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

3 **Authors:** James Skelton[1*], Andrew Loyd[1,2], Jason A. Smith[1], Robert A. Blanchette[3], Benjamin W. Held[3] &
4 Jiri Hulcr[1*]

5 [1] School of Forest Resources and Conservation, University of Florida, Gainesville, Florida 32603 USA
6 [2] Bartlett Tree Research Laboratories, Charlotte, NC 28278
7 [3.] Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108
8 *Corresponding authors: skelto3@gmail.edu, hulcr@ufl.edu

9

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

21 **Keywords:** Priority effects, Scolytinae, Ophiostomatales, Platypodinae, forest health, lignin, cellulose,
22 *Pinus taeda,* Basidiomycota

23

24

25 **INTRODUCTION**

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

37 Wood-boring bark and ambrosia beetles are widely believed to facilitate biomass recycling by
38 inoculating dead and dying trees with saprotrophic fungi (Ulyshen 2016). These beetles comprise more
39 than 7,000 species in the weevil subfamilies Platypodinae and Scolytinae that thrive throughout tropical
40 and temperate regions (Kirkendall et al. 2015). They are often the first insects to colonize the wood,
41 entering before the tree has died and have major impacts on fungal community development within
42 wood (Leach et al. 1934, Persson et al. 2011, Strid et al. 2014). The beetles have various relationships
43 with the fungi they carry, ranging from incidental commensalism, to highly co-evolved and reciprocally
44 obligate mutualisms (Harrington 2005, Hulcr & Stelinski 2017). Some beetles have specialized glandular
45 organs called mycangia for transporting and nourishing nutritional fungi and have become entirely
46 dependent on a fungal diet (Francke-Grosmann 1956, Batra 1963, Hulcr & Stelinski 2017). Likewise,
47 some fungi have evolved complete dependence on these beetles for dispersal and colonization of wood

48 tissues (Francke-Grosmann 1956, Batra 1963, Six 2003, Harrington 2005). The few species of beetles and
49 symbiotic fungi that kill healthy trees and impact agricultural and silviculture interests have been the
50 center of intense research efforts. In contrast, we know little about the ecological roles of the thousands
51 of other beetles that do not kill trees but are ubiquitous and abundant on every continent except
52 Antarctica.

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

70 While the nutritional fungi of most bark and ambrosia beetles typically do not degrade
71 lignocellulose, some ambrosia fungi do. The primary nutritional symbiont of beetles in the genera

72 *Ambrosiodmus* and *Ambrosiophilus* is the only known lignocellulose decaying ambrosia fungus -
73 *Flavodon ambrosius* (Polyporales) (Kasson et al. 2016). Several species of *Ambrosiodmus* are native to
74 the southeastern US, and at least three species are non-native. An Asian ambrosia beetle,
75 *Ambrosiodmus minor,* was first detected in Florida by state monitoring efforts in 2011, and has since
76 become one of the most frequently collected species across the state where it infests many species of
77 hardwood and coniferous trees (Hulcr et al. 2018). Although there have been more than 50
78 introductions of bark and ambrosia beetles in the United States (http:www.barkbeetles.info; accessed
79 Oct 15, 2018), *A. minor* is particularly likely to affect wood decomposition because of the combination of
80 its increasing abundance and its symbiotic wood-decay fungus.

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

90 **METHODS**

91 *Isolate Recovery and Identification:* All beetle-associated fungal isolates were obtained from the
92 University of Florida Forest Entomology Bark and Ambrosia beetle collection (Table 1). Isolation from
93 live beetles and culture methods followed Skelton et al. (2018). Identification of beetle-associated fungi
94 was based on BLASTn comparison of the ribosomal large sub unit (LSU) DNA sequence, using the primers

95 LR0R and LR5 (Vilgalys and Hester 1990). DNA extraction, PCR amplification and sequencing were
96 performed as described in Skelton et al (2018). We chose four common and globally-distributed beetle-
97 associated fungi to represent major groups of beetle associates in pines: *Ophiostoma ips*
98 (Ophiostmatales) which is commonly found associated with many pine-infesting bark beetle species,
99 *Raffaelea fusca* (Ophiostomatales) which is commonly associated with many pine and hardwood
100 infesting ambrosia beetles, *Ambrosiozyma monospora* which associates with many pine infesting
101 ambrosia beetles, and *Flavodon ambrosius* which is specifically associated with ambrosia beetles in the
102 related genera *Ambrosiodmus* and *Ambrosiophilus*. We also obtained cultures of two species of widely
103 distributed and abundant fungi that are pioneer colonizers that aggressively decay pine wood to
104 examine interactions between beetle-associated fungi and wood decay fungi; one white rotter,
105 *Ganoderma curtisii* f. sp. *meredithiae* (hereafter referred to as *G. curtisii*; Loyd et al. 2018a) and one
106 brown rotter (*Phaeolus schweinitzii*). The isolates were cultured from small pieces (<1cm^3) of context
107 tissue from inside the basidiomata of each wood decay fungus as described in Loyd et al. (2018b). The
108 cultures of *G. curtisii* and *Phaeolus schweinitzii* are archived and maintained at the Center for Forest
109 Mycological Research Fungal Collection (CFMR) in Madison, WI.

110 *Direct effects experiment:* To determine the direct contribution of each beetle-associated fungus to
111 decay of pine sapwood, we used a modified version of the standard wood decay protocol described in
112 Loyd et al. (2018b). Fresh sapwood was obtained from the lower trunk of a ~20 cm dbh loblolly pine
113 (*Pinus taeda*) from the University of Florida Austin Cary Forest, near Gainesville, Florida. The sapwood
114 was cut into 16 cm^3 cubes. Only wood displaying no signs of existing damage or disease was used. To
115 simulate beetle galleries, 4 holes (3 mm diameter) were drilled evenly spaced through two faces of the
116 cube to transect the grain of the wood, i.e. the simulated galleries were horizontal when the cube was
117 held in its original orientation in the tree. The cubes were then dried to completion at 50 °C for 7 d,
118 weighed for dry mass and stored in sealed plastic bags until used. Just prior to use, cubes were placed

119 individually in 237 ml jars with enough distilled water to hydrate to the original water content of the
120 fresh wood (42% water by mass), left overnight to absorb the water, and then autoclaved twice for 1 h
121 at 121 C and 103 kPa with 24 h in between autoclave cycles. After cooling, the cubes were inoculated
122 with fungal spore solutions (described below), jars were loosely capped and incubated at 25 °C in the
123 dark for 14 d to allow beetle-associated fungi to colonize the wood. Cubes were then transferred to
124 microcosms. Microcosms consisted of 237 ml jars containing ~120 ml of hydrated vermiculate, tamped
125 level, with two hydrated 800 mm^3 wood feeder strips (*Quercus nigra*) laid parallel across the surface of
126 the media. Microcosms were autoclave sterilized as described above. Strips were added to keep the
127 methodology consistent between this experiment and the "indirect effects" experiment described
128 below. The inoculated cubes were placed on the wood feeder strips, and microcosms were incubated at
129 25 °C in the dark for 90 d. Cubes were then removed from microcosms, lightly brushed to remove
130 vermiculite and fungal hyphae from the external surfaces, weighed for wet mass, dried to completion at
131 50 °C, weighed for final dry mass, and then stored in sealed plastic bags at -20 °C for later micrograph
132 imaging.

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

139 *Indirect effects experiment:* This experiment was designed to determine if pre-colonization of wood with
140 beetle-associated fungi would reduce subsequent decay from a common pine wood decay fungus, *G.*
141 *curtisii*. Tester cubes and microcosms were prepared as described above with one difference: the
142 wooden feeder strips in the microcosms were colonized with *G. curtisii* prior to the addition of the tester

143 cubes. Feeder strips were inoculated by placing a colonized agar plug at one end of each strip and
144 incubating in the microcosm at 25 °C in the dark for 14 d. In addition to each of the beetle-associated
145 fungi treatments, we also added an "all fungi" treatment in which *F. ambrosius* was combined with all
146 three fungi from all native beetles, with total spore solution volume and number of total spores were
147 held constant. Incubation and final mass were determined as described above. We included 8 replicates
148 for each treatment combination.

149 *Impact on wood structure:* A representative wood sample of each independent fungus and fungal
150 combination from the indirect effects experiment was processed for scanning electron microscopy to
151 assess damage to the structural components of the wood. Small sections of each sample were cut from
152 each sample wood block and infiltrated with TBS™ Tissue Freezing medium™ (Triangle Biomedical
153 Sciences, Durham, NC, USA) under a vacuum and then mounted on brass stubs at -20 °C in a freezing
154 microtome (International Equipment Company, Needham Heights, MA, USA). Samples were sectioned
155 to produce a smooth transverse view of the wood cells, and dehydrated by placing in 20, 30, 45 75 and
156 95 % ETOH for 5 min each. Following dehydration, samples were mounted on aluminum stubs and
157 coated with gold/palladium on a Cressington 108 auto sputter coater (Cressington Scientific
158 Instruments, Watford, United Kingdom). Samples were examined using a Hitachi S3500N (Hitachi,
159 Tokyo, Japan) scanning electron microscope.

160 *Interaction detail experiment:* While the experiments using tester cubes were designed to simulate the
161 interactions occurring within sap wood to determine their effects on biomass loss, they did not offer a
162 good visual assessment of the interactions. To provide a better view of the interactions that caused
163 significantly reduced decay in the indirect effects experiment, we devised a separate assay using tester
164 strips embedded in agar. Fresh pine sapwood (same as described above) was cut into 1 cm by 0.2 cm by
165 6 cm strips, with the grain of the wood running along the longest dimension. Strips were dried and
166 weighed, rehydrated by soaking in distilled water for 24 h, bulk autoclave sterilized as described above,

167 and then embedded in a 1% agar in standard 100 mm plastic petri dishes. After cooling, a rectangular
168 section of agar was removed from each end of the embedded strips and replaced with colonized
169 rectangular agar plugs, pressing the colonized surfaces of the plugs against the ends of the strips. Each
170 strip was inoculated with a wood decay fungus on one end, and the other end was inoculated with
171 either *O. ips*, *R. fusca*, or a sterile agar plug (negative control). In addition to *G. curtisii* from the previous
172 cube experiments, we also included a common pine infecting brown rot fungus (*Phaeolus schweinitzii*).
173 Petri dishes were sealed with plastic film, incubated at 25 °C in the dark for 120 d. Plates were examined
174 and photographed at 90 and 120 d to characterize the interactions between fungi. At 120 d strips were
175 removed from agar, rinsed and brushed gently to remove agar and hyphae from surfaces, dried to
176 completion at 50 °C, and weighed for final dry mass.

177 *Statistical analyses:* For all three experiments, our response variable was percent dry mass lost
178 calculated as the difference between initial dry mass and final dry mass, divided by initial dry mass and
179 multiplied by one hundred. Treatment effects were tested using linear models, implemented by the lm()
180 function in the stats package for R (R Developement Core Team 2010). The assumption of equal group
181 variance was tested using a Bartlett Test, implemented by the Bartlett.test() function. In the direct
182 effects experiment, we used the negative control as the reference group in the linear model. In the
183 indirect effects experiment, the group of tester cubes that received only *G. curtisii* was used as the
184 reference group and compared to all other groups which had been pre-colonized with a beetle-
185 associated fungus. We used a Kruskal-Wallis test (Kruskal.test() function in the stats package for R) to
186 compare the "all fungi" group to the reference group because a high degree of variance in that
187 treatment violated the assumption of homogeneity of variance. For the interaction detail experiment,
188 percent dry mass lost was modeled as a function of decay fungus (*G. curtissi* [reference group] or *P.*
189 *schweinitzii*), initial strip dry mass, beetle-associated fungus treatment (*O. ips, R. fusca*, or negative
190 control [reference group]), with interaction terms for initial mass and decay fungus, and beetle associate

191 and decay fungus. Initial strip mass was included in the model because decay in this assay was greatest
192 on surface of the strips that were exposed above the agar, thus percent mass loss was higher on smaller
193 strips which have a greater surface area to mass ratio.

194 **RESULTS**

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

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

211 *Impact on wood structure: Flavodon ambrosius* was the only beetle-associated fungus to cause
212 detectable structural damage to sapwood. Transverse sections of non-decayed wood (control) observed
213 with SEM showed the typical arrangement of *P. taeda* wood cells with intact tracheids of both early and

214 latewood (Figure 3A). Similar to the negative control blocks, wood in decay microcosms colonized by the
215 ambrosia fungi in the fungal phylum Ascomycota (*Ambroziozyma, Ophiostoma,* and *Raffaelea*) revealed
216 intact cells when transverse sections were observed with SEM. On the contrary, transverse sections of
217 *Pinus taeda* wood blocks that were colonized with the basidiomycetes *G. curtisii* or *Flavodon ambrosius*
218 showed evidence of simultaneous decay of tracheid cells typical of white rot decay fungi (Figures 3B-3C).
219 The simultaneous decay of *P. taeda* wood observed for both *G. curtisii* and *F. ambrosius* was only
220 observed in the earlywood cells, which tend to have larger cells with thinner cell walls compared to
221 latewood cells (Figure 3A).

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

232 Overall, *P. schweinitzii* caused significantly more mass loss than *G. curtisii* in this assay (Table 4).
233 There was also a significant interactive effect between *O. ips* and *P. schweinitzii* as *O. ips* reduced the
234 decay caused by *P. schweinitzii*, congruent with visual observations of reduced hyphal growth of *P.*
235 *schweinitzii* in the presence of *O. ips*. In contrast to the cube experiments, there was no significant effect
236 of either beetle-associated fungus on the decay caused by *G. curtisii* which probably reflected the

237 overall poor performance of *Ganoderma* in this assay; an average of 4.83% mass was lost on strips
238 compared to 12.5% on cubes.

239 **DISCUSSION**

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

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

255 Ascomycete symbionts of beetles interacted antagonistically with wood decay fungi, limiting
256 decay fungi from colonizing experimental wood samples. Competition between non-decaying
257 ascomycetes and wood-decaying basidiomycetes is well-known. For instance, the ascomycete
258 *Trichoderma* species is a proven biocontrol agent used to competitively exclude pathogenic wood-
259 decaying basidiomycetes through multiple competitive mechanisms (Hulme & Shields 1970, Bruce et al.

260 1995). An even more relevant example is the effective use of a pigment-less mutant of *Ophiostoma*
261 *piliferum* to competitively exclude the wood decay polypore *Phlebiopsis gigantea*, as well as blue
262 staining fungi in the order Ophiostomatales (Behrendt et al. 1995). These cases demonstrate that early
263 colonization of wood by ascomycetes can inhibit subsequent colonization of other pioneer decay fungi.
264 These priority effects that exclude or suppress decayers seem particularly relevant to beetle-associated
265 fungi that arrive very early in saprotroph community assembly as a result of their symbiosis with bark
266 and ambrosia beetles. Indeed, our results showed that some beetle-associated fungi exclude wood
267 decayers from colonizing portions of the wood, such as the ambrosia fungus *R. fusca* which prevented
268 *Ganoderma* from colonizing portions of tester strips. In other cases, the hyphae of wood decayers and
269 beetle-associated fungi intermingled, but the decayers showed reduced vigor. *Phaeolus schweinitzii* in
270 the presence of *O. ips* was noticeably sparser than controls without *O. ips,* and a slight inhibition of *G.*
271 *curtisii* by *O. ips* was observed on agar media (data not presented). Clearly the ascomycete associates of
272 bark and ambrosia beetles have the potential to impede subsequent colonization of wood by wood-
273 decaying basidiomycetes.

274 Competition between beetle-associated Ophiostomatales and wood decaying basidiomycetes
275 inhibited decay. Wood cubes incurred significantly less decay from *G. curtisii* when they were pre-
276 inoculated with either *O. ips* or *R. fusca.* Areas in which *R. fusca* had excluded *G. curtisii* showed none of
277 the structural degradation that occurred where *G. curtisii* had colonized experimental wood strips. We
278 also saw reduced decay from *P. schweinitzii* when co-inoculated with *O. ips* in the interaction detail
279 experiment. Ophiostomatalean fungi can rapidly reduce non-structural carbohydrates such as sugar and
280 extractives in wood (Wang et al. 2013), and competition between ascomycetes and basidiomycetes for
281 non-structural carbohydrates can reduce decay rates in non-beetle-associated systems (Hulme & Shields
282 1970). Thus, resource competition for labile carbohydrates is a plausible mechanism for the reduced
283 decay from wood decayers when they were co-inoculated with ophiostomatalean beetle associates. The

284 exclusion of *G. curtisii* from wood colonized by *R. fusca* suggests that this ambrosia fungus may compete
285 through chemical or physical means, in addition to, or instead of competition for labile carbon
286 resources.

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

299 The second source of disparity is guilt by association. Field experiments and surveys that relate
300 wood-boring insects and decay often involve not only bark and ambrosia beetles, but other wood borers
301 such as longhorn beetles (Cerambycidae). Several such studies provide evidence that longhorn beetles
302 cause significant losses in wood mass or density, however direct evidence for an association between
303 bark and ambrosia beetles and wood decay is generally lacking or inseparable from the effects of other
304 wood-boring insects (e.g. Leach et al. 1934, Leach & Orr 1937, Edmonds & Eglitis 1989, Müller et al.
305 2002, Angers et al. 2011). Longhorn beetles are much larger than bark and ambrosia beetles, and
306 consume or eject proportionately larger volumes of wood, which leads to significant losses in wood
307 mass through maceration. In contrast, even heavy infestations of the much smaller ambrosia beetles

308 result in less than half of one percent loss in sapwood volume (Zhong & Schowalter 1989). Our results
309 indicate that the negative effects on decomposition as a consequence of competition between beetle
310 associates and wood decay fungi could offset the small amount of physical maceration of sapwood by
311 ambrosia beetles, resulting in a net negative effect of ambrosia beetles on mass loss.

312 The third source of disparity between our conclusions and those of others is the intuitive but
313 tenuous inference that increasing fungal diversity in decaying logs will result in increased decomposition
314 of wood. Experimental and observational studies have firmly demonstrated that bark and ambrosia
315 beetles cause several fold increases in fungal diversity in freshly dead wood, including increased
316 diversity in decay fungi (Persson et al. 2011, Strid et al. 2014). However, the relationship between
317 diversity of decay fungi in wood and decay rate is not always positive and can in fact be negative as a
318 consequence of increased antagonistic interactions between decay fungi (Boddy 2000, Fukami et al.
319 2010, Dickie et al. 2012).

320 Unlike the ascomycete beetle-associated fungi, the introduced Asian basidiomycete *F.*
321 *ambrosius* caused significant decay of earlywood tracheid cells of pine sapwood, resulting in mass loss
322 similar to the common free-living pine decay fungus *G. curtisii* and visible degradation of wood structure
323 at microscopic and macroscopic levels. The amount of mass loss attributable to *F. ambrosius* was nearly
324 identical to previous work that used similar methods to measure decay of loblolly pine sapwood from
325 four species in the well-known wood decaying polypore genus *Ganoderma* (Loyd et al. 2018b). Our
326 results are also congruent with previous work that assessed decay of a hardwood (*Ailanthus*) in terms of
327 both mass loss and loss of wood hardness from two ascomycete symbionts of ambrosia beetles
328 (*Raffaelea subfusca* and *Fusarium* sp. AF-4) and *F. ambrosius* isolated from another introduced Asian
329 beetle, *Ambrosiophilus attratus* (Kasson et al. 2016). Our study had similar results showing minimal
330 decay from ascomycete beetle-associates, but significant mass and hardness loss from *F. ambrosius*.
331 Thus, unlike any other ambrosia symbiosis known, this unusual ambrosia fungus and it's introduced

332 beetle vectors could contribute directly to wood decay during the early stages of saprotroph community
333 development. Transcriptome and secretome analyses of other pioneering wood decay fungi have
334 elucidated mechanisms used to tolerate and metabolize resinous compounds in wood (Hori et al. 2014).
335 Having the ability to colonize freshly cut wood and capture resources appears to be a very effective way
336 for pioneer species of white rot fungi such as *P. gigantea* or *F. ambrosius* to exclude other fungi. A better
337 understanding of the genes involved with the transformation and detoxification of wood extractives by
338 *F. ambrosius* is needed.

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

352 **CONCLUSIONS**

353 Ascomycete associates of bark and ambrosia beetles can inhibit decay by competing with decay
354 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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456 Table 1: Collection information for fungal isolates used in microcosm experiments.

Species	Isolate name	extracted from	Location
Ambrosiozyma monospora	JH_14703	*Xyleborus pubescens* mycangium	Gainesville, FL, US
Flavodon ambrosius	MAJ001	*Ambrosiodmus minor* gallery	Gainesville, FL, US
Raffaelea fusca	JH_14643	*Xyleborus affinis* mycangium	Gainesville, FL, US
Ophiostoma ips	JH_14624	*Ips grandicollis* body surface	Gainesville, FL, US
Phaeolus schweinitzii	320NC	basidiomata	Charlotte, NC, US
Ganoderma curtisii f. sp. meredithiae	UMNFL50	basidiomata	Sarasota, FL, US

457

458 Table 2: Linear model for direct effects of beetle-associated fungi on mass loss in pine sapwood during a
459 90 d microcosm experiment. Residual standard error: 1.66 on 30 degrees of freedom, adjusted R-
460 squared = 0.8973, $F_{5,30}$ = 62.16, p < 0.001. * Treatments significantly different from the negative control
461 reference group (no fungi).

462

coefficients	estimates	t-value	p-value
intercept	1.4325	2.431	0.021
A. monosporum	2.9395	3.094	0.004*
F. ambrosium	13.0315	13.717	> 0.001*
G. curtisii	11.0450	13.256	> 0.001*
O. ips	3.0455	3.206	0.003*
R. fusca	3.1055	3.269	0.002*

463

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

coefficients	estimates	t-value	p-value
intercept	12.4775	20.215	< 0.001
A. monosporum	-0.8025	-0.919	0.3642
F. ambrosium	0.0675	0.077	0.9388
O. ips	-2.2487	-2.576	0.014*
R. fusca	-1.9675	-2.254	0.036*

468

469

470

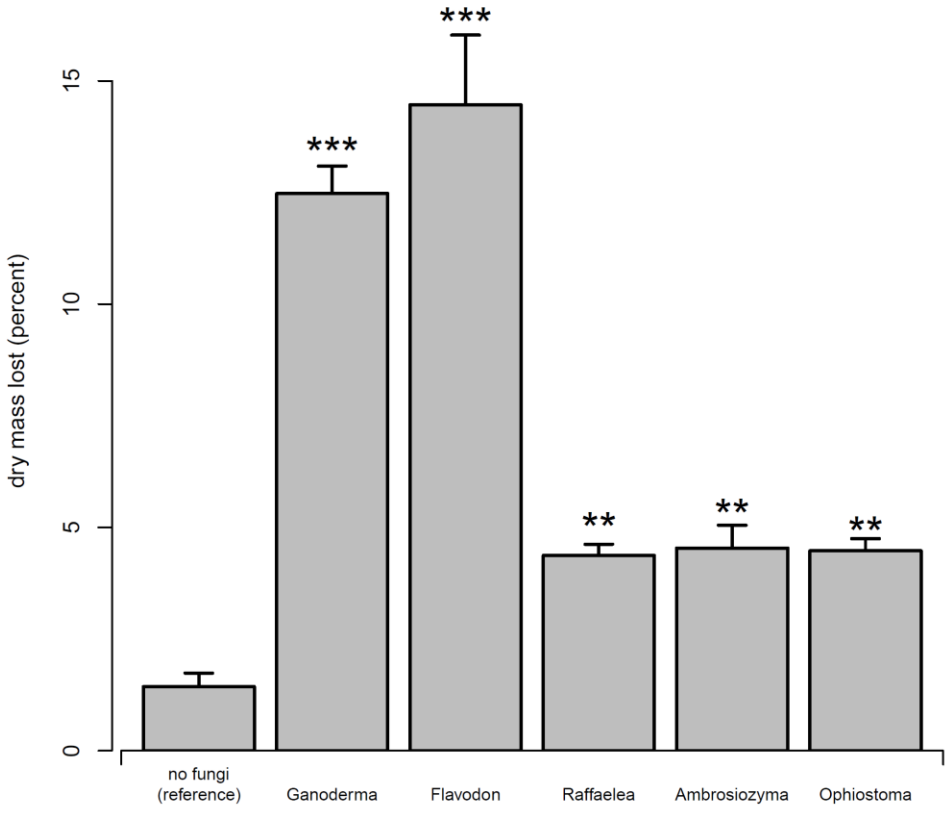
471

472 Table 4: Effects of co-inoculation of pine sapwood strips with beetle-associated fungi and two decay
473 fungi, *Ganoderma curtisii* and *Phaeoleus schweinitzii,* on decay in a 90 d interaction detail experiment.
474 Residual standard error: 2.38 on 44 degrees of freedom, adjusted R- squared = 0.8394, $F_{7,44}$ = 39.07, p <
475 0.001.

coefficients	**estimates**	**t-value**	**p-value**
intercept	10.1104	5.339	< 0.001
initial mass	-3.1860	-3.113	0.003*
decayer (*Phaeolus* vs. *Ganoderma*)	19.1531	7.206	< 0.001
treatment *O. ips*	1.8526	1.587	0.119
treatment *R. fusca*	0.8479	0.711	0.480
initial mass : decayer	-6.0653	-3.897	< 0.001*
decayer : *O. ips*	-4.6698	-2.909	0.005*
decayer : *R. fusca*	-1.7208	-1.047	0.301

476

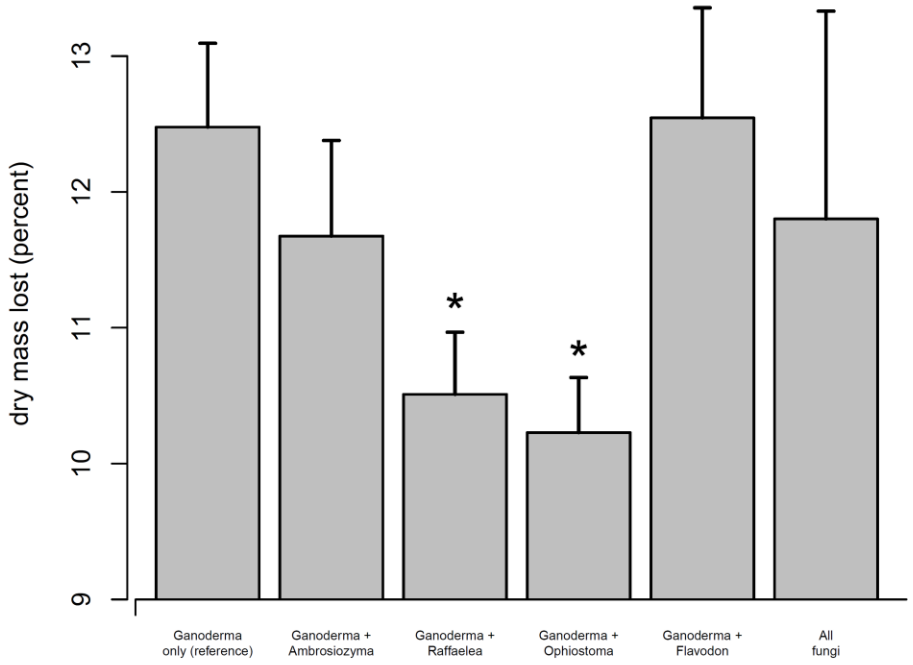
477 Figure 1: Direct effects of beetle-associated fungi on decay of pine wood (as percent dry mass lost) over
478 a 90 d microcosm experiment. All fungi assayed caused significantly more mass loss than negative
479 controls (no fungi), however, *Flavodon ambrosius* was the only beetle-associated fungus to cause mass
480 loss that was comparable to *Ganoderma curtisii,* a well-known decay fungus. Asterisks indicate
481 significant difference from no fungus reference group ** $p < 0.01$, *** $p < 0.001$.



482

483

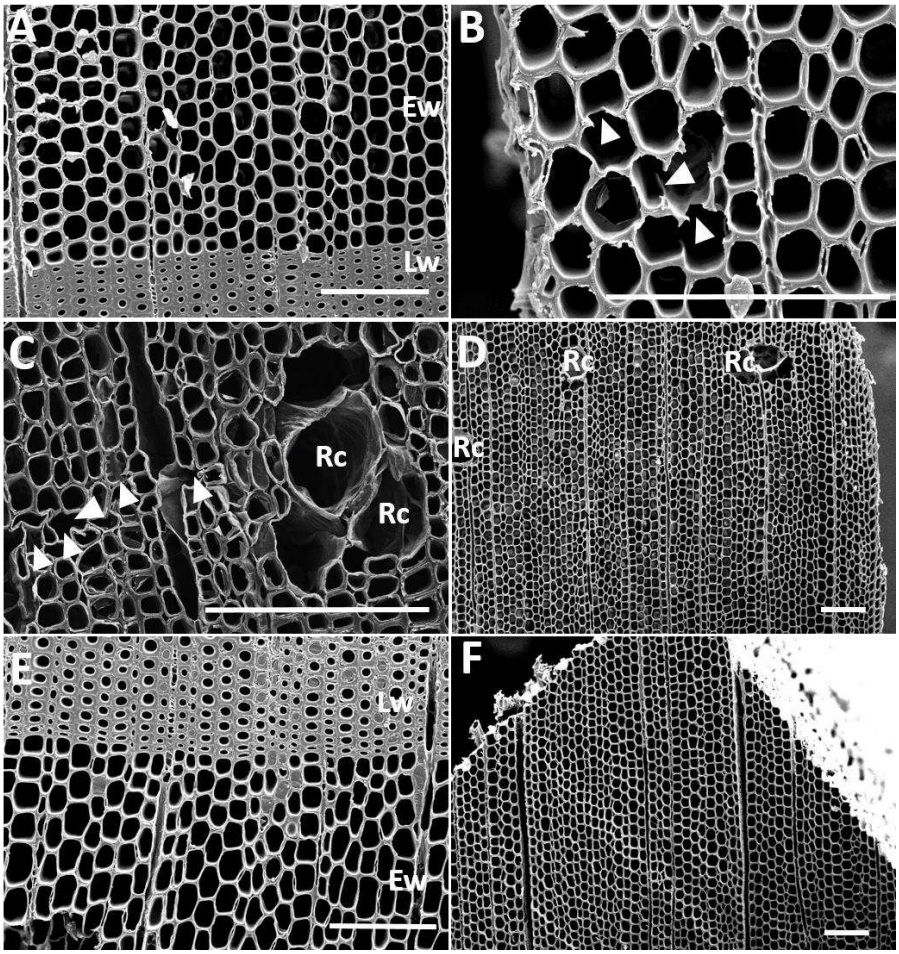
484 Figure 2: Co-inoculation of a wood decayer and beetle-associated fungi reduced decay in pine wood by
485 competing with decay fungus *G. curtisii*. Asterisks indicate treatments that were significantly different
486 from the reference group which was wood inoculated with only *G. curtisii*, *p < 0.05.



487

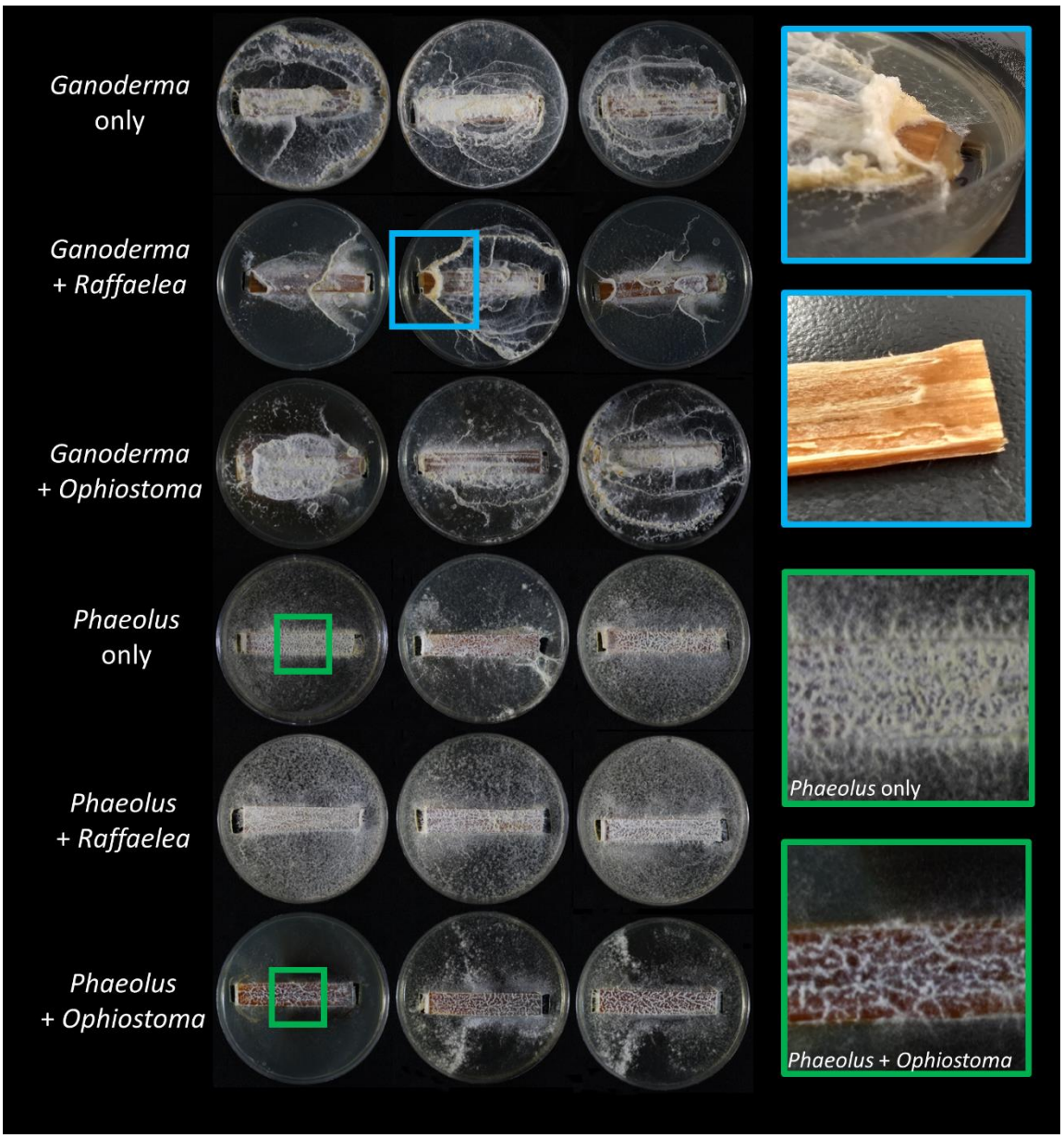
488

489 Figure 3: Transverse cross sections of pine (*Pinus taeda*) wood blocks colonized with ambrosia fungi or
490 the wood decay fungus *Ganoderma curtisii* observed with scanning electron microscopy (bars=200 µm).
491 A) Section of *P. taeda* wood not colonized with any fungi, where B) tracheids in the early wood
492 simultaneously decayed by *G. curtisii* indicated by the arrowheads; C) tracheids simultaneously decayed
493 by the ambrosia fungus *Flavodon ambrosius,* indicated by the arrowheads. D) Section of *P. taeda* wood
494 colonized with *Raffaelea fusca* showing no evidence of decay, E) section of *P. taeda* wood colonized with
495 *Ambrosiozyma monosporum* showing no evidence of decay, and F) section of *P. taeda* wood colonized
496 with *Ophiostoma ips* showing no evidence of decay. Ew = earlywood, Lw = latewood, Rc = resin canals



497

498 Figure 4: Beetle-associated fungi inhibit decay of pine sapwood from a white rot (*Ganoderma*) or brown
499 rot (*Phaeolus*) fungus. Blue boxes highlight area where *Raffaelea fusca* excluded *Ganoderma* from
500 colonization and prevented degradation of structural components. The same tester strip is shown with
501 mycelium intact (above) and after mycelium was removed (lower). Green boxes highlight differences in
502 the density of hyphal bundles on representative tester strips that were inoculated with *Phaeolus* only
503 (top) and *Phaeolus* plus *Ophiostoma ips*.



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