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Hidden worlds: Testing applicability of acoustic methods for examination of deadwood arthropod communities

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Abstract

1. Deadwood is an important component of forest health and terrestrial carbon storage. However, the diversity of arthropods inhabiting deadwood and the biotic factors influencing decledomposition remain poorly understood, partly due to the challenges associated with studying wood-dwelling organisms.
2. Ecoacoustic methods—non-invasive and non-destructive—offer a promising approach to investigating saproxylic arthropod communities.
3. We tested and optimised ecoacoustic techniques (including recording duration, sensor attachment methods, and soundproofing strategies) for saproxylic detecting abundance and diversity in a multisite experiment spanning Mississippi and Arizona, USA, and Comayagua, Honduras.
4. While linear mixed-effects models revealed no significant relationships between overall arthropod diversity and ecoacoustic diversity, models incorporating the abundance of particular taxa showed significant associations. Notably, the abundance of Hymenoptera and Blattodea correlated with ecoacoustic diversity measures. Effect size varied, ranging from small (−0.000015 ± 0.000005) to moderate (2.5569 ± 0.7623), depending on the diversity metrics included in the model.
5. This study represents the first application of ecoacoustic methods to access arthropod diversity in deadwood and highlights the potential of these tools for targeting studies of macroarthropods in decomposing wood environments.

KEYWORDS
biodiversity, brown food web, community ecology, forest entomology, Formicidae, Termitidae

INTRODUCTION

Forested ecosystems serve as important terrestrial carbon sinks, storing carbon in living trees, deadwood and soil (Pan et al., 2011). The majority of forest carbon (86%) is stored within living biomass and soil, but deadwood accounts for approximately 8% of global forest carbon and regions that have undergone widespread disturbance, such as fire, contain proportionally higher amounts of deadwood (Pan

et al., 2011). Following tree death, carbon stored within deadwood has two primary fates: incorporation into the soil, where it functions as a terrestrial carbon sink or release to the atmosphere, which is mediated by a variety of both biotic and abiotic factors (Cornwell et al., 2009; Ulyshen, 2016). Abiotic factors, such as temperature and moisture, are important drivers of deadwood decomposition at large scales (Harmon et al., 2004; Laiho & Prescott, 1999; Laiho & Prescott, 2004), but biotic factors are more predictive at local scales (Bradford et al., 2014). Specifically, fungi and insects are drivers of wood decomposition, but their relative impacts vary across biomes, and the latter is primarily driven by termite activity (Bradford et al., 2014; Schuurman, 2005; Stoklosa et al., 2016; Ulyshen et al., 2014; Ulyshen & Wagner, 2013). While understanding the contribution of insects and microbes to deadwood decomposition is increasing (Njoroge et al., 2025; Seibold et al., 2021), there is still a significant knowledge gap about the biodiversity within deadwood, in part due to the opacity of deadwood, the significant abundance of organisms, and their small size (Decaëns, 2010).

Diversity of organisms within ecosystems is often positively related to ecosystem function and services (Loreau, 1998; Tilman et al., 1997). Saproxylic arthropods (arthropods that rely on deadwood at some point in their life cycle) are a diverse and oftentimes cryptic group of organisms with significant ecological roles in forest ecosystems. Estimates of saproxylic arthropod diversity are high (Stokland et al., 2012; Ulyshen, 2018), but research is limited relative to other organisms as they are small, difficult to extract from deadwood, and the taxonomy for many species is not known rendering saproxylic insects difficult and time-consuming to study (Decaëns, 2010; Langor et al., 2008). Consequently, current estimates of total global diversity are derived from relatively few studies, most of which are concentrated in temperate climates (Seibold et al., 2015). Additionally, the effects of full assemblages of saproxylic arthropods on ecosystem services, such as wood decomposition and nutrient cycles, are rarely investigated, and instead, studies have focused on larger taxa such as termites and beetles (Cobb et al., 2010; Schuurman, 2005; Siegert et al., 2018; Stoklosa et al., 2016; Ulyshen et al., 2014; Ulyshen & Hanula, 2010; Ulyshen & Wagner, 2013). Because of the positive relationship between diversity and ecosystem functions, quantifying biodiversity is a goal of many ecology studies and management plans.

Research often focuses on larger organisms at least partly because of the challenges of extracting, identifying and quantifying the abundance of diverse taxa in deadwood. Studying saproxylic arthropods requires their extraction from wood (Hammond, 1997; Wikars et al., 2005), which is typically destructive (e.g., breaking apart wood or removing it from the field to rear invertebrates out) and renders the substrate sample functionally useless for future study. Inability to resample wood introduces inherent variation to experimental wood decomposition studies and may limit researchers' abilities to answer questions of succession in saproxylic communities. This field of research would greatly benefit from the development of non-destructive *in situ* methods for quantifying arthropod communities. Development of such methods would allow researchers to observe one experimental unit of decomposing wood and quantify the changing dynamics of saproxylic invertebrates present throughout the decomposition process.

Acoustic methods have become increasingly popular as *in situ* options for natural science research. These studies, often grouped under the broad term 'bioacoustics', allow researchers to observe target organisms and collect data remotely while minimally disturbing study subjects or their natural environment (Bertucci et al., 2016; Clark et al., 2018; Harris et al., 2016; Keen et al., 2017). Vulnerable species, such as whales and elephants, can be studied distantly, hopefully decreasing chances of detrimentally altering behaviour or drawing unwanted attention to poachable populations (Clark et al., 2018; Fournet et al., 2018; Keen et al., 2017). Bioacoustic methods have also been applied to arthropods, using both air (Kohlberg et al., 2024) and substrate-borne sounds (Johnson et al., 2007; Pekas et al., 2024). Pestiferous arthropod species have been the primary focus, with successful detection of urban (Lemaster et al., 1997; Mankin et al., 2002), agricultural (Escola et al., 2020) and forestry (Sutin et al., 2019) pests by acoustic means, and study of acoustics as pest deterrents (Aflitto & Hofstetter, 2014). Thus, there is the potential to expand bioacoustic applications to ecological research, particularly for challenging systems to study, such as deadwood.

A growing area of bioacoustics research aims to identify patterns/relationships between acoustic diversity and traditional diversity measures based on physical measures of species richness and evenness within a community of interest. Studies using bioacoustics methods to answer ecological questions, such as species diversity, are often considered 'ecoacoustic' studies (Sueur & Farina, 2015). Choruses of animals can be used to estimate overall biodiversity of areas, which is especially helpful in studies examining effects of habitat changes and disturbance. Gómez et al. (2018) utilised machine learning techniques with success to differentiate between three successionally distinct habitats as well as three separate sites within the same successional forest type. Similarly, Sueur, Pavoine, et al. (2008) found that ecoacoustic indices had higher diversity measures in intact forests compared to degraded forests within the same region. Research in the field initially focused on avian diversity using airborne sounds, but these methods are increasingly applied to other systems (Bertucci et al., 2016; Buxton et al., 2018; Harris et al., 2016). Ecoacoustic methods using developed acoustic indices have been applied to arthropod systems (Aide et al., 2017; Maeder et al., 2019; Maeder et al., 2022; Obrist et al., 2010; Tomar et al., 2017), but they have not yet been applied to saproxylic arthropods.

Much of the ecoacoustic research estimating diversity has examined either aquatic or airborne soundscapes (Bertucci et al., 2016; Buxton et al., 2018; Gómez et al., 2018; Harris et al., 2016; Sueur, Aubin, & Simonis, 2008; Sueur, Pavoine, et al., 2008), with a few focusing on soil arthropods (Maeder et al., 2019, 2022). Microphones and hydrophones are readily available commercially to record airborne and waterborne sounds respectively. Substrate-borne vibrations require different and additional hardware for recording compared to airborne sound recordings, and while some commercially available products exist, low-cost/entry-level methods have not been tested for recording diversity recordings. For substrate recordings, the sensor

is typically built using a piezoelectric diaphragm (small discs designed to turn vibrations into electronic sound, hereafter referred to as 'piezo sensor') (Cocroft, 2005; Maeder et al., 2019, 2022). Soundproofing is needed to exclude airborne sounds from recordings, and a way to connect the sensor to the substrate is necessary, either as a waveguide that can be inserted into the substrate (Maeder et al., 2019, 2022) or a paste/adhesive that conducts vibrations (Cocroft, 2005). Analysis of ecoacoustic measures may also require unique analysis procedures, such as different recording lengths. If proper soundproofing can be achieved, saproxylic arthropods may provide an ideal study system for applying ecoacoustic indices since most acoustic challenges in current studies involve non-target noises (Buxton et al., 2018).

Here we conducted both laboratory and field trials to test ecoacoustic methodology and performance in deadwood soundscapes. By combining field and laboratory trials, we were able to test methodology in a variety of scenarios, obtaining a more robust understanding of potential applications of ecoacoustics in deadwood studies. Methodology tested included soundproofing and waveguide requirements, and overall performance compared ecoacoustic measurements to 'traditional diversity indices'—Shannon-Wiener index, Gini-Simpson's index and order richness.

We tested the following hypotheses and predictions with our laboratory trials:

1. Soundproofing materials would vary in their efficacy of dampening and excluding external noise, with the prediction that a wooden box would perform best as this is an effective and commonly used material in other studies (Branding et al., 2024).
2. Waveguides would vary in their ability to transfer sound. If no differences are found, this would indicate the waveguide could be chosen by ease of use in the field.

We tested the following hypotheses and predictions with our field trials:

1. A longer duration of 2 min, as opposed to the standard 1 min length established by Farina (2018) for ecoacoustic studies tailored for ornithological research, is predicted to offer a more comprehensive representation of diversity within wood ecosystems. This prediction stems from the assumption that arthropod sounds might possess lesser distinctiveness compared to bird calls. Thus, extending the recording duration to 2 min would allow for a greater opportunity to capture subtle variations and nuances present within the acoustic landscapes.
2. Ecoacoustic indices will correlate with direct measurements of saproxylic biodiversity in wood (i.e., species diversity and richness).

METHODS

Recording materials

We created all recordings, both in laboratory and field studies, using a Zoom F4 Field Recorder (Zoom North America, Hauppauge, NY) (Figure 1). Although pre-made sensors exist for substrate-borne sounds, they are expensive; however, similar and effective equipment can be constructed on a lower budget (Aflitto & Hofstetter, 2014; Harvey et al., 2011; Maeder et al., 2019). Ceramic piezo sensors are low-cost vibrational transducers. The diameter of the polarised disc sensor attached to the monitored vibrating surface affects the resonant frequency of the sensor (Buxi et al. 2014; Tsai et al. 2009). The sensor performs best below its resonant frequency. For all trials we used a 27 mm piezo (resonant frequency 4.6 kHz +/− 0.5 kHz; CUI Devices, Lake Oswego, OR), soldered onto a single conductor, shielded wire (24 AWG, Single Conductor Shielded Cable; Tensility International Corp, Redmond, OR), which was then soldered onto an RCA input connector. Due to cable length and setup logistics, we placed soundproofing materials over the shielded wire soldered onto the piezo (Figure 1). This should not have affected our recordings negatively in any way, and may have further aided soundproofing our recordings, as sometimes cables can pick up sound.

Laboratory experiments

Soundproofing

We tested the relative efficacy of various soundproofing materials in controlled laboratory experiments. Soundproofing was necessary to eliminate unwanted ambient airborne sounds because of the sensitivity of the sensors built for this study. While soundproofing for insect recordings, both air- and substrate-borne, has been well studied (Branding et al., 2024; Kadyrov et al., 2024; Mankin et al., 1997; Vick et al., 1988), the ideal methods determined from this research do not translate well to field environments across vast spatial extents. Therefore, a primary consideration for our study was suitability in the field. We determined materials needed to be easily implemented and portable in the field and needed to be widely available to guarantee access regardless of study location. Therefore, we tested four readily available materials in laboratory trials: cardboard, Styrofoam, wood and clay ceramic. To test the effectiveness of each material at dampening and excluding external noise, we cut a pine (*Pinus taeda* L.) log that had been in cold storage for several years into four equal pieces (approximately 11 cm in length and 19 cm in diameter). We confirmed that no live insects were present in the log sections through visual assessment of spectrograms in combination with auditory confirmation of recordings to control for unexpected noises that may occur when doing soundproofing trials. We then attached a single 27 mm piezo to one of the log sections and recorded a set of five tones representative of different airborne frequencies (600 Hz, 650 Hz, 700 Hz, 750 Hz and 800 Hz) that encompass much of the frequency range observed in baseline recordings taken of talking and music playing, which were used as proxies for possible disturbances in the field. We played these frequencies in progression (from low to high frequency for approximately 30 s each) from an online tone generator (Online Tone Generator, accessed at https://www.szynalski.com/tone-generator/) accessed and played through a laptop (MacBook Pro,

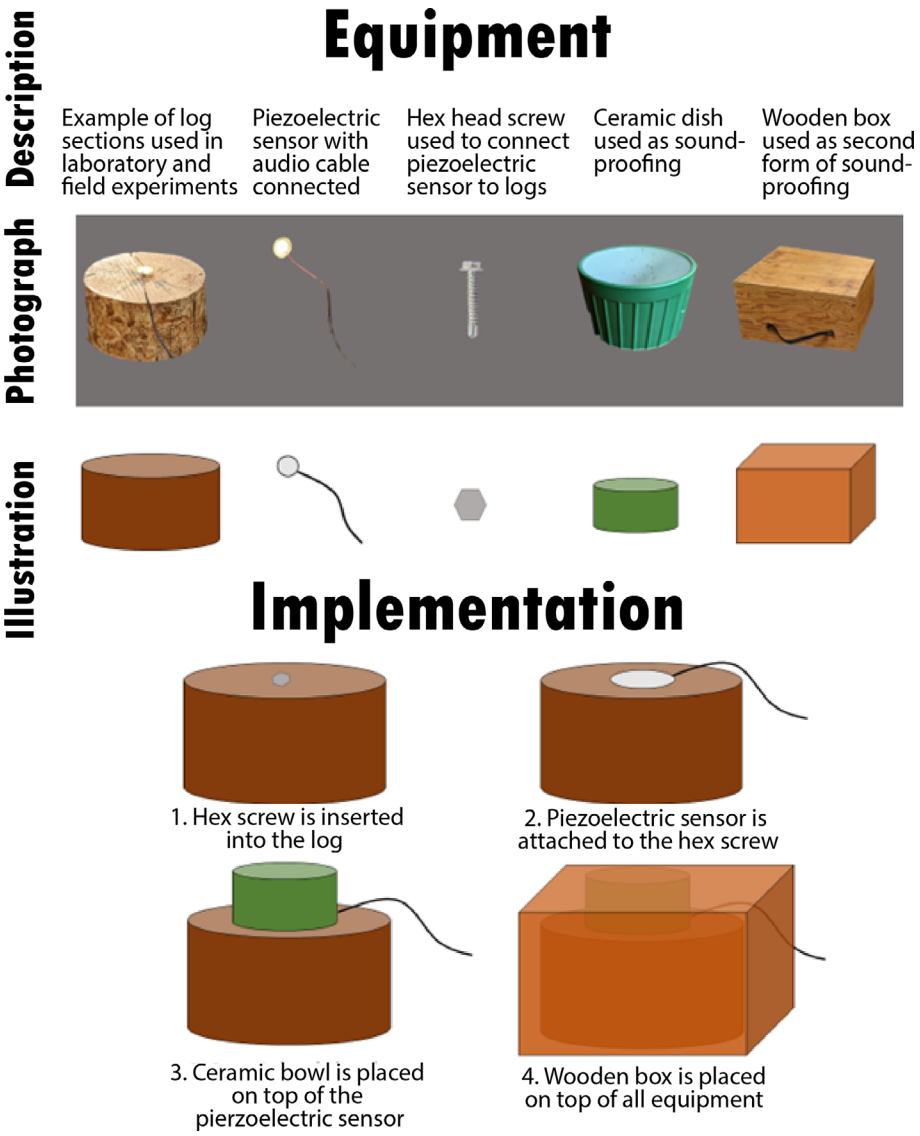

FIGURE 1 Process of applying soundproofing mechanisms in the field. First is a description of the equipment used (Description) that is associated with actual cropped images of the equipment (Photograph). Photographs are paired with a corresponding icon to represent the equipment (Illustration). These icons are then used in a pictorial schematic of the soundproofing methods in four steps (Implementation).

Retina, 15-inch, Mid-2015; Apple Inc., Cupertino, CA) that was placed on a separate laboratory bench at equal height 1.86 m away to reduce vibrations moving across the bench. The volume of the laptop and gain of the recorder remained constant throughout soundproofing trials, at 75% and 50%, respectively. The frequency of the tone was tested for accuracy by ensuring the recording unit registered the anticipated frequency (i.e., the recording had activity at 600 Hz when that frequency was selected on the tone generation website). Each soundproofing material (uncovered control, cardboard, Styrofoam, wood, and wood with a clay ceramic dish) was tested on each log section for a total of four trials per soundproofing material. We compared the effectiveness of each soundproofing mechanism across recordings by analysing the average power in decibels of a 30-s segment of each individual frequency recorded (i.e., not averaged across samples) (Supplementary Figures S1 and S2). We calculated average power (dB) with default settings in Raven Pro 1.4 (Bioacoustics Research Program, 2003), which uses a reference value of 1 for decibel comparisons.

Waveguide

In studies focusing on wood decay, logs exhibit diverse stages of decomposition, necessitating different levels of force for waveguide insertion. We tested four common materials as waveguides that could be relatively easily procured in remote sites and would allow for insertion into deadwood in both solid state (hard deadwood) and decayed state (soft deadwood): metal screws (Midwest Fastener 03284 screw, zinc plated, hex head, size #8 self-drilling screw, 2.5 cm in length;

Midwest Fastener Corp., Portage, MI), metal tubing (12.7 mm outer diameter, 9.5 mm inner diameter; unknown alloy and manufacturer), plastic tubing (19.1 mm HI-TEMP CPVC Pipe; Genova Products Inc., Davison, MI) and wooden dowel rods (9.5 mm diameter oak dowel; Madison Mill Inc., Ashland City, TN). We conducted trials by playing a known sound (*Dendroctonus barberi* Hopkins stridulation; Supplementary Figure S3) through the same four log sections used for the soundproofing trials. We played the sound through the wood using an audio player and exciter (Lukic et al., 2021) attached to the opposite cross-sectional face of the log as the waveguides being tested (Supplementary Figure S4). We inserted each waveguide material approximately 1 cm into the log sections (Supp Figure 4). The bark beetle stridulation was played on a 10-s loop and was recorded for a full loop for each trial and the average decibel of each recording was measured at a standardised location of the recording.

Field experiment

Field characteristics

We conducted field experiments at three field sites: one each in Honduras, Arizona, USA, and Mississippi, USA (Clay et al., 2024). Using these three sites allowed us to test hypotheses across diverse wood, invertebrate and climatic conditions. The site in Honduras was on the school forest of Universidad Nacional de Ciencias Forestales in Siguatepeque, Honduras (14°33′46.8″ N 87°48′54.0″ W). The stand consisted of yellow pine (*Pinus oocarpa* Schiede ex Schltdl.), of approximately 39 years in age, a basal area of 9 m^2/ha, and an average diameter at breast height (DBH) of 23 cm. The site in Arizona was in Centennial Forest, located about 10 km west of Flagstaff (35°09′39.6″ N 111°45′36.0″ W). The stand was approximately 90% ponderosa pine (*Pinus ponderosa* P. Lawson and C. Lawson) and 10% gambel oak (*Quercus gambelii* Nutt.), with a basal area of 31 m^2/ha, an average DBH of 27 cm, and an average stand age of 98 years. The Mississippi site was located at the Mississippi State University McNeill Research Unit in McNeill, MS (30°38′45.6″ N 89°38′27.6″ W). This site was composed of 90% loblolly pine (*Pinus taeda* L.) approximately 30 years in age with an approximate basal area of 43 m^2/ha, average DBH of 52 cm, and 10% mixed hardwoods. In 2017, two conspecific trees of similar DBH were harvested for use in this study and others evaluating wood decomposition. Harvested trees were further processed into cross-sections of approximately 10 cm in length, hereafter logs, which were deployed in a completely randomised grid (10 × 12 grid, of which half of the logs were randomly sampled the year prior to this study) at each site. Each site contained 60 logs at the time of ecoacoustic sampling, totaling 180 logs across all sites.

Ecoacoustics

The protocol for our field recordings was as follows: First, while in the field, we inserted a screw (Midwest Fastener 03284 screw, zinc plated, hex head, size #8 self-drilling screw, 2.5 cm in length; Midwest Fastener Corp., Portage, MI) into logs (approximately 2.5 cm in depth) as a waveguide and a ceramic piezo sensor was attached to the screw with a magnet. The magnets (Neodymium Magnets, D6H1; K&J Magnetics, Inc., Pipersville, PA) used for attachment were glued to the non-ceramic side of the sensor, to not disturb the active portion of the elements. Magnetic attachment methods for recording vibrations of wood-boring arthropods have been successful in other studies (Creemers, 2016) and allowed for screws to be inserted into the log before attachment to the piezo element and for quick attachment and removal of the sensors. This was important for our study because we had a relatively high number of recordings to take in a day.

Second, we placed a clay ceramic dish (10.2 cm in diameter, 6.3 cm in height; IQ Accessories, Inc., Minnetonka, MN) on top of the piezo and a wooden box over the log and the recording unit (Figure 1). A combination of a wooden box and clay ceramic dish was chosen for the soundproofing (Figure 1) because of ease of application/resource availability at field locations and anecdotal evidence of testing previously mentioned soundproofing methods in field environments. We conducted field trials prior to the previously mentioned laboratory trials, which is why the results (see below) were not considered in choosing these materials.

Finally, we took an acoustic recording of 5 min to allow for comparison of 1-min and 2-min analysis lengths (see next section). We chose an in-field recording length of 5 min to ensure that audio clips free of non-target noises could be selected from the middle of the recordings. An additional factor contributing to the long length of recording time was that the recording unit used had manual controls on the unit, so the recording had to be started before the soundproofing mechanisms were placed on top of the log. This resulted in a high amount of non-target background noises in the beginning of the recordings that reflected the time and associated sounds of the clay ceramic dish and box being put into place, the acoustician settling into a comfortable position and the vegetation surrounding the log settling after being mechanically disturbed. Thus, 5-min recording times ensured two unobstructed minutes of recording after initial setup and provided additional recording time to select from in case of unforeseeable noisy events during recording. Acoustic recording at each site took place over two consecutive days, recording 30 logs per day. Recordings began between approximately 8:00 and 9:00 AM local time at each site.

In the field recordings, we used a gain setting ranging between 50% and 75%. Initially, we selected a standard gain of 50%. However, in regions such as Honduras and Arizona, we elevated the gain to 75% to adequately capture the relatively subdued arthropod activity compared to the first sampled site (Mississippi). At all sites we used a recording frequency of 48 kHz, with a 24-bit WAV depth and a 320 kbps MP3 bit rate.

We used five ecoacoustic indices: Acoustic Complexity Index (ACI), Roughness, Entropy-Shannon's, Entropy-Simpson's and Spectral Peak Count (Table 1). We chose these indices based on their performance in previous ecoacoustic studies (Bertucci et al., 2016; Buxton et al., 2018; Harris et al., 2016; Maeder et al., 2019; Papin

TABLE 1 Description of ecoacoustic indices and how diversity is estimated for each.

Ecoacoustic index	Description
Acoustic Complexity Index (ACI)	Estimates diversity by binning acoustic recordings and comparing adjacent bins based on the acoustic intensity present (Pieretti et al., 2011).
Roughness	Estimates diversity by calculating the amount of change from 1 s to the next in terms of sound pressure level (Buxton et al., 2018).
Entropy—Shannon's	Estimates diversity based on changes in amplitude of the recorded sounds over time and the evenness of the distribution of those amplitude changes. Targeted to mimic Shannon-Wiener's diversity index (Sueur, Aubin, & Simonis, 2008; Sueur, Pavoine, et al., 2008).
Entropy—Simpson's	Estimates diversity based on changes in amplitude of the recorded sounds over time and the evenness of the distribution of those amplitude changes. Targeted to mimic Gini-Simpson's diversity index (Sueur, Aubin, & Simonis, 2008; Sueur, Pavoine, et al., 2008).
Spectral Peak Count	Estimates diversity based on the number of spectral peaks present in the recording (Gasc et al., 2013).

et al., 2019). We calculated ACI using a 5-s bin length, as this is an appropriate bin size used in other ecoacoustic studies (Maeder et al., 2019; Pieretti et al., 2011). Roughness is an index defined in Buxton et al. (2018), in which the sound pressure level of adjacent time segments is compared, the difference is summed and the median of these summed differences across observed frequencies is reported as the roughness index value. We calculated all other acoustic indices using the default settings in *seewave*, the sound analysis package in R used to calculate indices (Sueur, Aubin, & Simonis, 2008; Sueur, Pavoine, et al., 2008).

Determining best sound recording time

Since our methods may rely on subtle sounds such as arthropods walking and chewing within substrate, we analysed both a 1-min and a 2-min segment to test for potential benefits of extending the suggested 1-min recording length. For each recording, we deleted the first minute to eliminate the high amounts of noise created from set up (as described above). From this edited file, we created the two files used for analysis. The 1-min segment was the minute following the deleted first minute, and the 2-min segment was the 2 min following the deleted first minute (Figure 2). We did not experience interference within the 1- and 2-min windows in any recordings and no edits other than these described segmentations took place. We calculated the ecoacoustic indices, using methods mentioned in the previous section, for both 1- and 2-min recording lengths and analysed for statistically significant differences based on length of recording (see statistical analyses section below). We carried out comparisons between ecoacoustic and traditional diversity indices (see next section) using both 1- and 2-min recording lengths when significant differences were found between recording lengths, but when no significant difference was found the field standard of 1 min was used. We used only 1-min lengths for cumulative indices.

Arthropod collection for traditional biodiversity

After we obtained acoustic recordings from the field, we removed logs from the field site to extract arthropods. We hung each log in a cloth Berlese funnel with a 40-watt light bulb at the top for 5 days. The Berlese funnels had Whirl-Paks (Uline, Pleasant Prairie, WI) containing 70% ethyl alcohol attached at the bottom to preserve arthropods as they fell from the log. This method is commonly used for soil samples and later decay-class wood (Ferro et al., 2012; Owens & Carlton, 2015) and was used as an expedited alternative to the traditionally months-long process of extracting arthropods from wood, because our time at field locations in Honduras and Arizona was limited.

We sorted arthropods obtained from Berlese funnels taxonomically to order. Exceptions included subclass Acari, phylum Myriapoda, and insect larvae. We sorted Acari into functional groups of Oribatids (fungivores and scavengers) and non-Oribatids (largely predacious mites) (Kethley, 1990; Krantz & Ainscough, 1990; Norton, 1990; Philips, 1990). We sorted Myriapoda into class. We included insect nymphs in counts for orders because of their reliably identifiable features, but excluded insect larvae because of the overlap of larvae characteristics across insect orders. We keyed insects following Triplehorn and Johnson (2005) and non-insect arthropods as per Dindal (1990).

We calculated the Shannon-Wiener index, Gini-Simpson's index and richness at the order level, with mites included by functional group, Myriapoda included by class and excluded insect larvae. We conducted calculations using the *Vegan* package in R (Oksanen et al., 2022).

Statistical analyses

For all statistical analyses, we used an α of 0.05. Unless otherwise specified, all analyses were completed using the statistical computing software R, version 4.3.1, using the R Studio interface (Posit team, 2023; R Core Team, 2017). We edited audio files in the ecoacoustics software, Raven Pro (Bioacoustics Research Program, 2003). We performed all acoustic analyses in R version 4.0.3 (R Core Team, 2017). We used the sound analysis package, *seewave* (Sueur, Aubin, & Simonis, 2008; Sueur, Pavoine, et al., 2008), to calculate ecoacoustic indices.

We tested for statistically significant effects of soundproofing using a 1-way ANOVA with type II sum of squares to test for any interaction between the frequency played and soundproofing.

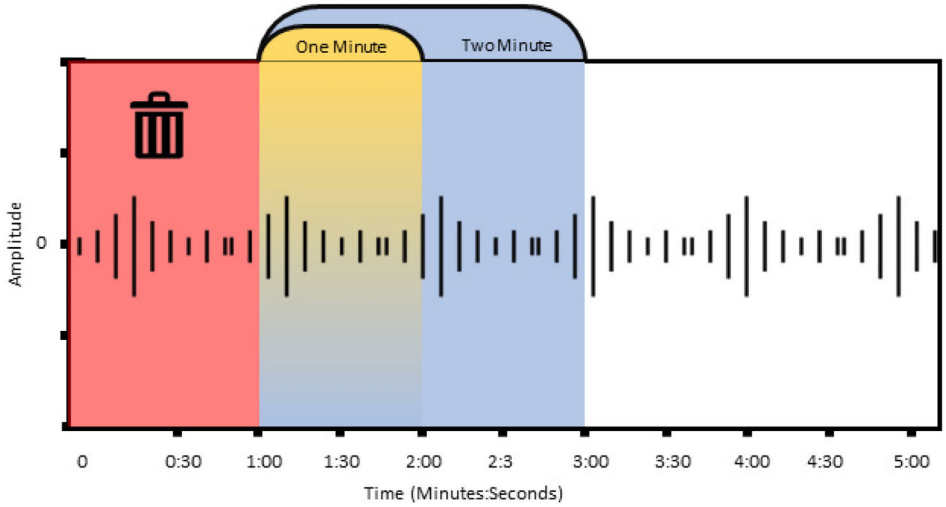

FIGURE 2 Editing process for selection of 1- and 2-min segments for audio analysis. The first 1 min (red with trash can symbol) was not used in analysis due to non-target sounds from set up and initial disturbance of log. The following 1 min (yellow) and 2 min (blue) were then examined for any non-target sound/noise interference. If none were detected these were used for analysis of ecoacoustics and traditional biodiversity measures. The remaining 2 min (white) provided recorded sound time available for use if the audio recordings in the first 3 min were not usable for any given reason.

Average dB was the dependent variable, with soundproofing material as the independent variable. All test assumptions were met.

Data from our waveguide trials did not meet normality assumptions, so we used a Kruskal-Wallis test to accommodate the non-normal distribution of measured values. Average dB was the dependent variable, with waveguide material as the independent variable.

To analyse for differences in ecoacoustic value depending on the length of recording time (1 min compared to 2 min), we used a 1-way ANOVA for three of the five ecoacoustic indices (Roughness, Entropy—Shannon's, and Entropy—Simpson's). When necessary, we transformed ecoacoustic indices to better adhere to the ANOVA assumption of normally distributed residuals. When assumptions could not be met with transformations, we used the non-parametric Kruskal-Wallis test. We log transformed roughness prior to the ANOVA test, and we tested both Shannon's and Simpson's entropy with the non-parametric Kruskal-Wallis test. We did not conduct ANOVA tests for ACI or Spectral Peak Count because these measurements are cumulative and thus should be expected to significantly differ with additional recording time.

We evaluated whether ecoacoustic measurements of biodiversity were related to physical measurements of biodiversity using linear mixed-effects models. We fitted linear mixed-effects models for each combination of ecoacoustic and traditional index (e.g., three linear mixed-effects models for the ecoacoustic measurement of ACI: one with the Shannon-Wiener index, one with Gini-Simpson's index and one with order richness) with the traditional index as a predictor and the ecoacoustic index as the response variable (Figure 3). We planned to fit site as a random effect in all models if there was a significant difference in any of the traditional or ecoacoustic variables between sites, which we tested with 1-way ANOVAs and Tukey multiple pairwise comparisons if significance was found through ANOVAs. Our initial models only included traditional biodiversity indices and ecoacoustic biodiversity indices, but upon graphical inspection of results, a handful of samples were identified as outliers that may have been driving statistical relationships. All identified outliers had relatively high quantities (>1000) of either termites or ants. In addition to being highly abundant, insects in these orders (Blattodea and Hymenoptera) are known to be relatively noisy, which could explain their influence as outliers (Bagheri et al., 2024; Hager & Kirchner, 2013). To address these outliers and explore the impact of these insects on ecoacoustic measures, we created several additional models per combination of traditional and ecoacoustic variables: we first added ant and termite abundance as predictors to the initial model with all samples included; we then created models with the dataset trimmed to four different levels of termite and ant abundance (samples trimmed to only include those with <1000, <100, <50, and 0 termites and/or ants). In this way, we tested whether including these various levels of analysis could describe the influence of dominant saproxylic taxa on these models, which would be important for researchers wanting to apply ecoacoustic methods to research in deadwood. We created these linear mixed-effects models using the *lme4* and *lmerTest* packages in R (Bates et al., 2015; Kuznetsova et al., 2017).

RESULTS

Laboratory experiments

There was no significant difference in average decibels recorded between any soundproofing methods (cardboard, Styrofoam, wood,

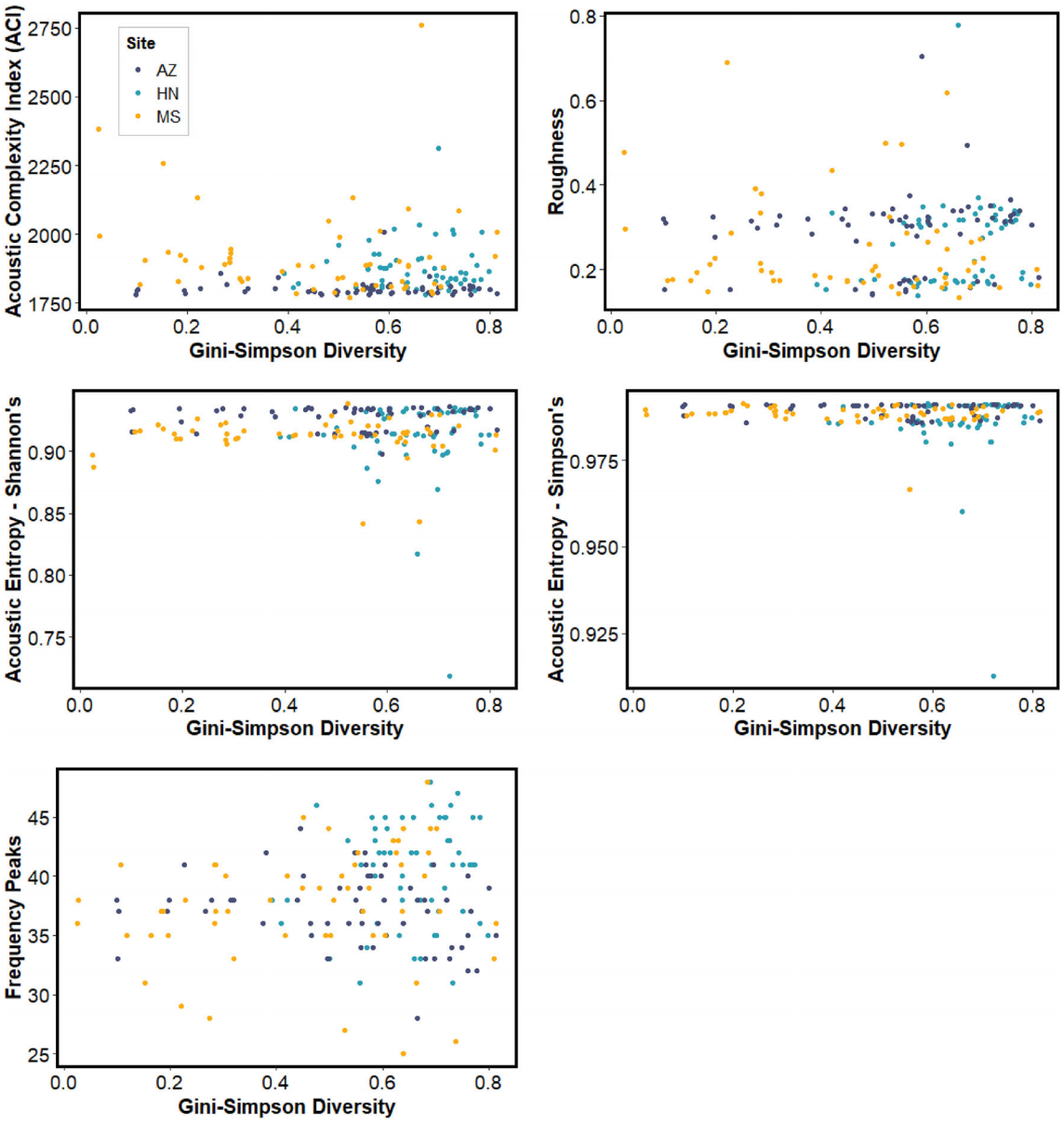

FIGURE 3 Visualisation of measured traditional (x axis) and ecoacoustic (y axis) biodiversity measures. These represent all calculations for all sites ($n = 3$) and have not been thinned. None of the predictors (i.e., the traditional diversity indices) for the five initial linear mixed-effects models were significant (Table 2).

and clay ceramic), including our control with no soundproofing ($F_{4,90} = 1.78$; $p = 0.14$), as determined by our ANOVA with type II sum of squares. In fact, control treatments and cardboard had the lowest mean average decibels (129 ± 10.2 and 129 ± 10.0, respectively), followed by the wooden box with the addition of the clay ceramic dish (130 ± 11.5), the wooden box (131 ± 10.5) and finally the Styrofoam box (134 ± 10.7). Additionally, there was no significant interaction between frequency and soundproofing ($F_{4,90} = 0.25$; $p = 0.91$).

Metal tubing and wooden dowels recorded the highest mean average decibel across laboratory trials (104 ± 3.2 and 101 ± 6.7 respectively). Plastic tubing had slightly greater overall means (100 ± 16.8) than metal screws tested (99 ± 2.5). However, there was no significant difference in waveguide performance based on average decibels (Kruskal-Wallis χ^2-squared = 3.38; df = 3; $p = 0.34$).

Field experiments

Ecoacoustics

We collected a total of 173 samples, comprising 60 samples from our Arizona field site, 58 from Honduras and 55 from Mississippi. All 173 samples were initially incorporated into the model for analysis before we removed samples for subsequent analyses focused on different thresholds of ant and termite abundance (<1000, <100, <50,

TABLE 2 Initial linear mixed effects models and associated test statistics grouped by the traditional diversity index fit as the response variable for the model. Models presented in this table were performed with the untrimmed dataset.

Ecoacoustic index	Predictors	Estimate	SE	*t*-value	*p*
ACI					
(a)	Gini-Simpson's diversity index	−39.909	48.989	−0.815	0.42
(b)	Shannon-Wiener index	−17.775	23.648	−0.752	0.45
(c)	Order richness	7.913	4.022	1.968	0.0533
Roughness					
(a)	Gini-Simpson's diversity	0.002	0.046	0.033	0.97
(b)	Shannon-Wiener index	0.002	0.022	0.072	0.94
(c)	Order richness	−0.004	0.003	−1.489	0.14
Entropy—Shannon's					
(a)	Gini-Simpson's diversity	−0.002	0.010	−0.207	0.84
(b)	Shannon-Wiener index	0.001	0.005	0.2	0.84
(c)	Order richness	0.000	0.001	−0.31	0.76
Entropy—Simpson's					
(a)	Gini-Simpson's diversity	−0.002	0.003	−0.691	0.49
(b)	Shannon-Wiener index	−0.001	0.001	−0.388	0.70
(c)	Order richness	0.000	0.000	−0.285	0.78
Frequency Peaks					
(a)	Gini-Simpson's diversity	1.478	1.861	0.794	0.43
(b)	Shannon-Wiener index	0.598	0.898	0.666	0.51
(c)	Order richness	−0.154	0.158	−0.97	0.33

and = 0 ants/termites present). Most of the samples removed from the dataset for subsequent models were from Mississippi (22 removed for <1000, 39 removed for <100, 40 for <50 and 51 for =0), but samples were trimmed for each model for Honduras as well (1 removed for <1000, 7 for <100, 16 for <50 and 56 for =0). Arizona sites were only removed in the highest level of trim, and only five samples were removed (5 removed for =0). The total number of samples included for subsequent trimmed models was 150 (<1000), 126 (<100), 116 (<50) and 60 (=0).

Noise attributed to electronic interference was recorded at ~8 kHz frequency across all recordings (Figure 4). This was above the range of recorded arthropod sounds (mostly > ~7 kHz), so this did not impact the frequency range of interest of these recordings. Sounds acoustically similar to ant stridulation (Masoni et al. 2021) and other unidentified arthropod vibrational activity were recorded (Figure 4).

Determining best sound recording time

No significant difference was found between 1- and 2-min recording lengths for Roughness (F value = 0.05; df = 1, 298; $p = 0.82$), Shannon's entropy (Kruskal-Wallis χ^2-square = 0.0005; df = 1; $p = 0.98$), or Simpson's entropy (Kruskal-Wallis χ^2-square <0.0001; df = 1; $p > 0.99$). Since there was no significant difference between the two recording lengths, the field-standard 1-min length was used for all further statistical tests.

Arthropod collection for traditional biodiversity

All 173 samples previously mentioned were used for analysis of arthropod abundance and diversity (Figure 3). Across the 173 samples, we collected a total of 121,395 arthropods from 24 orders. Mississippi had the highest average abundance of arthropods out of the three sites ($p < 0.001$ for both Arizona and Honduras pairwise comparison), accounting for a total of 72.92% of all arthropods collected, followed by Honduras and then Arizona, which were also significantly lower in terms of average abundance and accounted for 24.25% and 2.83%, respectively ($p = 0.0375$).

Significant variation was found for Shannon-Wiener diversity index, Gini-Simpson's diversity index and richness by site (all $p < 0.0001$). Arizona and Mississippi were not significantly different in Shannon-Wiener diversity ($p = 0.39$), but all other comparisons were significantly different. Samples from Honduras were significantly higher in biodiversity measures of order richness and Shannon-Wiener diversity but were the least diverse by measure of Gini-Simpson's diversity.

Order richness was marginally significant ($p = 0.053$) as a predictor of ACI but was not a significant predictor for other ecoacoustic indices (Table 2). Gini-Simpson's diversity and Shannon-Wiener indices were not significant predictors for the five initial linear mixed-effects models (Table 2). However, when Hymenoptera and Blattodea abundance were added to the models, these two measures were significant predictors in the models (Figure 5, Table 3, Supplementary

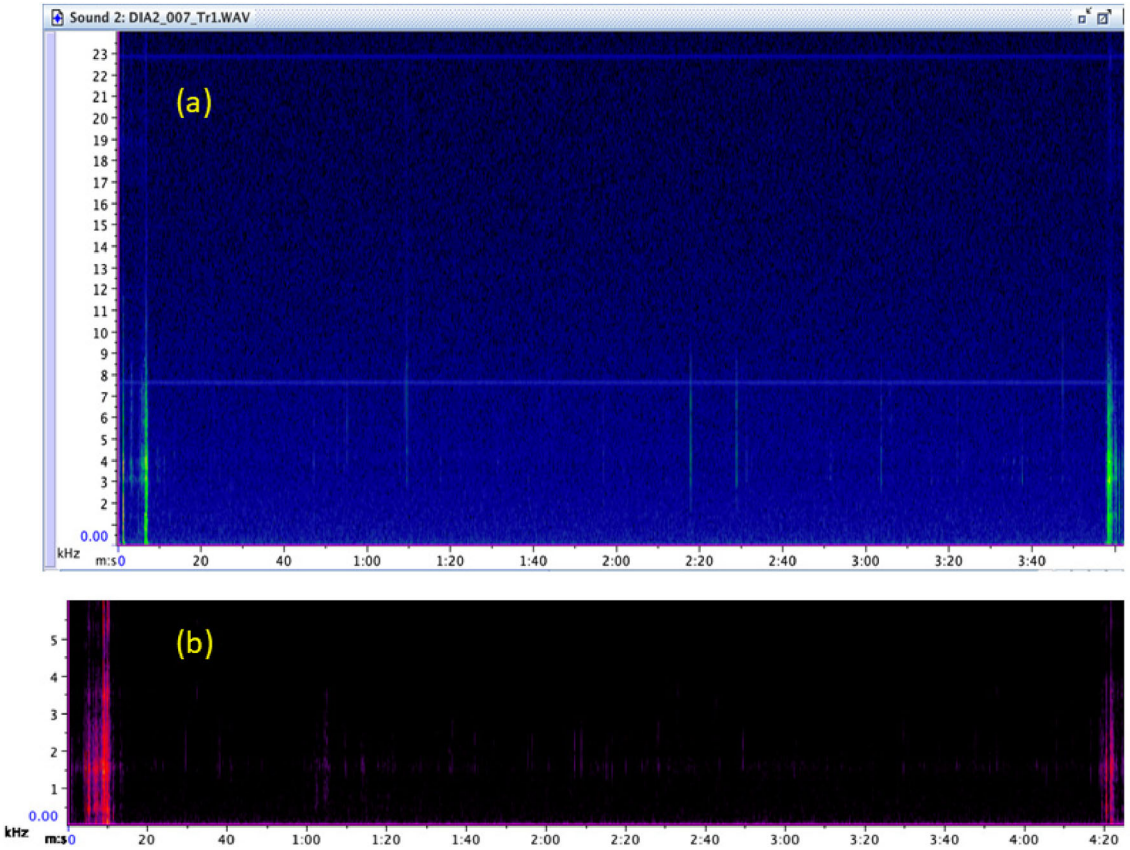

FIGURE 4 Examples of spectrograms from field recordings of deadwood arthropods. Full ranges of frequencies (a) were recorded and target arthropod sounds were found primarily in lower frequencies than sound generated from the electronic noise associated with the recorder (Harvey et al., 2011; Kadyrov et al., 2024; Masoni et al. 2021). Frequencies below 8 kHz (b) were analysed to: (1) best represent the common frequencies of arthropod vibrations and (2) eliminate the electronic noise at 8 kHz associated with the recorder.

Table S1, Figures S5–S18). Out of the 60 linear mixed-effects models that included ant and termite abundance, ant abundance was a significant predictor in 22, or 37% of, models. Ant abundance had a negative effect on ecoacoustic diversity in 13 of the 22 models (Supplementary Table S1), and all models in which ant abundance had a positive effect ($n = 9$), were fit with ACI as the ecoacoustic index. The only ecoacoustic index that did not have any models in which ant abundance was a significant predictor was Frequency Peaks. Out of those 60 linear mixed-effects models including abundance, termite abundance was significant in 52% ($n = 31$) of models. Of those 31 models, termite abundance had a positive effect on models using ACI and Roughness ($n = 13$), and all other effects were negative ($n = 18$). There were 16 instances where both ant and termite abundance were significant predictors in models.

DISCUSSION

Non-destructive methods of measuring biodiversity are increasingly important as climate change and anthropogenic activities alter habitats. Ecoacoustics represent not only a non-destructive sampling method but also potential means to sample historically challenging habitats like deadwood, which is opaque, occupied by thousands of tiny organisms and difficult to sample, particularly for all species at the same time (e.g., beetles can be optimally extracted through rearing, but this method does not allow for sampling of smaller shorter-lived taxa like collembola) (Hammond, 1997; Wikars et al., 2005). Here, we expand the application of ecoacoustics or non-destructive biodiversity sampling and demonstrate its utility and efficacy in deadwood (Buxton et al., 2018; Maeder et al., 2019; Pieretti et al., 2011). We demonstrated that commonly sourced materials all performed equally as soundproofing methods, with none of them excelling. Additionally, we found that deadwood ecoacoustic measures are best predicted by the abundance of Hymenoptera and Blattodea, specifically Formicidae and Termitidae respectively. Surprisingly, traditional overall deadwood community diversity measures were not good predictors of ecoacoustic diversity measures. Together, this may suggest that ants and termites are primary agents driving deadwood soundscape characteristics and ecoacoustic use in deadwood may be particularly well-suited to examining ant and termite ecology.

Our study demonstrated that each of the readily available soundproofing materials we tested performed similarly, and, in fact, there was no significant difference between our control and soundproofing methods. Thus, while acoustic interferences are a common issue with

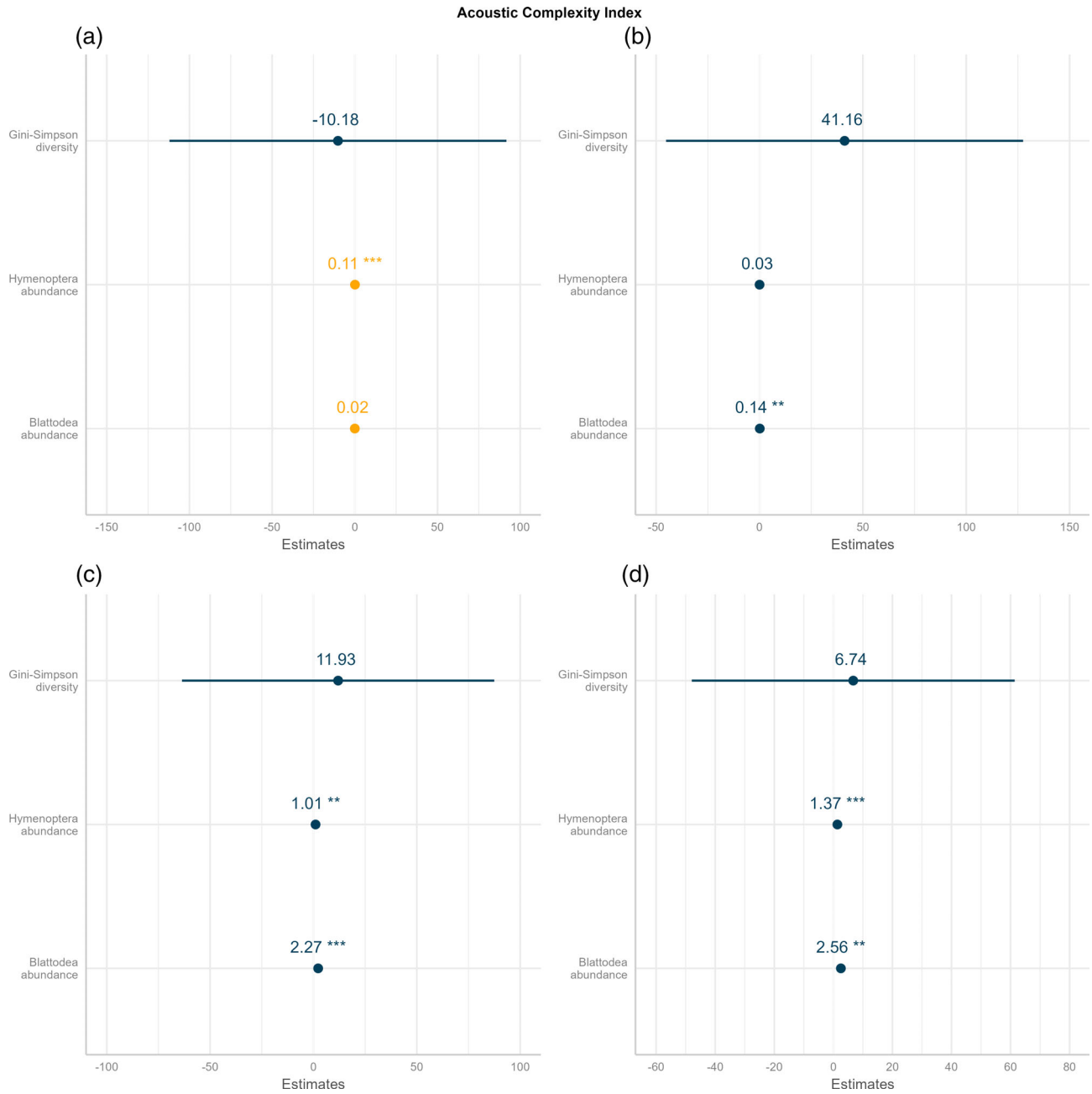

FIGURE 5 Results from linear mixed-effects models created to predict Acoustic Complexity Index values (obtained via recording deadwood samples) from Gini-Simpson diversity, Hymenoptera abundance and Blattodea abundance (obtained by rearing arthropods out of the same deadwood samples). Four models including Hymenoptera and Blattodea abundance data are shown, each modelled at four different cut-off/trim levels: No trim (a), samples with >1000 Hymenoptera/Blattodea trimmed (b), samples with >100 Hymenoptera/Blattodea trimmed (c), and samples with >50 Hymenoptera/Blattodea trimmed. The point associated with each predictor is the effect size for the predictor in that model, with the number representing the exact value. Bars on either side of the point represent the confidence interval for the predictor. Positive values represent a positive relationship between the predictor and Acoustic Complexity Index, with negative values representing negative relationships. Asterisks represent statistical significance of predictors in the represented model (*: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$). When both negative and positive predictors are present (a), the colour of effect size is different between positive and negative predictors.

ecoacoustic studies (Buxton et al., 2018), and despite our anecdotal experiences with materials in the field prior to sampling, our results suggest that the application of soundproofing for ecoacoustic measurements in deadwood may not be necessary. However, these trials were limited to several static pitches, and differences in soundproofing materials relative to no soundproofing material may occur with natural and more variable sounds that are often encountered in field settings (i.e., bird song, human voices and footsteps). Future research should explore alternative noise control methods, such as recording the background/ambient noise in the area to then decouple from the target recording in post-

TABLE 3 Linear mixed effects models and associated test statistics grouped by the traditional diversity index fit as the response variable for the model.

Ecoacoustic index	Predictors	Estimate	SE	*t*-value	*p*
ACI					
(a)	Gini-Simpson's diversity index	−10.183	51.597	−0.197	0.84
	Hymenoptera	0.107	0.026	4.156	0.0001
	Blattodea	0.015	0.010	1.570	0.12
(b)	Shannon-Wiener index	−2.123	24.790	−0.086	0.93
	Hymenoptera	0.107	0.026	4.154	0.0001
	Blattodea	0.016	0.010	1.625	0.11
(c)	Order richness	5.303	3.688	1.438	0.17
	Hymenoptera	0.101	0.026	3.826	0.0002
	Blattodea	0.017	0.009	1.896	0.06
Roughness					
(a)	Gini-Simpson's diversity	0.016	0.052	0.305	0.76
	Hymenoptera	9.752E-07	2.551E-05	0.038	0.97
	Blattodea	6.460E-06	9.950E-06	0.649	0.52
(b)	Shannon-Wiener index	9.313E-03	0.025	0.375	0.71
	Hymenoptera	1.469E-06	2.557E-05	0.057	0.95
	Blattodea	6.862E-06	9.878E-06	0.695	0.49
(c)	Order richness	−0.004	0.003	−1.298	0.29
	Hymenoptera	3.815E-06	2.560E-05	0.149	0.88
	Blattodea	4.112E-06	8.659E-06	0.475	0.64
Entropy—Shannon's					
(a)	Gini-Simpson's diversity	−0.002	0.011	−0.222	0.82
	Hymenoptera	−1.516E-05	5.313E-06	−2.854	0.01
	Blattodea	−2.341E-07	2.039E-06	−0.155	0.91
(b)	Shannon-Wiener index	0.001	0.005	0.220	0.83
	Hymenoptera	−1.514E-05	5.320E-06	−2.846	0.01
	Blattodea	1.615E-07	2.031E-06	0.080	0.94
(c)	Order richness	4.332E-04	0.001	0.524	0.60
	Hymenoptera	−1.576E-05	5.536E-06	−2.847	0.01
	Blattodea	−3.036E-08	1.846E-06	−0.016	0.99
Entropy—Simpson's					
(a)	Gini-Simpson's diversity	−0.002	0.003	−0.475	0.64
	Hymenoptera	−1.723E-06	1.634E-06	−1.055	0.30
	Blattodea	1.956E-07	6.305E-07	0.310	0.76
(b)	Shannon-Wiener index	−1.993E-04	0.002	−0.126	0.90
	Hymenoptera	−1.729E-06	1.638E-06	−1.055	0.29
	Blattodea	2.906E-07	6.278E-07	0.463	0.64
(c)	Order richness	3.948E-05	2.466E-04	0.160	0.87
	Hymenoptera	−1.817E-06	1.707E-06	−1.064	0.29
	Blattodea	3.132E-07	5.703E-07	0.549	0.58
Frequency Peaks					
(a)	Gini-Simpson's diversity	0.950	2.069	0.459	0.65
	Hymenoptera	−2.267E-04	0.001	−0.219	0.83
	Blattodea	−2.373E-04	3.950E-04	−0.601	0.55
(b)	Shannon-Wiener index	0.320	0.995	0.321	0.75

TABLE 3 (Continued)

Ecoacoustic index	Predictors	Estimate	SE	*t*-value	*p*
	Hymenoptera	−2.232E-04	0.001	−0.216	0.83
	Blattodea	−2.615E-04	3.937E-04	−0.664	0.51
(c)	Order richness	−0.151	0.164	−0.920	0.36
	Hymenoptera	5.796E-05	0.001	0.054	0.96
	Blattodea	−2.960E-04	3.574E-04	−0.828	0.41

Note: Models presented in this table were performed with the untrimmed dataset and included the abundance of orders Hymenoptera and Blattodea as predictors.

processing (Lamont et al., 2022). Moreover, we found no significant differences in waveguide performance; thus, researchers can select from these waveguide materials as needed based on ease of application in their study system. If study material is pliable/soft enough, waveguides could be permanently attached, or even soldered, to piezo which may improve the longevity of constructed piezo. Of note, over the course of our research several piezo sensors lost functionality due to wear and tear of connecting the piezo via magnet. As such, we recommend researchers bring multiple piezo sensors, especially to remote field sites, where new piezo sensors may be unattainable.

While the methods employed in this study worked well, future studies could continue to assess methodology to further develop methods that translate well into remote field sites with limited accessibility, like those used here. While our sensor building was budget-focused, an important consideration in the future is the longevity of sensors to reduce replacement in the field. This could include creating a casing to better protect the piezo sensor from wire movement and potential breaking. If this is done, each sensor could be compared to commercial accelerometers in a controlled environment to ensure the sensitivity of sensors is on par with market standards. Additionally, future research could assess potential impacts of the soundproofing materials on recordings by assessing the attenuation of signals across observed arthropod sounds (Branding et al., 2024). Impacts of soil textures and moisture content on target frequency attenuation (Lu & Sabatier, 2009) could also be considered if an open-bottom soundproofing method is used as it was in this project.

Interestingly, we found significant relationships between ecoacoustic indices and abundant taxa. In particular, Hymenoptera and Blattodea abundance had a significant effect on approximately 62% of models when included at various trim levels (not trimmed, <1000, <100, <50) to account for potential outliers. In models with full abundance included, ants (Hymenoptera) had significant positive effects on models created with ACI and significant negative effects on models created with Entropy—Shannon's. At the first trim level (<1000), termites (Blattodea) became the significant predictor in models. Models fit predicting ACI had significant positive effects from termites, whereas models fit to predict frequency peaks had significant negative effects from termites. More intensive trim levels (<100 and < 50) were more variable with patterns of significance, but all ecoacoustic indices modelled had some significance with ant or termite abundance (Supplementary Table S1, Figure S5). Although there are many reasons why different trends of significance for ants and termites exist in these models, one hypothesis that could explain these trends is the functionality of acoustic communication of the two orders. Ants are well-known to communicate using sound; this includes drumming and stridulation (Bagheri et al., 2024; Hill, 2009; Schönrogge et al., 2017). Inquilines, myrmecophiles, and nest parasites often must also mimic the sound production of ants to avoid detection, highlighting the ample use of sound by ants (Schönrogge et al., 2017). While termites also exhibit vibrational and drumming behaviour (Hager et al., 2019), it is often used as an alarm response (Delattre et al., 2019). This may explain why ant abundance but not termite abundance showed significant relationships with ecoacoustic measurements: if termites primarily produce sound in response to threats, while ants use sound more consistently and for a wider diversity of functions like nest-mate recruitment and species and caste identification (Schönrogge et al., 2017 and references therein), ant activity and diversity may generate complex soundscapes, although this remains to be tested.

Surprisingly, this study did not find significant relationships between traditional biodiversity indices and ecoacoustic indices. One potential hypothesis for the low relationship between the traditional biodiversity indices and the ecoacoustic indices is that because sound often transmits farther in solids, or may be more easily detected, organisms may remain 'quieter' as a potential predator avoidance mechanism (Hill, 2009). Alternatively, a number of methodological caveats may have also contributed to the lack of relationship between traditional and ecoacoustic biodiversity indices. For example, manipulation and disturbance of the deadwood prior to recording (inserting waveguide, walking near log, covering in soundproofing material) may have altered organismal behaviour and been perceived as a threat or predator. This may have caused some organisms to remain quieter and others to increase sound production (e.g., termite alarm drumming, thus leading to no relationship (Hertel et al., 2011)). Additionally, errors in either the sound recordings or in the direct traditional diversity measurements may have also contributed. While one focus of these methods was ease of use and relative affordability, future application would likely benefit from additional equipment. The recorder used for this research had a built-in preamplifier, which was the only one used in the study. Adding an external preamplifier for future trials would be of interest and may improve recordings since many of the signals being recorded in saproxylic arthropod communities are weak

(Maeder et al., 2019, 2022). When applying these methods in the future, we suggest inserting waveguides prior to the planned recording date to ensure time for arthropods to resume normal activity prior to recording. A possible way to maximise efforts and efficiency in experimental studies is for researchers to use a ferromagnetic screw to attach the sample label such as a metal tag, which could be later used as the waveguide for sound recording.

Recent studies applying ecoacoustics to soil arthropod communities have found a correlation between ACI and arthropod taxa richness (Maeder et al., 2019; Maeder et al., 2022) and have shown ecoacoustic measurements tend to be higher in soil samples from restored forests compared to degraded forests (Robinson et al., 2023). These successes, ever-changing technology, and a growing ecoacoustic field provide ample opportunities to apply and improve upon these methods. Moving forward, methods found in this manuscript could be applied to studies interested in the abundance of relatively large target taxa, such as termites or ants. Feeding behaviours of ants and termites could be monitored with continuous recordings, pairing acoustic activity with samples of microbial communities of host deadwood to examine multi-trophic effects of microbial decomposer communities on wood consumption by saproxylic macroinvertebrate communities. Using similar methods to identify target taxa from background soundscapes is another research pathway to explore. Work in this area would need to isolate target taxa to record reliable signals in the substrate. Because we were focused on recording entire soundscapes of arthropod activity in this research, we did not explore this. The impact of wood species and decay class on arthropod signals would also be important to consider in these applications. Another avenue for continued research includes examining wood from different tree species and conducting multiple recordings over an extended time. All samples in this study used deadwood from pine trees (family Pinaceae, genus *Pinus*) as the substrate of interest. Using only pine wood neglects saproxylic arthropods that specialise in hardwood species (Stokland et al., 2012). Waveguides could be left in logs long-term to explore insect activity and colonisation over the course of wood decay, providing long-term temporal data that, to date, have been difficult to obtain due to the destructive nature of sampling. These methods are also great candidates for machine learning methods. Many studies that have used ecoacoustics rely on combining multiple indices with machine learning techniques to construct models with increased predictive power (Buxton et al., 2018; Gómez et al., 2018; Towsey et al., 2014).

Overall, methods presented in this manuscript show potential for use monitoring specific macroinvertebrates but need improvement before application towards projects examining full saproxylic arthropod assemblages. The quick processing time of acoustic recordings and high transportability of the recording equipment make these methods ideal for application even in distant, rough terrain, which may help alleviate challenges associated with studying saproxylic invertebrates. Ecoacoustics may also aid in sampling threatened habitats, where removal of wood could be detrimental to biodiversity (Ranius & Fahrig, 2006), or in remote habitats where it is not feasible to remove the substrate of interest and extract invertebrates. However, our results suggest that more applied research is needed before ecoacoustic methods can be used reliably to estimate saproxylic arthropod diversity.

AUTHOR CONTRIBUTIONS

Kristy M. McAndrew: Conceptualization; data curation; formal analysis; investigation; methodology; visualization; writing – original draft; writing – review and editing. **Natalie A. Clay:** Data curation; funding acquisition; investigation; project administration; writing – review and editing. **Courtney M. Siegert:** Funding acquisition; investigation; project administration; writing – review and editing. **Juliet D. Tang:** Funding acquisition; investigation; project administration; writing – review and editing. **Richard W. Hofstetter:** Investigation; methodology; resources; writing – review and editing. **David D. Dunn:** Methodology; resources; writing – review and editing. **Oscar Leverón:** Investigation; resources; writing – review and editing. **John J. Riggins:** Conceptualization; funding acquisition; investigation; methodology; project administration; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data and code used in this study are available on Zenodo (McAndrew et al., 2025).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Spectrogram analysis for the lab soundproofing trials. Each frequency band is highlighted for measurement of average power, with measurements for each visible in the table below the spectrogram.

Figure S2. Visual comparison of spectrograms from the five treatments tested in the lab soundproofing trials.

Figure S3. Spectrogram for the recording of a *Dendroctonus barberi* beetle. This recording was played through a log in experimental laboratory trials to test performance of different waveguides.

Figure S4. Laboratory set up for testing waveguide performance. Metal tubing, plastic tubing, a wooden dowel rod and a hex screw were each tested by placing them approximately 1 cm into one cross-sectional face of a log (a). To test performance, a piezoelectric element was attached to a waveguide material while a recording of a bark beetle sound was played through the opposite cross-sectional face of the log (b).

Figure S5. Estimates from linear mixed effects models created to predict Acoustic Complexity Index values (obtained via recording deadwood samples) from Shannon diversity, Hymenoptera abundance and Blattodea abundance (obtained by rearing arthropods out of the same deadwood samples). Estimates are shown for all models, including Hymenoptera and Blattodea abundance data, which were modelled at four different cut-off/trim levels: no trim (a), samples with >1000 Hymenoptera/Blattodea trimmed (b), samples with >100 Hymenoptera/Blattodea trimmed (c) and samples with >50 Hymenoptera/Blattodea trimmed (d).

Figure S6. Estimates from linear mixed effects models created to predict Acoustic Complexity Index values (obtained via recording deadwood samples) from arthropod richness, Hymenoptera abundance and Blattodea abundance (obtained by rearing arthropods out of the same deadwood samples). Estimates are shown for all models, including Hymenoptera and Blattodea abundance data, which were modelled at four different cut-off/trim levels: no trim (a), samples with >1000 Hymenoptera/Blattodea trimmed (b), samples with >100

Hymenoptera/Blattodea trimmed (c) and samples with >50 Hymenoptera/Blattodea trimmed (d).

Figure S7. Estimates from linear mixed effects models created to predict acoustic roughness values (obtained via recording deadwood samples) from Gini-Simpson diversity, Hymenoptera abundance and Blattodea abundance (obtained by rearing arthropods out of the same deadwood samples). Estimates are shown for all models, including Hymenoptera and Blattodea abundance data, which were modelled at four different cut-off/trim levels: no trim (a), samples with >1000 Hymenoptera/Blattodea trimmed (b), samples with >100 Hymenoptera/Blattodea trimmed (c) and samples with >50 Hymenoptera/Blattodea trimmed (d).

Figure S8. Estimates from linear mixed effects models created to predict acoustic roughness values (obtained via recording deadwood samples) from Shannon diversity, Hymenoptera abundance and Blattodea abundance (obtained by rearing arthropods out of the same deadwood samples). Estimates are shown for all models including Hymenoptera and Blattodea abundance data, which were modelled at four different cut-off/trim levels: no trim (a), samples with >1000 Hymenoptera/Blattodea trimmed (b), samples with >100 Hymenoptera/Blattodea trimmed (c) and samples with >50 Hymenoptera/Blattodea trimmed (d).

Figure S9. Estimates from linear mixed effects models created to predict acoustic roughness values (obtained via recording deadwood samples) from arthropod richness, Hymenoptera abundance and Blattodea abundance (obtained by rearing arthropods out of the same deadwood samples). Estimates are shown for all models, including Hymenoptera and Blattodea abundance data, which were modelled at four different cut-off/trim levels: no trim (a), samples with >1000 Hymenoptera/Blattodea trimmed (b), samples with >100 Hymenoptera/Blattodea trimmed (c) and samples with >50 Hymenoptera/Blattodea trimmed (d).

Figure S10. Estimates from linear mixed effects models created to predict acoustic entropy—Shannon's values (obtained via recording deadwood samples) from Gini-Simpson diversity, Hymenoptera abundance and Blattodea abundance (obtained by rearing arthropods out of the same deadwood samples). Estimates are shown for all models, including Hymenoptera and Blattodea abundance data, which were modelled at four different cut-off/trim levels: no trim (a), samples with >1000 Hymenoptera/Blattodea trimmed (b), samples with >100 Hymenoptera/Blattodea trimmed (c) and samples with >50 Hymenoptera/Blattodea trimmed (d).

Figure S11. Estimates from linear mixed effects models created to predict acoustic entropy—Shannon's values (obtained via recording deadwood samples) from Shannon diversity, Hymenoptera abundance and Blattodea abundance (obtained by rearing arthropods out of the same deadwood samples). Estimates are shown for all models, including Hymenoptera and Blattodea abundance data, which were modelled at four different cut-off/trim levels: no trim (a), samples with >1000 Hymenoptera/Blattodea trimmed (b), samples with >100 Hymenoptera/Blattodea trimmed (c) and samples with >50 Hymenoptera/Blattodea trimmed (d).

Figure S12. Estimates from linear mixed effects models created to predict acoustic entropy—Shannon's values (obtained via recording deadwood samples) from arthropod richness, Hymenoptera abundance and Blattodea abundance (obtained by rearing arthropods out of the same deadwood samples). Estimates are shown for all models, including Hymenoptera and Blattodea abundance data, which were modelled at four different cut-off/trim levels: no trim (a), samples with >1000 Hymenoptera/Blattodea trimmed (b), samples with >100 Hymenoptera/Blattodea trimmed (c) and samples with >50 Hymenoptera/Blattodea trimmed (d).

Figure S13. Estimates from linear mixed effects models created to predict acoustic entropy—Simpson's values (obtained via recording deadwood samples) from Gini-Simpson diversity, Hymenoptera abundance and Blattodea abundance (obtained by rearing arthropods out of the same deadwood samples). Estimates are shown for all models including Hymenoptera and Blattodea abundance data, which were modelled at four different cut-off/trim levels: no trim (a), samples with >1000 Hymenoptera/Blattodea trimmed (b), samples with >100 Hymenoptera/Blattodea trimmed (c) and samples with >50 Hymenoptera/Blattodea trimmed (d).

Figure S14. Estimates from linear mixed effects models created to predict acoustic entropy—Simpson's values (obtained via recording deadwood samples) from Shannon diversity, Hymenoptera abundance and Blattodea abundance (obtained by rearing arthropods out of the same deadwood samples). Estimates are shown for all models including Hymenoptera and Blattodea abundance data, which were modelled at four different cut-off/trim levels: no trim (a), samples with >1000 Hymenoptera/Blattodea trimmed (b), samples with >100 Hymenoptera/Blattodea trimmed (c) and samples with >50 Hymenoptera/Blattodea trimmed (d).

Figure S15. Estimates from linear mixed effects models created to predict acoustic entropy—Simpson's values (obtained via recording deadwood samples) from arthropod richness, Hymenoptera abundance and Blattodea abundance (obtained by rearing arthropods out of the same deadwood samples). Estimates are shown for all models, including Hymenoptera and Blattodea abundance data, which were modelled at four different cut-off/trim levels: no trim (a), samples with >1000 Hymenoptera/Blattodea trimmed (b), samples with >100 Hymenoptera/Blattodea trimmed (c) and samples with >50 Hymenoptera/Blattodea trimmed (d).

Figure S16. Estimates from linear mixed effects models created to predict frequency peaks values (obtained via recording deadwood samples) from Gini-Simpson diversity, Hymenoptera abundance and Blattodea abundance (obtained by rearing arthropods out of the same deadwood samples). Estimates are shown for all models including Hymenoptera and Blattodea abundance data, which were modelled at four different cut-off/trim levels: no trim (a), samples with >1000 Hymenoptera/Blattodea trimmed (b), samples with >100 Hymenoptera/Blattodea trimmed (c) and samples with >50 Hymenoptera/Blattodea trimmed (d).

Figure S17. Estimates from linear mixed effects models created to predict frequency peaks values (obtained via recording deadwood

samples) from Shannon's diversity, Hymenoptera abundance, and Blattodea abundance (obtained by rearing arthropods out of the same deadwood samples). Estimates are shown for all models including Hymenoptera and Blattodea abundance data, which were modelled at four different cut-off/trim levels: no trim (a), samples with >1000 Hymenoptera/Blattodea trimmed (b), samples with >100 Hymenoptera/Blattodea trimmed (c) and samples with >50 Hymenoptera/Blattodea trimmed (d).

Figure S18. Estimates from linear mixed effects models created to predict frequency peaks values (obtained via recording deadwood samples) from arthropod richness, Hymenoptera abundance and Blattodea abundance (obtained by rearing arthropods out of the same deadwood samples). Estimates are shown for all models including Hymenoptera and Blattodea abundance data, which were modelled at four different cut-off/trim levels: no trim (a), samples with >1000 Hymenoptera/Blattodea trimmed (b), samples with >100 Hymenoptera/Blattodea trimmed (c) and samples with >50 Hymenoptera/Blattodea trimmed (d).

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