Fungal Diversity Associated with Zones of the AWPA Fungal Decay Hazard Map

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ABSTRACT

Wood deterioration, due to decay fungi, shortens the useful life span of wood and wood-based materials. Prescriptive preservative treatment is the most effective way to reduce the detrimental effects of these persistent microorganisms, particularly in soil contact and areas of critical use. A thorough understanding of decay hazard in soil contact is critical for proper selection and use of wood in ground contact. Current AWPA guidelines in the Use Category System specify 5 zones of severity regarding wood decay fungal hazard but contain very little information on diversity and abundance of these fungi in soil and wood. The goal of this work is to provide baseline data on fungal diversity and species composition in different zones of the existing AWPA hazard map. Results thus far indicate that fungal communities do differ between zones, but species richness decreases as zone severity increases. Preliminary discussion of the potential reasons for this trend are discussed.

Keywords: Biodeterioration, decay hazards, wood decay fungi.

INTRODUCTION

Service life prediction of wood and wood-based materials gives the end user an approximation of performance based on historical data and field testing, and can also be used to inform material comparisons and life cycle analyses [1]. The Use Category system of the American Wood Protection Association (AWPA) uses fungal decay hazard to describe deterioration rates in the United States [2, 3]. The AWPA Fungal hazard map is a graphical depiction of ground contact decay hazards in the United States. The map is divided into 5 zones with areas of low biodeterioration hazard classified as zone 1, up to severe biodeterioration hazards which are classified as zone 5. The zones of the map represent a substantial amount of discussion that has occurred among experts in AWPA over the last 40 years, and are primarily based on condition assessments of utility poles carried out in the 1950's by the Rural Electrification Association (REA). A more detailed history of the AWPA hazard map was discussed in an AWPA proceedings paper by Kirker et al. [4].

Another commonly used metric to determine decay severity is the Scheffer index, which is a calculated value based on the number of days with rainfall above a threshold value, and mean temperature above a threshold value [5]. There are several studies that indicate good agreement with Scheffer indices and above ground decay hazard [6, 7]. However, efforts to apply the Scheffer index to ground contact decay hazards have not been fully successful [8], indicating that additional factors need further study. Additionally, large scale surveys of utility pole service records indicate that utility poles have differential survival rates in different AWPA hazard zones [9].

Improved molecular methods for characterization of microbes in the environment now allow for fast, accurate and repeatable characterizations of fungi and bacteria in soil, wood and other environmental substrates [10-12]. A rather large missing component of current efforts to characterize decay hazard is biodiversity data to inform the effort. Amplicon based sequencing is a powerful tool for conducting these types of analyses and has been used by FPL researchers to characterize soil exposed to preservatives long term [13] and most recently wood exposed to leaf litter [14].

The goal of this research is to provide baseline biological data on fungal biodiversity within the different zones of the AWPA hazard map to determine if areas with higher fungal diversity have corresponding higher decay rates in ground contact. Additional community analyses will identify indicator species that are associated within the different zones to examine possible compositional differences in species assembly from areas of low to high decay hazard.

MATERIALS AND METHODS

Fifteen southern pine field stakes (18 x 1 x 1 inch) per overstory type (pine or hardwood) were sent to 14 National Forests (N = 30) and across the 5 AWPA hazard zones in the summer of 2016. Table 1 lists National Forests included in this study along with supplemental decay hazard information. Field stakes were placed by foresters in the ground approximately 8 inches deep in each forest type in three "W" shaped transects with 5 stakes per transect at 10 paces per stake. Soil samples (taken at the time of stake installation) were shipped back to FPL for amplicon-based fungal community and nutrient analysis. Stakes were exposed to the weather conditions of the site for approximately 1 year and returned to researchers by overnight shipping. Two National Forests were unable to locate field stakes and were left out of the study.

Field stakes were visually rated using AWPA E-7 rating criteria and documented as sound or broken (either arrived broken or were so decayed they could easily be broken by hand) and whether there was visual evidence of termite feeding, white rot, brown rot or soft rot decay. Stakes were then frozen at -20°C until DNA extraction could be completed.

Frozen field stakes were drilled using a ¼ inch paddle bit in four spots at one-inch intervals from the ground line down in order to acquire approximately 2 grams of sawdust from each stake. Sawdust from the 5 stakes per transect was pooled and considered one replicate. Sawdust was mixed and two 0.1g samples were added to tubes containing 800 ul 2% CTAB + 0.1% beta mercaptoethanol. Samples were ground using a hand-held drill and plastic pestle for approximately 30 seconds each and incubated 2hr at 65°C on a hotplate. DNA in solutions was separated from sawdust by centrifugation at 14,000 x g for 4 minutes, then the two samples were applied to the spin column of a Promega (Madison, WI) Wizard SV DNA extraction kit and DNA was extracted following manufacturer's instructions. Extracted DNA was cleaned following the manufacturer's instructions using the PowerClean Pro Cleanup kit (Qiagen, Hilden, Germany). Soil samples were processed using Powersoil DNA extraction kits (Qiagen, Hilden, Germany) following manufacturer's instruction, otherwise the procedure for sample preparation is the same as wood. Nutrient analysis was performed by WI State Soils laboratory, but those data are not presented here.

Table 1: Sampling sites included in this study and supplemental decay hazard information. AWPA hazard zone information is from 2019 U-1 listings and Scheffer Index is calculated from nearest NOAA weather station to the sampling site for the year corresponding to their exposure. Figure codes correspond to Figure 1.

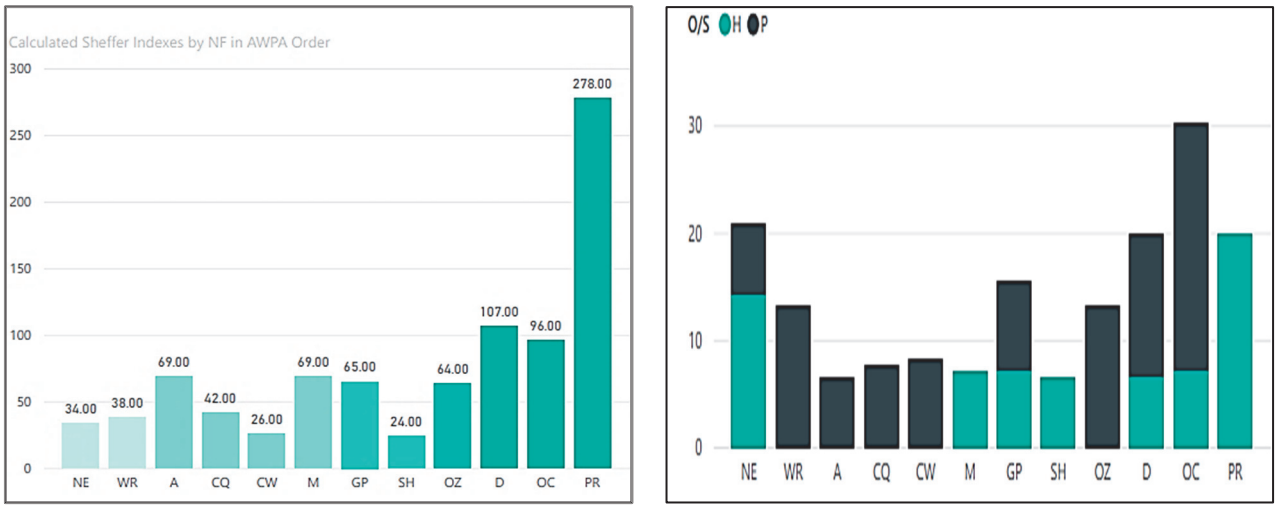
National Forest	AWPA Hazard Zone	Scheffer Index	Figure Code
Allegheny NF	2	69	A
Bienville NF	4	93	B
Chippewa NF	2	27	CW
Daniel Boone NF	4	69	DB
Desoto NF	5	107	DE
El Yunque NF (San Juan, PR)	5	278	PR
Gifford Pinchot NF	3	66	GP
Nebraska NF	1	34	NE
Nicollet-Chequamegon NF	2	42	CQ
Ocala NF	5	96	OC
Ozark St. Francis NF	4	64	OZ
Shatsta Trinity NF	3	24	SH
UW Arboretum	2	67	M
Valley View Test Site	2	68	WI
White River NF	1	38	WR

PCR was conducted using Phusion taq polymerase (New England Biolabs, Ipswich, MA) following the conditions provided by Bueé *et al*. [15] in triplicate (5 ng per reaction). Primers ITS1F and ITS2 were synthesized by IDT with the addition of 72 different combinations of dual-index barcoded primers, designed by the UW Biotech Center DNA Sequencing Facility (Madison, WI) to generate 400 bp product. Primer dimers removed after triplicate PCRs were combined by Select a Size DNA Clean and Concentrator kit (Zymo Research, Irvine, CA) by selecting for 200 bp and lower products to be eliminated. DNA was then quantified using Quant-iT ds DNA Assay Kit (ThermoFisher Scientific, Waltham, MA) and a hybrid multimode reader (Synergy H1, Biotek, Winooski, VT). Samples were pooled in equimolar concentrations (25ng) then submitted to UW Biotech Center DNA Sequencing Facility for MiSeq (Illumina, San Diego, CA) sequencing analysis.

New indices were calculated following Scheffer [5] protocols using weather data collected from the National Oceanic and Atmospheric Administration (NOAA) website over the time period that stakes were exposed. Average monthly temperatures and days with greater than or equal to 0.01 inch of rain were accumulated from 1 – 3 weather stations near each National Forest from August 2016 through July 2017. Data was averaged over the stations and a Sheffer index value was calculated using Scheffer's formula [5]:

Equation 1: $CI = (T-35) \times (D-3)$

**T is average temperature in Fahrenheit and D = days with precipitation greater than 0.01 inches.*



A **B**

Figure 1: (a) Calculated Scheffer indices for each sampling location based on August 2016 through July 2017 NOAA weather data. (b) Percentage of failed (being broken on return or able to be broken by hand) field stakes at each site after 12 months of exposure separated by overstory type, with pine totals in black and hardwood totals in green. The national forests on the X-axis are arranged from lowest to highest decay hazard based on the current AWPA hazard map.

PRELIMINARY RESULTS AND DISCUSSION

All wood samples and additional soil samples collected before stakes were installed have been analyzed using amplicon-based sequencing. Soil chemical analysis and physical properties have been collected for all soil samples. The results of amplicon-based sequencing are operational taxonomic units (OTUs), which correspond to a distinct species [16]. For this sequencing data, all OTUs with identity scores less than 97% have been discarded. The result is 8,179 operational taxonomic units, which can be considered analogous to species of fungi. Repetitive and non-informative OTUS were also filtered from the dataset for a total of 7,312 OTUs. The first step in analyzing the data set was to make broad comparisons based on species composition. Non-metric multidimensional scaling (NMDS) in PC-ORD [17] was used to generate plots that offer a visual

representation of fungal communities based on species composition. The first comparison was fungi present in wood versus those in soil (Figure 2). There was very little overlap between these communities which is not entirely surprising given that wood would represent a more specialized substrate with spatially restricted access. Future analysis will include separation of wood and soil data sets to focus independently on the two substrates.

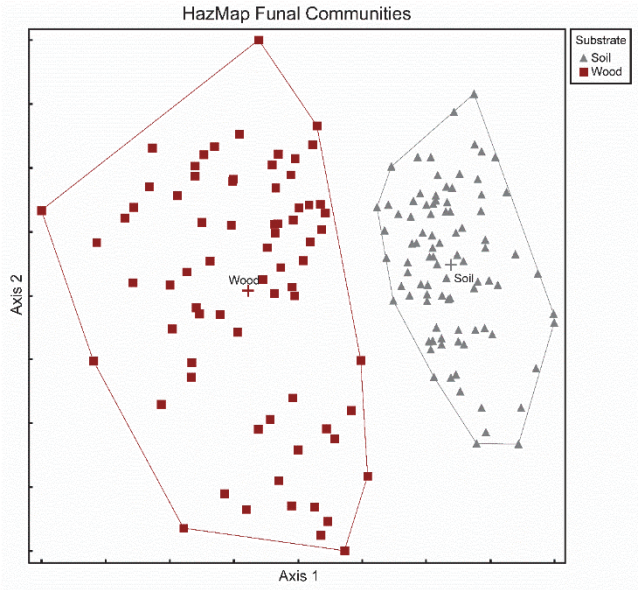


Figure 2: Non-metric multidimensional scaling (NMDS) ordination showing relative differences between fungal community composition in wood and soil. Groups clustered separately indicating that they are dissimilar in overall species composition. Greater spacing between wood samples also indicates more variability in fungal species composition relative to soil.

Ordinations of fungal community by site are shown below (Figure 3). More temperate sites clustered more closely indicating that they are more similar in regard to soil fungal species composition and more subtropical sites (Ocala, Desoto and San Juan) clustered more to the left of the ordination, which indicates that the species composition is unique from the other sites. Additional analyses are underway to compile site specific indicator species list that are associated with a given site.

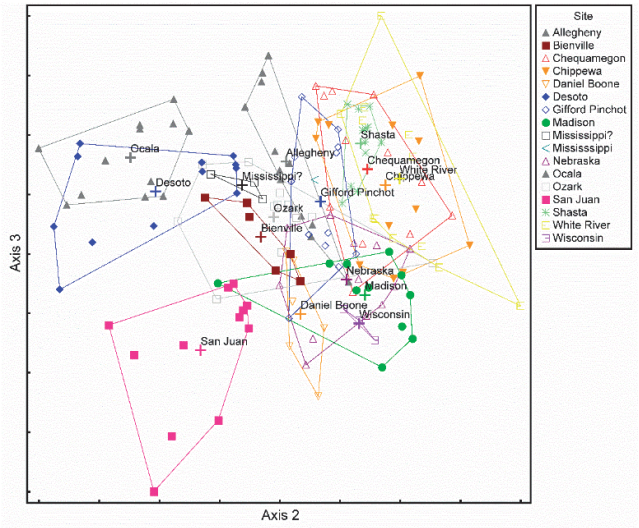


Figure 3: Non-metric multidimensional scaling (NMDS) ordination showing relative differences between fungal community composition between sites. Groups clustered separately indicating that they are dissimilar in overall species composition. Higher hazard sites cluster more closely indicating that they are more similar in fungal species composition than those in lower decay hazard classes.

Ordinations by AWPA hazard zone are shown below (Figure 4). The trend follows a similar pattern to what is noted on the site comparisons. The less severe zones (1-3) tend to group together and zones 4 and 5, which represent a more severe decay hazard, had more distinct fungal species composition. There is also a clear delineation from left to right along axis 2 showing a gradual shift in species composition from areas of high hazard to low.

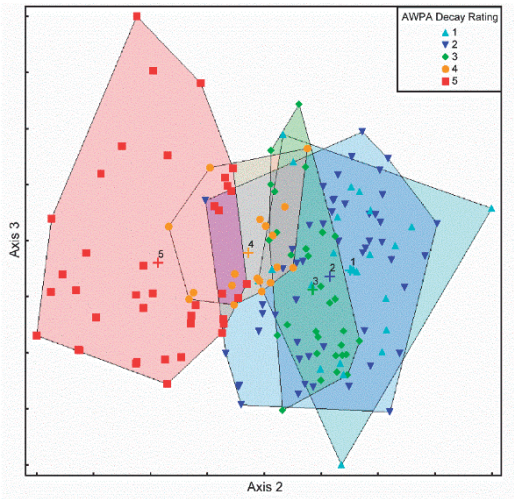


Figure 4: Non-metric multidimensional scaling (NMDS) ordination showing relative differences between fungal community composition between AWPA fungal decay hazard zones. Groups clustered separately indicating that they are dissimilar in overall species composition. Axis 2 gives a clear indication that there are differences in fungal species composition in more severe decay hazard zones.

The previous ordinations are graphical depictions of species composition and do not provide a quantitative measure of fungal abundance. In addition to the NDMS ordinations, PC-ORD was used to calculate values for species richness, evenness, Shannon diversity and Simpson diversity of each treatment group. Those metrics are defined below [18]:

Species richness

The number of different species in an ecological community. It is derived from summing all non-zero elements in a column or row.

Evenness

The similarity in number of species of between ecological community. It is derived from dividing the Simpsons index (H, shown below) by the natural log (ln) of the richness value.

Shannon Wiener Diversity (H)

A calculated diversity index that accounts for relative abundance of species rather than total number of species. It assumes all species are represented in a sample, and that they are randomly sampled. Shannon Wiener is also sometimes referred to as an information statistic index. The equation is shown below as Equation 2.

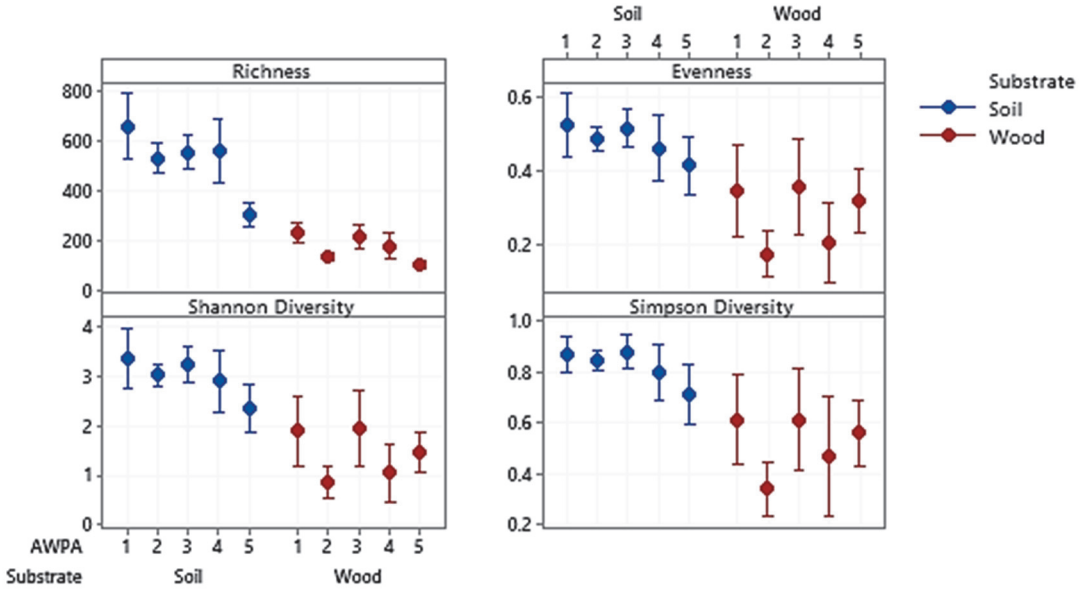
$$\textbf{Equation 2: } H = \sum_{i=1}^{R} p_1 ln p_i$$

Simpson Diversity

The Simpson index (D) is commonly referred to as a dominance index because it gives more weight to common or dominant species. The result is that a few rare species with low abundance will not affect the outcomes. The equation for the Simpson index is shown as Equation 3.

$$\textbf{Equation 3: } D = 1 - \frac{\sum n(n-1)}{N(N-1)}$$

The results are shown in the figure below (Figure 5). Lower diversity was noted in the wood samples, which is not surprising given the nutritional and spatial limitations of wood. No clear trend was noted between fungal diversity measures, but a general downward trend was noted and that was more distinct in soil than wood. These results would imply that fungal species richness is not simply higher in areas of more severe decay hazard. These results are complimentary to previous studies looking at competitive interactions in wood decay fungi [19], that indicate more diverse assemblages are present in more moderate temperate climate, and more severe tropical climates consist of well adapted species that outcompete and colonize the substrate more quickly and completely, which could lead to the diversity patterns observed within this study. Based on these results, the fungal communities in more severe climates are not necessarily more diverse, but are comprised of different species, which may be more aggressive [19], combative [20], or temporally restricted [21] in their habits. More work needs to be done to further investigate these concepts, but the outcomes could change how we view wood protection in sub-tropical and tropical climates.



Individual standard deviations are used to calculate the intervals.

Figure 5: Overall trends in species richness, evenness, and diversity (Shannon and Simpson) for both soil and wood split by AWPA hazard zone (1-5). Generally, higher values were obtained from soil than wood and only slight upward trends in diversity and evenness were noted in severe hazard zones. Individual standard deviations are used to calculate the intervals.

CONCLUSIONS

The data presented within this paper are results of analyses combining wood and soil fungi into one group. Future work will be focused on splitting the current dataset and analyzing wood and soil separately. It is very important to understand the role of soil in this process, as it provides an inoculum pool for wood in soil contact and represents a key route for exposure to decay fungi. Further studies on the role of soil contact in colonization and fungal succession are underway at FPL.

The primer set that was selected for this study is non-selective, meaning that it will amplify any and all fungal material present in the sample. As a result, there exists an entire spectrum of fungal species that range from generalists (molds, plant pathogens) down to more specialized modes of nutrient uptake (ECM fungi, wood decay fungi). In order to better isolate and look at these fungi based on their ecological role, Funguild [22] is being used to further characterize the fungi based on the functional guild in the environment, which describes their niche within the ecosystem.

Future work will fully characterize the fungal data for both soil and wood samples and indicator species lists will be compiled based on predominant fungal taxa within different zones of the AWPA fungal decay hazard map. This data will serve as baseline data for future studies to look at temporal changes of decay hazard across the US.

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