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### Note

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Turning an invasive hardwood into an asset: Inoculating *Ligustrum lucidum* logs with a medicinal mushroom, *Trametes versicolor*, accelerates wood decomposition under field conditions

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#### Ahstract

Glossy privet (Ligustrum lucidum W. T. Aiton) is a highly aggressive tree that has become globally invasive in a wide range of habitats and can quickly form dense thickets, shading and outcompeting native vegetation. Slowly decomposing slash following removal of dense infestations can create additional management challenges, including fire risk concerns, which curtailed planned invasive plant removal projects on certain tracts within the Balcones Canyonlands Preserve near Austin, TX. This prompted a pilot study to explore whether wood-decaying fungi could be used to hasten the recycling of L. lucidum logs back into the forest ecosystem. We inoculated 25 freshly cut L. lucidum logs with Trametes versicolor (Fr.) Pilat at two study sites and monitored the inoculated logs and 5 untreated control logs over a 3-yr period (February 2015 to March 2018). We found that inoculation significantly accelerated wood decay. By the end of our study, 100% of logs with >3 inoculation points were in advanced stages of decay, while only one of the control logs showed noticeable signs of decay. Inoculating logs in the field was easy and suitable for novices, requiring little or no previous experience to achieve successful results. An added benefit of using T. versicolor is its medicinal properties and potential for bioremediation. We have continued to inoculate logs with comparable success and are integrating them into forest restoration projects. Applied on a larger scale, these wooddecaying fungi have the potential to transform nonnative deadwood from a threat to an asset.

### Introduction

Glossy privet (*Ligustrum lucidum* W. T. Aiton) is a fast-growing evergreen shrub or tree native to China, Japan, and Korea (Maddox et al. 2010). Introduced as an ornamental and hedge plant, this invasive species occupies a wide range of habitats, including open woodlands, closed-canopy forests, grasslands, riparian areas, rain forests, and waste areas. It can easily escape cultivation and has been recorded on every continent except Antarctica (CABI 2018). Highly aggressive, it spreads by animal-dispersed seeds, particularly birds, as well as by sprouts from roots and stumps. As a result, *L. lucidum* can form dense, impenetrable monocultures, replacing native trees and reducing biodiversity if not controlled (CABI et al. 2018; Coutts-Smith and Downey 2006; Global Invasive Species Database 2018). *Ligustrum lucidum* invasions have been shown to alter below- and aboveground communities, having effects such as lower total seed density and richness in the soil seedbank (Ferreras et al. 2015), suppression of native tree recruitment (Lichstein et al. 2004), reduced understory cover (Hoyos et al. 2010), more homogenous vertical structure, increased canopy cover, lower litter depth, and declining bird species abundance, richness, and diversity (Ayup et al. 2014).

Recommended management of heavy *L. lucidum* infestations includes gradual removal combined with replacement with native plants (Johnson 2009). The resulting deadwood can be slow to degrade, creating additional management challenges. To address this issue, slash may be transported offsite or piled and burned to reduce wildfire risks. Pile burning can cause long-term soil damage (Page-Dumroese et al. 2017) and persistent canopy gaps (Rhoades and Fornwalt 2015). Page-Dumroese et al. (2017) provide alternative methods of burning forest residues to create biochar for use as a soil amendment and to sequester carbon. All of these methods remove biomass from the ecosystem, which can be disadvantageous, particularly for areas that have previously experienced significant biomass losses (e.g., due to land clearing). Alternatively, mechanical mulching and chainsaw felling of the closely related Chinese privet (*Ligustrum sinense* Lour.), with residues left onsite, have been shown to be effective strategies to increase percent cover, species richness, diversity, and evenness of herbaceous plants (Hudson et al. 2014).

### **Management Implications**

We found that inoculating freshly cut *Ligustrum lucidum* (glossy privet) logs with *Trametes versicolor* (turkey tail) is an easy and effective method for expediting wood decomposition and treating slash generated from removal of invasive hardwoods. Slash is typically left to decompose on its own, transported offsite, piled and burned, or mulched. We offer an alternative approach that accelerates in situ recycling of the woody debris. Inoculating logs with a native wood-decaying fungus allows the biomass to remain in the ecosystem and provide ecological services while reducing fire risk concerns. Inoculated logs can in turn be used to assist with habitat restoration, including erosion control, water retention, and seedling establishment.

While the plant communities were similar 5 yr after treatment, mulched plots had higher percent cover and number of plants than hand-felled plots (Hudson et al. 2014). However, mulching may not be feasible due to costs and/or inaccessibility. Further, Marcot (2017b) recommends "large chunks" of decaying wood to provide the "most beneficial, long-term ('time-released') ecological services."

In areas where hand felling is the preferred option, selective introduction of wood-destroying fungi can be used to accelerate decomposition of the debris (Pavlík et al. 2008; Pavlík and Pavlík 2013). Saprotrophic fungi are primary recyclers that break down surface detritus on the forest floor, where it then becomes accessible to other organisms (Arnstadt et al. 2016; Marcot 2017a). The process of wood decomposition contributes to soil development and productivity, nutrient cycling, moisture retention, and forest regeneration (Bednarz et al. 2013; Lonsdale et al. 2008; Marcot 2017b; Pavlík et al. 2008; Piškur et al. 2011). Turkey tail (Trametes versicolor Pilat) is a saprotrophic fungus with a circumpolar distribution (Lepp 2012; Stamets 2000; Veena and Pandey 2012). It is a nonparasitic but aggressive decomposer of dead wood, growing mainly on hardwoods and infrequently on conifers (Cotter 2014). A white rot fungus, T. versicolor is among the only organisms known to completely digest lignin (van der Wal et al. 2015), with the resulting wood becoming soft and spongy with a whitish color (Gao et al. 2010). Trametes versicolor also has welldocumented medicinal properties, which include antitumor, antimicrobial, immune-enhancing, antioxidant, and antiradical compounds (Chu et al. 2002; Hobbs 2004; Janjušević et al. 2017), as well as the ability to degrade several organic pollutants (Gao et al. 2010) and herbicides (da Silva Coelho-Moreira et al. 2013).

The Balcones Canyonlands Preserve near Austin, TX, is managed to conserve its diverse ecosystems, which support rare and endangered songbirds, karst invertebrates, and plants. Management activities focus on promoting healthy forests, from the forest floor to the tree canopy. Due to the rapid growth of *L. lucidum*, this invasive hardwood can quickly form dense stands, particularly along riparian corridors, which pose a threat to these endangered habitats. Thus, removal of *L. lucidum* and other nonnative, invasive plant species is a critical component of preserve management (Balcones Canyonlands Preserve 2007). At the same time, wildfires that occurred during a severe drought in 2011 have increased concerns about wildfire risks and curtailed planned invasive plant removal in areas where slash could not be easily removed or mulched. To explore a possible solution to this dilemma, we conducted a 3-yr pilot study to evaluate whether inoculating

*L. lucidum* logs with a native strain of *T. versicolor* would accelerate wood decomposition and present a viable option for treating slash of an invasive woody species.

### **Materials and Methods**

### Study Area

We conducted our study from February 27, 2015, to March 5, 2018, on two tracts within the Balcones Canyonlands Preserve (30.40°N, 97.86°W), located on the eastern Edwards Plateau west of Austin, TX, USA. This region is characterized by shallow soils over Cretaceous limestone plateaus dissected by steep canyons and has a subtropical subhumid climate. We chose two forested riparian study sites (Site 1, Site 2) that were ~6 km apart, both of which were dominated by mature Ashe juniper (Juniperus ashei J. Buchholz) and oak (Quercus spp.) trees on gentle east-facing slopes at elevations of ~230 to 260 m above sea level and <100 m from an intermittent creek. Total annual rainfall amounts for the Austin area during the years of our study were 1,523 mm (2015), 987 mm (2016), and 882 (2017), and mean annual temperatures ranged from 21.2 to 22.4 C, with a low of 9.4 C in January to a high of 31.8 C in July (Camp Mabry, Weather Underground 2015-2017). We chose sites 1 and 2 because of their similar environments, proximity of dense L. lucidum stands, and accessibility. Site 2 also has a team of volunteers who were able to assist with log inoculations and monitoring.

### Preparation of Trametes versicolor Inoculum

Although T. versicolor is ubiquitous in North America and many parts of the world, we chose to clone a local subvariety of T. versicolor to create our spawn for inoculation. This was done to ensure that the mycelium had already adapted to and proliferated in the climate of our study sites. Furthermore, the *T. versicolor* cloning sample was harvested specifically from a dead L. lucidum stump to ensure that T. versicolor would grow on the intended wood type. We used standard cloning procedures typical in commercial mushroom production (Stamets 2000). The sample was found growing on a dead L. lucidum stump in October 2014 and brought into the lab for cloning on the same day. Exterior debris was removed from the sample, and the sample was then placed in the steam of a 99.99% efficiency laminar flow hood, where it was manually split to expose the fleshy interior of the fruit body. Using sterile equipment, we extracted three grain-sized flakes of tissue with a surgical scalpel from the interior of the fruit body near its base and placed them on three separate sterile agar petri dishes. The dishes were sealed with Parafilm® tape; labeled A, B, and C; and placed in a dark room at 24 C for 72 h to allow the mycelium to begin growing on the surface of the agar. It is typical that, in the first tissue transfer from the wild harvest samples, other contaminants also will be present growing on the agar. Pure T. versicolor mycelium was isolated away from these contaminants by excising another grain-sized portion of mycelium from the leading edge of mycelial growth on the original agar dish 3 d later. Seventy-two hours after the original transfer, and with mycelial growth visually present on all three agar dishes, the dishes were returned to the laminar flow hood. The leading edge of mycelium was transferred from each of the three dishes onto three new sterilized agar dishes. Five days later all three dishes showed mycelial growth without signs of contamination from other organisms. The dish with the fastest mycelial growth (dish B) was chosen to continue for spawn production.

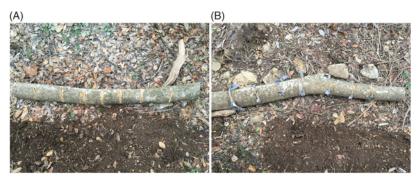


Figure 1. Inoculation of Ligustrum lucidum logs with Trametes versicolor at Site 1, Balcones Canyonlands Preserve, Austin, TX, February 27, 2015. (A) A log with 7 shallow cuts made using a circular saw; (B) a log after cuts were inoculated with sawdust spawn and covered with wet newspaper.

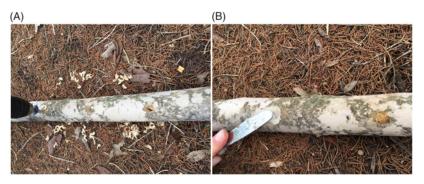


Figure 2. Inoculation of freshly cut Ligustrum lucidum log with Trametes versicolor at Site 2, Balcones Canyonlands Preserve, Austin, TX, March 15, 2015. (A) A log with holes drilled using a 12-mm drill bit with stop; (B) the holes filled with sawdust spawn and being covered with plug wax.

Roughly 1.4 L of sorghum [Sorghum bicolor (L.) Moench] grain was left to hydrate 12 h in tap water, drained for 45 min, and divided equally among four 1-L heat-resistant glass jars that were then capped with synthetic filter disks (99.97% filtration down to 0.3 microns). The four jars were sterilized in a pressure cooker for 2 h at 103.4 kPa (~121 C). The pressure cooker was left to cool for 24 h and was then opened in front of a laminar flow hood, and the jars were placed in the sterile stream of air. Four separate 1 cm by 1 cm mycelial patches were cut from dish B, following sterile protocol, with one mycelial patch being placed in each of the four sterilized grain jars. The filter disks were then replaced. The jars were placed in a dark 24 C room for 10 d to allow the mycelium to colonize the grain. The jars were shaken by hand on days 2, 5, and 7 to speed colonization by evenly distributing the myceliated grains throughout the jars.

Under sterile conditions, the fully colonized grain was transferred to four separate commercial mushroom production bags, each containing 2 kg of hydrated and sterilized oak sawdust, and the bags were then heat-sealed. The four bags were immediately shaken to evenly distribute the grain, placed in a dark 24 C room, and left to colonize the oak sawdust for 14 d. On day 15, the four fully colonized bags were placed in 2 C cold storage until ready for use in field inoculation of the *L. lucidum* logs. City of Austin staff received the spawn shipment on February 20, 2015, and stored it in the refrigerator at 7 C until the day of log inoculation.

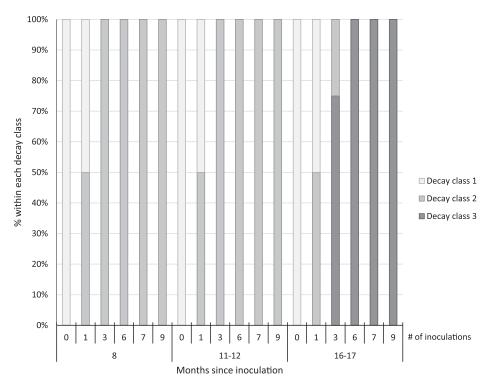
### Inoculation of Ligustrum lucidum Logs

We inoculated 25 freshly cut *L. lucidum* logs that were 3.8 to 11.4 cm in diameter and 53 to 123 cm in length. We placed the 25 inoculated logs and 5 untreated control logs ~10 to 15 cm apart, end to end, along slope contours and buried the lower half of each

log in the soil. We began the study at Site 1 on February 27, 2015, where City of Austin staff made a row of 7 shallow cuts ~1.9 to 2.5 cm below the depth of the bark, spaced ~10 to 15 cm apart, into the upper half of 9 logs by running a battery-operated circular saw perpendicular to the length of each log (Figure 1A). We covered the open cuts with a thin layer of sawdust spawn topped with newspaper wetted with creek water collected from the site (Figure 1B). For the controls, we scored 2 logs in the same manner as the treated logs but did not inoculate them. We placed the 2 controls on either end of the row of 9 treated logs.

At Site 2, City of Austin staff and volunteers inoculated 16 logs on March 15, 2015. While working with volunteers, we used a battery-operated drill and a 12-mm soft-steel drill screw-tip bit with stop (Figure 2A). We packed each hole with sawdust spawn and covered the top with plug wax (Figure 2B). To evaluate the labor intensity needed for effective treatments, we varied the density of inoculations at Site 2 by a factor of 3, with sets of 4 logs treated with 1, 3, 6, and 9 inoculation points, respectively. We then placed 1 untreated log on the south end and 2 untreated logs on the north end of the row of 16 treated logs as controls. No further treatment was provided at either site.

We conducted four post-inoculation visits at Site 1 on November 23, 2015, February 24, 2016, June 22, 2016, and March 5, 2018, and at Site 2 on November 15, 2015, January 31, 2016, August 16, 2016, and March 4, 2018. On each visit, we measured the decomposition stage of each log by assigning a decay class based on visual and tactile criteria (Russell et al. 2015). For the first three visits, we conducted nondestructive examinations of the surface of each log by looking for visual evidence of wood decay, such as presence of mycelia and fruit bodies, and assigned a decay class using Grove et al. (2009, p. 390) as follows: (1) "few signs of wood



**Figure 3.** Decay class of 30 freshly cut *Ligustrum lucidum* logs (25 treatments, 5 controls) at two sites within the Balcones Canyonlands Preserve, Austin, TX, at 8, 11–12, and 16–17 mo following inoculation with *Trametes versicolor*, by the number of inoculation points (0, 1, 3, 6, 7, 9) per log. Controls were not inoculated. Decay classes were assigned based on a nondestructive visual assessment using Grove et al. (2009, p. 390): (1) "few signs of wood decay," (2) "early signs of wood decay or discolouration," and (3) "fungi may be prominent."

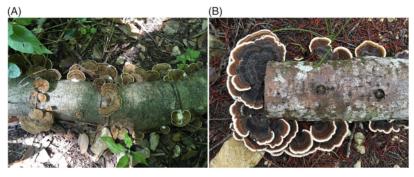


Figure 4. Trametes versicolor fruit bodies on Ligustrum lucidum logs at Site 1 (16 mo post-inoculation, A) and Site 2 (17 mo post-inoculation, B), Balcones Canyonlands Preserve, Austin, TX.

decay," (2) "early signs of wood decay or discolouration," and (3) "fungi may be prominent." On the last visit, we used a 5-mm galvanized steel nail (13.1-cm long) to penetrate each log to estimate the level of decay based on the five decay classes adapted from Mäkipää and Linkosalo (2011, p. 1137): "(1) still hard wood—[nail] penetrates a few millimeters into the wood; (2) still fairly hard wood—[nail] penetrates 1–2 cm into the wood; (3) outer layer of the log fairly soft, core still hard—[nail] penetrates 3–5 cm into the wood; (4) soft throughout—[nail] penetrates all the way into the wood; (5) very soft—wood structure of logs already collapsed." We took three measurements and used the average for each log.

In the analysis of decay of the *L. lucidum* logs, the response variable consists of the final five decay classes described. Because the decay classes were ordered (from low to high), we used an ordinal logistic regression (JMP software, v. 14.0.0, SAS Institute, Cary, NC) to examine how the number of inoculation points (0, 1, 3, 6, 7, 9) impacted decay in *L. lucidum*.

### **Results and Discussion**

We observed early signs of wood decay (decay class 2) in the form of white mycelia on the ends and underneath the treated logs at both sites at 8 and 11 to 12 mo post-inoculation, and few signs of decay on the controls at either site (Figure 3). Within 16 to 17 mo post-inoculation, 100% of the logs with 6 to 9 inoculation points were covered with fruit bodies (decay class 3; Figures 3 and 4), as were 75% of the logs with 3 inoculation points. No mycelia or fruit bodies were observed on the control logs (Figure 3).

Three years after inoculation, all logs with >3 inoculation points were in decay classes 3 to 5 (Figures 3 and 5). At Site 1, 100% of the treated logs (7 inoculation points) were in decay classes 4 to 5. One of the control logs was developing T. versicolor fruit bodies and in decay class 2, but the other control remained in decay class 1. At Site 2, we found 3 of the treated logs with 1 inoculation point were still in decay classes 1 to 2, while the remaining 13 treated logs at Site 2

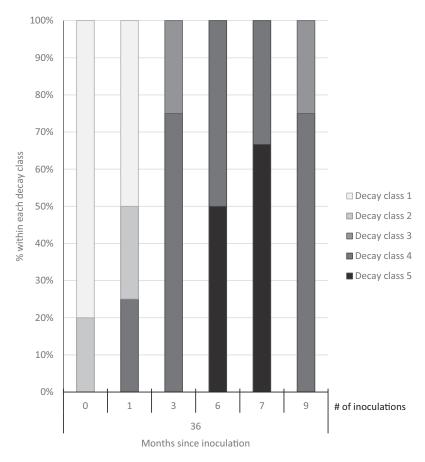


Figure 5. Decay class of 30 freshly cut *Ligustrum lucidum* logs (25 treatments, 5 controls) at two sites within the Balcones Canyonlands Preserve, Austin, TX, 3 yr after inoculation with *Trametes versicolor*, by the number of inoculation points (0, 1, 3, 6, 7, 9) per log. Controls were not inoculated. Final decay classes were adapted from Mäkipää and Linkosalo (2011, p. 1137): "(1) still hard wood—[nail] penetrates a few millimeters into the wood; (2) still fairly hard wood—[nail] penetrates 1–2 cm into the wood; (3) outer layer of the log fairly soft, core still hard—[nail] penetrates 3–5 cm into the wood; (4) soft throughout—[nail] penetrates all the way into the wood; (5) very soft—wood structure of logs already collapsed."

were in decay classes 3 to 5. None of the control logs at Site 2 had progressed beyond decay class 1 by the end of the study (Figures 5 and 6). The ordinal logistic regression (Table 1) shows that including the number of T. versicolor inoculations as a covariate significantly improves the model (P = 0.0006). With each unit of increase in the number of inoculation points, there was a 0.48 increase in the odds of the log being in a higher decay class (P = 0.0005) when site was held constant (Table 2).

## Critique of Our Pilot Study and Suggestions for Future Research

Wood-decaying fungi play an essential role in forest ecosystems. Their unique ability to digest wood releases nutrients (Marcot 2017b), contributes to carbon and water storage (Arnstadt et al. 2016), and provides habitat to a host of other organisms, including free-living nitrogen-fixing bacteria and mycorrhizal fungi (Lonsdale et al. 2008). The end product of the wood decomposition process is soil development needed for tree growth and forest regeneration. We explored whether wood-decaying fungi could be used to speed up this process to recycle an invasive hardwood back into the ecosystem. While the data set from our pilot study is limited, we found that inoculating *L. lucidum* logs with *T. versicolor* significantly accelerated wood decomposition at both of our study sites. By the end of our 3-yr study, all of the logs

with >3 inoculation points were in advanced stages of decay. Interestingly, one of the control logs was covered with T. versicolor fruit bodies and beginning to decompose, so T. versicolor could have spread from the row of inoculated logs to the control log, or it could have been colonized from native spores or mycelia already present at the site. None of the other control logs showed noticeable signs of decay. Based on our preliminary results, we recommend spacing inoculation points every  $\sim 15$  cm to promote more rapid growth of T. versicolor and wood decay.

While the potential for incorporating mushroom cultivation into sustainable forest management has been recognized (Garibay-Orijel et al. 2009; Lonsdale et al. 2008; Marcot 2017a, 2017b), we have found few published studies that have tested the feasibility of using wood-decaying fungi to accelerate decomposition of wood debris in the field (Pavlík and Pavlík 2013; Piškur et al. 2011). Piškur et al. (2011) evaluated the mycoremediation potential of oyster mushrooms [Pleorotus ostreatus (Jacq.) P. Kumm.] a thermal power plant site covered with fly ash by inoculating fresh European beech (Fagus sylvatica L.) wood chips and comparing the results with noninoculated wood chips 6 and 17 mo later. Although temperature, relative humidity, and lignin degradation increased in the first 6 mo, after 17 mo they concluded that the naturally occurring processes of wood degradation at sites not inoculated with P. ostreatus were as efficient as those at the inoculated sites. Pavlík and Pavlík (2013) inoculated F. sylvatica and

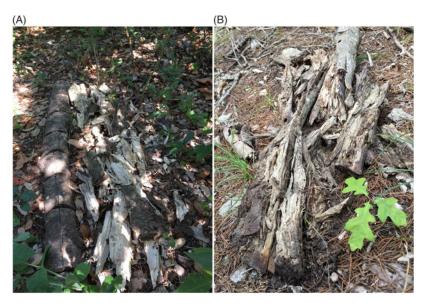


Figure 6. Comparison of control (not inoculated) Ligustrum lucidum log (left) and decomposed L. lucidum log 3 yr following inoculation with Trametes versicolor at Site 1 (A) and decomposed L. lucidum log 3 yr following inoculating with T. versicolor at Site 2 (B), Balcones Canyonlands Preserve, Austin, TX.

**Table 1.** Whole-model test of full versus reduced ordinal logistic regression examining the impact of the number of *Trametes versicolor* inoculation points (0, 1, 3, 6, 7, 9) on decay of *Ligustrum lucidum* logs 3 yr after inoculation within the Balcones Canyonlands Preserve, Austin, TX.

Model	-Log likelihood	df	χ²	Р
Difference	7.51	2	15.01	0.0006
Full	35.46			
Reduced	42.96			

**Table 2.** Parameter estimates of ordinal logistic regression model examining the impact of the number of *Trametes versicolor* inoculation points (0, 1, 3, 6, 7, 9) on decay of *Ligustrum lucidum* logs 3 yr after inoculation within the Balcones Canyonlands Preserve, Austin, TX.

Term	Estimate	SE	χ²	Р
Intercept (1)	0.2501	0.6878	0.13	0.7161
Intercept (2)	0.9378	0.7014	1.79	0.1812
Intercept (3)	2.1689	0.8025	7.31	0.0069
Intercept (4)	4.8238	1.1045	19.07	< 0.0001
Inoculations	0.4850	0.1394	12.11	0.0005
Site	0.1595	0.3792	0.18	0.674

European aspen (*Populus tremula* L.) logs with *P. ostreatus* and reported that all logs produced fruit bodies and resulted in effective and rapid wood decomposition during their 5-yr study; none of the control logs formed *P. ostreatus* fruit bodies.

Trametes versicolor fruit bodies were flourishing on 75% of our treated logs with >3 inoculation points following a 16- to 17-mo incubation period. Used medicinally for centuries, fruit bodies can be consumed in teas and soups. Wild strains have been shown to inhibit tumor growth and are used to treat a wide variety of cancers (Hobbs 2004). Research has shown that T. versicolor boosts the immune system by increasing natural killer cells, contains antiviral agents that may inhibit HIV replication, and has strong antibiotic compounds effective against several human pathogens (Chu et al. 2002). More recent research shows promise of antioxidant and antiradical activity in treating neurodegenerative diseases (Janjušević et al. 2017). Trametes versicolor is also known to break

down a host of pollutants, including polycyclic aromatic hydrocarbons (Gao et al. 2010), contaminants in wastewater (Shreve et al. 2016; Torán et al. 2017), and several herbicides (da Silva Coelho-Moreira et al. 2013). We have anecdotally observed T. versicolor fruit bodies growing on an L. lucidum stump 3 yr after it was cut and the stump was painted with imazapyr (50% v/v), and along the root of a stump  $\sim$ 1 yr after it was cut and treated with triclopyr (50% v/v). These observations show promise for successful inoculations following herbicide treatment and bioremediation potential of T. versicolor.

While not part of our study, our volunteers have since inoculated ~35 additional L. lucidum logs, all with prolific fruit bodies and comparable results. They have also inoculated photinia (Photinia spp.) logs with T. versicolor and subsequently observed abundant fruit bodies and accelerated wood decay. Given its widespread distribution and aggressive growth, we expect T. versicolor would colonize and accelerate decomposition of most dead invasive hardwoods. Similarly, we chose the ubiquitous *T. versicolor* because we found it growing on L. lucidum logs within our study area. However, for any given site, we recommend conducting inventories (Garibay-Orijel et al. 2009) to identify, collect, and culture native saprotrophic fungi suitable for inoculations. We provide detailed methods we used to culture a local strain of T. versicolor so they can be repeated. Alternatively, cultures of *T. versicolor* and other medicinal mushrooms are commercially available and can be custom made by commercial mycology labs. We have had successful results using commercially available T. versicolor spawn and recommend that future research investigate whether this could have an effect on native fungal communities.

We used freshly cut *L. lucidum* logs in our study because we wanted to test this method under likely field scenarios. We anticipated that a crew would inoculate the logs on the same day of tree felling rather than having to return on a different day, especially at a remote site. This method worked well in our study. Pavlík et al. (2008) also found that *P. ostreatus* grew best on freshly cut *F. sylvatica* wood. Other investigations we believe would help inform land management decisions include inoculation of older logs, standing dead trees and cut stumps (e.g., following girdling or herbicide treatment via stem injection or application to cut

stumps), and small stems with leaves and seeds, along with documenting the successional cascade following inoculation and comparing results with native deadwood.

In addition to accelerated decay, we are incorporating inoculated L. lucidum logs into forest restoration and remediation efforts. We use them as check logs (Jacke and Toensmeier 2005) to help protect soils and control erosion; improve soil structure, diversity, and nutrients; infiltrate and store water; and provide habitat for soil biota and small wildlife. We bury the lower half of the inoculated check logs into the soil along slope contours in small groupings to capture and disperse runoff. Where native mulch is available, we add this to the upslope side of the logs to help absorb more water. By the end of our 3-yr study, our inoculated logs were spongy and moist, even during the hot, dry summer, demonstrating the importance of fungi for retaining moisture in the understory. We are taking advantage of these moisture reservoirs by planting seeds of native trees and shrubs on either side of inoculated logs to provide a source of water for the seedlings. These are some of the many ways that L. lucidum logs inoculated with T. versicolor can be used as a functional land management tool to help restore degraded environments.

### **Conclusions and Management Implications**

Our experiment demonstrates that inoculating freshly cut L. lucidum logs with T. versicolor significantly accelerates the rate of wood decomposition and suggests a promising approach for more widespread application. The accelerated decay rate would allow for in situ recycling of the dead biomass to support soil formation, input of organic matter, and nutrient cycling, improving growing conditions for forest trees and promoting biodiversity conservation (Marcot 2017b). The accelerated decomposition of wood debris in turn reduces fire risk concerns. Inoculated logs can be strategically placed to assist with erosion control, water retention, pollutant filtering, and seedling establishment. An added benefit of inoculating logs with an aggressive wood-decaying fungus like *T. versicolor* is the proliferation of medicinal mushrooms, a potential nontimber forest product and revenue source for local communities (Garibay-Orijel et al. 2009). Inoculating logs in the field was a favorite activity of our volunteers, requiring little or no previous experience to achieve successful results. Applied on a larger scale, these medicinal saprotrophs have the potential to transform nonnative woody plants from a threat to an asset.

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### References

- Arnstadt T, Hoppe B, Kahl T, Kellner H, Krüger D, Bauhus J, Hofrichter M (2016) Dynamics of fungal community composition, decomposition and resulting deadwood properties in logs of *Fagus sylvatica*, *Picea abies* and *Pinus sylvestris*. For Ecol Manag 382:129–142
- Ayup MM, Montti L, Aragón R, Grau HR (2014) Invasion of *Ligustrum lucidum* (Oleaceae) in the southern Yungas: changes in habitat properties and decline in bird diversity. Acta Oecol 54:72–81

- Balcones Canyonlands Preserve (2007) Balcones Canyonlands Preserve Land Management Plan, Tier IIA, Chapter IV, Vegetation Management. Austin, TX: Balcones Canyonlands Preserve. 13 p. https://www.traviscountytx.gov/images/tnr/Docs/draft\_plan/tier\_iia-4\_vegetation\_management.pdf. Accessed: February 18, 2019
- Bednarz JC, Huss MJ, Benson TJ, Varland DE (2013) The efficacy of fungal inoculation of live trees to create wood decay and wildlife-use trees in managed forests of western Washington, USA. For Ecol Manag 307: 186–195
- [CABI] Centre for Agriculture and Bioscience International (2018) Datasheet: Ligustrum lucidum (broad-leaf privet). Invasive Species Compendium. https://www.cabi.org/ISC/datasheet/30751. Accessed: November 23, 2018
- Chu KKW, Ho SSS, Chow AHL (2002) Coriolus versicolor: a medicinal mushroom with promising immunotherapeutic values. J Clin Pharmacol 42:976–984
- Cotter T (2014) Organic Mushroom Farming and Mycoremediation: Simple to Advanced and Experimental Techniques for Indoor and Outdoor Cultivation. White River Junction, VT: Chelsea Green Publishing. 382 p
- Coutts-Smith AJ, Downey PO (2006) Impact of Weeds on Threatened Biodiversity in New South Wales. Adelaide, Australia: Cooperative Research Centre for Australian Weed Management Technical Series No. 11. 107 p
- da Silva Coelho-Moreira J, Maciel GM, Castoldi R, da Silva Mariano S, Inácio FD, Bracht A, Peralta RM (2013) Involvement of lignin-modifying enzymes in the degradation of herbicides. Pages 165–187 *in* Price JA, Kelton JA, eds. Herbicides: Advances in Research. London: TechOpen
- Ferreras AE, Giorgis MA, Tecco PA, Cabido MR, Funes G (2015) Impact of Ligustrum lucidum on the soil seed bank in invaded subtropical seasonally dry woodlands (Córdoba, Argentina). Biol Invasions 17:3547–3561
- Gao D, Du L, Yang J, Wu W-M, Liang H (2010) A critical review of the application of white rot fungus to environmental pollution control. Crit Rev Biotechnol 30:70–77
- Garibay-Orijel R, Córdova J, Cifuentes J, Valenzuela R, Estrada-Torres A, Kong A (2009) Integrating wild mushrooms use into a model of sustainable management for indigenous community forests. For Ecol Manag 258:122–131
- Global Invasive Species Database (2018) Ligustrum lucidum. http://www.iucngisd.org/gisd/speciesname/Ligustrum+lucidum. Accessed: November 23, 2018
- Grove SJ, Stamm L, Barry C (2009) Log decomposition rates in Tasmanian *Eucalyptus obliqua* determined using an indirect chronosequence approach. For Ecol Manag 258:389–397
- Hobbs CR (2004) Medicinal value of turkey tail fungus Trametes versicolor (L.:Fr.) Pilát (Aphyllophoromycetideae): a literature review. Int J Med Mushrooms 6:195–218
- Hoyos LE, Gavier-Pizarro GI, Kuemmerle T, Bucher EH, Radeloff VC, Tecco PA (2010) Invasion of glossy privet (*Ligustrum lucidum*) and native forest loss in the Sierras Chicas of Córdoba, Argentina. Biol Invasions 12:3261–3275
- Hudson JR, Hanula JL, Horn S (2014) Impacts of removing Chinese privet from riparian forests on plant communities and tree growth five years later. For Ecol Manag 324:101–108
- Jacke D, Toensmeier E (2005) Edible Forest Gardens. Volume 2, Ecological Design and Practice for Temperate-Climate Permaculture. White River Junction, VT: Chelsea Green Publishing. Pp 333–334
- Janjušević L, Karaman M, Šibul F, Tommonaro G, Iodice C, Jakovljević D, Pejin B (2017) The lignicolus fungus *Trametes versicolor* (L.) Lloyd (1920): a promising natural source of antiradical and AChE inhibitory agents. J Enzyme Inhib Med Chem 32:355–362
- Johnson S (2009) Privet species—are we sitting on species time bombs? Pages 307–341 in Proceedings of the 15<sup>th</sup> Biennial NSW Weeds Conference. Narrabri, NSW, Australia: Narrabri Shire Council
- Lepp H (2012) Mycogeography: Cosmopolitan and pan-tropical species. Australian Fungi. https://www.anbg.gov.au/fungi/mycogeography-cos-pan. html. Accessed: February 16, 2019
- Lichstein JW, Grau HR, Aragón R (2004) Recruitment limitation in secondary forests dominated by an exotic tree. J Veg Sci 15:721–728

- Lonsdale D, Pautasso M, Holdenrieder O (2008) Wood-decaying fungi in the forest: conservation needs and management options. Eur J Forest Res 127:1–22
- Maddox V, Byrd J, Serviss B (2010) Identification and control of invasive privets (*Ligustrum* spp.) in the middle southern United States. Invasive Plant Sci Manag 3:482–488
- Mäkipää R, Linkosalo T (2011) A non-destructive field method for measuring wood density of decaying logs. Silva Fenn 45:1135–1142
- Marcot BG (2017a) A review of the role of fungi in wood decay of forest ecosystems. Portland, OR: USDA Forest Service, Pacific Northwest Research Station Research NotePNW-RN-575. 31 p
- Marcot BG (2017b) Ecosystem processes related to wood decay. Portland, OR: USDA Forest Service, Pacific Northwest Research Station Research Note PNW-RN-576. 43 p
- Page-Dumroese DS, Busse MD, Archuleta JG, McAvoy D, Roussel E (2017) Methods to reduce forest residue volume after timber harvesting and produce black carbon. Scientifica 2017, 10.1155/2017/2745764
- Pavlík M, Hrasko M, Pavlikova A (2008) Oyster mushroom *Pleurotus ostreatus* (Jacq.) P. Kumm.—its cultivation and utilization in the Slovak forestry. Pages 246–254 in Proceedings of the Sixth International Conference on Mushroom Biology and Mushroom Products. Bonn, Germany: World Society for Mushroom Biology and Mushroom Products
- Pavlík M, Pavlík Š (2013) Wood decomposition activity of oyster mushroom (*Pleurotus ostreatus*) isolate in situ. J For Sci 59:28–33

- Piškur B, Bajc M, Robek R, Humar M, Sinjur I, Kadunc A, Oven P, Rep G, Petkovšek SAS, Kraigher H, Jurc D, Pohleven F (2011) Influence of Pleurotus ostreatus inoculation on wood degradation and fungal colonization. Bioresour Technol 102:10611–10617
- Rhoades CC, Fornwalt PJ (2015) Pile burning creates a fifty-year legacy of openings in regenerating lodgepole pine forests in Colorado. For Ecol Manag 336:203–209
- Russell MB, Fraver S, Aakala T, Gove JH, Woodall CW, D'Amato AW, Ducey MJ (2015) Quantifying carbon stores and decomposition in dead wood: a review. For Ecol Manag 350:107–128
- Shreve MJ, Brockman A, Hartleb M, Prebihalo S, Dorman FL, Brennan RA (2016) The white-rot fungus *Trametes versicolor* reduces the estrogenic activity of a mixture of emerging contaminants in wastewater treatment plant effluent. Int Biodeterior Biodegrad 109:132–140
- Stamets P (2000) Growing gourmet and medicinal mushrooms. Berkeley, CA: Ten Speed Press. 614 p
- Torán J, Blánquez P, Caminal G (2017) Comparison between several reactors with *Trametes versicolor* immobilized on lignocellulosic support for the continuous treatments of hospital wastewater. Bioresour Technol 243:966–974
- van der Wal A, Ottosson E, de Boer W (2015) Neglected role of fungal community composition in explaining variation in wood decay rates. Ecology 96:124–133
- Veena SS, Pandey M (2012) Physiological and cultivation requirements of Trametes versicolor, a medicinal mushroom to diversify Indian mushroom industry. Ind J Agric Sci 82:672–675