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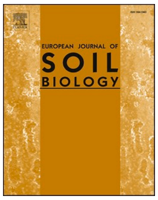

Original article

Continuous measurement of red wood ant (*Formica rufa*) outdoor behaviour using passive acoustic monitoring

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ABSTRACT

Ants serve as ecosystem engineers that maintain important ecological processes within forests. Given their ecological importance, it is a clear scientific shortcoming that we lack non-invasive methods to survey their behaviour inside common opaque habitats such as mounds, litter, and soil. In this study, we assess if acoustic signals from red wood ant (*Formica rufa*) mounds are useful to infer temporal changes in ant activity within forested ecosystems. We found that acoustic indices used previously as a proxy for soil fauna in soil ecological studies (Acoustic Complexity Index, Bioacoustic Index) can indeed separate sounds generated by the ant's daily routines (biophony) from other forest sounds. Yet, we also show that these indices are problematic proxies for soil diversity as they increase not only due to an increased number of species but also due to an increased number of the same species. Acoustic measures that incorporated the strength of acoustic signals, Average Power Density (APD) and Peak Power Density (PPD) also increased with increasing ant abundance and constituted the conceptually best proxy for ant activity. For example, the PPD could i) track diurnal changes in *Formica rufa* activity with a high temporal resolution (minutes) and ii) detect altered behavioural responses to temperature changes. We conclude that microphones detecting biophony can provide high-resolution information about *in situ* ant behaviours in forested ecosystems. Thus, passive acoustics monitoring offers a promising avenue as a non-invasive monitoring tool for soil macrofauna studies.

1. Introduction

Ants are important components of soil fauna as they act as ecosystem engineers [1]. They also represent a group of insects that include some of the potentially most destructive invasive species [2]. As ants affect the flow of material and energy within ecosystems, ant behaviours may have cascading effects on soil structure and other animals and plants [3–5]. Their ecological importance has fuelled the development of numerous monitoring methods attempting to quantify their abundance in natural settings. Methods include different soil extraction techniques (sifting, aspirators, Berlese-Tullgren funnels, Winkler funnels, etc.), baiting, and various forms of traps [6,7]. All these methods have a shortcoming in that they are often invasive and provide only 'snapshots' of abundance at the time of sampling. Alternatively, image-based methods represent the current state-of-the-art technique that can be applied to study *in situ* behaviour [8]. However, photos or video-based techniques are challenging to apply during periods with limited vision (night, rainstorms, fog, etc.) or in substrates where ants are well camouflaged or hidden inside opaque habitats (inside mounds, litter, and soil). In the latter habitats, video and photos cannot be applied without causing major disturbances to the studied system. Hence, there is an urgent need for developing non-invasive techniques that can provide continuous information about behaviours also in opaque environmental matrices if our knowledge about ants and other soil-dwelling organisms' behaviour should progress.

A growing number of studies suggest that traditional monitoring techniques that currently are resource-demanding, both economically and timely, can be replaced by acoustic methods [9], such as passive acoustic monitoring (PAM). This approach relies on autonomous recording units placed out in the field [10,11]. It allows for otherwise remote or inaccessible areas to be monitored while also offering the opportunity to generate broad-scale and high-resolution time series, which is important in biodiversity monitoring [11]. Additionally, PAM recordings can either target specific signals that transfer information

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between individuals (bioacoustics) or signals that infer the diversity of important ecological processes from soundscapes of whole ecosystems (ecoacoustics) [12]. Besides being often economically feasible, PAM has the additional advantage of being non-invasive [13]. So far, bioacoustics- or ecoacoustics-based techniques have mainly been applied to aboveground and aquatic animal groups, including birds, amphibians, wolves, and whales [14–17]. However, some pioneer works have also used acoustic approaches to detect insect infestations inside trunks and hayfields [18,19], and sound-based techniques have been touted as the future of soil monitoring [20,21]. Nevertheless, applications of ecoacoustics approaches that target soil fauna in natural settings are, to date, limited to a few studies [22–27]. While these pioneer studies have discovered relationships between soil fauna diversities and ecoacoustics indices, there is an urgent need for experimental work that can establish the causality behind observed trends.

Interpreting soil sounds from the perspective of soil fauna activity is particularly challenging given that methods cannot rely on recognising characteristic audible sounds that animals intentionally produce (by singing, howling, or croaking), which otherwise serve as a basis for bioacoustics approaches [28–30]. Instead, soil studies depend on ecoacoustics approaches relying on often faint or obscure acoustic signals made intentionally by soil fauna (stridulation, drumming, etc.) or unintentionally created during daily routines (such as burrowing, chewing, and locomotory actions). These collective sound sources (biophony) need to be separated from abiotic sounds (geophony) [31], which is a challenging task inside a forested ecosystem. Sounds from earthworm burrowing can be separated from background noise in laboratory settings [32], highlighting that biophony from specific macrofauna can be recognised. However, studies on soil macrofauna in natural settings have not targeted species-specific biophony but rather relied on a linkage between soil fauna diversity and ecoacoustics indices developed to describe various forms of variability in soundscapes [23,24]. Ecoacoustics indices have also been applied when listening for the effect of specific soil fauna (earthworms) in outdoor settings [22]. Yet, the causality between the correlation of soil fauna composition and soil soundscapes is not straightforward. First, it is difficult to visualise how an ecoacoustics index that targets variability in soundscapes can separate between a high-diversity soil and soil with many individuals of the same species. Second, the correlation between soil fauna composition and soil soundscapes may not necessarily be a direct result of biophony but rather from the impact that soil macrofauna has on the soil matrix itself, as studies have shown that earthworms make the soil louder by altering the soil structure [22]. Shortcomings with ecoacoustics indices as proxies for soil fauna have recently been recognised, and it has been suggested that the use of cluster-based acoustic classifiers or installations of hardware (plates) on top of the soil can improve our ability to separate between invertebrates moving on top of soils [33]. Nevertheless, identifying distinct acoustic signatures from individual ants within a chorus of sounds from many ants will likely be difficult, also for advanced cluster-based classifiers.

Little is known about the extent to which biophony from ants can be separated from background noise within forests and what ecoacoustics variables can be used to inform about their behaviour. In this study, we evaluated the use of PAM for red wood ants (*Formica rufa*-group) behaviour. To illustrate the strengths and weaknesses of different approaches to handling ecoacoustics data from a perspective of soil fauna activity, we applied indices previously used in pioneer soil fauna diversity studies [23,25–27] and variables used in bioacoustics literature [34–36] to evaluate their predictive power for ant activity. We focused on red wood ants as the experimental organism for several reasons. First, red wood ants are widespread in European forest ecosystems, and the high likelihood of this species group being present in areas of interest makes it suitable for environmental monitoring [37]. Second, red wood ants are known to have a diurnal activity pattern [38], and assessing temporal acoustic changes inside their mound (where sounds from other soil fauna can be considered negligible) offers a suitable 'ground truth' for trying out if acoustic measurements can detect this cyclic behaviour. Third, red wood ants are known to be ecosystem engineers by changing soil processes and functions [1,39], dispersing seeds [40] and providing pest control [41]; hence, their behaviours are important for ecosystem processes, and any effects on their performance may have cascading ecosystem consequences. We hypothesised i) commonly used ecoacoustics variables can separate ant biophony from background noise in the laboratory and natural settings, and ii) biophony can reveal the known peak in activity (acrophase) during daytime for *Formica rufa*. In addition, we explored how ant biophony could reveal how temperature affected *Formica rufa* activity over a daily to seasonal time scale.

2. Methods

2.1. Acoustic equipment

Our PAM system consisted of a recorder (Zoom F3), a preamplifier (sE Electronics DM2 TNT), an adapter (6.3" jack female to XLR male), an EKO FASI contact microphone, and a gold-plated copper wire ($\o = 1$ mm and 100 mm long) attached perpendicular to the microphone to act as a waveguide. One exception from this technical setup was that the soundscapes of abandoned and active mounds (described below) were recorded with a Zoom H4n Pro. The power source (20k, 30k or 96k Ah power banks) were connected directly to each recorder. Trials in laboratory settings were conducted with the microphone mounted on the side of the arena using the built-in suction cup of the microphone. In forest settings, the waveguide was inserted into the top of each ant mound.

All files were recorded in Waveform Audio Format (.WAV) but were compressed to.flac format to save storage space. Zoom H4n recordings were made with a 24-bit depth, whereas Zoom F3 recordings were made with a 32-bit depth and later reduced to 24-bit to make them comparable. For all recordings, we had a sampling rate of 48 kHz to ensure that the entire audible range was covered.

2.2. Experimental design

We focused on evaluating four acoustic variables: average power density (APD), peak power density (PPD), Acoustic Complexity Index (ACI), and Bioacoustic Index (BI). The rationale for evaluating ACI and BI was their previous use in soil fauna diversity studies [23,25,27] and their use in studies assessing impacts of other soil macrofauna [22]. We used APD and PPD as their use has been highlighted in more general bioacoustics literature [34–36]. The ADP is the average power in a selected area of a spectrogram, found by summing the power spectral density values from all bins divided by the number of time-frequency bins in the selection [42]. The PPD (or max power) refers to the highest power spectral density value within the selection [42]. While both of these 'power-based' variables show strong co-variance, PPD is more sensitive to random short energy bursts, while APD is less sensitive to rare extreme values. The ACI and BI are indices that describe variations in the soundscape rather than accounting for the strength of signals. Here, ACI was developed to be sensitive to irregular biophony, also in the presence of constant anthropophony [14,31,43]. The ACI is calculated as the absolute difference in amplitude between one time sample and the next within a frequency band (normalised to the amplitude of the first sample) [14,31]. The BI is a function of both amplitude and number of occupied frequency bands between 2 and 11 kHz, where each score is calculated relative to the quietest 1 kHz frequency band, making it a measure of increased disparity between loudest and quietest bands that could be affected by increased biophony [31,44]. In summary, ACI calculations are based on the notion that biotic sounds are characterised by variability in intensity, as seen in, for example, bird songs [14], whereas BI focus on specific frequency bands that birds use [44].

Our first hypothesis was tested both in laboratory and natural settings using APD, PPD, ACI, and BI as dependent variables and ant abundance as the independent variable. In our laboratory trial, ants were placed inside an insulated box having an arena (9 × 9 × 7 cm) with a replaceable paper bottom (removed between trials to avoid chemical cues from previously tested ants affecting individuals tested at a later stage). We generated a response curve for each acoustic variable by randomly changing the density of *Formica rufa* individuals (0, 1, 5, 10, 25 and 50 ants) in the arena. The ants were recorded for 180 s. Each density treatment was replicated five times with new ants, and the limit of detection (LOD) was calculated as 3 × standard error of the calibration curve/the slope of the regression line. In this context, LOD indicates at what abundance ants start to generate enough sounds to increase the ecoacoustics variables well above the background noise. Field-testing of hypothesis 1 was conducted in a region in northern Sweden characterised by summer (June–August) average temperatures of around 15 °C and precipitation of around 66 mm per month [45]. Mounds were located in sites with boreal forests dominated by Scots pine (*Pinus sylvestris*) with blueberry (*Vaccinium myrtillus*) and lingonberry (*Vaccinium vitisidae*) understory. We compared the acoustic variable of active red wood ant mounds ($N = 3$) with the soundscape of abandoned ant mounds ($N = 3$) at a site in Hissjö (63°92′89″256 N 20°15′46″657 E). Mounds were considered abandoned when no ants were observed at the surface of the mound after an hour of observation.

To test hypothesis 2, we monitored *in situ* soundscapes in *Formica rufa* mounds ($N = 24$) located within a boreal forest for a week in June (Carlslid, 63°80′27″95 N; 20°33′39″42 E, $N = 12$) and in September (Ekorrträsk, 64°49′94″26 N; 19°05′81″53 E, $N = 12$). We used temperature and moisture loggers (Tomst TMS-4) at the base of each mound.

2.3. Statistical analysis

The integrity of the audio files was assessed visually and audibly in Raven Pro 1.6.5 to make sure there were no recorder malfunctions or other artificial disturbances. After the initial control, a discrete Fourier transform (DFT) of size 512 with a boxcar window function was used to transform the soundwaves into power spectra. This was done using the function spectrogram of the package soundfile for Anaconda [46] suite for Python 3 [47] from which we calculated the average power density (APD) and the highest power density (PPD) over 600 s. The ecoacoustics indices, ACI and BI, were also calculated over 600-s periods using the package soundecology [48] for R [49].

The statistical validity of the first hypothesis was tested with linear regression using the package glmmTMB [50] from R-Studio. Here, we tested the correlation between the different response variables (APD, PPD, ACI, and BI) and the presence of ants in the laboratory trials and the mounds to assess if acoustic information from ants of different abundances could be detected and separated from background forest noise.

To test that there were significant temporal changes in biophony from the *Formica rufa* mounds (a prerequisite for hypothesis 2), we used a generalised linear mixed effects (GLME) model with PPD as a dependent variable. We focused on this ecoacoustics variable as it provides a theoretical rationale for responding proportionally to the increased strength of biophony. In the GLME model, we included mound temperature (Temp), time of the day (Time), and field site/season of the measurement as environmental factors. Due to the experimental design, season and location were confounded, but for simplicity, we refer to this factor as Season. To avoid confounding effects of other daily changes, we added 'Time' as a predictor and mapped it to a sinus function to linearise the effect. 'Season' was included as a fixed effect factor and ant mound (Mound) as a random effects predictor. Potential mound-specific responses to temperature (i.e. as a result of mound size or microsite conditions) were accounted for by allowing a random slope for the temperature effects. The residuals presented high kurtosis and were, therefore, modelled with a Pearson type VII distribution (family = t_family in the formula). The final specification of the model was:

$$\text{Response} \sim \text{Temp} \times \sin(\text{Time}) \times \text{Season} + (1+\text{Temp}|\text{mound}),\ \text{family} = \text{t\_family} \qquad \text{Eq. 1}$$

To evaluate the model fit, the simulated residuals were inspected using the DHARMa [51] package for R. The explanatory power of the model was calculated using Nakagawa R^2 [52,53] and the significance of the factors was further tested with an ANOVA.

To test Hypothesis 2, we used the R-package Cosinor2 [54] to assess circadian rhythm trends in ants based on PPD. Here, we identified the timing of the peak activity (acrophase) for June and September (hereinafter referred to as summer and autumn). In our models, we used the function population-mean cosinor [55], where a cosinor model was fitted to each mound that acted as an individual, and the population response was described as the mean of all the individual cosinor models [56]. In addition to the acrophase, we also extracted the rhythm-adjusted daily mean (midline estimating statistic of rhythm; MESOR) for the PPD. Significant differences between the seasons for MESOR and acrophase were tested using F-ratios [57]. The output from the cosinor for the acrophase was expressed in radians as time was mapped to a trigonometric function. This angle was back-transformed to time using the expression:

$$\varphi' = (-P/2\pi)\varphi, \qquad \text{Eq. 2}$$

Where P is the period (24 h), and φ is the acrophase [58]. We used ggplot2 to visualise the fitted values from the models and means.

3. Results

We found that all four acoustic variables increased in response to increasing ant densities in the laboratory setting (APD: $F_{1,28} = 6.57$, $p < 0.05$; PPD: $F_{1,28} = 4.24$, $p < 0.05$; ACI: $F_{1,28} = 4.90$, $p < 0.05$; and BI: $F_{1,28} = 24.55$, $p < 0.01$, Fig. 1a–d, diagnostic qq-plot in Figs. S1a–d). The linear model explanatory power was greatest for BI, which explained 45

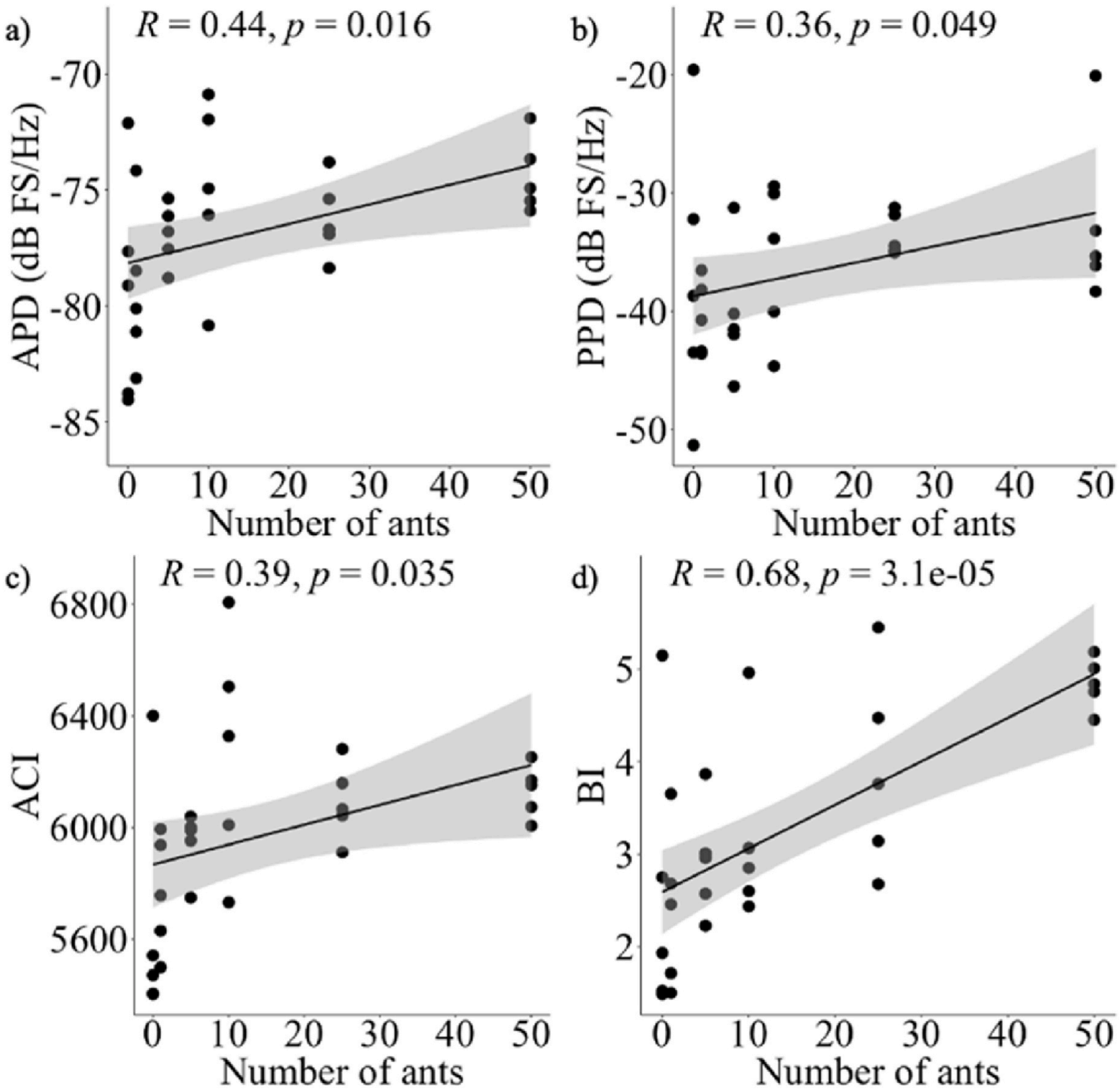

Fig. 1. Relationship between the number of ants and a) APD, b) PPD, c) ACI, and d) BI. Each dot represents one independent measurement, and the black line shows the linear relationship between the acoustic measurement and the number of ants, and the shaded area shows the 95%-confidence.

% of the variation, whereas the rest of the acoustics measures' explanatory power was lower (APD: 16 %, PPD: 10 %, and ACI: 12 %). The calculated lowest level of detection (LOD) for APD, PPD, and ACI was 24 ants, 28 ants, and 26 ants, respectively. The lowest LOD of 17 ants was found for the BI.

All four applied acoustic variables generated higher scores (p-value <0.05) in the active ant mounds in comparison to the abandoned mounds (Fig. 2a–d and Table 1, diagnostic qq-plot in Figs. S2a–d). The APD in active mounds (−72 ± 7 dB FS/Hz) was higher ($F_{1,\ 1407}$ = 567.90, $p < 0.001$) than that of the abandoned mounds (−79 ± 2 dB FS/Hz) (Fig. 2a). Likewise, we found that PPD was higher ($F_{1,\ 1407}$ = 1842.70, $p < 0.001$) in active mounds (−27 ± 9 dB FS/Hz) compared to abandoned mounds (−48 ± 9 dB FS/Hz) (Fig. 2b). Both acoustic indices, ACI and BI, showed similar patterns. ACI for active mounds (21167 ± 3904) was higher ($F_{1,\ 1441}$ = 444.93, $p < 0.001$) than for abandoned mounds (18148 ± 113) (Fig. 2c), and we found that BI was higher ($F_{1,\ 1441}$ = 312.96, $p < 0.001$) for active mounds (2.8 ± 1.7) compared to abandoned ones (1.6 ± 0.3) (Fig. 2d).

The explanatory power for the linear regression was highest for PPD (57 %), but a considerable proportion of the variance (29 %) was also explained by the APD. Our models indicate a stronger correlation between the presence of ants and the power density measurements in a natural setting and a lower correlation with the indices, ACI and BI (Table 1).

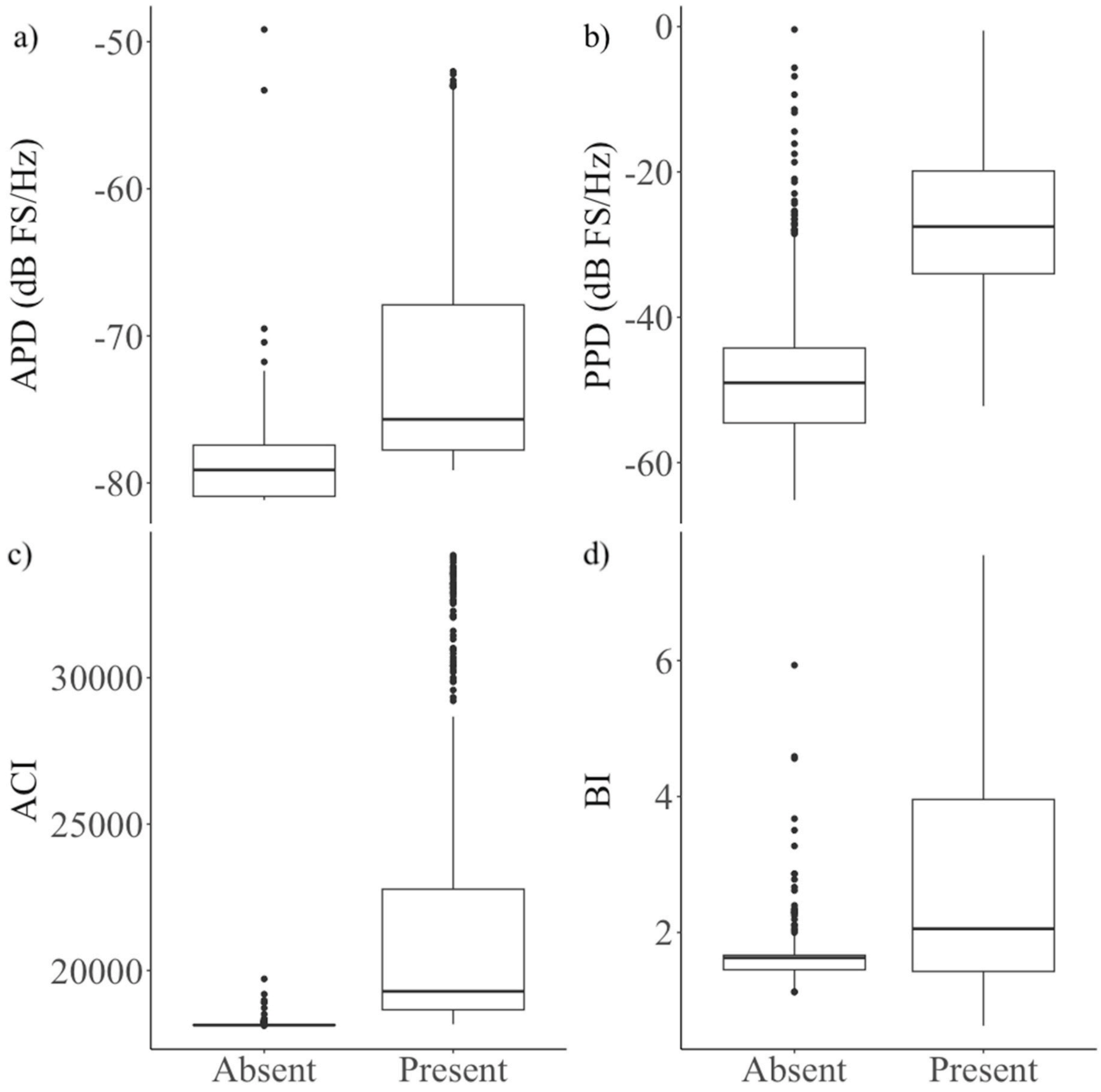

Fig. 2. Relationship between active ant mounds (labelled 'present') and abandoned ant mounds (labelled 'absent') for a) APD, b) PPD, c) ACI, and d) BI. The black line is the median, the box is the interquartile range, the whiskers are the complete data range, and the dots are outliers.

Table 1
Mean and standard deviation (±) of the four response variables: measurements of APD, PPD, ACI, and BI in abandoned and active ant mounds. Also shown are ANOVA outputs and R^2 for the linear models testing for a correlation between the response variables and a categorical independent variable (ants present or ants absent).

Response variables	Abandoned Mounds		Active mounds		F-statistics	P-value	R^2
APD *(dB Fs/Hz)*	−79	±2	−72	±7	$F_{(1, 1407)=}$567.90	<0.001	0.29
PPD *(dB Fs/Hz)*	−48	±9	−27	±9	$F_{(1, 1407)}$ = 1842.70	<0.001	0.57
ACI (unitless)	18148	±113	21167	±3904	$F_{(1, 1441)}$ = 444.93	<0.001	0.24
BI (unitless)	1.6	±0.3	2.8	±1.7	$F_{(1, 1441)}$ = 312.96	<0.001	0.18

Given that our model for PPD had the highest explanatory power in natural settings, we conducted our statistical test of temporal trends and environmental variables using this variable. A positive correlation was found between PPD and temperature ($X_1^2 = 172.47$, $p < 0.001$). This effect was modulated by the time of the day (Temp x Time, $X_1^2 = 52.51$, $p < 0.001$). Here, the temperature effects were higher during the autumn measurements (Temp x Season, $X_1^2 = 68.26$, $p < 0.001$). Moreover, the interaction between temperature and time had different effects during different seasons (Temp × Time × Season, $X_1^2 = 39.18$, $p < 0.001$). We also found a main effect of Season ($X_1^2 = 18.17$, $p < 0.001$) and Time ($X_1^2 = 52.13$, $p < 0.001$), but no effect of their interaction (Time × Season, $X_1^2 = 0.38$, $p > 0.05$). The model for the PPD accounted for 94.2 % of the variance, where 54.8 % was explained by the fixed effects (Time, Season, and Temp) when using Nakagawa's R^2 (diagnostic qq-plot in Fig. S3).

The diurnal variation for PPD followed a circadian rhythm with the time of the overall peak values (acrophase) occurring during the day-time (Fig. 3 and Table 2). Here, we found that PPD in summer (−28.19 ± 6.56 dB FS/Hz) was higher ($F_{1,16} = 9.66$, $p < 0.05$) than in the colder autumn (−38.43 ± 4.41 dB FS/Hz). The timing of the acrophase also

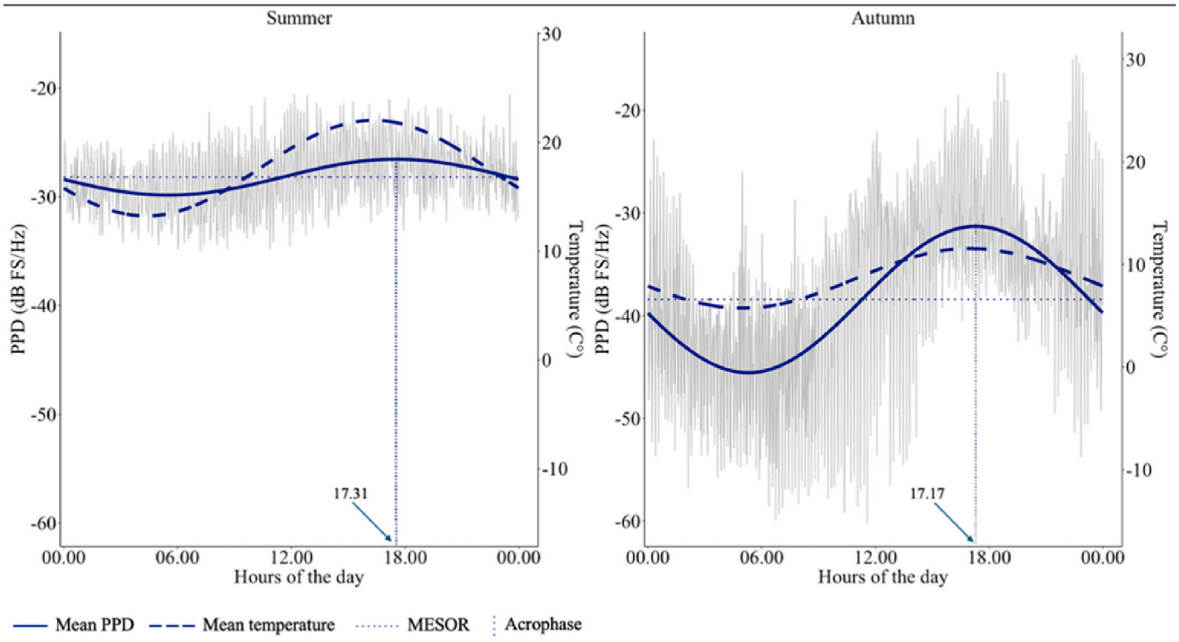

Fig. 3. Diurnal ant activity trends reconstructed using the PPD. The panel is split into two seasons: summer and autumn. The solid-coloured line is the fitted model for each season, the dashed line is the average daily temperature over the recording period, the horizontal dotted line is the midline estimating statistic of rhythm (MESOR), and the vertical dotted line is the acrophase. The arrow and time indicate the acrophase. The grey shading represents the range in means for each studied mound for each of the seven days that the experiment lasted.

Table 2
Circadian rhythm metrics estimated using the temporal trends in the PPD applied to soundscapes of *Formica rufa* mounds. Estimates include the midline estimating statistic of rhythm (MESOR) and the time period in a cycle during which the cycle peaks (acrophase). Mean values (± standard deviation for MESOR and min/max-values for acrophase) are reported for summer and autumn. Statistics (F- and P-values) comparing the two seasons are also shown. Note that the statistics for the acrophase are conducted on the time mapped to a sinus function, as explained in the methods.

	Summer		Autumn		F-statistics	P-value
MESOR dB FS/Hz	−28.19	±6.56	−38.43	±4.41	$F_{(1,16)} =$ 9.66	<0.05
Acrophase (time CEST)	17:31	14:38 19:14	17:17	16:56 17:42	$F_{(1,16)} =$ 33.94	<0.001

differed between seasons ($F_{1,16} = 33.94$, $p < 0.001$, Table 2), where it occurred around 17:31 CEST (with the earliest peak at 14:38 and the latest at 19:14) in summer but came on average 14 min earlier in autumn (17:17 CEST with the earliest peak at 16:56 and the latest at 17:42).

4. Discussion

In line with our first hypothesis, our results suggest that biophony from red wood ants can be separated from background noise in both laboratory and natural settings. Biophony from *Formica rufa* made sufficient contributions to the laboratory and forested soundscapes that all tested acoustic variables (APD, PPD, ACI, and BI) increased in their presence. Indeed, our findings support the assumption that ecoacoustics variables applied to PAM recordings can inform about soil fauna activity in outdoor settings, an assumption that is implicit in previous studies linking soil soundscape to soil fauna diversity [23,24]. Yet our results also highlight some shortcomings with ecoacoustics approaches. Indices (ACI and BI) used previously as a proxy for soil diversity [23,25–27] increased in response to increased ant abundance; hence, a high concentration of *Formica* ants in soil may falsely indicate high soil fauna diversity.

Biophony from ants inside mounds is characterised by a low variance in intensity. That the acoustic signals were more obscured in the mounds than in our open laboratory arena may explain why the ACI and BI were less able to register the biophony of ants in the mounds than APD and PPD. These later two variables, both accounting for the strength of biophonic signals, are also conceptually more suitable for monitoring changes in *Formica rufa* activity due to their abilities to respond to a chorus effect where sounds of many sources can be recognised by higher scores. These power-based variables both increased proportionally to the artificial increase in ant abundance in our laboratory trials and had the best model performances in the outdoor comparison of abandoned vs active mounds.

The diurnal cycle in activity for *Formica rufa* based on PPD resembles diurnal trends previously described in the literature, with the highest activity (acrophase) near noon and the lowest during the night [38]. That the diurnal cycle in the PPD shows expected temporal patterns across multiple sites and seasons suggests that mound acoustics can capture important temporal variation in ant activity in line with our second hypothesis. Indeed, this finding further supports various works arguing that PAM could be used to detect soil fauna diversity [19,22–24,26] but also highlights the importance of considering diurnal cycles when conducting snapshot recordings of soil. While little is known about daily cycles in soil soundscapes in general, we note that tropical soils also have reported strong diurnal trends with a reported early morning peak in activity for the BI [26]. This latter finding, in combination with our results, suggests that soil fauna activities may vary in response to biomes, temperature and seasons, indicating that acoustic-based surveys of soil fauna may need to adopt ecosystem-specific and season-specific sampling times to capture periods of peak activities.

Temporal trends using the PPD proved sensitive enough to detect increased activity in response to external factors such as increased temperature, a well-documented behavioural response for *Formica rufa* [38]. Because ant metabolism is stimulated by increased temperature, changes in air temperature directly affect their locomotion and walking speed [38,59]. This metabolism-temperature linkage can explain the positive activity response to increased temperature over 24 h, especially in autumn, that we inferred from the PPD. Confirmation of the temperature dependence of ant activity using the PPD further indicates the potential of PAM of soil fauna.

The behaviour of soil macrofauna is of paramount importance due to its possible cascading impacts on plants and other animals [60], and the phenology of soil fauna behaviour is a largely understudied field of research [61]. Studies on the phenology of soil fauna behaviours are, at least partly, held back by the relative scarcity of methods that can continuously monitor *in situ* activities over time and, thus, be used to develop long-term phenology datasets. In this context, our acoustic-based and non-invasive methodology, generating continuous information about belowground activity at timescales ranging from seconds to months, seems like an advancement for the field.

4.1. PAM to advance our understanding of red wood ant behaviour

Our reconstructed temporal changes largely confirmed what is already known about red wood ant behaviour. For example, the diurnal change in activity that we show has previously been described by studies using trapping techniques or ocular observations [62]. Similarly, our inferred higher red wood ant activities during the summer in comparison to the autumn confirms previous knowledge regarding how colder air temperatures reduce their movements when monitored using laboratory-based acrographs [59]. While our confirmation of previously known red wood ant behaviours serves as a validation that ecoacoustics can detect ecologically important behaviour, a justified question reads: what can PAM provide that other methods cannot? We see three advantages with our ecoacoustics method. First, our approach informs about activities ongoing in real time inside the mound, which cannot be detected by traditional video techniques without modifying the mound. Second, the autonomy of the system facilitates the monitoring of several replicates over a weekly time scale without overfilling memory cards. This is an important feature as continuous video recordings, the only other technique able to continuously monitor ant behaviour, rapidly generate needs for data storage that exceeds the capacity of most memory-card systems available on the market. Third, the continuous and high temporal resolution (seconds) of our recordings allowed us to assess synchronicity in behaviours between mounds that are difficult to measure using traditional baiting and trapping techniques.

While synchronous behaviours within an ant mound have been studied and explained by harmonised group activity driven by interactions with neighbouring individuals [63,64], less is known about temporal rhythms shared between mounds. We note a high general synchronism in PPD scores between mounds, as especially seen for the acrophase in autumn, but also intriguing periods where some individual mounds move out of phase with each other. For example, during summer, the inferred timing of the acrophase could differ by up to almost 6 h between mounds, while during autumn, these differences were down to at most 46 min. The overall reduced between-mound difference in the autumn was possibly a result of stronger temperature and light constraints on ant activities (a shorter 'window of opportunity'). However, we believe that important insights into the ecology of ants may be found by assessing mechanisms behind synchronous and asynchronous behaviours in their mounds. Here, synchronised behaviour seems key to understanding regional drivers of their behaviours, while exploring why mounds from the same area may adopt contrasting behaviours despite experiencing the same weather and light conditions will likely provide insights about ant mobility at a local scale relevant for forest managers and researchers interested in soil biodiversity. To date, the interpretation of ecoacoustics information from ant communities is a research field in its infancy, and thus, at this early stage, we hesitate to interpret variations observed between individual mounds in our study. In our study, small differences in recorder performance or placement of the waveguide may explain some mound disparities. However, the general agreement in temporal trends reconstructed for mounds from the same area makes us confident that the outlined approach captures ecologically relevant *Formica rufa* behaviours.

5. Conclusion

In this study, we showed that it is possible to detect ant biophony in natural outdoor settings. Commonly used acoustic indices have variable sensitivity to ant biophony but can clearly separate between the presence and absence of ants in outdoor settings. Power-based ecoacoustics variables can handle chorus effects and muffled biophonic signals and, thus, be used for capturing diurnal trends in ant activity and response to environmental stressors. We believe that the described ecoacoustics approach provides a promising avenue for studying the ecology of not only ants but also other soil macrofauna that dwell inside soil, litter, or other opaque matrices.

CRediT authorship contribution statement

L. Vang Sørensen: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **S. Rodríguez-Martínez:** Writing – review & editing, Software, Formal analysis, Data curation. **M. Rollo:** Writing – review & editing, Methodology, Investigation, Data curation. **J. Klaminder:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization.

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2024.103687.

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