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Relationship between wood-inhabiting fungi and *Reticulitermes* spp. in four forest habitats of northeastern Mississippi

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ABSTRACT

Fungi from coarse woody debris samples containing or lacking termites were isolated, and identified from upland and bottomland hardwoods and pines in northeast Mississippi. Samples yielded 860 unique fungal isolates, with 59% identified to genus level. Four phyla, six classes, 10 orders, 14 families, and 50 genera were recovered. The fungal groups encountered by decreasing taxonomic diversity were Imperfect Fungi, Ascomycota, Zygomycota, Basidiomycota, and unknown fungi. The most frequently encountered fungi were *Penicillium* (81 occurrences), *Nodulisporium* (57), *Cladosporium* (37), *Trichoderma* (34), *Xylaria* (29), *Talaromyces* and *Pestalotia* (27 each), and *Stachylidium* (26). The true wood decay fungi only accounted for 0.9% of the fungi isolated. The only statistical interactions associated with termites were the genus *Nodulisporium*, the class Coelomycetes, and the order Xylariales which all correlated with the absence of termites. Of particular interest is the strong correlation of the Xylariales and absence of termites. These white rot ascomycetes may have inhibitory effects on termites. In addition, the correlation of the genus *Nodulisporium* may be related as many species of this genus are considered asexual stages of *Xylaria* and *Hypoxylon*. There were also a number of significant interactions between wood species, habitat and presence of certain fungi. Most prior research has found an attraction of termites to wood infested with different types of wood-associated fungi. This study, however, found no positive statistical correlations between the presence of termites and any given group of fungi. An increased understanding of these interactions may help locate and isolate biologically active compounds that may influence termite behavior.

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1. Introduction

Subterranean termites belonging to the genus *Reticulitermes* are a key pest of wooden structures in North America. The Eastern subterranean termite, *Reticulitermes flavipes* ranges from Ontario, Canada down throughout the eastern United States and is the major termite pest of residential buildings excluding coastal areas invaded by the Formosan Subterranean termite, *Coptotermes formosanus*. Termites mechanically and chemically digest the structural components of wood, and in the southern United States they are major contributors to wood degradation (Shelton and Grace, 2003). Remedial treatment for termite infestations, repair of damaged structures, and control exceeds $1.5 billion dollars in the United States annually (Su and Scheffrahn, 1998). In the forest environment, subterranean termites are major contributors to soil turnover and nutrient cycling and interact with their surroundings to ensure coarse woody debris is broken down and converted to soil humus. In conjunction with termites, soil decay microorganisms also play critical roles in wood decomposition and nutrient cycling (Savory, 1954; Basham, 1959; Kaarik, 1975). Among the many microorganisms involved in wood deterioration, key are the saprophytic decay fungi. The environmental conditions favorable for termites are also favorable for fungi, and hence the two groups of organisms are commonly found in close association (Kofoid, 1934).

Decay fungi are among the first organisms to invade wood as it is exposed to the soil (Butcher, 1968). Termites compete with fungi for available nutrients and moisture (Sands, 1969), and competition for resources between termites and fungi may lead to habitat partitioning, symbiosis, or pathogenic relationships. Various wood decay fungi have been shown to have an effect on the behavior of subterranean termites. The brown-rot decay fungus, *Gleophyllum trabeum* Pers: Fries (Mur.), was the focus of the majority of early

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studies (Amburgey, 1979). Wood decayed by *G. trabeum* has been found to contain a chemical identical to a trail-pheromone (Matsumara et al., 1969) and also influences shelter tube orientation and food selection (Amburgey and Beal, 1977). A logical extension of this work combined *G. trabeum* decayed wood containing attractive compounds with slow-acting stomach poisons that could be ingested by the termites and transported back to the colony by workers (Esenther and Gray, 1968; Beard, 1974; Olstaff and Gray, 1975; Esenther and Beal, 1978; Su et al., 1995; Su and Scheffrahn, 1996). These decayed blocks impregnated with pesticides served as the precursors to the bait matrices that are used today.

Since then, a wide range of fungi have been found that effect termite behavior and physiology, and these effects vary by termite species. It was originally thought that brown-rotted wood was attractive to termites while wood degraded by white rot fungi was repellent, but it is now apparent that this association is more complex and varied (Grace et al., 1992). How termites respond to decayed wood differs with fungal species (Matsuo and Nishmoto, 1974; French et al., 1981; Cornelius et al., 2002), wood species (Lenz et al., 1991; Cornelius et al., 2004), as well as environmental conditions (Cornelius et al., 2003). Cornelius et al. (2002) compared the response of *R. flavipes* and *C. formosanus* to sawdust infected with three different types of rot fungi (brown, white, and litter rot fungi), and found that both termite species preferred sawdust colonized with any of the three fungi over untreated sawdust. There was also some preference among the three fungi, with white rot and litter rot colonized sawdust preferred over the brown rot colonized sawdust. In addition, the tunneling activity of *C. formosanus* was increased in sand treated with an extract from fungus-infected sawdust compared to the extract from uncolonized sawdust. It has been suggested that wood decayed by fungi may increase the nutritional value by making the digestible sugars more available, by degrading natural inhibitors found in the wood and/or by the production of fungal chemicals that attract the termites (Waller et al., 1987). Since this study was conducted, Little et al. (2012) investigated the feeding preference of *R. flavipes* for wood containing blue stain fungi and bark beetle pheromones. Their results indicated that *R. flavipes* preferentially fed on both air dried and kiln dried SYP blue-stained sapwood as well as kiln and air dried SYP sapwood treated with bark beetle pheromones and that *R. flavipes* is strongly attracted to beetle-killed trees.

Given the close association between termites and decay microorganisms, the successes found in applying *G. trabeum* and the limited nature and variable responses of past research, it is reasonable to hypothesize that other unstudied fungi may elicit termite responses that might be useful in controlling infestations or preventing attack. Identifying these fungi and their behavioral modifying effects on termites is consistent with both the mission of the U.S. Forest Service and the reduced risk policy on pesticides of the U.S. Environmental Protection Agency. With these facts in mind, the objectives of this study were to collect and identify wood-inhabiting fungi found in pine and oak coarse woody debris from upland and bottomland forest habitats in Mississippi, and to correlate the relationship between these fungi and the presence or absence of subterranean termites.

2. Material and methods

2.1. Field procedures

Study sites were located on the U.S. Department of Interior's Noxubee National Wildlife Refuge located 16-km south of Starkville, MS. Four habitats were sampled to reflect contrasting forest ecosystems: bottomland pine, bottomland hardwood, upland pine, and upland hardwood. Forest type (i.e., pine and hardwood), soil type, and elevation were used to distinguish habitats. Bottomland sites were restricted to elevations $\leq$ 76 m and contained bottomland soils typical of northeast Mississippi, while upland sites were restricted to elevations $\geq$ 91 m and contained upland soil types (Brent, 1973, 1986). A 15-m elevation buffer separated the bottomland and upland sites to provide greater ecological contrast between the lower wetter habitats and the higher drier habitats. The two common habitat collection sites, bottomland hardwood and upland pine, were 16 ha in size and contained dominant trees $\geq$15 cm diameter at breast height indicative of each stand type. The two uncommon habitat collection sites, bottomland pine and upland hardwood, were only 4 ha in size. Stands satisfying these criteria were located and numbered on digital maps with only square stands of 4 ha or 16 ha considered. Five stands (plots) per habitat were randomly selected for sampling. Latitudes and longitudes of plot centers were downloaded into a GPS unit to aid in their location on the ground. Sampling was conducted over a 5-wk period, one plot per habitat per week starting in May.

All coarse woody debris (CWD) > 5-cm diameter on the ground and capable of easy dissection with an axe was examined for the presence of termites while progressing outward from plot centers in a concentric fashion: spiraling outward from the center. The first four CWD samples containing termites or termite damage and the first four CWD samples without termites or termite damage were collected in each plot. Only pines or oaks were sampled in their respective forest type. Sample length and diameter were recorded, as well as the length and diameter of the wood containing termites. Termites representing each caste were collected and placed in 70% ethanol.

2.2. Fungal isolations

Wood samples were stored at room temperature before being processed. Decay fungi were cultured on Russell's Agar (25 g agar, 30 g malt extract, and 5 g peptone per liter with 0.3 ml of 1% *o*-phenyl phenol added after sterilization), a media more selective toward wood rotting basidiomycetes (Russel, 1956; Johnson and Curl, 1972). Ten 1–2 cm long wood pieces were removed from each sample and flame sterilized. The wood samples were each placed on separate plates of Russell's agar. After sufficient mycelial growth, each unique culture was transferred to five clean plates of malt extract agar to obtain pure cultures. If several types of fungi were found growing on the initial plate, they were separated and sub-cultured. Unique sub-cultures were grouped according to morphological characteristics found in existing keys (Nobles, 1958; Fergus, 1960; Ellis, 1971, 1976; Gilbertson, 1974, 1980; Gilbertson and Ryvarden, 1986; Chamuris, 1988; Farr et al., 1989; Hanlin, 1990, 1997, 1998; Wang and Zabel, 1990; Domsch and Gams, 1993; St.-Germaine and Summerbell, 1996; Barnett and Hunter, 1998; Watanabe, 2002). Fungi were mounted onto slides and viewed under a light microscope at 200×, 400×, and 1000× magnifications in order to search for taxonomically relevant morphological structures. Unidentified cultures were also analyzed using Fatty Acid Methyl Ester (FAME) analysis or molecular procedures.

2.3. FAME analysis

FAME analysis compares fatty acid presence and concentrations from unknown fungal mycelium extracts to fatty acid profiles of wood decay fungi in a database of ATCC cultures. Samples for FAME were first grown on Sabourand dextrose agar then transferred into Sabourand dextrose broth (Difco, Sparks MD). Mycelia was filtered, rinsed with sterile water and patted dry. FAME extractions on 0.1 g mycelia were preformed according to the manufacturer's specifications (MIDI Inc., Newark, DE) in preparation for gas chromatography. A negative control (with no fungal material) and positive control (consisting of a known fungus with the fatty acid profile stored in the database) were analyzed with each set of samples. The sample extract was injected into an Agilent 6890 gas chromatograph (GC) with a 7683-model auto-sampler connected to a computer with MIDI Sherlock Software (MIDI Inc., Newark, DE). The GC used an Agilent Ultra 2 capillary column; hydrogen (40 ml/min) at 275.79 kPa (40 psi) as the carrier, compressed air (450 ml/min) at 413.685 kPa (60 psi), and nitrogen (45-ml/min) at 413.685 kPa (60 psi). Samples were analyzed on the GC for 20.8 min at an initial temperature of 170 °C, ramped 5 °C per min to 260 °C. The oven temperature increased at 40 °C per min to 290 °C with an equilibration time of 1.5 min. The injector temperature was 250 °C and detector temperature was 300 °C. The FAME profiles of unknown fungi were compared to those in the database using the Sherlock software. The correct genus and species as determined by FAME analysis of the wood fungi were documented and added to a spreadsheet database.

2.4. Molecular identification

Because the FAME database for wood decay basidiomycetes is very limited, efforts were made to identify the remaining fungi by molecular methods. Unknown fungi were transferred to malt broth and placed on a shaker to grow sufficient mycelia for use in PCR. Whole genomic fungal DNA was extracted from 20 mg frozen mycelium using a DNeasy Plant Mini Kit (Qiagen Inc.). A portion of the internal transcribed spacer (ITS) region was amplified by the universal fungal primer set ITS-1 and ITS-4 using an Eppendorff thermocycler (White et al., 1990). All amplified DNA was visually verified on 2% agarose gels. Restriction enzyme cuts were made on the amplified DNA using *Alu* I, *Hinf* I, *Hae* II, and *Taq* I. The four restriction cuts along with the ITS and a 50-base pair ladder were loaded onto a high resolution 2.5% agarose gel and the resulting fingerprint was used to compare the unknown fungal species to known fungal fragment patterns. The correct genus and species of the identified fungi were documented and added to the collection data in a spreadsheet database.

2.5. Statistical procedures

The experiment used a split plot arrangement in a completely randomized design, where whole plots had a factorial treatment structure. The whole plots were created with factorial combinations of two factors, habitat (upland, bottomland) and tree type (pine or oak). Twenty sites were sampled, five per habitat-tree type combination. Each whole plot was further divided into subplots by termite presence. Eight wood samples, four with termites and four without termites, were collected at each site and evaluated for presence or absence of the fungi in question. Binary responses, for presence of fungi, were assumed to follow a binomial distribution.

The relationship between each factor (habitat, tree type, and termite presence) and fungal group (phylum, class, order, genus, and species) were analyzed using the GLIMMIX macro of SAS (SAS, 1999). The GLIMMIX macro analyzes the binary response variable, fungal presence, using a Mixed Model approach with sample site assumed a random effect. A statistical significance level of 0.10 was used. Differences in means of estimated probabilities of fungal presence were evaluated using pair-wise comparisons of the least squares means based on a *t*-distribution in case of significant effects.

Significant effects and interactions were analyzed at the 10% significance level ($P = 0.10$). This level was used to locate any statistical significance between the presence of wood associated fungi and the presence or absence of termites. Because of the exploratory nature of the study, it was deemed essential not to overlook possible relationships between fungal presence and other factors studied. It is acknowledged that this action increases the chance of type I error, but it is viewed as a lesser concern compared with possibly missing relationships that can be studied in more detail at a later time.

3. Results

A total of 160 coarse woody debris (CWD) samples yielded 860 unique fungal isolates. On average, 5.38 isolates were recovered from each sample, slightly more from pine than oak (Table 1). Four phyla, six classes, 10 orders, 14 families, and 50 genera were recovered. The fungal taxonomic groups encountered listed by decreasing taxonomic diversity were Imperfect Fungi (Table 2), Ascomycota (Table 3), Zygomycota (Table 4), Basidiomycota (Table 5), and unknown fungi which have been grouped based on cultural characteristics (Table 6).

The 415 Imperfect Fungi cultured in this study were from two classes, four orders, five families, and 32 genera. Representatives of this group were found in every habitat and approximately 48% of the fungi isolated were imperfect fungi. Hyphomycetes was the largest and most frequently encountered class (362 occurrences) containing two orders, three families, and 27 genera; although this taxonomic representation is incomplete because not all recovered fungi could be identified. Statistical analysis found a significant relationship between fungi belonging to order Moniliales and habitat, with more fungi within the Moniliales occurring from bottomland sites (Table 7). The most commonly encountered genera from the family Moniliaceae were *Penicillium* (81 occurrences), *Nodulisporium* (57 occurrences), *Trichoderma* (34 occurrences), *Arthrographis* (14 occurrences), *Aspergillus* (13 occurrences) and *Exophilia* (11 occurrences). *Penicillium* was the most frequently encountered fungus in this study. Although this genus is a common laboratory contaminant, care was taken to ensure that the isolates counted in this study originated from the wood material. The most common species, *Penicillium tholmii*, was encountered 33 times from all habitats and is considered a soil-born fungus (Domsch and Gams, 1993).

Nodulisporium was the second most commonly encountered fungus. Many species of this genus are considered the asexual stages of *Xylaria* and *Hypoxylon*, both Ascomycetes commonly associated with wood. Statistical analysis carried out at the genus level for commonly occurring genera within the imperfect fungi group found only the genus, *Nodulisporium* spp., statistically significant for a three-way interaction between termite presence, wood type, and habitat (Table 7). *Nodulisporium* spp. was encountered more frequently either in bottomland oak habitats or upland pine habitats, both without termites. Further analysis of these fungi at the genus and species level is needed to determine if these fungi influence termite presence or absence.

Table 1
Summary statistics of fungi isolated from coarse woody debris (CWD) samples.

Parameter	Bottom hardwood	Bottom pine	Upland hardwood	Upland pine	Total
Number of CWD	40	40	40	40	160
Number of isolates	200	219	184	257	860
Average per sample	5.00	5.48	4.60	6.43	5.38

Table 2
Systematic listing of representative members of the imperfect fungi recovered from four forested habitats. A species name is listed only if it was encountered at least five times. The data listed are the number of times a taxonomic group was isolated from the coarse wood debris with and (without) termites.

Fungi	Upland pine + (−) termites	Upland oak + (−) termites	Bottom pine + (−) termites	Bottom oak + (−) termites	Total
Imperfect fungi	59 (53)	38 (49)	63 (59)	38 (56)	415
Class Hyphomycetes	46 (47)	34 (38)	57 (51)	37 (52)	362
Order Moniliales	46 (47)	34 (38)	57 (51)	37 (50)	360
Family Moniliaceae	27 (30)	23 (27)	44 (37)	30 (37)	255
Acremonium	2 (1)	0 (1)	0 (0)	0 (2)	6
Arthrographis	0 (0)	2 (4)	5 (0)	2 (1)	14
Aspergillus	1 (4)	0 (0)	1 (2)	3 (2)	13
Aspergillus versicolor	0 (4)	0 (0)	1 (2)	2 (2)	11
Chrysosporium	2 (0)	0 (0)	0 (2)	0 (0)	4
Exophilia	2 (0)	1 (0)	1 (3)	4 (0)	11
Exophilia jeanselmii	0 (0)	1 (0)	0 (0)	4 (0)	5
Geotrichum candidum	0 (0)	0 (0)	4 (0)	2 (1)	7
Gliocladium	0 (0)	0 (0)	3 (2)	0 (0)	5
Nodulasporium	7 (17)	4 (4)	4 (4)	4 (13)	57
Oidiodendron	0 (0)	0 (2)	0 (0)	0 (0)	2
Paecilomyces	4 (0)	0 (0)	2 (0)	1 (0)	7
Penicillium	5 (7)	12 (8)	19 (19)	5 (6)	81
Penicillium tholmii	0 (0)	5 (2)	14 (12)	0 (0)	33
Rhinocladiella	0 (0)	0 (0)	0 (0)	1 (0)	1
Scopulariopsis	0 (0)	1 (0)	0 (0)	1 (4)	6
Scytalidium	0 (0)	0 (1)	0 (0)	0 (0)	1
Sporobolomyces	0 (0)	0 (0)	2 (0)	0 (0)	2
Trichoderma	4 (1)	2 (6)	3 (5)	7 (6)	34
Verticillium	0 (0)	1 (1)	0 (0)	0 (2)	4
Family Dermatiaceae	15 (14)	7 (7)	11 (14)	5 (11)	84
Aurobasidium	2 (0)	0 (2)	4 (1)	0 (0)	9
Aurobasidium pullulans	2 (0)	0 (0)	2 (1)	0 (0)	5
Chalariopsis	0 (0)	0 (0)	0 (0)	0 (1)	1
Cladosporium	8 (9)	0 (4)	2 (8)	2 (4)	37
Humicola	0 (2)	0 (0)	4 (0)	0 (1)	7
Humicola fuscoatra	0 (2)	0 (0)	4 (0)	0 (0)	6
Lacellina	0 (0)	0 (0)	0 (1)	1 (0)	2
Philaophora	1 (0)	0 (0)	0 (0)	0 (0)	1
Pithomyces	0 (0)	1 (0)	0 (0)	0 (0)	1
Stachylidium	4 (3)	6 (1)	1 (4)	2 (5)	26
Family Tuberculariaceae	0 (1)	0 (0)	0 (0)	1 (1)	3
Fusarium	0 (1)	0 (0)	0 (0)	1 (1)	3
Order Mycelia Sterilia	0 (0)	0 (0)	0 (0)	0 (2)	2
Papulospora	0 (0)	0 (0)	0 (0)	0 (2)	2
Class Coelomycetes	13 (6)	4 (11)	6 (8)	1 (4)	53
Order Sphaeropsidales	5 (2)	0 (4)	5 (4)	1 (4)	25
Family Sphaeropsidaceae	5 (2)	0 (4)	5 (4)	1 (4)	25
Coniothyrium	4 (2)	0 (0)	0 (0)	0 (0)	6
Phylosticta	0 (0)	0 (4)	0 (0)	0 (0)	4
Pyrenochaetes	1 (0)	0 (0)	5 (4)	1 (4)	15
Pyrenochaetes globosa	1 (0)	0 (0)	5 (2)	1 (1)	10
Pyrenochaetes terestris	0 (0)	0 (0)	0 (2)	0 (3)	5
Order Melanconiales	8 (4)	4 (7)	1 (4)	0 (0)	28
Family Melanconiaceae	8 (4)	4 (7)	1 (4)	0 (0)	28
Monochaetia	0 (0)	0 (0)	0 (1)	0 (0)	1
Pestalotia	8 (4)	4 (7)	1 (3)	0 (0)	27

The most commonly encountered genera in the family Dematiaceae were *Cladosporium* (37 occurrences) and *Stachylidium* (26 occurrences). *Cladosporium* was the third most commonly encountered genus in this study. Another common air mold, three species of *Cladosporium* were identified but a majority of the isolates (31) were not identified to the species level. Fungi in the class Coelomycetes are dematiaceous hyphomycetes and produce an acervulus on woody or leafy tissue. Representative genera of this class encountered in this study were *Pestalotia*, *Pyrenochaetes*, *Coniothyrium*, and *Phyllosticta*. The factors wood type and termite presence were found to interact and statistically influence the presence of fungi belonging to class Coelomycetes (Table 7), which were often recovered from oak wood samples without termites (5 with termites present versus 19 with termites absent). The presence of *Pestalotia* (Table 7) was also determined to be influenced by habitat and encountered more frequently in upland systems.

Though Zygomycetes were not an initial consideration of this research, it became apparent that they are important members of the fungal community found in forest ecosystems (Table 3). These fungi are frequently encountered in soil, dung and plant debris. Three genera and species of Zygomycetes were isolated in this study, but there were no noticeable interactions between these fungi and termites. *Mortierella* was the most common zygomycete genera (10 occurrences) encountered in this study. These fungi were widespread over termite infested and non-infested wood samples, but there was a noticeable effect by habitat. Fungi in the Order Mucorales were more commonly encountered in upland pine

Table 3
Systematic listing of representative members of the Zygomycota recovered from four forested habitats. A species name is listed only if it was encountered at least five times. The data listed are the number of times a taxonomic group was isolated from the coarse wood debris with and (without) termites.

Fungi	Upland pine + (−) termites	Upland oak + (−) termites	Bottom pine + (−) termites	Bottom oak + (−) termites	Total
Zygomycota	12 (18)	2 (7)	9 (4)	7 (7)	66
Class Zygomycetes	12 (18)	2 (7)	9 (4)	7 (7)	66
Order Mucorales	12 (18)	2 (7)	9 (4)	7 (7)	66
Family Mortierellaceae	1 (7)	0 (0)	2 (0)	0 (0)	10
Mortierella chlamydospora	0 (7)	0 (0)	0 (0)	0 (0)	7
Family Mucoraceae	0 (0)	0 (1)	0 (1)	0 (0)	2
Mucor	0 (0)	0 (0)	0 (1)	0 (0)	1
Rhizopus	0 (0)	0 (1)	0 (0)	0 (0)	1

habitats and were more commonly recovered from pine versus oak (Table 7). Two of the unknown groups from this study, Groups 3 and 4, are believed to be Zygomycetes.

Ascomycete fungi were commonly encountered (97 occurrences), but the diversity of species was low (6 species). Ascomycetes are sometimes associated with wood decay; several species of *Xylaria* and *Hypoxylon* are classified as white-rot decay fungi. Many other ascomycetes are described as cellulolytic fungi, obtaining their food source from the structural elements of wood. No significant interactions were noted at the phylum and class level. Of the Ascomycetes cultured in this study, there were two classes, three orders, five families, and six genera. There was statistical significance at the order level between the presence of fungi of the Order Xylariales and the absence of termites. *Xylaria* was the most frequently encountered Ascomycete (29 occurrences) and was significantly influenced by habitat, occurring more in upland habitats compared to bottomland (Table 7). Most of the *Xylaria* isolates were not identified to species. Also at the order level Eurotiales was determined to be influenced by wood type, occurring more in oak than pine (Table 7). The second most commonly encountered Ascomycete was in this order, *Talaromyces flavus* with 27 occurrences. The presence of *T. flavus* was determined to be influenced by habitat, with more representatives of this species occurring in bottomland habitats. Other encountered genera were *Sordaria* (13 occurrences), *Eupenicillium* (10) and *Chaetomium* (10).

Basidiomycota was the least encountered phyla with only 11 occurrences. Identification of members of this phylum is very difficult because these fungi do not typically produce spores or fruiting bodies on artificial media and they are morphologically similar. All identified taxa in this group were confirmed by both fatty acid analysis and molecular profiles. Of the two genera isolated in this study, both are true wood decay fungi in the class Hymenomycetes and order Aphyllophorales. *Gleophyllum* is a brown-rot wood decay fungus in the family Polyporaceae. Two species were identified, *G. sepiarium* (7 occurrences) and *G. trabeum* (1 occurrence). *Stereum* is white-rot wood decay fungus in the family Stereaceae. This species was isolated twice. There were no statistical interactions between any of the Basidiomycota groups and habitat or presence/absence of termites. Unknown Group 6 is a likely basidiomycete based on the presence of clamp connections.

Of the total 860 total isolates obtained in this study, 157 unknown isolates were grouped into ten groups based on morphological and cultural similarities (Table 6). These unknown groups were treated as artificial taxonomic groupings to determine any significant effects or interactions due to the presence of representatives of these groups on wood type, habitat, or termite presence. No significance was found when looking at termite presence. Unknown group 3 (Table 6), which was determined to be a zygomycete, had a positive correlation between the presence of members of this group and habitat as it was more common in upland habitats (Table 7). Unknown group 5 was found to be significantly influenced by wood type, with more representatives isolated from oak compared to pine (Table 7). Representatives of each unknown group have been retained for further study and

Table 4
Systematic listing of representative members of the Ascomycota recovered from four forested habitats. A species name is listed only if it was encountered at least five times. The data listed are the number of times a taxonomic group was isolated from the coarse wood debris with and (without) termites.

Fungi	Upland pine + (−) termites	Upland oak + (−) termites	Bottom pine + (−) termites	Bottom oak + (−) termites	Total
Ascomycota	13 (15)	8 (18)	8 (10)	11 (14)	97
Class Plectomycetes	2 (0)	3 (4)	6 (4)	8 (10)	37
Order Eurotiales	2 (0)	3 (4)	6 (4)	8 (10)	37
Family Trichocomaceae	2 (0)	3 (4)	6 (4)	8 (10)	37
Eupenicillium	0 (0)	3 (4)	0 (0)	1 (2)	10
Talaromyces flavus	2 (0)	0 (0)	6 (4)	7 (8)	27
Class Pyrenomycetes	9 (15)	5 (14)	2 (6)	3 (4)	58
Order Hypocreales	0 (0)	2 (0)	0 (0)	0 (0)	2
Family Nectriaceae	0 (0)	2 (0)	0 (0)	0 (0)	2
Nectria	0 (0)	2 (0)	0 (0)	0 (0)	2
Order Sordiales	4 (10)	1 (1)	1 (4)	3 (3)	27
Family Chaetomiaceae	1 (3)	0 (0)	1 (1)	1 (3)	10
Chaetomium	1 (3)	0 (0)	1 (1)	1 (3)	10
Chaetomium globosum	1 (3)	0 (0)	0 (1)	1 (3)	9
Family Sordariaceae	3 (6)	0 (0)	0 (3)	1 (0)	13
Sordaria	3 (6)	0 (0)	0 (3)	1 (0)	13
Order Xylariales	5 (5)	2 (13)	1 (2)	0 (1)	29
Family Xylariaceae	5 (5)	2 (13)	1 (2)	0 (1)	29
Xylaria	5 (5)	2 (13)	1 (2)	0 (1)	29

Table 5
Systematic listing of representative members of the Basidiomycota recovered from four forested habitats. The data listed are the number of times a taxonomic group was isolated from the coarse wood debris with and (without) termites.

Fungi	Upland pine + (−) termites	Upland oak + (−) termites	Bottom pine + (−) termites	Bottom oak + (−) termites	Total
Basidiomycota	6 (1)	0 (1)	0 (2)	2 (0)	12
Class Hymenomycetes	6 (1)	0 (1)	0 (2)	2 (0)	12
Order Aphyllophorales	6 (1)	0 (1)	0 (2)	2 (0)	12
Family Gloeophyllaceae	4 (1)	0 (1)	0 (2)	2 (0)	10
Gleophyllum	4 (1)	0 (1)	0 (2)	2 (0)	10
Gleophyllum sepiarium	3 (0)	0 (0)	0 (2)	2 (0)	7
Gleophyllum trabeum	1 (0)	0 (0)	0 (0)	0 (0)	1
Family Stereaceae	2 (0)	0 (0)	0 (0)	0 (0)	2
Stereum complicatum	2 (0)	0 (0)	0 (0)	0 (0)	2

possible molecular analyses for identification. An additional 112 isolates were complete unknowns; they could not be distinguished by morphological or cultural characteristics and were of rare occurrence, usually occurring only once or twice.

4. Discussion

The underlying objective of this research was to locate interactions among termites and different fungi that occur in the forest ecosystem. Voucher specimens have been retained for each individual genus or species of fungi for further study. The only statistical interactions found associated with termites were the genus *Nodulisporium*, the class Coelomycetes, and the order Xylariales which were all correlated with the absence of termites. Of particular interest is the strong correlation of the Xylariales and absence of termites. This suggests that these white rot ascomycetes may have inhibitory effects on termites. On wood, *Xylaria hypoxylon* is considered an early colonizer and cannot colonize or move into space already occupied by another fungus. However once established, it can maintain its space for many years and prevent other fungi from overgrowing its position (Chapela et al., 1988). Perhaps once established, it can also inhibit termites from its occupied wood. It is recommended that these fungi be utilized in bioassays to measure termite response to *Xylaria*-infested wood. It is possible that fungi belonging to this genus produce extracellular metabolites that affect termite feeding. Another interesting result is the correlation of the genus *Nodulisporium* and the absence of termites. Many species of this genus are considered asexual stages of the ascomycetes *Xylaria* and *Hypoxylon*. The presence of the other interacting factors makes it difficult to determine if this genus actually repels termites, but it should also be a selection for future behavioral studies.

Another ascomycete of particular note was *Chaetomium globosum*. Although this fungus was encountered infrequently, it was often encountered in wood samples that did not contain termites, but was not determined to be statistically significant due to low occurrence (isolated 2 times with termites and 7 times without termites). This species is a soft rot fungus and a common cellulolytic mold in homes. It is known to produce antimicrobial metabolites and has been used as a biocontrol agent for seed-borne fungi (Di Pietro et al., 1991). Because of its high antimicrobial activity, it may also be a good candidate for further study.

One possible limitation of this study is the exclusion of bark beetle damage from our observations. Little et al. (2012) found strong attraction of *R. flavipes* to beetle-killed SYP in laboratory tests, suggesting that *R. flavipes* preferentially feeds on CWD infested with blue stain fungi following bark beetle attack. Significant bark beetle damage was not observed in the samples collected for this study, nor was blue stain fungi (*Ophiostoma* spp., *Ceratocystis* spp., *Leptographium* spp., etc.) isolated from the samples. A more intensive sampling of CWD would be necessary to further investigate those relationships. Blue stain fungi were likely excluded by our choice of isolation media (Russell's agar), which contains 2-phenyl phenol which is labeled for prevention of stain fungi in sawn timber (Ash and Ash, 2004).

The true wood decay fungi only accounted for 0.9% of the fungi isolated. All basidiomycetes isolated were in the Order Aphyllophorales, which contains the majority of wood decay fungi, and in either the Polyporaceae or Stereaceae families. There were no statistical associations between any specific basidiomycetes and termites. One possible explanation for the lack of basidiomycetes encountered in this study may be the stage of decay of the wood sampled. All CWD samples were in more advanced stages of decay and already softened when they were collected. It is possible that only a few dominant decay species remained on the wood at these later stages of decay. In addition, basidiomycetes do not grow as rapidly as other fungi often resulting in a failure to colonize the media before the more aggressive fungi have proliferated.

Most prior research found an attraction of termites to wood infested with different types of wood-associated fungi. However,

Table 6
Grouped unknown fungi recovered from four forested habitats. The data listed are the number of times a group was isolated from the coarse wood debris with and (without) termites.

Fungi	Upland pine + (−) termites	Upland oak + (−) termites	Bottom pine + (−) termites	Bottom oak + (−) termites	Total
Unknown groups	28 (26)	20 (17)	15 (12)	15 (24)	157
Unknown 1	8 (7)	6 (1)	0 (0)	0 (0)	22
Unknown 2	2 (2)	1 (4)	2 (6)	4 (3)	24
Unknown 3	8 (6)	0 (3)	3 (0)	1 (0)	21
Unknown 4	4 (5)	2 (3)	3 (3)	4 (7)	31
Unknown 5	0 (1)	7 (5)	0 (1)	2 (7)	23
Unknown 6	0 (0)	3 (1)	0 (0)	0 (5)	9
Unknown 7	0 (1)	0 (0)	7 (0)	1 (2)	11
Unknown 8	2 (4)	1 (0)	0 (1)	0 (0)	8
Unknown 9	4 (0)	0 (0)	0 (1)	3 (0)	8
Unknown 10	0 (0)	0 (0)	4 (0)	1 (0)	5

Table 7
Summary of significant main effects and interactions found in this study. Majority of occurrences column describes the dominant conditions containing these fungal types.

Taxon identification	Significant effect(s)	*P*-value	Majority of occurrences
Order Moniliales	H	0.0344	Bottomland
Class Coelomycetes	WT * TP	0.0516	Oak without termites
Genus *Pestalotia*	H	0.0875	Upland
Genus *Nodulisporium*	H * WT * TP	0.0323	Upland pine without termites and bottomland oak without termites
Order Mucorales	WT * H	0.0343	Upland pine
Order Erotiales	WT	0.0365	Oak
Species *Talaromyces flavus*	H	0.034	Bottomland
Order Xylariales	TP	0.0476	Termites absent
Order Xylariales	H	0.054	Upland
Group unknown 3	H	0.0492	Upland
Group unknown 5	WT	0.0515	Oak

*denotes the presence of interaction(s) between main effects.
H = habitat; WT = wood type; TP = termite presence or absence.

this study found no positive statistical correlations between the presence of termites and any given group of fungi. In fact, the only correlations were negative ones. An increased understanding of these interactions may help locate and isolate biologically active compounds that may influence termite behavior. Habitat difference accounts for some variation. Those fungi with significant main effects or interactions will be used in behavioral bioassays to determine termite response to fungus-infested wood in a controlled laboratory environment.

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