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Acoustic analysis of saproxylic arthropod diversity in North and Central American pine forests

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in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Agricultural Life Sciences
in the Department of Biochemistry, Molecular Biology, Entomology, and Plant Pathology

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Biodiversity of arthropods living in dead wood is often understudied despite their potential effects on ecological processes such as wood decomposition and nutrient cycling. More time-efficient and less destructive methods are needed to study these saproxylic organisms to fully understand their global diversity. Because ecoacoustic methods have never been applied to saproxylic communities before, field and analytical methods such as waveguides, and soundproofing were developed, tested, and optimized. After developed methods were implemented in the field, Pearson's correlation tests were conducted to compare ecoacoustic index performance to traditional biodiversity indices. We found five significant correlations, all of which occurred at our Mississippi site, and all but one of which were negative correlations. Ecoacoustic indices performed best when correlated with order richness. Significance present in our study shows potential for ecoacoustics as a non-destructive method to study saproxylic arthropods, but methods still have room for improvement to optimize field application.

DEDICATION

To my loving parents Robert and Debbie McAndrew. Your support throughout my college career has helped me get to this point, and you have always encouraged me to keep on working towards my goals – even when it gets tough. You have fostered a love for the outdoors and gardening, both of which have fueled my fascination with insects. I would not be where I am today without you both.

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CHAPTER I

LITERATURE REVIEW

Introduction

The study of ecology explores interactions between living organisms and their environment. Although ecology is a vast discipline, with many subsets, many modern ecological studies have grown from a foundational study establishing the existence of trophic levels (Lindeman, 1942). Lindeman's research propelled ecology forward, allowing exploration of communities, as ecological interactions are often complex, involving multiple trophic levels which are affected by various biotic and abiotic factors (Power, 1992; Seibold et al., 2016; Siegert et al., 2018; L. H. Yang & Gratton, 2014). These interactions allow continuation of biogeochemical cycles and ecosystem services, which function to keep ecosystems from otherwise inevitable progression towards entropy. Thus, study of these interactions and their effects are the root of ecology.

Studying diversity of organisms in ecosystems provides insight to overall functionality of an ecosystem, but also to independent ecosystem services that are often overlooked and understudied (Loreau, 1998; Tilman et al., 1997). Different groups of organisms within ecosystems are often grouped as functional types or guilds which rely on similar resources and thus, carry out and facilitate similar natural processes, such as pollination, nitrogen fixation, and decomposition (Blondel, 2003; Steneck & Dethier, 1994). Within each of these functional groups can exist generalist species (those that can utilize a broad range of resources) and specialists (those that rely on a narrow range of resources). These generalities and specialties exist throughout and within functional groups present in ecosystems. For example, a 2019 study found that three ecologically similar (two of which were congeneric also making them taxonomically similar) beetles living within dead wood in the same forest system were relative specialists, all relying on dead wood of different species (Jaworski et al., 2019). Despite these three beetle species being specialists, it is estimated that the majority of beetles that rely on dead wood (approximately 75% in northern Europe) show only generic preferences between broadleaf and coniferous trees (Stokland et al., 2012). High species diversity that consists of both generalists and specialists and is typically associated with species niche and functional overlap resulting in functional redundancy driving ecosystem processes (Mooney et al., 1995). Diversity typically increases community stability through increased resistance and resilience that results in maintenance of ecosystem functions, such as wood decomposition, even following disturbances (Nyström, 2006; Park et al., 2019; Tilman, 1996; G. Yang et al., 2020). For this reason, determining biodiversity in an ecosystem is a goal of many ecology studies and management plans.

Dead Wood Importance

Dead wood accounts for approximately 8% of carbon stored in forest systems globally (Pan et al., 2011), and although historically thought of as primarily a management obstacle (Maser et al., 1979), is regarded as an important forest resource (Harmon, 2001). Prior to the late 1900s, dead wood was believed to be a hazard in forest stands, both to human and forest stand health because of a perceived ability to spread disturbances both biotic and abiotic (Harmon, 2001; Maser et al., 1979; Thomas, 2002). Forest management required removal of dead wood, and inventory methods developed included coarse woody debris estimates as a way to estimate fuel loads (Van Wagner, 1968). More recently, forest managers have realized the importance of dead wood to nutrient cycling, preservation of biotic diversity, and terrestrial carbon storage, and have since advocated for proper management of dead wood (Harmon, 2001; Harmon et al., 1986; Merganicova et al., 2012; Paletto et al., 2012). Public opinions of dead wood appear to support endeavors for proper management. A survey in southern Europe found that, for those surveyed, a majority of people preferred the "natural" look of the recreational forest land achieved through retainment of dead wood over the managed look of recreational areas with dead wood removed (Pastorella et al., 2016). Increased understanding of ecological importance of dead wood by forest managers and acceptance of dead wood presence by the general public opens the door to more research exploring the vital role of dead wood within forested ecosystems and organisms utilizing it.

Despite improved outlooks towards dead wood by both citizens and managers, the relatively new acceptance of dead wood retention in forest management leaves the scientific literature exploring the role of dead wood in biodiversity patchy at best and in need of expansion (Riffell et al., 2011; Seibold, Bässler, et al., 2015). Sampling methods to quantify coarse woody debris in forest plots are commonly used for stand inventories in both the United States and Europe (Rondeux & Sanchez, 2010; USDA, 2019). The United States Forest Service quantifies both coarse woody debris (dead wood greater than 3 in or 10 cm in diameter) and fine woody debris (dead wood less than 3 in or 10 cm in diameter) in annual Forest Inventory Analysis plots, utilizing transect methods for estimates (USDA, 2019). European foresters began accounting for dead wood in national inventories beginning in the 1990s (Paletto et al., 2012; Rondeux & Sanchez, 2010). Inventories of woody debris provide insight to natural amounts of dead wood and appropriate management strategies across forest types, which was lacking in past management/sanitation practices. This is particularly important for determining management strategies for the biodiversity of wood-inhabiting organisms (Gossner et al., 2016; Humphrey et al., 2004; Jaworski et al., 2019; Seibold, Brandl, et al., 2015).

Presence of dead wood in forest systems has benefits for the biotic communities within the systems, both directly through providing habitat and indirectly through impacting nutrient cycles like N and C (Harmon et al., 1986; Laiho & Prescott, 1999, 2004). Based on studies carried out in Europe, it is estimated that 20-30% of all forest insect species are saproxylic, meaning they rely on dead wood at some point in their life (Ulyshen, 2018). Dead wood diversity (species, decay class, size) and amount of dead wood in a forest stand positively correlate with biological diversity of both saproxylic and non-saproxylic species (Jaworski et al., 2019; Lassauce et al., 2011; Purahong et al., 2018; Riffell et al., 2011; Seibold et al., 2016; Seibold, Bässler, et al., 2015). In both southeastern United States pine forests and European broadleaf forests, the abundance of litter and soil arthropods is significantly higher in close proximity to or beneath coarse woody debris than distanced from the coarse woody debris (Jabin et al., 2004; Ulyshen & Hanula, 2009). Despite understanding the significant influence dead wood has on arthropod communities, most studies focus on few groups of taxa as opposed to the biodiversity of full assemblages of saproxylic arthropods.

Dead wood can also improve regeneration and health of future cohorts of trees. This is achieved through dispersal of nutrients through wood decomposition, which occurs at low levels but for a long period of time relative to other litter types (Laiho & Prescott, 1999, 2004), and through the structural and nutritional foundation of nurse logs seedlings may develop on (Harmon & Franklin, 1989; Orman & Szewczyk, 2015). These realized benefits of dead wood aside, the relatively recent universal acknowledgement of dead wood importance leaves many avenues unexplored in the field, notably the biotic processes affecting decomposition.

Dead wood decomposition rates are an important factor of ecology of dead wood because the rate will determine contributions to the ecosystem both through creation of structural habitat for organisms and the time frame of take-up and dispersal of nutrients. The rate of wood decay can be estimated by abiotic factors at large scales, but these abiotic factors are not as accurate at predicting localized wood decomposition rates. The most notable abiotic factors driving rate of wood decomposition are temperature and moisture (Harmon et al., 1986; Laiho & Prescott, 1999, 2004). However, when analyzing wood decomposition at local scales, Bradford et al. (2014) found that wood colonizing fungi was more representative of changes in decomposition rate, accounting for 73% of the 75% mass loss variance the model explained. Influence of arthropods has also been documented, although the relative importance of arthropod influence compared to fungal influence is debated. In Southeastern US forests, insects have been shown to contribute 9-10% to the decomposition of fine woody debris, such as twigs (Stoklosa et al., 2016). However, in a 2014 study in the same region termite presence or exclusion from experimental wooden bolts did not cause a significant difference in mass loss, although volume of wood was effected (Ulyshen et al., 2014). A study conducted in Africa found significant effects of termites on decomposition, but only from fungus-growing termite species (Schuurman, 2005). Knowledge of biotic factors driving dead wood decay rates are of global importance since dead wood accounts for a significant amount of terrestrial carbon storage (Bradford et al., 2014; Pan et al., 2011). Better understanding of factors influencing wood decay, especially the scantly studied effects of arthropods, would aid in representation of the ecological role of dead wood and importance at both local and global scales.

Studying Saproxylic Arthropod Communities

Saproxylic arthropods, although estimated to be speciose, are rarely quantified as a full assemblage in dead wood studies. Existing studies linking saproxylic arthropods and wood decomposition typically focus on large arthropods with the ability to excavate relatively large tunnels throughout dead wood (Schuurman, 2005; Stoklosa et al., 2016; Ulyshen et al., 2014; Ulyshen & Wagner, 2013). Little work has been done exploring the ability of arthropods without ecosystem engineer habits, to influence wood decomposition. Limited exploration of saproxylic arthropods and their contribution to wood decay may be, in part, due to the methods required to study these organisms. Methods used vary but are often destructive and time consuming (Hammond, 1997; Wikars et al., 2005). Destructiveness of sampling renders the samples useless for future study, making ongoing monitoring of arthropod activity in a single sample impossible. Common methods such as extraction from dead wood, using funnels or rearing chambers, and flight intercept traps require at least several months to provide a trustworthy representation of arthropods present. Methods utilizing extraction also require allocation of space, typically outside of the prepared research area, for a prolonged period. Additionally, to combat the destructiveness of sampling, extraordinarily large sample sizes may be required to study a system over the course of multiple years, which is necessary to offer a full representation of the wood decay process (Siegert et al., 2018; Ulyshen et al., 2014). Finally, an additional constraint to these studies lies in the identification of the arthropods found. Wood-dwelling arthropods include taxonomic representatives from all extant classes of arthropods (Hammond, 1997; Stokland et al., 2012; Ulyshen, 2018). With such taxonomic diversity present, there is a steep training curve experienced for workers identifying these extracted samples, which represents another avenue in which these studies are labor and time intensive.

Current estimates of the number of saproxylic invertebrate species in existence vary greatly, demanding continued study to determine the quantity and diversity of these organisms and how their diversity impacts the wood decay process (Stokland et al., 2012; Ulyshen, 2018). However, these studies are traditionally expensive, in both time and expertise, to carry out. Expedited extraction times can be used to estimate arthropods present, but the extensive sorting and identification still produces time and expense related roadblocks for projects. Addition of a non-invasive and time-efficient method to accompany traditional methods may lessen the burden of these studies, facilitating movement beyond these roadblocks. Development and utilization of an *in situ*, non-destructive method could create opportunities for expansion in research in this field, which has ample opportunities for expansion.

Acoustic Exploration of Biotic Communities

Bioacoustic methods have been increasing in popularity in various study environments. Bioacoustics is a term used to describe any use of acoustic monitoring or recordings in the broad field of biological sciences (Fletcher, 2007; Pijanowski et al., 2011). One of the many benefits of acoustic monitoring is its non-invasiveness. These methods can be applied with minimal disruption of the typical behavior of target organisms of the study (Clark et al., 2018; Fournet et al., 2018; Keen et al., 2017). Methods can also be applied remotely, which is especially helpful for monitoring of vulnerable or cryptic species. These methods benefit natural sciences because lab simulations are incapable of recreating natural environments, but there are often methodological obstacles when attempting to study a target organism in their natural environment. Acoustic monitoring, which is easily implemented in the field, has allowed for advancements in both behavioral and life history studies. Acoustic monitoring has been used to monitor whale pods movement as well as how whale pods adapt to ship noise in harbors, providing insight to animal adaptations to human disturbance through passive, non-invasive monitoring (Clark et al., 2018; Fournet et al., 2018). Algorithms are able to detect the low frequency vocalizations of forest elephants through non-invasive acoustic monitoring with 83.2% accuracy (Keen et al., 2017). The passive nature of monitoring elephants is especially important for the reduction of researcher traffic that may aid poachers in locating vulnerable species. Both above-mentioned studies highlight an additional benefit of acoustic studies given current technology: recordings can be analyzed at frequencies and volumes outside of the realm of human hearing, opening the door to communication and behaviors previously unable to be studied by scientists.

Bioacoustic methods are also used for arthropod monitoring efforts, namely for pest species (De La Rosa et al., 2005; Escola et al., 2020; Mankin et al., 2002; Sutin et al., 2019). Bioacoustics can detect both airborne (Escola et al., 2020) and substrate borne (Mankin et al., 2002; Sutin et al., 2019) sounds of arthropod pests. Airborne sounds produced through stridulation (sounds created by insects by rubbing together body parts) can be recorded using microphones and methods common with other airborne acoustic recording techniques. However, when arthropods live within substrate (such as wood or soil) application of acoustic sensors specifically designed to record vibrations from substrate are necessary. Acoustic sensors created for substrate dwelling invertebrates are commonly used in commercial pest settings and can accurately detect termites in structural wood when within 2.2 meters of attachment (Lemaster et al., 1997). More recently, international shipping ports are developing acoustic monitoring methods to monitor for invasive wood boring insects, and have detected two common families of wood boring beetle larvae with 87.5% accuracy using lab tests (Sutin et al., 2019). Harvey et al. (2011) distinguished between larval stridulation of three beetle species in substrate borne recordings through difference in frequencies present and duration of the sound created. Similar methods have been used to monitor urban trees for infestation by invasive Formosan termites, with trained human listeners able to detect whether or not a tree was likely infested even with an urban soundscape present in the recordings (Mankin et al., 2002). Passive acoustic monitoring methods have been implemented to detect the airborne sounds of cicada pests in coffee bean farms in Brazil with 96.41% accuracy (Escola et al., 2020). Success of these studies demonstrates the potential utility available through bioacoustics to monitor insects that are otherwise difficult to detect.

Ecoacoustics

A rich and growing base of knowledge exists around detection and manipulation of individual arthropod taxa with acoustics. However, little research has focused on analyzing the soundscape created by a community of arthropods (all sounds created by all living creatures within a recorded area) to determine community-level information. A newer field has developed within bioacoustics that uses environmental recordings to assess community-level information. This field, termed ecoacoustics, has mostly been used to estimate avian diversity (Depraetere et al., 2012; Pieretti et al., 2011; Pijanowski et al., 2011; Villanueva-Rivera et al., 2011). Successful uses of ecoacoustic methods in ornithology include differentiating between disturbed and pristine habitats based on bird diversity (Sueur et al., 2008), rapid estimation of biodiversity across multiple land use types (Depraetere et al., 2012), and predicting diversity through combining multiple ecoacoustic indices (Buxton et al., 2018).

Ecoacoustic indices can estimate species diversity by using sound diversity, with some created to mimic existing diversity indices (ex: Shannon’s and Simpson’s diversity indices). The relative infancy of the field of ecoacoustics, paired with the efforts of many different researchers to develop indices at once has led to an abundance of acoustic indices existing within the literature. Buxton et. al (2018) cited 69 separate indices from the peer reviewed literature. Among these 69 indices are several that are used frequently, and with good results. Acoustic complexity index (ACI) is one of the most commonly used, and notably accurate, indices (Bertucci et al., 2016; Buxton et al., 2018; Harris et al., 2016; Maeder et al., 2019; Papin et al., 2019; Pieretti et al., 2011). This index estimates diversity through binning acoustic recordings and comparing adjacent bins based on the acoustic intensity present (Pieretti et al., 2011). The entropy index was created to mimic more traditional diversity measures, and like ACI, has been successful in a variety of applications such as differentiating between intact and disturbed forests, estimating wolf pack size, and correlation with temperate marine reef diversity (Harris et al., 2016; Papin et al., 2019; Sueur et al., 2008). The entropy index can also be calculated in two different methods, one which mimics Shannon's diversity index, and one that mimics Simpson's diversity index, and is calculated through analyzing the changes in amplitude of a sound over time, and the evenness of the distribution of those changes in amplitude over the set time of recording. Another common index, spectral peak count, looks at the number of spectral peaks present in a recording, which has correlated with human observed acoustic activity (Buxton et al., 2018; Gasc et al., 2013; Towsey et al., 2014). As previously stated, many indices exist within the literature, but different indices are likely better or worse suited to different systems depending on the conditions of those systems and the specific questions tested. Most acoustic indices exist in the free analysis program R, under the package seewave (R Core Team, 2017; Sueur et al., 2008).

Although primarily developed for and used with ornithological studies, these ecoacoustic indices are being used for other taxa in the natural resources field with success. Papin et al.

(2019) used several of these indices and found positive correlation between index values, particularly Acoustic Richness and the Spectral Entropy Index, and grey wolf pack size, although deviations were high. Ecoacoustic indices have shown great promise in marine habitats, with high correlative results between ecoacoustic indices and species estimates (Bertucci et al., 2016; Buxton et al., 2018; Harris et al., 2016). To date, little work has been done to utilize these indices in the study of organisms living in substrate. Substrate recordings are great candidates for utilizing ecoacoustic indices because they offer recordings with little interference from noise outside of the target ecosystem, which is a noted issue in current use of indices studying a specific target taxa (Eldridge et al., 2018). Maeder et al. (2019) found a positive relationship between the Acoustic Complexity Index and diversity of soil invertebrates collected, showing promise for the use of these methods studying substrate-dwelling arthropods. Use of ecoacoustics to study saproxylic arthropods could save time and money when applied to study methods through the non-destructive and passive nature of their construction and deployment. The time saved through *in situ* methods could expedite studies, and the non-destructive nature of the sampling could allow for a single piece of woody debris to be continually monitored.

Conclusion

Characterizing species diversity and interactions are fundamental to answering ecological questions and developing best management practices. Biodiversity measurements are often included in ecology studies due to their fundamental nature, and those measurements can be used to infer effects of diversity on ecosystem functioning. However, when a researcher is unable to observe biodiversity due to opacity of an environment or the illusiveness of a group of organisms, such as dead wood and saproxylic arthropods, innovation is necessary to make efficient estimates.

Defining biodiversity patterns in dead wood is essential for understanding ecosystem processes like wood decomposition and nutrient cycling and expansion of ecoacoustic methods into wood decomposition studies provide an opportunity for growth in studies of saproxylic arthropods (Seibold, Bässler, et al., 2015; Ulyshen, 2013). The effects of these arthropods on nutrient cycling (such as Nitrogen, Phosphorus and Carbon) are currently difficult to study due to the lengthy time requirements, but existing research validates insect effects wood decomposition and nutrient cycling processes (Siegert et al., 2018; Ulyshen et al., 2017). The low time cost of ecoacoustic methods could allow for more studies to occur in various landscapes, expanding the current set of knowledge, which is currently centered around temperate and boreal forest ecosystems (Seibold, et al., 2015). Better knowledge in these areas can reflect in the applied field with better forestry management practices for dead wood utilization to support biodiversity and the processes saproxylic biodiversity supports.

CHAPTER II

UTILIZING ECOACOUSTIC METHODS TO EXAMINE SAPROXYLIC ARTHROPOD DIVERSITY

Introduction

Arthropod Diversity

Saproxylic arthropods are a diverse and oftentimes cryptic group of organisms with significant ecological roles in forested ecosystems. Although the diversity of arthropods that live in or rely on dead wood (saproxylic arthropods) is estimated to be high (Stokland et al., 2012; Ulyshen, 2018), they are difficult and time consuming to study leaving current estimates of total global species present uncertain and mostly extrapolated from relatively few and far between study sites. Because it is relatively difficult to obtain diversity information for wood-dwelling arthropods, the effects of full assemblages of saproxylic arthropods on ecosystem services, such as wood decomposition and nutrient cycles has yet to be reliably quantified. Information gained through further study of saproxylic arthropods could better define their role in forest ecosystem services, such as those mentioned above, potentially identifying organisms tied to ecosystem functionality and productivity.

Researchers exploring saproxylic invertebrate diversity would greatly benefit from time efficient *in situ* methods. Improved methods of study for saproxylic arthropods could expand the literature on arthropod involvement in the brown food web and nutrient cycling. The effects of termites, which have potential to act as ecosystem engineers through extensive tunneling and movement of soil, have been well documented (Clay et al., 2017; Little et al., 2012; Riggins et al., 2014; Schuurman, 2005; Siegert et al., 2018; Stoklosa et al., 2016; Ulyshen et al., 2014). However, little work exists documenting the effect of physically smaller arthropods, or the effects of overall diversity of arthropods on systems such as the brown food web (in relation to dead wood) (Seibold, Bässler, et al., 2015). The destructive and time-consuming nature of current sampling methods creates roadblocks when designing experiments to document arthropod effects on wood decomposition throughout the decomposition process, which may be partially to blame for the gaps in knowledge in this field of study. Studying saproxylic arthropods requires extraction from the substrate they live within (Hammond, 1997; Wikars et al., 2005). This process renders the substrate sample functionally useless for future study, meaning a different section of log would need to be sampled at each stage of decomposition, introducing high amounts of variability to a study through both biotic and abiotic factors. Past and current studies combat this through high sample numbers (Siegert et al., 2018; Ulyshen et al., 2014), which may lead to an additional time constraint. Developing non-destructive *in situ* methods would allow for studies to observe one decomposing unit of wood and quantify the changing dynamics of saproxylic invertebrates present throughout the decomposition process.

Bioacoustics

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. The ability to record remotely is a major advantage of these studies. Vulnerable species, such as whales and elephants, can be studied distantly, hopefully decreasing chances of detrimentally altering behavior or drawing unwanted attention to poachable populations (Clark et al., 2018; Fournet et al., 2018; Keen et al., 2017).

Current efforts to study wood-dwelling arthropods are plagued with difficulties due to the cryptic nature of the target taxa as well as the time and expertise required to identify and study the arthropods present. Ability to assess populations passively provides new avenues for study of saproxylic arthropods as well. Acoustic recordings can be taken with minimal disruption of the substrate, allowing study without altering behavior, and potentially without as many time-consuming extractions. Time and money saved could manifest in expansion of current studies, or expansion of the scope of saproxylic arthropod studies. However, desired taxonomic specificity should be carefully considered when planning to implement acoustic methods. Although taxonomic specificity can be high and allow researchers to distinguish between species in a controlled environment (Harvey et al., 2011; Sutin et al., 2019), studies conducted in natural environments may be limited to studying a single species (Escola et al., 2020; Mankin et al., 2002). Valuable information can still be gained from these more complex scenarios but the community-level information, without pairing with non-acoustic methods, would lack specific taxonomic data. Researchers should be aware of this tradeoff and implement acoustic methods appropriately, being certain desired objectives are achievable in their given study environment.

Although many existing studies examine arthropods based on sounds, few have attempted to glean diversity information from these recordings. Ecoacoustics is an emerging branch of bioacoustics that assesses recordings, not for individual target taxa, but for community-level information, most commonly diversity. Arthropod studies utilizing acoustics, up to this point, mostly focus on detecting sounds produced by a known target organism (Escola et al., 2020; Harvey et al., 2011; Lemaster et al., 1997; Mankin et al., 2002; Sutin et al., 2019). These studies, utilizing both airborne and substrate-borne sounds, focus on insect pest detection. Applying ecoacoustics to exploration of substrate dwelling arthropods would differ from these common detection methods by not focusing on the detection of one or several sounds produced by known target organisms, but instead analyzing all the sounds occurring in the recorded substrate. Following the recording of the full scope of arthropod sounds, the variation of sounds, the different frequencies present, and the amount of acoustic activity in the recording can be used as variables to provide estimates of arthropod diversity present.

Although ecoacoustics is a booming area of research, methods had not previously been applied to arthropod communities in dead wood before our study onset, necessitating modification of existing method to fit our needs. Ecoacoustic methods available at the start of our study were established, primarily, for use in ornithology. Despite development for use in ornithology, methods have shown promise in various study areas within the natural sciences discipline. Ecoacoustic indices have been used to estimate wolf pack size, with accuracy up to 7 to8 individuals (Papin et al., 2019). 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 (Bertucci et al., 2016; Gómez et al., 2018; Harris et al., 2016; Sueur et al., 2008). Gómez et al. (2018) utilized machine learning techniques with success to differentiate between three successionally distinct habitats as well as three separate sites within the same successional forest type. Researchers that have found significance between observed biotic diversity and acoustic diversity while using ecoacoustic methods have mostly used airborne sounds, though underwater recordings of marine reefs have also had success (Bertucci et al., 2016; Buxton et al., 2018; Harris et al., 2016). Studying wood-dwelling arthropods will require analysis of substrate-borne vibrations. This requires different hardware for recording, such as the use of soundproofing equipment and piezoelectric elements (piezo from here on) to record substrate-borne sounds as opposed to a more standard microphone that picks up air-borne sounds and, potentially different analysis procedures such as different recording lengths.

The ultimate purpose of establishing ecoacoustic methods for use in study of saproxylic arthropods is to create an effective and efficient method for studying this cryptic, yet ecologically important group of organisms. We also want these methods to be widely accessible, so we kept cost effectiveness of methods and ease of application in mind throughout experimental set up and testing. To test for success in both implementation and practice five objectives were developed for this study:

Objective 1

Determine which of two common piezo sized, 12 mm diameter piezo or 27 mm diameter, will best capture arthropod sounds in dead wood, since different diameter piezo amplify different ranges of frequencies. I hypothesize that 12 mm diameter piezo will better record arthropod sounds, as the resonant frequency range is closer to the reported ideal range for soil arthropod recordings (Mankin et al., 2002).

Objective 2

Determine an effective and field appropriate sound proofing method using items that can be locally purchased or built including cardboard boxes, Styrofoam coolers, wooden boxes, and a ceramic bowl. I hypothesize that a wooden box will perform best out of the tested soundproofing methods.

Objective 3

Determine the most feasible wave guide based on effectiveness of sound recording and ability to easily attach to logs in the field. Materials tested will include a wooden dowel rod, metal tubing, a nail, and a screw. I hypothesize that metal screws will be the most feasible waveguide out of the tested materials.

Objective 4

Develop in-field recording and post-field editing protocols through obtaining both one-minute and two-minute recording lengths from in-field recordings to compare ability of each to capture arthropod acoustic data. I hypothesize that two-minute recording lengths with perform better in analysis of diversity than one-minute recording lengths, and I plan to measure this by comparing the number of significant tests for each (one-minute vs. two-minute).

Objective 5

Determine ability of ecoacoustic indices to predict diversity of saproxylic arthropods through correlative tests between ecoacoustic indices and traditional biodiversity indices. I hypothesize that ecoacoustic indices will correlate with traditional diversity indices.

Methods

Objective 1

Sensors

Although pre-made sensors exist for substrate-borne sounds, they are sold at a high retail price that is often not feasible for research lab budgets (e.g., contact microphones marketed for field use). This high entry cost may act as an inhibitor for labs to venture into acoustic studies. However, similar and effective equipment can be constructed on a lower budget (Aflitto &

Hofstetter, 2014; Harvey et al., 2011; Maeder et al., 2019). Piezoelectric elements (piezo hereafter) are a low-cost transducer that can be used to record vibrations. The diameter of the polarized ceramic disc present on piezo determines the resonant frequency range, which is the range of frequencies at which a piezo best performs and can most easily record sound. To explore potential benefits of different resonant frequency ranges we used two different sizes of piezo: a 12 mm piezo (resonant frequency 9kHz +/- 0.5kHz) and a 27 mm piezo (resonant frequency 4.6kHz +/- 0.5kHz) (Table A.1). Piezos were soldered onto a single conductor, shielded wire (Table A.1), which was then soldered onto an RCA input connector. Piezo efficiency was tested through recordings taken in the field to be able to analyze based on what would be encountered in the field, as opposed to using controlled sounds in a lab setting.

Objective 2

Soundproofing

Soundproofing was necessary to eliminate unwanted ambient airborne sounds due to the sensitivity of the sensors built for this study. Soundproofing needed to be easily implemented and portable in the field. Because of the international and intercontinental travel associated with this project, the soundproofing materials also needed to be readily available at all three field sites (Siguatepeque, Honduras, Flagstaff, Arizona, McNeill, Mississippi) to ensure researchers would be able to access materials regardless of location.

We tested four readily available materials in laboratory trials: cardboard, Styrofoam, wood, and ceramic. To test effectiveness, we attached a single 27 mm piezo to a log 10.80 cm in height and 19.05 cm in diameter - similar in size to experimental logs used in the field study. With that sensor, we recorded a set of five airborne frequencies (600 Hz, 650 Hz, 700 Hz, 750 Hz, and 800 Hz) played in progression (from low to high frequency) from a laptop (Table A.1) placed 1.86 m away from the log. We repeated this progression a total of five times, changing the soundproofing method applied after each repetition (uncovered control, cardboard, Styrofoam, wood, and wood with a ceramic bowl (Figure 2.1)). The volume of the laptop and gain of the recorder (Table A.1) remained constant through all five repetitions, at 75% and 50% respectively. We compared the effectiveness of each of the soundproofing mechanisms across recordings by analyzing the average power in decibels of a 30 second segment of each frequency recorded (Figures A.1, A.2).

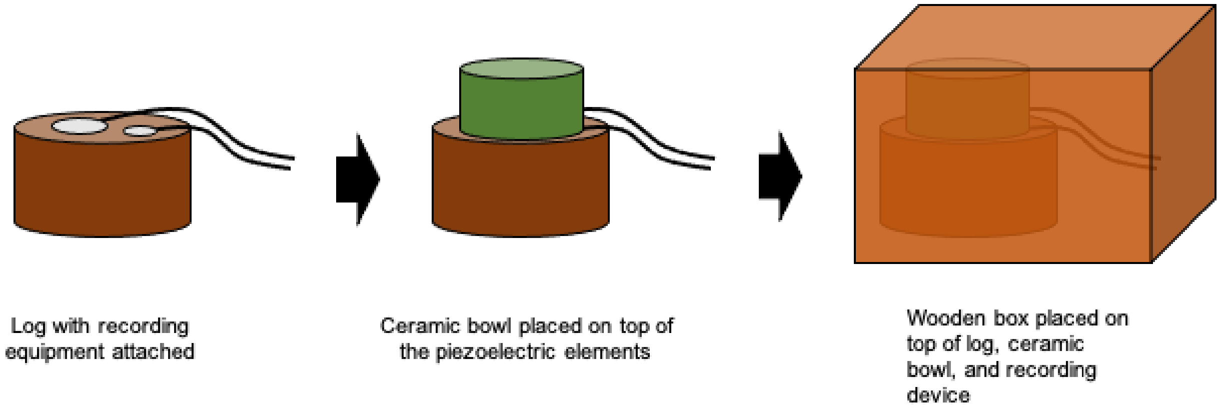

Figure 2.1 Process of applying soundproofing mechanisms in the field

These soundproofing mechanisms were also tested in the field by connecting the sensor to a log and listening to the sensor with headphones while someone caused disturbances that would be common in the field such as walking loudly, whistling, and talking. Although field tests were unable to be quantified due to the inherent variability of human-produced sounds, these were still important in the decision process because they reflected sounds that were likely to be encountered in the field as disturbances and were assessed based on the appearance of spectrograms (level of amplitude of bird songs and whistling) and through auditory comparison.

Objective 3

Waveguide

Waveguides are directional guides for electronic pick-up of sound waves, used with the intent of preserving as much of the vibrational signal as possible to be transformed into an electrical signal. A common real-world example of a waveguide is the needle of a record player following the grooves of a record, allowing the vibrations created to travel further up the arm and create electronic sound. When recording in substrate waveguides are necessary to attach to or, ideally, penetrate the target substrate and ensure the target acoustic signals are being transmitted to the piezo. Therefore, determining an object to use as a waveguide and an appropriate method for connecting the piezo to the chosen waveguide is an important determination. The logs that the waveguide(s) would be inserted or connected to would be in varying states of decay, meaning some would require a higher amount of force to insert the waveguide than others. Difference in force needed to insert may require different methods of implementation depending on the waveguide used, creating an unwanted source of variation.

Much of the waveguide testing addressed feasibility of application. Metal nails, metal screws, metal tubing, and wooden dowel rods were all initially considered. We conducted initial tests by playing a known sound through a log in a controlled laboratory environment and concluded that all tested materials were effective waveguides. However, not all could be easily implemented. Both the wooden dowel rod and the metal tubing required either a tool to puncture into the wood or large volumes of glue to attach them. If being inserted into a structurally intact log, the nail would require either a hammer to insert it, or an electric drill to create a pilot hole to insert the nail into. The metal screw would require insertion by an electric drill but would create a tighter connection than the nail since the screw has threads, which will be in contact with the log and create a greater connection in terms of surface area.

In-field Recording Protocol

We conducted this experiment at three field sites: one in Honduras, Arizona, and Mississippi. The site in Honduras was on the school forest of Universidad Nacional de Ciencias Forestales (UNACIFOR) in Siguatepeque, Honduras (14°33’46.8” N 87°48’54.0” W). The stand consisted of yellow pine (*Pinus oocarpa*), of approximately 39.3 years in age, a basal area of 9.4 m^2/ha, and an average 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*) and 10% gambel oak (*Quercus gambelii*), with a basal area of 31 m^2/ha, an average DBH of 26.6 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*) approximately 30 years in age with an approximate basal area of 43 m^2/ha, average DBH of 52.4 cm, and 10% mixed hardwoods. These three sites were established in 2017 for a research project on wood decomposition dynamics following bark beetle attacks. For the purpose of the study we harvested two trees, one bark beetle attacked and one non-attacked, from the nearest available infested forest stands relative to the field sites. Harvested trees were further processed into cross-sections 10.16 cm in height, hereafter logs, that were deployed in a completely randomized grid at each site. Each site contained sixty logs at the time of application of ecoacoustic sampling.

Although no previous publications exist that establish ecoacoustic methods for the study of arthropods in dead wood, ecoacoustic publications studying other taxa were explored to determine a recording length for analysis. The standard recording length for analysis is one minute (Farina, 2018), and this practice has been followed among most studies utilizing ecoacoustics. However, most of these studies analyze air-borne sounds (Depraetere et al., 2012; Gómez et al., 2018; Pieretti et al., 2011; Sueur et al., 2008; Towsey et al., 2014). Since our methods may rely on subtle sounds such as arthropods walking and chewing within substrate, we analyzed both a one-minute and two-minute segment to test for potential benefits of extending the suggested one-minute recording length.

The in-field recording length of five minutes was chosen to ensure that audio clips free of non-target noises could be selected from the middle of the recordings. Because of the length of the piezo cables, the audio recorder had to be positioned beneath the soundproofing box. 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 due to the ceramic dish and box being put into place, the acoustician settling into a comfortable position, and the needles, leaves, and vegetation surrounding the log settling after being mechanically disturbed. The five-minute recordings allowed for time at the beginning of the recording for the noise of set-up while allowing for two unobstructed minutes of recording in the middle of the recording. Five minutes also provided ample time in case of unforeseeable noisy events in the areas surrounding the field sites, such as trains passing. Acoustic recording at each site took place over two days, recording thirty logs per day.

A gain between 50% and 75% was used in the field for recordings. 50% was chosen as the standard, but in Honduras and Arizona the gain had to be increased to 75% due to the arthropod activity being quiet relative to Mississippi, which was the first site sampled.

Arthropod Collection

After acoustic recordings were obtained from the logs, logs were removed from the field site to extract arthropods. Each log was hung in a cloth berlese funnel with a 40-watt light bulb at the top for five days. The Berlese funnels had Whirl-Paks (Table A.1) attached at the bottom containing 70% ethyl alcohol to preserve arthropods as they fell from the log.

Objective 4

In-lab Audio Protocol

Two SD cards were used simultaneously for recording, allowing two methods of digitization to occur at once. One SD card collected stereo recordings, keeping the two piezo audios separate. The other SD card collected mono recordings, combining the two piezo audios into one sound. Using two SD cards also created an instant back-up for audio data in case of SD card malfunction or loss.

For each recording, the first minute was deleted to eliminate the high amounts of noise created from set up as described above. From this edited file, two files were created. The one-minute segment was the minute following the deleted first minute, and the two-minute segment was the two minutes following the deleted first minute (Figure 2.2). No edits other than these described segmentations took place.

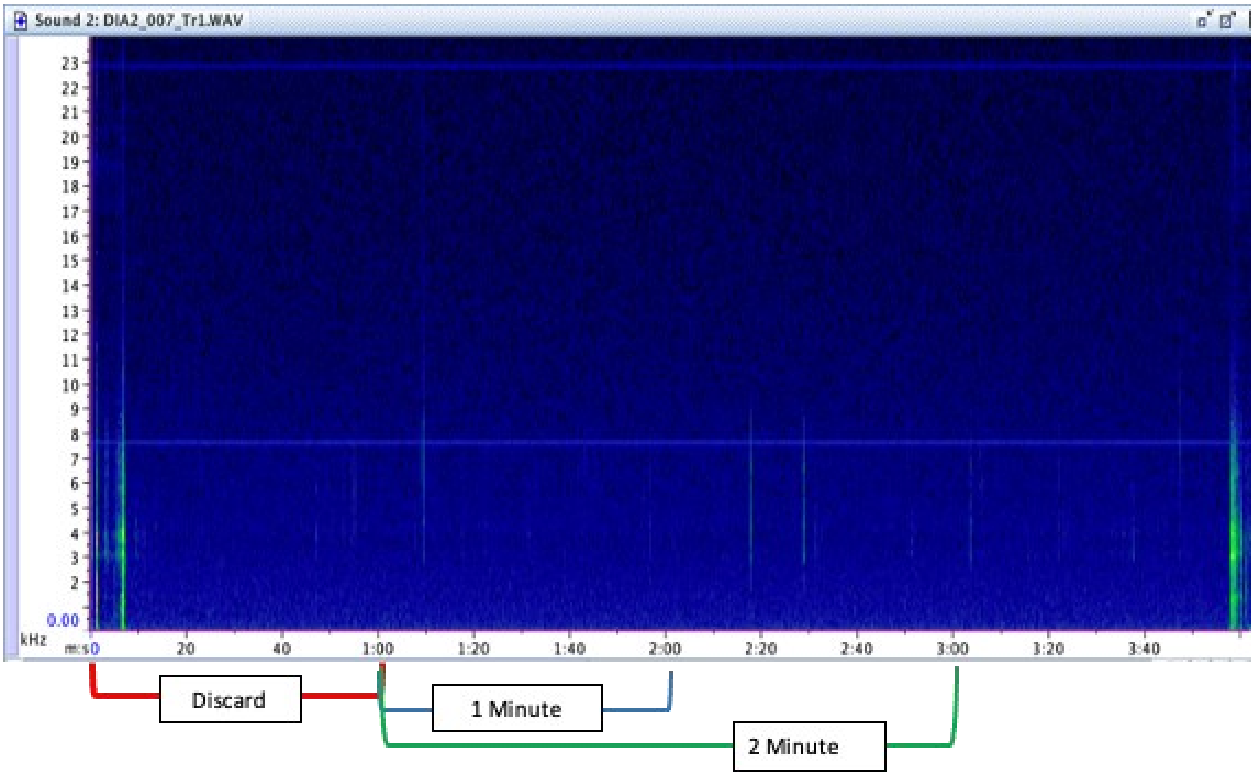

Figure 2.2 Editing process for selection of one and two-minute segments for audio analysis

Objective 5

Arthropod Sorting

Arthropods obtained from Berlese funnels were sorted taxonomically to order level. Exceptions included subclass Acari, phylum Myriapoda, and insect larvae. Subclass Acari was separated into functional groups of Oribatida, the fungivores and scavengers, and non-Oribatida, the largely predacious mites (Kethley, 1990; Krantz & Ainscough, 1990; Norton, 1990; Philips, 1990). Phylum Myriapoda was only sorted to Class to save time, since little diversity in orders occurred within samples. Insect nymphs were included in counts for Orders because of their reliably identifiable features, but insect larvae were excluded because of the overlap of larvae characteristics across different insect Orders. Insects were keyed following Triplehorn & Johnson (2005), and non-insect arthropods were keyed as per Dindal (1990). Due to the long processing time associated with invertebrate identification and quantification, not all samples were utilized for analysis. In anticipation of long sorting times, a randomized subsample was created identifying 30 samples from each site to sort for analysis. Following the completion of those 30 samples, a second tier of randomization identified additional samples to sort from each site to increase the samples size for analysis. Workers sorted through this second tier of subsamples until time of analysis, switching between sites in attempt to keep representation by site equal. The subsample included 33 samples from the Arizona field site, 33 samples from the Honduras field site, and 37 samples from the Mississippi field site for a total of 103 samples (N=103).

Shannon's biodiversity index, Simpson's biodiversity index, and richness were calculated at order level, with subclass Acari included by functional groups, phylum Myriapoda included by class, and insect larvae excluded (Equations 2.1 - 2.6).

$$Shannon's\ H = \sum((p_i)\ln(p_i)) \tag{2.1}$$

$$Simpson's\ D = \sum {p_i}^2 \tag{2.2}$$

$$Richness = count\ of\ unique\ orders\ present \tag{2.3}$$

$$p_i = \frac{n_i}{N} \tag{2.4}$$

$$n_i = sum\ of\ individuals\ in\ order\ i \tag{2.5}$$

$$N = sum\ of\ all\ orders \tag{2.6}$$

Analysis

We performed all analyses in R version 4.0.3 (R Core Team, 2017). The sound analysis package, seewave (Sueur et al., 2008) was used to apply ecoacoustic indices to each recording. We used five ecoacoustic indices: Acoustic Complexity Index (ACI), Roughness, Entropy - Shannon's, Entropy - Simpson's, and Spectral Peak Count. 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 et al., 2019). ACI was calculated using a five second bin length, as this is an appropriate bin size used in other ecoacoustic studies (Maeder et al., 2019; Pieretti et al., 2011). All other acoustic indices were calculated using the default settings in seewave.

We tested the null hypothesis that ecoacoustic measurements of biodiversity were not related to physical measurements of biodiversity using correlation analysis. Specifically, we calculated Pearson's correlation coefficient for each combination of ecoacoustic and traditional index (ex: ACI compared to Shannon's index, to Simpson's index, and to order richness), using an α of 0.05 to test for significance.

Results

Objective 1

Sensors

The smaller diameter (12 mm) piezo did not successfully record arthropod sounds (Figure 2.3), causing us to reject the hypothesis proposed for objective one. The channel used to record the smaller piezo was excluded from analysis due to the lack of arthropod sounds recorded.

Objective 2

Soundproofing

In general, all soundproofing methods provided some level of soundproofing, but which method was best depended on the frequency being tested. In three of the five frequencies tested in the lab trial the wooden box had the lowest decibel value of the five treatments, at 78.4 dB for 600 Hz, 92.9 dB for 750 Hz, and 91 dB for 900 Hz (Table 2.2). For two of the three frequencies in which the wooden box was the best method (750 Hz and 800 Hz), the wooden box with a ceramic cup had the second lowest dB value (100.1 dB and 92 dB respectively), and cardboard was the second most effective for the other frequency (650 Hz; 78.7 dB) (Table 2.2). For the two frequencies in which the wooden box was not the most effective soundproofing method (600 Hz; 64.1 dB and 700 Hz; 83.7 dB), the carboard box was the most effective (Table 2.2). For both frequencies, 600 Hz and 700 Hz, when cardboard was the most effective soundproofing method, the wooden box with a ceramic cup was the second most effective measuring 68.6 dB and 85.1 dB respectively. The combination of the wooden box and the ceramic cup was most frequently the soundproofing method with the second lowest dB measurement and was notably effective in the field trials.

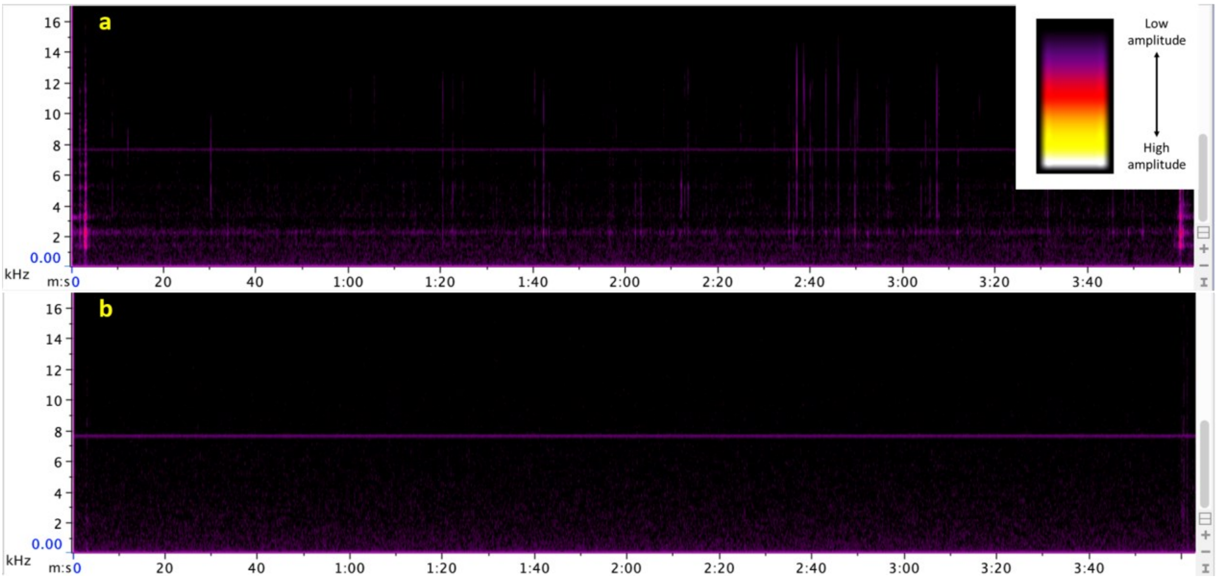

Figure 2.3 Spectrogram comparison between 12 mm and 27 mm piezo

Tandem recordings of 27 mm piezo (a) and 12 mm piezo (b) obtained through use of dual channel recording with the Zoom F4 Field recorder (Table A.1). The uniform horizonal line appearing at 8kHz is attributed to electronic noise from the recording unit. The vertical lines throughout the spectrogram are associated with arthropod noise (and, at the beginning and end of the spectrogram, human made noise). The 12 mm piezo was unable to properly record target arthropod taxa that the 27 mm piezo did.

The wooden box had the highest percentage of lowest dB measurements during the lab trial, performing the best out of the five treatments 60% of the time. Based on the lab trial alone, we cannot accept or reject the null hypothesis of different soundproofing methods outcompeting the wooden box without further replication of the test. Anecdotal evidence from field trials, based on listening to recordings and comparing the amplitude presented in spectrograms, suggested that the addition of a ceramic cup improved soundproofing against bird songs and human-caused disturbances. So, when considering both the lab trial data and the field trials, the soundproofing method of wooden box with the addition of the ceramic cup was chosen for application in the field for the purposes of our study.

Table 2.1 Decibel of airborne tones with varying soundproofing mechanisms

Frequency	Soundproofing Methods				
	Control	Styrofoam	Cardboard	Wood	Wood + Cup
600 Hz	75 dB	69 dB	64.1 dB	69.4 dB	68.6 dB
650 Hz	85.5 dB	83.9 dB	78.7 dB	78.4 dB	83.1 dB
700 Hz	90.9 dB	91.5 dB	83.7 dB	86.1 dB	85.1 dB
750 Hz	104.4 dB	104.1 dB	104.6 dB	92.9 dB	100.1 dB
800 Hz	96.9 dB	93.6 dB	94.6 dB	91 dB	92 dB

Recorded power of soundproofing methods during the lab trial to determine most effective soundproofing methods for use in the field.

Objective 3

Waveguide

All waveguides tested were effective in transferring sound when compared by ear, but methods of application for each differed. The need for either a wood puncturing tool or glue deemed the use of both the dowel rod and the metal tubing inappropriate since the puncturing tool would cause a large amount of disturbance and the glue would necessitate drying time and may not be effective if the weather conditions were wet. The insertion of nails and screws were similar in method, but with an electric drill being necessary in the field (for all logs when using a screw, and for intact logs when using the nail). When comparing the insertion methods, the tightness of the connection for the metal screw was deemed beneficial, leading to the choice of metal screws being used as waveguides. This choice supports our third hypothesis that screws would be the most feasible waveguide material, preventing us from rejecting the hypothesis for objective three.

The piezo element was attached to the screwhead with a magnet. The magnet was glued to the non-ceramic side of the piezo element, to not disturb the active portion of the element. This method of attachment allowed for the screw to be inserted into the log before attachment to the piezo element as well as quick attachment and removal of the sensor.

Arthropod sorting

Overall, we collected a total of 72,087 arthropods from 24 orders across the three experimental sites. Of the three sites Mississippi had the highest abundance of individual arthropods, accounting for 74.02% of the total number observed, followed by Honduras which accounted for 23.11% of the total, and finally Arizona which accounted for 2.87% of the total arthropods. Of the three sites, Honduras had the highest order richness, with 23 of the 24 orders appearing in all the samples. Mississippi had the second highest order richness, with 20 of the 24 orders appearing, followed by Arizona, with representatives from 13 of the 24 orders. The most common orders in both Honduras and Mississippi were Collembola, and both Oribatida mites and non-Oribatida mites (other mites hereafter). In Honduras, these three orders appeared in all 33 samples, and Hymenoptera was the second most common order, present in 32 out of 33 samples. Collembola, Oribatida mites and other mites also appeared in every sample in Mississippi, with Hymenoptera again being the second most common order, appearing in 33 or the 37 samples. Mites were the most common taxa in Arizona as well, with non-Oribatida mites present in all 33 samples, and Oribatida mites present in 29 of the 33 samples. Order Psocoptera was the second most common order, appearing in 27 of the 33 samples. Arizona was the only site that did not have order Collembola as one of the most common orders, as it only appeared in 19 of the 33 samples. Honduras had three unique orders not found in other sites: Dermaptera, Isopoda, and Trichoptera, each of which were found in one sample each. Order Orthoptera was unique to Mississippi. No orders were unique to Arizona.

The five most abundant orders across samples were Blattodea, Hymenoptera, Collembola, Oribatida, and other mites. These five orders accounted for 95.74% of the individuals present. The two most abundant orders present across samples were Blattodea

(41.42%) and Hymenoptera (17.17%). Order Collembola accounted for 15.42% of the total, Oribatida 14.04%, and other mites 7.68%. When looking at abundance by site, these five orders remained the top five most abundant for Mississippi and Honduras and accounted for 98.02% and 94.03% of total arthropod collected per site, respectively. The top five most abundant orders in Arizona samples did not include Blattodea and Hymenoptera, but instead contained orders Psocoptera and Diplopoda and accounted for 96.95% of total arthropod collected in Arizona (Figure 2.4).

Order Blattodea collected in samples belonged to epifamily Termitoidae, which includes termites. Epifamily Termitoidae was most abundant at the Mississippi, which contained 95.95% of Blattodea individuals in 59% of samples. Termites collected in Mississippi represented more than 23 times the amount of those collected in Hondruas, where they were present in 27% of samples. No termites were collected in Arizona. Order Hymenoptera occurred in all three sites, appearing in 89% of samples from Mississippi, 96% of samples from Honduras, and 6% of samples from Arizona. Hymenoptera collected in Honduras were 392 times greater than those collected in Arizona in terms of abundance, and the abundance of Hymenoptera collected in Mississippi was over 6 times greater than Honduras. Order Collembola was present in all Honduras and Mississippi samples, and 19 of 33 Arizona samples. The total number of Collembola collected in Mississippi outnumbered Honduras 2.7 fold and Arizona nearly 50 fold. Oribatida was present in all Honduras and Mississippi sites and 87% of samples from the Arizona site. Honduras contained the majority of Oribatida collected with abundance 2.5 times greater than that in Mississippi and 18.8 times greater than that in Arizona. Other mites was the only order present in all samples from all sites. Honduras also contained the majority of other mites, with samples containing 1.5 times more than Mississippi samples and approximately 6 times more than Arizona samples.

Figure 2.4 Most common five orders across field sites

Total counts of the top five arthropod orders of each site found in the subsample of the Arizona, Mississippi and Honduras.

Biotic diversity was calculated for each sample (n=103) and compared across sites using an ANOVA. Significant variation was found for Simpson's diversity index ($p < 0.0001$), Shannon's diversity index ($p < 0.0001$), and Richness ($p < 0.0001$). Following the significant ANOVA procedures, Tukey multiple pairwise-comparisons tests were conducted for each index to assess which sites were significantly different and make meaningful comparisons. Samples from Honduras contained the highest biodiversity across all three diversity measurements but did not significantly differ from Arizona in terms of Simpson's diversity index (*p* = 0.10) or from Mississippi in terms of order richness (*p* = 0.06) (Table 2.3). Arizona had the second highest diversity for both Simpson's and Shannon's diversity indices but did not significally differ from Mississippi for Shannon's diversity index (*p* = 0.51).

Table 2.2 Calculated means (± SD) for overall biodiversity by site accounting for the three biodiversity indices used

	Simpson's Diversity Index	Shannon's Diversity Index	Richness
	Mean: 0.431 ± 0.173	Mean: 1.143 ± 0.376	Mean: 8.184 ± 3.268
Arizona	0.423**a** ± 0.155	1.080**a** ± 0.366	4.640 ± 1.900
Honduras	0.342**a** ± 0.096	1.380 ± 0.221	10.500**a** ± 2.120
Mississippi	0.516 ± 0.201	0.991**a** ± 0.400	9.300**a** ± 2.320

Same letter following mean denotes lack of statistical difference between sites (based on a Tukey multiple pairwise-comparison) within a given index.

Objectives 4 & 5

Ecoacoustics

Results of ecoacoustic indices varied widely between tests and, through ANOVA tests, significant differences between sites with ACI (*p* value = 0.0001), entropy calculated for Shannon's calculation (*p* value = 0.0148), and frequency peaks (*p* value = 0.0029) were confirmed (Table 2.4). The ecoacoustic indices roughness and entropy calculated for Simpson's did not significantly differ across site (*p* values = 0.06 and 0.14 respectively) (Table 2.5).

Frequency peaks aligned well with observed order richness, with Honduras having the highest value (40.5 ± 3.89), followed by Mississippi (37.3 ± 5.33), and then by Arizona (37.2 ± 3.11). However, Mississippi and Arizona did not significantly differ based on a Tukey multiple pairwise-comparison (*p* value = 1.00). ACI also aligned well with observed richness, with Mississippi and Honduras having the two highest values which did not statistically differ from one another (*p* value = 0.09; 1925 ± 178.79, 1867 ± 59.05), followed by Arizona which did not statistically differ from Honduras (*p* value = 0.07; 1804 ± 38.28).

Table 2.3 Calculated means (± SD) for ecoacoustic indices by sitethat significantly differed across sites.

	ACI	Entropy - Shannon's	Frequency Peaks
	Mean: 1867.721 ± 123.95	Mean: 0.917 ± 0.025	Mean: 38.284 ± 4.491
Arizona	1804**a** ± 38.28	0.927**a** ± 0.10	37.2**a** ± 3.11
Honduras	1867**ab** ± 59.05	0.913**ab** ± 0.04	40.5 ± 3.89
Mississippi	1925**b** ± 178.79	0.911**b** ± 0.01	37.3**a** ± 5.33

Same letter following mean denotes lack of statistical difference between sites (based on a Tukey multiple pairwise-comparison) within a given index.

Table 2.4 Calculated means (± SD) for ecoacoustic indices by site that did not significantly differ across site.

	Roughness	Entropy - Simpson's
	Mean: 0.257 ± 0.103	Mean: 0.988 ± 0.008
Arizona	0.29 ± 0.106	0.990 ± 0.002
Honduras	0.25 ± 0.076	0.986 ± 0.014
Mississippi	0.23 ± 0.166	0.988 ± 0.001

The ANOVA conducted found no significant difference in means based on site.

Pearson's correlation analysis was calculated, by site, for each combination of biotic diversity index and ecoacoustic index. Significant correlations were only found in Mississippi data, with more significant correlations occurring with the one-minute length segments than with two-minute length segments (Table 2.6). Significant correlation occurred between order richness and roughness and entropy (Shannon's), and Shannon's diversity and roughness for one minute length recordings, and between order richness and entropy (Shannon's) and Shannon's diversity index and frequency peaks for two-minute length recordings. The correlation between frequency peaks and Shannon's traditional index analyzed based on two-minute length recordings was a positive correlation (r = 0.40, *p* value = 0.02). All other significant correlations observed presented as negative correlations. No significant correlations were found between the biodiversity and ecoacoustic indices of Honduras or Arizona sites (Tables A.2, A.3).

Table 2.5 Correlation values for one- and two-minute recording lengths at the Mississippi field site with the five tested ecoacoustic indices and three calculated biodiversity indices

		Mississippi					
		1 Minute			2 Minute		
		Simpson's	Shannon's	Richness	Simpson's	Shannon's	Richness
ACI	r	0.03	-0.02	0.29	0.08	-0.06	0.25
	p-value	0.85	0.89	0.09	0.64	0.72	0.14
Roughness	r	0.21	-0.41	-0.39	0.03	-0.26	-0.25
	p-value	0.22	0.02*	0.02*	0.85	0.14	0.16
Entropy – Simpson's	r	0.19	-0.20	-0.15	0.19	-0.24	0.08
	p-value	0.26	0.24	0.42	0.26	0.16	0.67
Entropy – Shannon's	r	0.10	-0.05	-0.41	0.19	-0.13	-0.34
	p-value	0.57	0.79	0.02*	0.26	0.46	0.05*
Frequency Peaks	r	-0.15	0.11	-0.07	-0.32	0.40	0.05
	p-value	0.37	0.53	0.70	0.05	0.02*	0.79

**p*-value < 0.05 ** *p*-value < 0.01

We rejected the hypothesis for our fourth objective based on the greater number of statistical tests with significant correlation found between ecoacoustic indices and traditional occuring in one-minute length recordings as opposed to two-minute length recordings. However, due to the presence of significance in Pearson's correlation tests we failed to reject our hypothesis for objective five

Discussion

Methods outlined in this study are yet another example of how broad the application and benefits of ecoacoustics are (Buxton et al., 2018; Maeder et al., 2019; Pieretti et al., 2011). Study of wood dwelling arthropods has long been burdened by the amount of time and destructive measures needed to examine them (Hammond, 1997; Wikars et al., 2005). Ecoacoustics offers a non-invasive method to gain knowledge about these arthropods. Given one-minute length recordings out-performed two-minute recordings, future applications could reduce total recording time and still expect to achieve quality results.

It would also be of interest to carry out similar analyses after performing a longer-term extraction for saproxylic invertebrates since the extraction methods used in this study were expedited. Arthropods essential to the soundscape may have been excluded from the diversity calculations based on sorted samples if the arthropods had not yet emerged by the end of the five-day extraction period. To date no published literature exists examining the sequence in which arthropods are collected during extraction from wood, and because of that it is not possible to estimate how much data may be missing from the five-day extraction period.

The use of a wooden box with the addition of a ceramic cup was a functional soundproofing method for this study. For future application of these methods further testing should be conducted since our preliminary testing was limited to mostly anecdotal conclusions as opposed to statistical. This is especially true since soundproofing methods appear to perform differently based on frequencies played. Frequencies of unwanted disturbances should be considered and kept in mind during testing soundproofing materials.

It is possible the insertion of waveguides by electric drill disturbed arthropod activity prior to recording. This could have affected acoustic arthropod activity, since insects such as termites may communicate vibrationally when disturbed or a threat is perceived (Hertel et al., 2011). There is also a chance that in the time between drill-caused disturbance and acoustic recording, arthropods may have exited the log due to the disturbance. While this would not affect correlation calculations between ecoacoustic and traditional indices since they would be absent during both recording and extraction, this may affect overall traditional biodiversity indices. When applying these methods in the future, it is advised to insert waveguides prior to the planned recording date, potentially even at the time of deployment of experimental logs. This would give time for arthropods to resume normal activity following the disturbance prior to recording.

Results from this study show promise for the future use of acoustics in the field of saproxylic invertebrates. Methods created and utilized for this study were moderately effective in modeling biotic diversity calculated from samples of saproxylic arthropods but showed limited utility due to the only significant results occurring from our Mississippi site. The most promising application for these ecoacoustic indices appears to be with order richness, since over 60% of the significant correlations occurred with order richness. ACI performed well in a similar study examining soil arthropod diversity, where it positively correlated with arthropod richness (Maeder et al., 2019), but did not perform as anticipated with our study. Instead, the ecoacoustic index of roughness performed well, as did acoustic entropy calculated to mimic Shannon's. Although when analyzed separately by site correlation values were only significant in Mississippi samples, this could be due to the higher sample size from the Mississippi site (n=37 vs n=33). Arizona samples had fewer arthropods present within the samples and, based on field listening, were the quietest of the sites. This could suggest that acoustic methods are more accurate with samples that have higher abundance of arthropods present.

Several avenues exist for additional research utilizing these methods. All samples utilized in this study used dead wood from pine trees (family Pinaceae, genus *Pinus*) as the substrate of interest. Using only pine wood neglects saproxylic arthropods that specialize on hardwood species (Stokland et al., 2012). Waveguides could be left in logs long-term to explore insect activity and colonization over the course of wood decay. Leaving waveguides intact for continued recording would allow study of the colonization of wood by arthropods in a way that has been impossible to this point due to the destructive nature of sampling. If the study were contained to a single geographic location, these monitoring methods could also explore effects of applied treatments over time, providing a timeline of effects on relative arthropod diversity throughout a plot or block.

These methods are also great candidates for machine learning methods. Many of the studies that have utilized ecoacoustics rely on combining of multiple indices, through use of machine learning techniques, to construct models with increase predictive power (Buxton et al., 2018; Gómez et al., 2018; Towsey et al., 2014). We plan to utilize machine learning in the future, with the subsample used for this manuscript as a training set and the samples that have not yet been analyzed as a test set.

Overall, methods presented in this thesis show potential but need improvement. The quick processing time of acoustic recordings could allow for expansion of studies into new regions, benefiting the field since to date most saproxylic insect studies take place in boreal and temperate forests (Seibold, Bässler, et al., 2015). Application of machine learning techniques could reveal better suited regressions, potentially allowing acoustic-based estimates of biodiversity with greatly reduced volumes of extracted samples needed for estimates, saving time and labor efforts. Additionally, ecoacoustic indices may perform better in different circumstances, such as different species or decay classes of wood. Ecoacoustics may also aid in sampling threatened habitats, where removal of wood could be detrimental to biodiversity, or in remote habitats where it is not feasible to remove the substrate of interest. However, more applied research is needed before ecoacoustic methods can be used reliable to estimate saproxylic arthropod diversity.

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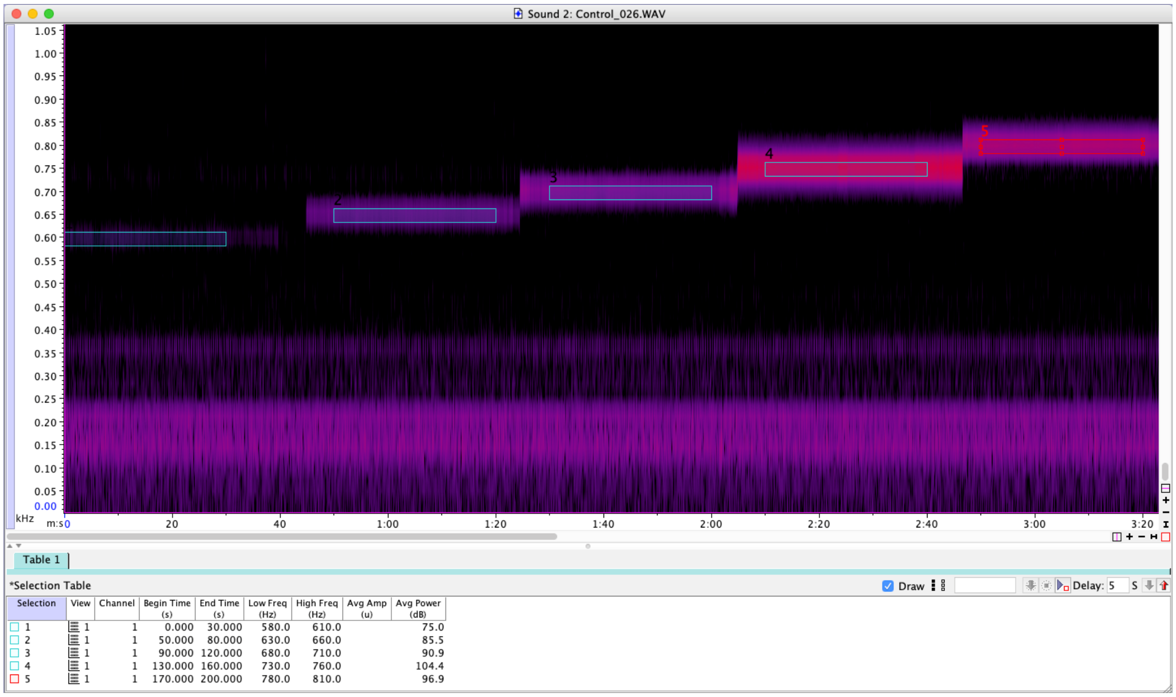
APPENDIX A

SUPPLEMENTARY TABLES AND FIGURES FOR CH. 2: UTILIZING ECOACOUSTIC METHODS TO EXAMINE SAPROXYLIC ARTHROPOD DIVERSITY

Table A.1 Product names and manufacturer information for equipment used. Equipment and products listed in order of mention in thesis

Product	Manufacturer	Manufacturer Location
12 mm 9kHz piezoelectric buzzer element	PUI Audio, Inc.	Dayton, OH
27 mm 4.6kHz piezoelectric buzzer element	CUI Devices	Lake Oswego, OR
24 AWG, Single Conductor Shielded Cable	Tensility International Corp	Redmond, OR
MacBook Pro (Retina, 15-inch, Mid 2015)	Apple Inc.	Cupertino, CA
Zoom F4 Field Recorder	Zoom North America	Hauppauge, NY
Neodymium Magnets; D6H1	K&J Magnetics, Inc.	Pipersville, PA
Whirl-Paks	Uline	Pleasant Prairie, WI



*Selection Table

Selection	View	Channel	Begin Time (s)	End Time (s)	Low Freq (Hz)	High Freq (Hz)	Avg Amp (u)	Avg Power (dB)
☐ 1	1	1	0.000	30.000	580.0	610.0		75.0
☐ 2	1	1	50.000	80.000	630.0	660.0		85.5
☐ 3	1	1	90.000	120.000	680.0	710.0		90.9
☐ 4	1	1	130.000	160.000	730.0	760.0		104.4
☐ 5	1	1	170.000	200.000	780.0	810.0		96.9

Figure A.1 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.

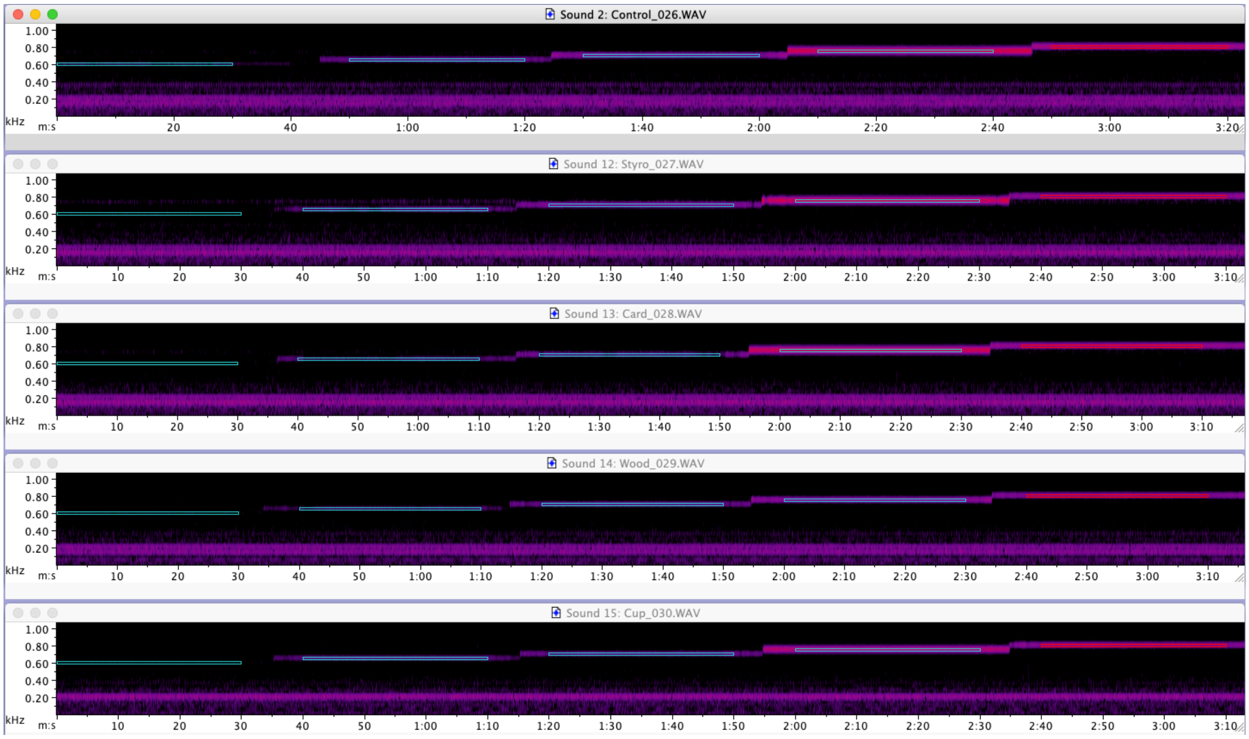


Figure A.2 Visual comparison of spectrograms from the five treatments tested in the lab soundproofing trials.

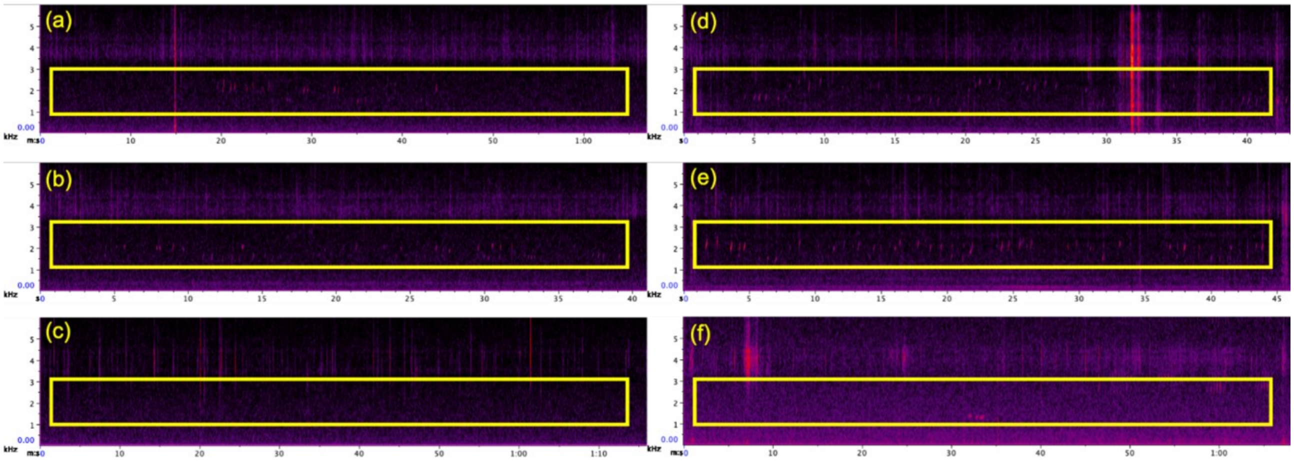


Figure A.3 Field trials for soundproofing methods.

Cardboard (a, d), Styrofoam (b, e), and a wooden box (c, f) were tested both on top of grass (a-c) and on top of pine needles (d-f). Target human made noises are highlighted with yellow boxes.

Table A.2 Correlation values for one- and two-minute recording lengths from the Arizona field site with the five tested ecoacoustic indices and three calculated biodiversity indices

		Arizona					
		1 Minute			2 Minute		
		Simpson's	Shannon's	Richness	Simpson's	Shannon's	Richness
ACI	r	-0.01	0.00	-0.02	-0.02	0.02	-0.01
	p-value	0.93	0.99	0.93	0.91	0.93	0.95
Roughness	r	-0.05	0.13	0.17	-0.05	0.13	0.17
	p-value	0.78	0.48	0.33	0.76	0.47	0.34
Entropy – Simpson's	r	0.01	0.09	0.20	0.01	0.09	0.19
	p-value	0.98	0.60	0.27	0.94	0.63	0.28
Entropy – Shannon's	r	-0.05	0.15	0.29	-0.04	0.13	0.28
	p-value	0.78	0.41	0.10	0.83	0.46	0.12
Frequency Peaks	r	0.21	-0.17	-0.05	0.14	-0.18	-0.21
	p-value	0.23	0.34	0.77	0.43	0.33	0.24

Table A.3 Correlation values for one- and two-minute recording lengths from the Honduras field site with the five tested ecoacoustic indices and three calculated biodiversity indices.

		Honduras					
		1 Minute			2 Minute		
		Simpson's	Shannon's	Richness	Simpson's	Shannon's	Richness
ACI	r	0.07	-0.08	0.16	0.11	-0.10	0.15
	p-value	0.72	0.67	0.37	0.53	0.56	0.40
Roughness	r	-0.29	0.30	-0.03	-0.28	0.30	-0.01
	p-value	0.10	0.09	0.86	0.11	0.09	0.95
Entropy – Simpson's	r	0.06	0.02	-0.03	0.04	0.03	-0.03
	p-value	0.75	0.93	0.87	0.83	0.85	0.89
Entropy – Shannon's	r	0.00	0.07	-0.01	-0.02	0.09	-0.01
	p-value	1.00	0.69	0.95	0.92	0.61	0.94
Frequency Peaks	r	-0.11	0.03	-0.14	-0.18	0.14	-0.06
	p-value	0.54	0.88	0.43	0.33	0.44	0.72