Investigating Soil Effects on Outcomes of a Standardized Soil–Block Test

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Abstract

Soil physical and chemical properties play important roles in mass loss during soil–block tests but the relationship between soil properties and the decay caused by brown-rot and white-rot fungi remains unclear. The objective of this study was to investigate the soil effects on the decay resistance of pine (*Pinus* spp.) and poplar (*Liriodendron tulipifera* L.) blocks. The properties of soil from nine different sources (six from Idaho, one from Mississippi, one from Wisconsin, and one from Oregon) were characterized for soil texture, sieved bulk density, water-holding capacity, pH, organic matter, and carbon and nitrogen concentrations. The moisture content and mass loss of decayed wood samples after 8 weeks of fungal exposure were measured. At the end of the study, block moisture ranged from 30 to 200 percent and mass loss ranged from 20 to 60 percent. Despite using a range of soils, there were no direct correlations between soil properties and wood-block moisture content or mass loss. Moreover, among all the soil properties examined, no significant effect of a single soil property on wood-block moisture content and mass loss was measured. Instead, the combined effects of soil physical and chemical properties may interact to govern the decay of wood blocks in the laboratory soil–block test.

Introduction

The soil–block test is a standardized laboratory method for rapidly assessing the durability of wooden materials and determining the effectiveness of wood preservative–treated wood against fungal attacks. This origin of this test was inspired by a lab experiment that was initially aimed to establish laboratory termite colonies, but instead, the wood samples in contact with soil were rapidly decayed by fungi (Leutritz 1946). Further studies highlighted that surface mineral soils are often high in nutrients that are favorable to many wood-decay organisms (Duncan 1958). The agar–block method is similar to the soil–block tests, but agar is used instead of soil and gives equally valid results if proper nutrients and moisture are maintained (European Committee for Standardization [CEN] 2020). Compared to the agar–block test, using soil is an easier method for regulating water content in wood during the decay period, thus allowing for more rapid and uniform results (Duncan 1958).

The effect of soil properties on soil–block wood decay tests was studied extensively by Duncan in 1958 (Duncan 1958) and this work influenced the specification of soil properties in the American Wood Protection Association (AWPA) standard soil–block tests (AWPA 2016). The general guidelines of selection of the appropriate soil type are (1) a water-holding capacity (WHC) of 20 to 40 percent, (2) pH of 5.0 to 8.0, (3) sieved bulk density of at least 0.76 g/cm^3, and (4) from a forest that has not had any chemicals applied. Even with these guidelines in hand, a later study conducted by Amburgey (1978) highlighted a significant influence of soil type on the soil–block test results. More recently, Castillo-Monroy et al. (2014) compared the effects of seven different soils on the final moisture content and mass losses of southern pine blocks (*Pinus* spp.) and found that none of the soil characteristics correlated with wood-block mass loss in the laboratory. Because one of the most important variables in the soil–block test is the soil, it is of paramount importance to determine which soil properties are important drivers of laboratory fungal decay. For example, soil moisture content is an important property that allows for adequate wetting of the test blocks to maximize fungal growth (Highley and Scheffer 1970). Soil moisture is also the only factor that could be changed

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without modifying the original soil and can be increased to 130 percent of the water-holding capacity, based on the standard AWPA E10 (AWPA 2016).

The objective of this study was to identify key soil properties that affect laboratory wood-block biological decay using locally sourced soil from Idaho. The soils collected from six different sites in Idaho, along with soils from established wood-durability testing labs, including those from Oregon, Mississippi, and Wisconsin, were characterized for their physical and chemical properties and were also used as a medium for soil–block testing per standard AWPA E10. Wood-block moisture content and mass loss were recorded after 8 weeks of brown-rot and white-rot exposure. The relationship between soil properties and the decay caused by fungi in a standard soil–block test was also clarified.

Materials and Methods

Materials

Soils used in this study were collected from four different states: Idaho, Oregon, Mississippi, and Wisconsin. Once collected the soils were transferred to the University of Idaho, Moscow, where they were screened through a 2-mm sieve, and air-dried before further use. Two brown-rot fungi, *Gloeophyllum trabeum* (American Type Culture Collection [ATCC] 11539, Madison 617) and *Rhodonia placenta* (ATCC 11538), and two white-rot fungi, *Trametes versicolor* (ATCC 42462) and *Irpex lacteus* (ATCC 11245), were used to test the durability of wood samples. Ponderosa pine (*Pinus ponderosa* Lawson & C.Lawson, approximately 6 mm by 28 mm by 34 mm, (radial) R by (tangential) T by (longitudinal) L) and poplar (*Liriodendron tulipifera* L., approximately 3 mm by 28 mm by 34 mm, R by T by L) were used as feeder strips for brown rot and white-rot fungi tests, respectively. Wood blocks (14 mm by 14 mm by 14 mm, L by T by R, end-matched) were cut from clear sapwood of southern pine (*Pinus* spp. mainly consisted of tracheids, $n = 108$ blocks) and poplar (mainly fibers and vessels, $n = 108$ blocks), for brown-rot and white-rot fungi, respectively. These two species were selected per AWPA E10 standard because of their low durability and medium density.

Characterization of soil properties

Soil textural analysis.—The percentages of sand, silt, and clay in soils as well as the textural classification were determined using a hydrometer by sedimentation method (Bouyoucos 1962). Specifically, 40 g of each sieved soil was first mixed with 100 mL of 5 percent sodium hexametaphosphate, and the mixture was shaken at room conditions for 16 hours. The suspension solution was transferred to a cylinder and the total volume was calibrated. Subsequently, the soil solution was equilibrated at room temperature for 2 hours and stirred thoroughly, followed by the addition of 2 mL isoamyl alcohol. A hydrometer was placed into the sample and the scale of the graduated cylinder at 40 seconds was recorded. The sample was left undisturbed for another 6.5 hours to record the second hydrometer reading. Sand and clay percentages were calculated from measurements taken on the soil suspension with a hydrometer at 40 seconds and 6.5 hours while silt percentages were obtained by subtracting the percentages of sand and clay from 100 percent. The soil textural class was determined following the USDA guidelines for textural classification (USDA NRCS 2020).

Sieved bulk density.—Soil sieved bulk density was determined by compressing the sieved soils in a glass vial with predetermined weight and volume, followed by oven-drying (105°C for 24 hours) and weighing. The sieved bulk density was calculated as the following: sieved bulk density (g/cm^3) = mass of oven-dried soil sample/volume of soil.

Organic matter.—Soil organic matter was determined by loss-on-ignition methods (Nelson and Sommers 1996). The air-dried soil samples were first oven-dried at 105°C overnight, cooled in a desiccator, and weighted (denoted as W_1), followed by combusting at 400°C for about 8 hours in a muffle furnace (Lindberg blue M BF51748A, Thermo Fisher Scientific, Waltham, Massachusetts, USA). The sample after combustion was further cooled in the desiccator and weighted as W_2. The organic matter of the soil was calculated by the following equation:

$$\text{Organic matter}\,(\%) = (W_1 - W_2)/W_1 \times 100\%.$$

Soil carbon and nitrogen analysis.—Soil carbon and nitrogen content were obtained by grinding 10-g soil samples within a Spex 8000D Mixer-Mill for 3 minutes, which were weighted to a precision of 0.0001 g. Ground soils were then combusted at 950°C using a Leco CN828 analyzer (LECO Corp., St. Joseph, Michigan, USA). Total percentages of carbon and nitrogen in the resulting gasses of a combusted sample were calculated based on the reported weight of the sample.

pH measurement.—The pH of each soil sample was measured using a pH meter with a glass electrode (Accumet AB 150, Fisher Scientific, USA). Briefly, a soil sample was mixed with water at a weight ratio of 1:2, followed by magnetic stirring for 1 minute. The suspension solution was left overnight for equilibrating and was filtered through two layers of Kimber wipe paper. The obtained supernatant was used for pH measurement.

Water-holding capacity.—Soil WHC was determined per standard AWPA E10 (AWPA 2016). An appropriate amount of soil was filled into a Buchner funnel (Ø = 50 mm) that was prefitted with a rapid-filtering paper. The soil was compacted by dropping the filled funnel three times at a height of 10 mm; the extra soil on the top was then scraped off. The funnel was then placed in a beaker and deionized (DI) water was added in to allow for the wetting of soil overnight. The extra water in the soil was removed by a vacuum pump and the mass difference between wet and dry soils was used to calculate the WHC.

Soil–block test

The main steps involved in AWPA standardized soil–block test are (1) preparation of soil culture bottles, (2) inoculation of sterilized wood samples to actively growing soil culture bottles, and 3) determination of mass loss rate due to fungal decay. A total of 216 soil culture bottles were prepared for each soil type. Bottles were filled with different amounts of DI water, based on 130 percent of WHC of each soil. Then 150 g of soil was transferred into the container, followed by placing two pieces of feeder strips on the top of the soil. The container was covered with a lid that had cotton plugs on the top and then was wrapped with aluminum foil. The culture bottles were autoclaved for 45 minutes and cooled under the hood. Upon cooling down,

each bottle was inoculated with two plugs of fungi (~2 mm by 2 mm by the thickness of the plug) that were cut from the outer edge of the actively growing fungus on a petri dish. The plugs were placed upside down on the soil next to the feeder strips. The containers were closed with the lids, wrapped with aluminum foil again, and placed in the incubator until the fungal mycelium was fully covered on the surface of the feeder strips (about 3 to 5 weeks, depending on soil). Once the bottles were prepared, the test blocks, pine for brown rot and poplar for white rot, were sterilized in the autoclave for 30 minutes and were placed on the top of the feeder strips with cross-sections facing down. The culture bottles were capped again and were incubated in the environmental chamber (25°C and 75% relative humidity) in the dark at for 8 weeks (Fig. 1). At the end of the incubation period, the test blocks were removed from the culture bottles and the adhering mycelia on the surface of wood samples were wiped off. The wet mass of the decayed samples was immediately measured to determine their moisture content. The samples were then oven-dried at 50°C for 48 hours to obtain the final mass of the decayed samples. The mass loss (%) due to fungal exposure was calculated by taking the difference between the initial and final oven-dried mass of the tested blocks.

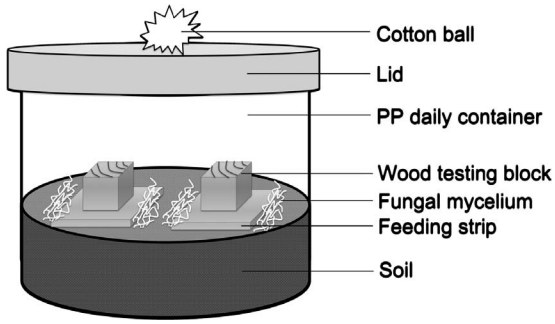

Figure 1.—A schematic drawing of a typical culture bottle and its setup in a standardized AWPA E10 soil–block test.

Statistical analysis

The data of soil properties, wood block moisture content and mass loss were independently collected without bias. These data were normally distributed per Shapiro-Wilk test but do not have equal variances, thus the Kruskal-Wallis test was used with SAS software (SAS System 9.4, SAS Institute Inc., Cary, NC). Significance levels were reported at the 95 percent confidence level ($P < 0.05$). Principal component analysis (PCA) on the properties of soil collected from multiple sites was also performed. Since multiple samples were collected at each site, the mean of each property at each site was calculated. This resulted in a matrix of soil properties in the columns and sites in the rows. We used the R function princomp with the argument cor = TRUE, which scaled each column to perform PCA.

Results and Discussion

Soil properties

The properties of soil collected from various sources are presented in Table 1. All the soils meet the requirement of the AWPA E10 standard by having (1) a WHC of 20 to 40 percent, (2) pH of 5.0 to 8.0, (3) sieved bulk density of at least 0.76 g/cm^3, and (4) been collected from a forested area that had not been exposed to chemicals. Exceptions to meeting these standard requirements are three of the six soils from Idaho (ID#1, ID#2, ID#4) and one from Mississippi (MS). In particular, the sieved bulk density of soil ID#1 was 0.68 g/cm^3 while the MS soil was 0.74 g/cm^3. Both of these sieved densities are slightly lower than the recommended value of 0.76 g/cm^3. In addition, the soil from ID#2 and ID#4 had averaged WHCs that were 19.5 and 18 percent, which are 0.5 and 2 percent lower than the suggested minimum WHC of 20 percent, respectively.

Soil property differences across the nine sites were further examined by PCA, as shown in Table 2 and Figure 2. The first principal component (PC, explaining 70.2% variation) is affected by all the properties, and four of them (organic matter, sieved bulk density, %C, and %N) in particular contribute more. Organic matter and percentage of carbon have similar weights because carbon is one of the major compositions in the organic matter. In terms of PC2 (explaining 22.9% variation), it is mainly affected by soil pH and WHC. In the score plot, Oregon (OR) and MS samples are located away from the other sites, suggesting that the combined soil properties from these two sites are similar but different from the other sites. The Wisconsin soil (WI) is located among ID sites, indicating that the WI samples are similar to ID samples, despite coming from a different state. Additionally, the soil textural class does not have an obvious association with the sites. Many of the Idaho soils are also influenced by volcanic ash and this is not reflected in the soil analyses. Volcanic ash is known to

Table 1.—Properties of soils from various locations in Idaho (ID) and established durability testing labs in Oregon, Mississippi, and Wisconsin (OR, MS, and WI).

Soil source	Series	Horizon[a]	Soil type/ texture	Particle size (%)			Sieved bulk density (g/cm^3)	Water-holding capacity (%)	pH	Organic matter (%)	%C	%N
				Sand	Silt	Clay						
ID#1	Santa	A	Silt loam	28.8	57.5	13.8	0.69 ± 0.05	62.1 ± 1.6	6.3 ± 0.07	2.27 ± 0.13	7.29	0.36
ID#2	Santa	Bw	Silt loam	21.85	63.8	14.4	1.23 ± 0.01	19.5 ± 0.8	6.5 ± 0.05	0.34 ± 0.05	0.45	0.05
ID#3	Santa	Bw2	Silt loam	23.8	61.9	14.4	0.83 ± 0.01	48.5 ± 2.4	6.56 ± 0.04	0.82 ± 0.04	1.78	0.12
ID#4	Kruse	BC	Sandy loam	65.6	25	9.4	1.49 ± 0.02	18.1 ± 0.6	6.24 ± 0.01	0.27 ± 0.05	0.18	0.02
ID#5	Santa	B+xb2	Loam	28.8	50	21.2	1.09 ± 0.03	39.58	6.49 ± 0.03	0.35 ± 0.1	0.13	0.02
ID#6	Hobo	Bw	Silt loam	28.8	65	6.2	0.80 ± 0.02	47.3 ± 1.7	6.4 ± 0.05	1.25 ± 0.05	4.31	0.16
OR	N/A	N/A	Loamy sand	83.8	7.5	8.8	0.89 ± 0.03	22.9 ± 1.3	6.06 ± 0.02	1.83 ± 0.19	4.77	0.11
MS	N/A	N/A	Silt loam	27.5	48.8	23.8	0.74 ± 0.01	31.35	5.6 ± 0.07	2.27 ± 0.2	5.81	0.32
WI	N/A	N/A	Silt loam	24.4	54.4	21.2	1.12 ± 0.01	39.78	6.61 ± 0.06	0.7 ± 0.04	1.86	0.17

[a] Each soil horizon with different letters has different physical, chemical, and biological characteristics.

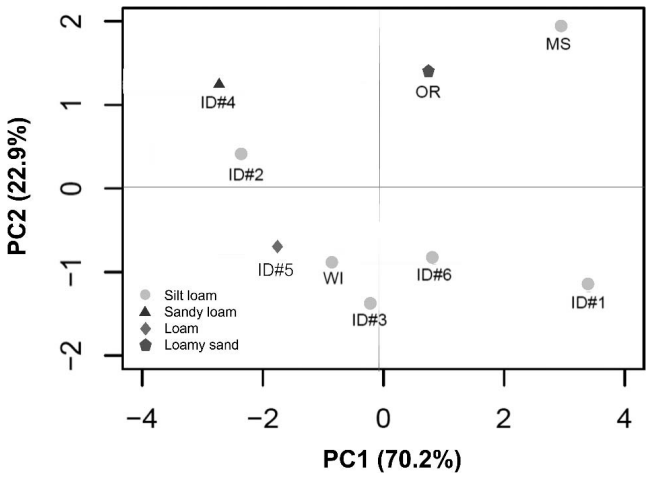

Figure 2.—Score plots of the first two principal components (PC1 by PC2) for soils from various locations. Principal component analysis was performed on the mean properties of soils from nine sites (see "Statistical analysis" section).

increase WHC and can change the availability of some soil nutrients (Shoji and Takahashi 2002).

Four of the six soils from Idaho had different sieved bulk density, pH, WHC, and organic matter, as well as carbon and nitrogen contents, but were all classified as silt loam. This soil textural class is commonly used in the well-established wood durability testing lab at the Department of Sustainable Products, Mississippi State University and Forest Product Lab at Madison, Wisconsin. It is also worth mentioning that the soil from Oregon was actually a mix of sandy loam soil, manure, and other compost materials, and was identified as loamy sand by the hydrometer method in this study.

Soil effect on AWPA E10 soil–block durability test

Effect of individual soil property on the final moisture content and mass loss of decayed wood samples exposed to different fungi.—The effect of different soil properties on wood-block moisture content and mass loss associated with brown-rot or white-rot decay is presented in Figures 3 and 4. Overall, the results from this study are consistent with previous reports that soil type significantly influenced the decay of wood samples caused by different fungi (Amburgey 1978, Colín-Urieta et al. 2019). For example, silt loam soils are associated with a significantly higher mass loss after 8 weeks of fungal exposure across all the tested fungi than those in loam, sandy loam, and loamy sand soil. This is consistent with previous findings (Duncan 1958). Silt loam soils generally have good porosity which leads to good air–gas exchange (USDA NRCS 2020). This makes this soil textural class a good choice for soil–block tests because of its wide availability and generally good WHC (Duncan 1958).

Table 2.—Principal component (PC) loading scores of soil properties from nine sites and proportion of variance explained.

Soil properties	PC loading					
	PC1	PC2	PC3	PC4	PC5	PC6
Organic matter	0.472	0.180	0.072	0.315	0.158	0.785
pH	−0.273	−0.680	−0.052	0.611	−0.263	0.132
Sieved bulk density	−0.439	0.204	−0.721	0.137	0.456	0.136
%C	0.474	0.062	−0.058	0.552	0.347	−0.586
%N	0.455	−0.046	−0.676	−0.139	−0.560	−0.032
Water-holding capacity (%)	0.280	−0.676	−0.106	−0.430	0.514	0.065
Proportion of variance explained	0.702	0.229	0.038	0.020	0.010	0.001
Cumulative proportion	0.702	0.931	0.969	0.989	0.999	1.000

Despite the fact that a minimum sieved bulk density of 0.76 g/cm^3 is recommended in the standard protocol (AWPA E10; AWPA 2016), this factor did not influence the degree of wood decay. For instance, although the sieved bulk density of soils ID#1 and MS was lower than the suggested threshold, these two soils had the highest average wood-block mass loss among all the tested soils.

Soil organic matter and each soil source contain differing amounts of plant and fungal available nutrients. Especially, soil sources can be strongly related to carbon and nitrogen concentrations (Bianchi et al. 2008, USDA NRCS 2010). In this study, we found that higher soil nitrogen resulted in significantly higher mass loss, as shown in Table 1 and Figure 4. This is because nitrogen in the soil can promote the fungal decay of wood (Leutritz 1946). For example, in a wood-stake study using *Pinus taeda* L. and *Populous tremuloides* Michx. on a transect from northern Finland to southern Poland, it was noted that as the stakes decayed, the fungal hyphae were able to move nitrogen into the wood as

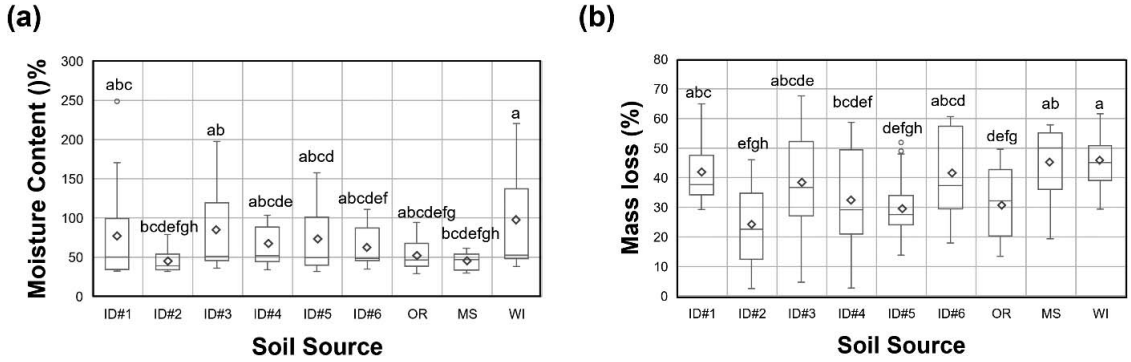

Figure 3.—(a) Moisture contents and (b) mass loss of decayed wood samples after 8 weeks of fungal exposure across nine soils with different origins. The same letter above each bar indicates no significant difference among soil sources at a significance level of 0.05.

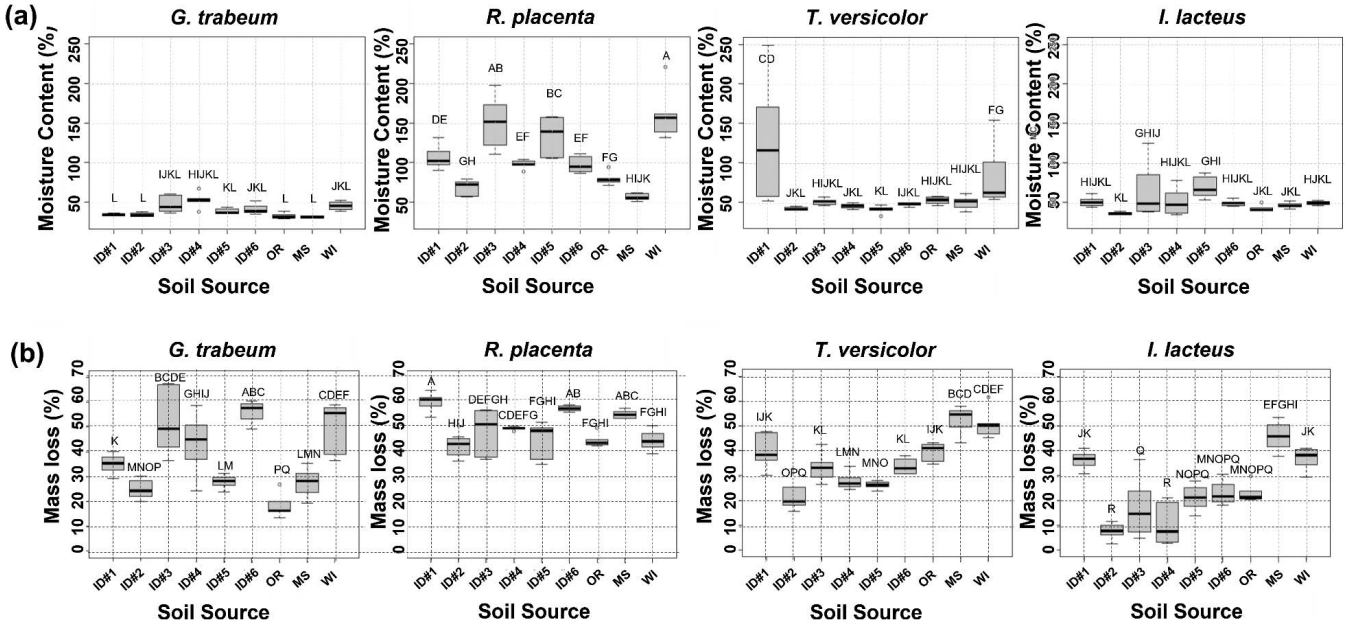

*Figure 4.—(a) Moisture contents and (b) mass loss of decayed wood samples after 8 weeks of brown rot (*Gloeophyllum trabeum *and* Rhodonia placenta*) and white rot (*Trametes versicolor *and* Irpex lacteus*) fungal exposure using different soils. The same letter above each bar indicates no significant difference among soil sources at a significance level of 0.05.*

decay progressed (Jurgensen et al. 2006). Therefore, soils with inherently higher nitrogen content may increase the mass loss of wood blocks.

The standards AWPA E10 and AWPA E22 suggest the soils used for wood-block tests have a WHC of 20 to 40 percent. However, we found that the lower WHC limit had a greater influence on wood-block mass loss in the soil–block test than the upper limit. For example, ID#2, ID#4, ID#5, and OR soils had a WHC close to the lower boundary of the recommended value of 20 percent. This led to some of the lowest average wood-block moisture contents. Likewise, in these four soils, wood-block mass loss is significantly lower than those in the other soils, indicating the importance of soil water in the decay process of wood (Arango et al. 2021). Moreover, low organic matter concentration and low WHC of the soil led to lower wood-block mass loss and moisture content (Duncan 1958). Contrary to the results from this study, a sandy loam soil with lower WHC than recommended resulted in significantly greater mass loss than those soils with a higher WHC (Amburgey 1978). Duncan (1958) concluded that as long as soil WHC was 20 to 40 percent, soil texture and organic matter concentration do not significantly influence wood-block mass loss. Nevertheless, no obvious relationships between soil WHC and wood-block moisture content or mass loss of decayed wood could be identified here as shown in Supplemental Figure S1, due to a desire to avoid pseudoreplication through applying one bulk soil characterization to each wood decay–soil replicate.

The pH of soil used in wood-block tests can alter the growth rate of fungi leading to changes in mass loss of wood blocks. Alkaline (pH $>$ 8.0) and acidic (pH $<$ 5.0) soils may produce varying results because of the interactions with both microbial processes and soil chemical properties (Little et al. 2010). The pH values of all the tested soils were within 6 to 6.7 except for soil from Mississippi, which is more acidic, with a pH of 5.6. No strong relationship between wood-block mass loss and soil pH was identified (Fig. S1), which is similar to the results of Duncan (1958). Although the soil pH range used in this research was relatively narrow, this condition is appropriate for brown-rot and white-rot decaying fungi growth as they prefer an acidic environment (Tudor et al. 2013).

Overall soil effect on the final moisture content and mass loss of decayed wood samples exposed to different fungi.—Generally, each fungus responded to the soils differently among the nine soils tested. This is no surprise as research from field placement of wood blocks indicates that fungal species may be more important than abiotic or soil conditions in wood block decomposition (Maynard et al. 2018). For example, the moisture content of *R. placenta* – decayed wood blocks is markedly higher than those exposed to *G. trabeum*, *T. versicolor*, and *I. lacteus* (Fig. 4a), possibly due to its preference for wetter conditions (Gonzalez and Morrell 2012). Also, a generally higher mass loss due to fungal *R. placenta* decay was observed, regardless of soil source. In contrast, *I. lacteus*–exposed wood blocks had the lowest mass loss in most of the soil tested, except in soils ID#1, MS, and WI, which had few common soil properties except that they had a silt loam texture.

The decay process is also affected by the amount of wood moisture. We found that the moisture contents of decayed samples after 8 weeks of fungal exposure were at least twice higher than the fiber saturation point of 20 to 30 percent. While the optimum moisture content for wood decay is reported to be typically around the fiber saturation point, other reported moisture contents of wood blocks range up to 330 percent (Castillo-Monroy et al. 2014).

From our results, we found that wood-block moisture contents varied from 30 to 200 percent, and that most wood-block mass loss occurred when the moisture content was greater than 30 percent. Also, a quadratic relationship (mass loss $= -0.0023 \times$ moisture content$^2 + 0.6402 \times$ moisture

content + 8.8592) was observed between mass loss and moisture content (Fig. S1) and this model explains 29 percent of the variability in the mass loss ($P > 0.0001$). The fungal decay process generally increases the moisture content of attacked wood blocks due to the slight water condensation on the blocks and fungal respiratory activity in breaking down carbohydrates into carbon dioxide and water (Leutritz 1946). Furthermore, wood-block moisture content is related to soil moisture content and therefore pore distribution and size and movement of water from soil to wood block is important for promoting the decay process. This moisture accumulating process is presumed to stop at the point at which it is no longer limiting the fungal growth (Zabel and Morrell 2012). These results indicate not only the moisture content of wood substrates but also other soil properties might also play important roles in the suitability of the soil–block test.

Conclusion

Soils collected from nine sources with different physical and chemical properties significantly affect the deterioration of wood in a standardized AWPA soil–block test. Generally, the silt loam soil ID#1, which was higher in WHC, organic matter, and carbon and nitrogen concentration was associated with a higher mass loss for both brown-rot and white-rot decay than the other soils tested. However, there are no direct correlations between each soil property and wood-block moisture content or mass loss based on the data presented. In summary, soil physical and chemical characteristics play an important role in wood degradation in a standardized soil–block test and it is the combined effects of many soil properties that act together to govern the decay of wood blocks in soil. In the future, soils selected for soil–block testing should emphasize soil textural class: for example, silt loam with a WHC of 60 percent and organic matter concentration of at least 2 percent. Future research should also perform soil analysis on each culture bottle used for decay tests, thus allowing for using all the replicates for statistical analysis other than their means.

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Supplemental Material

Supplemental material for this article is available at 10.13073/FPJ-D-22-00020.S1.

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