

Insect-Microbial Interaction

Termite Presence and Feeding on Loblolly Pine Wood Differs Among Four Root-Infecting Bluestain (ophiostomatoid) Fungal Species

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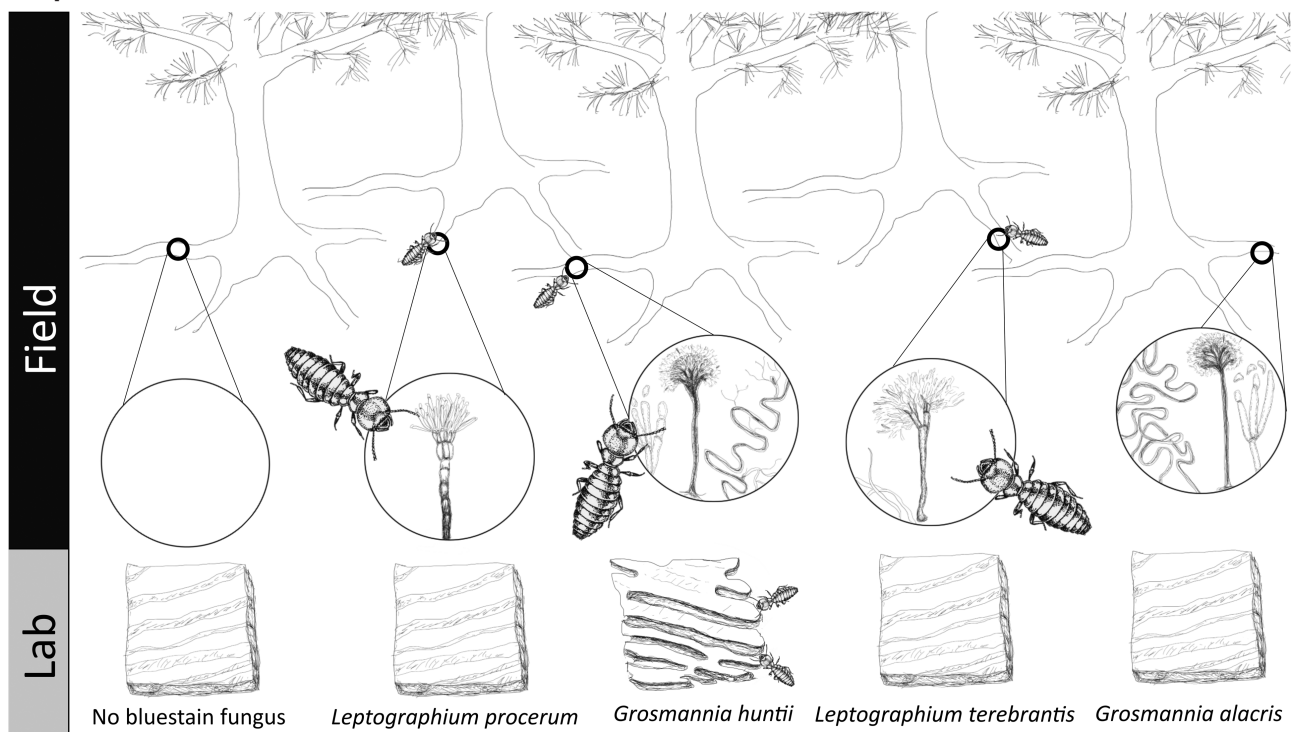
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Abstract

Bark beetles and root weevils can impact forests through tree death on landscape scales. Recently, subterranean termites have been linked to these beetles via the presence of bluestain fungi (Ascomycota: Ophiostomataceae), which are vectored to trees by beetles. However, only a small subset of bluestain species have been examined. Here, we tested whether termite-bluestain association patterns in the field reflect termite feeding preference in laboratory choice trials. We documented the presence of four bluestain fungi (*Leptographium procerum* (W.B. Kendr.), *L. terebrantis* (Barras & Perry), *Grosmannia huntii* (Rob.-Jeffer.), and *G. alacris* (T.A. Duong, Z.W. de Beer & M.J. Wingf.) in the roots of 2,350 loblolly pine trees in the southeastern United States and whether termites were present or absent on these roots and paired this with laboratory choice feeding trials. Termites were found 2.5-fold on tree roots with at least one bluestain fungus present than tree roots without bluestain fungi. Although termites in this study and others were associated with *L. procerum*, *L. terebrantis*, and marginally *G. huntii*, termites only showed preferential feeding on wood inoculated with *G. huntii* in laboratory trials. This suggests that increased termite presence on wood with bluestain fungi may be driven by factors other than increased wood palatability. Termites could thus disproportionately affect wood turnover rates for specific pools (e.g., bark beetle and root weevil attacked trees) and in some cases (e.g., *G. huntii*) accelerate wood decomposition. This study supports the growing evidence that the association between subterranean termites and bluestain fungi is spatially and taxonomically widespread.

Graphical Abstract



Key words: subterranean termites, root weevils, sap-stain fungi

Forest ecosystems are complex, dynamic systems that cover 31% of Earth's surface and store ca. 861 Gt C, rendering them the largest terrestrial carbon sink (Harmon et al. 1986, Pan et al. 2011). Species interactions within forests can impact forest ecosystem functions (Wardle 2002). For example, plant–microbe–insect interactions can change plant quality such as nutritional content and have cascading effects on both above- and belowground food webs (Van der Putten et al. 2009, Biere and Bennett 2013). Decomposer and belowground species interactions have received significantly less attention than aboveground interactions likely because of the small size of organisms and the opaque soil habitat, which makes direct observations difficult (Decaens 2010). However, a large portion of C and other nutrients are stored in belowground systems and detrital pools. For example, dead wood alone accounts for 8% of C (69 Gt) stored by forest globally (Pan et al. 2011). Understanding species interactions in belowground systems is essential to better understand forest ecosystem function and nutrient cycling dynamics.

Subterranean termites are ecosystem engineers whose activity can directly impact decomposition processes and rates of nutrient cycling in forest ecosystems (Eggleton and Tayasu 2001, Jouquet et al. 2011, Bradford et al. 2014, Maynard et al. 2015). When present, termites are the primary decomposers of wood along with fungi and thus understanding the factors that drive their forest-scale dispersion patterns and feeding activity are essential for understanding forest nutrient and carbon cycling (Griffiths et al. 2021). Termite interactions with fungi can influence termite foraging behavior and distribution (Viana-Junior et al. 2018 and references therein). For example, termites often increase consumption of wood infected with the brown rot fungus *Gleophyllum trabeum* (Pers.) Murrill, and white rot fungus *Phanerochaete chrysosporium* Brudsaal (Esenher et al. 1961; Amburgey 1979; Cornelius et al. 2002, 2012;

Little et al. 2013a) and in the field, this can influence termite presence on deadwood (Viana-Junior et al. 2018). However, some evidence suggests that this relationship may be driven by the advanced state of wood decay caused by decay fungi rather than due to the fungi themselves and termites can suppress decay fungi growth like *G. trabeum* when decay fungi and termites are in competition for cellulose in deadwood (Jayasimha and Henderson 2007a, b; Gazal et al. 2014). Presence of some fungi in deadwood can also decrease termite presence and wood consumption via increasing toxic secondary metabolites (Amburgey and Beal 1977, Kamaluddin et al. 2016, Viana-Junior et al. 2018). A recent review by Viana-Junior et al. (2018) found only 45 studies that included termite–fungi interactions and the vast majority were focused on decay fungi and specifically *G. trabeum*. Given the ubiquity and diversity of termites and fungi in deadwood, this suggests that our understanding of the ecology of termite–fungi interactions is still nascent.

Increasingly, studies are demonstrating that termites can be found in association with not only dead decaying trees, but also living trees in their trunks and roots (Harris 1969, Lai et al. 1983, Grace 1987, Osbrink et al. 1999, Riggins et al. 2014, Clay et al. 2017). Recent evidence also suggests that termites may recruit to dead and living trees when certain non-decay fungi are present. Specifically, bluestain fungi (Ascomycota: Ophiostomataceae) which are vectored by root weevils and bark beetles (Coleoptera: Curculionidae) (Little et al. 2012a, b; Little et al. 2013a; Riggins et al. 2014; Clay et al. 2017; Siegert et al. 2018). Bark beetles and root weevils (subfamilies Scolytinae and Molytinae respectively) are some of the most prevalent aboveground and belowground herbivores of coniferous forests that can have massive impacts on forest productivity (Leather et al. 1999, Aukema et al. 2010, Vega and Hofstetter 2015). Bark beetles, such as the southern pine beetle (*Dendroctonus frontalis* Zimmermann) and mountain

pine beetle (*D. ponderosae* Hopkins), kill millions of coniferous trees annually. For example, from 2015 to 2017, southern pine beetle killed 25% of forests in Honduras and from 1990 to 2008 a mountain pine beetle outbreak affected more than 47 million ha of forest in the western United States (Raffa et al. 2008, Billings 2015, Gomez et al. 2020). These aboveground herbivores ultimately generate massive amounts of deadwood that decomposes and enters belowground systems. Similarly, belowground, root weevils can cause major conifer mortality, particularly for young trees (Leather et al. 1999). Bark beetle and root weevil impacts on trees and forest ecosystems extend beyond tree death, beetles and their phoretic mites vector suites of ascomycete fungi to trees (Ostrosina et al. 1997, 1999; Klepzig et al. 2001; Eckhardt et al. 2007; Aukema et al. 2010; Zanzot et al. 2010). Some of these fungi infect the sapwood of the tree staining it blue or black, which is where the ophiostomatoid fungi get their characteristic name: bluestain fungi (Wingfield et al. 1993). Mites and beetles may feed upon the fungi, but the impact these bluestain fungi have on subsequent species interactions and their effects on ecosystem function are less understood.

Bluestain fungi do not affect the structural integrity of the tree (e.g., do not decompose cellulose, hemicellulose or lignin of wood, Humar et al. 2008), but some species can have important interactions with termites (Little et al. 2012a, b; Little et al. 2013a; Riggins et al. 2014; Clay et al. 2017). Specifically, some subterranean termites recruit to and preferentially feed upon wood infected with at least some species of bluestain fungi. Previous studies in the southeastern United States that examined the relationship between termites and aboveground bark beetles have demonstrated that both native (*Reticulitermes* spp.) and non-native (*Coptotermes formosanus* Shiraki) subterranean termites recruited to and preferentially fed on wood containing *Ophiostoma minus* (Hedgcock) bluestain fungi (Little et al. 2012a, b; Little et al. 2013a). Additionally, field studies in the southeastern United States have demonstrated that termites will recruit to living trees inoculated with *Leptographium terebrantis* (Barras & Perry) and *L. procerum* (W.B. Kendr.) in both the roots and the base of the tree (Riggins et al. 2014, Clay et al. 2017). However, there are hundreds of bluestain fungi species vectored by bark beetles and root weevils and multiple subterranean termite species (Wingfield et al. 1993, Eggleton and Tayasu 2001). Thus, more resolution is needed to determine whether the association between bluestain fungi and termites is taxonomically widespread.

Here we examined the relationship between subterranean termites and four species of bluestain fungi vectored by root weevils. This study built upon the study of Riggins et al. (2014), which examined *Leptographium* field associations with subterranean termites in the roots of loblolly pine trees in the southeastern USA. Here, we expanded this research to investigate the association between subterranean termites and two additional bluestain fungi in the genus *Grosmannia* in loblolly pine tree roots in pine stands from Alabama to Texas. Additionally, we performed laboratory choice feeding assays to determine native southeastern USA subterranean termite (*Reticulitermes* spp.) feeding preferences between yellow pine wood inoculated with one of the four bluestain fungal species (*L. procerum*, *L. terebrantis*, *G. alacris* [T.A. Duong, Z.W. de Beer & M.J. Wingf.], *Grosmannia huntii* [Rob.-Jeffer.] or sterile controls. We then compared these field observations (both the previously published *Leptographium* and newly examined *Grosmannia* species) with results from laboratory experiments to determine if field associations match termite feeding preferences. If termite feeding preferences match field observations, this may suggest wood inoculated with bluestain fungi are more nutritious. To test the hypothesis that wood inoculated with bluestain fungi is more nutritious than wood

without bluestain fungi, we analyzed the carbon and nitrogen content of bluestained wood and compared them to uninfected wood. If termite field associations with bluestain fungi on tree roots do not coincide with laboratory feeding preferences and wood chemistry results, this may suggest termites use the bluestain fungi as a cue for some other important resource such as suitable habitat.

Materials and Methods

Field Study

From 2000 to 2012, 2,350 loblolly pine (*Pinus taeda*) root samples were collected in Texas, Louisiana, Mississippi, and Alabama, USA for multiple studies to investigate the pathogenicity of *Leptographium* and *Grosmannia* fungi (Eckhardt et al. 2004a,b, 2007). One hundred fourteen forest sample sites were distributed across states, and consisted of even proportions of military installations, National Forests, and industry monoculture plantations (Fig. 1; see methods in Riggins et al. 2014). Root samples were excavated within each 1/16th acre plot (252.9 m²) from six dominant trees in the plot center as per forest health monitoring protocols (Dunn 1999). Root samples were collected with hand tools by excavating two >3 cm diameter lateral root segments from opposite sides of the tree to the approximate crown drip line (Ostrosina et al. 1997). At the time of root collection, the presence or absence of subterranean termites was recorded.

After roots were excavated, the presence and identity of fungi were determined from laboratory inspection and cultures (following methods of Klepzig et al. 1991, Ostrosina et al. 1997). Excavated roots were sectioned in situ into 20 cm segments starting 16 cm from each tree's root collar. Each segment was isolated in a plastic bag and placed on ice. Once in the laboratory, root segments were stored at 4°C for 2–3 d then visually inspected for presence or absence of four ophiostomatoid fungi: *L. procerum*, *L. terebrantis*, *G. huntii*, and *G. alacris*, which stain wood blue. Their presence or absence and identity was then verified by plating 160 surface sterilized root pieces from root segments onto 40 plates per tree (4 pieces per plate). Plates consisted of malt extract agar (MEA) and cycloheximide-streptomycin malt agar (CSMA) and were incubated at 25°C under 24 h fluorescent lighting (460 μmol/m²/s). After 2 wk, plates were examined under a dissecting microscope for the presence of ophiostomatoid fungi. Ophiostomatoid fungi, growing on the media or root segments were transferred to CSMA or MEA amended with streptomycin only (SMEA) and serially transferred until axenic cultures were obtained. Isolates were then transferred to MEA for identification similarly to the process described by Ostrosina et al. (1997) and isolates were archived on agar slants. Neither *Grosmannia* species ever occurred alone on roots, which confounds its results with other bluestain species. Consequently, they were excluded from the original study because additional experiments and longer-term lab testing were needed to tease apart the relationship between *Grosmannia* species and termite presence on tree roots and their relationship to termite feeding activity (Riggins et al. 2014). We included the *Grosmannia* species here and compare them with the laboratory feeding trials (below) to more comprehensively examine the relationship between subterranean termites and diversity of bluestain species and associate field patterns with laboratory feeding assays.

Statistical analysis

We tested the null hypothesis of no difference in presence or absence of subterranean termites on loblolly pine tree roots with *G. huntii* and *G. alacris* using logistic regression in SPSS v. 23 (IBM

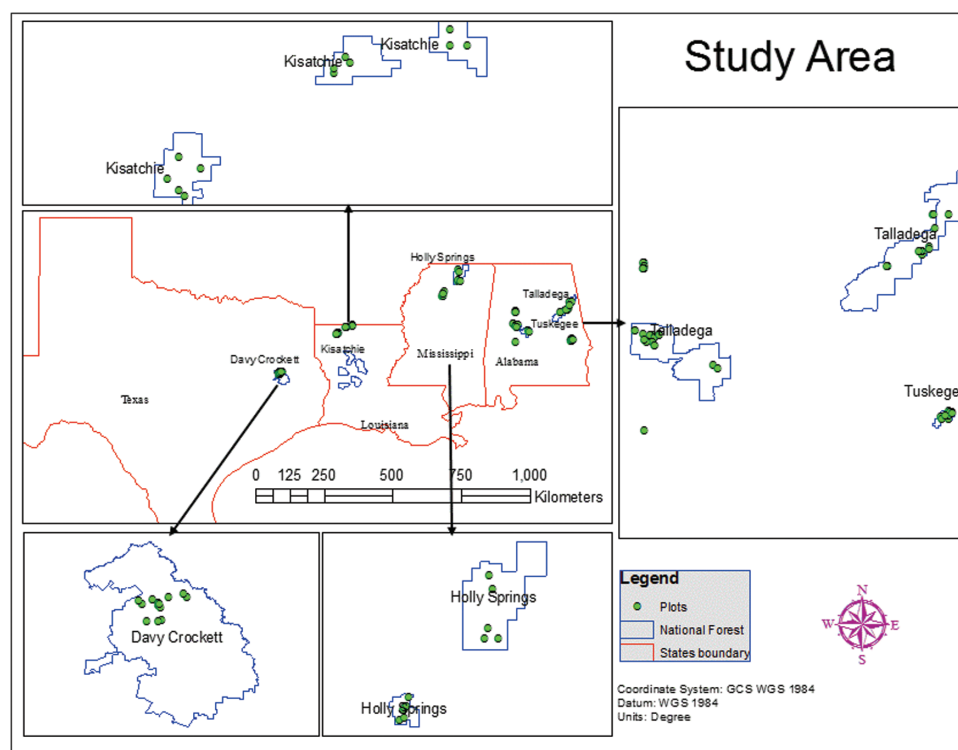


Fig. 1. Map of the 2350 loblolly pine (*Pinus taeda*) root samples collected in Texas, Louisiana, Mississippi, and Alabama. Dots (green colour can be seen in figure online) represent sampling sites.

Corp. 2013). We did not test *L. terebrantis* and *L. procerum* here because these species had previously been analyzed and found to be positively correlated with termite presence in the same field study (Riggins et al. 2014). Additionally, we tested whether the presence of multiple species of fungi on tree roots impacted the presence of termites. Specifically, we used Chi Square Test to test the null hypothesis of no difference in proportion of roots with termites present among roots that varied in number of fungal species present (0, 1, 2, 3, or 4). Significant results were followed by Bonferroni Corrected for multiple comparisons ($\alpha = 0.005$) pairwise comparisons of Z-scores and calculated *P*-values (Beasley and Schumacker 1995, Garcia-Perez and Nunez 2003).

Laboratory Study

From the field study, we identified two ophiostomatoid species whose association with the presence of subterranean termites was undetermined (not previously examined): *Grosmannia alacris* and *G. huntii*, and two fungi that were positively correlated with presence of subterranean termites *Leptographium procerum* and *L. terebrantis* (Riggins et al. 2014). Pure strains of these fungi collected from the root excavation studies that had been archived above were cultured on pine twig agar (PTA: water agar 15 g/liter with twice-autoclaved loblolly pine [*P. taeda*] twigs embedded into the agar).

To determine if termites preferentially fed upon wood inoculated with the four bluestain fungal species identified from the field study, we pre-inoculated sterilized wood wafers with one of the four focal bluestain fungi and used sterile wood as a control. Fungal isolates were transferred from slants and cultured on PTA. Once the fungi were vigorously growing, 12- 0.5 cm diameter plugs were taken and placed onto fresh MEA in 237 ml plastic containers. Wafers (2.54 × 2.54 × 0.635 cm) were cut from green undried loblolly pine sapwood that was visibly free of fungi and damage and were autoclaved for sterilization before exposure to fungal treatments. A sterilized plastic

2.5 × 2.5 cm mesh was placed over the agar and then wafers were placed on top, to separate wafers from plug surfaces. All handling of fungi was conducted under a laminar flow hood in sterile conditions. Containers were covered and the fungi was cultured in an incubator at 28°C for 5 d until evenly covering wafers. Controls were treated similarly but plugs and agar received no fungi. All controls were free of visible contaminants and all bluestain-treated wafers appeared to be free of contaminating fungi. Wafers were then removed from the containers and allowed to air dry for 2 wk. Wafers were weighed before deployment in termite trials.

Termite choice trials were set up following a modified American Wood Protection Association (AWPA) Standard E1-09 choice test and were used to evaluate wood wafers subjected to subterranean termite attack (AWPA 2011). Choice trials were performed during two time periods: the first in October 2014 testing only *G. huntii* ($n = 5$ choice trials) and *G. alacris* ($n = 5$ choice trials), and the second April 2015 testing *L. terebrantis* ($n = 10$ choice trials), and *L. procerum* ($n = 10$ choice trials). *Reticulitermes* spp. collections were made prior to laboratory trials by locating logs >50 m apart containing termites. Logs containing termites were collected from the field and placed in 31 gallon (117.3 liters) metal trash cans. In the first choice trials (*G. huntii* and *G. alacris*), five *Reticulitermes* spp. collections were made 2 wk prior to deployment in choice trials from the John W. Starr Memorial Forest in Mississippi (33°20' 7.03"N, -88°52'39.73"W). In the second choice trials (*L. terebrantis*, *L. procerum*), seven *Reticulitermes* spp. collections were done 1 d prior to deployment in choice trials from the John W. Starr Memorial Forest. Termites were mechanically extracted from logs and 1 g of termites was used in each jar of the trial. Replicates consisted of a 100 × 100 mm sterilized glass jars prefilled with 150 g sterilized and dried silica sand and 30 ml of deionized water. Two hours after water was added to sand, one control wafer and one treatment wafer were added to opposite sides of the jar. Each jar consisted of a choice

between wood inoculated with one of the four focal bluestain fungi species discovered in the field study and an untreated control wafer. However, a few choice trials had rapid termite death for unknown reasons and these trials were excluded from analyses resulting in the following sample sizes: *G. huntii* ($n = 5$ choice trials), *G. alacris* ($n = 5$), *L. terebrantis* ($n = 7$), and *L. procerum* ($n = 8$). Colonies were never used more than twice as replicates within a given bluestain fungi choice test (termite individuals were only used in a single choice test). After 28 d, wafers were removed from trials, cleaned of sand and termite generated mud tubes using a soft paint brush, and allowed to air dry as described previously. Wafers were photographed and after 2 wk, the wafers were re-weighed to determine mass loss due to termite feeding. Percent mass loss was calculated as:

$$((M_i - M_f) / M_i) \times 100$$

Where M_i is initial mass of wood and M_f is final mass of wood after 28 d in choice tests. Termite consumption of wood was also measured using a modified AWP Standard E1-09 visual rating system (AWPA 2011). The following modifications were used: A rating of 10 indicated no termite damage, 9 indicated only surface nibbles. Wood was rated for termite cross-sectional consumption as 8: $\leq 3\%$, 7: 3–10%, 6: 10–30%, 5: 30–50%, 4: 50–60%, 3: 70–80%, 2: 80–90%. Wood was rated as 1 if all that remained was tiny wood fragments, and 0 if no wood remained. Bluestain fungi do not decay wood (Humar et al. 2008) but may have an effect on moisture content that could impact change in mass, likely by increasing mass of bluestained wood from increased moisture content leading to Type II error. The visual ratings only use termite consumption as a measure and should not be impacted by fungi-derived changes in mass to wood.

Statistical analysis

To test the null hypothesis of no difference in wood consumption between control and treatment wafers by termites we performed Wilcoxon Signed Rank test in SPSS v. 23. To test the null hypothesis of no difference in visual termite feeding on wood wafers with and without fungi, we used Wilcoxon Signed Rank test.

Chemistry Analysis

To determine if wood inoculated with each of the four focal bluestain fungi has altered nutritional quality from control wood we chemically analyzed wood for C, N, and C:N. Specifically, we

inoculated five wafers for each species and fungi free controls as described above that were not used in choice trials. Wafers were oven-dried at 60°C and ground to particle size < 0.25 mm and stored in air-tight containers until analysis. Wood C and N nutrient content was determined using an elemental combustion analyzer (ECS 4010 CHNS-O, Costech).

Statistical analysis

To test the null hypothesis of no difference in carbon and nitrogen and C:N ratio among bluestain fungi inoculated wood ($n = 5$ per species) and control wood ($n = 5$), we used Kruskal–Wallis test. Significant results were followed by Dunn's post hoc tests in SPSS.

Results

Field Study

Of the 2,350 tree roots sampled, 17% and 11% of trees had *G. huntii* and *G. alacris* present respectively. Termites were found on 75% of all trees sampled in the study regardless of fungal presence, but termite presence differed based on presence of bluestain fungi. Specifically, termites were found on $\geq 71\%$ of tree roots that had at least one fungus present, whereas termites were only found on 28% of tree roots that did not have any bluestain fungi present ($\chi^2 = 16.41$, $df = 3$, $P = 0.001$). Trees with ≥ 1 bluestain fungi species all had similar termite occurrence likelihoods ($\sim 85\%$) except for trees that had 3 fungal species ($\sim 71\%$), which were $\sim 17\%$ less likely to have termites present than trees with one or two species present ($P \leq 0.019$, Fig. 2a). Very few trees had four bluestain species present ($n = 43$), but 86% of these trees had termites (Fig. 2a). *Grosmannia huntii* and *G. alacris* were present on 22% and 11%, respectively of tree roots with two bluestain fungi species were present, and 32% and 29%, respectively of tree roots with three bluestain fungi species present (Fig. 2b).

Termites trended toward a positive association with *G. huntii* (Logistic Regression: $\chi^2 = 2.820$, $df = 1$, $n = 2,350$, $P = 0.093$). When *G. huntii* was absent, termites were found on 74% of tree roots compared to when *G. huntii* was present and termites were found in 78% of tree roots. Termites showed no difference in presence in tree roots that had *G. alacris* versus tree roots that did not ($\chi^2 = 0.291$, $df = 1$, $n = 2,350$, $P = 0.590$). Specifically, 75% of the tree roots that had a fungus present other than *G. alacris* had termites, and

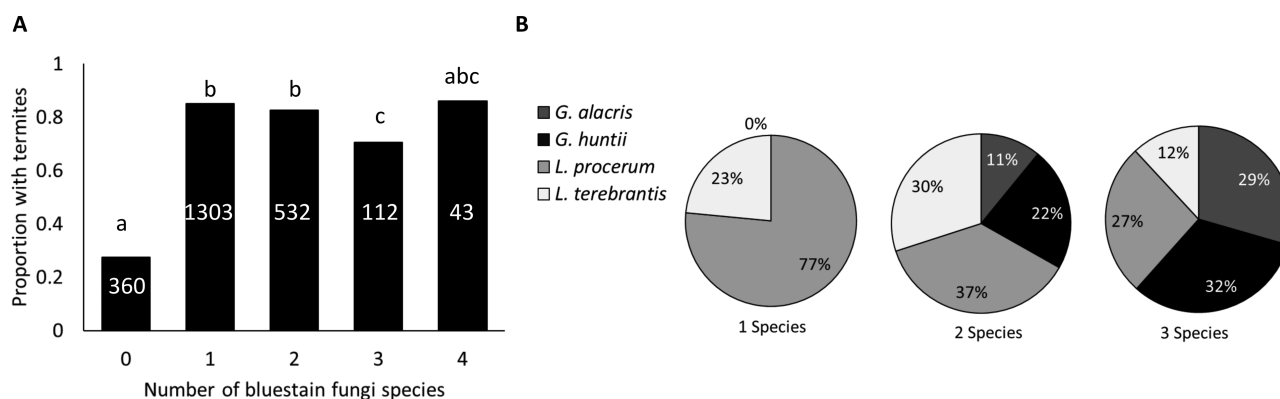


Fig. 2. (A) The proportion of tree roots with termites present in the field study across the number of bluestain fungi species present. Numbers in white text on bars represent the total number of tree root observations (i.e., out of 2,350 roots sampled) for each number of fungi species present. Different letters represent statistically different termite occurrences across number of fungal species present. (B) Relative percent of each bluestain fungal species encountered when 1, 2, or 3 fungal species co-occurred on tree roots. For 0 bluestain fungal species on tree roots all species had 0% and for 4 bluestain fungal species all species had 100% and were thus not depicted. *G. alacris* is depicted in dark grey and *G. huntii* is depicted in black.

73% with *G. alacris* had termites present. However, these results are confounded by the presence of other fungi. Neither *G. huntii* nor *G. alacris* were found alone on tree roots in any of the 2,350 tree roots sampled. *G. huntii* was found with one other fungi in 61% of the roots sampled, two other fungi in 28% of roots and three other fungi 11% of roots. This respective pattern was 45%, 38% and 17% for *G. alacris* in tree roots sampled.

Lab Study

After nearly 1 mo in laboratory choice feeding trials, termites had a median consumption of 16.4% (interquartile range: 11.6) of all control wafers. Termites had marginally significant preference for *G. huntii* over controls (mass loss: $\chi^2 = -1.753$, $df = 5$, $P = 0.080$, visual rating $\chi^2 = 10.00$, $df = 5$, $P = 0.066$) and consumed ca. 45% more wood inoculated with *G. huntii* than controls (Fig. 3a) and had an median visual rating of 4 (IQR: 3) of *G. huntii* which was 1.8-fold lower (indicating greater wood attack) than paired controls (visual rating: 7 IQR 1; Fig. 3b). Termites did not differ in their consumption between control wood and *G. alacris*, (mass loss: $\chi^2 = 0.674$, $df = 5$, $P = 0.500$, visual rating: $\chi^2 = 11.00$, $df = 5$, $P = 0.343$), *L. procerum* (mass loss: $\chi^2 = 0.700$, $df = 8$, $P = 0.484$, visual rating: $\chi^2 = 20.00$, $df = 8$, $P = 0.309$), or *L. terebrantis* (mass loss: $\chi^2 = 0.000$, $df = 7$, $P = 1.000$, visual rating: $\chi^2 = 8.500$, $df = 7$, $P = 0.197$; Fig. 3) inoculated wood.

Chemistry

Wood carbon, nitrogen, and C:N ratios were determined for wood inoculated with each of the four bluestain fungi and wood without fungi. Percent N ranged from 0.01% to 0.18% and differed among treatments ($\chi^2 = 15.968$, $P = 0.003$). Wood without fungi (median $\pm$ IQR: 0.129% $\pm$ 0.050%) and wood inoculated with *G. huntii*

(median $\pm$ IQR: 0.145% $\pm$ 0.007%) had the highest percent N with ~14 times the N as *L. terebrantis* (median $\pm$ IQR: 0.010% $\pm$ 0.0009%, $P < 0.018$, Fig. 4b). However, percent N of *G. huntii* wood did not differ from *G. alacris* ($P = 1.000$) or *L. procerum* ($P = 0.335$) and there were no other differences in percent N among treatments and controls ($P \geq 0.05$, Fig. 4b). Percent carbon was similar among fungal treatments and control wood ($\chi^2 = 3.852$, $P = 0.426$) with an overall median and IQR of 46.34% $\pm$ 1.10% (Fig. 4a). C:N ratios ranged from 215 to 5815 and differed among treatments ($\chi^2 = 16.191$, $p = 0.003$). Differences in C:N followed the same pattern as percent N with *G. huntii* and wood without bluestain fungi having the lowest median C:N (median $\pm$ IQR: 308.68 $\pm$ 67.78 and 274.63 $\pm$ 115.59, respectively) and being significantly lower than *L. terebrantis* ($P = 0.018$ and $P = 0.009$, respectively). All other comparisons between treatments and controls were not different ($P \geq 0.05$, Fig. 4a and c).

Discussion

Complex interactions among organisms can drive often unappreciated community and ecosystem dynamics. Subterranean termite distribution and activity on dead and living wood is likely to impact forest nutrient cycling and decomposer system dynamics. Here, we examined how bluestain fungi vectored by root weevils impact termite presence in the field and termite feeding preference in laboratory choice trials. Field results from sampling tree roots for *G. huntii* and *G. alacris* were generally supported by the laboratory feeding trials, despite *G. huntii* and *G. alacris* never being found alone in tree roots. Specifically, in both the laboratory and the field, termites showed increased feeding and presence respectively on wood with *G. huntii*, but did not differ in feeding or presence on wood with *G. alacris* in the lab or field respectively. Previously, Riggins et al. (2014) had found increased termite presence on tree roots with *L. procerum* and *L. terebrantis* in the field. However, laboratory feeding trials presented herein did not show a difference in termite preference between wood with or without these fungi. These results demonstrate the complexity of the system. The increased presence of termites associated with bluestain fungi in general (Fig. 2) combined with the mixed fungal effects on termite feeding activity (Fig. 3) suggest that the mechanisms driving the connection between bluestain fungi and termites are not yet fully understood.

The results from the field study demonstrate that termites are likely attracted to roots with bluestain fungi (Fig. 2). The results of this study combined with those of Riggins et al. (2014) suggest that there may be a general pattern of increased presence of subterranean termites across a diversity of bluestain fungi species, but not all (e.g., *G. alacris*). These results are similar to those of Clay et al. (2017) where trunks of healthy loblolly pine trees were inoculated with either *O. minus*, *O. ips* (Rumb.) Nannf., *L. terebrantis*, *L. procerum* or a combo of *O. minus* + *L. terebrantis* had increased subterranean termite presence in the trunks of live trees relative to controls. A potential explanation for termite attraction to wood with bluestain fungi present would be increased access to nutrients like N, which is particularly limiting in wood (Filipiak 2018). However, while *G. huntii* had the highest median N, it did not differ from control wood in C, N, and C:N (Fig. 4). Moreover, the other bluestain species tended to have similar or lower N than controls and *G. huntii* and termites still recruited to *L. terebrantis* and *L. procerum* in the field despite lower N. This suggests that N content of bluestain inoculated wood is not driving increased termite recruitment or feeding. Termites have shown attraction to other nutrients such as P and Na that were not measured here but may be higher in wood

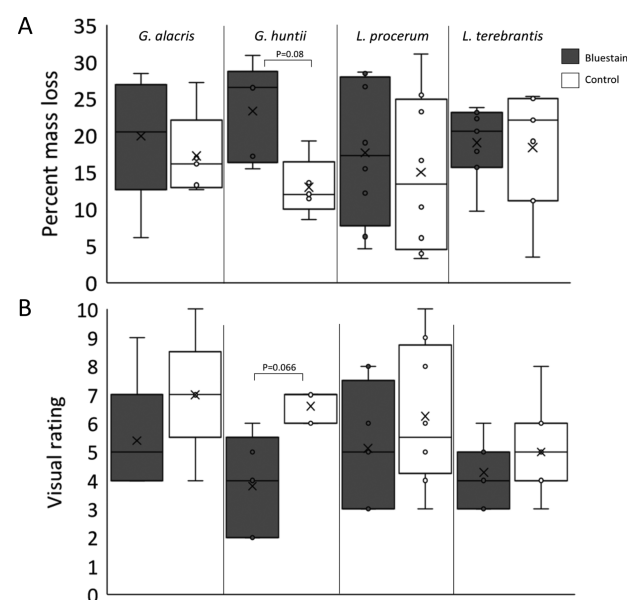


Fig. 3. (A) Boxplots of percent mass loss of pine wood wafers in termite choice trials. White boxes indicate the amount of termite consumption (% mass loss) of pine wood wafers without fungi present (controls). Grey boxes represent termite consumption of pine wood wafers with fungi present (Bluestain fungi). Each division in the graph represents the paired (control and focal fungi species) trial for one of the four focal fungal species discovered in the field study. X's on graphs represent the mean. (B) Boxplots of visual ratings of termite consumption on wood in paired choice trials (divisions). Lower visual rating values indicate greater termite consumption.

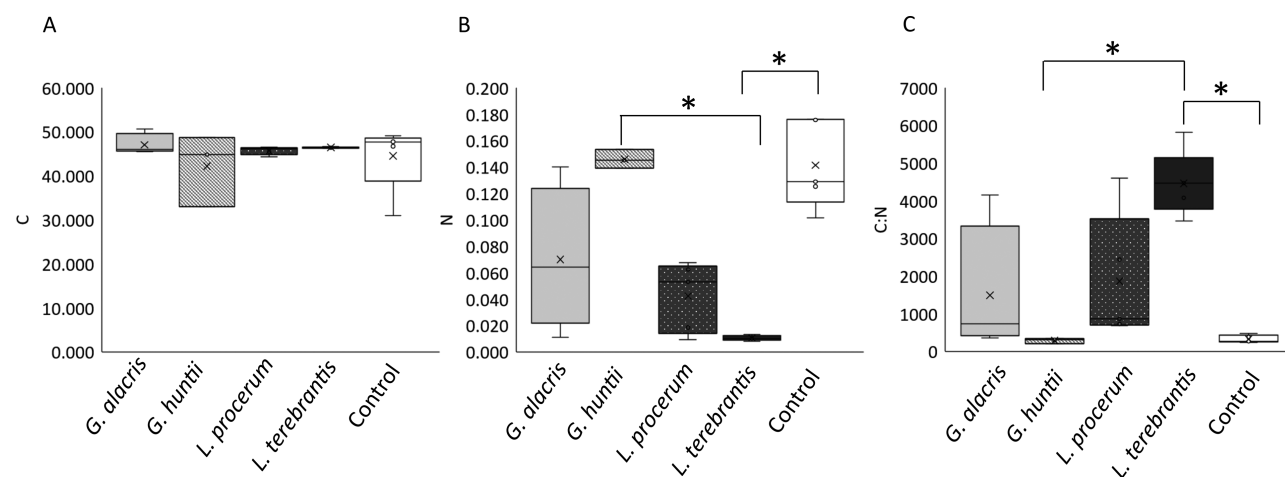


Fig. 4. Average percent carbon (A), nitrogen (B), and C:N (C) of loblolly pine wood inoculated with one of the four focal bluestain fungi species: *G. huntii*, *G. alacris*, *L. procerum*, *L. terebrantis* or wood without fungi as controls. Stars denote significant differences ($\alpha = 0.05$).

with bluestain fungi (Botch et al. 2010, Kaspari et al. 2014). But, if limited nutrients were driving this pattern, increased termite presence in wood in the field should be matched by increased consumption in choice tests, which they were not in this study. However, termite presence still coincided with bluestain fungi based on the field data regardless of differences in feeding activity and nutrients suggesting that nutrients may help explain part (e.g., if *G. huntii* is of higher quality for other essential elements or macronutrients) but not the full mechanisms driving termite dispersion and feeding activity. Although the mechanism remains unclear, these studies suggest that the presence of bluestain fungi in both aboveground and belowground systems can impact subterranean termite distributions and potentially feeding activity.

Subterranean termites rely on dead wood for both habitat and food (Eggleton and Taysu 2001). Termites frequently move their colonies to food sources but dead wood is ephemeral (although less so than other detritus) (Thorne et al. 1999, Bulmer and Traniello 2002). Locating large dead wood sources at an optimal time could lower energetic and temporal costs of foraging and thus potentially increase termite fitness. Only a few of the above mentioned bluestain species are associated with increased termite feeding (i.e., *O. minus*, and *G. huntii*, although *O. ips* has not been tested, Little et al. 2012a, b, 2013a). This suggests that termites may be associating with bluestain inoculated wood for reasons other than strictly increased palatability of wood (e.g., Fig. 4). Given that trees attacked by bark beetles and root weevils generally die shortly after (Lewis and Hartley 2006), termites may be using bluestain fungi presence or some other associated variable as a cue for suitable food or habitat. The mechanism for termite location of wood infected with bluestain fungi remains unclear. Initial laboratory tests indicate that termites are not attracted to the bluestain through olfaction (N.A. Clay and J.J. Riggins, unpublished trail following data). Termite discovery of bluestain infected wood may be opportunistic and manifest through increased recruitment of other worker termites relative to wood without bluestain fungi.

Increased termite feeding on wood with bluestain fungi may increase rates of dead wood turnover in forest ecosystems. Here we found that *G. huntii* wood had a median mass loss of 26.5% (IQR: 9.3%) and median visual rating of 4 (IQR: 3) versus paired controls that had 12.0% (IQR: 2.1) and 7 (IQR: 1) mass loss and visual rating respectively. The percent mass loss in our study is similar or greater than that measured by other studies for wood with some

decay fungi. For example, Cornelius et al. (2012) found that for three decay fungi *Phanerochaete chrysosporium* Burdsall, *G. trabeum*, and *Pycnoporus cinnabarinus* (Jacq.:Fr.) Karst, termites consumed an average of 10% of wood with these fungi (fungi did not differ in termite consumption) versus 4.8% of control wood after 30 d. In another study Becker and Lenz (1975) found that in choice trials termites consumed an average of 10–11%, 8–10%, 8–11%, and 3–5% on wood with *G. trabeum*, *Coniophora puteana* (Schum. Ex Fries) Karst., *Serpula lacrymans* (Wulfen) J. Schröt., and *Poria vaillantii* (D.C. ex Fr.) Cke., respectively in 17 d versus less than 1% on controls. The magnitude of termite consumption on wood with decay fungi from that study linearly scaled to 28 d would be similar to what we found. However, Amburgey and Smythe (1977a) found variation in termite consumption of wood with different strains of *G. trabeum* ($n = 3$) and *P. incrassate* (Berk. and Curt.) Burt ($n = 2$). Specifically, in 7 of 11 trials of all strains (some were single fungi choice trials and some were both fungi and control choice trials) and 6 of 9 trials with *G. trabeum* and 7 of 8 trials with *P. incrassate*, termites consumed more control wood than wood with decay fungi after 1 wk. In Little et al. (2013a) which directly compared experimental wood stakes in a field study inoculated with either *G. trabeum* or four species of bluestain fungi, termites degraded wood stakes with *O. minus* and *O. ips* at rates greater than or equal to *G. trabeum*. However, termite degradation of stakes with *L. terebrantis* and *L. procerum* failed to differ from controls similar to what we found in our laboratory feeding trials (Little et al. 2013a). Additional work on decay fungi like *G. trabeum* indicates that the increased decay of wood, making wood easier to consume and digest, caused by fungi is likely the mechanism increasing termite consumption on wood with decay fungi (Amburgey and Smythe 1977b, Gazal et al. 2014, Oberst et al. 2018). Given bluestain fungi do not degrade wood, increased consumption of wood with bluestain fungi by termites is likely not mediated by the same mechanisms as decay fungi.

Termite distributions can greatly impact rates of carbon cycling in forest ecosystems (Jouquet et al. 2011, Maynard et al. 2015, Griffiths et al. 2021). Once termites locate suitable habitat and wood food source, they can accelerate rates of woody debris decomposition (Bradford et al. 2014, Griffiths et al. 2021). Wood decomposition may be further enhanced if termites also preferentially feed on bluestained wood (i.e., *O. minus* and *G. huntii*). Recently, Siegert et al. (2018) found that after just 1 yr, termites were found four times

more in wood with *O. minus* than controls and bluestained logs had accelerated biogeochemical processes resulting in decreased C and increased N content relative to control logs without initial fungi inoculation. This suggests that this multitrophic interaction between bark beetle and root weevil-attacked trees, bluestain fungi, and subterranean termites may span above- and below-ground boundaries to influence nutrient cycling and likely subsequent decomposer community structure.

This study builds upon a growing body of research suggesting that the relationship between bluestain fungi and subterranean termite species may be widespread both geographically (e.g., at least the southeastern USA) and taxonomically: in both native and non-native termite species (*R. flavipes* and *C. formosanus*) and across five of the six bluestain species tested so far (*O. minus*, *O. ips*, *L. procerum*, *L. terebrantis*, *G. huntii*, but not *G. alacris*) (Little et al. 2012a, b; Little et al. 2013a; Riggins et al. 2014; Clay et al. 2017; Siegert et al. 2018). If termites are recruiting to root systems with bluestain species present, this is likely to decrease the stability and structural integrity of these trees (Lai et al. 1983, Grace 1987, Osbrink et al. 1999) resulting in increased tree falls and coarse woody debris inputs in forests with prevalent root weevil activity. Better understanding this relationship and its consequences on forest ecosystems will enable effective forest management strategies that include and consider the monitoring and managing fuel loads, nutrient cycling, and productivity or yield in unmanaged and managed stands (Blanco and Lo 2012).

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