Fungal communities in woodpecker cavities at Pringle Falls Experimental Forest:

Preliminary results from post-treatment
woodpecker surveys and fungal sequencing on Lookout Mountain.

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Pringle Falls Experimental Forest (PFEF) was established in 1931 as a natural laboratory for research on ponderosa pine (*Pinus ponderosa*) management and silvics in the eastern Oregon Cascades. Between 2011 and 2015, thinning and prescribed burning treatments were conducted on Lookout Mountain at PFEF for a project entitled *Forest dynamics after thinning and fuel reduction in dry forests*. The larger goals of this project were to evaluate the short- and long-term effects of thinning and fuel reduction treatments on forest vegetation (Youngblood 2009). To evaluate treatment effects on wildlife Saab and Lehmkuhl (2011) established surveys to measure cavity excavating birds pre- and post-treatment. Surveys focused on white-headed woodpecker (*Leuconotopicus albolarvatus*), which is a species of concern in dry forests of the northwestern U.S.

Pre-treatment surveys were conducted on Lookout Mountain in spring 2011. Only six woodpecker nests were documented in the pre-treatment area and no white-headed woodpecker nests were found within the area to be treated. Thus, a decision was made that post-treatment monitoring on Lookout Mountain should focus on new research questions, if possible. Ideally, monitoring of cavity excavators post-treatment would explore questions of management interest that could be meaningfully examined within a small geographic area.

Currently, biologists lack information on fungi that cause wood decay for woodpecker cavity excavation in western North America. This is because sporocarps needed for fungal species identification are rare (Lorenz et al. 2015) and the genomics techniques for detecting these fungi have only been developed in the last few years (Lindner and Banik 2009, Lindahl et al. 2013, Jusino et al. 2014). Sequencing of wood decay fungi at woodpecker excavations at PFEF would provide valuable information for managers on fungi associated with cavity creation. Measuring vegetation associated with fungal occurrence in different treatments would provide additional insights into management techniques that promote fungal colonization of snags. Such studies could be conducted at smaller spatial scales than studies of woodpecker nest occupancy. Accordingly, a decision was made to sample wood decay fungi at Lookout Mountain in 2017 while also surveying treatments for woodpecker nests. The objectives of this report are to present preliminary results on occurrence of avian cavity excavators post-treatment on Lookout Mountain at PFEF, and fungi sequenced from their nest cavities.

Methods

Study area

The Lookout Mountain unit on the Pringle Falls Experimental Forest is located 45 km (30 miles) southwest of Bend, Oregon, on the Deschutes National Forest. Ponderosa pine is the dominant tree species on most of the Lookout Mountain units, with Douglas-fir (*Pseudotsuga menziesii*), grand fir (*Abies grandis*), white fir (*Abies concolor*), sugar pine (*Pinus lambertiana*), western white pine (*Pinus monticola*), and mountain hemlock (*Tsuga mertensiana*) at higher elevations and on northeast aspects.

Prior to 2011, Lookout Mountain had undergone little major disturbance since about 1845 when a stand-replacement fire resulted in the establishment of the current cohort of trees (Youngblood 2009). In 2011, 2012, and 2013, thinning and fuel reduction was implemented on Lookout Mountain in five treatments: (1) thin from below to 100% Upper Management Zone (UMZ), (2) thin from below to 75% UMZ, (3) thin from below to 50% UMZ, (4) free thin for all tree diameters to 75% UMZ, with 0.1 ha canopy gaps, and (5) no thin (control). All thinning was based on stand density index values for ponderosa pine for the Deschutes National Forest and

was followed by mowing and underburning from 2013-2015. Control units were not mowed or burned. Additional details on treatments are available in Youngblood (2009).

As noted above, pre-treatment surveys for cavity excavating birds in 2011 focused on white-headed woodpecker. Past studies in central Oregon indicated that this species is most common <1600 m in this region (Frenzel 2004) and pre-treatment woodpecker surveys were restricted to elevations below 1646 m on Lookout Mountain (Saab and Lehmkuhl 2011). In 2017 we also restricted our surveys to elevations below 1646 m to enable comparisons with the 2011 survey effort.

Woodpecker surveys

We searched for woodpecker nests during three post-treatment visits to Lookout Mountain on May 10-12, May 25-28, and June 7-9, 2017. We focused nest searches on four woodpecker species: white-headed woodpecker (WHWO), black-backed woodpecker (*Picoides albolarvatus*; BBWO), hairy woodpecker (*Leuconotopicus villosus*; HAWO), and northern flicker (*Colaptes auratus*; NOFL). We noted sapsucker (*Sphyrapicus* spp.) nests when we encountered them but did not actively search for their nests.

To search for nests of focal species we walked transects spaced ~250 m apart throughout all Lookout Mountain units that were surveyed for woodpeckers in 2011. We conducted surveys between sunrise (~0500 hrs) and 1600 hrs. When we encountered territorial or breeding behavior in focal species we paused transect surveys and observed woodpecker behavior until a nest was located or until the bird was lost to view for more than ~30 minutes, at which point we resumed surveying transects. We followed birds off the transect line whenever necessary to track them to nests but did not approach nests until after the nesting season, unless nests were found unexpectedly while walking through the units.

We alternated the order that transects were surveyed each day to ensure that all portions of Lookout Mountain were visited in morning and afternoon hours. Our focal species are typically territorial and nest >300 m from conspecifics. Once we located nests for each species in a particular area, we did not return to nest search again unless breeding behavior of other focal species was detected in the area. On the other hand, other portions of Lookout Mountain in which we detected breeding behavior but did not immediately locate nests were visited repeatedly and nest-searched until a nest was located, or the breeding season ended.

Woodpeckers may initiate excavation at many cavities in a breeding season, but only one cavity is eventually completed and used for laying eggs and raising young. We omitted cavity starts from sampling. We considered cavities complete if adults were observed excavating a cavity that was large enough for them enter fully, or nestlings were observed inside cavities at a later date. We did not use cavity inspection cameras to view nest contents and confirm egg laying because inspection cameras are associated with decreased nest survival (Newlon and Saab 2011). During vegetation sampling in August and September 2017, we measured cavity depth and confirmed that all sampled cavities were indeed complete excavations.

Vegetation sampling

In August and September 2017 and after the woodpecker nesting season, we returned to nests to measure vegetation characteristics. We restricted vegetation sampling (and fungal sampling, described below) to nests in pines. For safety reasons, we also restricted our sampling to nests that could be sampled from the ground or from climbing ladders and in which the nest

snag appeared stable. We omitted nests from sampling if they were too high to be accessed by ladders (n = 3) or if the snag appeared unstable while setting up a climbing ladder (n = 2).

At all accessible snags we measured cavity and tree height with a clinometer and snag diameter at breast height (DBH) with a DBH tape (see protocols, Appendix 1). We noted the presence of visible fungal fruiting bodies (sporocarps) and woodpecker foraging evidence on the bole of nest snags. We also visually estimated the proportion of limbs, branches, bark, and top missing from each snag and the proportion of each bole that was blackened with fire. We non-destructively measured wood hardness at each nest following Matsuoka (2000) and Lorenz et al. (2015). We measured surrounding tree and snag density (trees/ha) and canopy cover (with a spherical densiometer) and visually estimated the shrub and woody debris cover on the ground surrounding each nest snag. We also measured cavity sill width, cavity horizontal depth, and cavity vertical depth with a ruler (Appendix 1).

For making comparisons with unexcavated snags, we paired each nest snag with a randomly selected 'control' snag within 75 m. Control snags matched attributes of nest snags but lacked cavities (Appendix 1). We measured all of the aforementioned features at control snags, except cavity width and depth (because control snags lacked cavities). For comparison with nests, we measured wood hardness on control snags at the same height and orientation as its paired cavity. We marked each nest and paired control snag with a tree tag placed in a nearby stump or log and took a series of nine photos at each site (Appendix 2). We compared attributes of nest and control snags using paired sample t-tests and considered attributes different at α = 0.05.

Fungal sampling and sequencing

For sequencing of wood decay fungi, we aseptically collected a sample of wood from all nest and control snags included in vegetation sampling. We first collected a sample of wood shavings from inside nest cavities with a sterile sharpened spoon following Jusino et al. (2014). We used the spoon to scrape inside the cavity above the nest chamber, a region also called the cavity dome (Jusino et al. 2014). Second, we used a sterile 9 mm wood drill bit to aseptically collect wood shavings from the bole of each nest snag 2-5 cm above the nest cavity opening. This sample was collected by drilling horizontally into each nest snag with a cordless drill, with the drill bit parallel to the ground. We collected two wood samples from this region. The first sample contained wood extracted as we drilled from the bark surface to ~3 cm deep. The second sample was collected from wood extracted as we drilled from 3-10 cm deep. We kept these samples separate (and sterilized between samples). Our first sample (0-3 cm deep) simulated sill or cavity entrance wood. Our second sample (3-10 cm deep) represented wood in the nest body region, or cavity chamber wood.

Our third sample was collected from the bole of control snags. As noted above, control snags matched attributes of nest snags but lacked cavities. As a result, we could not collect a spoon scraping from control snags. For comparison with nests we sampled wood from control snags at the same height and orientation as its paired nest using the sterile wood drill bit. Spoons and drill bits were sterilized in the field between every sample using a 20% sodium hypochlorite bath, isopropyl spray, and flame sterilization.

We transferred shavings from nest cavities (obtained via spoon scraping) immediately to sterile 1.5 mL tubes containing sterile cell lysis solution (CLS; Lindner and Banik 2009). Tubes were placed on ice until returning to the field station, where they were frozen at -18° C. Shavings from drill samples transferred immediately to sterile 50 mL tubes and placed on ice. Upon

returning to the field station each day, drill samples were sterilely transferred to CLS in 1.5 mL tubes (protocols for wood sampling and storage are in Appendix 1). All samples were then maintained at -18° C until DNA extraction, polymerase chain reaction (PCR), and sequencing in September and October 2017.

To identify fungal taxa at nests and paired control snags we used ion torrent semiconductor sequencing with 400 base-pair technology. We included a custom mock community control in every run to parameterize downstream bioinformatics, which were run using AMPtk (https://github.com/nextgenusfs/amptk). Identification of fungal taxa was carried out to the furthest extent possible based on sequences in GenBank (NCBI) and UNITE (Kõljalq et al. 2005). To compare species composition, we used nonparametric multidimensional scaling (NMDS) in the Vegan package of R (Oksanen et al. 2012) to plot and visualize differences in fungal community composition. We used nonparametric permutational multivariate ANOVA (PERMANOVA) tests (Anderson 2001) to test for significant differences in fungi communities as a function of sampling source (cavity spoon scraping, nest snag bole sample, or control snag bole sample).

Results

Woodpecker surveys

We surveyed 30 km of transects for woodpeckers and located 35 nests within the treated Lookout Mountain units (Figure 1). The most common species nesting in treated units were hairy woodpecker (n = 13 nests) and white-headed woodpecker (n = 10 nests), followed by black-backed woodpecker (n = 5 nests) and northern flicker (n = 5 nests; Appendix 3, 4). There were two additional locations on the Lookout Mountain units where we observed breeding behavior of white-headed woodpeckers but did not locate a nest. We suspect in these two cases white-headed woodpeckers attempted to nest but we were unable to find it before nest failure.

Vegetation surveys

Nests occurred in all harvest treatments but no nests of focal species were found in control units (Table 1, Figure 1). Most nests were excavated in ponderosa pine snags (80%) and easily accessible by climbing ladders (80% of nest cavities were <5 m high). Median cavity height was 2.7 m (mean 3.5 m; Table 2). Diameter of nest snags averaged 50.5 cm and was similar to control snags ($\bar{x}$ = 57.4 cm; t_{23} = 1.22, P = 0.2332). The only vegetation attribute that differed between nest and control sites was hardness of wood within the tree's interior, measured 3-10 cm inside the bole (Table 2; t_{23} = 9.55, P < 0.0001). Average hardness of wood at nests was 1.7 nm compared to 6.2 nm for control sites.

Fungal sampling and sequencing

We sequenced 840 fungal taxa from our samples. Fungal communities differed between samples taken from woodpecker cavities, the bole of the nest snags, and control snags (Figure 2; r^2 = 0.35, pseudo-F = 18.4, P < 0.0001). Fungal communities were most diverse in cavity samples with a median of 59 fungal taxa detected per cavity (and 336 total taxa unique to cavity samples), compared to a median of 13 for boles of nest snags (with 43 unique taxa) and 32 for control snags (with 123 unique taxa). The most commonly occurring taxa within excavated cavities were not wood decay fungi, but rather fungi in class *Eurotiomycetes* (found in 75% of cavities) and *Cladosporium cladosporioides* (found in 71% of cavities; Table 3). Half of cavities (54%) contained *Rhizopogon* spp., an ectomycorrhizal symbiont of conifers that may have been

brought into cavities by rodents. These taxa were not present in samples from the bole of nest or control snags.

The wood decay fungus *Fomitopsis pinicola* was the most prevalent wood decay fungus sequenced from woodpecker nests (found in 58% nests, and in both cavity and bole samples). Nest snags containing *F. pinicola* were taller ($\bar{x}$ = 24 m) than those lacking *F. pinicola* ($\bar{x}$ = 6.7 m; t_{22} = 3.15; P = 0.0046) and typically had intact tops. A t-test for differences in snag diameter was marginally non-significant (t_{22} = 1.82, P = 0.0829; $\bar{x}$ = 56 cm for *Fomitopsis*-infected nests and $\bar{x}$ = 42 cm for non-*Fomitopsis* snags) indicating a slight trend for *F. pinicola* to be present in larger snags. There was evidence that nest snags containing *F. pinicola* were spatially clustered at Lookout Mountain based on a post-hoc Ripley's K-function (Figure 3).

Fomitopsis-infected nest snags had lower fungal diversity than snags lacking *F. pinicola*. Median number of fungal taxa in *Fomitopsis*-infected nests was 6 (mean 12), compared to a mean of 25 taxa for samples not containing *Fomitopsis*. Compared to its prevalence in nest snags, *F. pinicola* was sequenced from only two control trees (8%). Both of these control trees were within 200 m of a nest snag containing *F. pinicola*, further indicating spatial clustering of this taxa on Lookout Mountain.

Discussion

Our post-treatment surveys revealed many more woodpecker nests post-treatment compared to pre-treatment. Pre-treatment surveys conducted in 2011 located only 6 nests for all cavity excavators within the treatment area, and no white-headed woodpecker nests. During post-treatment surveys we found 35 active nests across all species and 10 nests of white-headed woodpecker. This represents a five-fold increase in woodpecker nest occurrence. While formal hypothesis tests are not possible due to small sample sizes and pseudoreplication in study design (treatments were only conducted within the Lookout Mountain unit at PFEF), these results suggest that cavity excavators responded positively to treatments at PFEF.

Nests for all focal woodpecker species were more numerous post-treatment. Considered together, hairy woodpecker and northern flicker nests increased from 5 to 17, while white-headed and black-backed woodpecker nests were only found post-treatment. These focal species represented both generalist and specialist guilds and forage on a variety of substrates – northern flicker and hairy woodpecker are habitat generalists that commonly forage on the ground (NOFL) or in dead wood (HAWO), while black-backed and white-headed woodpecker are habitat specialists that forage predominately on dead wood (BBWO, WHWO) and by bark and foliage gleaning (WHWO) (Murphy and Lehnhausen 1988, Elchuk and Wiebe 2003, Lorenz et al. 2016). These woodpeckers are alike mostly in their use of snags for nesting. Many studies have shown they are attracted to post-fire habitats during the breeding season, presumably because burned forests contain high densities of snags (Raphael and White 1984, Saab and Dudley 1998, Tarbill et al. 2015). Thus it is likely that woodpeckers were attracted to treatments on Lookout Mountain due to the availability of snags from burning the units.

In western coniferous forests, research suggests that woodpeckers select snags for nesting because snags are more likely to contain decayed wood for cavity excavation compared to live trees. This is true even for snags that lack visible signs of decay or fungal sporocarps (Lorenz et al. 2015). Fungal fruiting bodies are rare on woodpecker nest snags in the northwest, and therefore the wood decay fungi that cause wood softening for cavity excavation not known with certainty. This is the first study to use genomic techniques to identify wood decay fungi at woodpecker nests in Oregon. We found *Fomitopsis pinicola* was most prevalent fungal taxa

sequenced from nests, present in 58% of nest snags. In contrast to nests, *F. pinicola* was present in only two control trees (9%).

F. pinicola is a common wood decay fungus world-wide. It is classified as a brown rot fungus meaning it primarily consumes cellulose and hemicellulose in wood, rather than lignin (Rayner and Boddy 1988, Pelaez et al. 1995). It contains antimicrobial and antifungal abilities that can suppress growth of competitors on dead wood (Keller et al. 1996, Guler et al. 2009. Dresch et al. 2015) even in hot and dry environments, such as during extreme heat characteristic of forest fires (Carlsson et al. 2014). In addition to being a strong competitor, *F. pinicola* is considered a generalist species and predominately colonizes dead trees (Rayner and Boddy 1988).

In several past studies *F. pinicola* has been used as a means to artificially create woodpecker nest snags via artificial inoculation (Bull and Partridge 1986, Brandeis et al. 2002, Bednarz et al. 2013). In all of these studies, snag inoculation with *F. pinicola* has largely failed to create woodpeckers nest snags. For example, Bednarz et al. (2013) and Bull and Partridge (1986) collectively inoculated 430 trees with *F. pinicola* in the northwest to encourage cavity excavation but found no woodpecker nest cavities in these snags five to nine years later. In contrast, at PFEF snags infected with *F. pinicola* were excavated by woodpeckers two to four years after tree death. There are many possible reasons why past artificial inoculation efforts have failed to attract woodpecker cavity excavations. *F. pinicola* may not be an important decayer, even at PFEF. As noted above, the presence of fungal DNA in our samples provides no information on the decay activities of a given taxa. Our sequencing only provides information on presence and absence of fungal taxa. It is possible other fungi were responsible for wood softening in PFEF snags. Another hypothesis is that in past studies, priority effects in artificially inoculated snags prevented *F. pinicola* from becoming established and decomposing wood.

It is also possible that *F. pinicola* is an important decayer only under limited conditions. At PFEF we found *F. pinicola* predominately in larger ponderosa pine snags killed as a result of treatment activities. Nests in smaller diameter snags, broken topped snags, and cut stumps (indicative of tree death prior to treatments) did not contain *F. pinicola*. Thus, *F. pinicola* was limited to a subset of snags with fairly unique characteristics at PFEF. Moreover, snags infected with *F. pinicola* at PFEF were spatially clustered, indicating pockets of infection, rather than widespread occurrence of this fungus on Lookout Mountain. It is also noteworthy that during concurrent sampling of both ponderosa pine and Douglas-fir snags in central Washington, *F. pinicola* has been detected in <10% of 49 sampled nest snags (unpublished data). Nest snags are smaller on average on these Washington sites, providing support for the idea that *F. pinicola* is more influential in decay of large snags. But even large ponderosa pine snags in Washington have lacked *F. pinicola*. Overall, we conclude that while *F. pinicola* was common in nests excavated in large ponderosa pine snags at PFEF, it is not necessarily an important decay fungus (for woodpecker excavations) throughout the Pacific Northwest.

In an effort to resolve some of these uncertainties, we are planning additional analyses of wood samples collected at PFEF. Tests of cellulobiase activity have been conducted by collaborators at Yakima Valley College following methods of Garcia et al. (2016) (J. Seveyka, pers. comm.) and tests of Dilute Alkali Solubility (DAS; Schilling et al. 2015) is planned by collaborators at the University of Minnesota in winter 2018 (J. Schilling, pers. comm.). After analyzing results from these tests, we hope to have information on dominant rot type in each sample, providing insights into decay pathways and taxa causing decay. Also, sequencing of 146 additional samples from sites in central Washington in winter 2018 will shed light on the

prevalence of all fungal taxa across a larger geographic area. Our conclusions from this preliminary report may be modified as this new data becomes available.

Appendices

Appendix 1. Protocols and datasheets used for vegetation surveys and fungal sampling at Lookout Mountain, Pringle Falls Experimental Forest, Oregon, in 2017.

Appendix 2. Photos of plots from nest and control snags sampled for fungi at Lookout Mountain, Pringle Falls Experimental Forest, Oregon, in August and September 2017. Nine photos were taken at each sampled nest and control snag: one photo of the snag's tree tag marker, one photo each taken 1 m from base of snag in north, east, south, and west directions, and then one photo each taken 10 m from nest snag base, looking back at snag from north, east, south, and west directions. See appendix 1 for field protocol.

Appendix 3. Nest cards compiled during nest searches for woodpeckers on Lookout Mountain, Pringle Falls Experimental Forest, Oregon, in May and June 2017. Note coordinates often differ from coordinates in Appendix 4 because nest locations were typically recorded from a distance during nest searches. During sampling in August and September 2017, nests selected for fungal sampling had a new and more accurate coordinate taken at the nest snag, available in Appendix 4.

Appendix 4. Summary of site and vegetation characteristics at woodpecker nest and control snags at Lookout Mountain, Pringle Falls Experimental Forest, Oregon, in 2017.

Contributions

This report was compiled by T.J. Lorenz, U.S. Forest Service, Pacific Northwest Research Station. Sequencing was conducted by M.A. Jusino with support from D.L. Lindner and M.T. Banik at the U.S. Forest Service, Center for Forest Mycology Research, Northern Research Station. The study was designed by T.J. Lorenz with input from M.A. Jusino. Nest searching was conducted by T. Lorenz with assistance from P.C. Fischer. Fungal sampling and vegetation surveys were conducted by N. Weprin, B. Drahota, J. McLeod, and S. Brehm. P.D. Anderson was influential in securing funding for field work, which was provided by the U.S. Forest Service, Pacific Northwest Research Station.

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Table 1. Occurrence of nests for all focal woodpecker species, and white-headed woodpecker specifically, by treatment year and type at Lookout Mountain on Pringle Falls Experimental Forest, Oregon, in 2017. Focal species were white-headed woodpecker, hairy woodpecker, black-backed woodpecker, and northern flicker.

Treatment year (burn/thin)	Number (proportion) of nests (all focal species)	Number of white-headed woodpecker nests	Area surveyed (ha)[1]
2013/2011	10 (0.30)	4	199
2014/2012	6 (0.18)	2	165
2015/2013	17 (0.52)	4	310
Control (no burn/thin)	0 (0.00)	0	111

Treatment type	Number (proportion) of nests (all focal species)	Number of white-headed woodpecker nests	Area surveyed (ha)[1]
100 UMZ	8 (0.24)	1	221
50 UMZ	7 (0.21)	3	184
75 UMZ	8 (0.24)	2	158
75 UMZ/canopy gaps	10 (0.30)	4	111
Control (no treatment)	0 (0.00)	0	111

[1] Estimate of area within each treatment type surveyed for focal species. This is not equivalent to the total area treated within each unit or treatment type at Lookout Mountain.

Table 2. Mean (±standard deviation) of site characteristics for woodpecker nest snags compared to control snags (that lacked woodpecker cavities) at Lookout Mountain, Pringle Falls Experimental Forest, Oregon, in 2017.

	Nest snags (n = 24)	Control snags (n = 24)
DBH (cm)	50.5 (±18.8)	57.4 (±28.5)
Cavity height (m)	3.5 (±2.8)[1]	na
Snag height (m)[2]	15.5 (±14.2)[1]	26.3 (±11.6)
Height of char on bole (m)	8.4 (6.7)	5.2 (±6.0)
Cavity wood hardness (newton meters)[2]	1.7 (±0.8)	6.2 (±2.2)
Cavity 'sill' wood hardness (newton meters)	3.9 (±2.7)	3.1 (±1.8)
Percent canopy cover	17.2 (±11.6)	17.5 (±12.3)
Percent large woody debris cover on ground	6.6 (±6.0)	3.7 (±3.3)
Percent small woody debris cover on ground	10.2 (±7.6)	7.5 (±7.7)
Percent shrub cover	31.3 (±24.9)	29.0 (23.7)
Number of snags with conks present on bole	7	7
Number of snags with woodpecker forage sign on bole	24	22

[1]Cavity and tree height were estimated for all 35 nest sites; other characteristics in this table were only measured at the 24 trees also sampled for fungi.

[2] Indicates this characteristic differed at $\alpha < 0.05$ in a paired-sample t-test.

Table 3. The most prevalent fungal taxa (present in at least 40% of samples) sequenced from the bole of nest snags (n = 24), inside woodpecker nest cavities (n = 24), and from the bole of control snags (snags lacking woodpecker excavations; n = 24) at Lookout Mountain, on Pringle Falls Experimental Forest, Oregon, in 2017.

Sequence identities, percent similarities, accession number, and classification of fungal operational taxonomic units (OTUs)	**Proportion of sites containing each taxa**
Samples from bole of nest snags, for snags not containing *Fomitopsis pinicola*	
GS|99.3|KM493073;k:Fungi,p:Basidiomycota,c:Microbotryomycetes,o:Sporidiobolales	0.50
US|0.9368|KM493531;k:Fungi,p:Basidiomycota,c:Microbotryomycetes,o:Sporidiobolales	0.50
GS|99.2|KC171331;k:Fungi,p:Basidiomycota,c:Tremellomycetes,o:Tremellales,f:Tremellaceae,g:Cryptococcus	0.50
GS|99.6|KX100391;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,f:Vibrisseaceae,g:Phialocephala	0.40
GS|99.7|KM493763;k:Fungi,p:Basidiomycota,c:Cystobasidiomycetes	0.40
GS|97.0|KM494067;k:Fungi,p:Basidiomycota,c:Tremellomycetes,o:Tremellales	0.40
Samples from bole of nest snags, for snags containing *Fomitopsis pinicola*	
GS|100.0|EU218884;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Polyporales,f:Fomitopsidaceae	1.00
Samples from inside woodpecker nest cavities	
GS|100.0|KT220144;k:Fungi,p:Ascomycota,c:Eurotiomycetes	0.75
GS|100.0|JQ936096;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Capnodiales,f:Cladosporiaceae,g:Cladosporium,s:Cladosporium cladosporioides	0.71
GS|100.0|JF749180;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Capnodiales	0.63
GS|100.0|EU218884;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Polyporales,f:Fomitopsidaceae	0.58
GS|100.0|KU134841;k:Fungi,p:Ascomycota,c:Taphrinomycetes,o:Taphrinales,f:Protomycetaceae,g:Saitoella,s:Saitoella complicata	0.54
GS|100.0|KP152486;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Dothideales,f:Dothioraceae,g:Sydowia,s:Sydowia polyspora	0.54
GS|98.8|EU552113;k:Fungi,p:Ascomycota,g:Coniozyma,s:Coniozyma leucospermi	0.54
GS|99.6|KT219601;k:Fungi,p:Ascomycota,c:Leotiomycetes,f:Myxotrichaceae	0.54
GS|100.0|HM123665;k:Fungi,p:Ascomycota,c:Sordariomycetes	0.50
GS|98.3|KP411571;k:Fungi,p:Ascomycota,c:Leotiomycetes,f:Myxotrichaceae,g:Pseudogymnoascus,s:Pseudogymnoascus pannorum	0.50
GS|100.0|GU083211;k:Fungi,p:Ascomycota,c:Leotiomycetes,f:Myxotrichaceae,g:Pseudogymnoascus	0.50
US|0.8142|KP891819;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales	0.46
GS|100.0|JX136606;k:Fungi,p:Ascomycota,c:Dothideomycetes	0.46
US|0.8271|KM056294;k:Fungi,p:Ascomycota	0.46
GS|99.2|UDB020417;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Penicillium	0.46
GS|100.0|KY102338;k:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,g:Candida,s:Candida ponderosae	0.46
US|0.8853|KU062445;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Capnodiales,f:Mycosphaerellaceae	0.46
US|0.8474|KP891704;k:Fungi,p:Ascomycota	0.46
SS|1.0000|KF983527;k:Fungi	0.46
GS|100.0|HQ914255;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Boletales,f:Rhizopogonaceae,g:Rhizopogon,s:Rhizopogon salebrosus	0.46
GS|100.0|LC203712;k:Fungi,p:Basidiomycota,c:Tremellomycetes,o:Tremellales,f:Tremellaceae,g:Naganishia,s:Naganishia friedmannii	0.42
US|0.8255|KU062190;k:Fungi,p:Ascomycota,c:Leotiomycetes	0.42
GS|99.6|KT692578;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,f:Sclerotiniaceae,g:Botrytis,s:Botrytis cinerea	0.42
GS|100.0|KY103298;k:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,g:Debaryomyces,s:Debaryomyces vindobonensis	0.42
Samples from control snags	
US|0.9387|KU164637;k:Fungi,p:Basidiomycota	0.46
GS|97.9|HM595536;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,f:Hyaloscyphaceae,g:Lachnellula	0.42
US|0.8078|KY105766;k:Fungi,p:Basidiomycota,c:Microbotryomycetes	0.42

Figure 1. Locations of woodpecker transects and nests at Lookout Mountain, Pringle Falls Experimental Forest, Oregon, in spring 2017. Nests with a white outline were sampled for fungi in fall 2017, and red circles indicate white-headed woodpecker (WHWO) nests, yellow circles indicate black-backed woodpecker (BBWO) nests, blue circles indicate hairy woodpecker (HAWO) nests, brown circles indicate northern flicker (NOFL) nests, and black circles indicate nests of Williamson's and red-breasted sapsucker. Coordinates of nest locations are in Appendix 4. Treatments included control units (no thin or burn) and three levels of thinning: 50% Upper Management Zone (UMZ; or Upper Density Limit, Cochran et al. 1994) thin, 75% UMZ thin, and 75% UMZ thin with gaps in the canopy. All thin treatments were followed by prescribed burning two years after thinning.

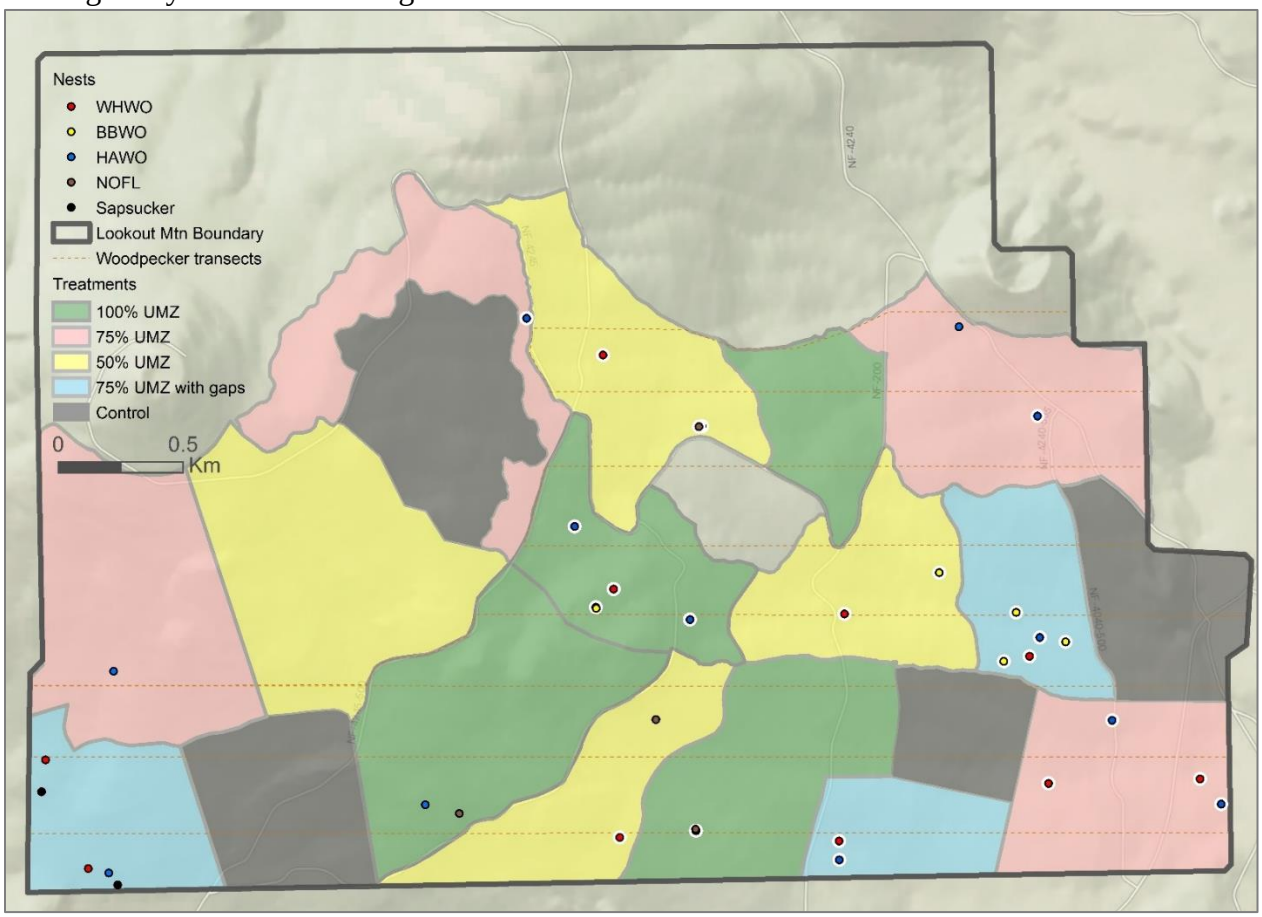

Figure 2. Nonparametric Multidimensional Scaling (NMDS) ordinations of the communities of fungi sequenced from within woodpecker cavities (NT cavity), from the bole of woodpecker nest trees (NT drill), and from control snags not excavated by woodpeckers (CT drill). The dots in the center represent the means of the points on the two NMDS axes, and the bars represent one standard error from the mean.

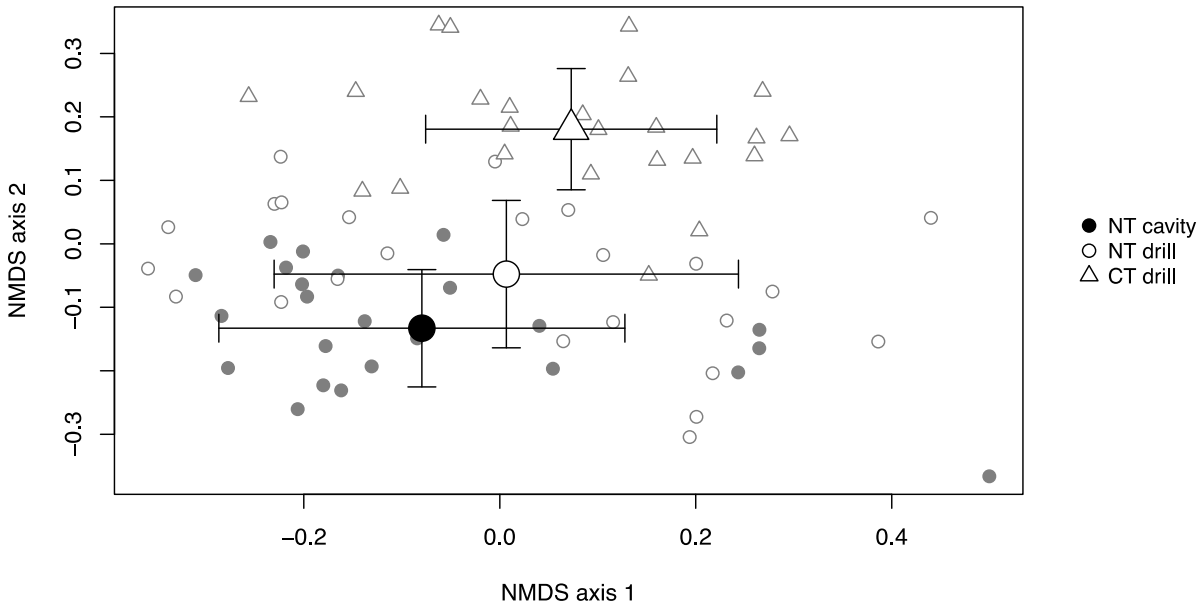

Figure 3. Plot of Ripley's K function for analysis of spatial clustering of woodpecker nests containing *Fomitopsis pinicola* at Lookout Mountain, Oregon, with confidence envelope from 99 permutations. Observed K that is greater than the confidence envelope indicates significant spatial clustering at the associated distance.