evenness* and $ACIt_f$ *evenness* values that range from 0 to 1 are transformed into nine classes from 0 to 9, before being combined into an EE code. Potentially, there are 1000 codes that can result from the combination of these three code digits, ranging from 000 (this code is present only in theory and represents no sound at all) to 999 (saturated acoustic spectrum such as white noise).

To clarify the meaning of EE codes, we present three examples:

1) a code of 199 indicates a low intensity sequence (**1**99), with even temporal distribution (1**9**9) and a broad distribution of information across frequencies (19**9**).
2) a code of 191 indicates a low intensity sequence (**1**91), with even temporal distribution (1**9**1), and a narrow frequency distribution (19**1**).
3) a code of 119 indicates a low intensity sequence (**1**19), with narrow temporal distribution (1**1**9) and a broad distribution of information across frequencies (11**9**).

Diversity of Ecoacoustic Events (H_{EE}') *(Shannon and Weaver, 1949; Farina et al. 2018)*

$$H_{EE}' = -\sum e_i log e_i \tag{8}$$

Where e_i is the relative abundance of each *EE* in the entire collection (all data aggregated for each sampling location).

Acoustic Signature Dissimilarity ASD *(Orloci, 1967; Legendre and Gallagher, 2001; Farina et al., 2018)*

$$ASD = \frac{2}{n^2} \sum_{i=j}^{n} \sum_{j=1}^{n} Dchord(x_i, x_j) \tag{9}$$

where x_i and x_j are the successive processing steps; n is the total number of AS which varies depending on the temporal aggregation parameter (i.e., for temporal aggregation 1 second, $n = 600$); the function $Dchord(x_i, x_j)$ is formulated by:

$$Dchord(x_i, x_j) = \sqrt{\sum_{f=1}^{n_f} \left(\frac{y_{if}}{\sqrt{\sum_{f=1}^{n_f} y_{if}^2}} - \frac{y_{if}}{\sqrt{\sum_{f=1}^{n_f} y_{jf}^2}} \right)^2} \tag{10}$$

where y_{if} and y_{jf} are the elements of the ith and j^{th} $ACIt_f$, and n_f the number of spectral lines (in this case 512). The chord distance is a maximum of $\sqrt{2}$ when the acoustic signature of two sampling units are completely different and is 0 in the case of an identical distribution.

The fractal dimension of the EE, D$_{EE}$ and of ASD, D$_{ASD}$ *(Mandelbrot, 1983; Feder, 1988; Li et al., 2009; Monacchi and Farina, 2019)*

$$D = 1 - b \tag{11}$$

where b is the slope of the regression equation between the logarithm of the scale of temporal processing (1, 5,10,15, 20 second) and the logarithm of number of *EEs* and of the values of *ASD*, respectively. *D* ranges from 1 to 2. *D* is equal to 1 when the number of *EEs* or *ASD* does not change across the scale. Higher *D* values are rendered if details (number of *EEs*) emerge quickly as the observational scale decreases, demonstrating thus a more complex pattern. *D* is not a proxy for biodiversity, but is an indicator of acoustic complexity. A high value of *D* may be obtained if several species are present with a narrow spectral frequency (the majority of insects). However, where acoustic communities have species that use a broad spectral frequency, such as songbirds in temperate biomes, *D* may be high despite the fact that such communities are not rich in species.

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106927.

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