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# Intraspecific interactions among wood-decay fungi alter decay rates and dynamics of interspecific interactions

Mark T. Banik [*,1], Daniel L. Lindner [1,**], Michelle A. Jusino, [1]

*USDA Forest Service, Northern Research Station, Center for Forest Mycology Research, One Gifford Pinchot Drive, Madison, WI, USA*

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ABSTRACT

Interactions among wood-decay fungi can have a profound effect on fungal community composition, decay rates and ultimately the chemical composition of the material remaining after the decay process. Interspecific interactions among fungi as they decay wood have been well-studied but almost nothing is known about the effect of intraspecific interactions between individual genets on the decay process. In this study we examine the effect of both intra- and interspecific competition on wood mass-loss for five species of wood-decay fungi: *Cerrena unicolor*, *Fuscoporia gilva*, *Irpex lacteus*, *Stereum ostrea* and *Trametes versicolor*. Four of the five species studied showed a significant increase in mass loss when five individual isolates (genets) of the same species simultaneously colonized aspen test wafers *in vitro*. The dynamics of interspecific interactions were also significantly impacted by the presence of multiple genets of each species. Taken together, these results demonstrate that intraspecific interactions can change the outcome of interspecific interactions and thus the functioning of the overall community.

## 1. Introduction

Many factors interact to affect the process of wood decay. Temperature, moisture, substrate, animal activity and the microbial community all have an effect on how fast the decay will proceed and the recalcitrance of any remaining organic matter (e.g., whether material will quickly be returned to the atmosphere as $CO_2$ or will enter slow soil carbon pools). The microbial decay community is composed primarily of fungi, with bacteria playing a lesser role in the decomposition of lignified substrates; interactions among community members and the environment result in a range of decay rates and final products of decomposition. The fungal community primarily responsible for the breakdown of woody substrates is composed mostly of basidiomycete fungi with the two primary outcomes being brown rotted wood, which is rich in recalcitrant lignin residues, or white rotted wood, in which the lignin has been mostly degraded (Goodell et al., 2020; Rayner and Boddy, 1988).

Interactions between species of wood decay fungi as they compete for resources have been extensively studied and the range of possible outcomes is varied. The competition between fungi that decay wood can be thought of as a battle for available resources with the outcome generally regarded as deadlock or replacement and a range of possible outcomes in between (Boddy 2000; Hiscox et al., 2018; Rayner and Boddy 1988). These categories describe the effect of the interactions on the organisms themselves but do not describe how these interactions affect the decay process. The decay process itself, which experimentally is commonly measured as the mass loss that occurs as a woody substrate is utilized as a carbon source, can be altered in several ways by competition between fungal species. Multiple species interacting to decompose a substrate have been reported to enhance, decrease or have no effect on decomposition rate, with the exact effect highly dependent on the fungal species involved (Boddy 2000; Fukami et al., 2010; Lindner et al., 2011; Maynard et al., 2017; Tiunov and Scheu 2005; Venugopal et al., 2017). Not only do the fungi have intrinsically different decay rates but these decay rates are also dependent on the makeup of the fungal community around them. The exact effect of competition between fungal species on mass loss is highly unpredictable due to the intransitive nature of the interactions (Boddy 2000; Sturrock et al., 2002).

Although intraspecific interactions may have ecological

* Corresponding author.
** Corresponding author.
*E-mail addresses:* mark.t.banik@usda.gov (M.T. Banik), daniel.l.lindner@usda.gov (D.L. Lindner).
[1] indicates shared first authorship based on equal contributions.

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consequences at least as significant as interspecific interactions (Des Roches et al., 2018), they have rarely been examined in fungi in the context of wood decay. Mycologists have traditionally examined intraspecific interactions in terms of haploid pairings as a means of determining conspecificity. They have also used confrontational cultural techniques as a tool for determining conspecificity and for defining individual genets in ecological studies (Adams et al., 1981; Chamuris and Falk 1987; Williams and Todd 1985). Such interactions are responsible, via the formation of melanized zone lines in colonized wood, for the delimitation of fungal genets in nature (Adams et al., 1981; Rayner and Todd 1977). Besides physical barriers such as the construction of zone lines, organic compounds produced during fungal interactions also play a crucial role in fungal community establishment. Diffusible organic compounds (DOCs) and volatile organic compounds (VOCs) are means by which fungi may affect the growth of other fungi without being in physical contact. These compounds have been primarily shown to have an impact on other fungal species (El Ariebi et al., 2016) but have also been shown to reduce the hyphal extension rate of other members of the same species (Heilmann-Clausen & Boddy, 2005). The types of DOCs and VOCs produced are often specific to the interaction in which they occur, perhaps accounting for the intransitivity of fungal interactions (Evans et al., 2008).

Very little is known about intraspecific interactions in woody substrates and what effect they may have on the wood decay process and subsequent mass loss. This may depend on how many genets of the same species interact while decaying a particular substrate. In logs, some authors have reported one individual of one wood decay species (Huss 1993; Keirle et al., 2014); others have reported 3–4 individuals (Kauserud and Schumacher, 2002), 6–12 (Jenssen et al., 2020; Chamuris and Falk 1987) or 15 or more genets of a single species inhabiting the same substrate (Kay and Vilgalys 1992; Williams and Todd 1985). All of these reports involve different species of decay fungi which may affect the number of individuals occurring within a given substrate. The number of individuals per log may also be related to how common the species is in the ecosystem and if it primarily colonizes new resources via spore dissemination or mycelial growth.

Current ecological studies mainly focus on using tools such as high throughput amplicon sequencing (HTS) to assess the makeup of fungal communities in woody substrates (Gilmartin et al., 2022; Jusino and Boddy 2022; Jusino et al., 2022; Kubartová et al., 2012; Maillard et al., 2022; Palmer et al., 2018; Wang et al., 2020). Using such tools, the effects of interactions among individual genets in natural systems cannot be determined at this time. Questions such as how prevalent are different genets in a log and are genet-genet interactions more common than interspecies interactions, are very difficult to answer on a large scale using currently available techniques. The purpose of this study was to determine if interactions among individual genets of the same species of wood-decay fungi have an effect on wood-decay as measured by mass loss in a defined model system. In addition, during interspecies interactions involving five species, we examined the effect of genet-to-genet variability on the resulting mass loss. Finally, we investigated the impact of the presence of multiple genets of the same species on the wood-decay process when multiple species are interacting.

## 2. Materials and methods

### 2.1. Fungal culture isolation and identification

Collections of *Cerrena unicolor, Fuscoporia gilva, Irpex lacteus, Stereum ostrea* and *Trametes versicolor* were made between August 2018 and February 2019 from a 4-acre mixed hardwood woodlot and adjacent 10-acre mixed conifer planting located in Dane County, Wisconsin, USA. Fungi from a limited geographical area were chosen because their gene pools have evolved under the same climatic conditions and also could be reasonably assumed to have interacted with each other in nature. All isolations were made from separate pieces of substrate (i.e. from separate trees), thus limiting the possibility of obtaining multiple isolates of the same genet. In addition, because the species chosen are known to be reliant on spore dispersal as a means of dissemination and are not known to spread via rhizoidial cords or similar means of substrate colonization (they are "unit restricted"), the collection of non-unique genets is very unlikely when isolated from substrates derived from different individual trees. Isolations were made either from basidiomata contexts or from rotted wood associated with basidiomata. Isolations were made on malt extract agar (MEA; 1.5% malt extract, 2.0% agar) and modified Taylor's medium (Taylor 1971; recipe in supplemental) by placing an approximately 1 mm × 3 mm piece of the appropriate isolation material toward one edge of a 40 mm Petri dish containing one of the media. Six isolations per medium were done for each collection of which 4 were sub-cultured to individual storage tubes containing MEA.

Identity of the cultures was verified by amplifying their ITS regions with primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al., 1990) using previously described reagents and protocols (Lindner and Banik 2009). The procedure was modified for use of pipet tip swabs of the cultures for generating template DNA and thus an additional 3 μl of water was added to the reaction mix to replace the 3 μl of purified DNA which was supplanted by the tip swipe. Following amplification, the resulting products were Sanger sequenced according to Lindner and Banik (2009). Sequences were identified via BLAST search of the NCBI database as well as being compared among themselves. Twenty-five isolates, all from unique collections, comprising five individual isolates of each species (*T. versicolor*, *F. gilva*, *S. ostrea*, *I. lacteus* and *C. unicolor)*, were chosen as test isolates and were randomly designated as genets A-E.

### 2.2. Decay tests

Decay tests were performed with the use of feeder dowels placed in a 5 x 5 dowel matrix to serve as the inoculum source for aspen test wafers (Fig. S1). The feeder dowels were approximately 6.35 mm in diameter and 5 mm long and were separated from each other by approximately 1 mm in the 42 mm square 25 dowel matrix. The dowels were sterilized prior to use by autoclaving twice at 24-h intervals. For autoclaving, the dowel matrices were placed in glass Petri dishes, with water added to approximately half the height of the dowels. For colonization of the feeder dowels with the chosen fungal isolates, 8–10 approximately 3 mm × 3 mm x 5 mm pieces of colonized MEA were distributed around the edges of 25 mm × 100 mm glass Petri dishes containing approximately 30 mL of MEA. After mycelial growth had completely covered the agar surface, matrices holding sterile feeder dowels were aseptically placed on the surface in the center of the plates. These were then incubated at 26 C until the dowels appeared completely colonized, 2–4 weeks depending on the fungal isolate. The colonized dowels were aseptically removed from their matrices with sterile forceps into sterile empty Petri dishes in preparation for their distribution into the appropriate treatment matrices. Completely colonized feeder dowels were used as inoculum sources as a way to standardize the amount of inoculum in the competitive interactions.

Each test setup was composed of 25 colonized dowels randomly placed in matrices to complete the following treatments: (a) each dowel colonized by the same genet, 5 genets per species (randomly labeled A-E for each species); (b) five dowels of five different genets of the same species; (c) five dowels of one genet from each of five species; (d) one dowel of each of 25 different genets for a total of 36 treatments. The five dowels of one genet from each of five species treatment was performed using 5 variations out of a potential 3125 different combinations. The five variations placed the genets of each species with the same identification letter (A-E) in competition. All treatments were replicated 5 times except for the treatment composed of one dowel colonized by each of the 25 genets which was replicated 10 times. After the appropriate colonized dowel matrices were assembled, they were placed on 30 mL of

**Table 1**
Percentage mass loss observed in aspen test wafers as a result of colonization by 5 different genets (one genet per wafer) of *C. unicolor* (CO), *F. gilva* (FG), *I. lacteus* (IL), *S. ostrea* (SO) and *T. versicolor* (TV), and when co-colonized with 5 genets of the same species.

| Species | Isolate (genet) | Average % mass loss ± standard deviation for each genet and pairwise comparisons (letters indicate statistical significance) | ANOVA results of average % mass loss among genets of each species | Average % mass loss ± standard deviation when: (a) each genet grown separately; (b) all 5 genets of the same species co-colonized | Welch's t-test of mass loss when genets were grown separately vs mass loss when genets were co-colonized | % change in mass loss when genets were co-colonized vs when they were grown separately |
|---|---|---|---|---|---|---|
| *Cerrena unicolor* | CU genet A | 11.75 ± 1.29 b | df=4, F=6.15, **p = 0.0021** | (a) 12.57 ± 2.25 | | |
| | CU genet B | 10.98 ± 2.84 b | | | | |
| | CU genet C | 14.12 ± 0.41 a,b | | | | |
| | CU genet D | 10.34 ± 3.10 b | | | | |
| | CU genet E | 15.66 ± 1.02 a | | | | |
| | CU genets A-E | | | (b) 16.25 ± 0.32 | t = 6.458, df=26, **p<0.0001** | 29.3+ |
| *Fuscoporia gilva* | FG genet A | 17.43 ± 3.09, c | df=4, F=18.15, **p < 0.0001** | (a) 23.21 ± 3.79 | | |
| | FG genet B | 22.32 ± 1.16, b | | | | |
| | FG genet C | 27.76 ± 0.88, a | | | | |
| | FG genet D | 24.68 ± 2.55, a,b | | | | |
| | FG genet E | 23.91 ± 1.28, b | | | | |
| | FG genets A-E | | | (b) 23.29 ± 2.63 | t = -0.051, df=8, p=0.9602 | 0.3+ |
| *Irpex lacteus* | IL genet A | 17.85 ± 0.76 a | df=4, F=3.428, **p = 0.0274** | (a) 16.94 ± 1.22 | | |
| | IL genet B | 18.13 ± 0.69 a | | | | |
| | IL genet C | 15.78 ± 2.97 a | | | | |
| | IL genet D | 15.50 ± 0.43 a | | | | |
| | IL genet E | 17.47 ± 0.87 a | | | | |
| | IL genets A-E | | | (b) 20.83 ± 0.42 | t = 9.795, df=26, **p<0.0001** | 23.0+ |
| *Stereum ostrea* | SO genet A | 9.79 ± 2.88 c | df=4, F=65.96, **p < 0.0001** | (a) 14.60 ± 6.07 | | |
| | SO genet B | 14.14 ± 1.86 b | | | | |
| | SO genet C | 25.09 ± 1.17 a | | | | |
| | SO genet D | 11.67 ± 0.50 b,c | | | | |
| | SO genet E | 12.31 ± 0.75 b,c | | | | |
| | SO genets A-E | | | (b) 18.49 ± 0.59 | t = 3.305, df=26, **p=0.0027** | 26.6+ |
| *Trametes versicolor* | TV genet A | 24.78 ± 2.30 c | df=4, F=6.6401, **p = 0.0014** | (a) 28.45 ± 3.36 | | |
| | TV genet B | 31.19 ± 5.17 a,b | | | | |
| | TV genet C | 32.58 ± 1.46 a | | | | |
| | TV genet D | 27.86 ± 2.38 a,b,c | | | | |
| | TV genet E | 25.83 ± 1.66 b,c | | | | |
| | TV genets A-E | | | (b) 31.10 ± 1.15 | t = 2.751, df=23, **p=0.0111** | 9.3+ |

2% water agar medium in a 25 mm × 100 mm Petri dish and sterile aspen test wafers were aseptically placed on top. The sterile aspen test wafers were 48 mm square and 5 mm thick. The wafers were previously dried at 70C for three days, weighed and then autoclaved twice in glass Petri dishes in the presence of water as described above. The complete assembly was incubated for 12 weeks at 26C. After incubation, fungal mycelia covering the test wafers were gently scraped off with a razor blade and the wafers were dried at 70C for three days and weighed.

### 2.3. Mass loss analysis

The mass loss for each aspen test wafer was determined by subtracting the dry weight of the wafer after 12 weeks of incubation from the dry weight of the wafer prior to colonization. This was then converted to percent mass loss by dividing the change in weight by the weight of each wafer prior to colonization. The average percent mass loss for all wafers from a given treatment was determined.

### 2.4. Test wafer DNA analysis

Nine test wafers from the five individuals of each of five species treatment (5 genets per wafer, each a different species) that were homogeneous in appearance were sampled to determine the identity of the fungi present. Sampling was conducted after the wafers had been dried and weighed to determine mass loss. Sampling was done by drilling cross sectionally into the wafer with a 3 mm diameter bit to a depth of about 15 mm, collecting the resulting shavings in a 1.5 mL sterile microcentrifuge tube and covering them with sterile cell lysis solution (CLS; Lindner and Banik, 2009). The DNA was then extracted, amplified, and Sanger sequenced following the procedure of Lindner and Banik (2009). The sequences were then identified by comparing them to the sequences for the original test fungi.

### 2.5. Statistical analyses

Percent mass loss observed for the 25 individual (single) genets when singly colonizing aspen wafers was used as baseline data for examining the impact of various interactions in the more complicated co-colonized treatments. Mass loss observed in the single genet colonized wafers represents the inherit decay potential of each species without the compounding effects of interactions with other genets or species. Therefore, by comparing the mass loss observed in the co-colonized treatments to the appropriate combination of mass loss from the individual genets, we can obtain on estimate of how interactions among genets are affecting mass loss. Likewise, by comparing the mass losses observed in several permutations of one genet of each species co-colonizing to the maximum complexity of five genets of five species co-colonizing, we can estimate the impact of within species genets on intraspecific interactions.

All statistical analyses were performed using R (R core team). The variation in mass loss within each species for the 5 genets sampled individually per species was determined using one-way ANOVA tests, implemented using function *aov* in the multcomp package. Significant ANOVA tests were followed by Tukey's post-hoc pairwise comparisons using an alpha value of 0.05, implemented by function *TukeyHSD* and given common characters using function *multcompLetters4* in the multcompView package. Box plots were generated to visualize the variation

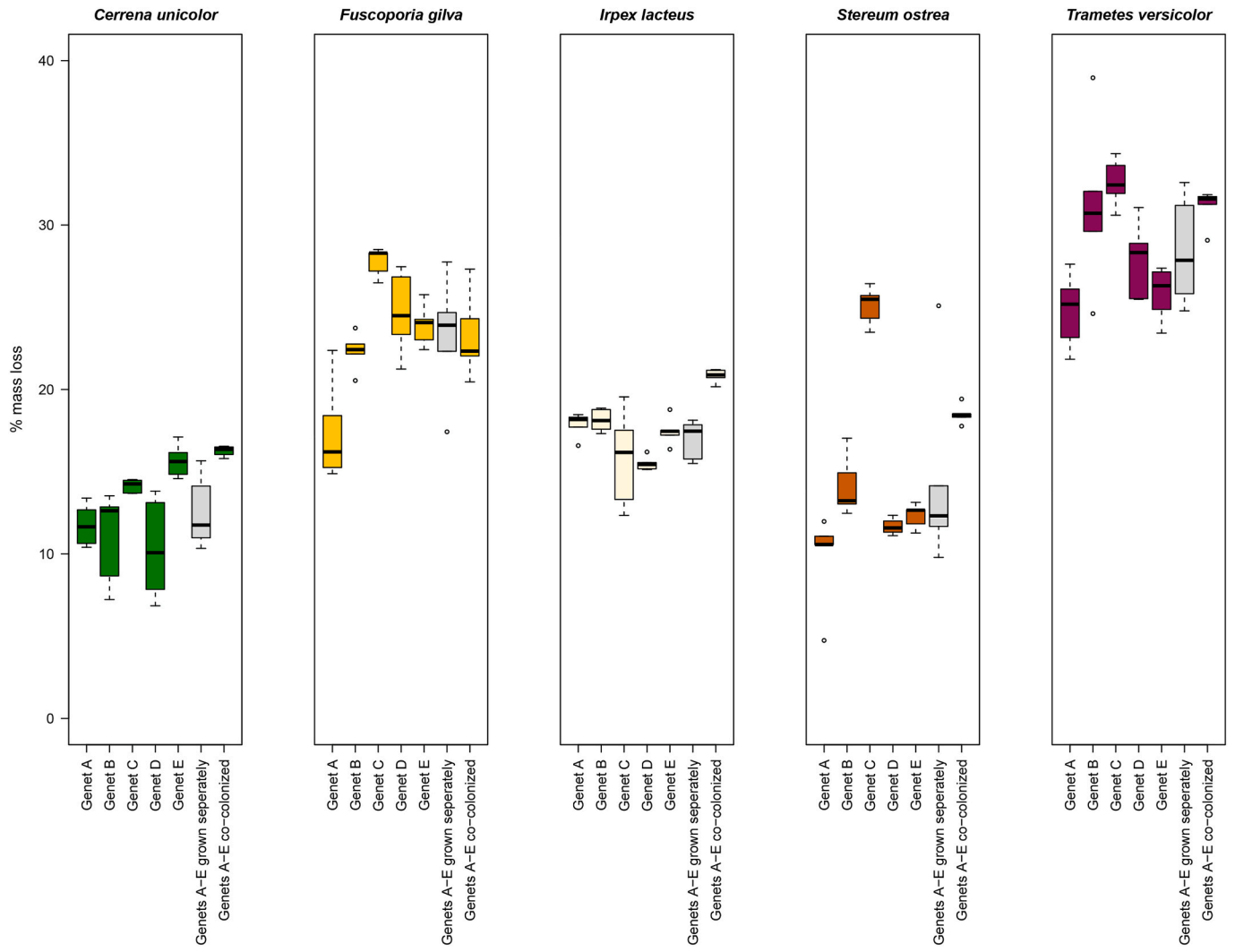


**Fig. 1.** Percentage mass loss in aspen wafers when colonized by 5 genets of 5 species of wood-decay fungi alone and when co-colonized by other genets of the same species. Error bars represent one standard deviation. Gray boxes represent the percentage mass loss of genets A-E individually for each species.

in mass loss within each species for the 5 genets sampled individually per species compared to and the co-colonized treatment that consisted of 5 genets of the same species. The individual genet mass loss vs co-colonized mass loss values for each species were then compared using Welch's t-tests, implemented by function *t.test*, with var.equal = FALSE. We followed similar logic to compare the percentage mass loss observed in aspen test wafers when colonized simultaneously by either one genet each of the 5 species to those co-colonized by 5 genets of each of 5 species.

## 3. Results

The collection information for each genet used is presented in Table S1. ITS sequence length varied between 618 and 716 bp depending on the species. ITS sequence similarity was greater than 99% among all five genets of a species for each of the five species used in this study. Sequences for each genet are deposited with GenBank with accession numbers listed in Table S1.

The mass loss for treatments involving only one species is shown in Table 1. Average percent mass loss for the five genets of each species ranged from 10.3 to 15.7 (*C. unicolor*), 17.4–27.8 (*F. gilva*), 15.5–18.1 (*I. lacteus)*, 9.8–25.1 (*S. ostrea*) and 24.8–32.5 (*T. versicolor*). Species exhibited significant differences in mass loss among their respective genets as demonstrated by ANOVA tests, with *I. lacteus* exhibiting the least variation, and *S. ostrea* the most (Table 1; Fig. 1). Variation in percent mass loss was compared for genets grown separately on test wafers vs when all five genets of the same species co-colonized test wafers, using Welch's t-tests (Table 1). All species except *F. gilva* exhibited a significant increase in mass loss when the five genets of each species co-colonized test wafers compared to when each individual genet colonized test wafers separately (Table 1; Fig. 1).

Mass loss for treatments involving multiple species and multiple genets are shown in Table 2. The observed mass loss for the five permutations of one genet of each of five species treatment ranged from 17.7% to 20.1% with significant differences between many of the permutations (Table 2, Fig. 2). The percentage mass loss for permutations co-colonized by multiple species (5 genets per wafer) was significantly different from the values obtained by growing each of the five species separately (1 genet per wafer) in some cases (Table 2). The observed mass loss was greater than expected in 1 permutation and less than expected in 1 permutation. Wafers co-colonized by all 25 genets averaged 23.2% mass loss, which is significantly higher than mass loss observed when all 25 genets were grown separately (one genet per wafer; average of 19.2% mass loss; $p = 0.0077$) or when one genet of 5 different species were co-colonized (5 genets per wafer; average 18.9% mass loss; $p = 0.01188$)(Table 2, Fig. 2).

Zone line formation was absent in all wafers colonized by only a single genet. When five genets of one species colonized each wafer, zone

**Table 2**
Percentage mass loss observed in aspen test wafers when colonized simultaneously by one genet each of *C. unicolor* (CU), *F. gilva* (FG), *I. lacteus* (IL), *S. ostrea* (SO), and *T. versicolor* (TV) (5 genets per wafer; 5 different species), or 5 genets of each of 5 species (25 genets per wafer; 5 different species).

| Treatment | Isolates (genets) co-colonized on wafers | Number of reps | Average percentage mass loss with co-colonization (letters indicate significant differences) | ANOVA results of average percent mass loss with co-colonization | Average percentage mass loss based on individual genets grown separately | Welch's t-test comparing % mass loss when genets were co-colonized vs grown separately | Welch's t-test comparing % mass loss when one genet of each of 5 species were co-colonized (5 genets per wafer) vs when 5 genets of 5 species were co-colonized (25 genets per wafer) |
|---|---|---|---|---|---|---|---|
| one genet of each of 5 species (5 genets per wafer) | Genet A: CU, FG, IL, SO, TV | 5 | 19.06 ± 0.35 ab | df=4, F=7.905, **p=0.0005** | 16.31 ± 5.76 | t = 2.355, df=24, **p=0.0268** | |
| | Genet B: CU, FG, IL, SO, TV | 5 | 17.68 ± 0.29 c | | 19.35 ± 7.63 | t = -1.089, df=24, p=0.2867 | |
| | Genet C: CU, FG, IL, SO, TV | 5 | 19.22 ± 1.10 ab | | 23.07 ± 7.36 | t = -2.480, df=27, **p=0.0195** | |
| | Genet D: CU, FG, IL, SO, TV | 5 | 18.58 ± 0.47 bc | | 18.01 ± 7.42 | t = 0.382, df=24, p=0.7058 | |
| | Genet E: CU, FG, IL, SO, TV | 5 | 20.10 ± 0.92 a | | 19.04 ± 5.29 | t = 0.936, df=27, p=0.3571 | |
| 5 genets of each of 5 species (25 genets per wafer) | Genets A-E: CU, FG, IL, SO, TV | 10 | 23.23 ± 3.99 | | 19.16 ± 7.02 | t = 3.366, df=9, **p=0.0077** | t = 2.891, df=13, **p=0.01188** |

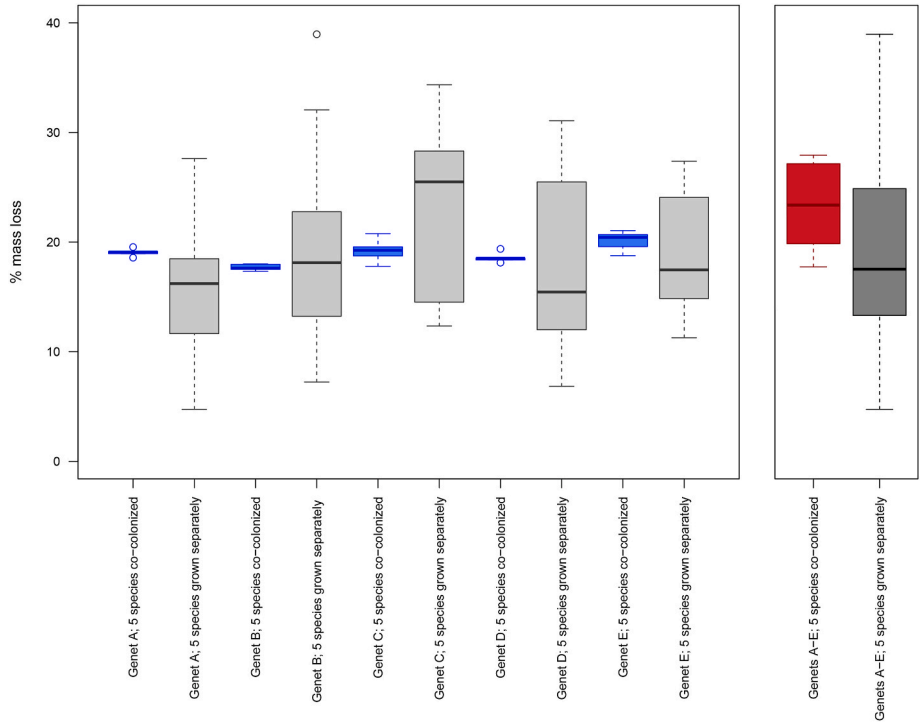


**Fig. 2.** Percentage mass loss observed in 5 permutations of aspen wafers co-colonized by 1 genet of each of 5 species of wood decay fungi (blue boxes, n = 5). The red box is the percentage mass loss observed when 25 genets, five of each of five species, co-colonized aspen wafers (n = 10). Error bars represent one standard deviation. Gray boxes represent the percentage mass loss expected for their respective treatments and is based on averaging the individual percentage mass loss for each of the genets in that treatment.

lines were distinct and dark for *F. gilva*, distinct and lighter colored for *I. lacteus* and *T. versicolor*, and diffuse and lightly colored for *C. unicolor* and *S. ostrea* (Fig. 3). The average number of sectors observed from the five wafers of each of the species was 19.8 (*F. gilva*), 14.2 (*T. versicolor*), 17.0 (*I. lacteus*), 13.2 (*C. unicolor*) and 9.4 (*S. ostrea*). An average of 2.4, 0.8, 1.6, 1.6, and 0.6 sectors were observed for each of the five permutations of the five species by one genet treatments, respectively (Fig. 4). Zone lines were generally well defined and darkly colored but the few sectors that were present were often quite small. Sampling in the homogenous area between sectors yielded only the DNA sequences of *I. lacteus*. The ten replicated wafers colonized by all 25 genets (25 genets per wafer) exhibited an average of 7.9 sectors per wafer, which were

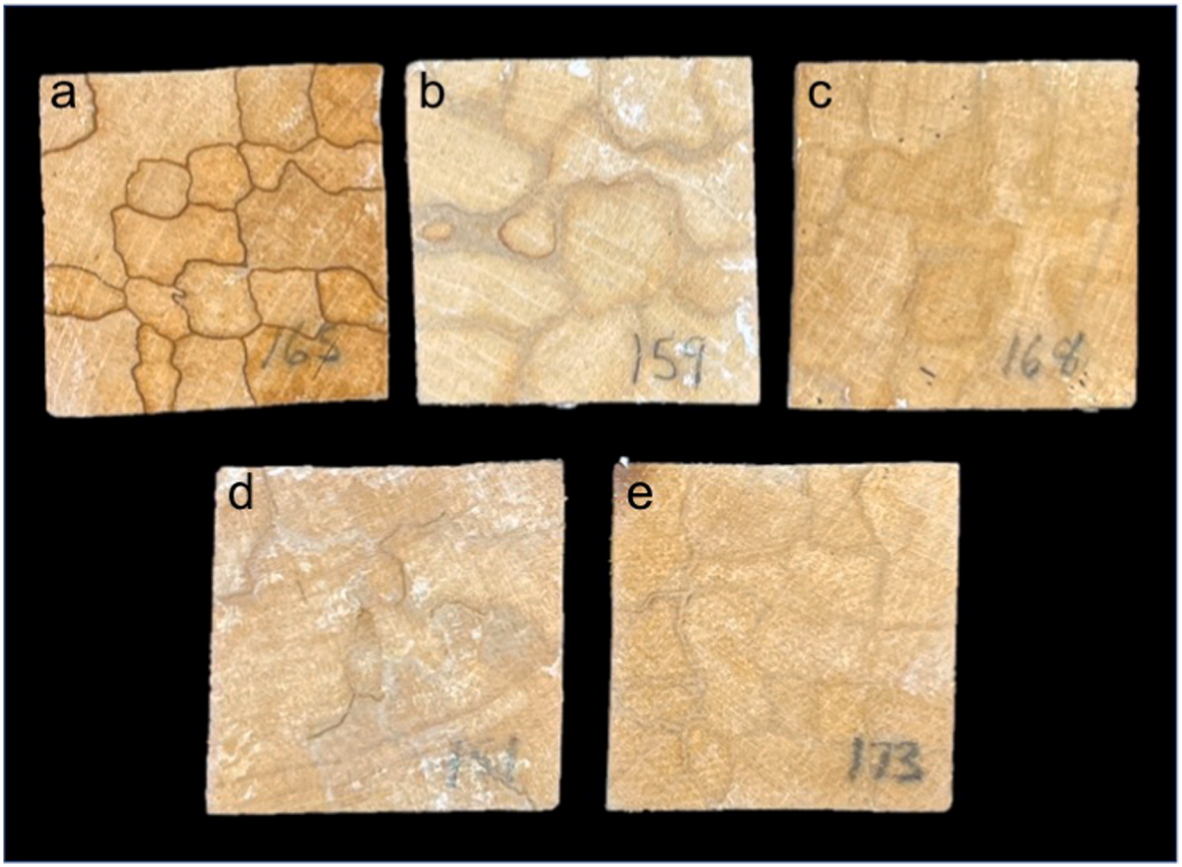


**Fig. 3.** Representative aspen wafers colonized by five genets of *Fuscoporia gilva* (a), *Trametes versicolor* (b), *Cerrena unicolor* (c), *Stereum ostrea* (d), or *Irpex lacteus* (e). For all species, wafers colonized by only one isolate exhibited no zone lines (not shown).

usually separated by dark, well-defined zone lines, although lighter colored zone lines were also present (Fig. 4).

## 4. Discussion

We examined the impact of intraspecific interactions of wood-decay fungi on mass loss using five genets of five species. Four of the five species studied exhibited a significant increase in mass loss during co-colonization by genets of the same species when compared to the mass loss observed when the five genets were grown separately. Multiple mechanisms could account for these results. Because mass loss increased during co-cultivation, competitive interactions seem to be less important than facilitative/synergistic interactions, which could include direct and indirect interactions, behavioral changes, and upregulating activities. The increase in mass loss when five individuals of the same species co-decay is perhaps related to the increase in production of lignin modifying enzymes observed when co-culturing certain species of white rot fungi (Chi et al., 2007; Dong et al., 2012; Qi-he et al., 2011). It is conceivable that individuals of the same species could benefit from utilizing the same suite of enzymes to decay wood even as they construct zone lines to keep their mycelia physically separated. The four species that exhibited an increase in mass loss when individuals co-decayed all produced diffuse zone lines which might be more amenable to this type of enzyme sharing. It is also possible that sensing competition, these species employ a "use it or lose it" approach to substrate utilization and thus allocate more resources toward consumption and mycelial growth while devoting fewer resources to building zone lines to protect the resource. It is notable that *F. gilva*, which formed the most intense zone lines in intraspecies interactions, was the only species that showed no significant change in mass loss during co-colonization by all five genets. The intensity of the zone lines may have prevented the type of cooperative decay seen in the other species or alternatively more resources were allocated to establishing zone lines and less to wood degradation (e.g. the idea that competition or antagonistic interactions lead to decreased mass loss, as suggested in Fukami et al., 2010). Fungi that produce distinct zone lines may be employing the strategy of first protecting the resource that has been colonized before utilizing it at their own pace.

The increase in decay rate observed during co-colonization by genets of the same species for most of the species studied here would appear to be in opposition to the findings of Heilmann-Clausen and Boddy (2005), who reported decreased fungal hyphal extension in the presence of wood colonized by their own species for four different primary wood decay fungal species. However, as they pointed out, their experiment looked at the effect from colonized wood block exudates (DOCs, Diffusible organic compounds) on growth on agar and not on decay rates. It is unknown if a reduction in hyphal extension rate concomitantly reduces actual mass loss. In addition, the colonized wood which was the source of the DOCs was autoclaved post colonization which could have unknown impacts on the DOCs produced, including a shift toward the release of inhibitory compounds (Heilmann-Clausen & Boddy, 2005).

The impact of interactions on mass loss when multiple species and genets are involved in decay was also studied. As a baseline, mass loss in test wafers co-colonized by one genet of each of the five species was determined. This was significantly different from the values of the five genets growing separately for some permutations of genets, but not all, depending on the combination; the mass loss could be either more or less than expected. Test wafers from the one genet of each of five species treatment were mostly homogeneous in appearance after incubation, exhibiting very little zone line production, which might indicate a lack of colonization by multiple species. Only the DNA of *I. lacteus* was

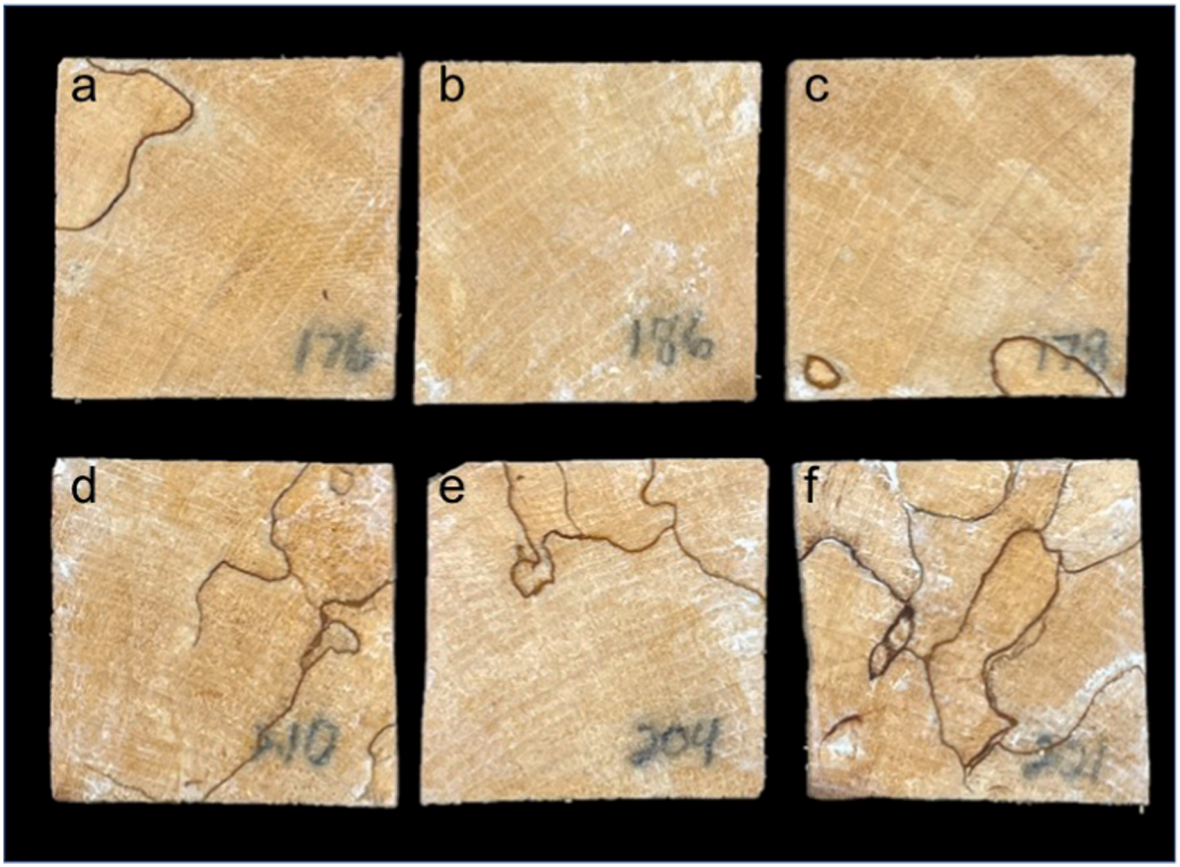


**Fig. 4.** Three representative aspen wafers after 12 weeks of simultaneous colonization by one genet of five fungal species (a-c, fewer zone lines) or 5 genets of 5 fungal species (d-f, more complex zone lines.

recovered from the homogeneous area, even though this species does not decay wood as fast as *T. versicolor* or *F. gilva*. Thus, it appears that *I. lacteus* was able to suppress the growth of the other more aggressive species and become the major component in the decay of these wafers, which were inoculated by only one genet of each species. However, when all 25 genets, five of each species, were employed as inoculum, it appears *I. lacteus* no longer dominates the interaction. These wafers have multiple zone lines and the mass loss is significantly higher than either that observed from the one genet of each of five species treatment or the mass loss of the 25 individuals when grown alone. Increasing the complexity of the community by adding more genets of each species significantly increased the mass loss observed compared to the expected from one genet of each species as well as the expected from all 25 individuals. This may indicate that the more aggressive decayers, *T. versicolor* and *F. gilva*, are dominating these interactions. This finding is in contrast to previous studies that have suggested the decay rates in complex assemblages tend toward those of the slower decaying species (Venugopal et al., 2017; Yang et al., 2016).

One explanation for the dominance of *I. lacteus* in the one genet of each of five species treatment could be the production of VOCs by this species suppressing the growth of the other species. The effect of VOCs on fungal interactions has been well documented (Evans et al., 2008; El Ariebi et al., 2016). The suppression of other species without the formation of zone lines fits this scenario. A similar dominance has been observed when *Flavodon subulatus*, a close relative of *Irpex*, was also observed to suppress other saprotrophic fungi in logs into which it was inoculated (Hulcr et al., 2021; Jusino et al., 2020). It has also been shown that the types of VOCs produced by a fungus is dependent on the fungi it is interacting with (Evans et al., 2008). The lack of dominance of *I. lacteus* in the treatment employing five genets of each of five species is perhaps a result of a shift in VOC production brought about by interacting with members of its own species. Hiscox et al. (2017) reported that adding a third competitor to an otherwise stable paired interaction can lead to unpredictable results due to the intransitivity of fungal interactions resulting in an increase in fungal diversity in colonized wood. A similar pattern was observed in the current study, only in this case the addition of intraspecies individuals seems to be destabilizing otherwise predictable multispecies interactions, resulting in the establishment of a more complex community. Thus, the presence of multiple individuals of the same species may have a large effect on the outcome of interspecies competition.

Fungal community composition in decaying wood substrates has been proposed to be largely determined by interspecies interactions between decay fungi (Boddy 2000; Hiscox and Boddy 2017; Venugopal et al., 2017). While interspecies interactions are undoubtedly one of the main biological drivers, the role of intraspecies interactions may also be an important component. In most cases in the current study, colonization by multiple individuals of the same species led to an increase in decay rate. Adding multiple individuals of each species changed the dynamic of the interspecies interactions and decay outcomes. Whereas *I. lacteus* was dominant among the five species tested in the absence of intraspecies competition, this was no longer the case when other individuals of *I. lacteus* were present. This lack of dominance by *I. lacteus* was also accompanied by a concomitant increase in decay rate when compared to situations where intraspecies competition was absent. It seems likely that in the case of *I. lacteus*, intraspecies competition reduced its ability to compete with other species, although the exact mechanism responsible for the change was impossible to determine at this time. Maynard et al. (2017) asserted that species richness can act as means of maintaining species richness by limiting competitive exclusion by the most dominant species. In the case of the current study, it appears that intraspecies individual richness can fulfill the same role, with more

individuals of *I. lacteus* limiting its ability as a species to dominate the interactions.

This study uses mass loss in test blocks as a metric for determining the effect of intraspecies interactions on wood decay. We have speculated on some of the mechanisms that may have led to the observed results. However, disentangling different potential mechanisms, such as how DOC and VOC production, zone line formation and growth rate interact in solid wood substrates, is beyond the scope of the present study. To better determine the mechanisms that underlie these observations, one would need to be able to track both species as well as individual genets in solid wood substrates. While tracking of individual species could potentially be accomplished via qPCR, few methods exist to track individual genets, and those that do are expensive and time consuming, in addition to being difficult to apply in solid substrates. Advances in these techniques would facilitate research to determine the roles that individual genets play in the breakdown of woody substrates.

This study examined only a limited number of wood-decay species using only a small number of permutations of potential genet interactions. Examining the impact of variable numbers of conspecific genets on large species assemblage outcomes would undoubtedly be more similar to real world situations. Including a more diverse assemblage of fungi, especially brown rot fungi and Ascomycota (including species typically found as endophytes), could be important in determining impacts of intraspecies and interspecies interactions on decay outcomes. Inclusion of brown rot species could be especially critical due to the potential importance of brown rot decay on soil structure and carbon sequestration. In addition, the increase in decay rate observed when co-culturing genets of the species should be further investigated for its potential to improve industrial processes such as bio-pulping and bioremediation.

## 5. Conclusions

Using *in vitro* decay assays, we demonstrated that intraspecific interactions of wood decay fungi have a major impact on mass loss in colonized wood. During interspecific co-colonization assays, we observed that mass loss varied depending on the genets used, even though the assemblage of species was unchanged, thus highlighting the importance of utilizing biological replication in the form of different genets when examining interspecies interactions. We further determined that in mixed species assemblages involving more than one genet of each species, intraspecific interactions can be an important driver of fungal community composition. Mass loss in these complex communities is then indirectly affected by the community structure imposed by intraspecific interactions. Many previous studies have highlighted the critical impact interspecific interactions among wood decay fungi have on determining fungal community composition and thus ultimately decay outcomes. This paper demonstrates that intraspecific interactions can also have outcomes as variable as interspecific interactions. Additionally, intraspecific interactions may change the outcome of interspecific interactions in terms of fungal community composition and subsequently carbon cycling.

## Data availability

The corresponding Sanger sequences for all isolates used in this paper were deposited with NCBI GenBank, accession numbers OP984636-OP984660.

## Declaration of competing interest

The authors declare no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2023.101314.

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