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Termites and flooding affect microbial communities in decomposing wood

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ABSTRACT

Wood properties and microbial community characteristics were compared between loblolly pine (*Pinus taeda* L.) logs protected or unprotected from termites (Blattodea: Rhinotermitidae: *Reticulitermes* spp.) and other arthropods for two years in seasonally flooded and unflooded forests in the southeastern United States. Significant compositional differences were observed between treatments and between flood patterns for both bacterial and fungal communities. Bacteria were 8–9-fold more abundant in unprotected logs compared to protected logs in both flooded and unflooded forests, with the greatest abundance seen in unprotected and unflooded logs. Wood nitrogen and lignin contents were unaffected by treatment, flood pattern or levels of termite damage visible in unprotected logs. We conclude that termites alter the composition of both bacterial and fungal communities and thus have the potential to indirectly affect wood decomposition and related processes through interactions with the microbial community.

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1. Introduction

Wood decomposition is largely driven by microbes capable of producing the enzymes necessary to digest cellulose and lignin. Although the activities of these organisms are largely controlled by physical conditions such as temperature, moisture and substrate chemistry, decomposition models based on physical conditions alone rarely explain more than 75% of the variation in observed decay rates (Adair et al., 2008; Gholz et al., 2000; Liu et al., 2013; Meentemeyer, 1978; Moorhead et al., 1999; Trofymow et al., 2002). Differences in microbial community composition, richness and assembly history potentially explain much of the remaining variation (van der Wal et al., 2013; van der Wal et al., 2015). For example, Fukami et al. (2010) caused three-fold differences in fungal species richness and decay rates by manipulating the arrival order of fungal colonists. Although randomness no doubt plays an important role in the arrival order of fungi under natural conditions, these communities may be influenced by the activities of other organisms as well. Many insect species share decomposing wood with microbes, for example, and interactions between these organisms are predicted to alter decomposer communities and wood decomposition rates (Jacobsen et al., 2015; Ulyshen, 2016; Ulyshen et al., 2016).

There are a number of potential mechanisms by which invertebrates may alter microbial communities in decomposing wood. Some beetle species have been shown to facilitate fungal colonization by vectoring spores (Strid et al., 2014) or by creating tunnels into wood (Leach et al., 1937). Other invertebrates are thought to influence interspecific interactions between fungi (Crowther et al., 2011; Crowther et al., 2013), feed preferentially on certain fungal taxa (Ingham, 1992) or inhibit fungal activity in general (Warren and Bradford, 2012). Effects of insects on individual fungal species vary depending on the insects involved. In Sweden, Weslien et al. (2011) found *Fomitopsis pinicola*, a brown rot fungus, to be facilitated by a species of bark beetle but inhibited by a species of cerambycid beetle, for example. Overall effects on community metrics have been less studied but Müller et al. (2002) reported a negative correlation between the amount of damage caused by a bark beetle and fungal richness in Finland. While most efforts to explore such relationships have taken place in temperate regions where beetles dominate the wood-feeding insect community, there is a shortage of such studies from tropical or subtropical regions where termites are often the most numerous wood-dwelling insects (King et al., 2013) and can consume larger

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volumes of wood than all other insects combined (Ulyshen et al., 2014). Termites may be especially likely to alter microbial communities given their abundance, their movement of large amounts of soil into wood (Ulyshen and Wagner, 2013; Ulyshen et al., 2014) and their production of anti-microbial compounds. Salivary gland secretions, for instance, are known to have antimicrobial effects in termites and have been shown to protect against fungal pathogens (Bulmer et al., 2012). The fecal material produced by termites and closely related wood cockroaches also appear to have antifungal properties (Chouvenc et al., 2013; Rosengaus et al., 2013) with extended disease resistance arising from growths of Actinobacteria (Chouvenc et al., 2013). Most research in this area has focused on mechanisms by which termites are protected from entomopathogenic fungi whereas no experimental efforts, to our knowledge, have explored how the microbial community at large is affected by these insects under natural conditions.

The implications of interactions between insects and microbes for wood decomposition and nutrient balance also remain unexplored. A number of researchers have proposed a link between invertebrate activity in decomposing wood and nutrient mineralization (Swift, 1977a, b; Swift and Boddy, 1984; Takamura, 2001; Takamura and Kirton, 1999), and have further suggested these organisms may play a key role in releasing nutrients immobilized in fungal tissues (Boddy and Watkinson, 1995). In addition, many wood-feeding insect species are known to promote nitrogen fixation by endosymbiotic and free-living prokaryotes, with poorly understood effects on forest nitrogen dynamics (Ulyshen, 2015). By facilitating the establishment of rot fungi or by improving aeration (e.g., oxygen limitation constrains lignin degradation, see van der Wal et al. (2013)), invertebrates also have the potential to indirectly promote the breakdown of lignin, one of the most recalcitrant components of wood. Indeed, recent research found lignin content to be lower in logs colonized by phloem-feeding cerambycid beetles than in logs without them, suggesting these insects facilitated fungal colonization (Ulyshen et al., 2016).

The objective of the current study was to explore how the exclusion of subterranean termites (*Reticulitermes* spp.) from decaying logs affected wood properties (nitrogen, lignin and water contents) and microbial community characteristics (bacterial and fungal abundance, richness and composition) after two years in both seasonally flooded and unflooded forests in the southeastern United States.

2. Materials and methods

2.1. Location and design

This project was part of a larger study aimed at quantifying the contributions of arthropods to wood decomposition in flooded and unflooded forests in the southeastern U.S. (for a complete description, see Ulyshen, 2014). In total, 200 loblolly pine (*Pinus taeda* L.) logs measuring 55.9 cm long and 23.1 ± 0.2 cm in diameter were cut from trees felled for this purpose. Half the logs were enclosed within stainless steel mesh bags (0.38 mm openings) to exclude termites and other insects whereas the other logs were left unenclosed. These treatments are hereafter referred to as "protected" and "unprotected", respectively. Twenty widely separated (i.e., 0.1–20 km apart) transects were established in mature mixed hardwood/pine forests in northern Mississippi (Oktibbeha, Noxubee and Winston counties). Half of these were situated near streams and flooded from late winter to early spring every year ("flooded") whereas the remainder were situated at slightly higher elevations and never flooded ("unflooded"). Each transect consisted of five plots separated by 10 m. In early November 2010, each plot within each transect received one protected and one unprotected log. Those placed in the flooded forests were secured to a metal stake driven into the ground using metal wire and eye screws. One pair of logs was sampled every six months from each transect beginning and ending at 6 and 31 months, respectively, for a total of five sampling periods. The current study made use of the forty logs (i.e., 10 replicates for each combination of flood pattern and treatment) collected after 24 months in October 2012.

2.2. Wood properties

Data were collected on the nitrogen, lignin and water contents as well as *Reticulitermes* damage from the centers of the forty logs using the following methods. Each log was cut in half and one surface was cleaned of debris with an air hose and photographed. A 20 × 20 grid was superimposed over these images and the percentage of cells containing *Reticulitermes* damage was determined for each log. An electric drill was then used to drill six holes, each measuring 1 cm wide and 9 cm deep. One hole was drilled through the pith whereas the other five were drilled 4.5 cm away from the center hole in the shape of a pentagon. All the wood fragments created during the drilling for each log were collected and the water, nitrogen and lignin contents of these samples were determined. Water content was calculated as a percentage of wet weight after drying the samples at 102 °C for 24 h (i.e., wet weight − dry weight/wet weight) (Ulyshen et al., 2014). After grinding the dried samples into a powder using a Wiley mill, we measured N content (%) using a Perkin Elmer Series II 2400 CHN analyzer at the University of Georgia's Chemical Analysis Laboratory. The lignin contents (%) of these samples were determined (Complex Carbohydrate Research Center, University of Georgia) by pyrolysis-molecular beam mass spectrometry (pyrolysis-MBMS), a method used in previous studies to investigate chemical changes in decomposing wood (Kelley et al., 2002). For this analysis, each sample was prepared in duplicate by weighing about 2.5–3.5 mg into a stainless metal cup, which was single-shot pyrolyzed (Frontier Lab) at 500 °C. The volatile compounds were analyzed for lignin by molecular beam mass spectrometer (Extrel Core Mass Spectrometers). The raw data were processed through The UnscramblerX 10.1 software to obtain the principal components and uncorrected lignin. A standard, NIST 8492 (Lignin content, 26.2%) was pyrolyzed and analyzed in the same manner and in the same batch as the unknown samples. The lignin values from our samples were corrected based on this standard. The average lignin values for the duplicate samples were then calculated and used in data analyses.

2.3. Microbial community characteristics

Estimates of bacterial abundance were made for each sample as follows. A weighed amount (0.15–0.2 g) of wood from each sample was sonicated, diluted (100 or 1000-fold in distilled water), and spread onto petri dishes containing R2A agar (Reasoner and Geldreich, 1985). We used R2A agar because it is less nutrient-rich than many bacteria media (Reasoner and Geldreich, 1985) and may therefore be more suitable for bacteria associated with nutrient-poor substrates like wood (Dr. Susan T. Bagley, personal communication). After several days of incubation at 25 °C, the number of bacterial colonies visible on each plate was counted. Estimates for the number of colony forming units per mg wood, hereafter referred to as "abundance", were then calculated for each sample.

To compare the richness and composition of bacterial and fungal communities between flood patterns and treatments, terminal restriction fragment length polymorphism (T-RFLP) data (i.e., the presence or absence of recognizable taxonomic units, hereafter

referred to as "species") were collected. Although this method is known to overlook rare species and underestimates the richness of microbial communities, it is suitable for making the kinds of general comparisons we sought in the current study (Bent et al., 2007; Blackwood et al., 2007; Fierer, 2007). Two ~0.1 g subsamples were taken from each sample of wood chips, placed in separate sample tubes containing CTAB buffer and pulverized in a bead mill for two 3-minute cycles. After adding 200 μl Tris-EDTA buffer with 20 mg/mL lysozyme (to lyse bacterial cell walls), the samples were sonicated for 5 min. Bacterial and fungal DNA were then extracted with a Machery Nagel Nucleospin Tissue Kit or Plant Kit (Machery Nagel, Easton, PA, USA) and amplified using fluorescently-labeled 16S and ITS primers. Primers used for bacteria were forward: 5′-AGAGTTTGATCCTGGCTCAG-3′ and reverse: 5′-ACGGGCGGTGTGTRC-3' (Liesack and Dunfield, 2004) and for fungi were forward: 5′- CTTGGTCATTTAGAGGAAGTAA-3' (Gardes and Bruns, 1993) and reverse: 5′- TCCTCCGCTTATTGATATGC-3' (White et al., 1990). The forward primers of both sets were labeled with the Beckman WellRed-D4 fluorescent tag. DNA amplification was carried out in an Eppendorff Mastercycler with the following settings for bacteria (16S rRNA gene-based): an initial hot start at 94 °C for five minutes (10 μl DNA template only), melting at 94 °C for 45 s, annealing at 57 °C for 60 s, and extension at 72 °C for two minutes for 30 cycles with a final extension at 72 °C for seven minutes. The settings for fungi (ITS region-based) were: an initial hot start at 94 °C for four minutes (10 μl DNA template only), melting at 94 °C for 35 s, annealing at 55 °C for 55 s, and extension at 72 °C for one minute for 39 cycles with a final extension at 72 °C for 10 min. After the initial hot start, 40 μl of a master mix containing (final concentration) 2 mM Tris reaction buffer, 1.5 mM $MgCl_2$, 0.02 mM of each primer, 0.02 mM bovine serum albumin, 0.02 mM deoxynucleotide triphosphates (dNTPs), ultra-pure water, and 2.5U of Taq polymerase was added to each sample.

After verifying adequate amplification on 2% agarose gels, restriction digests were carried out using *Msp* I and *Taq* I digest enzymes for bacterial and fungal DNA, respectively. After verifying adequate digestion on 2% agarose gels, the samples were purified using a PCR cleanup kit (Machery Nagel, Easton, PA, USA). Samples (5 μl) plus 40 μl Sample Loading Solution (Beckman Coulter) and 0.8 μl of 600 bp Size Standard (Beckman Coulter) were analyzed on a Beckman Coulter GeXP Capillary Electrophoresis system (Beckman Coulter, Fullerton, CA, USA) to produce T-RFLP fragment data. The restriction fragment sizes and bin widths were estimated by the Beckman Coulter software. To distinguish baseline noise from signal peaks, the software removed all peaks below an arbitrarily chosen peak-height threshold value of 5000 Relative Fluorescent Units (RFU). Single peaks were not deleted. Once the software generated the data matrix, all peaks and bins were visually checked and manually adjusted if necessary. The final bacterial and fungal datasets consisted of matrices containing binary presence/absence data. The number of "species" present in each sample was summed to determine species richness and data matrices containing information on the presence or absence of each species were used to compare community composition between flood pattern and treatments.

2.4. Statistical analysis

We conducted two main analyses to investigate how termites influence wood properties [% N, % lignin, % water] and microbial communities [bacterial abundance, bacterial richness, fungal richness and community composition]. The first considered the complete dataset whereas the second was limited to unprotected logs only. For the complete dataset, we conducted two-way ANOVAs to compare response variables between treatments (i.e., protected and unprotected logs) and between flood patterns (flooded and unflooded). The interaction term was included in all models. For these models, data on % N and bacterial abundance were arcsine square root- and log-transformed, respectively, prior to analysis to improve normality. Otherwise untransformed data were used and only untransformed data are presented in figures. To compare among the four treatment × flood pattern combinations, we applied Tukey's multiple comparison test to least square means. To compare community composition between flood patterns and treatments, permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) was carried out using PC-ORD. After eliminating any species present in fewer than three samples, the Jaccard distance measure was used on presence-absence data for bacteria and fungi separately and both tests involved 5000 permutations. To help visualize any compositional differences, nonmetric multidimensional scaling (NMDS) was carried out on the same datasets using the "slow and thorough" option in the autopilot mode of PC-ORD (Kruskal, 1964; Mather, 1976; McCune et al., 2002). The results are presented in ordination diagrams in which each experimental unit is depicted by a symbol and distances between symbols correspond to community dissimilarity (i.e., similarity decreases with increasing separation).

In our second set of analyses, limited to unprotected logs, we were interested in exploring how wood properties and microbial community characteristics varied with the amount of termite damage visible in cross-section. This was accomplished using ANCOVAs, with continuous and categorical variables being damage and flood pattern, respectively. In addition to the six main response variables considered in the analyses of the complete dataset, here we also used ordination scores from our NMDS analyses as metrics for bacterial and fungal community composition. In each case we used scores from the axis explaining the greatest amount of variation; axis 2 ($R^2 = 0.51$) for bacteria and axis 1 ($R^2 = 0.46$) for fungi. Because the interaction between damage and flood pattern was insignificant for all response variables, this term was excluded from the final models. As for the ANOVA models, data on % N and bacterial abundance were arcsine square root- and log-transformed, respectively, prior to analysis. Otherwise untransformed data were used and only untransformed data are presented in figures.

3. Results

3.1. Termite activity

As intended, termites were completely excluded from all protected logs placed within the mesh bags.

By contrast, termites caused a considerable but highly variable amount of damage to unprotected logs in both flooded and unflooded forests. Only three of the 20 unprotected logs lacked visible damage in cross-section. Although termites caused almost twice as much damage, on average, in unflooded (42.2 ± 8.1) compared to flooded (24.8 ± 7.1) forests, the effect of flooding was not statistically significant ($F_{1,18} = 2.6$, $P = 0.1$).

3.2. Wood properties

Based on ANOVA, % N and % lignin did not vary significantly between treatments or flood patterns and no interactions were detected (ANOVA results not shown but see Fig. 1). By contrast, % water varied significantly between treatments ($F_{1,36} = 4.1$, $p = 0.05$) and flood patterns ($F_{1,36} = 8.5$, $p < 0.01$), being lower in protected

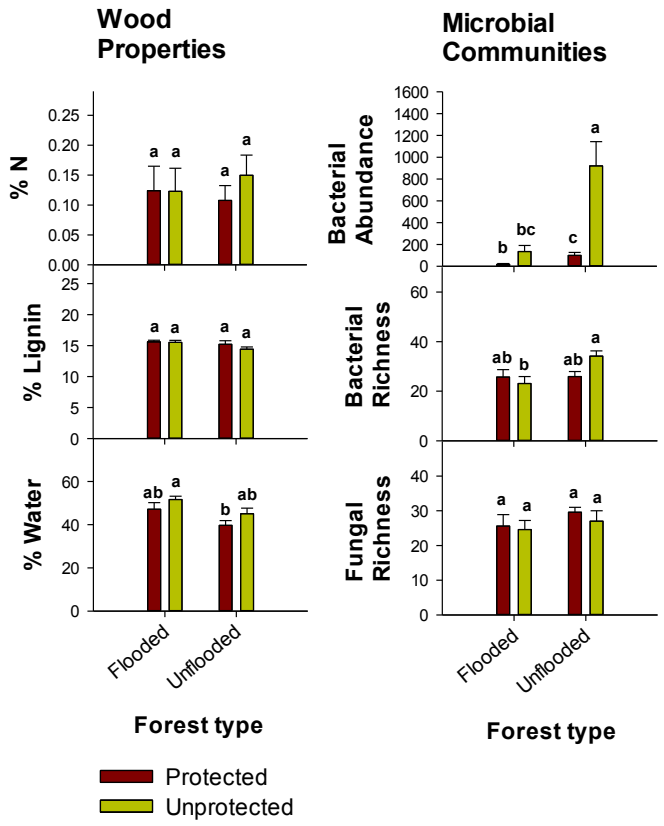

Fig. 1. Mean ± SE (n = 10, untransformed data) wood properties and microbial community characteristics at the centers of logs that were either protected or unprotected from termites for two years in seasonally flooded and unflooded forests. Bars with different letters above them are significantly different ($\alpha < 0.05$) based on Tukey's multiple comparison test. Bacterial abundance refers to the number of colony forming units per mg of wood.

logs and in unflooded forests. When compared for each flood pattern separately, the exclusion treatment did not significantly affect wood water content (Fig. 1). No significant differences were detected between wood properties and the amount of termite damage (Fig. 2).

3.3. Microbial community characteristics

Based on ANOVA, bacterial abundance varied significantly between treatments ($F_{1,36} = 16.3$, $p < 0.001$) and between flood patterns ($F_{1,36} = 24.0$, $p < 0.0001$), being lower in protected logs and in flooded forests. The treatment effect was only significant in unflooded forests based on Tukey's multiple comparison test (Fig. 1). Bacterial richness varied significantly between flood patterns ($F_{1,36} = 5.0$, $p = 0.03$) and there was a significant treatment × forest interaction ($F_{1,36} = 4.6$, $p < 0.04$). Bacterial richness tended to be higher in protected logs in flooded forests whereas the opposite pattern was seen in unflooded forests (Fig. 1). Bacterial richness was significantly higher in unprotected logs in unflooded forests compared to unprotected logs in flooded forests (Fig. 1). Fungal richness did not vary significantly between treatments or flood patterns and there was no significant interaction between these effects. Except for bacterial nmds scores, no significant differences were detected between microbial communities and the amount of termite damage (Fig. 2).

The results from PERMANOVA show bacteria communities, based on T-RFLP patterns, differed significantly between treatments

Table 1
Results from PERMANOVA for bacteria (top) and fungi (bottom). Treatment refers to whether logs were protected or unprotected from insects. Flood pattern refers to whether logs were placed in flooded or unflooded forests.

	df	MS	F	p
Bacteria				
Treatment	1	0.50	2.60	<0.001
Flood pattern	1	0.52	2.66	<0.001
Treatment × Flood pattern	1	0.28	1.43	0.09
Residual	36	0.19		
Fungi				
Treatment	1	1.60	9.14	<0.001
Flood pattern	1	0.88	5.02	<0.001
Treatment × Flood pattern	1	0.45	2.59	0.01
Residual	36	0.17		

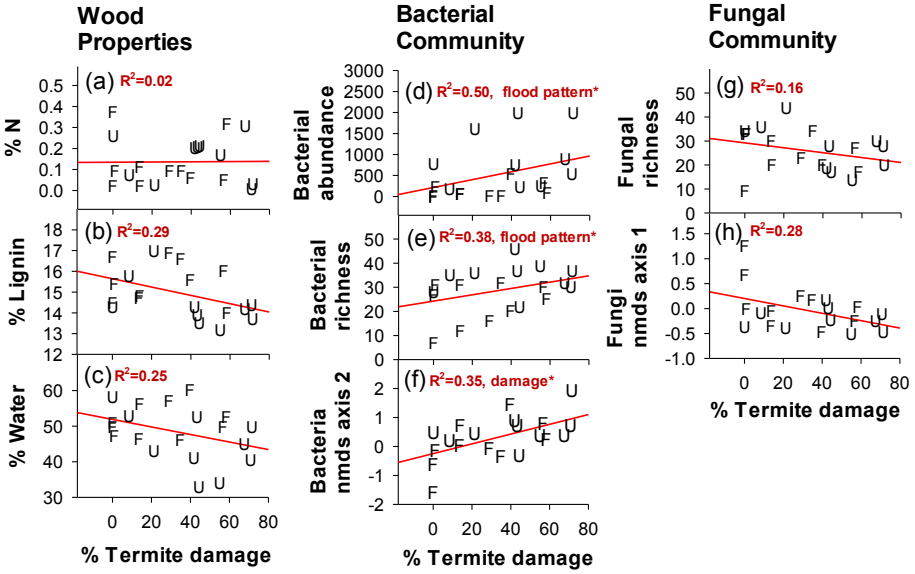

Fig. 2. Relationships between visible termite damage in unprotected logs and % nitrogen (a), % lignin (b), % water (c), bacterial abundance (d), bacterial richness (e), bacteria nmds scores for axis 2 (f), fungal richness (g) and fungi nmds scores for axis 1 (h). Untransformed data are presented in every case. Symbols denote flooded (F) and unflooded (U) forests. Note: significant differences between flood patterns or significant relationships with termite damage are indicated within graphs, asterisks denote significance ($\alpha < 0.05$).

and flood patterns (Table 1). Fungal communities, based on T-RFLP patterns, also differed between treatments and flood patterns and there was a significant interaction between the two factors (Table 1). Nonmetric multidimensional scaling yielded two dimensional solutions for both the bacterial and fungal datasets and a final stress of 22.3 and 17.2, respectively. The differences in bacteria communities detected by PERMANOVA are not easily visualized in the NMDS ordination (Fig. 3). The interaction between treatment and flood pattern detected for fungal communities by PERMANOVA can be visualized, however. There is considerable overlap between flood patterns for unprotected logs whereas the communities associated with protected logs formed comparatively distinct groupings for each flood pattern (Fig. 3).

4. Discussion

Bacterial and fungal communities differed compositionally between logs protected or unprotected from termites. Moreover, bacteria were significantly more abundant in unprotected logs, especially in unflooded forests where termites were more active (Ulyshen, 2014). While these findings speak only to species capable of being cultured under laboratory conditions, they suggest bacteria may be strongly favored by termite activity. Similarly, Chouvenc et al. (2011) found the galleries of *Reticulitermes flavipes* (Kollar) to support diverse microbial assemblages including 100-fold more bacteria than non-gallery material in laboratory arenas. Fungi were not common in the galleries, possibly due to the fungistatic properties of termite fecal material used in gallery construction (Chouvenc et al., 2008). More recently, *Coptotermes formosanus* Shiraki was shown to be protected from entomopathogens by Actinobacteria (*Streptomyces* spp.) growing on the fecal nest material constructed by the species (Chouvenc et al., 2013). The feces associated with the nests of the wood cockroach *Cryptocercus punctulatus* Scudder also appear to provide protection against entomopathogenic fungi (Rosengaus et al., 2013). In addition to the defensive properties of bacteria associated with feces, termites also reduce their exposure to entomopathogens through meticulous grooming and the production of antimicrobial secretions (Rosengaus et al., 2011, and references therein). It remains unclear how these defensive mechanisms affect the broader fungal community or wood decomposition in general. In the current study, fungal richness was not significantly affected by the exclusion of insects and this was true in both flooded and unflooded forests. We also did not see a significant relationship between fungal richness and the level of termite damage visible in cross section.

That we did not find a reduction of nitrogen in unprotected logs is noteworthy considering the longstanding expectation (Swift, 1977a) and observation (Takamura, 2001) that insects accelerate nutrient mineralization from decomposing wood. It is possible that insects simultaneously act to promote nitrogen fixation while also increasing its mineralization with their net effect (i.e., the nitrogen content of wood) depending on the species involved, physical conditions and stage of decomposition (Ulyshen, 2015). Any influence of arthropods on cord-forming basidiomycetes may also alter the nitrogen content of decomposing wood as these organisms are capable of translocating nutrients from outlying sources (Boddy, 1999).

In addition to the effects of insect exclusion, our findings suggest seasonal flooding also affects microbial communities. Both bacterial and fungal communities exhibited compositional differences between flood patterns, for instance. For fungi, PERMANOVA detected a significant interaction between flood pattern and treatment. It appears from Fig. 3 that the fungal communities associated with protected logs in the two flood patterns were more distinct than their unprotected counterparts. These findings suggest insects may dampen the effects of flooding on fungal community composition. Although not statistically significant, almost twice as much termite damage, on average, was reported from the centers of unprotected logs in unflooded forests compared to those from flooded forests. This difference may explain why some of the between-treatment differences (e.g., bacterial abundance and richness) were stronger in unflooded compared to flooded forests.

Exclusion studies are widely used to isolate the ecological effects of particular organisms and have been used extensively in research on leaf litter decomposition. The current study represents the first use of exclusion methods to explore the effects of wood-dwelling arthropods on microbial communities, however. The results from alternative methods—such as opportunistic surveys of microbes occurring in the presence or absence of termites—can be difficult to interpret. For example, Kirker et al. (2012) recently surveyed wood-inhabiting fungi from naturally-occurring logs in the southeastern United States. Although the presence of Xylariales (white rot ascomycetes) was strongly correlated with the absence of termites, it is difficult to know with certainty whether termites were the inhibitors or the inhibited. Exclusion efforts have some important drawbacks as well, however. Most notably, the exclusion method

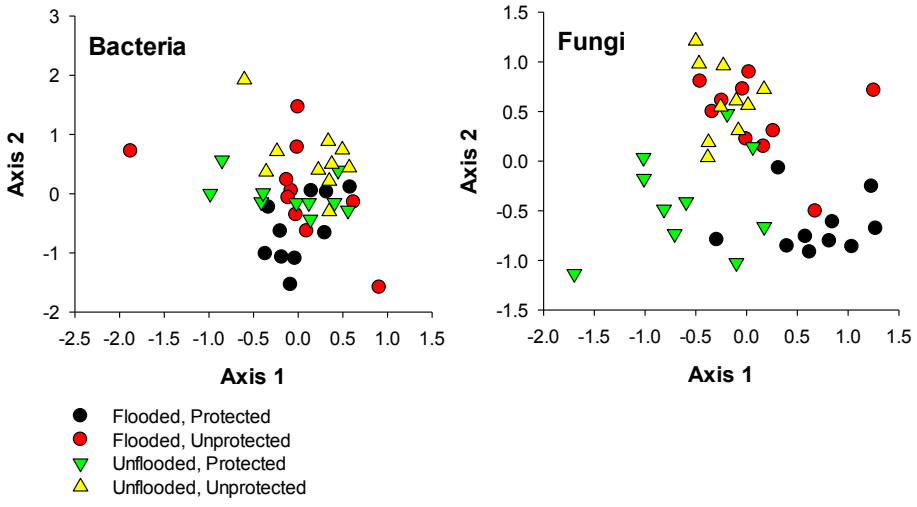

Fig. 3. NMDS ordinations for bacterial (left) and fungal (right) T-RFLP patterns.

itself has the potential to affect the response variable of interest beyond the exclusion of the focal organisms. The most important mechanism by which an exclusion method is likely to affect microbial communities is by altering moisture content. In our study, water content was slightly lower in protected logs compared to unprotected logs in both flooded and unflooded forests. These differences were probably due to the mesh bags and not termite exclusion as these insects appear to negatively affect wood water content (Fig. 2c, Ulyshen et al., 2014). Because these differences were rather small, however, we consider the likelihood that our conclusions were affected to be low. Moreover, our analysis of termite damage, which was limited to unprotected logs, lends additional support to our main conclusions. The relationships observed between % termite damage and the various wood properties and microbial community characteristics were largely consistent with our comparison of protected vs. unprotected logs. We therefore feel confident that the mesh bags allowed us to isolate the effects of termites as intended. We nevertheless acknowledge the possibility that mesh bags have the potential to confound results by altering the microclimate experienced by enclosed logs. We thus encourage future researchers interested in such questions to consider alternative physical exclusion methods such as open-topped pans with screened bottoms that are less likely to alter microclimate (Ulyshen et al., 2016).

Although many insect species were excluded by the mesh bags (Ulyshen, 2014), termites were probably largely responsible for the differences we observed considering they are by far the dominant wood-feeding insects in our study region (Ulyshen et al., 2014). Previous studies have reported significant positive effects of termites on wood decomposition in our study area (Stoklosa et al., 2016; Ulyshen, 2014; Ulyshen et al., 2016). While these organisms have the potential to affect wood decomposition through both direct (e.g., consumption and digestion) and indirect (e.g., altered microbial communities) means, the results from the current study suggest the former mechanism is more important in southeastern U.S. forests. Although both bacterial and fungal communities were affected by termites and seasonal flooding, we found limited evidence that arthropods influence wood decomposition by affecting microbial communities. Most notably, lignin content did not vary between treatments in flooded or unflooded forests. This is consistent with a recent study that also showed no effect of termites on lignin content in decomposing logs belonging to a different tree species (Ulyshen et al., 2016). That study found a significant decrease in lignin associated with beetle activity, however, suggesting that other insects active within decomposing wood may have indirect effects on the process.

5. Conclusions

We sought to explore how subterranean termite activity in decomposing pine logs affects microbial communities in both seasonally flooded and unflooded forests in the southeastern United States. We found the exclusion of termites over the first two years of decomposition resulted in significant compositional differences in both bacterial and fungal communities. Moreover, bacterial abundance was significantly greater for unprotected than protected logs. Further research is needed to determine which species of bacteria and fungi are most influenced by termite activity and to better explore the implications of these interactions for wood decomposition and related processes.

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References

Adair, E.C., Parton, W.J., Del Grosso, S.J., Silver, W.L., Harmon, M.E., Hall, S.A., Burke, I.C., Hart, S.C., 2008. Simple three-pool model accurately describes patterns of long-term litter decomposition in diverse climates. Glob. Change Biol. 14, 2636–2660.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.

Bent, S.J., Pierson, J.D., Forney, L.J., 2007. Measuring species richness based on microbial community fingerprints: the emperor has no clothes. Appl. Environ. Microbiol. 73, 2399–2401.

Blackwood, C.B., Hudleston, D., Zak, D.R., Buyer, J.S., 2007. Interpreting ecological diversity indices applied to terminal restriction fragment length polymorphism data: insights from simulated microbial communities. Appl. Environ. Microbiol. 73, 5276–5283.

Boddy, L., 1999. Saprotrophic cord-forming fungi: meeting the challenge of heterogeneous environments. Mycologia 91, 13–32.

Boddy, L., Watkinson, S.C., 1995. Wood decomposition, higher fungi, and their role in nutrient redistribution. Can. J. Bot. 73, S1377–S1383.

Bulmer, M.S., Denier, D., Velenovsky, J., Hamilton, C., 2012. A common antifungal defense strategy in *Cryptocercus* woodroaches and termites. Insectes Sociaux 59, 469–478.

Chouvenc, T., Efstathion, C.A., Elliott, M.L., Su, N.-Y., 2013. Extended disease resistance emerging from the faecal nest of a subterranean termite. Proc. R. Soc. B 280, 20131885.

Chouvenc, T., Elliott, M.L., Su, N.-Y., 2011. Rich microbial community associated with the nest material of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Fla. Entomol. 94, 115–116.

Chouvenc, T., Su, N.-Y., Elliott, M.L., 2008. Interaction between the subterranean termite *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) and the entomopathogenic fungus *Metarhizium anisopliae* in foraging areas. J. Econ. Entomology 101, 885–893.

Crowther, T.W., Boddy, L., Jones, T.H., 2011. Outcomes of fungal interactions are determined by soil invertebrate grazers. Ecol. Lett. 14, 1134–1142.

Crowther, T.W., Stanton, D.W.G., Thomas, S.M., A'Bear, D., Hiscox, J., Jones, T.H., Voříšková, J., Baldrian, P., Boddy, L., 2013. Top-down control of soil fungal community composition by a globally distributed keystone consumer. Ecology 94, 2518–2528.

Fierer, N., 2007. Tilting at windmills: a response to a recent critique of terminal restriction fragment polymorphism data. Appl. Environ. Microbiol. 73, 8041–8042.

Fukami, T., Dickie, I.A., Wilkie, J.P., Paulus, B.C., Park, D., Roberts, A., Buchanan, P.K., Allen, R.B., 2010. Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. Ecol. Lett. 13, 675–684.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for Basidiomycetes – application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118.

Gholz, H.L., Wedin, D.A., Smitherman, S.M., Harmon, M.E., Parton, W.J., 2000. Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. Glob. Change Biol. 6, 751–765.

Ingham, R.E., 1992. Interactions between invertebrates and fungi: effects on nutrient availability. In: Carroll, G.C., Wicklow, D.T. (Eds.), The Fungal Community: its Organization and Role in the Ecosystem, second ed. Marcel Dekker, Inc., New York, pp. 669–690.

Jacobsen, R.M., Birkemoe, T., Sverdrup-Thygeson, A., 2015. Priority effects of early successional insects influence late successional fungi in dead wood. Ecol. Evol. 5, 4896–4905.

Kelley, S.S., Jellison, J., Goodell, B., 2002. Use of NIR and pyrolysis-MBMS coupled with multivariate analysis for detecting the chemical changes associated with brown-rot biodegradation of spruce wood. FEMS Microbiol. Lett. 209, 107–111.

King, J.R., Warren, R.J., Bradford, J.B., 2013. Social insects dominate eastern US temperate hardwood forest macroinvertebrate communities in warmer regions. PLoS ONE 8, e75843.

Kirker, G.T., Wagner, T.L., Diehl, S.V., 2012. Relationship between wood-inhabiting fungi and *Reticulitermes* spp. in four forest habitats of northeastern Mississippi. Int. Biodeterior. Biodegrad. 72, 18–25.

Kruskal, J.B., 1964. Nonmetric multidimensional scaling: a numerical method. Psychometrika 29, 115–129.

Leach, J.G., Orr, L.W., Christensen, C., 1937. Further studies on the interrelationship of insects and fungi in the deterioration of felled Norway pine logs. J. Agric. Res. 55, 129–140.

Liesack, W., Dunfield, P.F., 2004. T-RFLP analysis. In: Spencer, J.F.T., Ragout de

Spencer, A.L. (Eds.), Environmental Microbiology: Methods and Procedures. Humana Press, Totowa, NJ, pp. 23–37.
Liu, W., Schaefer, D., Qiao, L., Liu, X., 2013. What controls the variability of wood-decay rates? For. Ecol. Manag. 310, 623–631.
Mather, P.M., 1976. Computational Methods of Multivariate Analysis in Physical Geography. John Wiley & Sons, London.
McCune, B., Grace, J., Urban, D.L., 2002. Analysis of Ecological Communities. MjM Software Design, Gleneden Beach, Oregon.
Meentemeyer, V., 1978. Macroclimate and lignin control of litter decomposition rates. Ecology 59, 465–472.
Moorhead, D.L., Currie, W.S., Rastetter, E.B., Parton, W.J., Harmon, M.E., 1999. Climate and litter quality controls on decomposition: an analysis of modeling approaches. Glob. Biogeochem. Cycles 13, 575–589.
Müller, M.M., Varama, M., Heinonen, J., Hallaksela, A.-M., 2002. Influence of insects on the diversity of fungi in decaying spruce wood in managed and natural forests. For. Ecol. Manag. 166, 165–181.
Reasoner, D.J., Geldreich, E.E., 1985. A new medium for the enumeration and sub-culture of bacteria from potable water. Appl. Environ. Microbiol. 49, 1–7.
Rosengaus, R.B., Mead, K., Du Comb, W.S., Benson, R.W., Godoy, v.G., 2013. Nest sanitation through defecation: antifungal properties of wood cockroach feces. Naturwissenschaften 100, 1051–1059.
Rosengaus, R.B., Traniello, J.F.A., Bulmer, M.S., 2011. Ecology, behavior and evolution of disease resistance in termites. In: Bignell, D.E., Roisen, Y., Lo, N. (Eds.), Biology of Termites: a Modern Synthesis. Springer, New York, pp. 165–191.
Stoklosa, A.M., Ulyshen, M.D., Fan, Z., Varner, M., Seibold, S., Müller, J., 2016. Effects of mesh bag enclosure and termites on fine woody debris decomposition in a subtropical forest. Basic Appl. Ecol. 17, 463–470. http://dx.doi.org/10.1016/j.baae.2016.03.001.
Strid, Y., Schroeder, M., Lindahl, B., Ihrmark, K., Stenlid, J., 2014. Bark beetles have a decisive impact on fungal communities in Norway spruce stem sections. Fungal Ecol. 7, 47–58.
Swift, M.J., 1977a. The ecology of wood decomposition. Sci. Prog. 64, 175–199.
Swift, M.J., 1977b. The roles of fungi and animals in the immobilisation and release of nutrient elements from decomposing branch-wood. In: Lohm, U., Persson, T. (Eds.), Soil Organisms as Components of Ecosystems, pp. 193–202.
Swift, M.J., Boddy, L., 1984. Animal-microbial interactions in wood decomposition. In: Anderson, J.M., Rayner, A.D.M., Walton, D.W.H. (Eds.), Invertebrate-microbial Interactions. Cambridge University Press, Cambridge, pp. 89–131.
Takamura, K., 2001. Effects of termite exclusion on decay of heavy and light hardwood in a tropical rain forest of peninsular Malaysia. J. Trop. Ecol. 17, 541–548.
Takamura, K., Kirton, L.G., 1999. Effects of termite exclusion on decay of a high-density wood in tropical rain forests of peninsular Malaysia. Pedobiologia 43, 289–296.
Trofymow, J.A., Moore, T.R., Titus, B., Prescott, C.E., Morrison, I., Siltanen, M., Smith, S., Fyles, J., Wein, R., Camiré, C., Duschene, L., Kozak, L., Kranabetter, M., Visser, S., 2002. Rates of litter decomposition over 6 years in Canadian forests: influence of litter quality and climate. Can. J. For. Res. 32, 789–804.
Ulyshen, M.D., 2014. Interacting effects of insects and flooding on wood decomposition. PLoS ONE 9, e101867.
Ulyshen, M.D., 2015. Insect-mediated nitrogen dynamics in decomposing wood. Ecol. Entomol. 40 (Suppl. 1), 97–112.
Ulyshen, M.D., 2016. Wood decomposition as influenced by invertebrates. Biol. Rev. 91, 70–85.
Ulyshen, M.D., Müller, J., Seibold, S., 2016. Bark coverage and insects influence wood decomposition: direct and indirect effects. Appl. Soil Ecol. 105, 25–30.
Ulyshen, M.D., Wagner, T.L., 2013. Quantifying arthropod contributions to wood decay. Methods Ecol. Evol. 4, 345–352.
Ulyshen, M.D., Wagner, T.L., Mulrooney, J.E., 2014. Contrasting effects of insect exclusion on wood loss in a temperate forest. Ecosphere 5 article 47.
van der Wal, A., Geydan, T.D., Kuyper, T.W., de Boer, W., 2013. A thready affair: linking fungal diversity and community dynamics to terrestrial decomposition dynamics. FEMS Microb. Ecol. 37, 477–494.
van der Wal, A., Ottosson, E., de Boer, W., 2015. Neglected role of fungal community composition in explaining variation in wood decay rates. Ecology 96, 124–133.
Warren, R.J., Bradford, M.A., 2012. Ant colonization and coarse woody debris decomposition in temperate forests. Insectes Sociaux 59, 215–221.
Weslien, J., Djupström, L.B., Schroeder, M., Widenfalk, O., 2011. Long-term priority effects among insects and fungi colonizing decaying wood. J. Animal Ecol. 80, 1155–1162.
White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: a Guide to Methods and Applications. Academic Press, San Diego, pp. 315–322.