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Lonicera maackii alters decay dynamics of coarse woody debris

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LONICERA MAACKII ALTERS DECAY DYNAMICS OF COARSE WOODY DEBRIS

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by

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Michaela J. Woods ENTITLED *Lonicera maackii* alters decay dynamics of coarse woody debris BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

Woods, Michaela J. M.S., Department of Biological Sciences, Wright State University, 2018. *Lonicera maackii* alters decay dynamics of coarse woody debris.

Since industrialization, anthropogenic carbon emissions have led to excess atmospheric carbon dioxide that may alter the stability of ecosystem processes. Microorganisms are essential in mitigating excess carbon and play a notable role in the breakdown of organic material. This process, decomposition, is essential in forested ecosystems where microorganisms can recycle nutrients and store carbon in soil organic matter or release it through respiration. Fungi participate in decomposition through the release of enzymes responsible for carrying out the chemical reactions that break down plant material. Species introductions have the potential to alter decomposition dynamics. In the Midwestern US, the invasive shrub species *Lonicera maackii* has overtaken many forests and is likely altering decay dynamics and the destiny of carbon within the region. Thus, it is essential to monitor the decay of woody debris under invasion pressures of *L. maackii* in order to monitor nutrient cycling in this region. I placed blocks of native *Quercus rubra* and economically important *Pinus radiata* in an *L. maackii* invaded forest for one year to determine environmental, enzymatic and fungal drivers of decomposition. Decomposition was faster for oak wood than pine wood, and decomposition rate was not directly altered by *L. maackii*. Instead, *L. maackii* increased the moisture of the decomposing wood, leading to higher amounts of hydrolytic enzyme activity which

structured fungal communities within decaying wood. This insinuates that despite not altering decomposition rates directly, *L. maackii* is priming native woody debris for faster decomposition and therefore increasing the rate of nutrient turnover. Thus, *L. maackii* imposes shifts to fungal communities and their functionality and the soil environment. These changes could become especially important in later stage decay where there will likely be perceptible differences in decay rates as altered by *L. maackii*. The changes *L. maackii* imposes on decomposition will likely lead to faster carbon release from forested ecosystems and shorter retention times. Consequently, to ensure effective management strategies that mitigate excess carbon dioxide from the atmosphere, monitoring decomposition of woody material in invaded forests is imperative.

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INTRODUCTION

Anthropogenic carbon dioxide (CO_2) emissions induce a warmer climate globally by adding excess CO_2 into the atmosphere, leading to increased concern for the stability of ecosystem processes (IPCC 2014). In the span of 10 years, atmospheric CO_2 has risen 23 ppm to an average around 408 ppm in 2018 (Dr. Pieter Tans, NOAA/ESRL: www.esrl.noaa.gov/gmd/ccgg/trends and Dr. Ralph Keeling, Scripps Institution of Oceanography: www.scrippsco2.ucsd.edu). The role microorganisms play in mitigating this excess carbon is well documented (Allison et al., 2010; de Graaff et al., 2006; Lal, 2008; Singh et al., 2010) but their role in contributing to increases in atmospheric CO_2 is an area of developing research. In forest ecosystems, soil microorganisms play a particularly important role in the carbon cycle through the process of decomposition, which releases stored organic carbon via the breakdown of plant material. Outside of naturally occurring carbon release, additional carbon emissions from temperate forest soil processes have the potential to contribute 6.3 Gt of excess carbon to the atmosphere and therefore including them in carbon cycle modeling is important for accurate representations of the global carbon cycle (Guo and Gifford, 2002). However, achieving accurate estimates for soil carbon fluxes requires a global, systematic experiment controlling for confounding variables (Crowther et al., 2016). For my master's thesis, I quantified decomposition in an internationally available pine substrate. This research was part of a global project, with a local component including decay of an oak substrate

for paired decomposition measurements.

A key group of decomposers are fungi that live within the soil and break down plant material by releasing extracellular enzymes. The chemistry of decaying leaf litter or wood within forested ecosystems can alter fungal functionality, as can the living plant and animal communities within the environment. In Midwestern forests, there is a dense invasion of *Lonicera maackii* (Amur honeysuckle) which can greatly alter nutrient dynamics in the soil and understory. I monitored decomposition of angiosperm wood, *Quercus rubra* (red oak), in comparison to a gymnosperm wood, *Pinus radiata* (Monterey pine), in the Wright State woods to determine drivers of decay for coarse woody debris in a temperate Midwestern forest. I conducted a standard litter bag experiment and let wood decay for one year while monitoring canopy coverage. I measured decay rates, enzymatic activities, and fungal species within the decaying wood. Taken together, results from this work will be important for global models seeking to understand carbon dynamics but also for understanding the role *L. maackii* plays in altering decomposition of dead woody debris.

Background

Fungal Decomposer Guilds and Function

Multiple fungal guilds, or fungi grouped by similar functionality, can partake in decomposition. The first species to establish in leaf litter are typically foliar fungi, or endophytes, from leaves of the forest canopy. These fungi obligately inhabit leaves in part of their life cycle (Carroll, 1988). When leaves senesce, the role of endophytes switches to that of a saprophyte, where they begin decaying the plant material. Several months after senescence, the overall species composition in leaf litter shifts to fungi that

were already present in the soil (Song et al., 2016). Thus, the decomposer community will shift over time, progressing from soil fungal saprophytes in the spring to endophytes in the fall, which may establish in decomposing litter as leaves senesce. I expected endophyte establishment within decaying wood during the fall season of this study, with the majority of decomposition driven by saprophytes.

Saprophytes are the main contributors to decomposition, including a high number of fungi belonging to white rot and brown rot guilds. Unlike other decomposers, white rot breaks down the chemical compound lignin, a major component of woody materials (Zhang et al., 2016). In contrast, brown rot degrades two other large components of woody substrate, cellulose and hemicellulose, and can modify the structure of lignin (Ray et al., 2010). Together, these fungi decompose the majority of woody material or make it possible for other microbes to decompose the more labile substrates left behind. Species from these guilds are likely to be the most abundant in a survey of decomposer communities, though other fungi can participate in breaking down woody material using similar enzymes.

Fungi that demonstrate similar enzymatic activities used for decay include mycorrhizas and pathogens. In particular, ectomycorrhizal (ECM) fungi harbor saprotrophic functionality by secreting enzymes to facilitate their symbioses and perform nutrient exchanges (Courty et al., 2005). ECM fungi can change their function under varying nutrient availabilities by either emitting enzymes to obtain nutrients from plants in symbiosis, or by releasing enzymes to break down dead plant material for uptake of nutrients from decaying material (Bödeker et al., 2009).

ECM and saprophytic fungi demonstrate niche partitioning in the soil substrate, suggesting different strategies for degrading the same compounds and allowing for cohabitation in the same litter (Hobbie and Horton, 2007). Decaying wood can also harbor potential pathogenic fungi (van der Wal et al., 2017). While some pathogenic fungi obligately reside in living plants, others are facultative in that they can live in the soil or on decaying substrate using similar strategies as ECM and saprophytic fungi to participate in decomposition (Garbelotto, 2004). Together, these fungi are secreting extracellular enzymes to induce decay of plant material.

Fungal Enzyme Utilization

Enzymes that fungi use to break down woody substrate include phenol oxidase, peroxidase, β-glucosidase, acid phosphatase and leucine aminopeptidase. Phenol oxidase and peroxidase perform oxidative reactions, oxidizing lignin and phenolic compounds, respectively (Talbot et al., 2015). Leucine aminopeptidase is responsible for polypeptide degradation, while β-glucosidase and phosphatase are observed in cellulose degradation with phosphatase being more prevalent where phosphorous is limited (Pritsch et al., 2004; Talbot et al., 2015). White rot fungi produce higher amounts of phenol oxidase than ECM fungi and other saprophytes, and peroxidase is produced at similar rates among ECM fungi and saprophytes (Talbot et al., 2015). Saprophytes produce higher amounts of β-glucosidase and phosphatase than ECM fungi, while leucine amino peptidase is produced at a high rate by white rot and other saprophytes with little activity from ECM fungi (Talbot et al., 2015). This suggests that where there is a high prevalence of white rot fungi there will be more phenol oxidase and leucine aminopeptidase, and where there is more β-glucosidase, phosphatase, and peroxidase, there should be a higher

abundance of saprophyte. Since enzymes facilitate decomposition, the quantity of enzymes present in substrate should correlate to the decomposition rate of the substrate. Indeed, in an experiment conducted on woody decay of 13 tree species, enzyme quantities were one of the strongest predictors of wood decay (Kahl et al., 2017).

Plant Species and Effect on Decomposition

Characteristics of Decomposition with Angiosperm and Gymnosperm Coarse Woody Debris

Decomposition of woody substrates occurs at different rates due to variation in their chemical composition. In a meta-analysis of wood decomposition rates, angiosperms decayed faster than gymnosperms despite the lower density of gymnosperm wood, possibly due to higher lignin concentrations in gymnosperms (Weedon et al., 2009). The organic polymer lignin is found in woody litter where it protects cellulose and hemicellulose from the enzymatic processes of most decomposers (Kirk and Farrell, 1987). Since *Quercus spp.* have lower levels of lignin than *Pinus spp.* wood (Rahman et al., 2013), *Quercus spp.* decomposition should occur at a faster rate than *Pinus spp.* decomposition.

The living organisms involved in decomposition also vary with species of woody substrate. While there is generally a similar biomass of fungal decomposers in angiosperm and gymnosperm wood, the community composition between these two wood types tend to be distinct. Previous studies have shown a greater prevalence of ascomycetes and endophytes on angiosperms and a higher prevalence of *Boletales*, which are typically ECM, on gymnosperm wood (Heilmann-Clausen et al., 2016). Additionally, more white rot and phenol oxidase will likely be present on *Pinus spp.* as these fungi are

well equipped to decay lignin. Introduced non-native species will change forest composition and the chemistry of introduced leaf litter will likely result in changes to the fungal communities.

Decomposition in the Presence of Invasive L. maackii

Invasive species alter many ecosystem processes using a variety of mechanisms (Ehrenfeld, 2010). *L. maackii*, an invasive shrub, is a dominant component of the understory in southwestern Ohio (Runkle et al., 2005). This shrub has negative effects on native plants including reducing their overall size, decreasing their flowering potential, and stopping plant growth completely (Miller and Gorchov, 2004). It can also increase soil carbon and nitrogen, changing microbial ecosystems and nutrient availabilities (Kolbe et al., 2015). *L. maackii* has the potential to release allelopathic chemicals belowground that change many biotic and abiotic factors of the soil and directly reduce the fitness of neighboring plants (Cipollini et al., 2008). Since *L. maackii* retains its leaves for a longer period of time than native plants, it reduces the amount of rain water that could saturate the forest floor, causing shifts in soil moisture with higher cation concentration and longer deposition of nutrients in the soil that would normally be washed away (McEwan et al., 2012). The measured soil moisture beneath honeysuckle shrubs is largely context dependent, however (Hartman and McCarthy, 2004; Kolbe et al., 2015; Pfeiffer and Gorchov, 2015; Woods et al. *in prep*)

In addition to altering the soil environment, *L. maackii* also changes decomposition rates through the input of its herbaceous litter. Across species, decomposition rates of invasive herbaceous litter is 117% higher than native species' herbaceous litter (Liao et al., 2008). Limited work examining the role *L. maackii* plays in

altering decomposition in forest ecosystems suggests *L. maackii* litter degrades more slowly directly underneath its shrubs than when placed further away from its shrubs (Arthur et al., 2012). Furthermore, litter from *L. maackii* exhibits higher decomposition rates by itself, but it does not synergistically increase decomposition rates of herbaceous litter from native trees when incubated together (Poulette and Arthur 2012). Despite these important effects on herbaceous litter decomposition, no published studies have examined how its own woody material decays, or how its herbaceous litter may shift decay of native wood. This is particularly important because *L. maackii* is likely introducing alternate nutrients and microorganisms into the soil community as well as the community residing in decaying wood within the ecosystem.

Despite the important role fungi play in decomposition, there is not a definitive explanation for how *L. maackii* influences fungal decomposer communities. Preliminary work indicates a higher concentration of endophytic fungi present in the *L. maackii* leaves that began the process of decay during senescence before exposure to soil fungi; however, the total fungal biomass was the same overall (Arthur et al., 2012). This study was limited in that it used an older technique to measure fungal biomass, phospholipid fatty acid analysis, which is limited in its ability to provide species level community information. Further studies that utilize current technologies to quantify shifts in the soil microbial community due to the presence of *L. maackii* are needed in order to fully elucidate its effects on overall ecosystem function.

Insect Alteration of Decomposition

Insects make up a large portion of fauna living within surface soil in forest ecosystems and are critically important in ecosystem processes including soil formation,

nutrient cycling and decomposition (Lavelle et al., 2006). Therefore understanding their role in decomposition is necessary to completely understand the processes occurring. Termites, in particular, are known for their ability to break down woody material. They have high activity in arid and semi-arid environments, such as tropical habitats, savannas, and deserts, and their mounds create a shift in local microbial communities (Jouquet et al., 2011). For example, insects can promote microbial decomposition by creating tunnels in the wood that increase the surface area for establishment of fungi and by carrying soil containing fungi (Ulyshen, 2016). Despite this importance, how insects contribute to overall decomposition rates of coarse woody debris is unclear. No difference to decomposing wood volume occurred when comparing specimens that were open to insects, partially excluded insects, or completely excluded insects, suggesting that insects may inhibit microbial decay and therefore leave the overall decomposition rate unchanged (Ulyshen and Wagner, 2014). Alternatively, insects could also increase decomposition rates by consuming fungal mycelia which causes saprophytes to increase their enzyme excretion (A'Bear et al., 2014a). In whole, insects may be an important driver of decay.

Specific Research Aims:

The overall goal of my MS thesis is to understand drivers of wood decay in a temperate deciduous forest. To do so, I addressed four specific research aims:

1. Quantify decomposition rate using wood mass loss as a proxy.
2. Identify which species of fungi are present during decay.
3. Quantify decomposition rates as a function of fungal enzymatic activity based on hydrolytic and oxidative enzymes.

4. Quantify effect of insects on decomposition.

METHODS

Global Survey

This study was conducted as part of a global collaboration, Global Decay, led by Dr. Amy Zanne (George Washington University). For this experiment, I followed the standardized protocol of the global study for deployment of wooden baits (Cheesman et al., 2018). To ensure comparable data and results with the global study, *P. radiata* was used as the substrate bait. *P. radiata* is endemic to western North America but is now restricted to five isolated populations in California (USA) and two islands off Baja California (Mexico; Axelrod, 1986; Grotkopp et al., 2004) despite this restriction in its natural range, *P. radiata* has been widely planted as a timber species across many of world's Mediterranean climates, including Chile, New Zealand, Australia, Spain, South Africa, Ecuador, Italy and Argentina (Mead, 2013). The global distribution of *P. radiata* further emphasizes the utility of understanding its decay dynamics across a variety of ecosystems. Furthermore, *P. radiata* has a consistent carbon concentration throughout decomposition, with 50% mass loss accounting for 49% carbon loss, making mass differences an appropriate proxy for carbon loss when this species is used as a substrate (Ganjegunte et al., 2004). Taken together, these two attributes make *P. radiata* an ideal species for understanding global patterns of decay.

Sample Sites

This study took place in the Wright State University's (WSU) Woods in Dayton, Ohio (39.785253 ºN, 84.05424 ºW) from April 2017 to April 2018. This area received 128.016 cm of precipitation in 2017 with an average high temperature of 28.89 ℃ in the summer of 2017 and a low of 3.89 ℃ in the winter of 2018 (Wright Patterson Air Force Base Weather Station). WSU's woods are dominated by oak and maple with no natural conifers (Runkle et al., 2005). *L. maackii* is found at varying densities throughout the woods at WSU and scales in growth from small shrubs to thicker more tree-like plants (Runkle, *personal observation*).

Transects

To evaluate differences in decomposition among *L. maackii* invasion status, I placed two transects of wooden block bait stations in an E-W orientation in the WSU woods. Bait stations were covered with 70% green shade cloth (Easy Gardener, Waco, TX, USA) to reduce UV exposure. Each station consisted of two baits of *P. radiata* (one insect partial exclosure and one complete exclosure) and one bait of *Q. rubra* (complete insect exclosure) per shade-cloth cover (Figure 1). Transect one was placed in a densely invaded area of *L. maackii* that had more bait stations within one square meter of a *L. maackii* shrub than transect two. Transects were 95 m in length with 20 bait stations pegged to the ground with tent stakes every 5 m along the transect. I placed stations directly on the

Figure 1: Bait station with one *Q. rubra* bait and two *P. radiata* blocks, one an insect partial exclosure. (The three other blocks will be examined another year past this experimental time frame.)

transect unless there was coarse woody debris, exposed rocks, or substantial water flow in the location of a station, then stations were placed 0.5 m or less from the transect. While placing the baits, leaf litter was removed from the forest floor and the duff layer was homogenized through scraping. In the event of coarse woody debris falling onto bait stations or water displacing bait stations over the course of the project, stations were left intact at their original location since these events are natural occurrences which happen in forest ecosystems during decay.

Wood Baits and Bait Stations

Each bait station along each transect contained two wooden baits of *P. radiata* (hereafter referred to as pine wood) and one bait of *Q. rubra* (hereafter referred to as oak wood). Planed and untreated baits were purchased from Home Depot. Each bait had dimensions of 2.9 cm X 8.8 cm X 12.7 cm and 1.905 cm X 8.89 cm X 12.7 cm respectively. To remove phenolic volatiles which are otherwise unpalatable to certain genera of nasute termites, baits were dried at 120 ^{o}C for 48 hours and then cooled to room temperature in a lab environment before deployment. Prior to deployment, each bait was weighed for future calculation of decomposition rates and placed into 300 μm nylon mesh (Industrial Netting Inc, Minneapolis, MN) with the edges rolled and stapled to create a standard litter bag (Figure 2). Forty bags used for decay of pine wood had 10 holes 5-7 mm in diameter on the underside to allow for termite access ('partial insect exclosures').

Figure 2: Nylon bag for wooden baits

Data Collection

To quantify the density and size of *L. maackii* along each transect, each shrub growing within a meter radius of each bait station was counted and basal diameter recorded. From this, basal radius was used to calculate basal area by squaring the radius and multiplying by π. If no shrubs were present in a square meter radius, an absence was recorded for that bait station. Light availability for each bait station was assessed using fish eye lens photos of the tree canopy taken monthly at each station (Figure 3) and processed with Gap Light Analyzer (GLA; Simon Fraser University, Cary Institute of Ecosystem Studies).

Figure 3: Photo of forest canopy to be analyzed with GLA.

Attack score	Termite attack	Fungal attack
0 (sound wood)	No perceptible termite attack	No perceptible fungal attack or softening
1 (perceptible but very limited changes)	Very superficial deterioration to 1–2 mm in depth at some points or over several cm^2	Discoloration and very superficial degradation or softening up to 1 mm in depth
2 (clear changes to a moderate extent)	Damage from 2 to 5 mm in depth over several cm^2, or with scattered points down to a depth exceeding 5 mm, or by different combinations of the two types	Softening to a depth of 2–3 mm deep over all or part of the stake
3 (severe changes)	Extended and deep destruction from 5 to 10 mm in depth, or tunnels reaching the centre of the stake, or by different combinations of the two	Marked decay in wood to a depth of 3–5 mm over a wide surface area or by softening to a greater depth (10–15 mm) over a small area
4 (breakage of the stake while up-rooting prior to inspection)	Breakage due to extent of termite tunnelling	Breakage due to fungal attack and softening

Table 1: Insect and fungal damage assessment (Davies et al., 1999)

One half of the baits were collected one year after deployment (20 of each treatment from each transect, 120 baits total). Each bait was removed from the ground and placed into a zip lock plastic bag and stored at -20 ºC until processed, between one and three weeks after retrieval. For processing, baits were removed from nylon bags and excess soil was eliminated. Insect and fungal damage were then identified using standard assessment methods (Table 1, Davies et al. 1999). A power drill with a 3/8-inch drill bit was used to extract approximately 5 g wet weight of saw dust from each bait. Half of the saw dust (~2.5 g) was stored at -20 ºC for enzyme reactions and half was stored at -80 ºC for DNA extractions. The wet mass of the saw dust extracted from each bait as well as the mass of each bait after it was drilled was recorded. Drilled baits were then dried at 105 ºC for 72 hours, cooled to room temperature and weighed for their final dry mass. The percent moisture of each drilled bait was calculated using the wet weight of the drilled bait and its dry weight. Using the percent moisture, the dry weight of the extracted saw dust was calculated and added to the dry weight of the bait to which it belonged to account for the total dry weight after decay.

Decomposition Rate

Decomposition rate (*k)* was assessed as a function of collection time in years (*t*), initial dry mass of substrate (W_o) and dry mass at collection time (W_t; Equation 1; Olson 1963).

$$k = -\frac{\ln\left(\frac{W_t}{W_o}\right)}{t}$$

Equation 1

Fungal Sequencing

DNA was extracted in triplicate from each bait (120 baits X 3 extractions) using 50 mg dry weight of saw dust with a Qiagen PowerPlant Pro Kit (Qiagen, Carlsbad, CA) following standard protocol. DNA was quantified using a Qubit 3.0 dsDNA BR assay (ThermoFisher, Waltham, MA), and quality was assessed using a Nanodrop One Microvolume UV-Vis Spectrophotometer (ThermoFisher, Waltham, MA). DNA was considered sufficient quantity when extractions were greater than 20 ng/ml. DNA was then stored at -80 ºC for up to three weeks, pooled, and cleaned using DNeasy PowerCleanPro Kit (Qiagen, Carlsbad, CA) two times with two additional ethanol wash steps using ethanol at 4 ºC. Cleaned DNA was diluted to 5 ng/ul using molecular grade water and loaded randomly into 96 well plates. Each sample was loaded into the plates in triplicate with samples from each bait station loaded onto the same plate. Plates were sealed and stored at -80 ºC until hand delivery to the Ohio State University Molecular and Cellular Imaging Center (MCIC; Wooster, OH) for MiSeq library preparation with the ITS1F and ITS2R region amplified.

Amplification of the ITS1 locus was completed with unique Nextera indices for sample indexing. Samples were amplified in two rounds of PCR with the initial round attaching the Illumina adapted sequence, and the second round to complete the sequence. PCR was conducted on Eppendorf epMotion5075 (Hauppauge, NY) with initial denaturation at 96 ºC for three minutes, then 25 cycles between 96 ºC for 30 seconds of denaturation, 55 ºC for 30 seconds of annealing, and 72 ºC for 30 seconds of elongation. Samples were then cleaned with Agencourt AMPure XP beads (Beckman Coulter Life Sciences, Indianapolis, IL) at room temperature where samples were incubated with

beads for five minutes and then placed on the magnetic stand for three minutes. Supernatant was removed from samples, and then samples were washed with 200 µl of 80% ethanol, incubated for 30 seconds and removed. This step was repeated once more. Beads were then air dried and removed from magnet. Thirteen µl of nuclease free water was used to suspend beads which were incubated for two minutes and then supernatant transferred to a new plate without any beads. PCR was repeated with the same conditions as the first round, but with 8 cycles instead of 25. Amplification of the ITS region was verified with gel electrophoresis. Samples were cleaned again following the same protocol as the first cleaning, and then were assessed for primer dimers using the Pippen Prep size selection (Sage Science, Beverly, MA) by running on 1.5% agarose gel. If there were primer dimers present, a cleaning step was repeated. Before sequencing, purified amplicon libraries were pooled in equimolar ratios.

Sequencing was performed on the Illumina MiSeq platform with PhiX mixed in the amplicon libraries to a final concentration at 14.3 pM. Libraries were sequences using 300PE MiSeq kit with standard Illumina primers and the run was clustered to 681 ± k/mm^2. The MiSeq instrument also conducted image analysis, base calling, and quality assessment. Finally, sequences were demultiplexed and adapters removed.

Bioinformatics

I analyzed sequences using the bioinformatics pipeline Quantitative Insights Into Microbial Ecology 2 (QIIME2; http://qiime2.org; Caporaso et al. 2010). Heterogeneity spacers were removed from the sequences with *cutadapt trim-paired* function in *cutadapt* (Martin, 2011), then sequences were denoised, chimeras removed, paired ends merged, and exact sequence variants (ESV) were identified with the *dada2 denoise-paired*

function of *DADA2* (Callahan et al., 2016). ESVs were used as a proxy for fungal species. Replicates of each sample were pooled into one sample using *feature-table group* function from *feature-table*. I assigned taxonomy to ESVs using 0.70 confidence threshold in *feature-classifier classify-sklearn* (Pedregosa et al., 2011). RDP classifier was trained with UNITE v. 2.3 database (https://doi.org/10.15156/BIO/587481; Koljalg et al. 2005) using *feature-classifier fit-classifier-naïve-bayes* function from *feature-classifier* to determine sequence identity based on 100% similarity as ESVs. After removing sequences which were not assigned to at least phylum level, sequences were rarefied to the least amount of sequences per sample using *feature-table rarefy* from *feature-table* for standardized values in statistical analyses. ESVs were then classified into functional guild using an online version of FUNGuild (http://funguild.org, accessed June 5, 2018; (Nguyen et al., 2016). All ESVs were parsed into functional guild. If ESVs were parsed into multiple guilds, sequences were assigned proportionally to each guild they represented. To determine richness of fungal communities at the ESV and the functional guild levels of each block, I calculated Shannon's diversity using *diversity* function, observed richness using *specnumber* function, and Chao1 richness using *specpool* function, each from the *vegan* package in R v 3.5.1 (Oksanen et al., 2017; R Core Team, 2018).

Enzyme Assays

To assess microbial function, the enzymes phenol oxidase, peroxidase, β-glucosidase phosphatase, and leucine-aminopeptidase were assayed to find their maximum potential activities. Each assay was conducted using homogenate slurries made with saw dust and 50 mM sodium acetate buffer at pH of 5.6 and were incubated in the

dark at room temperature (~22 °C). Incubation times were determined by measuring activities incrementally until the maximum potential enzymatic activity was attained. Assay absorbance and fluorescence values were measured using a BioTek Synergy HT microplate reader (BioTek, Winookski, VT, USA).

The oxidative reactions of phenol oxidase and peroxidase were conducted using of 3,4-Dihydroxyphenylalanine (L-DOPA). Each oxidative assay included eight replicates of each of the following: substrate blank, buffer blank, wood homogenate blank, and wood homogenate with substrate for each sample. The phenol oxidase reaction required 50 µl of a 25 µM L-DOPA mixed with 50 mg dry weight of each sample and 15 ml of sodium acetate buffer and were incubated for 5-7 days. Several samples required only 5 days before reaching peak activity, yet most required a full 7 days for peak reactive activity. Peroxidase assays also required 50 µl of a 25 µM solution of L-DOPA as well as 10 µl of 3% hydrogen peroxide but was instead mixed with 100 mg dry weight of each sample in 15 ml of sodium acetate buffer. The buffer and sample were incubated at 4 ℃ while shaking on a Labline 3520 orbital shaker (Marshall Scientific, Hampton, NH, USA) for 16 hours prior to use in reaction. Peroxidase activity reached maximum potential after 24 hours of incubation. After absorbance measurement on microplate reader at 450 nm, enzyme activities were calculated in µmol h^{-1} g^{-1}.

Hydrolytic enzyme reactions of β-glucosidase, phosphatase and leucine aminopeptidase were conducted using 50 mg dry weight of saw dust in 15 ml of 50 mM sodium acetate buffer incubated at room temperature. Each assay included eight replicates of a substrate blank, buffer blank, wood homogenate blank, and wood homogenate reaction as well as standard curves of either methylcoumarin for leucine

aminopeptidase or methylumbelliferyl for β-glucosidase or phosphatase with each wood sample and with buffer. β-glucosidase was assayed using MUB linked substrate, 100 µM MUB β-D-glycopyranoside, and was incubated at 22 ℃ for 30 minutes in the dark. Phosphatase was assayed with 250 µM MUB-phosphate and incubated in the dark at 22 ℃ for 1 hour. Leucine aminopeptidase was assayed using methylcoumarin linked substrate, 100 µM L-Leucine-7-amido-4-methylcoumarin hydrochloride and incubated in the dark at 22℃ for four days. After incubation of fluorescent assays, 10 µl of 1M NaOH was added to each well and plates were then read at 360 nm excitation and 450 nm emission on microplate reader 10 minutes after addition. Using a standard curve, excitation coefficients and fluorescent measurements, enzyme activities were calculated in µmol h^{-1} g^{-1}.

Statistical Analysis

Data Transformations

Prior to analysis, all variables were tested for normality. *L. maackii* was represented by two metrics: abundance (count of *L. maackii* per meter radius from bait) and average basal area of shrubs within a square meter radius (cm^2/m^2). *L. maackii* abundance and *L. maackii* basal area were square root transformed for normality. Canopy coverage was measured monthly and then averaged to accommodate variability throughout the year. Abiotic variables of slope (degree of elevation facing East), canopy coverage (% cover) and bait moisture (% water content) were normal without transformation. Phosphatase activity was normal without transformation. β-glucosidase, phenol oxidase and leucine amino peptidase activities were square root transformed for normality. Peroxidase was zero inflated with over dispersion and was represented by

generalized linear models with a negative binomial distribution. Shannon diversity, Chao1, and observed abundance of guilds and Shannon diversity and Chao1 of ESVs were normal, while observed richness of ESVs required log transformation. All analyses were conducted in the statistical programming environment R v.3.5.1 (R Core Team, 2018). All statistics were visualized using *ggplot2* unless otherwise noted (Wickham, 2009).

To account for differences in the amount of *L. maackii* along each transect, I conducted two sample t-tests examining *L. maackii* abundance and basal area. In the same fashion, I performed two sample t-tests to determine differences between canopy coverage, slope and bait moisture across both transects. T-tests were created using *lm* function from the *base* package (R Core Team, 2018).

Linear regressions were used to determine direct relationships between *L. maackii* parameters and abiotic factors. Thus, separate linear regressions were conducted for *L. maackii* basal area and *L. maackii* abundance, both as functions of canopy coverage, slope, and bait moisture. Interactions between abiotic factors were tested using ANOVA and Tukey's post hoc analysis with function *lsmeans* from *emmeans* (Lenth, 2018).

Decay rates and enzyme activities

Decay rates were zero inflated without over dispersion and were best represented by a Poisson distribution. To evaluate if *L. maackii* size or abundance affected decay rates, I used a generalized linear model with logit link and the *glm* function from the *stats* package. To determine if *L. maackii* size or abundance influences enzyme activities, variables were subject linear models using the *lm* function from the *stats* package. To inform if there were differences between decay rates and enzyme activities across

transects, data were assessed individually with a two-sample t-tests. I used a generalized linear model with a logit link to assess if abiotic factors (wood type, moisture, slope, canopy coverage) affected decay rates, and a linear model for enzyme activities as a function of the measured abiotic factors. Interactions between abiotic factors were also tested using ANOVA and Tukey's post hoc analysis. Finally, to determine if decay rates varied by enzyme activities, variables were subject to generalized linear models with logit link.

Fungal community: univariate

To determine if richness (Shannon, Chao1, observed) of ESVs were altered by abiotic variables, I used a linear regression with canopy coverage, slope, moisture, and wood type as predictor variables. To assess the effect of abiotic variables on abundance of fungal guilds (endophyte, mycorrhizae, pathogen, saprophyte), I used generalized linear models with logit links. Similarly, linear models were used to examine possible relationships between richness and *L. maackii* abundance, size, and transect and generalized linear models to examine relationships between guild abundance with *L. maackii* abundance, size, and transect. To evaluate the role fungal richness and communities play in altering decay rates, generalized linear models with logit links were applied. To evaluate the role fungal richness and communities play in altering enzyme activities, linear models were used.

Abiotic variables: multivariate

Principal component analysis (PCA) was applied to condense enzyme activities into linear principal components (PC) to determine if there was any grouping in space corresponding to wood type or transect using *prcomp* from the *stats* package (R Core

Team, 2018). Similarly, I applied PCA to the abiotic variables to examine possible grouping in space by the same predictors. To visualize PCAs, I used g*gplot2* and *ggbiplot* (Vu, 2011) with groupings based on a 95% confidence interval. To evaluate if any grouping in space was due to the predictor variables, I used analysis of similarity with the *ANOSIM* function with 999 permutations from the *vegan* package (Oksanen et al., 2017).

Fungal communities: multivariate

Differences in fungal communities at the family and guild level by *L. maackii* presence, transect, and wood type were visualized in two ways: stacked bar charts and non-metric multidimensional scaling (NMDS). Stacked bar charts for relative abundance values were created using *melt* function from *data.table* package (Dowle and Srinivasan, 2018) and visualized in *ggplot* (Wickham, 2009). NMDS was used to condense fungal communities at the ESV, family and the guild levels into linear components with *metaMDS* from *vegan* (Oksanen et al., 2017). I used *ANOSIM* to determine if grouping in space was significant by predictor variables, and to parse out which species were driving differences, I used *SIMPER* from the *vegan* package.

Canonical correspondence analysis was used to compare a matrix consisting of β-glucosidase, phenol oxidase, and leucine amino peptidase activities to the abiotic matrix including slope, canopy coverage, and block moisture using *cca* in *vegan* (Oksanen et al., 2017). Peroxidase was excluded from this analysis since 56 out of 120 samples with zero activity, as these reported zeros could not be used in the CCA matrix without eliminating data from each other variable. To determine if there were significant relationships between these matrices, ANOVA was performed using the *anova.cca* function from

vegan package with 999 permutations. *Ggplot2* was used to visualize groupings by either transect, wood type, or *L. maackii* presence using a 95% confidence interval.

RESULTS

Environmental characteristics and *L. maackii* metrics

Transect two had more abundant *L. maackii* overall ($F_{1,38} = 4.169$, $P = 0.048$, $R^2 = 0.075$) with larger and more variable basal area than transect one ($F_{1,38} = 8.72$, $P = 0.008$, $R^2 = 0.15$; Figure 4). Neither bait moisture ($P > 0.05$), slope ($P > 0.05$), nor canopy coverage ($P > 0.05$) differed between transects. Thus, the variable transect can be used to represent differences in *L. maackii* abundance and size alone.

Bait moisture was higher in oak wood than in pine wood ($P < 0.001$, $F_{2,117} = 37.28$; Figure 5). Bait moisture was weakly and negatively correlated with canopy coverage ($P = 0.031$, $F_{2,118} = 4.793$, $R^2 = 0.04$; Figure 5), without interactions with either *L. maackii* size ($P > 0.05$) or *L. maackii* abundance ($P > 0.05$). Bait moisture increased with *L. maackii* size alone ($P = 0.044$, $F_{2,118} = 4.11$, $R^2 = 0.03$; Figure 6), but not with *L. maackii* abundance ($P > 0.05$). Slope had no effect on moisture of the baits ($P > 0.05$) or *L. maackii* basal area or abundance ($P > 0.05$). *L. maackii* variables were also not correlated to canopy coverage ($P > 0.05$).

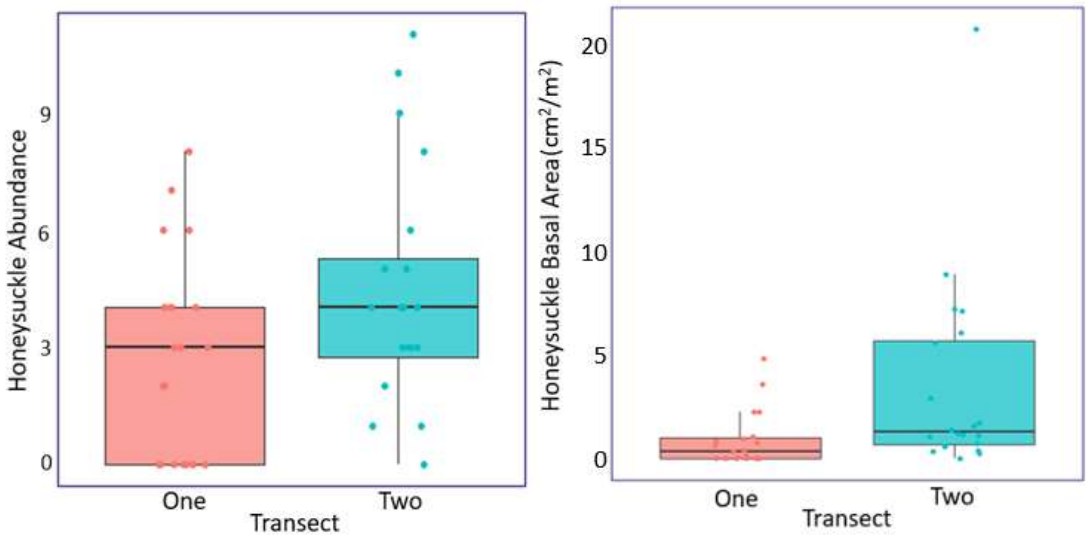

Figure 4. Boxplot representing differences between *L. maackii* abundance and *L. maackii* basal area between transect one and two. Transect one had a lower abundance of *L. maackii* (P = 0.048) and *L. maackii* was larger with more variation on transect two (P = 0.008).

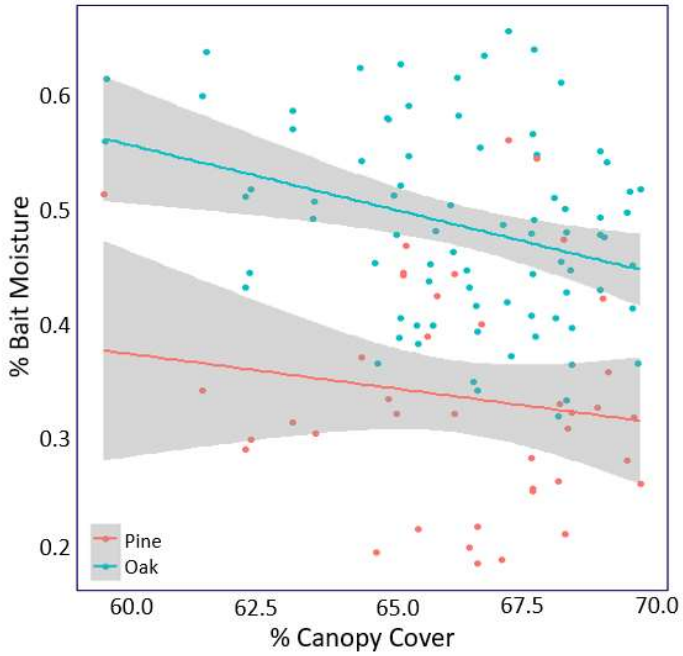

Figure 5. Linear regression of percent moisture of blocks by percent canopy coverage colored by wood type with pink representing pine wood and blue representing oak wood (P = 0.031, R^2 = 0.04). Shaded area represents 95% confidence interval.

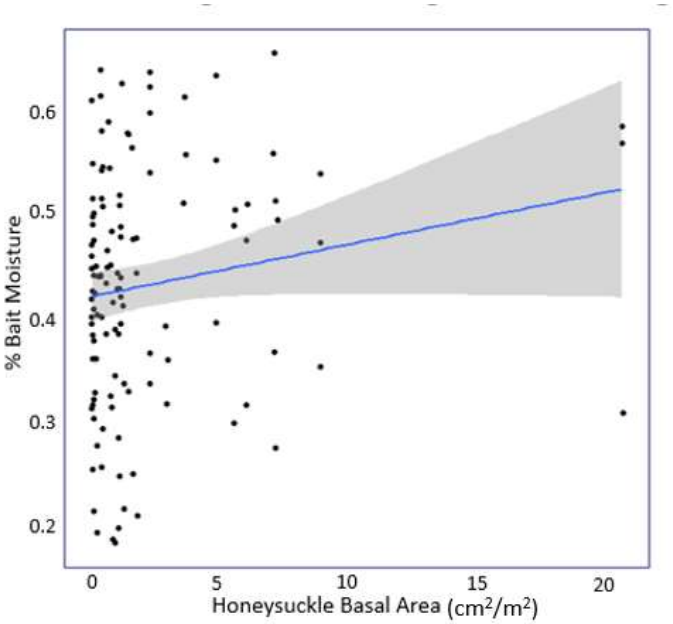

Figure 6. Linear regression of percent moisture of blocks by *L. maackii* basal area (P = 0.044, R^2 = 0.03). Shaded area is 95% confidence interval.

Decay rates by *L. maackii* and environmental characteristics

Neither *L. maackii* abundance (P > 0.05), size (P > 0.05) nor presence (P > 0.05) predicted decay rates. Oak wood decomposition rate ($\bar{x}$ = 0.019) was higher than pine wood decomposition rate ($\bar{x}$ = 0.007) overall ($F_{1,118}$ = 3.84, P = 0.052, R^2 = 0.02), regardless of insect access (P > 0.05). There was no significant relationship between decay rates and bait moisture (P > 0.05), canopy coverage (P > 0.05), or slope (P > 0.05).

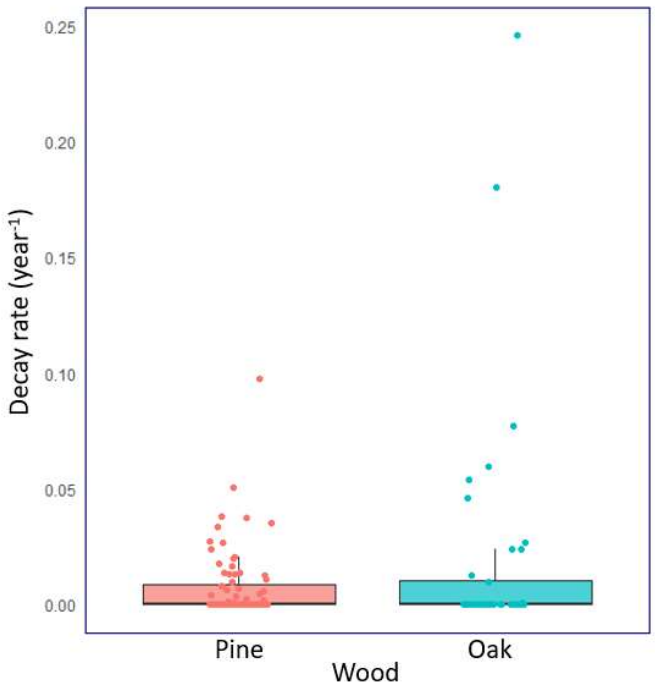

Figure 7. Box plot representing decay rates by wood type with pine wood in pink and oak wood in blue where oak wood decayed faster than pine wood (P = 0.052).

Enzyme activities as predicted by *L. maackii* and environmental characteristics

β-glucosidase ($F_{1,118}$ = 11.99, P < 0.001, R^2 = 0.09) and phosphatase activities (F_{118} = 4.402, P = 0.038, R^2 = 0.03; Figure 8) increased with increases in *L. maackii* basal area. Phosphatase activity was lower on pine wood on transect one than transect two but was the same on oak wood despite transect placement ($F_{1,116}$ = 10.08, P = 0.003, R^2 = 0.19; Figure 9A). There was no relationship between β-glucosidase activity and transect (P > 0.05), or *L. maackii* abundance (P > 0.05). Leucine aminopeptidase activity was driven by an interaction between the transect and the wood type, such that pine wood on transect two had higher activities than pine wood on transect one, regardless of insect access, and pine wood had higher activities than oak wood on both transects ($F_{5,107}$ = 6.903, P = 0.002; Figure 9B). Leucine aminopeptidase activity was not explained by *L. maackii* abundance (P > 0.05), size (P > 0.05), or presence (P > 0.05). Phenol oxidase

activities were related to the interaction between wood type and transect ($F_{2,112} = 15.15$, $P < 0.001$; Figure 10), where pine wood, regardless of insect access, was not significantly different between transects, but oak wood had higher phenol oxidase activity on transect one than on transect two.

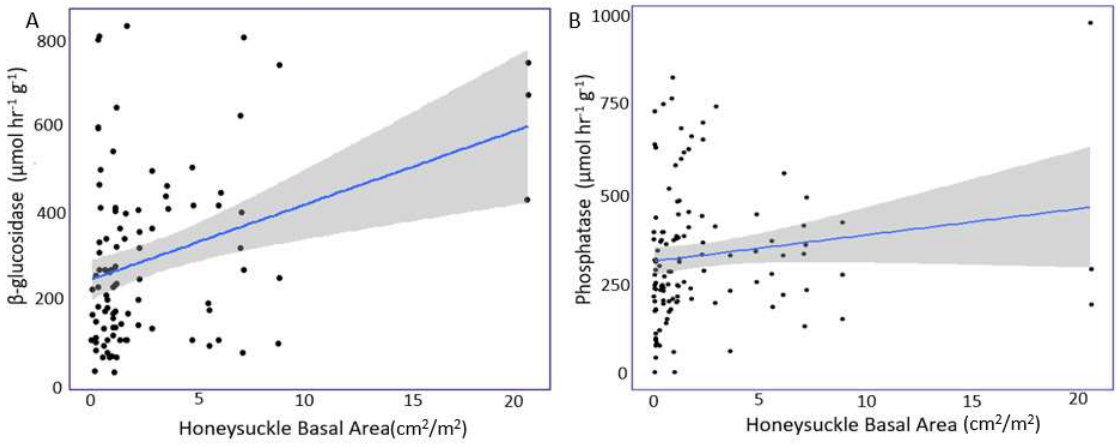

Figure 8. Linear regression demonstrating the increase of β-glucosidase activity (A; P = 0.008, $R^2 = 0.06$) and phosphatase activity (B; P = 0.038, $R^2 = 0.03$) with increasing *L. maackii* basal area. Shaded area represents 95% confidence interval.

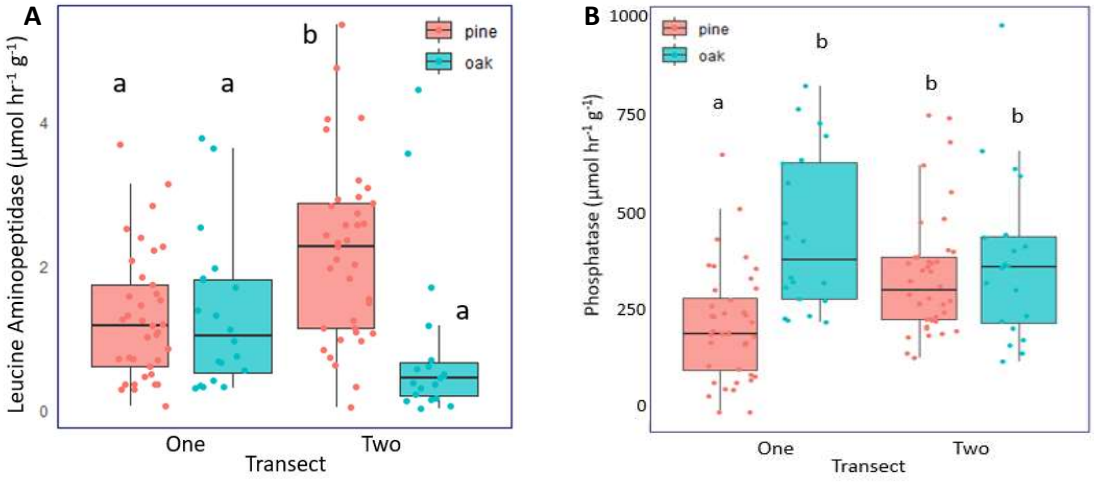

Figure 9. Box plots representing enzyme activities by transect and wood type. A: Leucine aminopeptidase activity increased on pine wood on transect two but remained the same on oak wood on each transect (P = 0. 002). B: Phosphatase activity was lower on pine wood on transect one, but the same as oak wood on transect two with no change

in activity on oak wood despite transect (P = 0.003). Pine wood is in pink and oak wood is in blue.

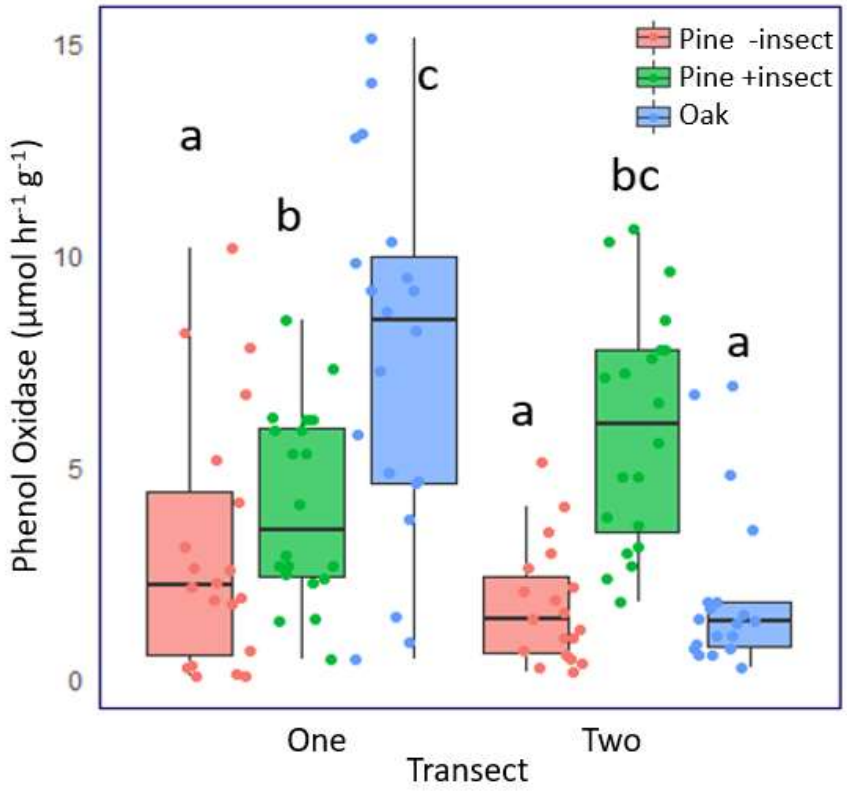

Figure 10. Boxplot showing peroxidase activity by wood type on each transect. Neither control pine wood (pink) nor insect pine wood (green) changed by transect, but oak wood (blue) had higher phenol oxidase activity on transect one than on transect two (P < 0.001).

Activities of β-glucosidase ($F_{1,118}$ = 68.81, P < 0.001, R^2 = 0.36) and leucine aminopeptidase ($F_{1,111}$ = 34.25, P < 0.001, R^2 = 0.23) were increased with bait moisture (Figure 11). Neither of these enzyme activities were affected by canopy coverage (P > 0.05), slope (P > 0.05), or wood type (P > 0.05). There was an interaction between moisture and wood type predicting phosphatase activity such that activity increased with bait moisture in pine wood but decreased in activity with increasing bait moisture in oak wood ($F_{3,116}$ = 8.424, P = 0.004, R^2 = 0.16; Figure 12). Phosphatase activity was higher in oak wood overall ($F_{2,117}$ = 8.424, P < 0.001, R^2 = 0.149), with insects contributing to a

higher activity than pine wood alone ($F_{2,117}$ = 8.424, P = 0.013, R^2 = 0.15; Figure 13B). The oxidative enzymes, phenol oxidase and peroxidase, had no relationship with bait moisture (P > 0.05), canopy coverage (P > 0.05) or wood type (P > 0.05). Phenol oxidase activity, however, was affected by slope, where activity was higher on flatter bait stations as opposed to those on a larger incline ($F_{1,116}$ = 13.08, P < 0.001, R^2 = 0.09; Figure 14). Peroxidase was not correlated with slope (P > 0.05). Alone, wood type was an important driver of β-glucosidase, leucine aminopeptidase and phenol oxidase activities. Both β-glucosidase ($F_{2,117}$ = 7.23, P = 0.001) and leucine aminopeptidase ($F_{2,111}$ = 11.6, P < 0.001) activities were higher in oak wood than pine wood, regardless of insect access (Figure 15). Phenol oxidase demonstrated higher activities where insects had access to pine wood compared to pine wood without insects and oak wood ($F_{2,115}$ = 10.02, P < 0.001; Figure 13A). There was no difference in peroxidase activity as predicted by wood type (P > 0.05).

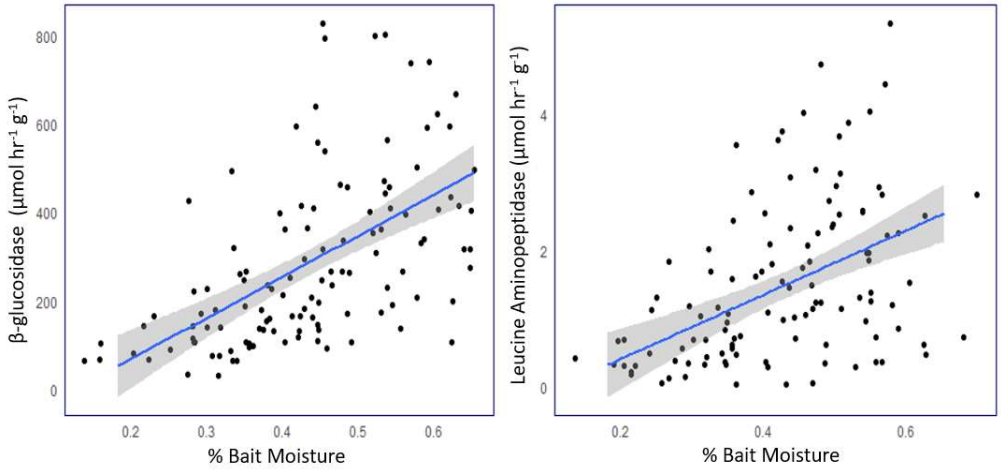

Figure 11. Bait moisture as a driver of β-glucosidase activity (P > 0.01, R^2 = 0.36) and leucine aminopeptidase activity (P > 0.01, R^2 = 0.23) in linear regression with the grey area indicating a 95% confidence interval.

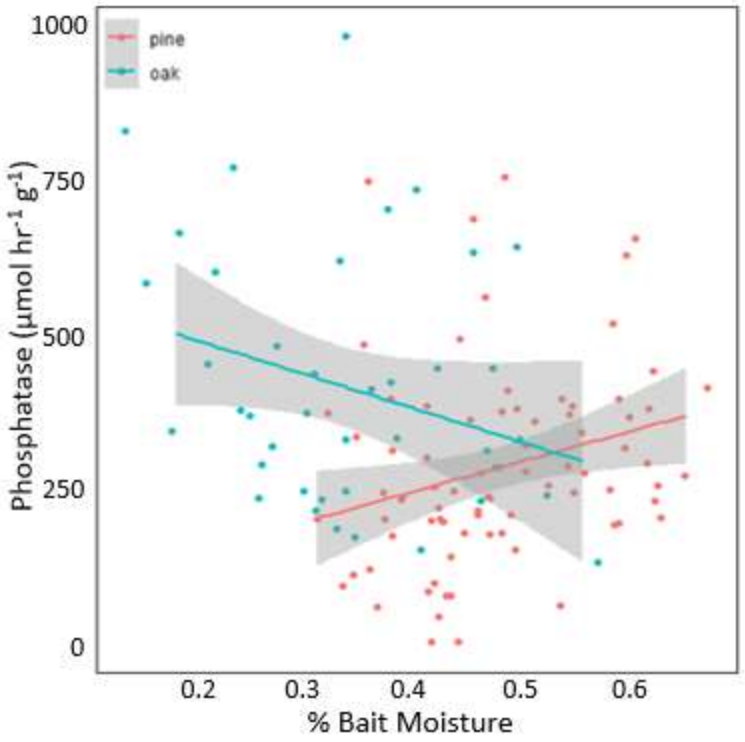

Figure 12. Linear regression of phosphatase activity and bait moisture with pine wood in pink and oak wood in blue where activity decreases with increasing moisture in oak but increases in activity with increasing moisture on pine wood ($P = 0.004$, $R^2 = 0.16$). Shaded area represents 95% confidence interval.

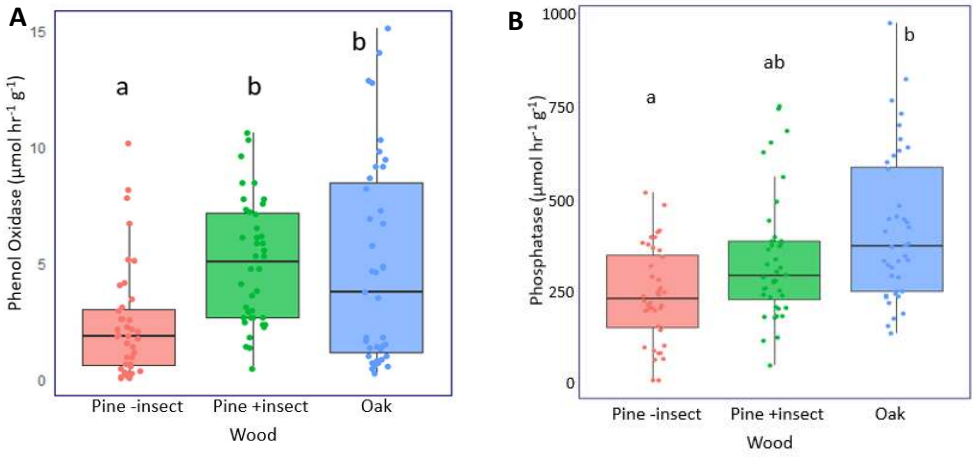

Figure 13. Phenol oxidase (A; $P < 0.001$) and phosphatase (B; $P = 0.013$) activities based on wood type where pine control is pink, insect pine is green, and oak is blue.

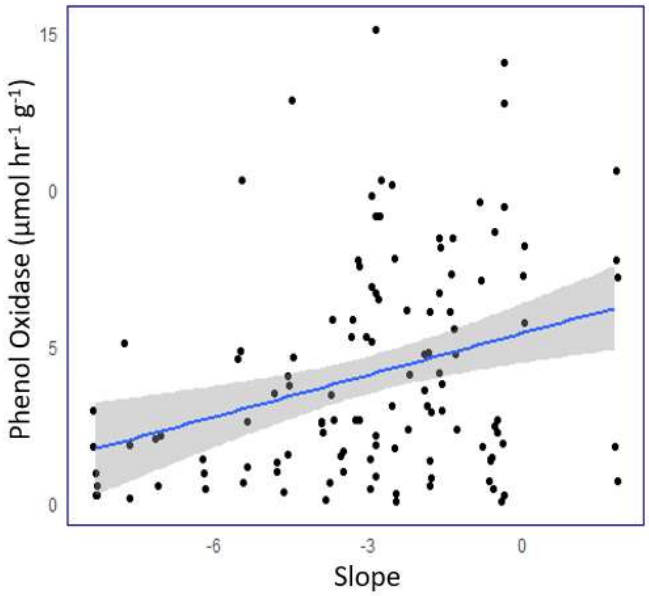

Figure 14. Phenol oxidase activity increased as landscape slope increased ($P < 0.001$, $R^2 = 0.09$). The shaded area represents a 95% confidence interval.

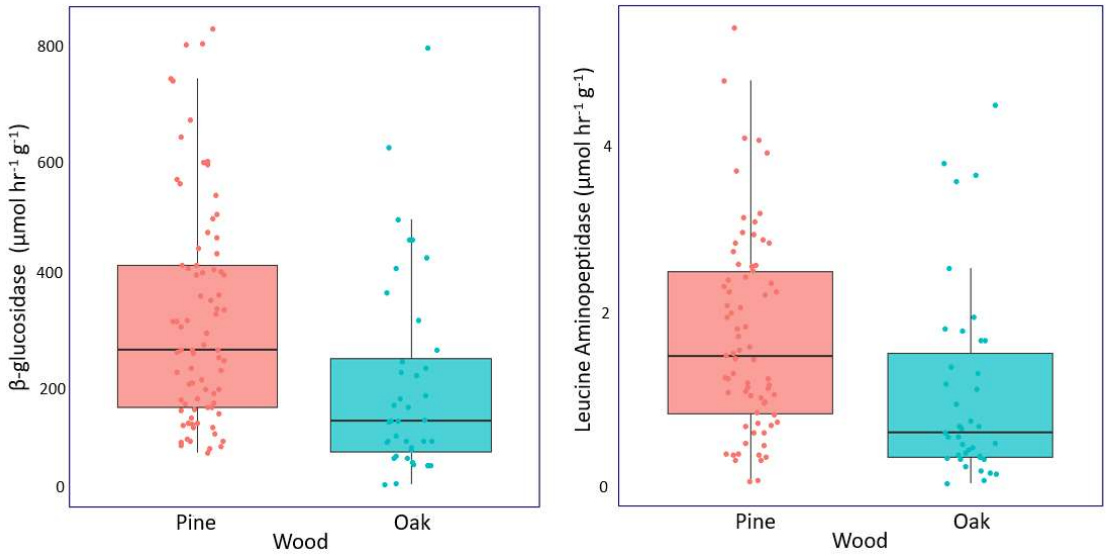

Figure 15. β-glucosidase activity ($P = 0.001$) and leucine aminopeptidase activity ($P < 0.001$) were higher on pine wood (pink) than oak wood (blue).

Enzyme activity as a predictor of decay rates

No enzyme activities measured in this study were able to predict decay rates ($P > 0.05$).

PCAs of enzyme activity and environmental variables

In the environmental PCA which condensed slope, moisture and canopy coverage into linear PCs, PC1 accounted for 39.4% of the variation, while PC2 accounted for 35.0%, in sum accounting for 74.4% of total variation (Figure 16). Canopy coverage scaled positively with PC1 and PC2, while moisture scaled negatively with PC1 and positively with PC2. Slope scaled negatively with PC1. There was a week relationship between environmental variables and wood type (R = 0.13, P = 0.001) and environmental variables with transect (R = 0.09, P = 0.001; Figure 16).

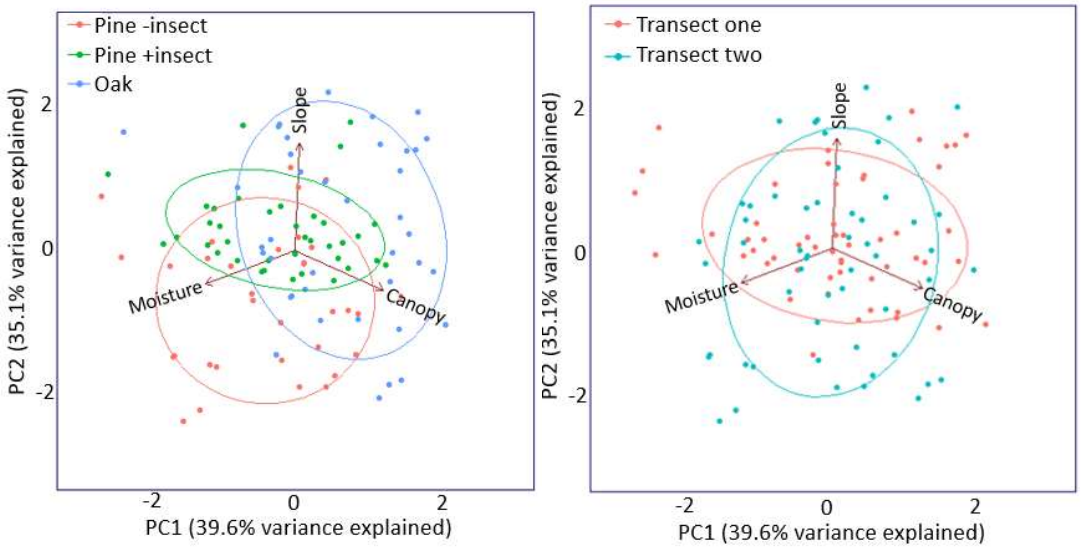

Figure 16. PCAs of environmental variables as grouped by wood type (R = 0.13, P = 0.001) or transect (R = 0.09, P = 0.01).

The enzyme activity PCA condensed peroxidase, phenol oxidase, phosphatase, leucine aminopeptidase and β-glucosidase activities into PCs. PC1 explained 46.3% of the variation and PC2 explained 27.3% of the variation in the enzymatic activity PCA, totaling 73.6% of the variation within the data being explained by this PCA (Figure 17). Peroxidase activity scaled positively with PC2, while phenol oxidase activity scaled negatively with both PC1 and PC2. β-glucosidase and leucine aminopeptidase scaled

negatively with PC1 and slightly positively with PC2. Grouping by wood type weakly yielded differences in enzyme activities (R = 0.10, P = 0.001), but grouping by transect did not expose differences (R = 0.02, P = 0.056; Figure 17).

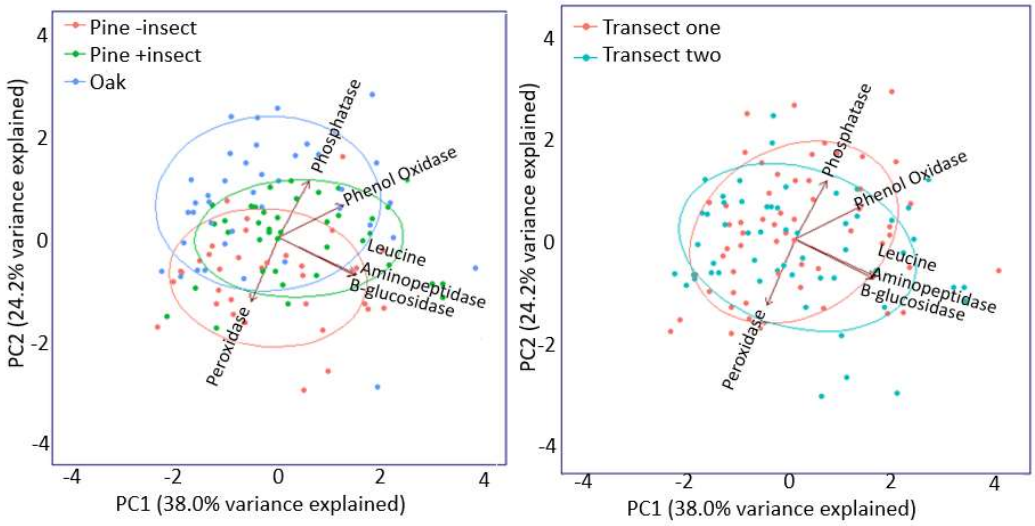

Figure 17. PCAs of enzymatic activities grouped by either wood type (R = 0.10, P = 0.001) or transect (R = 0.02, P = 0.056).

Fungal Communities

Rarefaction and taxonomy assignment

A total of 10,766,005 sequences were recovered. After quality filtering and chimera removal, 795,376 sequences were assigned taxonomic fungal ESVs from the UNITE database. Assigned sequences were rarefied to a sampling depth of 109 sequences per wooden bait, with an end total of 8,829 sequences accounting for 74 ESVs (Figure 18). Finally, only the top 99% of sequences were used in analysis to remove singletons and PCR artifacts which resulted in the use of 8,743 sequences and 49 ESVs. Remaining ESVs were classified into functional groups of either saprophyte, pathogen, endophyte, or mycorrhiza based on FUNGuild. Relative abundance values were calculated at the functional guild, ESV, and family levels to use in further analysis.

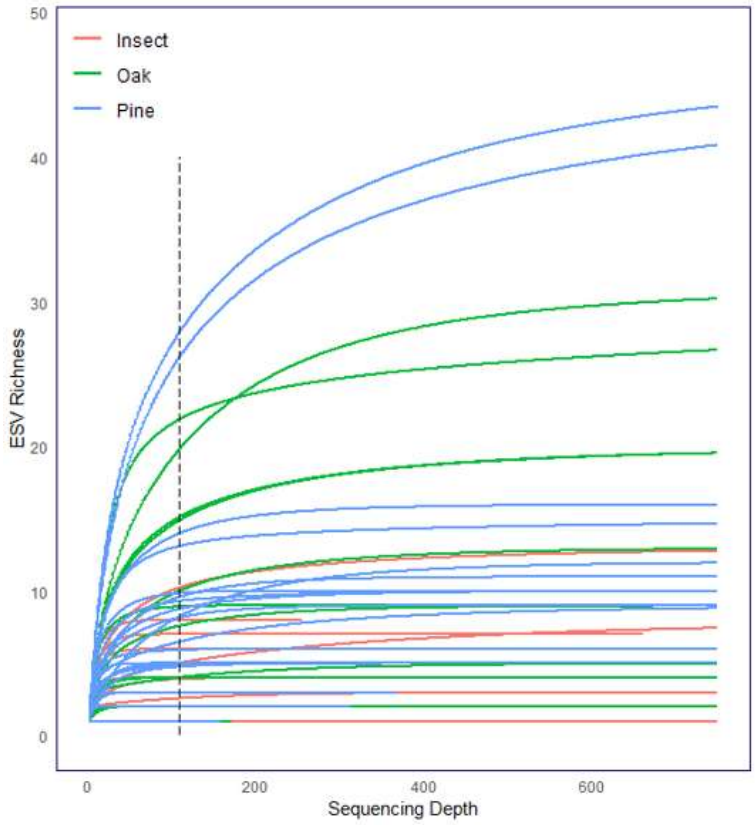

Figure 18. Rarefaction curve with each line representing the sequencing depth of each block. The dotted line represents 109 sequences of which depth was used to rarefy sequence abundance across data for use in further analysis. Pine wood with insects was represented in pink, pine without insect in blue, and oak wood in green.

Fungal community by environmental variables

Fungal guild relative abundance, Shannon's diversity, Chao1, and observed richness were not predicted by canopy coverage ($P > 0.05$), slope ($P > 0.05$), or bait moisture ($P > 0.05$). These fungal community representations were also not driven by wood type ($P > 0.05$), transect ($P > 0.05$), *L. maackii* size ($P > 0.05$) or abundance ($P > 0.05$).

The fungal families Marasmiaceae, Hyaloscyphaceeae, Tricholomataceae, Leotiaceae, Orbiliaceae, and Ceratobasidiceae drive differences in community composition by transect ($R = 0.10$, $P = 0.007$; Figure 19; Figure 21). Transect placement

may also drive differentiation between fungal ESVs (R = 0.07, P = 0.049; Figure 20; Figure 21) with *Arachnopeziza1*, *Pezoloma ericae*, and *Arachnopeziza aurata* driving this difference. Neither fungal families (P >0.05) nor ESVs (P > 0.05) differ based on wood type. Neither wood type nor transect significantly differentiated fungal guilds (P > 0.05; Figure 19).

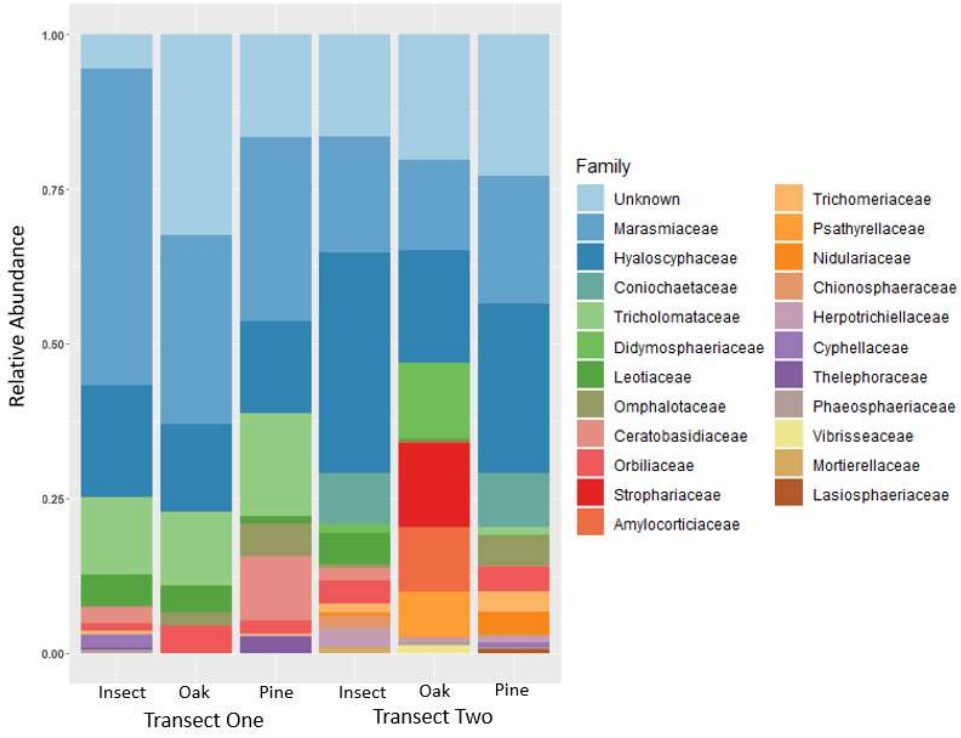

Figure 19. Relative abundance of fungal families by transect and wood type.

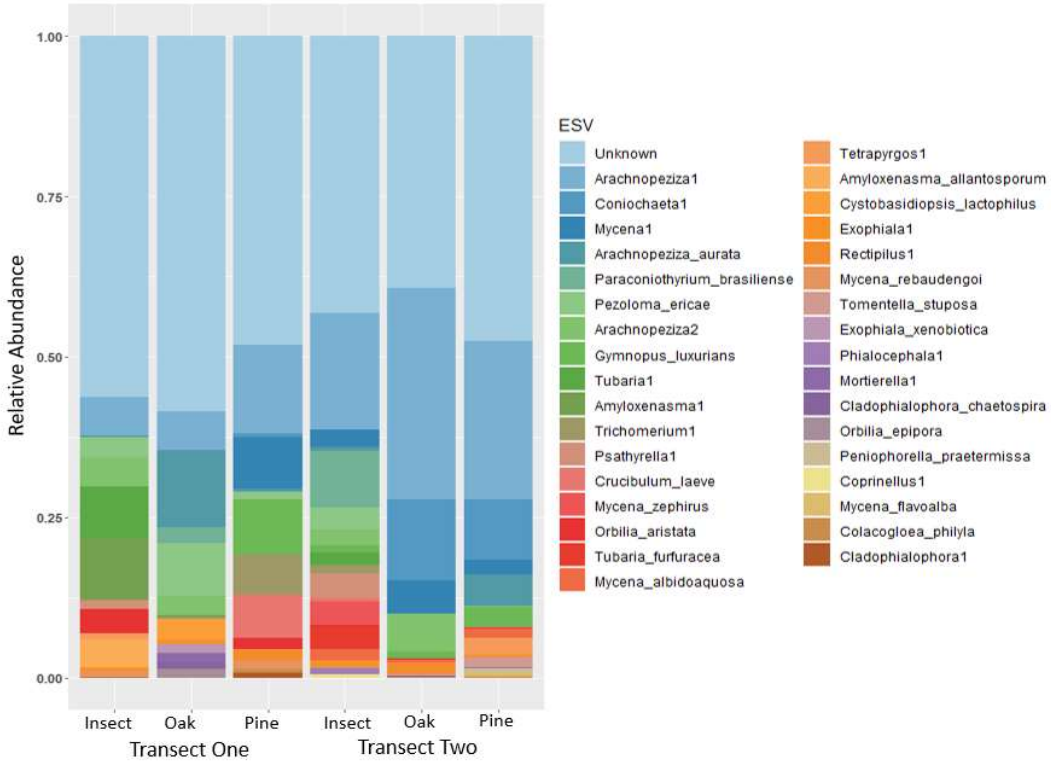

Figure 20. Relative abundance of fungal ESVs by transect and wood type.

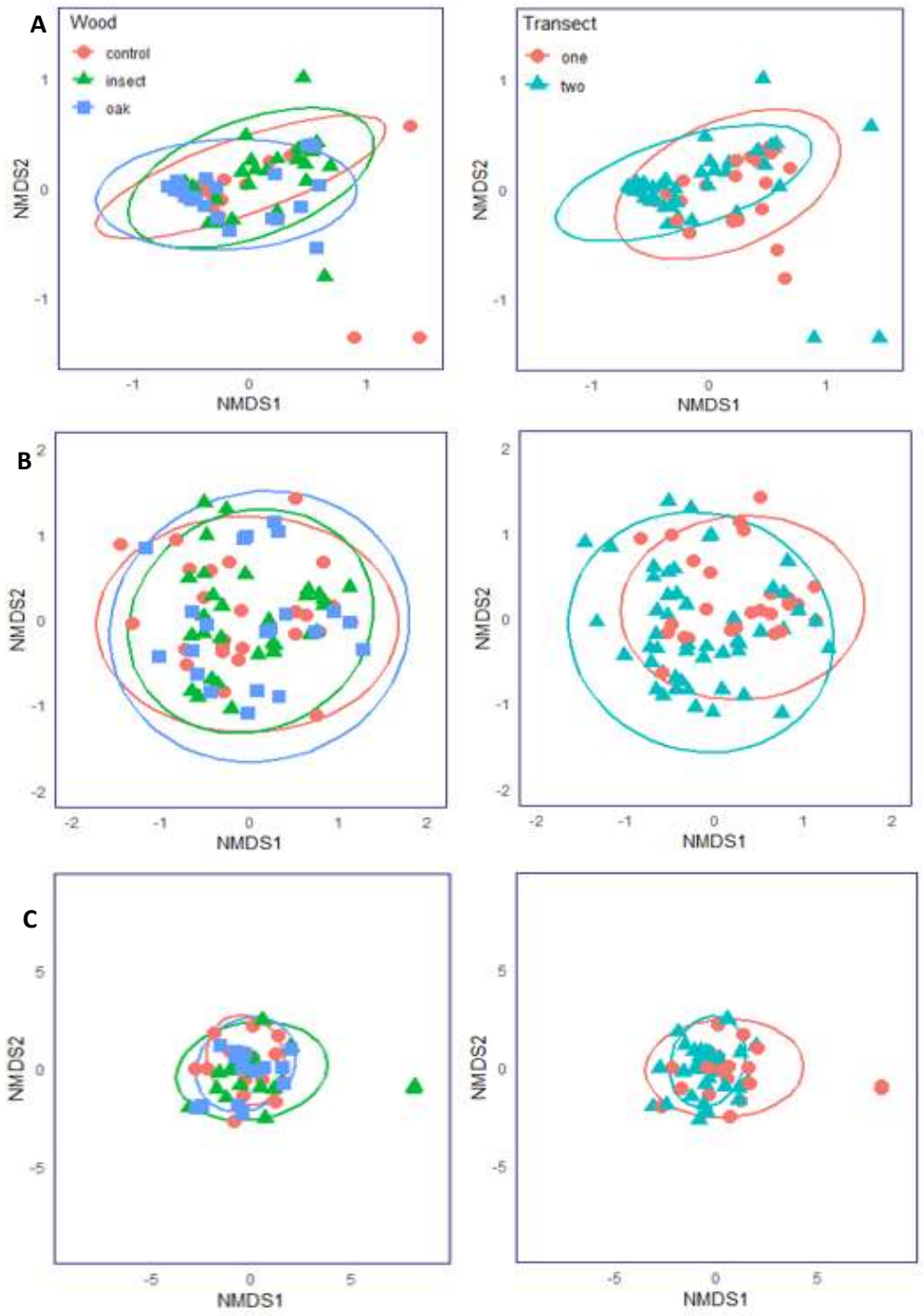

Figure 21. NMDS of fungal guilds (stress = 0.087; A), families (stress = 0.185; B), and ESVs (stress = 0.099; C). NMDS ordinations were grouped by either wood type with pine without insect in pink, pine with insect in green, and oak in blue; or ordinations were grouped by transect, with transect one in pink and transect 2 in blue. There is differentiation by fungal families (R = 0.097, P = 0.01) and ESVs by transect (R = 0.071, P = 0.049), but no other grouping is significant (P > 0.05).

Fungal community by enzymatic variables

Pathogen and saprophyte relative abundances predicted phenol oxidase activities with an interaction by wood type. Phenol oxidase activity on oak wood with increasing pathogen abundance while pine, with or without insects, changed with pathogen abundance ($F_{5,73}$ = 5.375, P = 0.041, R^2 = 0.22; Figure 22A). Additionally, greater saprophyte abundance decreased phenol oxidase activity in the oak without changing activity in pine despite insect access ($F_{5,73}$ = 7.504, P = 0.011, R^2 = 0.294; Figure 22B). Neither mycorrhiza (P > 0.05) or endophyte (P > 0.05) abundance nor diversity or richness metrics (P > 0.05) influenced phenol oxidase activity. No fungal community metrics altered the activities of β-glucosidase (P > 0.05), leucine aminopeptidase (P > 0.05), phosphatase (P > 0.05), or peroxidase (P > 0.05). The fungal community metrics also did not influence decay rates (P > 0.05).

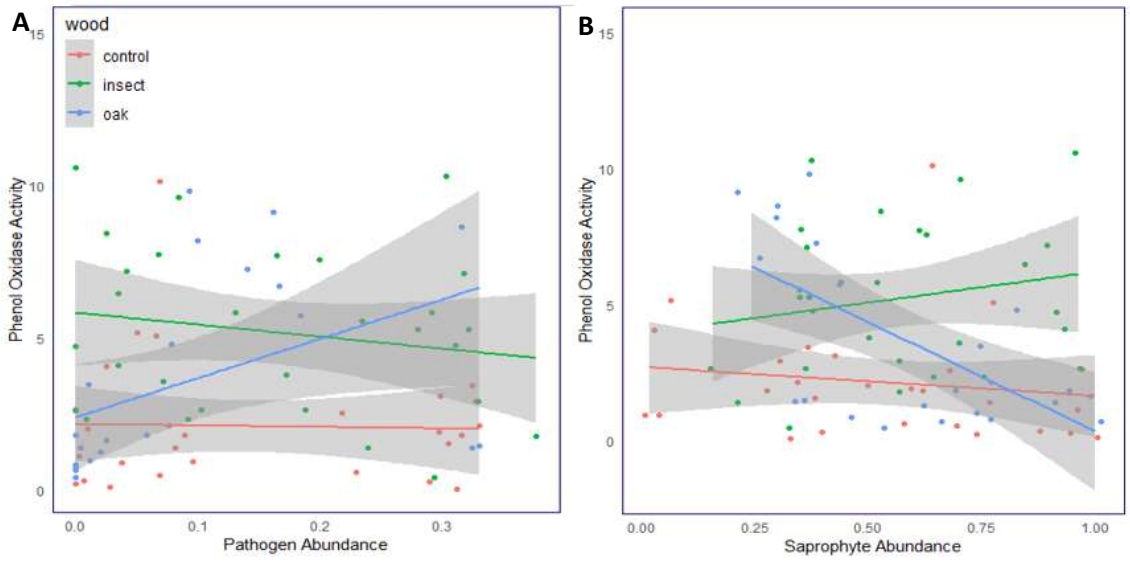

Figure 22. Phenol oxidase as a function of pathogen abundance (P = 0.041, R^2 = 0.219) and saprophyte abundance (P = 0.011, R^2 = 0.294) in linear regression where blue represents oak wood, pink represents pine wood without insect access, and green

represents pine wood with insect access and the shaded area conveys a 95% confidence interval.

CCA of fungal community with environmental and enzymatic variables

CCA at the ESV level demonstrated a significant relationship between environmental characteristics, enzymatic activities and fungal ESVs ($F_{7,62}$ = 1.22, P = 0.006). Phenol oxidase activity was a driver of community structure ($F_{1,61}$ = 1.38, P = 0.034), and moisture ($F_{1,61}$ = 1.31, P = 0.058) and phosphatase activity ($F_{1,61}$ = 1.31, P = 0.071) may also contribute to structuring the community (Figure 23). Particularly important to differences in community were the ESVs *Orbilia aristata* ($F_{1,36}$ = 2.44, P = 0.040), *Coniochaeta1* ($F_{1,36}$ = 2.53, P = 0.034), *Tomentella stuposa* ($F_{1,36}$ = 2.60, P = 0.030), and possibly *Exophiala1* ($F_{1,36}$ = 2.25, P = 0.053; Figure 23). CCA at the family level determined a relationship between environmental and enzymatic activities and fungal families present ($F_{7,62}$ = 1.395, P = 0.006). Moisture ($F_{1,61}$ = 1.73, P = 0.012), phenol oxidase activity ($F_{1,61}$ = 1.51, P = 0.39) and phosphatase activity ($F_{1,61}$ = 1.66, P = 0.028) were significant in structuring fungal families, particularly Coniochaetaceae ($F_{1,47}$ = 3.58, P = 0.007), Didymosphariaceae ($F_{1,47}$ = 2.56, P = 0.035), and the Amylocorticiaceae families ($F_{1,47}$ = 2.34, P = 0.047; Figure 24). There was no relationship between fungal guilds and environmental or enzymatic variables (P > 0.05).

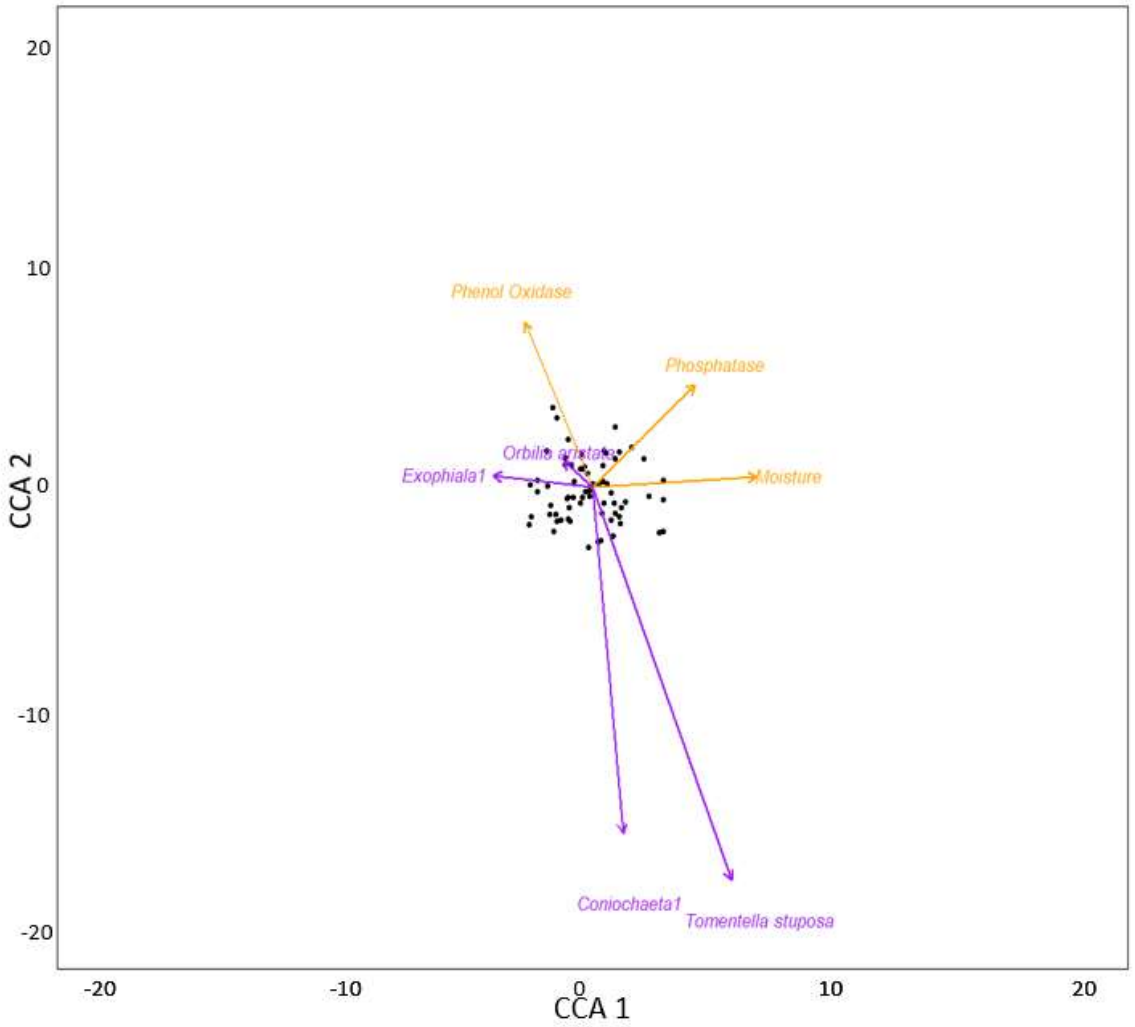

Figure 23. CCA comparing fungal ESVs to enzymatic activities and environmental factors. Environmental variables are represented in orange with Phenol oxidase activity was a driver of community structure ($F_{1,61} = 1.38$, $P = 0.034$), and moisture ($F_{1,61} = 1.31$, $P = 0.058$) and phosphatase activity ($F_{1,61} = 1.31$, $P = 0.071$) may also contribute to structuring the ESV community represented in purple.

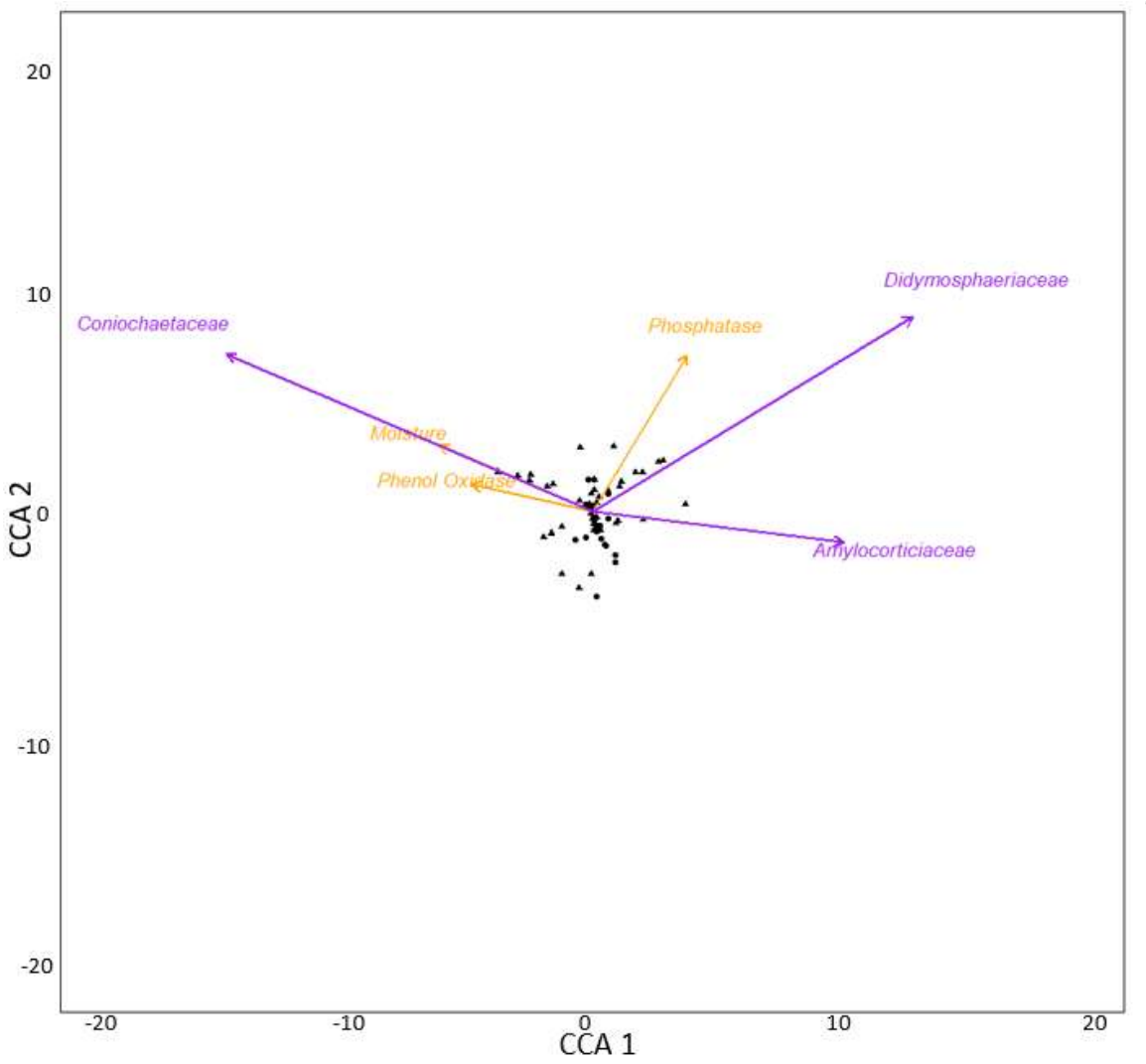

Figure 24. CCA comparing fungal families to enzymatic activities and environmental factors. Environmental variables are represented in orange with moisture ($F_{1,61}$ = 1.73, P = 0.012), phenol oxidase activity ($F_{1,61}$ = 1.51, P = 0.39) and phosphatase activity ($F_{1,61}$ = 1.66, P = 0.028) structuring family composition represented in purple.

DISCUSSION

Anthropogenic carbon emissions have been rising dramatically due to industrialization (IPCC 2014), but the role of microorganisms, which are critical in mitigating carbon cycling (Allison et al., 2010; de Graaff et al., 2006; Lal, 2008; Singh et al., 2010), remains largely unknown. While forested ecosystems are enormous carbon stocks that can store excess carbon through microorganism driven processes, the role that introduced species play in further altering carbon cycling is an area of developing research. Here, I show that *L. maackii* alters the soil environment, fungal community composition, and their enzymatic activities possibly resulting in increased rates of wood decay. Through these changes, *L. maackii* may harbor the ability to foster quicker carbon cycling within invaded forests, leading to faster CO_2 release and less carbon storage within the soil. Thus, measuring environmental properties, enzymatic activities, and fungal community structure during coarse woody debris in invaded forests is an essential step in elucidating ways that the invasive shrub is altering carbon cycling.

One of the most well established introduced species in Midwestern forests, *L. maackii,* has been well documented to alter ecosystem processes and patterns within forests (Cipollini et al., 2008b; Kolbe et al., 2015; McEwan et al., 2009). This is reflected in the work presented here such that larger *L. maackii* shrubs increased bait moisture (Figure 6), which is important for decomposition because higher substrate moisture increases decay rate (Van Der Wal et al., 2015). While the impact of *L. maackii* on soil moisture is highly context dependent (Hartman and McCarthy, 2004; Kolbe et al., 2015;

Pfeiffer and Gorchov, 2015), it has demonstrated an ability to increase soil moisture in the WSU woods where this study was conducted (Woods et al. *in prep*) and thus creates an overall wetter environment for decay on the forest floor. Furthermore, the relationship between *L. maackii* and bait moisture was driven by particularly large shrubs that are tree-like in size (basal area > 9 cm^2). This suggests that as honeysuckle establishes and thrives, it will continue to alter the microhabitat of the forest floor by increasing moisture of decaying litter.

Despite these changes in environmental conditions, *L. maackii* did not significantly alter wood decay rates in this study. Since leaf litter has reduced decay rates underneath shrubs (Arthur et al., 2012), wood decay was suspected to have lower decay rates in response to close proximity with shrubs. Alternatively, a synergistic relationship wherein the quick decay rate of *L. maackii's* leaf litter could increase the decay rate of surrounding litter has been proposed (Poulette and Arthur, 2012). Neither of these hypotheses were supported by data in this study which did not detect direct effects of *L. maackii* on decomposition rates. Perhaps with a longer decay period, the changes to enzymatic activity and fungal community composition as imposed by *L. maackii* would become apparent and be represented by decay rates of the woody debris.

Even though *L. maackii* did not explain decay rates, it did alter enzymatic activity. Both β-glucosidase and phosphatase increase in activity with increasing *L. maackii* size (Figure 8). In addition, there was higher leucine aminopeptidase and phosphatase activity on pine wood on transect two than transect one (Figure 9), suggesting that the larger and more abundant shrubs on this transect were driving faster release of these enzymes. Alternatively, there was higher phenol oxidase on oak wood on transect one than transect

two (Figure 10), and there was no change in peroxidase activity due to *L. maackii*, suggesting that *L. maackii* may have a stronger effect on hydrolytic enzymes than oxidative enzymes. Interestingly, similar results were found in *L. maackii* removal areas where *L. maackii* presence increased β-glucosidase activities and decreased or did not affect phenol oxidase or peroxidase activities (Woods et al. *in prep*). Perhaps this relationship is due to the lability of *L. maackii* leaf litter, in that it has high amounts of nitrogen and is easily broken down (Poulette and Arthur, 2012; Trammell et al., 2012). Additionally, the seasonal pulses of nutrients from this litter could stimulate higher β-glucosidase, leucine aminopeptidase and phosphatase activities by relieving nutrient limitations and thus priming the decay environment with fungi that are better equipped to utilize these enzymes.

In conjunction to altering enzymatic activities directly, *L. maackii* induced enzymatic activity mediated through increased bait moisture. β-glucosidase and leucine aminopeptidase activities increase with bait moisture (Figure 11). Similarly, phosphatase activity increased with moisture on pine wood, but this relationship was not true on oak wood (Figure 12). This is likely because oak wood retained more moisture than the pine wood making it able to sustain phosphatase release. Cases of hydrolytic enzymes increasing in activity with higher moisture are common (A'Bear et al., 2014b; Machmuller et al., 2016), though the trend does not extend into oxidative enzymes. Indeed, the only environmental variable to drive changes in phenol oxidase activity was the slope of the landscape, where flatter land leads to higher activity (Figure 14). Previous work suggests that this is common for phenol oxidase in that it is lower in sloped regions (Wickings et al., 2016).

Changes to fungal associated enzyme activities were directly altered by the size and/or abundance of *L. maackii* shrubs and indirectly through *L. maackii*'s alterations to the environment, suggesting that changes to functional guilds of fungi (endophytic, saprophytic, pathogenic, or mycorrhizal) may be driven by *L. maackii* invasion. Unfortunately, we did not detect a change in fungal guild abundance due to environmental differences, transect, or type of wood being decayed. On the whole, saprophytes comprised the majority of fungi present. While imperative to wood decay, brown rot and white rot were not well represented in this survey, though brown rot fungi from the family Amylocorticiaceae were important in community structure; however, the low abundance of these fungi prevented further exploration (Figure 24). Interestingly, phenol oxidase activity was higher with pathogen abundance on oak wood, but lower with saprophyte abundance on oak wood. Perhaps pathogenic fungi in this forest are better equipped to establish on more labile oak wood, increasing competition with saprophytes and decreasing their establishment success (Figure 22). This would indicate that saprophytes and pathogens have a similar ability to establish on pine wood. While endophytes specific to *L. maackii* shrubs were identified in a previous study (Arthur et al., 2012), the abundance of endophytes in this study was not representative of such changes.

Since the enzymes measured in this study are produced by decomposer fungi, the environmental variables that predicted enzymatic activity could also be responsible for structuring fungal communities. On the family and ESV levels, fungal communities were weakly structured by transect placement of the baits, and thus *L. maackii* size and abundance (Figure 21). Moisture was an important influence on community composition

at both the family and ESV level as well, indicating that *L. maackii's* amendments to the environment are also important in structuring decomposer communities. In particular, moisture was tightly linked to abundance of members of the Coniochaetaceae family (Figure 24), which typically function as endophytes or pathogens, further supporting that *L. maackii* may alter the abundance of these functional groups.

Again, since these enzymes are fungal derived, enzymatic activity likely predicts the structure of fungal community composition. Thus, community composition at the family and ESV levels were structured by phenol oxidase and phosphatase activities (Figure 23, 24). There was an association between phosphatase activity and the abundance of members of the Didymosphariaceae family, suggesting that members of this saprophytic family may demonstrate alternate enzymatic activities than other fungal families established on these baits. This could imply a potential for niche partitioning by nutrient use. The only ESV to demonstrate a significant association with enzyme activity, *Orbilia aristata,* was associated with phenol oxidase activity, which is perhaps unsurprising as it is a saprophyte and therefore able to breakdown recalcitrant material.

The conclusion that more ESVs were not related to enzyme activities may reflect the low number of ESVs recovered here (49 ESVs) which likely constrained my ability to adequately determine associations between fungi and the environment. The limited number of fungi recovered in this study may reflect the relatively short period of time this study covers (one year). With a longer decay period, changes to fungal abundances may become more apparent. In particular, with more fall seasons that would allow *L. maackii* leaf litter to interact with woody decay, more endophytes that are specific to *L. maackii* would have opportunity to establish. Additionally, changes to the microhabitat of the

wood as imposed by *L. maackii* may become more defined with a longer period of wood decay. This would lead to further niche partitioning and competition between fungi, potentially causing divergent community composition that would be perceptible in later stage wood decay. While the differences in fungal community composition by *L. maackii* may not be completely clear, it is apparent that the shrub is causing changes to enzymatic activities during decay. The differences in enzymatic activities caused by *L. maackii* are suggestive that fungal community composition may account for differences in functionality. A longer decay period would allow for more fungal establishment on the wood that would aid in a larger sample size of established fungi in which to compare to *L. maackii* metrics.

While transect, and therefore honeysuckle size, was able to weakly explain fungal community composition, wood type did not structure fungal communities (Figure 23). This was unexpected, as fungal groups such as white rot may be more suited for lignin heavy pine wood, but it suggests that the early establishment of fungi on woody litter is similar among species despite chemical makeup of woody material. Over a longer time period, community composition may become distinct between the two wood types based on the success of species in acquiring nutrients (Prewitt 2014).

Despite a lack of effect of wood type on fungal community composition, we did detect differences in enzyme activities based on wood type. Though oak wood did decay faster than pine wood overall (Figure 7), leucine aminopeptidase and β-glucosidase were higher on pine wood (Figure 9). This is likely because of the recalcitrance of the pine wood (Weedon et al., 2009). Fungi may have needed to secrete larger quantities of these enzymes on the pine wood to release the same amount of nutrients that a smaller quantity

of enzymes would release on oak wood. In contrast were phenol oxidase and phosphatase which were higher on oak wood than pine wood without insect access (Figure 13). Perhaps phenol oxidase activity is more indicative of early wood decay rates in that it can degrade recalcitrant carbon material and is necessary in breaking down the large carbon polymers present in the wood (Talbot et al., 2015). Phosphatase may be higher on the oak wood because it is tightly linked to faster carbon and nitrogen cycling (Ratliff and Fisk, 2016), which is induced by phenol oxidase and perhaps in part by leucine aminopeptidase and β-glucosidase on the oak wood. While enzyme activities were not significantly related to overall wood decay rates in this study, they may become increasingly important drivers of later stages of wood decay (Kahl et al., 2017).

Insect access to decaying pine wood was not dependent on environmental conditions, nor did it predict fungal community composition. Further, insect access was only indicative of increased phenol oxidase and phosphatase activities (Figure 13). This could be due to insects being mycophagus, eating the hyphae of the fungi producing these enzymes and stimulating enzyme release (A'Bear et al., 2014a). It was not surprising that insects did not alter the decay rates of wood in this ecosystem as there were no wood boring decomposer insects, such as termites, present in this survey. Instead, insects in this ecosystem may alter decay either by being mycophagus or by dispersing fungal spores onto decaying material (Ulyshen, 2016). *L. maackii* may increase the abundance of insects by providing habitat and increased food availability (Loomis et al., 2014), so perhaps with a longer time period of decay there could be a discernable difference in the ways insects alter decay dynamics as an interaction with *L. maackii*.

In summary, decomposition of coarse woody debris is being altered by *L. maackii* both by the shrubs' ability to increase environmental moisture and hydrolytic enzyme activity, and by potentially modifying the fungal communities responsible for decay. *L. maackii's* size is particularly important, with larger shrubs driving the changes to moisture and enzyme activities. The prolonged effect of *L. maackii* on forest ecosystems may become especially problematic as land managers shift from removal efforts to allowing shrubs to grow into larger tree-like shrubs. Continuing to monitor the effects these larger *L. maackii* shrubs have on important ecosystem processes like decomposition is imperative for management strategies which aim to mitigate potential carbon release from forested ecosystems. *L. maackii* is not alone in its ability to drastically alter ecosystem function (Dornbush, 2014; Tamura et al., 2017; Tamura and Tharayil, 2014; Zhang et al., 2010), other invasive plant species may transform functionality of forests, reducing carbon storage capacity by inducing increased rates of decay. To more fully understand the effects of invasive species on ecosystem processes, it is imperative that we continue to monitor the effects of invasive species on coarse woody decay to inform management strategies that best conserve ecosystem processes.

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