Title: Experimental evidence that fungal symbionts of beetles suppress wood decay by competing with decay fungi.

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Abstract: Throughout forests worldwide, bark and ambrosia beetles inoculate dead and dying trees with symbiotic fungi. We experimentally determined the effects of three common and widely distributed ascomycete symbionts, and one introduced Asian basidiomycete symbiont on the decay of pine sapwood. Ascomycetes caused less than 5% mass loss and no structural degradation, whereas the basidiomycete Flavodon ambrosius caused nearly 15% mass loss and visible degradation of wood structure. In co-inoculation experiments, the beetle symbionts Ophistoma ips and Raffaelea fusca reduced white and brown rot decay through competition with Ganoderma curtisii and Phaeolus schweinitzii, respectively. The inhibitory effects of O. ips and R. fusca on decay were negated when co-inoculated with F. ambrosius, suggesting that widespread introduction of this beetle symbiont could alter forest carbon fluxes. In contrast to the predominant forest biology narrative, most bark and ambrosia beetles introduce fungi that delay rather than facilitate tree biomass recycling.

Keywords: Priority effects, Scolytinae, Ophiostomatales, Platypodinae, forest health, lignin, cellulose, Pinus taeda, Basidiomycota
INTRODUCTION

Wood comprises as much as 98% of the living biomass in forests (Fittkau & Klinge 1973). Wood is mostly composed of lignocellulose which requires non-enzymatic and specialized enzyme suites for biological degradation. The ability to degrade wood is possessed primarily by fungi within the phylum Basidiomycota (Blanchette 1991, Floudas et al. 2012). Decay fungi are essential to forest productivity and biodiversity because they release the immense stores of energy and nutrients bound in wood to the surrounding biological community (Rayner & Boddy 1988). Because of the immensity and ubiquity of woody biomass world-wide, changing wood decomposition rates could have global effects on nutrient cycling and carbon sequestration (Floudas et al. 2012, Hibbett et al. 2016). Wood decomposition is modulated by ecological interactions between decay fungi and other organisms (Hulme & Shields 1970, Boddy 2000), and small changes in fungal colonization processes can lead to several-fold changes in decay rates (Fukami et al. 2010, Cline & Zak 2015).

Wood-boring bark and ambrosia beetles are widely believed to facilitate biomass recycling by inoculating dead and dying trees with saprotrophic fungi (Ulyshen 2016). These beetles comprise more than 7,000 species in the weevil subfamilies Platypodinae and Scolytinae that thrive throughout tropical and temperate regions (Kirkendall et al. 2015). They are often the first insects to colonize the wood, entering before the tree has died and have major impacts on fungal community development within wood (Leach et al. 1934, Persson et al. 2011, Strid et al. 2014). The beetles have various relationships with the fungi they carry, ranging from incidental commensalism, to highly co-evolved and reciprocally obligate mutualisms (Harrington 2005, Hulcr & Stelinski 2017). Some beetles have specialized glandular organs called mycangia for transporting and nourishing nutritional fungi and have become entirely dependent on a fungal diet (Francke-Grosmann 1956, Batra 1963, Hulcr & Stelinski 2017). Likewise, some fungi have evolved complete dependence on these beetles for dispersal and colonization of wood.
tissues (Francke-Grosmann 1956, Batra 1963, Six 2003, Harrington 2005). The few species of beetles and symbiotic fungi that kill healthy trees and impact agricultural and silviculture interests have been the center of intense research efforts. In contrast, we know little about the ecological roles of the thousands of other beetles that do not kill trees but are ubiquitous and abundant on every continent except Antarctica.

In the southeastern United States, the phloem of stressed, declining, or recently dead pines (Pinus spp.) is typically infested by native bark beetles in the genera Ips, Dendroctonus, Orthotomicus, and Hylastes, and the xylem is colonized by ambrosia beetles in the genera Xyleborus, Myoplatypus, and Gnathotrichus. These beetles are primarily associated with saprotrophic fungi in the orders Ophiostomatales (e.g. Ophiostoma, Leptographium, and Raffaelea), and Saccharomycetales (e.g. Pichia, Candida, and Ambrosiozyma), and incidental associations with various other fungi are common (Harrington 2005, Hofstetter et al. 2015, Hulcr & Stelinski 2017, Skelton et al. 2018). Fungi in Saccharomycetales and Ophiostomatales are not typically capable of degrading the structural components of wood, and therefore not likely to have a large direct effect on wood decomposition. However, these fungi could impede colonization and degradation by wood decay fungi by competing with pioneer decay fungi for simple carbohydrates or producing toxic secondary metabolites. Pre-colonization of wood by other non-beetle-associated fungi such as Trichoderma spp. reduces subsequent decay through competitive interactions with decay fungi (Hulme & Shields 1970), but similar effects have not been examined in beetle-associated fungi. Ophiostomatales may also influence colonization of fungi that cannot tolerate extractives such as pitch and other resinous compounds in freshly exposed xylem. Ophiostomatales and some other early colonizing fungi not only tolerate these compounds but can degrade them over time (Blanchette et al. 1992).

While the nutritional fungi of most bark and ambrosia beetles typically do not degrade lignocellulose, some ambrosia fungi do. The primary nutritional symbiont of beetles in the genera
Ambrosiodmus and Ambrosiophilus is the only known lignocellulose decaying ambrosia fungus. Flavodon ambrosius (Polyporales) (Kasson et al. 2016). Several species of Ambrosiodmus are native to the southeastern US, and at least three species are non-native. An Asian ambrosia beetle, Ambrosiodmus minor, was first detected in Florida by state monitoring efforts in 2011, and has since become one of the most frequently collected species across the state where it infests many species of hardwood and coniferous trees (Hulcr et al. 2018). Although there have been more than 50 introductions of bark and ambrosia beetles in the United States (http://www.barkbeetles.info; accessed Oct 15, 2018), A. minor is particularly likely to affect wood decomposition because of the combination of its increasing abundance and its symbiotic wood-decay fungus.

The objectives of this study were to examine the direct effects of native ascomycete associates, and the introduced basidiomycete associate on pine wood decomposition, and to assess secondary effects through competitive interactions with two common wood decaying fungi in pines of the southeastern United States. We hypothesized that native bark and ambrosia beetles can suppress early stages of decay by inoculating fresh wood with fungi that do not decompose structural elements of wood, but compete with pioneer colonizing wood decay fungi. We also hypothesized the inclusion of the non-native wood decaying ambrosia fungus F. ambrosius could offset the inhibitory effects of native beetle-associated fungi on early decomposition through functional redundancy with free-living decay fungi.

METHODS

Isolate Recovery and Identification: All beetle-associated fungal isolates were obtained from the University of Florida Forest Entomology Bark and Ambrosia beetle collection (Table 1). Isolation from live beetles and culture methods followed Skelton et al. (2018). Identification of beetle-associated fungi was based on BLASTn comparison of the ribosomal large sub unit (LSU) DNA sequence, using the primers
LR0R and LR5 (Vilgalys and Hester 1990). DNA extraction, PCR amplification and sequencing were performed as described in Skelton et al (2018). We chose four common and globally-distributed beetle-associated fungi to represent major groups of beetle associates in pines: *Ophiostoma ips* (Ophiostomatales) which is commonly found associated with many pine-infesting bark beetle species, *Raffaelea fusca* (Ophiostomatales) which is commonly associated with many pine and hardwood infesting ambrosia beetles, *Ambrosiozyma monospora* which associates with many pine infesting ambrosia beetles, and *Flavodon ambrosius* which is specifically associated with ambrosia beetles in the related genera *Ambrosiodmus* and *Ambrosiophilus*. We also obtained cultures of two species of widely distributed and abundant fungi that are pioneer colonizers that aggressively decay pine wood to examine interactions between beetle-associated fungi and wood decay fungi; one white rotter, *Ganoderma curtisii* f. sp. *meredithiae* (hereafter referred to as *G. curtisii*; Loyd et al. 2018a) and one brown rotter (*Phaeolus schweinitzii*). The isolates were cultured from small pieces (<1 cm³) of context tissue from inside the basidiomata of each wood decay fungus as described in Loyd et al. (2018b). The cultures of *G. curtisii* and *Phaeolus schweinitzii* are archived and maintained at the Center for Forest Mycological Research Fungal Collection (CFMR) in Madison, WI.

**Direct effects experiment:** To determine the direct contribution of each beetle-associated fungus to decay of pine sapwood, we used a modified version of the standard wood decay protocol described in Loyd et al. (2018b). Fresh sapwood was obtained from the lower trunk of a ~20 cm dbh loblolly pine (*Pinus taeda*) from the University of Florida Austin Cary Forest, near Gainesville, Florida. The sapwood was cut into 16 cm³ cubes. Only wood displaying no signs of existing damage or disease was used. To simulate beetle galleries, 4 holes (3 mm diameter) were drilled evenly spaced through two faces of the cube to transect the grain of the wood, i.e. the simulated galleries were horizontal when the cube was held in its original orientation in the tree. The cubes were then dried to completion at 50 °C for 7 d, weighed for dry mass and stored in sealed plastic bags until used. Just prior to use, cubes were placed
individually in 237 ml jars with enough distilled water to hydrate to the original water content of the fresh wood (42% water by mass), left overnight to absorb the water, and then autoclaved twice for 1 h at 121 °C and 103 kPa with 24 h in between autoclave cycles. After cooling, the cubes were inoculated with fungal spore solutions (described below), jars were loosely capped and incubated at 25 °C in the dark for 14 d to allow beetle-associated fungi to colonize the wood. Cubes were then transferred to microcosms. Microcosms consisted of 237 ml jars containing ~120 ml of hydrated vermiculate, tamped level, with two hydrated 800 mm³ wood feeder strips (Quercus nigra) laid parallel across the surface of the media. Microcosms were autoclave sterilized as described above. Strips were added to keep the methodology consistent between this experiment and the “indirect effects” experiment described below. The inoculated cubes were placed on the wood feeder strips, and microcosms were incubated at 25 °C in the dark for 90 d. Cubes were then removed from microcosms, lightly brushed to remove vermiculite and fungal hyphae from the external surfaces, weighed for wet mass, dried to completion at 50 °C, weighed for final dry mass, and then stored in sealed plastic bags at -20 °C for later micrograph imaging.

Simulated beetle galleries were inoculated by pipetting 100 µl of a spore solution containing 50 spores per µl into each gallery to deliver a spore load similar to what we have observed from naturally dispersing beetles. We inoculated 5 cubes per fungal isolate. Spore solutions were made by placing a colonized agar wedge in 0.5 ml of sterile water and vortexing at 2,000 rpm for 30 s, determining spore concentration by light microscopy and hemocytometer and diluting with sterile water to reach the target concentration. Five negative controls were sham inoculated with sterile water.

**Indirect effects experiment:** This experiment was designed to determine if pre-colonization of wood with beetle-associated fungi would reduce subsequent decay from a common pine wood decay fungus, *G. curtisii*. Tester cubes and microcosms were prepared as described above with one difference: the wooden feeder strips in the microcosms were colonized with *G. curtisii* prior to the addition of the tester
cubes. Feeder strips were inoculated by placing a colonized agar plug at one end of each strip and
incubating in the microcosm at 25 °C in the dark for 14 d. In addition to each of the beetle-associated
fungi treatments, we also added an “all fungi” treatment in which F. ambrosius was combined with all
three fungi from all native beetles, with total spore solution volume and number of total spores were
held constant. Incubation and final mass were determined as described above. We included 8 replicates
for each treatment combination.

Impact on wood structure: A representative wood sample of each independent fungus and fungal
combination from the indirect effects experiment was processed for scanning electron microscopy to
assess damage to the structural components of the wood. Small sections of each sample were cut from
each sample wood block and infiltrated with TBS™ Tissue Freezing medium™ (Triangle Biomedical
Sciences, Durham, NC, USA) under a vacuum and then mounted on brass stubs at -20 °C in a freezing
microtome (International Equipment Company, Needham Heights, MA, USA). Samples were sectioned
to produce a smooth transverse view of the wood cells, and dehydrated by placing in 20, 30, 45 75 and
95 % ETOH for 5 min each. Following dehydration, samples were mounted on aluminum stubs and
coated with gold/palladium on a Cressington 108 auto sputter coater (Cressington Scientific
Instruments, Watford, United Kingdom). Samples were examined using a Hitachi S3500N (Hitachi,
Tokyo, Japan) scanning electron microscope.

Interaction detail experiment: While the experiments using tester cubes were designed to simulate the
interactions occurring within sap wood to determine their effects on biomass loss, they did not offer a
good visual assessment of the interactions. To provide a better view of the interactions that caused
significantly reduced decay in the indirect effects experiment, we devised a separate assay using tester
strips embedded in agar. Fresh pine sapwood (same as described above) was cut into 1 cm by 0.2 cm by
6 cm strips, with the grain of the wood running along the longest dimension. Strips were dried and
weighed, rehydrated by soaking in distilled water for 24 h, bulk autoclave sterilized as described above,
and then embedded in a 1% agar in standard 100 mm plastic petri dishes. After cooling, a rectangular section of agar was removed from each end of the embedded strips and replaced with colonized rectangular agar plugs, pressing the colonized surfaces of the plugs against the ends of the strips. Each strip was inoculated with a wood decay fungus on one end, and the other end was inoculated with either *O. ips*, *R. fusca*, or a sterile agar plug (negative control). In addition to *G. curtisii* from the previous cube experiments, we also included a common pine infecting brown rot fungus (*Phaeolus schweinitzii*).

Petri dishes were sealed with plastic film, incubated at 25 °C in the dark for 120 d. Plates were examined and photographed at 90 and 120 d to characterize the interactions between fungi. At 120 d strips were removed from agar, rinsed and brushed gently to remove agar and hyphae from surfaces, dried to completion at 50 °C, and weighed for final dry mass.

**Statistical analyses:** For all three experiments, our response variable was percent dry mass lost calculated as the difference between initial dry mass and final dry mass, divided by initial dry mass and multiplied by one hundred. Treatment effects were tested using linear models, implemented by the `lm()` function in the stats package for R (R Development Core Team 2010). The assumption of equal group variance was tested using a Bartlett Test, implemented by the `Bartlett.test()` function. In the direct effects experiment, we used the negative control as the reference group in the linear model. In the indirect effects experiment, the group of tester cubes that received only *G. curtisii* was used as the reference group and compared to all other groups which had been pre-colonized with a beetle-associated fungus. We used a Kruskal-Wallis test (`Kruskal.test()` function in the stats package for R) to compare the “all fungi” group to the reference group because a high degree of variance in that treatment violated the assumption of homogeneity of variance. For the interaction detail experiment, percent dry mass lost was modeled as a function of decay fungus (*G. curtissi* [reference group] or *P. schweinitzii*), initial strip dry mass, beetle-associated fungus treatment (*O. ips*, *R. fusca*, or negative control [reference group]), with interaction terms for initial mass and decay fungus, and beetle associate...
and decay fungus. Initial strip mass was included in the model because decay in this assay was greatest on surface of the strips that were exposed above the agar, thus percent mass loss was higher on smaller strips which have a greater surface area to mass ratio.

RESULTS

Direct effects experiment: The introduced Asian basidiomycete, \textit{F. ambrosius}, was the only beetle-associated fungus to decay sapwood. All assayed fungi caused mass loss that was significantly greater than negative controls (Table 2). However, the ascomycete beetle-associated fungi (\textit{Ophiostoma, Raffaelea, and Ambrosiozyma}) caused less than 5% mass loss on average, indicating that they did not utilize the structural components of the wood but only consumed the available labile sugars and extractives. In contrast, \textit{Flavodon ambrosius} caused mass loss that was comparable to the common free-living wood decay fungus \textit{G. curtisi} (Figure 1) and similar to previous reports that used similar methods to measure decay from these fungi (Kasson et al. 2016, Loyd et al. 2018b).

Indirect effects experiment: Ophiostomatalean beetle-associated fungi reduced wood decay by competing with wood decay fungus. \textit{G. curtisi} caused an average decrease of 12.5% in dry mass of tester cubes in the absence of beetle-associated fungi. This loss was significantly diminished by pre-inoculation with either \textit{O. ips} or \textit{R. fusca}, but not \textit{A. monospora} or \textit{F. ambrosius} (Table 3; Figure 2). Pre-inoculating with all four beetle-associated fungi simultaneously resulted in higher variance in this group (Bartlett test; $\chi^2 = 20.665, p < 0.001$), but did not affect the mean mass loss compared to \textit{G. curtisi} -only reference treatment (Kruskal-Wallis test; $\chi^2 = 0.21, p = 0.64$). In both treatments involving \textit{F. ambrosius} and \textit{G. curtisi}, \textit{F. ambrosius} visibly excluded \textit{G. curtisi} from colonizing the tester cubes.

Impact on wood structure: \textit{Flavodon ambrosius} was the only beetle-associated fungus to cause detectable structural damage to sapwood. Transverse sections of non-decayed wood (control) observed with SEM showed the typical arrangement of \textit{P. taeda} wood cells with intact tracheids of both early and
latewood (Figure 3A). Similar to the negative control blocks, wood in decay microcosms colonized by the ambrosia fungi in the fungal phylum Ascomycota (Ambroziozyma, Ophiostoma, and Raffaelea) revealed intact cells when transverse sections were observed with SEM. On the contrary, transverse sections of Pinus taeda wood blocks that were colonized with the basidiomycetes G. curtisii or Flavodon ambrosius showed evidence of simultaneous decay of tracheid cells typical of white rot decay fungi (Figures 3B-3C). The simultaneous decay of P. taeda wood observed for both G. curtisii and F. ambrosi was only observed in the earlywood cells, which tend to have larger cells with thinner cell walls compared to latewood cells (Figure 3A).

Interaction detail experiment: We observed multiple antagonistic interactions between the two decay fungi and beetle-associated fungi. Ophiostoma ips grew relatively quickly at the onset of the experiment, but its growth was halted when it encountered either P. schweinitzii or G. curtisii, both of which continued to grow over O. ips. While there was no visible effect of O. ips on the growth of G. curtisii, the hyphal bundles of P. schweinitzii were thinner and sparser in the presence of O. ips compared to controls that only had P. schweinitzii (Figure 4). Raffaelea fusca completely excluded colonization of wood from G. curtisii, causing G. curtisii to make mounding walls of hyphae at the zone of contact. Because R. fusca was much slower growing, it colonized and defended relatively small sections of the tester strips, within approximately 1 cm of the inoculation point, but maintained its territory for the entire 120 d duration of the experiment. There was no visible response of P. schweinitzii to R. fusca. Overall, P. schweinitzii caused significantly more mass loss than G. curtisii in this assay (Table 4).

There was also a significant interactive effect between O. ips and P. schweinitzii as O. ips reduced the decay caused by P. schweinitzii, congruent with visual observations of reduced hyphal growth of P. schweinitzii in the presence of O. ips. In contrast to the cube experiments, there was no significant effect of either beetle-associated fungus on the decay caused by G. curtisii which probably reflected the
overall poor performance of *Ganoderma* in this assay; an average of 4.83% mass was lost on strips compared to 12.5% on cubes.

**DISCUSSION**

Bark and ambrosia beetles are widely believed to accelerate wood decay by introducing fungi during the early stages of saprotroph community assembly. However, the majority of fungi associated with these beetles are ascomycetes, which are not typically capable of degrading the lignified cellulose that comprises wood (Hofstetter et al. 2015, Hibbett et al. 2016, Hulcr & Stelinski 2017). The results of this study provide support for a new hypothesis; bark and ambrosia beetles suppress wood decay by introducing fungi that do not decay wood, but compete with wood decayers for labile resources. Because bark and ambrosia beetles are ubiquitous in forests world-wide and wood decay is a major flux in global carbon cycles, this effect could have significant impacts on global carbon budgets.

Common ascomycete symbionts of bark and ambrosia beetles do not decay wood. We observed an average loss of less than 5% dry mass from each of the three ascomycete symbionts when inoculated as monocultures. Although this was a significant loss compared to negative controls which had no fungi, the loss was not attributable to the degradation of the structural components of the wood; scanning electron micrographs revealed no structural damage attributable to any ascomycete symbionts. Our results are congruent with previous work showing that ascomycete beetle associates do not decay wood (Kasson et al. 2016).

Ascomycete symbionts of beetles interacted antagonistically with wood decay fungi, limiting decay fungi from colonizing experimental wood samples. Competition between non-decaying ascomycetes and wood-decaying basidiomycetes is well-known. For instance, the ascomycete *Trichoderma* species is a proven biocontrol agent used to competitively exclude pathogenic wood-decaying basidiomycetes through multiple competitive mechanisms (Hulme & Shields 1970, Bruce et al.
An even more relevant example is the effective use of a pigment-less mutant of *Ophiostoma piliferum* to competitively exclude the wood decay polypore *Phlebiopsis gigantea*, as well as blue staining fungi in the order Ophiostomatales (Behrendt et al. 1995). These cases demonstrate that early colonization of wood by ascomycetes can inhibit subsequent colonization of other pioneer decay fungi. These priority effects that exclude or suppress decayers seem particularly relevant to beetle-associated fungi that arrive very early in saprotroph community assembly as a result of their symbiosis with bark and ambrosia beetles. Indeed, our results showed that some beetle-associated fungi exclude wood decayers from colonizing portions of the wood, such as the ambrosia fungus *R. fusca* which prevented *Ganoderma* from colonizing portions of tester strips. In other cases, the hyphae of wood decayers and beetle-associated fungi intermingled, but the decayers showed reduced vigor. *Phaeolus schweinitzii* in the presence of *O. ips* was noticeably sparser than controls without *O. ips*, and a slight inhibition of *G. curtisii* by *O. ips* was observed on agar media (data not presented). Clearly the ascomycete associates of bark and ambrosia beetles have the potential to impede subsequent colonization of wood by wood-decaying basidiomycetes.

Competition between beetle-associated Ophiostomatales and wood decay basidiomycetes inhibited decay. Wood cubes incurred significantly less decay from *G. curtisii* when they were pre-inoculated with either *O. ips* or *R. fusca*. Areas in which *R. fusca* had excluded *G. curtisii* showed none of the structural degradation that occurred where *G. curtisii* had colonized experimental wood strips. We also saw reduced decay from *P. schweinitzii* when co-inoculated with *O. ips* in the interaction detail experiment. Ophiostomatalean fungi can rapidly reduce non-structural carbohydrates such as sugar and extractives in wood (Wang et al. 2013), and competition between ascomycetes and basidiomycetes for non-structural carbohydrates can reduce decay rates in non-beetle-associated systems (Hulme & Shields 1970). Thus, resource competition for labile carbohydrates is a plausible mechanism for the reduced decay from wood decayers when they were co-inoculated with ophiostomatalean beetle associates. The
exclusion of *G. curtisii* from wood colonized by *R. fusca* suggests that this ambrosia fungus may compete through chemical or physical means, in addition to, or instead of competition for labile carbon resources.

In contrast to our findings, most other studies of the effects of bark and ambrosia beetles on wood decay generally conclude that the beetles facilitate wood decay (reviewed in Ulyshen 2016). Much of the disparity between our study and others is a result of differences in perspective and interpretation, rather than conflicting experimental outcomes. These differences fall into three categories. First, many symbionts of bark and ambrosia beetles, commonly referred to as “blue-stain fungi”, cause extensive discoloration of sapwood and phloem surrounding beetle galleries. Observations of this discoloration is sometimes conflated with decomposition, either by the authors or subsequent readers of research articles that report dark staining as a consequence of beetle infestation (e.g. Švihra & Kelly 2004, Leach et al. 1934). The results presented in this paper and others (Kasson et al. 2016), indicate that wood staining is not equitable to wood decay; both *O. ips* and *R. fusca* caused discoloration of the wood with minimal loss of biomass and no signs of structural degradation, and thus we do not equate staining with decay.

The second source of disparity is guilt by association. Field experiments and surveys that relate wood-boring insects and decay often involve not only bark and ambrosia beetles, but other wood borers such as longhorn beetles (Cerambycidae). Several such studies provide evidence that longhorn beetles cause significant losses in wood mass or density, however direct evidence for an association between bark and ambrosia beetles and wood decay is generally lacking or inseparable from the effects of other wood-boring insects (e.g. Leach et al. 1934, Leach & Orr 1937, Edmonds & Eglitis 1989, Müller et al. 2002, Angers et al. 2011). Longhorn beetles are much larger than bark and ambrosia beetles, and consume or eject proportionately larger volumes of wood, which leads to significant losses in wood mass through maceration. In contrast, even heavy infestations of the much smaller ambrosia beetles
result in less than half of one percent loss in sapwood volume (Zhong & Schowalter 1989). Our results indicate that the negative effects on decomposition as a consequence of competition between beetle associates and wood decay fungi could offset the small amount of physical maceration of sapwood by ambrosia beetles, resulting in a net negative effect of ambrosia beetles on mass loss.

The third source of disparity between our conclusions and those of others is the intuitive but tenuous inference that increasing fungal diversity in decaying logs will result in increased decomposition of wood. Experimental and observational studies have firmly demonstrated that bark and ambrosia beetles cause several fold increases in fungal diversity in freshly dead wood, including increased diversity in decay fungi (Persson et al. 2011, Strid et al. 2014). However, the relationship between diversity of decay fungi in wood and decay rate is not always positive and can in fact be negative as a consequence of increased antagonistic interactions between decay fungi (Boddy 2000, Fukami et al. 2010, Dickie et al. 2012).

Unlike the ascomycete beetle-associated fungi, the introduced Asian basidiomycete *F. ambrosius* caused significant decay of earlywood tracheid cells of pine sapwood, resulting in mass loss similar to the common free-living pine decay fungus *G. curtisii* and visible degradation of wood structure at microscopic and macroscopic levels. The amount of mass loss attributable to *F. ambrosius* was nearly identical to previous work that used similar methods to measure decay of loblolly pine sapwood from four species in the well-known wood decaying polypore genus *Ganoderma* (Loyd et al. 2018b). Our results are also congruent with previous work that assessed decay of a hardwood (*Ailanthus*) in terms of both mass loss and loss of wood hardness from two ascomycete symbionts of ambrosia beetles (*Raffaelea subfusca* and *Fusarium* sp. AF-4) and *F. ambrosius* isolated from another introduced Asian beetle, *Ambrosiophilus attratus* (Kasson et al. 2016). Our study had similar results showing minimal decay from ascomycete beetle-associates, but significant mass and hardness loss from *F. ambrosius*. Thus, unlike any other ambrosia symbiosis known, this unusual ambrosia fungus and it's introduced
beetle vectors could contribute directly to wood decay during the early stages of saprotroph community development. Transcriptome and secretome analyses of other pioneering wood decay fungi have elucidated mechanisms used to tolerate and metabolize resinous compounds in wood (Hori et al. 2014). Having the ability to colonize freshly cut wood and capture resources appears to be a very effective way for pioneer species of white rot fungi such as P. gigantea or F. ambrosius to exclude other fungi. A better understanding of the genes involved with the transformation and detoxification of wood extractives by F. ambrosius is needed.

*Flavodon ambrosius* illustrates the importance of considering symbiont functional traits to understand the impacts of their hosts. When *F. ambrosius* was combined with the ascomycete beetle associates, we observed decay rates that were not significantly different from controls which received only wood decay fungi. This result indicates that inclusion of *Flavodon*-farming beetles into bark and ambrosia beetle assemblages could nullify the inhibitory effect of ascomycete associates on early decay in pine sapwood. Notably, *F. ambrosius* excluded *Ganoderma* from colonizing the cubes in the *F. ambrosius* treatment and the “all fungi” treatment, and thus the similar level of decay observed in cubes pre-inoculated with this fungus, either alone or in combination with other beetle associates, represent replacement and functional redundancy with the decayer, not a peaceful co-existence in the wood. Given that *Flavodon*-farming beetles have been widely introduced, are becoming widely established within their population, rapidly increasing in some areas along with their exotic aggressive pioneer wood decay fungus (Hulcr et al. 2018), these series of events could likely have a substantial impact on decomposition and the turnover of forest biomass, nutrients, and carbon.

**CONCLUSIONS**

Ascomycete associates of bark and ambrosia beetles can inhibit decay by competing with decay fungi. In contrast, the introduced basidiomycete ambrosia fungus *Flavodon ambrosius*, causes decay
similar to other well-known free-living white rot fungi and nullifies the inhibitory effects of ascomycete beetle symbionts, suggesting that widespread introduction of this fungus and its vectors could significantly impact carbon turnover rates in forest ecosystems. A more complete understanding of the influence that wood-boring beetles have on forest ecosystems requires detailed knowledge of the functional traits and ecology of their phylogenetically diverse symbionts.

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Table 1: Collection information for fungal isolates used in microcosm experiments.

<table>
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<th>Species</th>
<th>Isolate name</th>
<th>extracted from</th>
<th>Location</th>
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<td><em>Ambrosiozyma monospora</em></td>
<td>JH_14703</td>
<td><em>Xyleborus pubescens</em> mycangium</td>
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<td><em>Flavodon ambrosius</em></td>
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<td><em>Ambrosiadius minor</em> gallery</td>
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<td><em>Ips grandicollis</em> body surface</td>
<td>Gainesville, FL, US</td>
</tr>
<tr>
<td><em>Phaeolus schweinitzii</em></td>
<td>320NC</td>
<td>basidiomata</td>
<td>Charlotte, NC, US</td>
</tr>
<tr>
<td><em>Ganoderma curtisii f. sp. meredithiae</em></td>
<td>UMNFL50</td>
<td>basidiomata</td>
<td>Sarasota, FL, US</td>
</tr>
</tbody>
</table>

Table 2: Linear model for direct effects of beetle-associated fungi on mass loss in pine sapwood during a 90 d microcosm experiment. Residual standard error: 1.66 on 30 degrees of freedom, adjusted R-squared = 0.8973, F$_{5,30}$ = 62.16, p < 0.001. * Treatments significantly different from the negative control reference group (no fungi).

<table>
<thead>
<tr>
<th>coefficients</th>
<th>estimates</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>intercept</td>
<td>1.4325</td>
<td>2.431</td>
<td>0.021</td>
</tr>
<tr>
<td><em>A. monosporum</em></td>
<td>2.9395</td>
<td>3.094</td>
<td>0.004*</td>
</tr>
<tr>
<td><em>F. ambrosium</em></td>
<td>13.0315</td>
<td>13.717</td>
<td>&gt; 0.001*</td>
</tr>
<tr>
<td><em>G. curtisii</em></td>
<td>11.0450</td>
<td>13.256</td>
<td>&gt; 0.001*</td>
</tr>
<tr>
<td><em>O. ips</em></td>
<td>3.0455</td>
<td>3.206</td>
<td>0.003*</td>
</tr>
<tr>
<td><em>R. fusca</em></td>
<td>3.1055</td>
<td>3.269</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

Table 3: Effects of pre-inoculating pine sapwood cubes with beetle-associated fungi on decay caused by *Ganoderma curtisii* in a 90 d microcosm experiment. Residual standard error: 1.75 on 35 degrees of freedom, adjusted R-squared = 0.1749, F$_{4,35}$ = 3.067, p = 0.028. * Treatments significantly different from the negative control reference group (*Ganoderma* only).

<table>
<thead>
<tr>
<th>coefficients</th>
<th>estimates</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>intercept</td>
<td>12.4775</td>
<td>20.215</td>
<td>&lt; 0.001</td>
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<tr>
<td><em>A. monosporum</em></td>
<td>-0.8025</td>
<td>-0.919</td>
<td>0.3642</td>
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<tr>
<td><em>F. ambrosium</em></td>
<td>0.0675</td>
<td>0.077</td>
<td>0.9388</td>
</tr>
<tr>
<td><em>O. ips</em></td>
<td>-2.2487</td>
<td>-2.576</td>
<td>0.014*</td>
</tr>
<tr>
<td><em>R. fusca</em></td>
<td>-1.9675</td>
<td>-2.254</td>
<td>0.036*</td>
</tr>
</tbody>
</table>
Table 4: Effects of co-inoculation of pine sapwood strips with beetle-associated fungi and two decay fungi, *Ganoderma curtisii* and *Phaeoleus schweinitzii*, on decay in a 90 d interaction detail experiment. Residual standard error: 2.38 on 44 degrees of freedom, adjusted R-squared = 0.8394, F$_{7,44}$ = 39.07, p < 0.001.

<table>
<thead>
<tr>
<th>coefficients</th>
<th>estimates</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>intercept</td>
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<td>5.339</td>
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<tr>
<td>initial mass</td>
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<td>-3.113</td>
<td>0.003*</td>
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<tr>
<td>decayer (Phaeolus vs. Ganoderma)</td>
<td>19.1531</td>
<td>7.206</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>treatment O. ips</td>
<td>1.8526</td>
<td>1.587</td>
<td>0.119</td>
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<tr>
<td>treatment R. fusca</td>
<td>0.8479</td>
<td>0.711</td>
<td>0.480</td>
</tr>
<tr>
<td>initial mass : decayer</td>
<td>-6.0653</td>
<td>-3.897</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>decayer : O. ips</td>
<td>-4.6698</td>
<td>-2.909</td>
<td>0.005*</td>
</tr>
<tr>
<td>decayer : R. fusca</td>
<td>-1.7208</td>
<td>-1.047</td>
<td>0.301</td>
</tr>
</tbody>
</table>
Figure 1: Direct effects of beetle-associated fungi on decay of pine wood (as percent dry mass lost) over a 90 d microcosm experiment. All fungi assayed caused significantly more mass loss than negative controls (no fungi), however, *Flavodon ambrosius* was the only beetle-associated fungus to cause mass loss that was comparable to *Ganoderma curtisii*, a well-known decay fungus. Asterisks indicate significant difference from no fungus reference group ** *p* < 0.01, *** *p* < 0.001.
Figure 2: Co-inoculation of a wood decayer and beetle-associated fungi reduced decay in pine wood by competing with decay fungus *G. curtisi*. Asterisks indicate treatments that were significantly different from the reference group which was wood inoculated with only *G. curtisi*, *p* < 0.05.
Figure 3: Transverse cross sections of pine (*Pinus taeda*) wood blocks colonized with ambrosia fungi or the wood decay fungus *Ganoderma curtisii* observed with scanning electron microscopy (bars=200 µm).

A) Section of *P. taeda* wood not colonized with any fungi, where B) tracheids in the early wood simultaneously decayed by *G. curtisii* indicated by the arrowheads; C) tracheids simultaneously decayed by the ambrosia fungus *Flavodon ambrosius*, indicated by the arrowheads. D) Section of *P. taeda* wood colonized with *Raffaelea fusca* showing no evidence of decay, E) section of *P. taeda* wood colonized with *Ambrosiozyma monosporum* showing no evidence of decay, and F) section of *P. taeda* wood colonized with *Ophiostoma ips* showing no evidence of decay. Ew = earlywood, Lw = latewood, Rc = resin canals.
Figure 4: Beetle-associated fungi inhibit decay of pine sapwood from a white rot (*Ganoderma*) or brown rot (*Phaeolus*) fungus. Blue boxes highlight area where *Raffaelea fusca* excluded *Ganoderma* from colonization and prevented degradation of structural components. The same tester strip is shown with mycelium intact (above) and after mycelium was removed (lower). Green boxes highlight differences in the density of hyphal bundles on representative tester strips that were inoculated with *Phaeolus* only (top) and *Phaeolus* plus *Ophiostoma ips*. 