THESIS

FIRE-ASSOCIATED SHIFTS IN THE SOIL MICROBIOME IN WESTERN CONIFER FORESTS: IMPLICATIONS FOR ARMILLARIA ROOT DISEASE BIOCONTROL AND MANAGEMENT

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2024

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ABSTRACT

FIRE-ASSOCIATED SHIFTS IN THE SOIL MICROBIOME IN WESTERN CONIFER FORESTS: IMPLICATIONS FOR ARMILLARIA ROOT DISEASE BIOCONTROL AND MANAGEMENT

The research presented in this thesis integrates the current understanding of environmental disturbances, plant associated microbiomes, and microbial biological control of fungal forest pathogens to contribute to improved disease management. In Chapter 2, I examined how fire disturbances affect soil microbial communities in areas where Armillaria root disease, caused by the pathogen *Armillaria solidipes*, is prevalent by documenting changes to bacterial and fungal community diversity and composition following three distinct levels of burn severity (high, low, and unburned) in a conifer forest in northern Idaho, United States. Expected reductions in bacterial community alpha diversity were observed when comparing burned communities with unburned communities; however, fungal communities showed a lack of significant change in alpha diversity in response to burn severity at the sampling time of 15-months post-fire. However, in both bacterial and fungal soil communities, compositional changes corresponding to burn severity levels were observed. Further examinations characterized similarities and differences between burn severity-associated communities and *Armillaria* species-associated communities to determine how these microbial changes might influence Armillaria root disease. At high-severity burn sites, colonization by *A. solidipes* and the

associated microbial community was prevalent when compared with low-severity burn and unburned sites. In contrast, the presence and abundance of the weakly pathogenic species *A. altimontana* and its associated microbial community, including beneficial ectomycorrhizal fungi, appeared to be negatively impacted by high-severity burns. Further research is needed to determine which microbial taxa are critical for promoting or suppressing *A. solidipes* activity, yet the results from this study suggest that high-severity burns may create environments hospitable to this pathogen and thus monitoring for increased disease pressure following severe burns may be warranted.

Chapter 3 focuses specifically on beneficial members of the native soil microbial community that exhibit antagonistic activity against *A. solidipes*. Because these native species are adapted to the environmental conditions and community interactions, they are more likely than foreign microbial species to successfully establish a stable population required for effective biological control. I isolated putative native biological control agents from soil samples collected under different burn severity conditions and tested their *in vitro* capabilities to inhibit the growth of *A. solidipes* with dual culture confrontation tests. Effective *in vitro* pathogen inhibition was observed with 10 microbial isolates, including five bacterial isolates from the genera *Bacillus* and *Caballeronia* and five fungal isolates from the genera *Trichoderma* and *Mortierella.* Further examination of the sites these microbes and communities originated from and their compositional changes documented in Chapter 2 revealed that the presence or abundance of our effective biological control organisms did not differ based on burn severity. Importantly, this suggests that fire disturbances may not directly influence the use of these species in management methods for Armillaria root disease in similar conifer forests. However, considering the increased presence of *A. solidipes* observed following a high-severity burn, there may be

additional biotic or abiotic influences apart from biological control agents that are influencing the activity of *A. solidipes* after fire.

These studies enhance our understanding of how abiotic and biotic influences interact to affect the presence of virulent soilborne forest pathogens and associated soil microbes. Considering the effects of these interactions is critical for the development of sustainable long-term management strategies that will help to preserve these ecosystems facing increasing environmental and pathogen-related stressors. The overall goals of this research are to build upon the growing body of research examining how the soil microbiome contributes to disease development and to provide tangible results that can be incorporated by forest managers to help reduce damage caused by Armillaria root disease in fire-prone conifer forests.

ACKNOWLEDGEMENTS

Many thanks to my advisor, Dr. Jane Stewart, for your guidance and patience throughout this research. Your expertise and mentorship have immensely strengthened my foundation as a scientist and plant pathologist. I look forward to further growth as I continue my time as part of the Stewart Lab, particularly with your support in embracing challenges that push me beyond my comfort zone! To Dr. Mee-Sook Kim, thank you for your guidance and knowledge during the course of this project and for spending long days in the field with me. I am excited to continue having you as a mentor throughout my PhD and into the start of my career. I would also like to thank Dr. Ned Klopfenstein for your invaluable attention to detail and support throughout the writing process. Additional thanks to Dr. Amy Charkowski and Dr. Zaid Abdo for your support as committee members on this project, and to Dr. Patrick Bennett for your assistance in the field and editorial process. A huge thank you to Sara Ashiglar for finding and navigating our field sites, without which this project could not have happened, and to John Hanna, for your unmatched rhizomorph-finding abilities. I would also like to express my gratitude to my colleagues in the Stewart Lab for their interest and support of my work throughout this project. Special thanks to Dr. Jorge Ibarra Caballero for your help with the computational analysis of this project and for always having time to thoughtfully answer my many questions. To my colleagues and friends, Noah Lindeman and Grace Ganter, I am endlessly thankful to have been able to share both the toughest and most joyous parts of graduate school with you both.

Finally, I am especially grateful for my parents, Dr. Ulrike Axen and Dr. Kelly Fitz. Thank you, Mom and Pop, for your early and continued commitment to my education and encouragement of my many professional and personal interests. I hope to carry the confidence, leadership,

creativity, and devotion you both embody into a career and family of my own. Of my achievements, I am most proud to be your daughter.

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CHAPTER 1: LITERATURE REVIEW

1.1 The Genus *Armillaria*

1.1.1 Ecosystem Roles

The basidiomycete fungal genus *Armillaria* is composed of at least 40 species and has a worldwide geographic distribution, occupying ecosystems from boreal to tropical forests and occurring on hundreds of deciduous and coniferous tree host species in both natural forests and agronomic settings or timber plantations (Kim et al., 2022; Baumgartner et al., 2011; Shaw & Kile, 1991). *Armillaria* species occupy a range of ecological niches within ecosystems where they are present. For example, many species act as facultative necrotrophic plant pathogens, living saprophytically before infecting a host tree and returning to a saprophytic state on dead tree tissues after mortality occurs (Chen et al., 2023; Coetzee et al., 2018; Baumgartner et al., 2011). The saprophytic abilities of *Armillaria* species can provide benefits to the ecosystem by contributing to decomposition and nutrient cycling. However, *Armillaria* species can persist in this manner for many years, becoming a long-term inoculum source for spreading infection (Chen et al., 2023; Baumgartner et al., 2011). Pathogenic species of *Armillaria* vary in their virulence: some act as primary pathogens, leading to mortality of healthy hosts, while many are secondary pathogens that attack previously weakened trees (Shaw & Kile, 1991). Among 11 known *Armillaria* species in North America, primary pathogens include *A. solidipes, A. mellea*, *A. sinapina*, and *Desarmillaria caespitosa* (formerly *D. tabescens/A.tabescens* in North America) while examples of less aggressive pathogens or secondary/opportunistic pathogens species are *A. altimontana*, *A. gallica*, *A. nabsnona*, *A. calvescens*, *A. gemina*, and *A. cepistipes* (Kim et al., 2023b; Kim et al., 2022). A few *Armillaria* species even behave in a mycorrhizal manner,

functioning as a carbon source for parasitic orchids lacking chlorophyll (Kim et al., 2022; Coetzee et al., 2018; Shaw & Kile, 1991).

1.1.2 Geographic Distribution

Armillaria species are separated phylogenetically into three distinct clades that reflect their geographic distribution and ancestral origins: the African clade, Holarctic clade, and Australasian-South American clade (Coetzee et al., 2018). The Holarctic clade, on which the largest body of phylogenetic research has been performed, is further separated in five lineages or superclades based on phylogenetic analyses of the translation elongation factor 1-alpha gene region (*tef1*), which are named after the species *A. solidipes/ostoyae*, *A. gallica*, *A. mellea*, *A. mexicana*, and the recently reclassified *Desarmillaria* species (Coetzee et al., 2018; Klopfenstein et al., 2017). Within these groups, species differ in their distributions, with some occurring only in specific regions or countries and others having a transcontinental distribution (Coetzee et al., 2018; Klopfenstein et al., 2017). The Australasian-South American clade includes species from sub-Saharan Africa, Australasia, and South America and has three lineages named for *A. hinnulea*, *A. novae-zelandiae*, and *A. luteobubalina* (Coetzee et al., 2018; Koch et al., 2017). Finally, the African clade, which is basal to the other *Armillaria* clades (excluding *Desarmillaria* species), includes two major lineages (Coetzee et al., 2018; Koch et al., 2017).

1.1.3 Armillaria Root Disease

Pathogenic *Armillaria* species cause a white rot of tree roots and lower boles, degrading both lignin and cellulose components of the woody tissues and leading to decline in host tree vigor (Kim et al., 202; Baumgartner et al., 2011). The most obvious signs of Armillaria root disease are fruiting bodies, mycelial fans, and rhizomorphs. Fruiting bodies, commonly known as

"honey mushrooms," release wind-dispersed basidiospores, although these spores typically do not act as the primary inoculum source for spreading infection. Rather, *Armillaria* pathogens from the Holarctic and Australasian-South American clades often grow from an infected to a healthy host tree through root contact with subterranean *rhizomorphs*, melanized cord-like groupings of mycelium (Koch et al., 2017). Upon penetration of the outer bark, which occurs through both physical force exerted by the rhizomorph and the breakdown of woody components through secreted fungal compounds, hyphal growth occurs and the pathogen can decompose the underlying layers of the roots by releasing a variety of enzymes (Baumgartner et al., 2011). Mycelial fans may eventually develop, indicating an advanced stage of disease (Shaw & Kile, 1991). Plant symptoms that develop in response to root decay include wilting and stunted growth, as well as resinosis in coniferous hosts (Baumgartner et al., 2011; Shaw & Kile, 1991). *Armillaria* pathogens can lead directly to host mortality through girdling, preventing nutrient and water transport from the roots to the rest of the tree. Indirect causes of death may also occur, for example, windthrow due to the decline in structural integrity of the lower bole or increased susceptibility to lethal pests like bark beetles (Shaw & Kile, 1991). Landscape-scale mortality from Armillaria root disease can cause extensive structural changes to forest ecosystems as the pathogen spreads outward in a radial fashion from an initial infection point, creating clear pockets of disease (e.g., Ferguson et al., 2023). Infection and spread of Armillaria root disease is most severe on trees that are predisposed to infection because of preexisting stressors, such as insects or adverse environmental conditions (Shaw & Kile, 1991).

1.1.4 The Pathogen Armillaria solidipes

The North American species *A. solidipes* is a native pathogen that attacks mostly coniferous hosts throughout the northern and western regions of the continent (Kim et al., 2021;

Kim et al., 2022), with a recent first report of the pathogen on a deciduous host tree in North Carolina, United States (Kim et al., 2023a). *Armillaria solidipes* is the North American vicariant of the Eurasian species *A. ostoyae*, which are distinct species based on morphology and genetic differences at the *tef1* region (Klopfenstein et al., 2017; Antonín et al. in prep.). As an aggressive primary pathogen, *A. solidipes* can weaken and kill otherwise healthy trees via white rot of the root and boles, altering forest structure and function via expansive growth (Kim et al., 2021; Cruickshank, 2011; Ferguson et al., 2003). For example, a single genet of *A. solidipes* was found to span 965 ha in Oregon, United States, with an estimated age of over 2000 years (Ferguson et al., 2003). Current control methods for Armillaria root disease include stump removal, root collar excavation, and soil fumigation (Kim et al., 2022; Baumgartner et al., 2011). However, because of the enormous areas *A. solidipes* can cover, these methods are economically and logistically impractical for controlling damage at the landscape scale (Mesanza et al., 2016). Alternatives to these methods utilizing aspects of natural forest ecosystems, such as native microbial biological control species, have been proposed as potentially effective strategies for managing Armillaria root disease (Ibarra Caballero et al., 2023; Stewart et al., 2021; Warwell et al., 2019).

1.2 Soil Microbial Communities

1.2.1 Core Microbiomes

Microbial taxa that co-occur with a certain plant host under a range of environmental conditions compose the plant's *core microbiome* (Trivedi et al., 2020). Core microbiomes have been investigated throughout diverse fields of biology, including the microbiomes of various animal organs and environments (Neu et al., 2021). Plant core soil microbiomes are a subset of the microbial diversity found within the bulk soil environment, composed of species whose presence are promoted by the host plant, for example, via chemotactic attraction to plant

exudates (Wang et al., 2023; Stewart et al., 2021; Trivedi et al., 2020). Taxa within these communities play key roles that support the health of their plant hosts. For example, mycorrhizal fungi assist with accessing and transporting limiting nutrients to the plant, and many bacterial species secrete antibiotics that can impede the activity of pathogens and promote a baseline of disease resistance (Wang et al., 2023; Stewart et al., 2021; Trivedi et al., 2020). Importantly, these beneficial functions cannot be predicted by examining the activities of individual members of the core microbiome, but rather from the interactions within the microbial community, with the plant, and with the surrounding environment (Trivedi et al., 2020). Key beneficial taxa within the core microbiome help to confer disease resistance and may act as effective biological control agents (BCAs) through a variety of mechanisms, for example, through competition for limited space and the production of antagonistic compounds (Wang et al., 2023; Qiu et al., 2022). Further, because interactions within the core microbiome support their plant host, changes to the microbiome, via biotic or abiotic influences, can lead to reduced plant health (Qiu et al., 2022).

1.2.2 Pathobiomes and Disease Suppressive Soils

A microbial community composed of members that reduce plant health and positively contribute to disease development by a pathogen is known as a pathobiome (Stewart et al., 2021). The concept of a pathobiome, focused on microbial community interactions that influence disease progression, modifies the traditional pathology disease triangle, which depicts disease stemming from interactions between a single pathogen and a single host (Stewart et al., 2021). As plant pathogens establish, the abundance of beneficial microbes may be reduced and shifts from a healthy core microbiome to a pathobiome may occur with the onset of disease (Wang et al., 2023; Stewart et al., 2021). This effect has been demonstrated in multiple pathosystems. For example, Qiu et al. (2022) demonstrated that infection with the cotton (*Gossypium hirsutum)*

pathogen *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) resulted in changes to the composition of the core rhizosphere microbiome at the seedling stage when compared with uninfected plants. Compositional changes were also found in Erlacher et al. (2014), with a higher alpha diversity of Gammaproteobacteria in the microbial communities associated with lettuce plants infected with the pathogen *Rhizoctonia solani* than in the communities of uninfected plants.

In contrast to a microbial pathobiome, disease suppressive soils occur when antagonistic activity in the rhizosphere microbial community reduce disease incidence caused by soilborne pathogens (Palmieri et al., 2022; Trivedi et al., 2020; Carrión et al., 2019; Schlatter et al., 2017). General disease suppression is a preexisting soil characteristic that arises through microbial competition with a range of soilborne pathogens, preventing them from successfully establishing and giving the plant a basal level of disease protection (Stewart et al., 2021; Schlatter et al., 2017). In contrast, specific suppression occurs during a disease outbreak, when a single species or specific taxa is enriched within the microbiome and confers resistance (Stewart et al., 2021; Carrión et al., 2019). Specific suppression typically results from an enrichment of microorganisms that attack the pathogen or compete for a specific niche. Specific suppression can be transferred to a diseased soil by transplanting a small amount of the suppressive soil or the key microbial taxa responsible for the suppression (Stewart et al., 2021; Trivedi et al., 2020; Schlatter et al., 2017). However, it can also be lost from the soil when pressure from the pathogen or presence of the host plant are lessened, or intentionally removed via heat treatment (Carrión et al., 2019).

Identifying critical members of a disease suppressive soil and using management strategies to shift the soil microbiome away from a pathobiome and towards a higher composition of these beneficial taxa could be an effective method for managing soil-borne plant

diseases (Yu et al., 2022; Stewart et al., 2021; Trivedi et al., 2020). This can be achieved through inoculation with specifically designed microbial communities (see below) and by altering soil environmental characteristics that cater to the preferences of various microbial taxa, for example, using agricultural inputs (Stewart et al., 2021; Raaijmakers & Mazzola, 2016).

1.2.3 Synthetic Communities

Another method for altering the soil microbiome to assist in disease management is through the application of synthetic microbial communities, or SynComs, applied as soil inoculants. These SynComs are made up of microbial taxa that perform critical beneficial functions, and their use has been shown to promote plant growth and decrease disease incidence (Li et al., 2021; Liu et al., 2020; Trivedi et al., 2020). Inoculation with entire microbial communities, in contrast to using a single beneficial species, recognizes the community-level interactions that can influence the survival of microbial species and thus the effectiveness of the management technique. While members of the native microbiome are likely able to outcompete a single microbial species, inoculation with SynComs that already consist of strong community networks may be more successful (Wang et al., 2023; Trivedi et al., 2020). For example, Li et al. (2021) found that inoculation with a SynCom composed of four beneficial bacterial genera was more effective at reducing root rot incidence for milkvetch (*Astragalus membranaceous*) than any individual taxa, despite all genera having functions in plant growth promotion or antagonism against the pathogen *Fusarium oxysporum.* While the use of SynComs as a management strategy for Armillaria root disease is likely unfeasible at the landscape level, it may be possible to incorporate in agricultural or horticultural settings.

1.3 Biological Control of Armillaria Root Disease

1.3.1 Microbial Biological Controls

In addition to symbiotic species, key microbial players in a disease suppressive soil include BCAs, living organisms that inhibit pathogen activity through a variety of mechanisms, including the production of antibiotic compounds, competition for limiting resources, or by stimulating plant resistance mechanisms (Bonaterra et al., 2022; Palmieri et al., 2022; Köhl et al., 2019). Further, the application of biocontrol agents has been shown to inhibit the formation of microbial community structures associated with plant pathogens (pathobiomes) (Qiu et al., 2022; Erlacher et al., 2014). When used alone or concurrently with additional control methods, BCAs may be a more environmentally sustainable and cost-effective management option than chemical and cultural controls (Collatz et al., 2021; Rabiey et al., 2019). However, the efficacy of microbial species as BCAs is dependent on their ability to establish a population within the rhizosphere, as both physical protection against a pathogen and the production of antagonistic metabolites rely on having a sufficient population density (Prospero et al., 2021; Paulitz, 2000; Lewis & Papavizas, 1984). Thus, BCA microorganisms must be able to compete effectively with the native microorganisms present within the microbiome, which are adapted to the environmental conditions of the rhizosphere and likely have a competitive advantage (Mazzola & Freilich, 2017). Because of this, microbial strains derived from native soil communities who share this advantage are more successful as biological control agents against soil-borne pathogens than foreign strains (Chen et al., 2019; Mazzola & Freilich, 2017; Mesanza et al., 2016).

1.3.2 Bacterial Biological Controls

Bacterial BCAs inhibit fungal pathogens through a variety of mechanisms, including competition, production of antibiotics, parasitism, and induction of plant defense responses (Bonaterra et al., 2022; Pliego et al., 2011; Whipps, 2001). Further, a variety of effective bacterial BCAs have been identified for *Armillaria* pathogens, including from the genera *Pseudomonas*, *Streptomyces*, *Bacillus*, and *Erwinia* (Zhang et al., 2023; Kedves et al., 2021; Mesanza et al., 2016; de Vasconcellos & Cardosa, 2009; DeLong et al., 2002). For example, in Mesanza et al. (2016), three bacterial species isolated from a radiata pine (*Pinus radiata*) plantation heavily infested with fungal pathogens proved to be effective BCAs for *A. mellea*–*Pseudomonas fluorescens*, *Bacillus simplex*, and *Erwinia bilinigiae*. These species reduced *in vitro* rhizomorph formation and growth of the pathogen *A. mellea*, and during field trials, treatment with these species decreased seedling disease symptoms and mortality when compared to untreated seedlings (Mesanza et al., 2016).

Differences between certain *in vitro* and *in vivo* results with bacterial BCAs in Mesanza et al. (2016) highlight how lab-based testing of BCAs can fail to provide a realistic view of pathogen control under field conditions. For example, *Escherichia coli* provided protection to inoculated seedlings in field trials, but performed less well during dual culture confrontation tests (Mesanza et al., 2016). Dual culture confrontation tests involving the co-culturing of a pathogen and a putatively beneficial organism are frequently used for initial identification of BCAs (Raymaekers et al., 2020). However, these laboratory assays cannot account for the interactions between the BCA, pathogen, and environmental factors or other microorganisms that influence BCA success in rhizosphere colonization and thus in providing protection to the plant (Pliego et al., 2011). For this reason, confirming the results of *in vitro* assays with field trials is critical when evaluating the effectiveness of putative BCAs.

1.3.3 Trichoderma species

The fungal genus *Trichoderma* includes many species that are effective BCAs for various fungal plant pathogens, including those from the genera *Rhizoctonia*, *Alternaria*, *Fusarium*, and *Armillaria* (Asad, 2022; Kedves et al., 2021; Sood, 2020; Chen et al., 2019). *Trichoderma* species can contribute to the control of fungal pathogens through mycoparasitism, antibiotic production, competition, and plant growth promotion. Chen et al. (2019) used dual culture confrontation tests to demonstrate the *in vitro* efficacy of *Trichoderma* species as biocontrol agents for various *Armillaria* pathogens. Multiple species of *Trichoderma*, for example *T. virens*, show inhibitory activity towards *Armillaria* by covering the pathogen colony with mycelia and sporulating after 5 days of co-culturing. The results of these confrontation tests were further confirmed with field assays, in which *T. virens* and *T. atrobrunneum* spore suspension treatment reduced mortality of oak seedlings grown in a high pathogen load environment (Chen et al., 2019). Further transcriptome analyses of a confrontation test between *T. atroviride* and *A. ostoyae* in Chen et al. (2023) helped reveal how *Trichoderma* species may inhibit *Armillaria* pathogens by showing an upregulation of *T. atroviride* genes functioning in antibiotic production, synthesis of cell wall degrading enzymes, and detoxification of foreign chemicals.

1.4 Additional Influences on the Soil Microbiome

1.4.1 Fire

Managing soilborne pathogens by shifting the soil microbiome composition to promote the beneficial symbionts and biocontrol species important in disease suppressive soils may be hindered by other environmental and biological factors. For example, in the northwest United States where *A. solidipes* is prevalent, there has been an increase in the frequency and severity of

fires in recent decades (Halofsky et al., 2020), which have the potential to dramatically alter the diversity and composition of soil bacterial and fungal communities.

Many soil and fire characteristics, including intensity, residence time, organic layer thickness, and water content, interact to affect heat transfer from a fire to the soil, interfering with predictions of the impacts of a fire on a given microbial community (Hart et al., 2005). Despite this, common changes to soil microbial communities across ecosystems following fire have been reported. These include decreased microbial diversity and abundance, as well as compositional changes that favor the relative abundance of taxa with characteristics that allow colonization under the extreme post-fire conditions (Pérez-Izquierdo et al., 2023; Ammitzboll et al., 2021; Sáenz De Miera et al., 2020; Day et al., 2019; Pérez-Valera et al., 2017; Reazin et al., 2016). Further, fires can impact the soil microbiome directly, through heat-induced mortality, and indirectly by changing the vegetative community and edaphic properties (Pérez-Izquierdo et al., 2023; Hart et al., 2005). While reported changes to edaphic characteristics vary, common shifts across ecosystems and fire types include decreased organic matter and increased pH levels (Agbeshie et al., 2022; Certini, 2005), and these changes can be associated with an altered microbiome (Pérez-Izquierdo et al., 2023; Day et al., 2019; Pérez-Valera et al., 2017). Significant shifts in vegetation in forest ecosystems can occur after severe fires resulting in the mortality of dominant overstory trees, allowing herbaceous understory plants from the seed bank to grow. Severe fire and associated tree mortality have an especially detrimental impact on communities of symbiotic microbial species, including many genera of beneficial ectomycorrhizal (ECM) fungi (Day et al., 2019; Reazin et al., 2016; Dahlberg et al., 2001), and post-fire tree survival has been found to be an important factor in sustaining fungal community diversity and composition (Pérez-Izquierdo et al., 2021). However, some ECM fungi, including

the genus *Suillus*, have been found frequently post-fire due to their ability to effectively colonize post-disturbance ecosystems (Pérez-Izquierdo et al., 2021; LeDuc et al., 2013; Visser, 1995).

Other differential impacts to microbial taxa following fire have been reported. For example, communities residing in lower soil layers, which are shielded from intense heat by the organic horizon, show less change in diversity and composition than those in the upper soil layers (Ammitzboll et al., 2021; Pérez-Izquierdo et al., 2021). Fungal communities grouped by ecological lifestyle or taxonomy may also show contrasting responses to fire. For example, Pérez-Izquierdo et al. (2021) reported an increase in the richness of saprophytic fungi following fire, despite an overall decrease in fungal richness. However, Day et al. (2019) found similar decreases to richness and diversity following fire for both total fungal communities and saprophytic fungi. Additionally, Reazin et al. (2016) found compositional changes in fungal communities driven by an increase in the relative abundance of Ascomycetes, while Basidiomycete and Zygomycete abundance decreased after severe fire.

While these many factors interact to influence fire's effects on microbial communities, improving our understanding of how the soil microbial communities associated with pathogen-dominated ecosystems will likely change following fire disturbances is important for predicting how this will alter pathogen activity and the potential for using disease suppressive soils as a management tool.

1.4.2 Plant Exudates

In addition to fires, plants release root exudates which influence the soil microbiome (Yu et al., 2022; Liu et al., 2020; Trivedi et al., 2020). These exudates, such as amino acids and secondary metabolites, alter the chemical and physical environment of the rhizosphere, creating a gradient of soil characteristics from the bulk soil to the rhizoplane (Trivedi et al., 2020; Zhang et al., 2017). This gradient leads to an enrichment of certain microbial taxa near the roots who thrive in the conditions created by the plant exudates.

Host plants can fine-tune this interaction with their microbial community by selectively secreting exudates to promote the abundance of certain taxa. Following an abiotic or biotic stress, plants may use this 'cry for help' strategy to develop a stress-resistant microbiome, with an increased relative abundance of beneficial species that can help them to mitigate the effects of the stressor, a strategy which has been demonstrated in multiple agricultural pathosystems (Yu et al., 2022; Liu et al., 2020; Trivedi et al., 2020). Further, the promotion of a stress-resistant microbiome in response to pathogen attack suggests that the microbial species enriched in the roots and rhizosphere of infected plants may indicate which taxa are effective at preventing or resisting disease. For example, Dudenhöffer et al. (2016) found that barley (*Hordeum vulgare*) plants infected with the fungal pathogen *Fusarium graminearum* secreted exudates that were more attractive to certain Pseudomonad species with presumed disease suppression activity than exudates from uninfected plants, increasing the abundance of these beneficial bacteria in the rhizospheres of infected barley. Qiu et al. (2022) also demonstrated an increased relative abundance of multiple microbial taxa with potential biocontrol activity, including the bacterial genera *Streptomyces* and *Pseudomonas*, in the rhizosphere of cotton (*Gossypium hirsutum*) plants infected with the fungal pathogen *Fusarium oxysporum*. Additionally, Li et al. (2021)

demonstrated that a synthetic community composed of bacterial genera selected for by plants infected with *F. oxysporum* was effective at increasing plant growth and reducing mortality when additional plants faced inoculation with the pathogen. Similarly, Carrión et al. (2019) found that a community composed of *Chitinophaga* and *Flavobacterium*, which were abundant in the endophytic root microbiome associated with disease suppressive soils of sugar beets (*Beta vulgaris*) inoculated with the pathogen *Rhizoctonia solani*, could effectively protect against infection.

Following a fire, surviving plants may secrete exudates to form a microbial community that will help them recover from the environmental stress. In areas with a high incidence of root disease pathogens, it is possible that these recruited communities will include broadly beneficial taxa that can help protect the host from disease, making the microbial communities of living host plants post-fire a place to look for potential biocontrol species.

1.5 *Armillaria solidipes* and *A. altimontana*

1.5.1 A. altimontana *Benefits Tree Health*

In contrast to the aggressive pathogen *A. solidipes*, the species *A. altimontana* has historically been considered a weak or secondary pathogen. However, recent research by Warwell et al. (2019) on the Ida Creek Provenance Test in northern Idaho indicates that on sites where *A. solidipes* and *A. altimontana* co-occur, *A. altimontana* may benefit tree health. In this study, indicators of Armillaria root disease, such as mycelial fans and wood rot, were associated with 74.7% of *A. solidipes* isolates from western white pine (*P. monticola*) trees, while 98.2% of *A. altimontana* isolates were not associated with disease. Measurements on tree height, diameter at breast height (DBH), and survival rate were also taken from the time the plantation was

established in 1971 until 1987. By 1987, trees colonized with only *A. altimontana* had higher average height, DBH, and percent survival than trees colonized only with *A. solidipes*, and significantly higher average measurements for height and percent survival were observed compared to trees colonized with both species and with neither (Warwell et al., 2019). Finally, spatial distributions of the two *Armillaria* species across the site revealed that *A. altimontana* may be outcompeting *A. solidipes* over time. While the locations of individual genets indicated that both species were once found throughout the site, the 1987 survey and subsequent studies show a progressively decreasing prevalence of *A. solidipes* (Warwell et al., 2019).

1.5.2 Microbial Community Differences

Unique differences in the microbial communities associated with *A. solidipes* and *A. altimontana* may contribute to their differing ecological roles. Ibarra Caballero et al. (2023) reported no significant differences between the richness or diversity of the fungal and bacterial communities associated with the two fungi, though certain microbial taxa were found to be more relatively abundant in either community. For example, the bacterial family Enterobacteriaceae was more abundant in communities associated with *A. solidipes*, and three families of ECM fungi – Suillaceae, Rhizopogonaceae, and Atheliaceae – were more abundant in association with *A. altimontana* (Ibarra Caballero et al., 2023). The higher relative abundance of Enterobacteriaceae in *A. solidipes*-associated soils could indicate that members of this taxa are part of the pathobiome and thus contributing to disease progression and decline in host health. The ECM fungi associated with *A. altimontana* form symbiotic associations with their tree hosts, aiding with water and nutrient acquisition. Thus, the association of *A. altimontana* with these beneficial fungi may help to explain why the presence of *A. altimontana* is correlated with

healthier trees (Warwell et al., 2019) and suggests that these fungal families are important components of disease suppressive soils for *A. solidipes*.

1.6 Research Objectives

As fires in the Western United States continue to increase in severity and frequency due to anthropogenic activity and climate change, there is a critical need to understand how forest diseases will influence regenerating forests and develop management strategies to reduce any compounding effects of fire and biotic disease. Current methods to manage Armillaria root disease in these locations are ineffective at the landscape scale, leading to large areas of mortality and altered forest structure and composition where disease is present. Developing new management strategies that consider wildfire effects will help preserve and sustain these forest ecosystems.

This research aims to understand how different levels of burn severity impact the native soil microbial communities associated with the virulent forest pathogen *A. solidipes* in western conifer forests and how these impacts will affect the biological control and management of Armillaria root disease. Metagenomic analyses of fungal and bacterial communities from ecosystems where *A. solidipes* is prevalent that have experienced different levels of burn severity will show how microbial community composition and diversity are affected by fire, with particular consideration to the effects on taxa that constitute putative disease suppressive soils and thus may be important for restricting pathogen growth and disease spread. Isolation of fungal and bacterial species from soils collected on burn sites and *in vitro* confrontation tests with *A. solidipes* will aid in identifying novel BCAs that can be incorporated into sustainable management strategies. Finally, investigating how the presence or abundance of these BCAs changes under different burn severity levels will reveal how the use of these taxa in a

management strategy for *A. solidipes* may be impacted by prevalent environmental influences. Overall, the knowledge gained from this research will promote the development of more effective management strategies for Armillaria root disease by responding to the changes in microbial communities following fires.

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CHAPTER 2: FIRE-ASSOCIATED MICROBIAL SHIFTS IN SOILS OF WESTERN CONIFER FORESTS WITH ARMILLARIA ROOT DISEASE

2.1 Summary

Fires in coniferous forests throughout the northern United States alter ecosystem processes and ecological communities, including the diversity and composition of microbial communities living in the soil. In addition to its influence on ecosystem processes and functions, the soil microbiome can interact with soilborne pathogens to facilitate or suppress plant disease development. Altering the microbiome composition to promote taxa that inhibit pathogenic activity has been suggested as a management strategy for forest diseases, including Armillaria root disease caused by *Armillaria solidipes*, which causes growth loss and mortality of conifers. These forest ecosystems are experiencing increased wildfire frequency and burn severity that could influence *A. solidipes* activity and interactions of the soil microbiome with Armillaria root disease. In this research, we examine changes to the soil microbiome following three levels of burn severity in a conifer forest in northern Idaho, United States, where Armillaria root disease is prevalent. We further determine how these changes correspond to the soil microbiomes associated with the pathogen *A. solidipes,* and a putatively beneficial species, *A. altimontana*. At 15-months post fire, we found significant decreases in bacterial community richness and diversity associated with increasing burn severity, yet no significant changes to these metrics were found in fungal communities following fire. Shifts in the richness and alpha diversity of bacterial and fungal communities, especially community evenness, were also associated with various soil characteristics, including organic matter and nitrogen content. However, both

bacterial and fungal communities showed compositional changes associated with burn severity, including microbial taxa with altered relative abundance. Further, significant differences in the relative abundance of certain microbial taxa in communities associated with the three burn severity levels overlapped with taxa associated with various *Armillaria* species. For example, following severe burn, we observed a decreased relative abundance of the beneficial ectomycorrhizal fungi associated with the microbial communities of *A. altimontana* that may contribute to the antagonistic activity of this soil microbial community. Additionally, *A. solidipes* and associated microbial taxa were found to dominate following high-severity burns, suggesting that severe fires provide suitable environmental conditions for these species. Overall, our results suggest that shifts in the soil microbiome and an associated increase in the activity of *A. solidipes* following high-severity burns in similar conifer forests may result in priority areas for monitoring and proactive management of Armillaria root disease.

2.2 Introduction

Fires in forest ecosystems are important disturbance events that alter ecosystem processes and ecological communities, including the soil microbial community. Fire influences on soil microbial communities have broader implications for nutrient cycling, plant-microbe, and microbe-microbe interactions (Certini, 2005; Hart et al., 2005). Fires can alter microbial communities through direct, heat-induced mortality or indirectly through changes to vegetation and soil properties, yet predicting fire impacts on a given community is difficult due to interactions among edaphic and fire characteristics that affect heat transfer to the soil (Hart et al., 2005). Commonly observed changes to microbial communities following fire include reduced biomass, diminished diversity, and changes in community composition due to selection for taxa adapted to withstand fire or effectively colonize post-disturbance conditions (Pérez-Izquierdo et

al., 2023; Ammitzboll et al., 2021; Sáenz De Miera et al., 2020; Day et al., 2019; Pérez-Valera et al., 2017; Reazin et al., 2016). Such changes may be attributable to common post-fire edaphic changes, including increased soil pH and decreased organic matter (Agbeshie et al., 2022; Certini, 2005). Further, extensive mortality of dominant overstory plants following severe fires can be especially detrimental to populations of microbial symbionts (Pérez-Izquierdo et al., 2021; Hart et al., 2005).

Recent research has accentuated the importance of the soil microbiome in creating conditions that are either conducive or unfavorable to plant disease development, termed a pathobiome or a disease suppressive soil, respectively (Stewart et al., 2021; Trivedi et al., 2020; Carrión et al., 2019). Transitions from a healthy microbiome to a pathobiome with the onset of disease, including changes in the relative abundance of key microbial taxa, has been demonstrated in multiple agricultural settings (Qiu et al., 2022; Erlacher et al., 2014). Plants can also selectively secrete exudates in response to biotic or abiotic stressors to promote the abundance of microbial taxa in the rhizosphere that assist in resistance or recovery, thereby fostering the formation of a plant-associated disease suppressive soil (Yu et al., 2022; Liu et al., 2020; Trivedi et al., 2020; Dudenhöffer et al., 2016). Consequently, post-fire changes to microbial community composition can indirectly affect plant disease development by altering the diversity and abundance of taxa that play key roles in a pathobiome or disease suppressive soil.

Fungal pathogens that cause Armillaria root disease are responsible for mortality and growth reduction of woody plants in agronomic, horticultural, and natural settings worldwide (Kim et al., 2022; Murray & Leslie, 2021; Baumgartner et al., 2011; Cruickshank, 2000; Shaw & Kile, 1991). In the northwestern United States, the native species *A. solidipes* (formerly North American *A. ostoyae*) acts as a virulent pathogen, causing a white rot of primarily conifer hosts

that reduces structural integrity of the roots and lower bole, diminishes growth, and increases susceptibility to additional mortality agents (Kim et al., 2021; Shaw & Kile, 1991). The resulting tree mortality can alter forest structure and composition, with individual genets of *A. solidipes* capable of encompassing over 900 hectares of land (Ferguson et al., 2003). Current control methods for Armillaria root disease, such as root collar excavation and soil fumigation, are economically and logistically impractical at this forest landscape scale (Mesanza et al., 2016; Baumgartner et al., 2011).

In contrast to *A. solidipes*, the native species *A. altimontana* [formerly North American biological species (NABS) X] is considered a secondary pathogen (Kim et al., 2023). Evidence from research conducted at the Priest River Experimental Forest in Idaho, United States, suggests that *A. altimontana* may be beneficial to the health of western white pine (*Pinus monticola*) on sites where it co-occurs with *A. solidipes* (Warwell et al., 2019). A tree health assessment at 16-years post-planting showed that trees associated solely with *A. altimontana* had increased height, diameter at breast height (DBH), and survival rates compared with trees associated with *A. solidipes.* Trees associated with *A. altimontana* also had greater height and survival rates compared to trees associated with both *Armillaria* species and with neither *Armillaria* species (Warwell et al., 2019). Further, the distribution of these two *Armillaria* species across the field site indicated that *A. altimontana* was gradually outcompeting *A. solidipes* and thus reducing disease prevalence.

The benefits to tree health and competitive advantage of *A. altimontana* suggest a putative disease suppressive soil that could effectively control *A. solidipes*. When compared to the *A. solidipes* microbial communities, the soil microbial community of *A. altimontana* showed a higher relative abundance of three families of ectomycorrhizal (ECM) fungi (Caballero et al.,

2023). ECM fungi form symbiotic relationships with tree hosts and assist with the uptake and transport of key nutrients and water from the soil (Smith & Read, 2010), possibly contributing to the increased survival rates and tree growth associated with *A. altimontana* in Warwell et al. (2019). In contrast, a higher relative abundance of the bacterial family Enterobacteriaceae was found in the microbial community of *A. solidipes* when compared with an *A. altimontana*-associated community, suggesting a potential role for Enterobacteriaceae in the *A. solidipes* pathobiome (Ibarra Caballero et al., 2023). In coniferous forests where *A. solidipes* is prevalent, practices to shift the composition of the soil microbial community toward taxa from a putative disease suppressive community could be part of an effective strategy for landscape scale management of Armillaria root disease.

While previous research has demonstrated how soil microbial communities change in response to fire (e.g., Pérez-Izquierdo et al., 2021; Pérez-Valera et al., 2017) and examined the potential importance of microbial communities influencing the activity of *A. solidipes* (Ibarra Caballero et al., 2023), they have not yet examined how burn severity interacts with the soil microbiome to affect Armillaria root disease. Impacts from these interacting influences are increasingly important, as climate change and anthropogenic activity continue to raise fire severity and frequency in the regions of North America where *A. solidipes* is prevalent (Halofsky et al., 2020). The objective of this study was to determine how different levels of burn severity influence soil microbial communities in coniferous forest ecosystems where *A. solidipes* and *A. altimontana* co-occur, particularly how burn severity affects taxa that compose putative pathobiomes and disease suppressive soils for Armillaria root disease. To investigate these interactions, we examined differences in composition and diversity of bacterial and fungal communities in soils associated with three different burn-severity levels (high, low, and

unburned) at 15-months post-fire in a coniferous forest in northern Idaho, United States, where Armillaria root disease caused by *A. solidipes* naturally occurs. The results presented here can foster the development of novel management strategies for Armillaria root disease by determining how burn severity influences disease development through its impact on the soil microbiome.

2.3 Materials and Methods

2.3.1 Sample Collection

In July 2021, portions of a mature conifer forest within the Nez Perce-Clearwater National Forest in northern Idaho, United States, experienced a severe wildfire (Sand Mountain Fire), resulting in large areas of dead trees. During the fire, forest managers attempted to manage the spread of the fire with controlled burns, producing areas of low-severity burn in which the trees had experienced fire but remained alive. Unburned areas were also interspersed within the burn areas, resulting in a study site with three levels of burn severity: high, low, and unburned (Figure 2.1). Further, Armillaria root disease caused by *A. solidipes* is known to occur on the site (G.I. McDonald and J.W. Hanna, unpublished observations). Throughout all burn levels, the dominant forest type was composed primarily of western white pine (WWP), western redcedar (*Thuja plicata*), grand fir (*Abies grandis*), Douglas-fir (DF; *Pseudotsuga menziesii*), western larch (*Larix occidentalis*), and western hemlock (*Tsuga heterophylla*). Soils throughout the site are primarily silty loams mixed with volcanic ash.

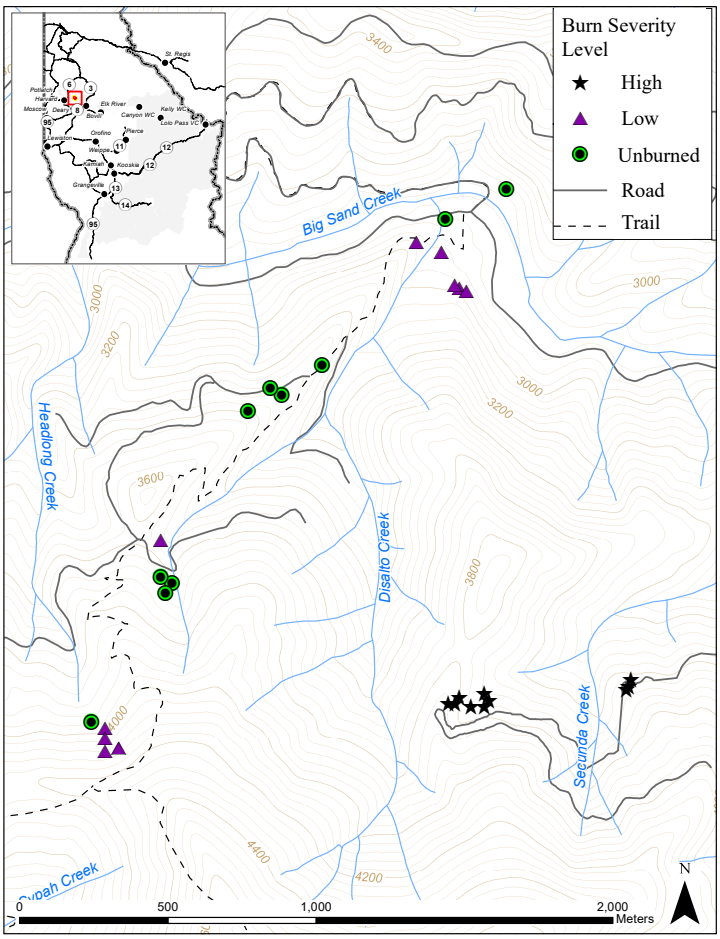

Figure 2.1. Site map of sampled trees in mature conifer forest within the Nez Perce - Clearwater National Forest (near Laird Park Campground) in northern Idaho, United States that experienced a fire (Sand Mountain Fire). Burn-severity level (high, low, unburned) associated with each tree is indicated by color and shape.

Within each burn severity area, five mature WWP (DBH 40 to 98 cm) and five mature DF (DBH 41 to 68 cm) trees were identified for sampling, resulting in 30 sampled trees. Sampled trees were spaced at least 20 meters apart. In October 2022, two soil cores were collected near the base of each tree, 2 meters from the bole and in opposite cardinal directions. The top 1-5 cm of each soil core, composed mostly of forest floor litter, were removed, producing cores of depth 5-20 cm. The two soil cores from each tree were homogenized and 2 g from the homogenized soil were placed in 4 mL of LifeGuard™ Preservation Solution (MO BIO

Laboratories, Inc., Carlsbad, CA, USA). The remaining soil samples and tubes containing soil in LifeGuard solution were stored at -20°C until later use.

Surveys for *Armillaria* species were performed in October 2022 and June 2023 by excavating main lateral roots (ca. 0.5 m deep and 1 m away from the bole) of each sampled tree to collect rhizomorph samples, and the outer bark of the roots and lower bole was removed to collect mycelial fan samples. Rhizomorph samples were placed in 15-mL Falcon ® tubes while mycelial fans were stored in paper bags. All *Armillaria* samples were stored in a refrigerator at 4°C for 2-4 days until isolation.

2.3.2 Armillaria Isolation and Identification

In a laminar flow hood, *Armillaria* mycelial fan samples were separated from the bark using a sterile blade, and a small section of fungal tissue was excised from each fan. These samples were plated in a Petri dish on modified malt extract agar media for *Armillaria* cultures (Armillaria MEA: 3% malt extract, 3% dextrose, 1.5% peptone, 1.5% agar; Kim et al., 2006).

For surface sterilization, rhizomorph samples were first soaked in a 20% commercial bleach solution (1.5% NaClO) for 8 minutes, then rinsed with sterile distilled water, and the samples were transferred to 3% H_2O_2 for 7-10 minutes. Samples were moved to the laminar flow hood and soaked in sterile distilled water for 2-10 minutes. Treated rhizomorphs were then cut into sections approximately 1-cm long and embedded vertically into Armillaria MEA in Petri dishes. All *Armillaria* sample plates were incubated in the dark at 20°C.

To obtain mycelia for DNA extractions, *Armillaria* isolates were subcultured onto sterile 0.22-µm pore MF-Millipore™ Membrane nylon filters (MilliporeSigma, Burlington, MA, USA) overlayed on modified Armillaria MEA (1.5% malt extract, 1.5% dextrose, 0.5% peptone, 1.2%

agar) in Petri dishes. SYNERGY™ 2.0 Plant DNA Extraction Kits (OPS Diagnostics, Lebanon, NJ) and manufacture protocols were used to extract DNA from the *Armillaria* mycelia. Extracted DNA was quantified with a Nanodrop™ 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and diluted with sterile deionized (DI) H_2O to a concentration of 10 ng/uL. Polymerase chain reaction (PCR) with primers EF983F (5'-GCYCCYGGHCAYCGTGAYTTYAT - 3') (Rehner & Buckley, 2005) and ARMEF-R (5'-TACCCGTTCGGCGATCAATCT-3') (Elías-Román et al., 2013) or 2218R (5'-ATCATGACACCRACRGCRACRGTYTG-3') (Rehner & Buckley, 2005) and an Eppendorf Mastercycler pro Thermal Cycler (Eppendorf, Hamburg, Germany) were used to amplify the translation elongation factor 1-alpha (*tef1*) region of the DNA. The PCR cycle was 1 minute at 94°C, 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds, before a final 10 minutes at 72°C. Amplicons were visualized using gel electrophoresis, cleaned with ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Santa Clara, CA, USA), and sent to Eurofins Genomics (Louisville, KY, USA) for sequencing using either the previously mentioned *tef1* primers or four *Armillaria*-specific sequencing primers (ARMEF-F3A, ARMEF-R2, ARMEF-FI2, and ARMEF-RI2) (Elías-Román et al., 2018). The resulting sequences were used with the BLASTn search engine of the NCBI (National Center for Biotechnology Information) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) database to identify the *Armillaria* species.

2.3.3 Soil DNA Extraction and Sequencing

For soil DNA extraction, one tube containing soil and LifeGuard™ Preservation Solution was thawed at room temperature and centrifuged for 5 minutes at 12,300 g to consolidate soil materials. The supernatant was discarded and approximately 250 mg of saturated soil was placed

in a sterile tube. DNA extractions were performed using Qiagen DNeasy Power Soil Pro kits (Qiagen®, Carlsbad, CA, USA) following manufacture protocols. Quantity and quality of the extracted DNA was assessed with a Nanodrop™ 1000 spectrophotometer and approximately 50 µL of each sample was sent to Novogene Corp. Inc (Sacramento, CA) for metabarcoding. Amplicon metabarcoding was performed using 515F (5'-GTGCCAGCMGCCGCGGTAA-3') (Caporaso et al., 2010) and 907R (5'-CCGTCAATTCCTTTGAGTTT-3') (Lane et al., 1985) primers to amplify the 16S V4-V5 region of bacteria, and ITS3 (5'-GCATCGATGAAGAACGCAGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990) primers to amplify the ITS2 rDNA region of fungi. These data are available from the NCBI database (BioProject ID PRJNA1098642), which includes both the bacterial and fungal sequencing results.

2.3.4 Characterization of the Soil Microbiome

The program FastQC (Andrews, 2010) was used to remove adapters and low-quality reads from the soil metabarcoding sequences. All remaining analyses were performed through R (R Core Team, 2022) using the interface R Studio (version 1.1.456; RStudio Team, 2016). The dada2 package (Callahan et al., 2016) was used to construct an amplicon sequence variant (ASV) table of bacterial and fungal ASV's. Identification of the 16S and ITS2 ASV's was performed by matching to the ITGDB (Hsieh et al., 2022) and UNITE (Abarenkov et al., 2024) databases, respectively. Sequencing of the ITS2 region of sample rDNA included ASV's from other eukaryotic organisms within the sample; these non-fungal ASV's were removed from further analyses.

The phyloseq package (McMurdie & Holmes, 2013) was used to calculate microbial community richness and alpha diversity, as measured by the Shannon's diversity index (*H'*) and

the inverse Simpson's index ($1/D$). Both indices account for the richness, number of unique ASV's, and evenness, how evenly abundant these ASV's are within the sample. However, Shannon's diversity index gives more weight to increased sample richness, while inverse Simpson's index prioritizes evenness (Nagendra, 2002). Using the car package (Fox & Weisberg, 2019), type III ANOVA analyses were used to determine the influence of burn severity (high, low, and unburned), host tree species (DF and WWP), and association with *Armillaria* species (*A. solidipes*, *A. altimontana, A. solidipes* and *A. altimontana*, *A. sinapina,* and none) on microbial community richness and alpha diversity. ANOVA assumptions were first assessed through diagnostic plots and Shapiro-Wilk (Shapiro & Wilk, 1965) and Levene (Levene, 1960) tests, and appropriate data transformations were applied as needed. Additional pairwise comparisons were performed with Tukey adjusted *p*-values in the emmeans package (version 1.8.4-1; Lenth, 2023) if there was a significant effect ($p < 0.05$) of burn severity, host species, or *Armillaria* species association.

Compositional differences between communities grouped by burn severity, host tree species, burn severity and host tree species (BurnSpecies), and *Armillaria* species (*A. solidipes*, *A. altimontana, A. solidipes* and *A. altimontana*, *A. sinapina,* and none) association were visualized through 3-dimensional non-metric multidimensional scaling (NMDS) plots created using the vegan package (version 2.6-4; Oksanen et al., 2022) and 2-dimensional NMDS and principal coordinate analysis (PCoA) plots created with the phyloseq package. A permutational multivariate ANOVA (PERMANOVA) was used to assess statistical differences among communities using the vegan package. Permutests were also used to check for homogeneity of variance among communities. Pairwise comparisons using PAST software (version 4.14;

Hammer & Harper, 2001) were performed on communities with statistically significant differences in composition and Bonferroni-adjusted *p*-values were calculated.

The ggplot2 package (Wickham, 2016) was used to create pie charts representing bacterial genera present at ≥ 2.5% relative abundance and fungal genera present at ≥ 5% relative abundance in BurnSpecies communities (Supplemental Figures 2.4 and 2.5). Bacterial and fungal ASV's were consolidated by genus; those present in significantly different relative abundance (adjusted *p*-value <0.05) among burn severity and *Armillaria* species (*A. solidipes*, *A. altimontana,* and none) association communities were identified using the metagenomeSeq package (Paulson et al., 2013). Taxa present in only one sample were removed to obtain a more accurate representation of genera responsible for differences. Relative abundance data were used to generate Venn diagrams depicting genera that proliferated in communities associated with each burn-severity level or *Armillaria* species when compared with the others.

2.3.5 Bacterial and Fungal Isolation from Soils

Within 8 weeks of collection, the homogenized soil samples were thawed and serial dilutions were performed. From each sample, 2 g of soil were added to 5 mL of sterilized DI H_2O, inverted 5 times to mix, and left to settle for 30 seconds. From the resulting supernatant, 200 µL was added to 1.8 mL of DI water, which was again inverted and left to settle. This process was repeated three more times, for dilutions of 1:10, 1:100, 1:1000, and 1:10000. For each dilution, 150 µL was placed on culture medium in a Petri dish and spread with a sterilized glass probe. Four plates of each dilution were made: two with nutrient agar (2.3% nutrient agar) and two with quarter-strength potato dextrose agar (PDA) with antibiotics (0.975% potato dextrose agar, 1.125% agar, 0.01% streptomycin, 0.005% chloramphenicol), for a total of 16 plates per soil sample. Plates were placed in a dark incubator at 23°C and monitored. As

microbial growth appeared, unique bacterial and fungal colonies were visually identified and transferred to new plates. These microbial isolates were incubated to allow growth in pure culture before subsequent DNA extraction.

A modified colony PCR procedure was used to extract DNA from bacterial cultures. A small sample of the bacterial colony was mixed with 40 µL of molecular grade H_2O followed by heating at 98°C for 15 minutes to lyse the cells. Tubes were vortexed and then briefly centrifuged. Amplification of the 16S V4-V5 region of bacterial DNA was performed with the Eppendorf Mastercycler pro Thermal Cycler with primers fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rP2 (5'-ACGGCTACCTTGTTACGACTT-3') (Weisburg et al., 1991). The PCR cycle was 95°C for 10 minutes, 35 cycles of 95°C for 2 minutes, 54°C for 1 minute, and 72°C for 2 minutes, before a final 10 minutes at 72°C.

To extract DNA from fungal cultures, a pipette tip was used to scrape a small amount of mycelia from the edge of the colony, which was added to a tube containing 100 µL of 5% Chelex™ 100 Resin solution (Bio-Rad Laboratories, Hercules, CA, USA). Tubes were heated at 98°C for 20 minutes, then briefly centrifuged. The resulting supernatant was used as the DNA template for PCR. The ITS region of fungal DNA was amplified with primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). The PCR cycle was 95°C for 2 minutes, 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute, before a final 2 minutes at 72°C.

Fungal cultures for which initial DNA extraction failed were subjected to a second round of extractions using a modified NaOH procedure from Wang et al. (1993). Again, a pipette tip was used to add a small amount of mycelia from each culture to a tube containing 300 µL 0.5 M NaOH and five glass beads, then vortexed for one minute and centrifuged for one minute at

15,000 g. From the resulting supernatant, 5 µL of DNA was added to a new tube with 495 µL 100 uM Tris-HCl pH 8.0. Primers and PCR settings from the Chelex ™ procedure were used to amplify the ITS region.

Cultures that failed extractions three times were removed from further analyses. Successful amplicons were visualized using gel electrophoresis, cleaned with ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific), and sent to Eurofins Genomics (Louisville, KY) for Sanger sequencing in the forward direction. These sequences were identified using the BLASTn search function of the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). For comparison with metabarcoding data, isolates that could not be confidently identified to the genus level were removed from analysis.

2.3.6 Influence of Soil Characteristics

Approximately 250 g of each soil sample was sent to Ward Laboratories, Inc. (Brighton, MI, USA) for analysis of soil chemical characteristics. All analyses of soil characteristics were conducted using the RStudio (version 1.1.456; RStudio Team, 2016) interface for R (R Core Team, 2022). Pearson's correlation analysis was used to determine which soil characteristics were highly correlated. One of two characteristics with a correlation coefficient above 0.80 or below -0.80 were removed to improve the strength of the model (Supplemental Table 2.1). Significant predictor variables from the filtered list were selected through stepwise analysis using Akaike Information Criterion (AIC), and these predictor variables were then used to create multiple linear regression models with alpha diversity indices as the dependent variable. Type III ANOVAs were ran on these regression models using the car package to evaluate the relationships between the soil characteristics and microbial richness and alpha diversity.

Diagnostic plots and Shapiro-Wilk tests were used with each model to confirm ANOVA assumptions had been met and data transformations were applied as necessary.

Type III ANOVA analyses were also used to determine the effect of burn severity on each soil characteristic. If linear models failed to meet the ANOVA assumptions, as evaluated by diagnostic plots and Shapiro Wilks and Levene tests, the necessary data transformations were applied, or Kruskal-Wallis tests (Kruskal & Wallis, 1952) were performed as a non-parametric alternative. If a significant effect (p <0.05) of burn severity was found, additional pairwise comparisons were performed using the emmeans package and Tukey-adjusted p-values were calculated.

2.4 Results

2.4.1 Armillaria Collection and Identification

A total of 18 rhizomorph and 11 mycelial fan samples were collected in October 2022 and June 2023 (Table 2.1). Pure cultures were obtained from most samples; however, four rhizomorph and three mycelial fan samples did not yield pure cultures due to contamination and/or absence of viable fungal tissue. Sequencing of the *tef1* region identified seven of the eight cultures obtained from mycelial fan samples as *A. solidipes* and one as *A. altimontana*. Of the 14 cultures obtained from rhizomorph samples, 10 were identified as *A. altimontana,* two *A. sinapina,* and two *A. solidipes*. Three rhizomorph isolates from June 2023 were duplicates of rhizomorph isolates collected in October 2022 from the same tree. In addition, both mycelial fan and rhizomorph samples were collected from four trees, including DF and WWP hosts from high-severity burn sites. On two of these trees, samples were identified as the same *Armillaria* species, however, on the other two trees the rhizomorph samples were identified as *A. altimontana* and the mycelial fan samples were identified as *A. solidipes*. Six of the seven trees

with *Armillaria* samples from high-severity burn sites were colonized by *A. solidipes* mycelial fans, which may indicate a preexisting Armillaria root disease pocket in the area and/or post-fire saprophytic colonization. In contrast, only two of the eight trees with *Armillaria* samples from low-severity burn or unburned sites were colonized by *A. solidipes*. GenBank accession numbers for the *Armillaria* isolate sequences can be found in Supplemental Table 2.8.

Table 2.1. Identification of *Armillaria* isolates associated with sampled trees. Isolate information, including GenBank accession numbers, can be found in Supplemental Table 2.8.

Tree Number	**Tree Species[A]: Burn Severity**	**Sample type: rhizomorph (R); mycelial fan (MF)**	***Armillaria* sp.[B] Identification (*tef1*)[C]**
3	DF:High	MF	SOL
4	DF:High	R, MF	ALT (R), SOL (MF)
5	WWP:High	R, MF	SOL
6	WWP:High	MF	SOL
7	WWP:High	R, MF	ALT (R), SOL (MF)
8	DF:High	R, MF	ALT
10	DF:High	MF	SOL
24	DF:Low	R	ALT
29	DF:Low	R	ALT
14	DF:Unburned	R	ALT
15	DF:Unburned	R	SOL
16	WWP:Unburned	R	SIN
20	WWP:Unburned	R	SIN
26	WWP:Unburned	R	ALT
27	DF:Unburned	MF	SOL

[A]DF = Douglas-fir (*Pseudotsuga menziesii*), WWP = western white pine (*Pinus monticola*)
[B]SOL = *A. solidipes*, ALT = *A. altimontana*, SIN = *A. sinapina*
[C]*tef1* = translation elongation factor 1-alpha gene

2.4.2 Soil Metabarcoding

All 30 soil DNA samples were used for sequencing of the ITS2 region; however, only 29 were used for the 16S region because of low DNA quality. A total of 3,870,864 reads were generated from sequencing of the 16S region, with an average of 133,478 reads per sample and a range of 64,209 – 148,974 reads. In total, 3,694,647 reads were produced from ITS2 sequencing,

with an average of 123,155 reads per sample and a range of 17,961 – 148,725 reads. The sequencing depth of each sample was confirmed with rarefaction curves (Supplemental Figure 2.1). After filtering out low-quality reads, merging paired ends, and removing identical reads and chimeras, a total of 2,927,776 16S reads remained, averaging 100,958 reads per sample and ranging from 41,478 – 121,438 reads. Processing resulted in a total of 2,678,572 reads of the ITS2 region, with an average of 89,286 reads per sample and a range of 13,550 – 110,994 reads. Matching to the ITGDB and UNITE databases identified 20,692 unique ASVs from the sequencing of the 16S region and 4,679 unique ASVs from sequencing of the ITS2 region. After ASVs from non-fungal organisms were removed from the ITS2 results, 4,041 unique ASVs remained.

2.4.3 Characterization of the Soil Microbiome

Alpha Diversity

Significant differences in bacterial community alpha diversity, as measured by richness and Shannon's diversity index, were identified based on burn severity ($F_{2,23} = 31.84$, $p < 0.0001$ and $F_{2,23} = 9.33$, $p = 0.001079$, respectively). Pairwise comparisons showed that differences in richness occurred between high-severity burn and unburned DF communities, high-severity burn and low-severity burn WWP communities, high-severity burn and unburned WWP communities, and low-severity burn and unburned WWP communities (Table 2.2, Figure 2.2A). Pairwise comparisons of Shannon's diversity showed significant differences between high-severity burn and unburned WWP communities and low-severity burn and unburned WWP communities (Table 2.2, Figure 2.2C). Both bacterial community richness and Shannon's diversity increased progressively as burn severity decreased. No statistically significant differences in bacterial richness or alpha diversity, as measured by Shannon's diversity index or inverse Simpson's

index, were identified based on host tree species, and no significant differences were identified in the bacterial community inverse Simpson's index based on burn severity.

No significant differences were found in the richness or alpha diversity of fungal communities based on burn severity or host tree species (Figure 2.2B,D), and no significant differences in richness or alpha diversity of fungal or bacterial communities occurred based on the association with different *Armillaria* species.

Table 2.2. Results from pairwise comparisons of richness and Shannon's diversity index (*H'*) of bacterial communities grouped by host tree species [Douglas-fir (DF) and western white pine (WWP)] burn severity level (high, low, unburned).

Host Tree Species	**Alpha Diversity Index**	**Burn Community Comparison**	**DF**	***t*-value**	**Tukey-adjusted *p*-value**
DF	Richness	High vs. low	23	-1.845	0.1778
DF	Richness	High vs. unburned	23	-3.970	0.0017
DF	Richness	Low vs. unburned	23	-2.125	0.1068
DF	*H'*	High vs. low	23	-0.441	0.8988
DF	*H'*	High vs. unburned	23	-2.477	0.0529
DF	*H'*	Low vs. unburned	23	-2.036	0.1262
WWP	Richness	High vs. low	23	-3.259	0.0093
WWP	Richness	High vs. unburned	23	-7.310	<0.0001
WWP	Richness	Low vs. unburned	23	-3.633	0.0038
WWP	*H'*	High vs. low	23	-0.239	0.9690
WWP	*H'*	High vs. unburned	23	-3.159	0.0117
WWP	*H'*	Low vs. unburned	23	-2.740	0.0302

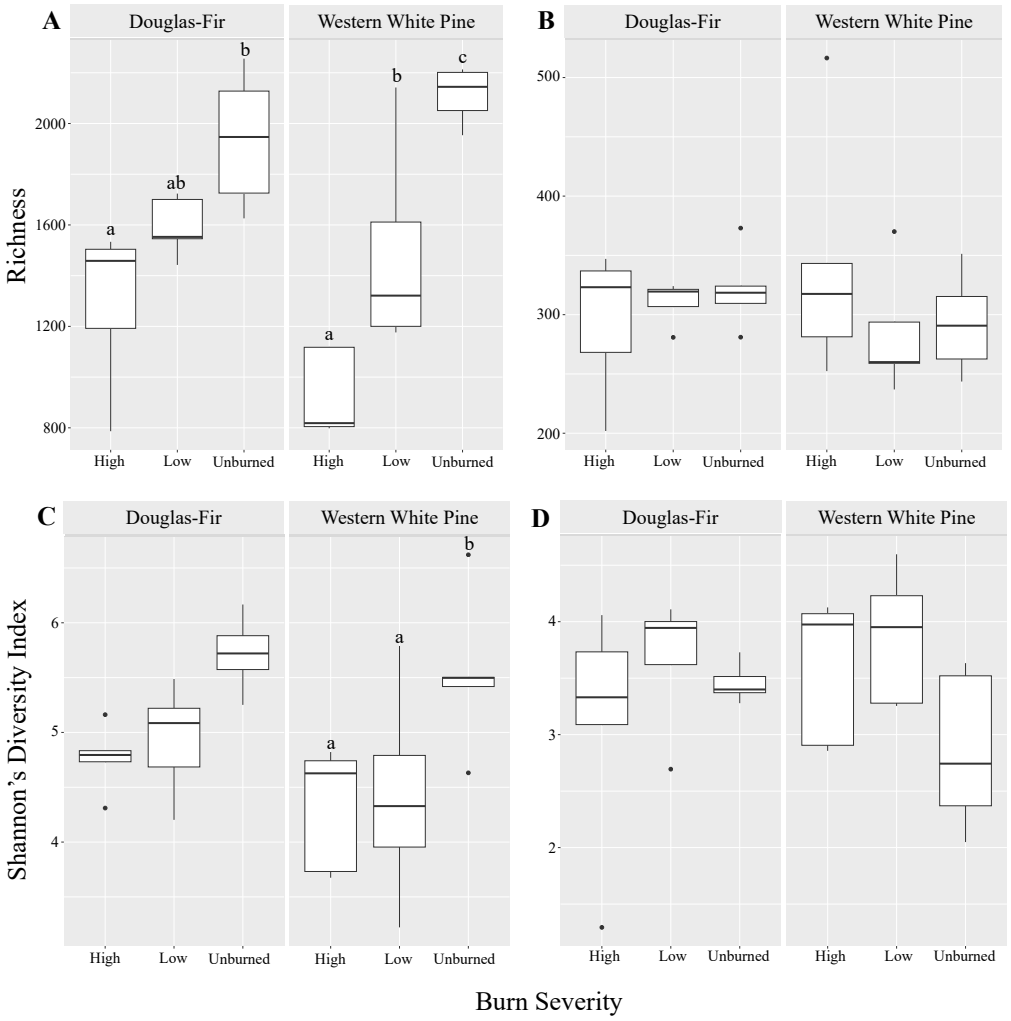

Figure 2.2. Alpha diversity, as measured by richness and Shannon's diversity index, of bacterial (A, C) and fungal (B, D) communities separated by burn-severity level (high, low, and unburned) and host tree species [Douglas-fir (*Pseudotsuga menziesii*) and western white pine (*Pinus monticola*)].

Beta Diversity

PERMANOVA tests revealed significant differences among bacterial and fungal community composition based on burn severity and BurnSpecies (Table 2.3). However, fungal BurnSpecies communities failed to pass the permutest, indicating that significant PERMANOVA results may be from unequal dispersion within the groups, rather than true compositional differences. Bonferroni-adjusted *p*-values generated from pairwise comparisons indicated

significant differences between high-severity burn and unburned groups and between low-severity burn and unburned groups for bacterial and fungal communities (Supplemental Table 2.2). Further, visual inspection of the NMDS plots suggests an increased variance in community composition associated with high-severity burns (Figures 2.3, 2.4), which was also supported by the PCoA plots (Supplemental Figure 2.2). Pairwise comparisons with Bonferroni adjustment found no significant differences between bacterial BurnSpecies communities. Due to permutest failure, pairwise comparisons were not run on fungal BurnSpecies communities. However, the NMDS plots show overlap among communities, especially low-severity burn and unburned communities (Figure 2.4C,D). No significant effects were found for host tree species or *Armillaria* species association on microbial community composition (Supplemental Figure 2.3).

Table 2.3. Results from PERMANOVA tests indicating significant differences between bacterial and fungal community composition when grouped by burn severity (high, low, unburned) and BurnSpecies [burn severity and host tree species, Douglas-fir (*Pseudotsuga menziesii*) or western white pine (*Pinus monticola*)].

Community	Group	Df	F-value	*P*-value
Bacteria	Burn Severity	2	2.3074	<0.001
	BurnSpecies	5	1.4332	0.003
Fungi	Burn Severity	2	2.1756	<0.001
	BurnSpecies	5	1.5603	0.002

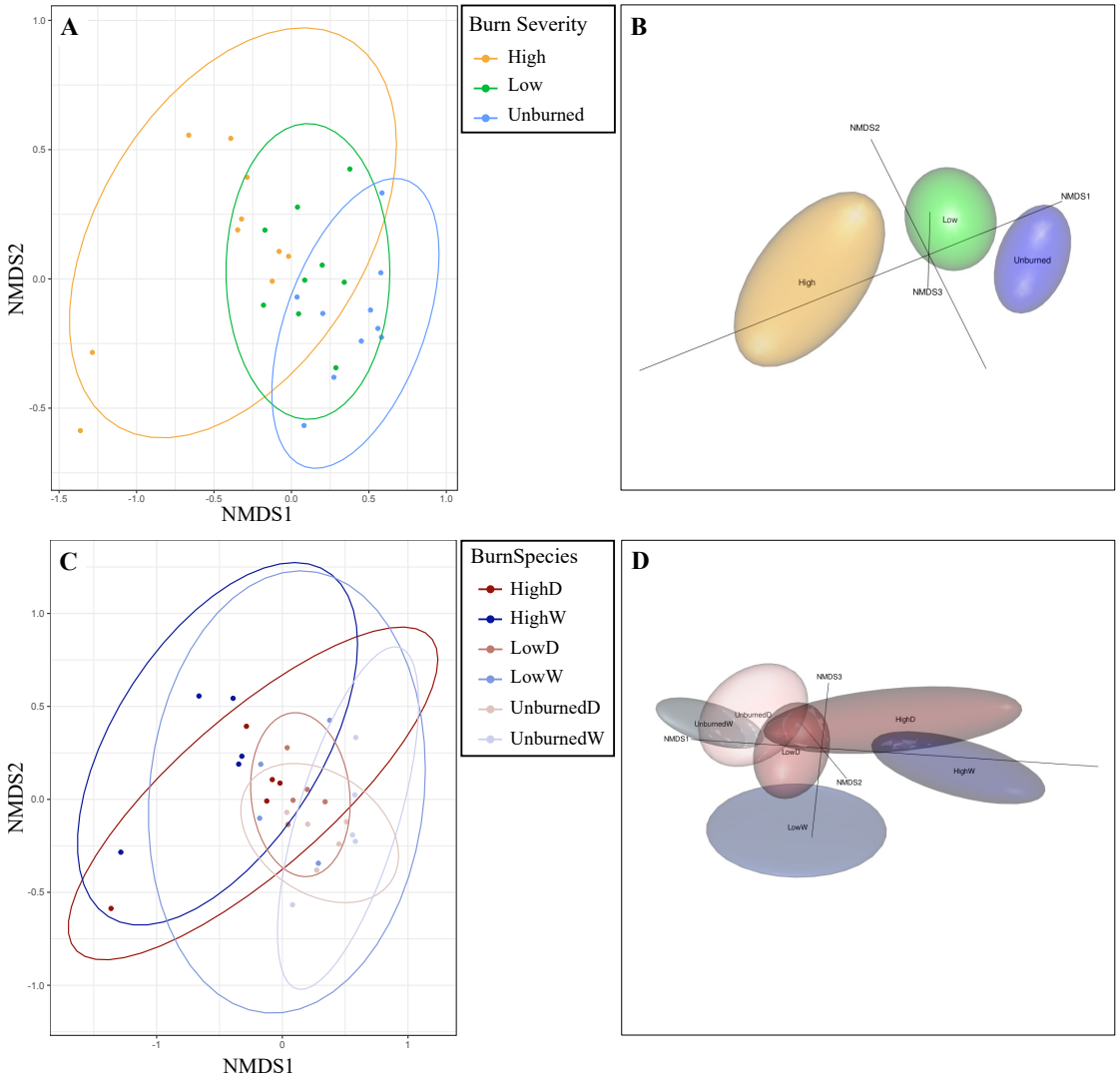

Figure 2.3. 2- and 3-dimension non-metric multi-dimensional scaling (NMDS) plots representing bacterial communities grouped by burn-severity level (high, low, unburned) (A, B) and BurnSpecies (C, D) (stress value = 0.08875). HighD, LowD, and UnburnedD = high-severity burn, low-severity burn, and unburned Douglas-fir (*Pseudotsuga menziesii*), respectively, HighW, LowW, and UnburnedW = high-severity burn, low-severity burn, and unburned western white pine (*Pinus monticola*), respectively.

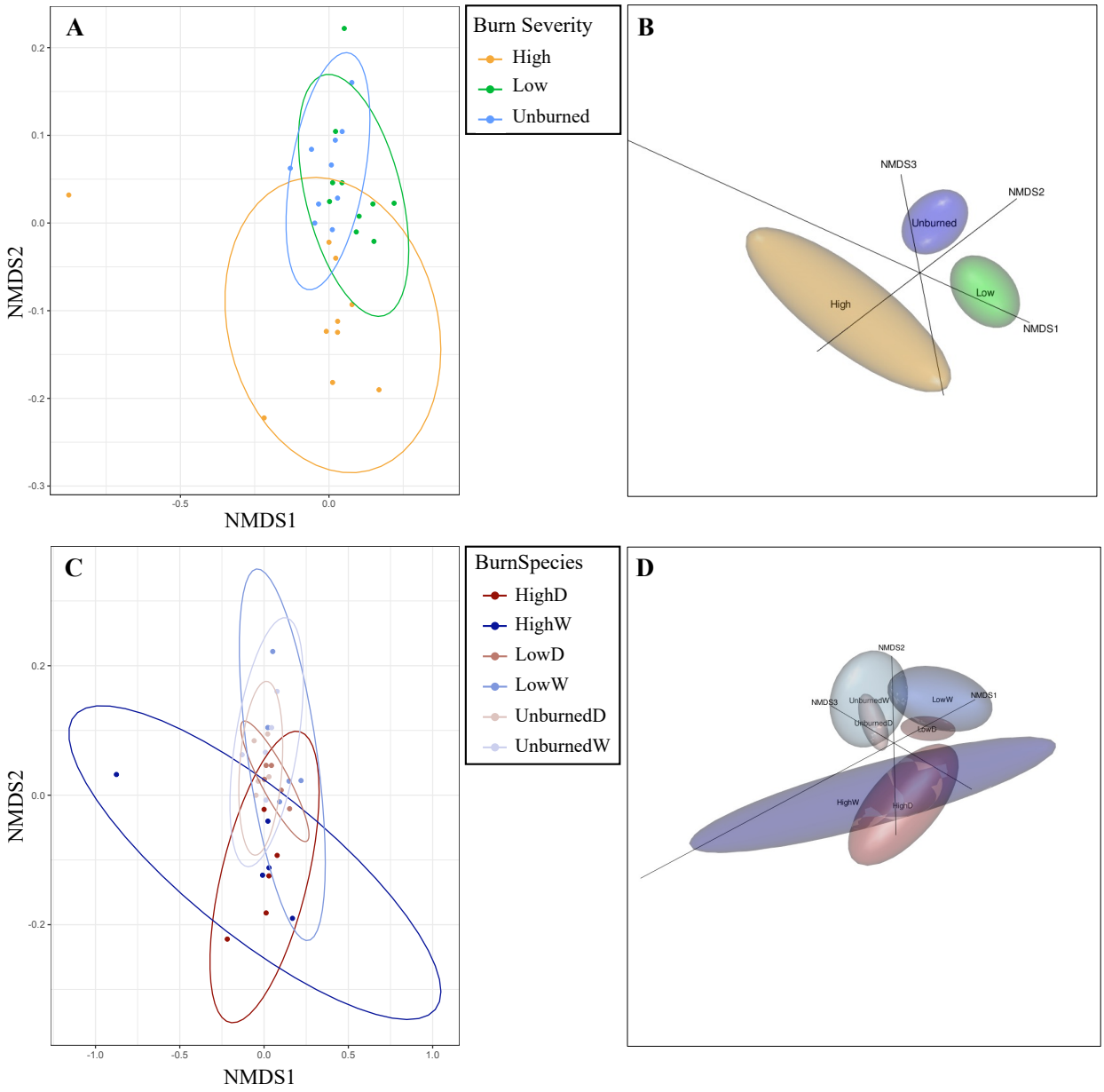

Figure 2.4. 2- and 3-dimension non-metric multi-dimensional scaling (NMDS) plots representing fungal communities grouped by burn-severity level (high, low, unburned) (A, B) and BurnSpecies (C, D) (stress value = 0.13742). HighD, LowD, and UnburnedD = high-severity burn, low-severity burn, and unburned Douglas-fir (*Pseudotsuga menziesii*), respectively, HighW, LowW, and UnburnedW = high-severity burn, low-severity burn, and unburned western white pine (*Pinus monticola*), respectively.

Taxonomic Trends

In total, 11 genera were found to be present in at least 1% relative abundance in bacterial BurnSpecies communities (Supplemental Figure 2.4). *Pseudomonas* followed by *Pseudoarthrobacter* and *Yersinia* were found in high proportions across all communities. The genus *Ewingella* was also present at a high relative abundance in unburned WWP and DF and low-severity burn WWP communities.

A total of 14 genera were present in at least 5% relative abundance in fungal BurnSpecies communities (Supplemental Figure 2.5). Apart from low-severity WWP communities, each had a high relative abundance of *Ilyonectria.* Both high- and low-severity burn communities had a higher relative abundance of *Penicillium* than unburned communities.

Because host tree species did not have a significant effect on microbial community alpha or beta diversity, communities from DF and WWP hosts were combined by either burn severity or *Armillaria* species association for the remaining analyses. For these analyses, we use the terms "proliferated", or "abundant" to indicate taxa that had significantly greater relative abundance in the specified microbial community, when compared to others (for example, high-severity burn communities when compared to low-severity burn communities or in *A. solidipes* communities when compared to *A. altimontana* communities). Further, we add the term "consistently" to indicate taxa that were more abundant in the specified microbial community when compared to both opposing communities (for example, high-severity burn communities when compared to both low-severity burn and unburned communities), which are represented by 'satellite' circles in Figures 2.5 and 2.6.

When comparing unburned, low-severity burn, and high-severity burn communities, unburned communities had the most relatively abundant and consistently abundant bacterial and fungal genera (Figure 2.5). In bacterial and fungal communities, both unburned and high-severity burn communities shared the fewest relatively abundant genera, while unburned and low-severity burn communities shared the most (Figure 2.5).

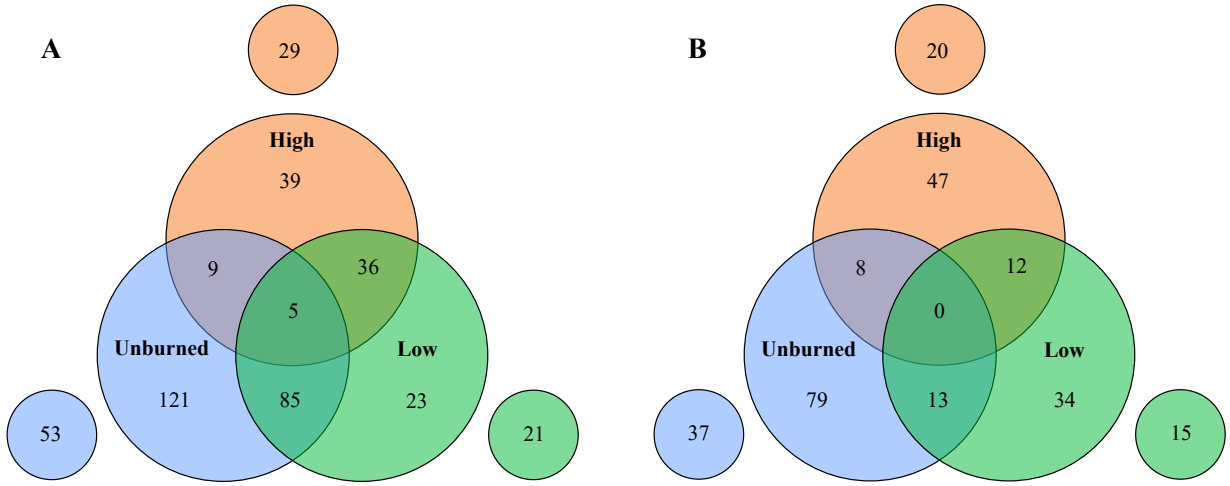

Figure 2.5. Numbers of bacterial (A) and fungal (B) genera that were significantly more abundant in microbial communities under each level of burn severity (high, low, and unburned) when compared with the other communities. Satellites indicate the number of genera that consistently proliferated in the associated burn severity when compared to the other two groups. List of genera can be found in Supplemental Tables 2.3 (bacteria) and 2.4 (fungi).

When comparing bacterial communities associated with each *Armillaria* species, *A. solidipes*-associated communities had the most consistently abundant genera (Figure 2.6A). In contrast, communities associated with only *A. solidipes* or *A. altimontana* had the same number of consistently abundant fungal genera (Figure 2.6B). For both bacterial and fungal communities, *A. solidipes*- and *A. altimontana*-associated communities shared the fewest relatively abundant genera (Figure 2.6).

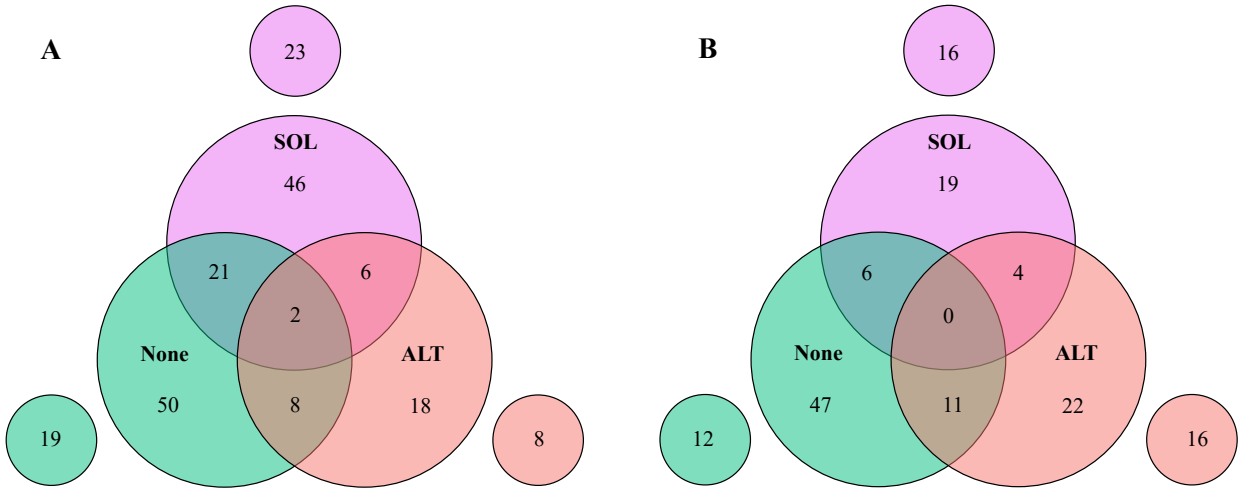

Figure 2.6. Numbers of bacterial (A) and fungal (B) genera that were significantly more abundant in microbial communities associated with *Armillaria solidipes* (SOL), *A. altimontana* (ALT), or neither (None) when compared with the other communities. Satellites indicate the number of genera that consistently proliferated with the indicated *Armillaria* sp. when compared to the other two groups. List of genera can be found in Supplemental Tables 2.5 (bacterial) and 2.6 (fungi).

2.4.4 Bacterial and Fungal Isolation from Soils

From approximately 800 initial cultures, microbial species isolated from soil samples were identified as belonging to 34 bacterial and 21 fungal genera (Supplemental Table 2.7). A large proportion of bacterial isolates were identified as either *Bacillus* species or *Pseudomonas* species, while *Penicillium* species and *Mortierella* species dominated the fungal isolates. Each of these genera were isolated from all three burn-severity levels. The number of isolates of bacterial and fungal genera were relatively evenly distributed among burn severity groups (Figure 2.7). GenBank accession numbers and taxonomic identification for bacterial and fungal isolates can be found in Supplemental Tables 2.9 and 2.10, respectively.

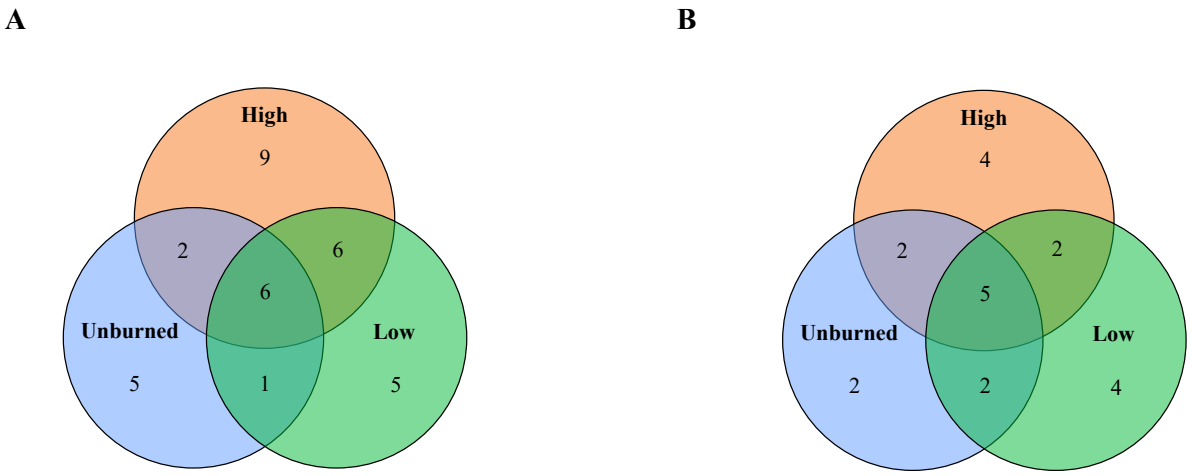

Figure 2.7. Numbers of bacterial (A) and fungal (B) genera isolated in culture from soils collected in high-severity burn, low-severity burn, and unburned areas. List of genera can be found in Supplemental Table 2.7.

2.4.5 Analysis of Soil Characteristics

Multiple linear regressions were used to determine the relationships between soil characteristics and bacterial and fungal community richness and alpha diversity, as measured by Shannon's diversity index and inverse Simpson's index (Table 2.4). For bacterial and fungal communities, every soil variable selected for the model using AIC was a significant predictor of inverse Simpson's index. Further, more soil characteristics were associated with bacterial community diversity compared to fungal community diversity. Opposite trends (positive versus negative) in changes to the alpha diversity of bacterial and fungal communities in response to changing edaphic characteristics were observed for soil N content, organic matter loss on ignition (LOI), cation exchange capacity (CEC), and K saturation.

Table 2.4. Soil characteristics with significant effects at the * 5% level; ** 1% level; *** 0.1% level on bacterial and fungal richness (R) and alpha diversity, as measured by Shannon's diversity index (*H'*) and inverse Simpson's index (1*/D).* Direction of the relationship (+ or -) produced by the linear model is indicated.

Soil Characteristic	**Bacteria**			**Fungi**		
	R	*H'*	1*/D*	R	*H'*	1*/D*
Soil pH		$+^{*}$				
Organic matter LOI[A]			$+^{**}$	$-^{*}$	$-^{*}$	$-^{*}$
Kg N per hectare	$-^{**}$	$-^{*}$	$-^{**}$		$+^{**}$	$+^{**}$
K ppm[B]			$-^{***}$			
Zn ppm			$-^{***}$			
Mn ppm			$+^{*}$			
Na ppm		$-^{**}$	$-^{*}$			
Cation exchange capacity		$+^{**}$	$+^{***}$		$-^{*}$	$-^{**}$
K saturation			$+^{***}$		$-^{**}$	$-^{**}$

[A]Loss on ignition
[B]Parts per million

Burn severity had a significant effect on soil N content (kg N per hectare) ($F_{2,27} = 4.33$, $p = 0.0235$) and Fe content (Fe parts per million (ppm)) ($F_{2,27} = 6.62$, $p = 0.0046$). Further pairwise comparisons found significantly higher N content in high-severity burn soils when compared to unburned soils ($t(27) = 2.928$, $p = 0.0182$). Pairwise comparisons indicated significant differences in soil Fe content between high-severity burn and unburned soils ($t(27) = -3.43$, $p = 0.0054$) and low-severity burn and unburned soils ($t(27) = -2.761$, $p = 0.0268$), with unburned soils having higher soil Fe content than burned soils.

2.5 Discussion

Our study provides a new understanding of the interactions among fire, soil microbial communities, and Armillaria root disease by examining changes to bacterial and fungal communities following low- and high-severity burns in coniferous forests where *Armillaria* species commonly occur. We observed compositional changes in microbial communities following fire that correspond with the microbial communities of various *Armillaria* species

Importantly, following high-severity burns, we observed a decreased relative abundance of many ECM fungi, which likely contribute to the benefits to plant growth associated with *A. altimontana* (Caballero et al., 2023; Warwell et al., 2019). Additionally, we found that *A. solidipes* and associated taxa were prevalent in high-severity burn areas, suggesting an increased need for post-fire monitoring and management for Armillaria root disease. These results further elucidate microbial taxa that may be critical components of the microbiomes associated with *A. solidipes* and *A. altimontana* in western United States coniferous forests and provide initial indications of how management strategies could be altered to accommodate changes in the soil microbiome following fire.

At 15 months post-fire, we observed that burn severity continued to have a significant impact on bacterial community richness and Shannon's diversity index. The observed changes to bacterial alpha diversity are consistent with previous research showing that fire, especially high-severity burns, is associated with decreased bacterial community richness and Shannon's diversity index across ecosystems (Ammitzboll et al., 2021; Sáenz De Miera et al., 2020; Pérez - Valera et al., 2017). Because decreases in fungal richness and diversity following fire have been previously reported (VanderRoest et al., 2024; Ammitzboll et al., 2021; Day et al., 2019; Reazin et al., 2016), we expected that fungal communities in our study would also follow this trend. In contrast, we found no change in fungal community richness or diversity following fire. Hopkins et al. (2021) similarly found no change in the richness or alpha diversity of fungal communities following burns in two pyrophilic ecosystems managed with frequent prescribed fire. Further, studies separating fungal communities by soil depth show insignificant changes in overall fungal richness and diversity in lower soil layers following fire (Ammitzboll et al., 2021; Pérez-Izquierdo et al., 2021). Additionally, Pérez-Izquierdo et al. (2021) found that the richness of

saprophytic soil ascomycetes in boreal forests increased following fire, despite decreased or unchanged total fungal community richness. Separating our fungal communities by ecological lifestyle or soil depth may have revealed similar trends in how specific fungal groups respond to burn severity.

Meta-analyses of typical changes to soil characteristics following fire report decreased organic matter, increased pH, and increased available nitrogen (Agbeshie et al., 2022; Certini, 2005). In contrast, we observed that only soil N content and Fe content varied significantly in response to burn severity. The discrepancies between the results of this study and previous studies are perhaps attributable to our sampling time, which was at 15-months post-fire, as another study reported a return to near pre-fire conditions after only 1 year (Pérez-Valera et al., 2017). Further, it seems reasonable that our results were again affected by pooling soils from different depths, given that Pérez-Izquierdo et al. (2023) found differences among soil properties in the organic layer following fire but insignificant changes in the underlying mineral soil. Our results suggest that post-fire changes to edaphic characteristics have a greater influence on microbial evenness than richness, while vegetative shifts can have the opposite effect. For example, we observed that inverse Simpson, which prioritizes community evenness, was influenced by more soil characteristic changes than were richness or Shannon's diversity index, especially for bacterial communities (Table 2.4). In contrast, our burn-severity levels (unburned, low, and high) were determined by post-fire tree survival and roughly corresponded with the tree-related burn severity metric used in Pérez-Izquierdo et al. (2023), which included factors such as tree mortality and stem soot height. In our study, these tree-related changes, represented by our burn severity levels, corresponded with changes in bacterial community richness and Shannon's diversity index, but not changes to inverse Simpson's index.

We observed that fire resulted in changes to the composition of our soil microbial communities, concurring with previous research (VanderRoest et al., 2024; Pérez-Izquierdo et al., 2023; Ammitzboll et al., 2021; Hopkins et al., 2021; Sáenz De Miera et al., 2020; Perez-Valera et al., 2017; Reazin et al., 2016). Previous studies have reported increased clustering of soil microbial communities in the upper soil layers following high-severity burns (Ammitzboll et al., 2021; Pérez-Izquierdo et al., 2021) as fire selects for taxa that can withstand high temperatures and post-disturbance conditions; however, no change or decreased clustering post-fire has also been observed (VanderRoest et al., 2024; Hopkins et al., 2021). We found that high-severity burn communities consistently showed higher variance in composition compared to our low-severity burn communities; this trend was also observed when communities were separated by host species, apart from WWP bacterial communities. We speculate that, in our study, exudates from trees that remained alive following low-severity burns may have promoted a stress-resistant soil microbiome, resulting in more similar microbial communities composed of beneficial taxa that could expedite tree recovery.

Fire is known to change the relative abundance of specific microbial taxa within the soil community as the disturbance selects for bacteria and fungi that are adapted to withstand heat or efficiently colonize under post-fire environmental conditions (VanderRoest et al., 2024; Ammitzboll et al., 2021; Hopkins et al., 2021; Day et al., 2019; Whitman et al., 2019; Pérez -Valera et al., 2017). For example, across a variety of ecosystems, the bacterial genera *Arthrobacter* and *Massilia* and the Phylum Firmicutes tend to increase in relative abundance following fire (VanderRoest et al., 2024; Ammitzboll et al., 2021; Sáenz De Miera et al 2020; Whitman et al., 2019; Pérez -Valera et al., 2017), as do the fungal genera *Pyronema*, *Penicillium*, and *Geopyxis* (Pérez-Izquierdo et al., 2021; Day et al., 2019; Whitman et al., 2019; Reazin et al.,

2016). In contrast, fungi from the family Umbelopsidaceae, including *Mortierella* species, and ECM fungi tend to decrease following fire (VanderRoest et al., 2024; Pérez-Izquierdo et al., 2021; Whitman et al., 2019; Reazin et al., 2016). In this study, we found some contrasting results in changes to specific taxa based on both our metabarcoding and culture-based analyses. For example, many of these fungal genera, including *Geopyxis*, *Pyronema*, and *Mortierella*, were present on our study sites yet did not drive significant differences among the composition of microbial communities in our metabarcoding analyses. *Arthrobacter* was more abundant in high-severity burn communities compared to low-severity burn communities, but also consistently proliferated in unburned communities compared to other burn-severity levels. Further, in our culture-based analyses, species belonging to *Penicillium* and *Mortierella* were isolated from all three burn-severity levels, reflecting the widespread distribution of these taxa in the soil. However, fire-associated changes in the presence or abundance of certain taxa reflected results from previous studies. For example, in this study, ASV's belonging to *Penicillium*, which was associated with fire in Day et al. (2019) and Whitman et al. (2019), proliferated in high-severity burn communities when compared with unburned communities. We also isolated *Massilia,* a genus that was previously found to be abundant in the burned communities of managed *Eucalyptus* forests (Ammitzboll et al., 2021), from soils of high- and low-severity burns, but not from unburned soils. Similarly, the fungal genus *Paraphoma* (recently separated from *Phoma*), which was associated with higher severity fire in Canadian boreal forests (Day et al., 2019), was isolated from a sample of high-severity burn soil in this study.

Changes to communities of ECM fungi following fire are especially relevant to Armillaria root disease management due to their potential role in a putative disease suppressive soil and promoting tree growth and survival. Decreases in relative abundance, richness, and

diversity of ECM fungi are typically observed following fire (VanderRoest et al., 2024; Day et al., 2019; Reazin et al., 2016, Dahlberg et al., 2001), especially severe fires that lead to the mortality of their tree hosts (Pérez-Izquierdo et al., 2023; Hart et al., 2005). In our study, changes to the relative abundance of specific ECM genera following fire varied in comparison with results from other studies. For example, the relative abundances of the ECM fungi *Russula* and *Cortinarius*, reported to be indicators of unburned conditions in boreal forests in Pérez-Izquierdo et al. (2021), were not significantly different between burned and unburned communities in our study. Further, this same study found that the ECM genus *Laccaria* was more relatively abundant in burned conditions; however, we found that *Laccaria* consistently proliferated in unburned communities of our study. We also found that the ECM genera *Inocybe* and *Tomentella* consistently proliferated in unburned communities when compared with both low-severity and high-severity burn communities, which concurs with the findings of Reazin et al. (2016) that showed decreased relative abundance of these genera following fire in ponderosa pine (*P. ponderosa*) forests. We also found fire-associated changes in the relative abundance of *Suillus* and *Truncocolumella*, two ECM genera in the family Suillaceae, which were previously reported to proliferate in soil microbial communities associated with *A. altimontana* (Caballero et al., 2023). In our study, *Suillus* showed higher relative abundance in low-severity burn communities when compared to unburned communities, while *Truncocolumella* showed higher relative abundance in unburned communities when compared to low-severity burn communities. Previous studies have indicated that *Suillus* species, which have wind-dispersed spores, are effective post-fire colonizers (Pérez-Izquierdo et al., 2021; LeDuc et al., 2013; Visser, 1995), potentially supporting their role as important ECM symbionts for tree regeneration following disturbance.

In agreement with Ibarra Caballero et al. (2023), we found no significant differences in the alpha or beta diversity of our microbial communities based on association with different *Armillaria* species. Further, we also found similarities between taxa enriched in the communities of *A. altimontana* or *A. solidipes.* This included a proliferation of *Suillus* and *Truncocolumella* in *A. altimontana*-associated communities when compared with *A. solidipes*-associated communities, providing support for the hypothesis that these beneficial symbionts are playing a role in the positive plant growth effects connected to *A. altimontana* (Warwell et al., 2019). Similarly, we found an increased abundance of the bacterial genus *Solirubrobacter* in *A. solidipes*-associated communities compared to *A. altimontana*-associated communities, which supports a result that Ibarra Caballero et al. (2023) found at the family level (Solirubrobacteraceae). In addition, three genera belonging to Enterobacteriaceae, a large bacterial family previously associated with *A. solidipes* communities (Ibarra Caballero et al., 2023), were significantly different among our microbial communities. However, instead of being affiliated with *A. solidipes*, our study found that two genera (*Trabulsiella* and *Buttiauxella*) consistently proliferated in *A. altimontana*-associated communities and one genus (*Cedecea*) consistently proliferated in communities associated with no *Armillaria* species. Interestingly, two bacterial genera that have been found enriched in the microbiome of plants under pathogen attack, *Chitinophaga* and *Chryseobacterium* (Carrión et al., 2019; Rolli et al., 2021), were consistently abundant in *A. solidipes*-associated communities in our study. While the majority of microbial communities where both *A. solidipes* and *Chitinophaga* were present were in high-severity burn soil samples, one community was from an unburned soil sample. This could possibly suggest that this particular tree (tree 27) was secreting exudates to promote a stress resistant microbiome against the pathogen.

Interpretations of comparisons among the microbial communities from different burn-severity levels and *Armillaria* species are complicated by the uneven distribution of *Armillaria* species across our burn-severity groups. For example, the majority of trees with only *A. solidipes* isolates were found in high severity burn areas, which may explain why fire-associated bacteria, including *Massilia* and *Arthrobacter*, were found to proliferate in *A. solidipes*-associated communities. Similarly, many trees associated only with *A. altimontana* were from low-severity burn or unburned areas. Further, in our high-severity burn sites, *A. solidipes* was likely existing as a saprophyte on trees killed by the fire, and this microbial community may differ from the community present when *A. solidipes* is acting as a pathogen. However, because an exact overlap among communities from various burn-severity levels and *Armillaria* species was not observed, we examined shared taxa that consistently proliferated in microbial communities grouped by fire severity and *Armillaria* species. There were no genera that consistently proliferated in both low-severity burn communities and *A. solidipes*-associated communities, while high-severity burn communities and *A. solidipes*-associated communities showed the most overlap in consistently abundant bacterial and fungal genera. Similarly, we found no shared bacterial or fungal genera that consistently proliferated in *A. altimontana*-associated communities and those that consistently proliferated after high-severity burns, while consistently abundant genera were shared among *A. altimontana*-associated communities and low-severity burn or unburned communities.

In conclusion, we found that incremental increases in burn severity altered the diversity and composition of soil microbial communities in a northern Idaho forest where Armillaria root disease from *A. solidipes* is present. Further, the high occurrence of *A. solidipes* in high-severity burn areas and the overlap between consistently abundant taxa in high-severity burn and *A.*

solidipes communities indicates that these high-severity burn sites may provide ideal conditions for *A. solidipes* and its microbial community to inhabit. Although *A. solidipes* has a saprophytic lifestyle in areas of intense burn, this could produce an inoculum source for both adjacent regions with remaining live trees and regenerating trees on the severely burned site, increasing the presence of Armillaria root disease over time. For this reason, severely burned conifer forests where *A. solidipes* occurs should be identified as potential priority areas for monitoring and preventative management for Armillaria root disease. In contrast, the association between the ECM genus *Suillus* and *A. altimontana* and its increased relative abundance following low-severity burns may suggest that controlled, low-severity burns have the potential to be part of a management strategy for *A. solidipes* by shifting the soil microbiome towards taxa that potentially comprise a disease suppressive soil. However, a deeper understanding of the soil microbiome in coniferous forests, including the importance of ECM fungi in the putative disease suppressive soils associated with *A. altimontana* and other specific microbial taxa associated with the virulence of *A. solidipes*, and the influences of burn severity on its ecological functions is needed to help integrate fire into an effective landscape-scale management strategy. Overall, our research provides insight on the importance of understanding the complex interactions among burn severity and soil microbial communities and the consequent management of prevalent forest diseases.

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CHAPTER 3: ISOLATION AND *IN VITRO* TESTING OF POTENTIAL NATIVE MICROBIAL BIOCONTROL AGENTS FOR *ARMILLARIA SOLIDIPES*

3.1 Summary

The use of living organisms to control plant pests and pathogens is prevalent in agricultural and natural ecosystems as a component of environmentally friendly and economically sustainable management strategies. Microbial biological control agents (BCAs) rely on a diverse repertoire of mechanisms to control various plant diseases. Further, the use of BCAs presents specific benefits for controlling soilborne root pathogens that are notoriously difficult to manage, including Armillaria root disease. In the northwest United States, Armillaria root disease is caused by the virulent pathogen *Armillaria solidipes*, which is responsible for landscape-scale pockets of infection that dramatically alter forest structure and function. Promoting the presence of native BCAs found within the soil microbiome and pre-adapted to existing environmental conditions is a promising option for managing landscape-scale infections. However, other influences on the soil microbiome, including increasingly frequent and severe wildfire disturbances in these areas, are likely to alter the microbial composition, including the presence or abundance of important BCAs. In this study, we build upon previous research by identifying potential BCAs for the pathogen *A. solidipes* from the native soil microbiome of a conifer forest in northern Idaho, USA, and examine how three levels of burn severity (high, low, and unburned) may alter the presence of these organisms within the soil. We identified five bacterial and five fungal isolates that effectively restricted the *in vitro* growth of *A. solidipes*, including isolates from the genera *Bacillus*, *Trichoderma*, and *Mortierella*. Further, we found no

significant differences in the presence or abundance of these putative BCA genera among our three levels of burn severity, indicating that fire may not affect the potential for these organisms to be incorporated into management strategies for Armillaria root disease. Additionally, we found initial evidence that the weakly pathogenic species *A. altimontana* exhibited superior saprophytic colonization when compared with *A. solidipes*, suggesting the need for further field testing of *A. altimontana* as a BCA. Overall, our results contribute to the growing body of knowledge identifying effective microbial controls for *Armillaria* pathogens and consider the effects of environmental stressors on using biological control in the sustainable management of forests diseases.

3.2 Introduction

Biological control, or the use of living organisms to control pests and pathogens, is used in agricultural and natural environments for plant disease management (Bonaterra et al., 2022; Palmieri et al., 2022; Prospero et al., 2021). Diverse biological control agents (BCAs), including insects, nematodes, bacteria, and fungi, can reduce the occurrence of various plant diseases (Balla et al., 2021). Effective use of BCAs relies on establishing a stable population of the BCA (Prospero et al., 2021; Paulitz, 2000; Lewis & Papavizas, 1984) and, in many cases, undergoing regulatory processes such as verifying the BCA will not cause harm to the broader ecosystem (Raymaekers et al., 2020; Hunt et al., 2008; Goettel et al., 2001). However, in contrast to traditional control methods, biological control can be a practical and sustainable management option. For example, control with pesticides is often highly effective, yet can damage the ecosystem by entering soil and waterways (Beaumelle et al., 2023; Rad et al., 2022), impacting non-target organisms (Duke, 2017; Dijksterhuis et al., 2011), or driving pesticide resistance in pest/pathogen populations (Hawkins et al., 2019; Lucas et al., 2015). Cultural control methods,

such as the breeding and planting of resistant plant species, can be expensive and time-consuming (Khan & Korban, 2022; Sniezko, 2006). BCAs, especially those already present within the ecosystem, present less risk to other organisms and ecosystem processes (Rabiey et al., 2019) and can be a less costly long-term solution (Collatz et al., 2021). Identifying effective BCA organisms can be done through a combination of lab-based tests and field assays. Dual culture confrontation tests are widely used for initial screening and involve the co-inoculation of a putative BCA organism and a pathogen on agar and determination of pathogen growth inhibition (Raymaekers et al., 2020; Pliego et al., 2011). However, subsequent field trials are needed to verify that BCAs which perform well in these *in vitro* settings will provide similar levels of control in natural ecosystems, as preexisting environmental factors can influence the activity and establishment of BCAs and thus alter their performance (e.g., Mesanza et al., 2016).

Bacterial and fungal BCAs can effectively inhibit microbial plant pathogens through various mechanisms, including competition, antibiosis, direct parasitism, and stimulation of plant growth and defense mechanisms (Bonaterra et al., 2022; Palmieri et al., 2022; Köhl et al., 2019). Highly competitive microbial species reduce the availability of limiting nutrients and space for other microbes, which can inhibit pathogen activity. For example, the production of iron-scavenging siderophores by *Trichoderma* species is suggested to contribute to the biocontrol activity of this genus against plant pathogens by reducing iron availability in the soil (Chen et al., 2019; Segarra et al., 2010). Additionally, some BCAs, especially many bacterial species, secrete toxic, low molecular weight antibiotic compounds (Palmieri et al., 2022; Fravel, 1988). These antibiotics contribute to pathogen control by reducing growth and virulence or, at sufficiently high concentrations, causing mortality (Arseneault & Filion, 2017). Microbial BCAs in direct contact with the pathogen may also consume the contents of pathogen tissues as a source of

nutrients, termed hyperparasitism (Terhonen et al., 2018). In addition to methods of inhibition which depend on interactions between the BCA and the pathogen, interactions between BCAs and host plants can result in indirect disease control. The presence of beneficial microbes can lead to induced systemic resistance (ISR), in which microbe associated molecular patterns (MAMPs) prime plant responses, including defense-related gene expression and enhanced constitutional barriers, for stronger defense against future pathogen attack (Köhl et al., 2019; Pieterse et al., 2014). Examples of MAMPs include essential microbial structural compounds or secreted proteins, very low concentrations of which can stimulate plant defense responses (Boller & Felix, 2009). Finally, plant growth promoting microorganisms, including rhizobacteria and mycorrhizal fungi, can further contribute to pathogen control by boosting plant health and vigor (Weng et al., 2022; Thakur & Singh, 2018; Compant et al., 2005).

The use of microbial BCAs to control notoriously hard-to-manage soilborne diseases in forest landscapes, including Armillaria root disease, has been studied (Thakur & Singh, 2018; Chen et al., 2019; Mesanza et al., 2016). Armillaria root disease pathogens, from the genera *Desarmillaria* and *Armillaria*, have a cosmopolitan distribution and cause a white rot on the roots and lower boles of tree hosts in horticultural and landscape settings leading to a decline in tree strength and health, increased susceptibility to other disease agents, and eventual tree mortality (Kim et al., 2022; Baumgartner et al., 2011; Shaw & Kile, 1991). Hosts for *Armillaria* species vary from deciduous to coniferous hosts, and virulence of *Armillaria* species ranges from weaker secondary pathogens to aggressive primary pathogens (Koch et al., 2017; Shaw & Kile, 1991). *Armillaria solidipes* is the virulent North American vicariant of *A. ostoyae* and is an aggressive pathogen of primarily coniferous tree hosts throughout North America (Kim et al., 2021; Shaw & Kile, 1991). Armillaria root disease caused by *A. solidipes* can grow to infect

large areas of forests (e.g., Ferguson et al., 2003), creating clear pockets of disease and mortality across the landscape with altered forest structure and composition, such as shifts in the dominant vegetation, and can persist saprophytically on these sites for long periods (Chen et al., 2023; Kim et al., 2022; Baumgartner et al., 2011). Current management methods for Armillaria root disease include root collar excavation, soil fumigation, stump removal, and planting of resistant tree species, though these methods are often logistically impractical for managing landscape-scale infections and adapting more holistic strategies may prove to be useful (Kim et al., 2022; Mesanza et al., 2016; Baumgartner et al., 2011). Utilizing native microbial species, including BCAs and microbial communities that can inhibit damage from pathogenic *Armillaria* species, has been suggested as a more effective and sustainable strategy for Armillaria root disease management at this landscape scale (Ibarra Caballero et al., 2023; Kim et al., 2022).

Many bacterial and fungal microorganisms have proven to be effective BCAs for *Armillaria* species during both dual culture confrontation tests and field trials. Examples of these BCAs include species from the bacterial genera *Pseudomonas*, *Streptomyces*, *Bacillus*, and *Erwinia* (Mesanza et al., 2016; de Vasconcellos & Cardosa, 2009; DeLong et al., 2002), among others (Kedves et al., 2021). Fungal species from the genus *Trichoderma*, well known for providing effective biocontrol of a variety of plant pathogens, can also inhibit *Armillaria* species (Kedves et al., 2021; Sood et al., 2020; Chen et al., 2019). Several of these genera are widely distributed soil inhabitants, including *Trichoderma* species, *Bacillus* species, and *Pseudomonas* species (Asad, 2022; Bonaterra et al., 2022; DeLong et al., 2002). BCA organisms already present within the native soil microbiome are adapted to the pre-existing environmental conditions and interactions with other soil microbes, increasing the likelihood of successful population establishment and effective disease management when compared to exotic,

introduced BCAs (Chen et al., 2019; Mesanza et al., 2016). Sufficient BCA population and colonization is needed for multiple modes of action of effective pathogen inhibition, such as for superior competitive abilities (Bonaterra et al., 2022; Köhl et al., 2019) and the production of antimicrobial compounds (Arseneault & Filion, 2017; Raaijmakers et al., 1999). Populations of native BCAs can be promoted by altering environmental conditions to support specific requirements (Stewart et al., 2021; Raaijmakers & Mazzola, 2016) or transplanting soils with the beneficial microorganisms (Li et al., 2021; Trivedi et al., 2020).

Microbiomes specifically associated with plants under the attack of pathogens may harbor high populations of native BCA organisms, making these microbiomes an important location to search for novel BCAs. Plant-secreted exudates in the rhizosphere alter conditions and attract certain microorganisms, changing the microbial community composition close to plant roots when compared with that of the bulk soil (Zhang et al., 2017; Doornbos et al., 2012). Further, when facing abiotic or biotic stressors, plants can selectively secrete exudates in a 'cry for help' strategy to promote populations of specific, beneficial microbial taxa (Liu et al., 2020; Trivedi et al., 2020). Antagonistic microbes with putative BCA activity are often included in the taxa promoted by the plant under these situations (Qiu et al., 2022; Li et al., 2021; Carrión et al., 2019; Dudenhöffer et al., 2016). This effect has been observed in relation to Armillaria root disease: Chen et al. (2019) identified *Trichoderma* species that effectively inhibited the activity of multiple *Armillaria* pathogens from soils in locations with high levels of disease, including soils associated with *Armillaria* rhizomorphs.

While utilizing the soil microbiome and native BCAs to control soilborne diseases is a potential management strategy, other environmental factors that affect the soil microbiome must be considered. For example, in northwestern United States forests where *A. solidipes* is

ubiquitous, fire disturbances are increasing in frequency and severity (Halofsky et al., 2020). Fire is known to alter the diversity and composition of the soil microbiome, often decreasing microbial richness and diversity, causing compositional changes, and altering the relative abundance of microbial taxa by selecting for those that have disturbance related adaptations or can effectively colonize post-disturbance ecosystems (Pérez-Izquierdo et al., 2023; Ammitzboll et al., 2021; Sáenz de Miera et al., 2020; Day et al., 2019; Pérez-Valera et al., 2017; Reazin et al., 2016). Fires that result in plant mortality can be particularly detrimental for symbiotic microbes, such as ectomycorrhizal (ECM) fungi, which rely heavily on their plant hosts for survival (Pérez-Izquierdo et al., 2021; Hart et al., 2005). Populations of native microbial BCAs are likely to also be affected by these burns, both directly and through the mortality of living plant hosts which may be supporting them. Fire disturbances may also alter the activity and abundance of soilborne pathogens themselves (e.g., Chapter 2). Therefore, in forest ecosystems where fire disturbances are common, understanding how populations of microbial BCAs and soilborne pathogens will be altered is critical for effectively utilizing these native species for disease control.

The goal of this research was to expand upon the current knowledge of BCAs for Armillaria root disease by examining how native microbial species with potential BCA activity control growth of the virulent pathogen *A. solidipes*. To investigate this, we collected bacterial and fungal species from forest soils in northern Idaho, United States and performed *in vitro* dual culture confrontation tests between *A. solidipes* and putative BCAs, identified through literature sources indicating biocontrol or plant growth promoting abilities. Further, we examined how three levels of burn severity (high, low, and unburned) altered the presence of these putative BCAs throughout our study site. Our results provide the foundation for additional field studies with our proposed BCAs, contributing to more effective management of Armillaria root disease

on the landscape scale, and suggest how disturbance events such as fire may alter the feasibility of using native microbial species in plant disease management.

3.3 Materials and Methods

3.3.1 Sample Collection

Armillaria and soil samples were collected from the Nez Perce-Clearwater National Forest in Idaho, United States, from areas of a mature forest composed of western white pine (WWP, *Pinus monticola*), western redcedar (*Thuja plicata*), grand fir (*Abies grandis*), Douglas-fir (DF, *Pseudotsuga menziezii*), western larch (*Larix occidentalis*), and western hemlock (*Tsuga heterophylla*). In July 2021, a fire (Sand Mountain Fire) resulted in areas of dead trees, while trees remained alive in areas of controlled burned around the fire's periphery. Unburned areas were also sampled, thus, our study site had three burn-severity levels (Figure 3.1). *Armillaria solidipes* is known to cause Armillaria root disease throughout this conifer forest (G.I. McDonald and J.W. Hanna, unpublished observations).

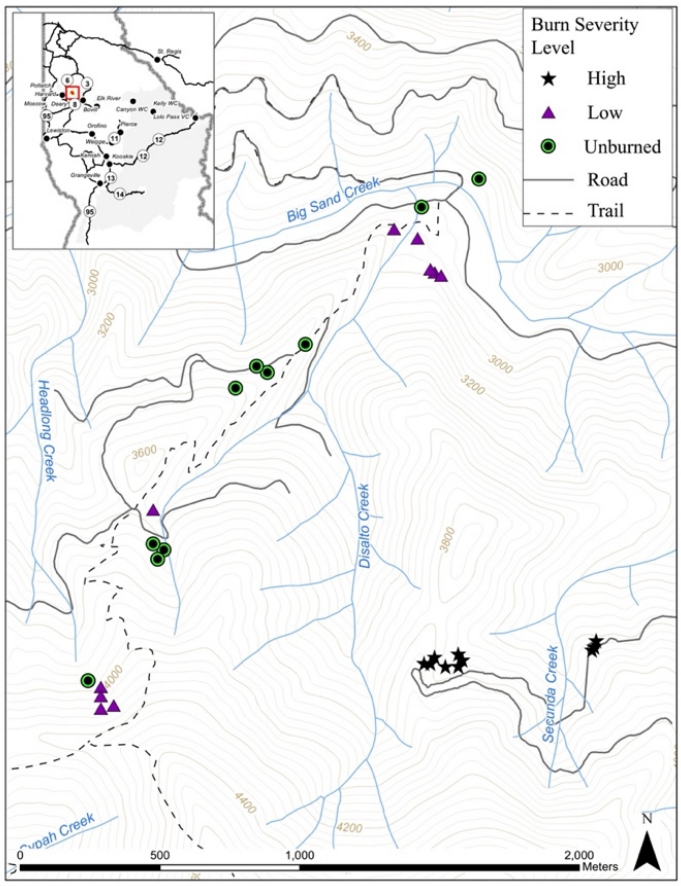

Figure 3.1. Study site in northern Idaho, United States, indicating sampled trees within three levels of burn severity (high, low, unburned).

In October 2022, five WWP (DBH 40 to 98 cm) and five DF (DBH 41 to 68 cm) trees, separated by ≥20 m, were sampled from each burn severity level, for a total of 30 trees. Soils cores of depth 20 cm were collected at 2 meters from the base of each tree in two cardinal directions and the top 5 cm of each core was discarded. Soil samples from a single tree were combined, homogenized and stored at -20°C.

Additionally, each tree was surveyed for *Armillaria* infection by removing soil from the main roots (ca. 0.5 m deep and 1 m away from bole). These exposed roots were examined for adhered rhizomorphs, while outer bark layers of roots and the lower tree bole were peeled back to check for mycelial fans. When encountered, rhizomorphs were carefully removed from the

roots and placed in 15 mL tubes. Mycelial fans attached to outer bark layers or underlying cambium were removed and stored in paper bags. Throughout the sampled areas, any *Armillaria* fruiting bodies present were also collected and stored in paper bags. All rhizomorph, mycelial fan, and fruiting body samples were stored at 4°C and subsequently isolated within 48 hours. Rhizomorph and mycelial fan samples were collected in October 2022 and June 2023, while all fruiting body samples were collected in October 2022.

3.3.2 Armillaria Isolation and Identification

Armillaria cultures were isolated from rhizomorph and mycelial fan samples according to methods described in Chapter 2. Briefly, soaking in 20% commercial bleach solution (1.5% NaClO) was used to sterile the outer surface of the rhizomorphs. Samples were then rinsed with water, soaked in 3% H_2O_2, and finally soaked in sterile distilled water. Under sterile conditions, the rhizomorphs were cut into 1 cm sections and inserted vertically into petri dishes with Armillaria malt extract agar (MEA) media (3% malt extract, 3% dextrose, 1.5% peptone, 1.5% agar). A sterile blade was used to collect a small amount of clean fungal tissue from the center of each of the mycelial fan samples. A sterile blade was also used to cut into each fruiting body sample, and a piece of clean fungal tissue was collected from the inner stipe using forceps. Mycelial fan and fruiting body tissues were also plated on Armillaria MEA in petri dishes, and all plates of *Armillaria* samples were then stored at 20°C.

Pure cultures of *Armillaria* isolates were subcultured on nylon membranes on top of modified Armillaria MEA (1.5% malt extract, 1.5% dextrose, 0.5% peptone, 1.2% agar). DNA was extracted from the *Armillaria* isolate mycelia using SYNERGY™ 2.0 Plant DNA Extraction Kits (OPS Diagnostics, Lebanon, NJ) and protocols. A Nanodrop™ 1000 spectrophotometer was

used to quantify the extracted DNA before diluting samples with sterile deionized (DI) water to 10 ng/uL. The translation elongation factor 1-alpha (*tef1*) region of *Armillaria* DNA was amplified with polymerase chain reaction (PCR) using the primers EF983F (5'-GCYCCYGGHCAYCGTGAYTTYAT - 3') (Rehner & Buckley, 2005) and ARMEF-R (5'-TACCCGTTCGGCGATCAATCT-3') (Elías-Román et al., 2013) or 2218R (5'-ATCATGACACCRACRGCRACRGTYTG-3') (Rehner & Buckley, 2005) and an Eppendorf Mastercycler pro Thermal Cycler (Eppendorf, Hamburg, Germany). The PCR cycle consisted of an initial 1 minute at 94°C before 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds, and a final 10 minutes at 72°C. Gel electrophoresis was used to view the resulting amplicons before cleaning with ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Santa Clara, CA). These cleaned DNA samples were sequenced at Eurofins Genomics (Louisville, KY) with four *Armillaria*-specific sequencing primers (ARMEF-F3A, ARMEF-R2, ARMEF-FI2, and ARMEF-RI2) (Elías-Román et al., 2018) or the *tef1* primers used during PCR. The BLASTn search from NCBI (National Center for Biotechnology Information) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used for species identification of each *Armillaria* isolate.

3.3.3 Armillaria solidipes Somatic Pairs Tests

Somatic pairing tests were conducted to identify which *A. solidipes* isolates obtained from fruiting bodies and used in further biocontrol testing represented unique genets (vegetative clones) of the pathogen. From the edge of colonies growing on Armillaria MEA, 8 mm plugs of two *A. solidipes* isolates were cut and placed 2 cm apart on a new petri dish with Armillaria MEA. Cultures were stored at room temperature and monitored for growth. If the mycelium of two isolates grew together, these were considered to be the same genet of *A. solidipes*, whereas

individuals belonging to two different genets were identified by the presence of a macroscopic separation between the growing isolates.

3.3.4 Bacterial and Fungal Isolation from Soils

Bacteria and fungi were isolated from soil samples and DNA sequencing was performed using methods outlined in Chapter 2. Briefly, four serial dilutions were performed with each sample, resulting in soil:H_2O concentrations of 1:10, 1:100, 1:1000, and 1:10000. Each sample dilution was plated on two petri dishes with nutrient agar (2.3% nutrient agar) and two with quarter strength potato dextrose agar (PDA) with antibiotics (0.975% potato dextrose agar, 1.125% agar, 0.01% streptomycin, 0.005% chloramphenicol). Colonies of unique bacteria and fungi were identified visually and subcultured to new plates for DNA extraction.

DNA was extracted from bacterial isolates via a modified colony PCR to amplify the 16S V4-V5 region of bacterial DNA. A small amount of each bacterial colony was diluted with 40 µL of molecular grade before heating at 98°C for 15 minutes. PCR was performed using primers fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rP2 (5'-ACGGCTACCTTGTTACGACTT-3') (Weisburg et al., 1991) and a cycle of 95°C for 10 minutes, 35 cycles of 95°C for 2 minutes, 54°C for 1 minute, and 72°C for 2 minutes, and a final 10 minutes at 72°C.

DNA was extracted from fungal cultures by placing mycelium in 100 µL of 5% Chelex™ 100 Resin solution and heating at 98°C for 20 minutes. PCR was performed to amplify the ITS region of fungal DNA with primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990) and a cycle of 95°C for 2 minutes, 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute, before a final 2 minutes at 72°C, using the Chelex/mycelium product as the DNA template. If this failed to

produce amplicons, a modified NaOH extraction procedure was attempted (Wang et al., 1993). Fungal mycelium was physically and chemically degraded using glass beans and 0.5 M NaOH. From the resulting solution, 5 µL was combined with 495 µL 100 uM Tris-HCl pH 8.0, and PCR was performed using this solution as the DNA template with identical primers and settings to the Chelex procedure.

For both bacterial and fungal isolates, DNA extraction was attempted three times, after which the culture was removed from further analyses. Gel electrophoresis was used to visualize amplicons and ExoSAP-IT™ PCR Product Cleanup Reagent was used to clean samples before sequencing at Eurofins Genomics (Louisville, KY). DNA was sequenced in the forward direction and the BLASTn search function of the NCBI (National Center for Biotechnology Information) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to assign taxonomy.

We were not able to confidently identify to species six bacterial isolates identified as *Bacillus* species from sequencing of the 16S. To better clarify the identify of these isolates, Geneious 9.0.5 (https://www.geneious.com) was used to create phylogenies with both maximum likelihood (ML) in PhyML (Guindon et al., 2010) and Bayesian inference (BI) in MrBayes 3.2 (Ronquist et al., 2012) methods. Reference sequences of *B. subtilis*, *B. halotolerans*, *B. velezensis*, *B. amyloliquefaciens*, *B. licheniformis*, *B. thuringiensis* and *B. mojavensis* were obtained from NCBI GenBank, and a sequence of *Paenibacillus polymyxa* was used as the outgroup. An HKY85 substitution model with 4 rate variation categories was used for both methods based on substitution model identification using IQ-Tree web server's model finder program (Nguyen et al., 2015). Markov chain Monte Carlo (MCMC) with 4 chains of length 1,100,000 was run, and the first 20% of trees were discarded as burn-in when generating the BI

consensus tree. The effective sample size for this analysis was >1600. An ML consensus tree was created from the 200 bootstraps. The combined ML/BI tree can be found in Figure 3.4.

3.3.5 Culture Confrontation Tests

Isolates of *A. solidipes* from fruiting body samples were used in dual culture confrontation tests. Potential BCA species were identified from bacterial and fungal isolates through previously published demonstrated antifungal activity or plant growth promoting abilities (Table 3.1). All putative BCAs were subject to additional rounds of sequencing, including both forward and reverse sequencing with fD1/rP2 primers for bacterial isolates and ITS1/ITS4 primers for fungal isolates. However, in multiple cases, identification and BCA selection based on the initial sequencing of the 16S or ITS rDNA regions resulted in incorrect genus assignment, which this additional sequencing later corrected; thus, some tested putative BCAs have no known previous reports of BCA or plant growth promoting activity. *Armillaria solidipes* isolates were randomly paired with potential biological control organisms based on pure culture isolate availability and timing. An 8 mm plug of *A. solidipes* was taken from the growing edge of an approximately 2-month-old culture grown on Armillaria MEA. The plug was placed 2 cm from the center of a petri dish with ½ strength potato dextrose agar (PDA; 1.95% potato dextrose agar, 0.75% agar) media and placed in a dark environment at room temperature.

Table 3.1. Putative bacterial and fungal isolates with species identification from sequencing of the 16S or ITS rDNA regions, respectively. If available, literature sources indicating previously demonstrated biocontrol agent activity or plant growth promoting abilities are also indicated.

Isolate ID	**ITS/16S Identification**	**Source Indicating BCA or Plant Growth Promotion Activity**
T2C6	*Bacillus* sp.	Ali et al., 2020; Fira et al., 2018; Mesanza et al., 2016
T5C23	*Bacillus* sp.	Ali et al., 2020; Fira et al., 2018; Mesanza et al., 2016
T2C24	*Bacillus* sp.	Ali et al., 2020; Fira et al., 2018; Mesanza et al., 2016
T7C12	*Bacillus* sp.	Ali et al., 2020; Fira et al., 2018; Mesanza et al., 2016
T7C18	*Bacillus* sp.	Ali et al., 2020; Fira et al., 2018; Mesanza et al., 2016
T16C10	*Bacillus* sp.	Ali et al., 2020; Fira et al., 2018; Mesanza et al., 2016
T14C9	*Caballeronia udeis*	Puri et al., 2020a; 2020b
T6C5	*Collimonas arenae*	Doan et al., 2019
T3C2	*Devosia* sp.	None
T6C4	*Lysobacter capsici*	Lin et al., 2021; Liu et al., 2019
T1C15	*Pseudomonas* sp.	Mesanza et al., 2016; Panpatte et al., 2016
T6C12	*Pseudomonas* sp.	Mesanza et al., 2016; Panpatte et al., 2016
T29C22	*Mortierella elongata*	Liao et al., 2019
T1C33	*Penicillium murcianum*	Srinivasan et al., 2020; Alam et al., 2011
T23C14	*Penicillium rubens*	Srinivasan et al., 2020; Alam et al., 2011
T1C29	*Trichoderma harzianum*	Asad, 2022; Sood, 2020; Chen et al., 2019
T20C20	*Trichoderma oblongisporium*	Asad, 2022; Sood, 2020; Chen et al., 2019
T17C24	*Trichoderma paraviridescens*	Asad, 2022; Sood, 2020; Chen et al., 2019
T20C14	*Trichoderma paraviridescens*	Asad, 2022; Sood, 2020; Chen et al., 2019
T2C45	*Umbelopsis* sp.	None
T14C20	*Umbelopsis* sp.	None
T2C44	*Umbelopsis vinacea*	None

After 28 days, 8 mm plugs of putative fungal BCAs were taken from the edge of colonies grown on ½ strength PDA and placed 2 cm from center of the petri dish in the opposite direction from the *A. solidipes* colony, resulting in a 4 cm distance between the *A. solidipes* plug and the putative BCA plug. Potential fungal BCA cultures were 3 weeks old at the time of plating. Three replicates of each *A. solidipes*/fungal BCA pairing were made, 3 control plates for each *A. solidipes* isolate, and 2 control plates for each putative BCA. In multiple cases, contamination reduced either the number of confrontation tests or the number of *A. solidipes* control plates by one. After adding the putative fungal BCA, plates were sealed with parafilm and returned to the dark environment. Confrontation tests were measured after 2, 5, 7, and 14 days using ImageJ software (Schneider et al., 2012), with biocontrol index (BCI; Chen et al., 2019) calculated as:

$$BCI = \frac{area\ of\ biocontrol\ colony}{area\ of\ biocontrol\ colony + area\ of\ A.\ solidipes\ colony} \times 100$$

For multiple putative fungal BCAs, the colony grew to completely cover the plate, making it impossible to determine where the BCA and *A. solidipes* colonies overlapped. In these cases, BCI was recorded as 100%. Average BCI for each putative fungal BCA was calculated from the replicates. Putative fungal BCAs with an average BCI above 80% on day 14 were considered successful.

For testing of putative bacterial BCAs, *A. solidipes* was plated in an identical manner to confrontation tests with fungal BCAs and grown for 7 days in a dark environment. Bacterial isolates were taken from a 7-day old culture grown on ½ strength PDA and applied in a half-inch line with a sterile bacterial loop 1 cm from the *A. solidipes* plug. At 28 and 56 days, tests were measured with ImageJ software (Schneider et al., 2012) and area inhibition percentage (AIP; Mesanza et al., 2016) was calculated as:

$$AIP = \frac{(A.\ solidipes\ area\ in\ control - A.\ solidipes\ area\ in\ confrontation\ test)}{A.\ solidipes\ area\ in\ control} \times 100$$

Three replicates of each *A. solidipes*/BCA pairing were used, with three control plates for each *Armillaria* isolate and two control plates for each BCA. Average AIP for each putative bacterial BCA was calculated from the replicates. Putative bacterial BCAs with an average AIP above 60% on day 56 were considered successful.

3.3.6 Wood Disk Confrontation Tests

Successful putative bacterial and fungal BCAs from culture confrontation tests were used for further testing against *A. solidipes*. To better simulate field conditions, additional confrontation tests were performed on wood disks instead of artificial media, using methods adapted from Simeto et al. (2023) (Figure 3.2). Wood disks were cut to a width of approximately 1.75 cm from Douglas-fir trees with an approximate DBH of 2.5 cm. The outer bark was removed with a knife and disks were dried in an oven at 90°C for 48 hours. The dry weight of each disk was recorded before disks were rehydrated in sterile deionized (DI) water for 20 hours. Finally, disks were autoclaved in glass petri dishes filled with 10 mL DI water for 30 minutes at 121°C. The autoclave cycle was repeated twice with 24 hours between cycles.

Jars were prepared by adding 100 mL Fox Farm Ocean Forest® Potting Soil mix to 473 mL wide mouth glass mason jars. Soil was moistened with 45 mL DI water and jars were autoclaved twice at 121°C for 30 minutes with 24 hours between cycles. Sterilized wooden disks were placed in the center of each jar with sterilized tweezers. *Armillaria solidipes* isolates were randomly paired with potential fungal or bacterial BCAs that were considered successful in dual culture confrontation tests. An 8 mm plug was taken from the growing edge of an approximately 2-month-old *A. solidipes* isolate growing on Armillaria MEA and placed against the side of the

wood disk, with the mycelium side of the plug touching the wood. Identical plugs were taken from 3-week-old potential fungal BCA cultures growing on ½ strength PDA and plugs were placed on the opposite side of the wood disk with the mycelium side touching the wood. For potential bacterial BCAs, a sterilized bacterial loop was used to apply a small amount of bacterial colony taken from a one-week-old culture growing on ½ strength PDA. An additional pairing of *A. altimontana*, a weakly pathogenic species with proposed BCA activity where it co-occurs with *A. solidipes* (Warwell et al., 2019), and *A. solidipes* was created using identical methods to other putative fungal BCAs; however, the plug of *A. altimontana* was taken from the growing edge of a 2-month-old culture growing on ½ strength PDA with antibiotics (1.95% potato dextrose agar, 0.75% agar, 0.01% streptomycin, 0.005% chloramphenicol) added, due to contamination issues, and the two *Armillaria* species were inoculated on the same day. Five replicates were created for each *A. solidipes*/putative BCA pairing, as well as five controls for each *A. solidipes* isolate and putative BCA, and 15 replicates of negative controls consisting of wood disks inoculated with ½ PDA agar plugs. Jars were sealed with parafilm and placed in the dark at room temperature for 14 weeks.

After 14 weeks, sterilized scalpels were used to remove microbial tissues adhered to the wood disk. These tissues were placed in 5 mL tubes and immediately frozen using dry ice (CO_2). Soil that had adhered to the underside of the wood disk due to mycelial growth, and any additional soil from approximately 1 cm in all directions from the wood disk, were collected in a plastic bag and immediately frozen with dry ice. Microbial tissue and soil samples were stored at -80°C. Wood disks were again dried in an oven at 90°C for 48 hours. The final dry weight of each disk was recorded, and dry weight loss was calculated by subtracting the final dry weight from the initial dry weight.

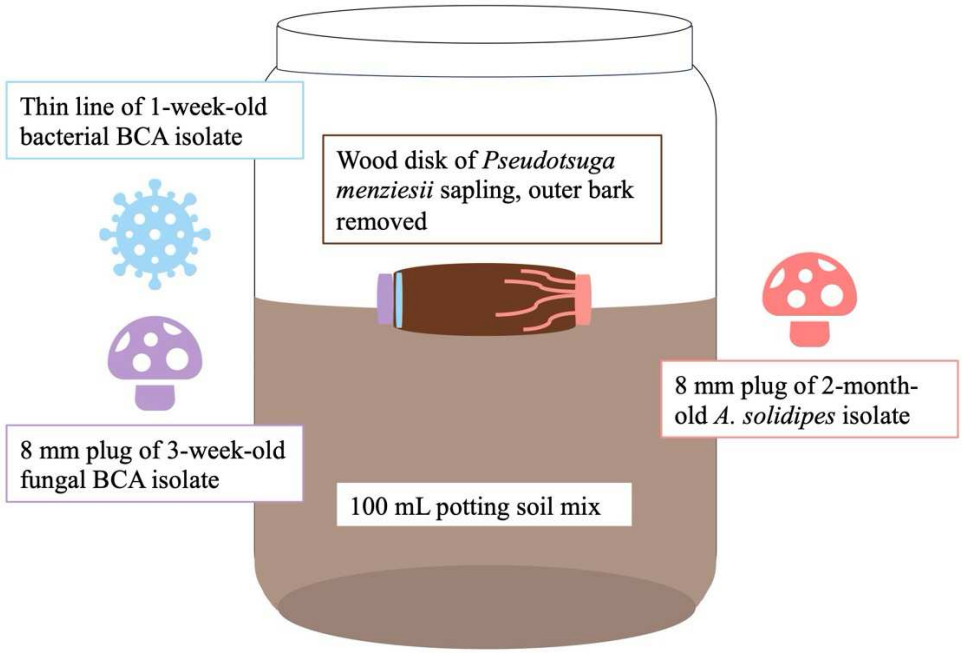

Figure 3.2. Diagram of wood disk confrontation test setup between *Armillaria solidipes* and putative bacterial or fungal biocontrol agents (BCAs). Each confrontation test contained only one putative BCA.

3.4 Results

3.4.1 Armillaria Isolation and Identification

In total, 18 fruiting body, 18 rhizomorph, and 11 mycelial fan samples were collected from the study site in October 2022 and June 2023. However, pure cultures could not be obtained from multiple rhizomorph and mycelial fan samples, resulting in 14 rhizomorph and 8 mycelial fan isolates. DNA sequencing identified 17 isolates obtained from fruiting bodies as *A. solidipes* and one as *A. altimontana*. Ten isolates from rhizomorphs were identified as *A. altimontana*, while two were identified as *A. solidipes* and two as *A. sinapina*. Seven of the mycelial fan samples were identified as *A. solidipes* and one as *A. altimontana*. Additionally, there were three

pairs of isolates obtained from rhizomorphs that were collected from the same tree at different timepoints and identified as the same *Armillaria* sp. (*A. altimontana*). Four trees were also associated with both rhizomorph and mycelial fan isolates, identified as the same *Armillaria* sp. (*A. solidipes* or *A. altimontana*) in two cases and different *Armillaria* species (*A. solidipes* mycelial fan and *A. altimontana* rhizomorph) in the others. A full table with isolate ID numbers, tree host, sample type, collection date, species identification, and GenBank accession numbers can be found in Table 3.2.

3.4.2 Armillaria solidipes Somatic Pairs Tests

Somatic pair testing of *A. solidipes* fruiting body isolates revealed that all isolates represented unique genets (individuals), except for isolates SJNF#016FB and SJNF#017FB. The mycelium of these two isolates fused, indicating that they are the same genet (vegetative clone) of *A. solidipes.*

3.4.3 Bacterial and Fungal Isolation from Soils

Approximately 800 initial microbial cultures were obtained from sub-culturing of serial dilution plates and were identified to 34 bacterial and 19 fungal genera based on sequencing of the 16S and ITS rDNA regions, respectively. Genera that included most of the tested putative BCAs were isolated from all three levels of burn severity, including *Bacillus*, *Pseudomonas, Lysobacter*, and all fungal genera. However, *Collimonas* species and *Caballeronia* species were only isolated from high-severity burn and unburned soil samples, while *Devosia* sp. was only isolated from a high-severity burn soil sample. A full list of isolated bacterial and fungal genera and GenBank accession numbers can be found in Chapter 2.

Table 3.2. Identification of *Armillaria* isolates from fruiting body, rhizomorph, and mycelial fan samples.

Armillaria Isolate ID	Tree Host #	Tree Host Species	Sample Type	Collection date	Species ID	GenBank Accession Number
SJNF#001FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694542
SJNF#002FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694546
SJNF#003FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694554
SJNF#004FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694555
SJNF#005FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694553
SJNF#006FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694552
SJNF#007FB	n/a	n/a	Fruiting body	October 2022	*A. altimontana*	PP694570
SJNF#008FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694563
SJNF#009FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694564
SJNF#010FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694551
SJNF#011FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694561
SJNF#012FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694562
SJNF#013FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694559
SJNF#014FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694567
SJNF#015FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694560
SJNF#016FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694558
SJNF#017FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694557
SJNF#018FB	n/a	n/a	Fruiting body	October 2022	*A. solidipes*	PP694556
SJNF#019MF	3	DF	Mycelial fan	October 2022	*A. solidipes*	PP694565
SJNF#020MF	4	DF	Mycelial fan	October 2022	*A. solidipes*	PP694548
SJNF#020R	4	DF	Rhizomorph	June 2023	*A. altimontana*	PP694577
SJNF#021MF	6	WWP	Mycelial fan	October 2022	*A. solidipes*	PP694550
SJNF#022MF	8	DF	Mycelial fan	October 2022	*A. altimontana*	PP694578
SJNF#022R	8	DF	Rhizomorph	October 2022	*A. altimontana*	PP694579
SJNF#022R-2	8	DF	Rhizomorph	June 2023	*A. altimontana*	PP694574
SJNF#023R	14	DF	Rhizomorph	October 2022	*A. altimontana*	PP694580
SJNF#024R	16	WWP	Rhizomorph	October 2022	*A. sinapina*	PP694568
SJNF#025R	20	WWP	Rhizomorph	October 2022	*A. sinapina*	PP694569
SJNF#026R	24	DF	Rhizomorph	October 2022	*A. altimontana*	PP694572
SJNF#026R-2	24	DF	Rhizomorph	June 2023	*A. altimontana*	PP694576
SJNF#027R	26	WWP	Rhizomorph	October 2022	*A. altimontana*	PP694581
SJNF#027R-2	26	WWP	Rhizomorph	June 2023	*A. altimontana*	PP694575
SJNF#028MF	5	WWP	Mycelial fan	June 2023	*A. solidipes*	PP694549
SJNF#028R	5	WWP	Rhizomorph	June 2023	*A. solidipes*	PP694544
SJNF#029MF	7	WWP	Mycelial fan	June 2023	*A. solidipes*	PP694547
SJNF#029R	7	WWP	Rhizomorph	June 2023	*A. altimontana*	PP694573
SJNF#030R	15	DF	Rhizomorph	June 2023	*A. solidipes*	PP694566
SJNF#031R	29	DF	Rhizomorph	June 2023	*A. altimontana*	PP694571
SJNF#032MF	10	DF	Mycelial fan	June 2023	*A. solidipes*	PP694545
SJNF#033MF	27	DF	Mycelial fan	June 2023	*A. solidipes*	PP694543

3.4.4 Culture Confrontation Tests

Due to the expected differences in our bacterial versus fungal growth rate, we used separate metrics and time scales to measure putative BCA performance, based on methods from Mesanza et al. (2016) and Chen et al. (2019), respectively. Of the 12 putative bacterial BCAs tested with dual culture confrontation tests, 5 proved to be successful at reducing *A. solidipes* colony size by over 60% at 56-days post-BCA inoculation when compared to pathogen control plates, despite average colony size of the bacterial BCAs remaining much lower than the area of *A. solidipes* colonies (Table 3.3, Figure 3.3). Of these 5 successful putative BCAs, 4 isolates were identified through sequencing of the 16S region as *Bacillus* sp. and one as *Caballeronia udeis*. The remaining unsuccessful BCAs had AIP values below 30% at 56 days post-BCA inoculation, with two resulting in negative AIP values indicating, on average, larger *A. solidipes* colonies in confrontation test plates than in control plates. In many cases, including for both successful and unsuccessful bacterial BCAs, AIP values at 56 days post-inoculation were lower than at 28 days.

Analysis of ML and BI phylogenies created with *Bacillus* species isolates revealed that three of our isolates clustered together with *B. mojavensis* and *B. halotolerans*, while the other three clustered together with *B. velezensis* and *B. amyloliquefaciens* (Figure 3.4). The three isolates that clustered with *B. velezensis/B. amyloliquefaciens* were highly effective at restricting *A. solidipes* growth, with AIP values over 70% on day 56 post-inoculation. The *Bacillus* sp. isolate that clustered with *B. mojavensis/B. halotolerans* had the lowest AIP value of the putative BCAs we considered to be effective, at 64.2% on day 56.

Table 3.3. Average area inhibition percentage (AIP) calculated from dual culture confrontation tests between putative bacterial biocontrol agents (BCAs) and the pathogen *Armillaria solidipes* at two timepoints post-BCA inoculation. Asterisks indicate isolates that were selected for further testing. Full measurements of BCA and *A. solidipes* colonies can be found in Supplemental Table 3.1.

Putative Bacterial BCAs	**GenBank Accession Number**	**Average Area Inhibition Percentage (AIP) [Standard Deviation]**	
		Day 28	**Day 56**
Bacillus sp.	PP583282	38.7 [3.3]	28.2 [13.7]
Bacillus sp.	PP559176	29.8 [26.3]	15.1 [24.0]
Bacillus sp.*	PP559175	70.5 [4.7]	64.2 [7.9]
Bacillus sp.*	PP583357	73.3 [11.5]	73.6 [20.5]
Bacillus sp.*	PP559178	79.7 [5.5]	85.0 [6.4]
Bacillus sp.*	PP559179	75.0 [4.1]	79.6 [5.4]
*Caballeronia udeis**	PP583404	77.9 [1.2]	73.4 [1.8]
Collimonas arenae	PP583343	35.1 [8.1]	6.6 [4.4]
Devosia sp.	PP559177	31.4 [1.6]	25.1 [12.1]
Lysobacter capsici	PP583342	16.7 [1.5]	-27.1 [6.0]
Pseudomonas sp.	PP583271	26.6 [13.3]	13.8 [14.7]
Pseudomonas sp.	PP583347	-18.4 [5.9]	-22.4 [11.8]

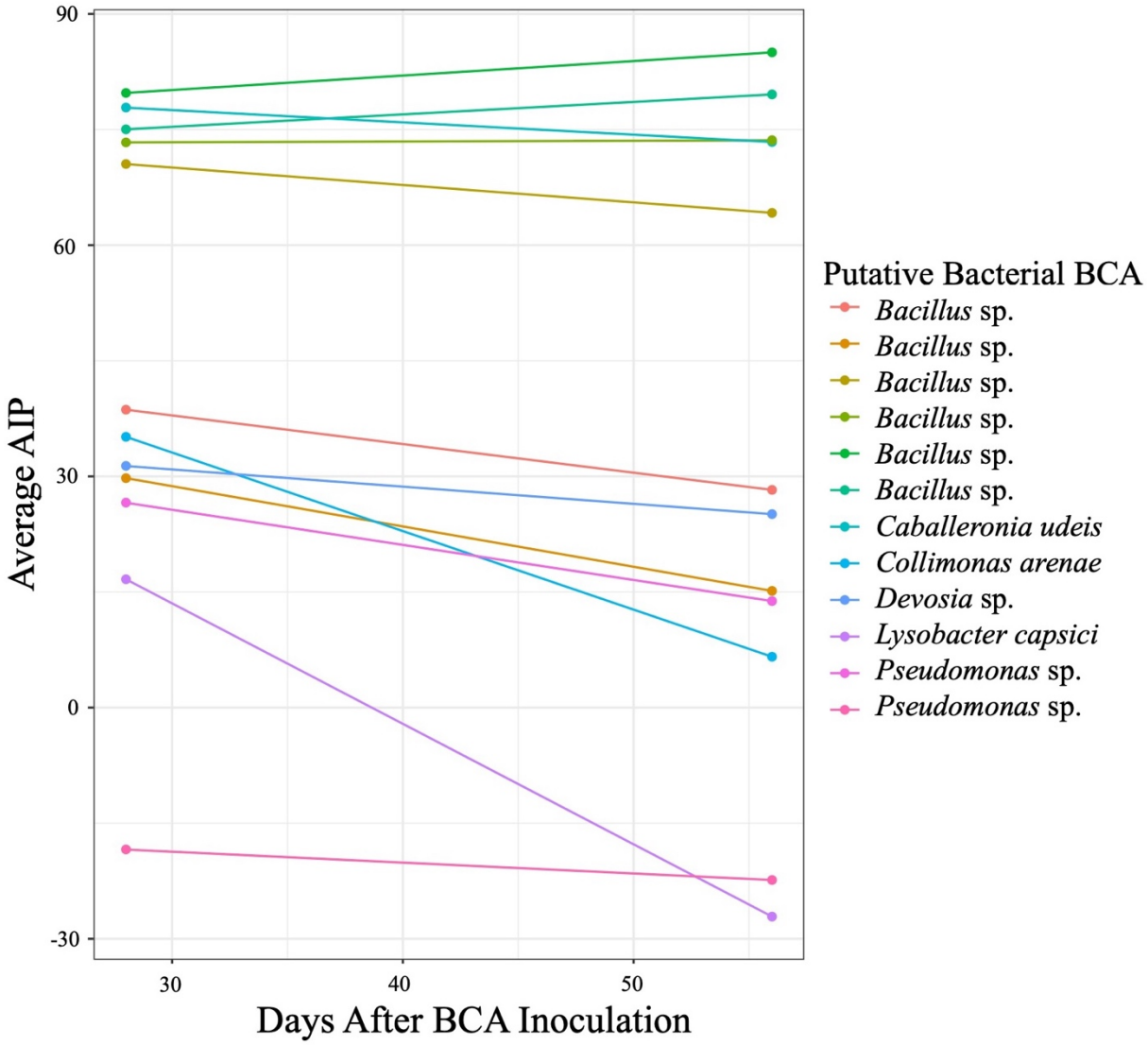

Figure 3.3. Average area inhibition percentage (AIP) from dual culture confrontation tests between putative bacterial biocontrol agents (BCAs) and the pathogen *Armillaria solidipes*, as measured at 28- and 56-days post-inoculation with the BCA. Full measurements of BCA and *A. solidipes* colonies can be found in Supplemental Table 3.1.

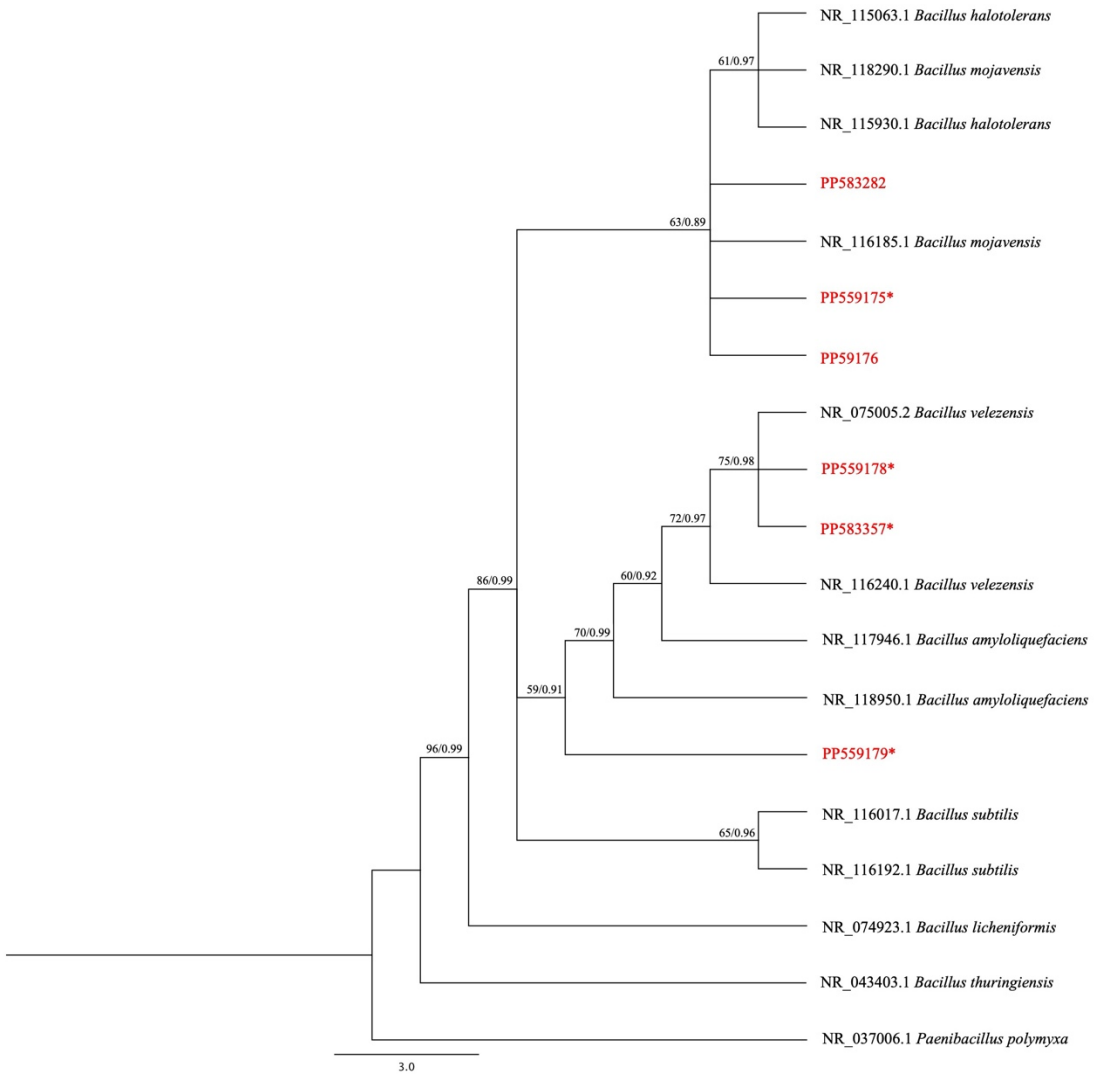

Figure 3.4. Phylogenetic analysis, including bootstrap values from maximum likelihood analysis/posterior probabilities from Bayesian inference, of the 16S rRNA region of *Bacillus* isolates used in our study (red) and reference isolates obtained from the NCBI GenBank database. Asterixis indicate successful *in vitro* biocontrol activity against the pathogen *Armillaria solidipes*. Isolate and reference names indicate GenBank accession numbers.

Of the 10 putative fungal BCAs tested through dual culture confrontation tests, 5 resulted in BCI values of over 80% at 14 days post-BCA inoculation (Table 3.4, Figure 3.5). Four of these 5 putative BCA isolates were identified as *Trichoderma* species and one isolate as *Mortierella elongata.* In contrast to bacterial BCAs, our effective putative fungal BCAs grew rapidly, colonizing both the Petri dish and the *Armillaria* colony, with some test replicates from 4 of the 5 effective putative BCAs showing complete coverage of the dish and colony (100% BCI) at 14-days post-inoculation (Supplemental Table 3.2). The 5 unsuccessful fungal BCAs had BCI values below 64% on day 14, and were identified as *Penicillium* species or *Umbelopsis* species.

Table 3.4. Average biocontrol index (BCI) calculated from dual culture confrontation tests between putative fungal biocontrol agents (BCAs) and the pathogen *Armillaria solidipes*, measured at four timepoints post-BCA inoculation. Asterisks indicate isolates that were selected for further testing. Full measurements of BCA and *A. solidipes* colonies can be found in Supplemental Table 3.2.

Putative Fungal BCA	**GenBank Accession Number**	**Average Biocontrol Index (BCI) [Standard Deviation]**			
		Day 2	**Day 5**	**Day 7**	**Day 14**
*Mortierella elongata**	PP573750	57.7 [8.3]	78.5 [3.4]	80.5 [3.7]	83.1 [2.5]
Penicillium murcianum	PP562365	13.2 [1.0]	28.5 [1.5]	36.1 [1.8]	45.8 [4.0]
Penicillium rubens	PP562366	22.1 [4.7]	52.0 [10.5]	55.1 [8.9]	57.4 [6.8]
*Trichoderma harzianum**	PP573516	66.2 [2.0]	84.6 [1.6]	84.6 [1.5]	100.0 [0]
*Trichoderma oblongisporium**	PP573675	47.6 [5.2]	71.7 [7.4]	76.7 [10.1]	89.7 [17.8]
*Trichoderma paraviridescens**	PP600952	47.6 [3.2]	72.9 [4.5]	77.0 [3.5]	100.0 [0]
*Trichoderma paraviridescens**	PP600951	43.3 [2.4]	71.1 [1.7]	77.1 [0.4]	100.0 [0]
Umbelopsis sp.	PP573536	10.8 [2.5]	22.0 [1.6]	30.5 [1.5]	42.2 [2.3]
Umbelopsis sp.	PP562368	14.2 [1.8]	27.5 [7.7]	36.4 [9.0]	48.7 [10.5]
Umbelopsis vinacea	PP562367	21.1 [1.9]	44.2 [6.3]	54.8 [4.6]	63.5 [2.9]

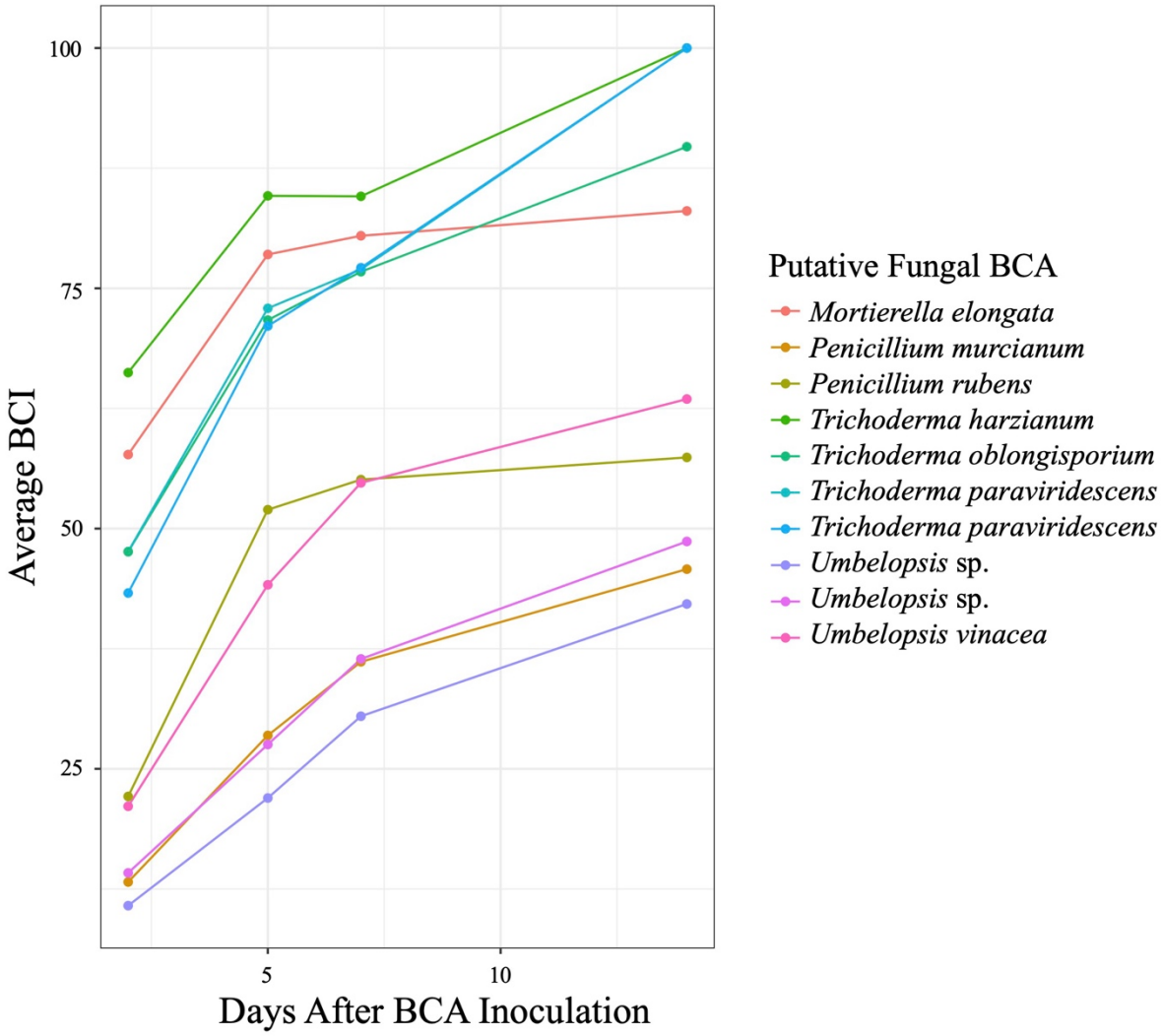

Figure 3.5. Average biocontrol index (BCI) from dual culture confrontation tests between putative fungal biocontrol agents (BCAs) and the pathogen *Armillaria solidipes*, as measured at 2-, 5-, 7-, and 14-days post-inoculation with the BCA. Full measurements of BCA and *A. solidipes* colonies can be found in Supplemental Table 3.2.

3.4.5 Wood Disk Confrontation Tests

Multiple issues arose during the 14-week course of the wood disk assays, and statistical analysis was not possible. Primarily, many jars had obvious fungal contamination by the end of this period with contaminant mycelia covering the wood disk and surrounding soil. Additionally,

there was a lack of *A. solidipes* growth and wood degradation on the wood disks. Finally, there was no trend in dry weight loss between the disks with little *A. solidipes* growth and those where there was strong growth of *A. solidipes* and the pathogen appeared to be degrading the disk. However, the confrontation tests between *A. solidipes* and *A. altimontana* did produce observational results that suggest the need for further research. *A. altimontana* grew well in the control jars, with four of five controls resulting in significant *A. altimontana* growth and apparent beginnings of wood degradation represented by mycelium clearly remaining within the wood disk following the removal of fungal tissues (Figure 3.6). Additionally, in two of these jars, *A. altimontana* formed dense mats of mycelia with early fruiting body formation (Figure 3.6A,B). When confronted with *A. solidipes*, *A. altimontana* grew to cover more than half of the wood disk in the three test jars where there was abundant fungal growth and no contamination (Figure 3.7). These results indicate that further examination of the potential for *A. altimontana* to act a BCA for *A. solidipes* through superior saprophytic colonization is warranted.

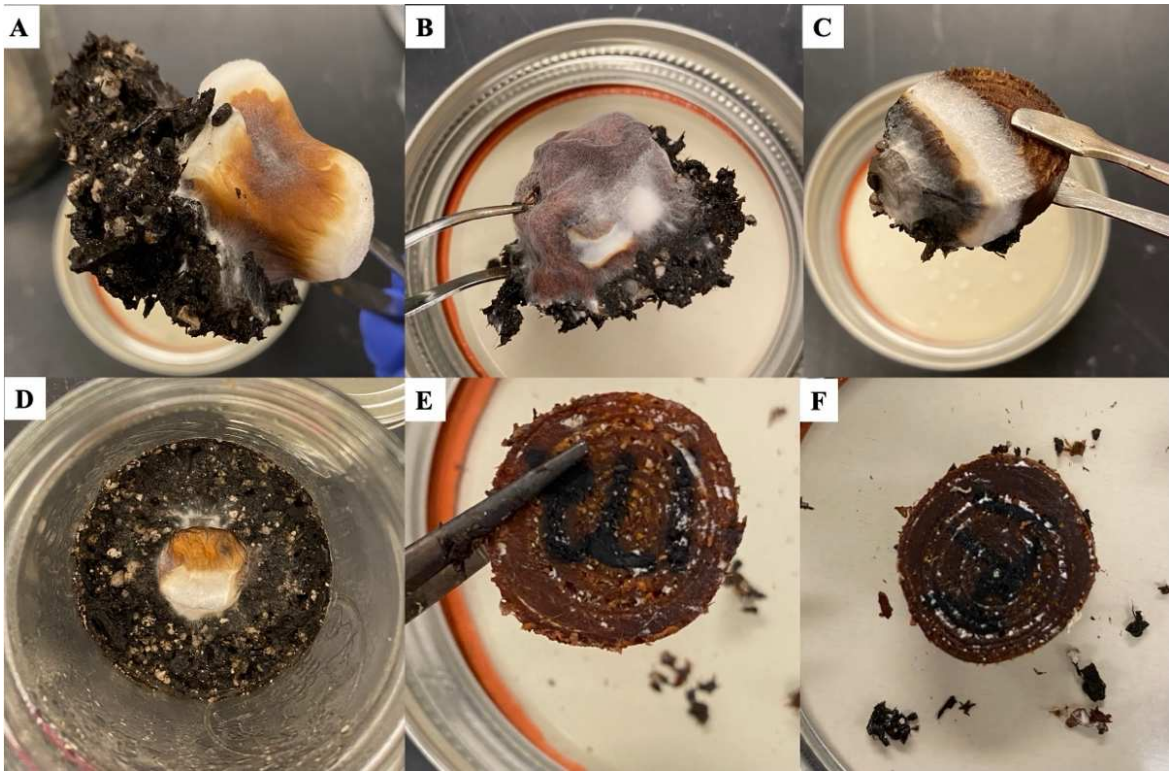

Figure 3.6. Control replicates of wood disk tests inoculated with only *Armillaria altimontana* and 14-weeks post-inoculation. A-D are wood disks prior to the removal of fungal mycelium, with A and B showing the dense mycelium producing a fruiting body-like structure. E and F show the wood disks after fungal tissues have been removed, with white fungal mycelium clearly remaining within the wood disk.

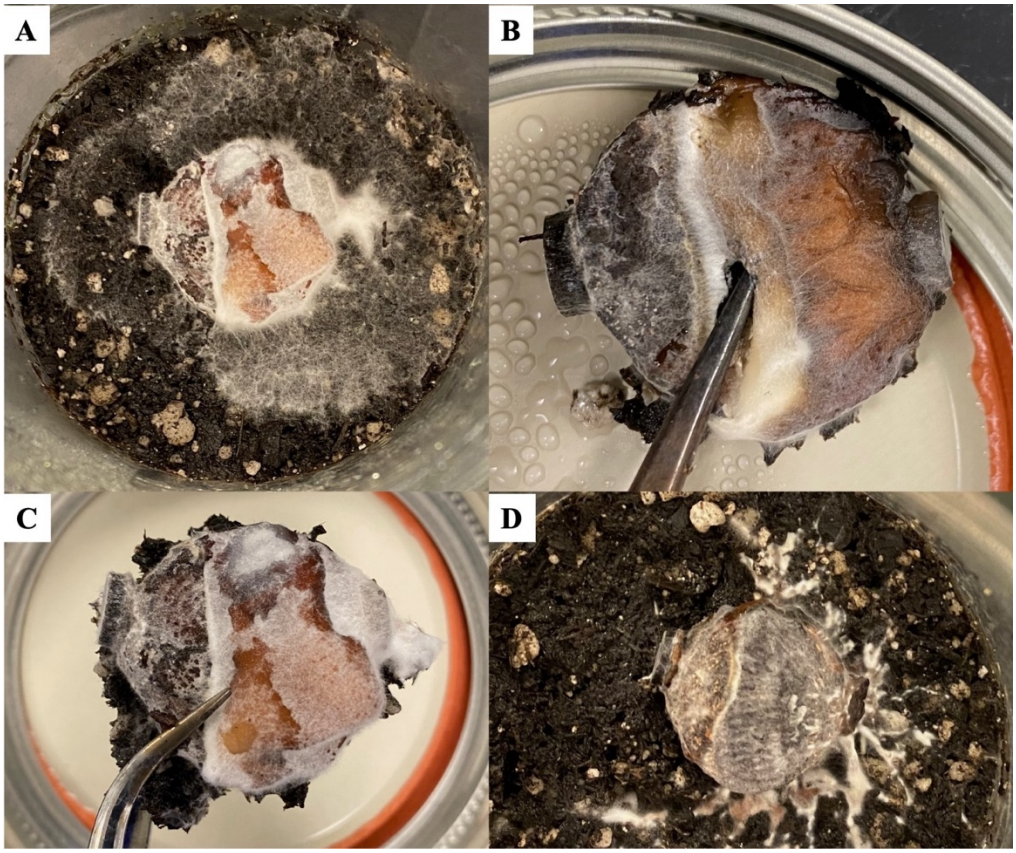

Figure 3.7. Confrontation tests on wood disks between *Armillaria solidipes* (left side of each disk) and *A. altimontana* (right side of each disk) at 14-weeks post-inoculation, showing the superior growth of *A. altimontana*. A and C are from the same confrontation test, while A/C, B and D are unique replicates.

3.5 Discussion

This research contributes to the expanding body of knowledge identifying microbe-microbe interactions that may play a role in effective management strategies for forest diseases. We observed effective *in vitro* inhibition of the Armillaria root disease pathogen *A. solidipes* by five bacterial and five fungal isolates obtained from the native soil microbiome in areas where *A. solidipes* infection is prevalent. These included commonly reported BCA organisms in the genera *Bacillus* and *Trichoderma*. We found that analysis of microbial isolations from the three levels of burn severity (high, low, unburned) did not indicate that these putative BCA taxa will be negatively impacted by severe fires in western United States forests. Further, our results

suggest the need for further assays to elucidate how the putatively beneficial species *A. altimontana* can contribute to the management of Armillaria root disease caused by *A. solidipes*.

The effectiveness of *A. solidipes* growth inhibition by our putative BCAs was assessed through *in vitro* dual culture confrontation tests, in which a putative BCA and a pathogen are co-inoculated on artificial media in the same Petri dish (Raymaekers et al., 2020). While this method is commonly used for initial biocontrol screening, we recognize that these assays cannot capture certain beneficial characteristics that may contribute towards pathogen antagonism in natural settings, including the potential for plant growth promotion and stimulation of plant resistance mechanisms. Additional field trials are necessary to more fully elucidate how our proposed effective BCAs will perform under natural settings.

Four of the six tested *Bacillus* species isolates showed effective inhibition of *A. solidipes* growth during dual culture confrontation tests, with the most effective isolates clustering with the species *B. velezensis* and *B. amyloliquefaciens* based on phylogenetic analysis of the 16S rRNA region. Our results are supported by numerous previous studies which have demonstrated effective *in vitro* and *in vivo* BCA activity for a variety of fungal plants pathogen by *B. velezensis* (Huang et al., 2023; Zhang et al., 2022), *B. amyloliquefaciens* (Luo et al., 2022; Chowdhury et al., 2015; Ji et al., 2013), *B. halotolerans* (Kang et al., 2023; Tsalgatidou et al., 2022), and *B. mojavensis* (Yi et al., 2022; Diabankana et al., 2021). Genomic analyses have revealed that these *Bacillus* species harbor large gene clusters related to the production of antimicrobial secondary metabolites (Tsalgatidou et al., 2022; Fan et al., 2018; Fira et al., 2018; Chowdhury et al., 2015), including fungal cell wall degrading enzymes, the expression of which has been confirmed by *in vitro* assays (Huang et al., 2023; Kang et al., 2023; Yi et al., 2022; Diabankana et al., 2021), although lower concentrations of these compounds have been reported

under field conditions (Chowdhury et al., 2015). Further, *Bacillus* species promote plant growth, for example by producing the hormone indole acetic acid (IAA) and iron-scavenging siderophores (Huang et al., 2023; Yi et al., 2022; Ali et al., 2020), and are widely reported to secrete surfactins, which are both antagonistic to fungal pathogens and can trigger induced systemic resistance in the plant (Kang et al., 2023; Fan et al., 2018; Chowdhury et al., 2015; Arguelles-Arias et al., 2009). Previous studies have also directly examined the BCA activity of *Bacillus* species against *Armillaria* pathogens; Mesanza et al. (2016) found effective inhibition of *A. mellea* growth by *B. simplex* during dual culture confrontation tests, and Zhang et al. (2022) reported that treatment with *B. velezensis* reduced disease symptoms on poplar seedlings inoculated with *A. solidipes*. However, further studies of the *B. velezensis/A. solidipes* interaction in Zhang et al. (2023) revealed that *A. solidipes* caused harmful effects to *B. velezensis* cells, including deformation and breaks in the cell walls, which could reduce the potential for *B. velezensis* to act as an effective BCA for *A. solidipes*. However, the inhibitory activity against fungal pathogens of *Bacillus* isolates demonstrated during our assays and previous studies suggests that native *Bacillus* species could be part of an effective biocontrol strategy for Armillaria root disease caused by *A. solidipes*.

In addition to *Bacillus* species, *Caballeronia udeis* proved to be an effective at restricting *A. solidipes* growth by over 70% at 56-days post-inoculation (Table 3.3). The genus *Caballeronia* was recently separated from *Burkholderia* and *Paraburkholderia* based on phylogenetic analysis of the 16S rRNA region (Dobritsa & Samadpour, 2016). There are multiple reports of *Caballeronia* species promoting the growth of forest and horticulture plants, likely due to nutrient acquisition and transportation abilities (Kim et al., 2021; South et al., 2021; Puri et al., 2020a; Puri et al., 2020b). Additionally, Puri et al. (2020a) used *in vitro* enzyme

assays to reveal that *C. sordidicola* exhibited high activity of enzymes with fungal cell wall degrading functions, including chitinases and glucanases. However, during these assays, *C. udeis* did not show chitinase or glucanase activity. Regardless, to our knowledge, this is the first report of direct pathogen inhibition from this genus.

Unexpectedly, our two isolates of *Pseudomonas* species showed poor inhibition of *A. solidipes* growth during our confrontation tests (Table 3.3, Figure 3.3). This contrasts with results reported in Mesanza et al. (2016), in which confrontation tests between the pathogen *A. mellea* and the BCA *P. fluorescens* resulted in AIP values of over 94%, and in Ali et al. (2020), in which *Pseudomonas* isolates inhibited the *in vitro* growth of various other fungal pathogens. Additionally, *Pseudomonas* species, especially *P. fluorescens* and closely related species, have been widely recognized as effective BCAs for a variety of soilborne plant pathogens. Characteristics such as aggressive colonization of the rhizosphere, effective competition for limiting resources, production of antimicrobial compounds, and stimulation of plant resistance mechanisms likely drive these benefits (Panpatte et al., 2016; Couillerot et al., 2009; Weller, 2007). However, the genus *Pseudomonas* shows much genomic and metabolic diversity (Silby et al., 2011), including among strains of the same species. Within the broader *P. fluorescens* complex, the genomes of certain species include genes responsible for the production of various antibiotic compounds, while other species lack these genes (Garrido-Sans et al., 2016). As we were not able to identify our *Pseudomonas* isolates to species based on sequencing of the 16S rDNA region, it is possible that the lack of antagonistic ability we observed may be attributable to our isolates not producing antifungal exudates that would have led to *in vitro* inhibition of *A. solidipes*.

As expected, the *Trichoderma* isolates had the highest BCI values of our putative fungal BCAs by day 14, with 100% BCI values for confrontation test replicates from each *Trichoderma* isolate (Supplemental Table 3.2). Species from the genus *Trichoderma* have long been recognized for possessing an array of characteristics that allow them to act as effective BCAs for a variety of plant pathogens, including multiple *Armillaria* species (Asad, 2022; Kedves et al., 2021; Chen et al., 2019). These include rapid growth and colonization (Gams & Bissett, 2002), which we observed over the 14-day monitoring period for our confrontation tests. Additionally, *Trichoderma* species also directly parasitize the tissues of other fungi, killing them with secreted antifungal enzymes, including cell wall degrading enzymes (Sood, 2020; Druzhinina et al., 2011); this likely occurred during our confrontation tests by day 14 when we observed *Trichoderma* mycelia growing over the *Armillaria* colonies. Previous research has closely examined how *Trichoderma* may inhibit the activity of *Armillaria* pathogens with transcriptome analyses during confrontation tests between *T. atroviride* and *A. ostoyae* (Chen et al., 2023). During this interaction, *T. atroviride* expressed an assortment of genes with biocontrol-related functions. These included genes associated with fungal cell wall degrading enzymes, with secondary metabolite (antibiotic) synthesis, and with ATP-binding cassette transporters which may transport the secondary metabolites (Chen et al., 2023). These results support the variety of mechanisms that *Trichoderma* species are reported to utilize as effective BCAs. Additionally, *Trichoderma* species have other characteristics which make them successful BCAs, but which we were unable to demonstrate with *in vitro* confrontation tests. These include superior competitive abilities for limiting resources in the soil, for example, producing siderophores to improve iron uptake, in addition to promoting plant growth and inducing plant resistance mechanisms (Sood, 2020; Chen et al., 2019; Harman et al., 2004).

In addition to the *Trichoderma* isolates, *Mortierella elongata* showed rapid growth in dual culture confrontation tests with *A. solidipes*, with an average BCI of 83.1% at 14-days post-inoculation. *Mortierella* species are ubiquitous soil inhabitants and endophytic fungi and are widely recognized to promote plant growth (Ozimek & Hanaka, 2020; Liao et al., 2019). Additionally, Johnson et al. (2019) reported that *Arabidopsis* seedlings inoculated with *M. hyalina* showed slower disease development, reduced disease severity, and lower mortality rates than uninoculated seedlings when facing the necrotrophic fungal pathogen *Alternaria brassicae*. This occurred despite *in vitro* growth inhibition of *M. hyalina* by *A. brassicae* and was attributed to modified plant defense responses when colonized by *M. hyalina* (Johnson et al., 2019). Further, Wani et al. (2016) found that *M. alpina* isolated from the endophytic community of *Crocus sativus* corms was able to inhibit the growth of 5 of the 6 tested fungal pathogens during dual culture confrontation tests, despite a lack of antifungal activity associated with *M. alpina* extracts. Based on the results from these previous studies, and because our BCI metric was based largely on putative BCA colony size, we suggest that *M. elongata* could help to control Armillaria root disease through the rapid growth and substrate colonization that it exhibited in this study and the previously demonstrated promotion of plant growth and modification of plant defense responses (Ozimek & Hanaka, 2020; Johnson et al., 2019).

In ecosystems where fire disturbances are expected to increase in frequency and severity, understanding how burns will impact the presence of native BCA species used for plant disease management is critical, as BCA taxa which are negatively impacted by fire may be ineffective in the long term at reducing disease. Apart from *Caballeronia udeis*, effective putative BCAs in our study, as evaluated through *in vitro* dual culture confrontation tests, belonged to genera that were isolated from all three burn severity levels (high, low, and unburned) (Chapter 2, Supplemental

Table 2.7), and the relative abundance of these genera did not differ between burn severity levels (Chapter 2, Supplemental Tables 2.3 and 2.4). These results reflect the ubiquitous presence of the genera *Bacillus*, *Mortierella*, and *Trichoderma* within soil microbiomes across environments (Asad, 2022; Bonaterra et al., 2022; Ozimek & Hanaka, 2020) and suggest that fire, including severe fire that results in tree mortality, may not change the availability of these native BCAs for *A. solidipes*. However, as reported in Chapter 2, high-severity burn sites appeared to harbor a higher presence of *A. solidipes* and its associated microbiome (Chapter 2, Table 2.1). Although very few studies have evaluated how fire changes the efficacy of BCA organisms, we speculate that these high-severity burns reduce the biocontrol activity of native BCAs or lend *A. solidipes* a competitive advantage over these species that allow the pathogen to overcome any antagonistic effects. However, in contrast to this suggestion, Reaves et al. (1990) reported that *Trichoderma* isolates from burned soils were more effective at preventing *A. solidipes* (*A. ostoyae*) rhizomorph development than those from unburned soils. More research is needed to determine how the activities of common microbial BCAs are impacted by burns, and thus how their antagonistic abilities may change following fire. Additionally, our microbiome analysis reported in Chapter 2 revealed a decreased abundance of beneficial ECM fungi following high-severity fires. We did not isolate any ECM fungi from our soil samples, but it is possible that dual culture confrontation tests with ECM isolates would not reveal BCA activity, as the benefits associated with these symbionts are due to their plant growth promoting and nutrient acquisition abilities (Smith & Read, 2010; Leake et al., 2004). However, we suggest that the reduced abundance of these ECM fungi on high-severity burn sites may have allowed for abundant colonization by *A. solidipes*, despite the presence of native BCAs within the soil microbiome.

Confrontation tests on wood disks cut from young Douglas-fir trees instead of artificial media were established to better replicate the environmental conditions of natural settings. However, due to contamination, lack of *A. solidipes* and putative BCA growth, and inconsistent trends in dry weight loss, we could not use these assays to support the results of our initial culture confrontation tests. Our methods for these assays were based on those outlined in Simeto et al. (2023), in which saprophytic fungi were inoculated on wood disks to assess wood decay via dry weight loss. However, there were notable differences which may have contributed to our lack of adequate results. For example, Simeto et al. (2023) allowed colonization of the wood disk to occur over a period of 27 weeks, while our colonization period was only 14 weeks. It is likely that a longer period would have allowed for further colonization and wood degradation, and thus higher dry weight loss. Additionally, while Simeto et al. (2023) included highly saprophytic fungal species, *Armillaria* species are difficult to inoculate with and are notoriously slow growing, which may for the lack of observed pathogen growth.

However, our wood disk confrontation tests between *A. solidipes* and *A. altimontana* were largely free of contamination and showed significant growth of both species. The weakly pathogenic species *A. altimontana* has been associated with improved tree health and reduced Armillaria root disease damage on sites where it co-occurs with *A. solidipes* (Ibarra Caballero et al., 2023; Warwell et al., 2019) During our wood disk assays, we found that an isolate of *A. altimontana* collected from our study site showed efficient saprophytic colonization of inoculated wood disks. *Armillaria altimontana* also appeared to have a competitive advantage over *A. solidipes* when the species were co-inoculated, as visual inspection of confrontation tests at 14 weeks post-inoculation showed *A. altimontana* covering more than 50% of the wood disks and having more biomass (Figure 3.7). It has been suggested that the benefits to tree health

attributed to *A. altimontana* may be due to the abundance of ECM fungi found in the soil microbiomes associated with this species (Ibarra Caballero et al., 2023; Chapter 2). However, our wood disk assay results suggest that further research is needed to determine whether *A. altimontana* itself may be an effective BCA for *A. solidipes* through superior competition and colonization, with or without the support of its associated microbiome.

In this research, we used *in vitro* dual culture confrontation tests between bacterial and fungal isolates from the native soil microbiome and the pathogen *A. solidipes* to support the previously demonstrated BCA capabilities of the microbial taxa *Bacillus*, *Trichoderma*, and *Mortierella*, as well as demonstrating effective pathogen inhibition from the bacterial species *C. udeis*. Importantly, our research from Chapter 2 did not indicate a significant difference in the presence or abundance of these putative BCA taxa in soils from different burn severity groups, suggesting that high severity fires may not directly impact the use of these microorganisms as part of a management strategy for *A. solidipes*. However, fire's effect on other members of the native soil microbiome and/or the pathogen itself may indirectly influence the efficacy of these putative BCAs. Further, we found that the weakly pathogenic species *A. altimontana* appears to show superior saprophytic colonization abilities when compared to *A. solidipes*, indicating a need for further research regarding *A. altimontana*'s BCA activity. Overall, our research adds to previous literature identifying microbial BCA organisms for important fungal plant pathogens and provides insight into considering the effects of major environmental disturbances on using these organisms in landscape scale management strategies.

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CHAPTER 4: CONCLUDING REMARKS

The native soil microbiome harbors abundant microbial taxa with abilities that can improve our management of prevalent forest diseases. A deeper understanding of the microbe-microbe, plant-microbe, and environmental interactions that underly microbe-based control strategies of devastating phytopathogens is critical for the efficient integration of these strategies into management plans. The research presented in this thesis focuses on how the relationships between the native soil microbiome, the potential biological control agents found within, fire as a major environmental disturbance, and the virulent pathogen *Armillaria solidipes* affect disease progression and pathogen activity in western conifer forests where Armillaria root disease is prevalent. The chapters are tied together by the common thread of understanding how the microbiome can be manipulated for effective, landscape-scale control of Armillaria root disease.

In Chapter 2, I used metagenomic analyses to identify how changes to the native soil microbiome following fire correspond with the microbial communities associated with different *Armillaria* species and considered the implications of this overlap to our management of Armillaria root disease. The results from this study indicate that high severity burns appear to support the presence of *A. solidipes* and its associated microbial partners, while reducing the presence of the putatively beneficial species *A. altimontana* and the symbiotic ectomycorrhizal (ECM) fungi associated with the *A. altimontana* microbiome. As a result, conifer forests that have experienced severe fire disturbances appear to be priority areas for the monitoring and preemptive management of Armillaria root disease. However, the increased relative abundance of the ECM genus *Suillus*, previously associated with *A. altimontana* and reported to be an early

post-disturbance colonizer, following low-severity fires suggest the potential for low-severity fires to be part of a management strategy for *A. solidipes*.

Future research to build off this work should involve further investigation of the beneficial relationships between ECM fungi on their host tree symbionts in areas severely affected by Armillaria root disease. An effective way to investigate this is through greenhouse experiments in which conifer seedlings with and without various diverse ECM partners are inoculated with *A. solidipes* and the effects on tree health and disease progression are assessed. By clarifying how and which ECM fungi contribute to disease suppressive soils for Armillaria root disease, results from such an experiment can be translated directly into tangible disease management methods, for example, by promoting the abundance of specific ECM taxa through inoculations or the transfer of synthetic microbial communities that include these symbionts. Further links can then be drawn to the microbial changes I observed in Chapter 2 following fire. For example, if *Suillus* species indeed reduce disease severity and tree mortality in these trials, the reports of early colonization by this genus following fire indicate that additional testing of low-severity, controlled burns to manage *A. solidipes* via the soil microbiome is warranted.

Chapter 3 builds upon the growing body of research identifying biological control agents (BCAs) that can be used instead of or alongside chemical and cultural control methods for the sustainable management of plant pathogens. *In vitro* dual culture confrontation tests were used to identify members of the native soil microbiome that could directly inhibit the growth of *A. solidipes* through antagonistic activity. I used these lab-based assays for initial BCA screening, yet I understand that these methods lack the ability to characterize many modes of action that contribute to pathogen inhibition and other environmental factors that influence successful biological control. Further field assays with the proposed BCAs I report in Chapter 3 are

certainly needed to ensure that *in vitro* results will show similar levels of efficacy under natural conditions. Regardless, antagonistic activity towards *A. solidipes* was demonstrated by the widely reported BCA genera *Bacillus* and *Trichoderma*, and by the less studied species *Caballeronia udeis* and *Mortierella elongata.*

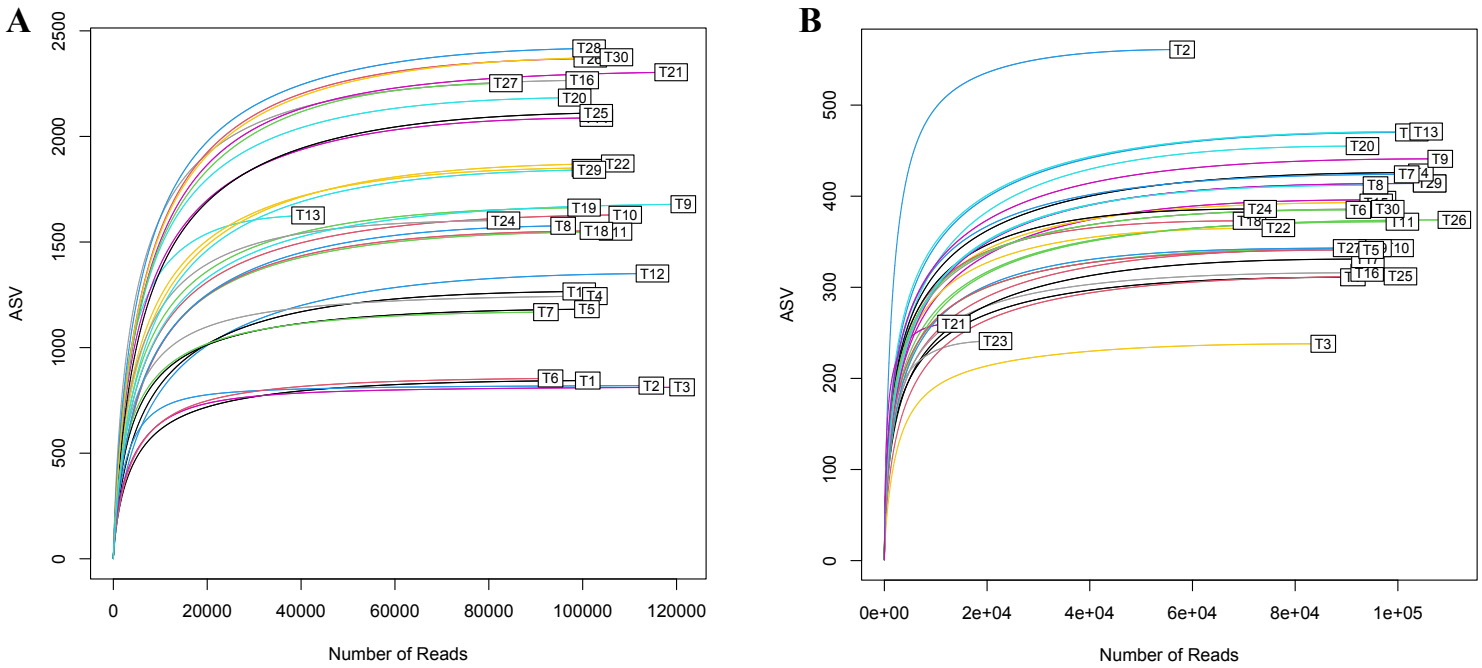
While I experienced major setbacks when trying to replicate these results under conditions more similar to the field environment, initial results from my wood disk confrontation tests presented in Chapter 3 support the previously reported benefits to tree health attributed to the weak pathogen *A. altimontana* in areas where *A. solidipes* is prevalent. My results indicate that *A. altimontana* may have a competitive advantage in saprophytic substrate colonization when compared with the abilities of *A. solidipes*, suggesting that *A. altimontana* may be used as a BCA for Armillaria root disease. I propose the use of further *in vitro* and *in vivo* confrontation tests between these two *Armillaria* species to determine how *A. altimontana* inhibits *A. solidipes,* both with and without the assistance of its microbial community.

Finally, the use of transcriptome analyses of effective *A. solidipes* inhibition by microbial BCAs is needed to better understand how BCA organisms restrict the activity of this pathogen and thus aid in the identification of novel BCAs with similar transcriptional signatures. I intend to continue with this aspect of the research through transcriptome analyses of dual culture confrontation tests between *A. solidipes* vs. *Mortierella elongata* and *A. solidipes* vs. *Trichoderma paraviridescens*. The results of this work will be integrated with Chapter 3 of this thesis into a final publication from this work, with an expected publication date in Fall 2024.

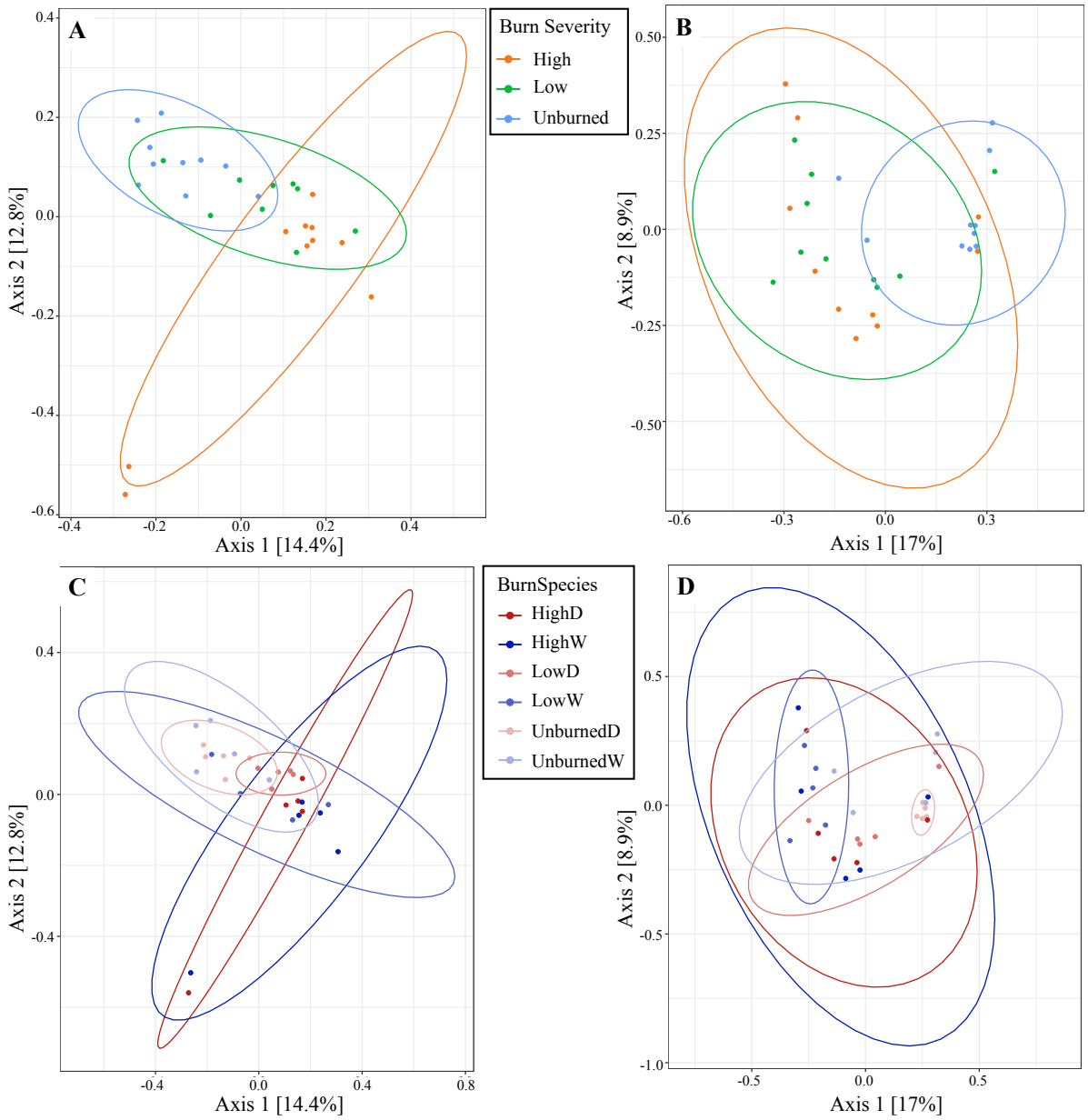
Overall, both studies presented in this thesis contribute to our expanding understanding of the complex interactions between the microbiome, the environment, and phytopathogens that underly plant diseases. As anthropogenic activity and climate change are expected to continue

increasing the severity and frequency of environmental disturbances such as fire, it is especially critical to consider how these stressors will affect all aspects of forest ecosystems and how we can mitigate potentially harmful repercussions from changes to the soil microbiome. It is important to me that this work is not only scientifically interesting, but also helps in the development of novel solutions for Armillaria root disease management. I hope that forest managers in Rocky Mountain regions can incorporate the results presented in this thesis into management strategies to preserve and protect our forest ecosystems for future generations.

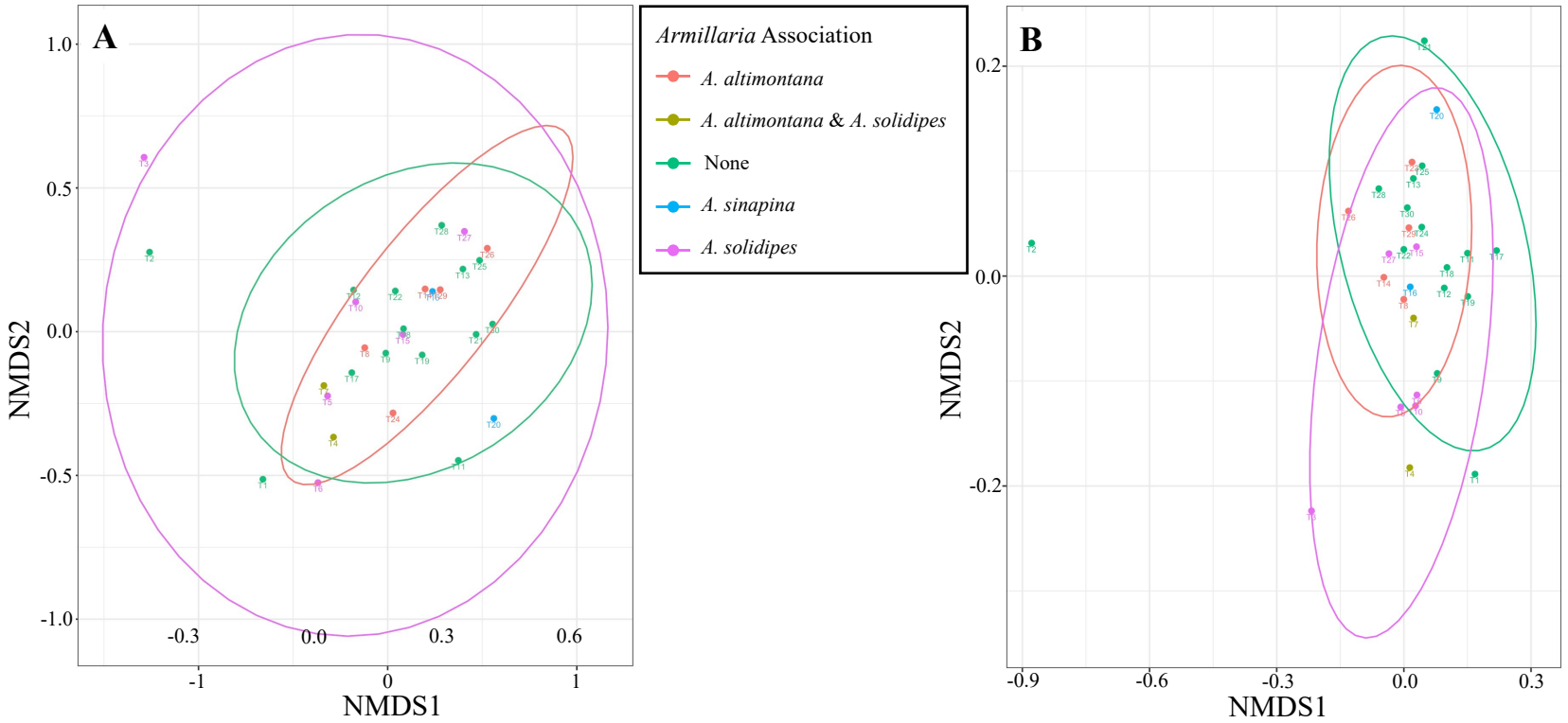
APPENDIX



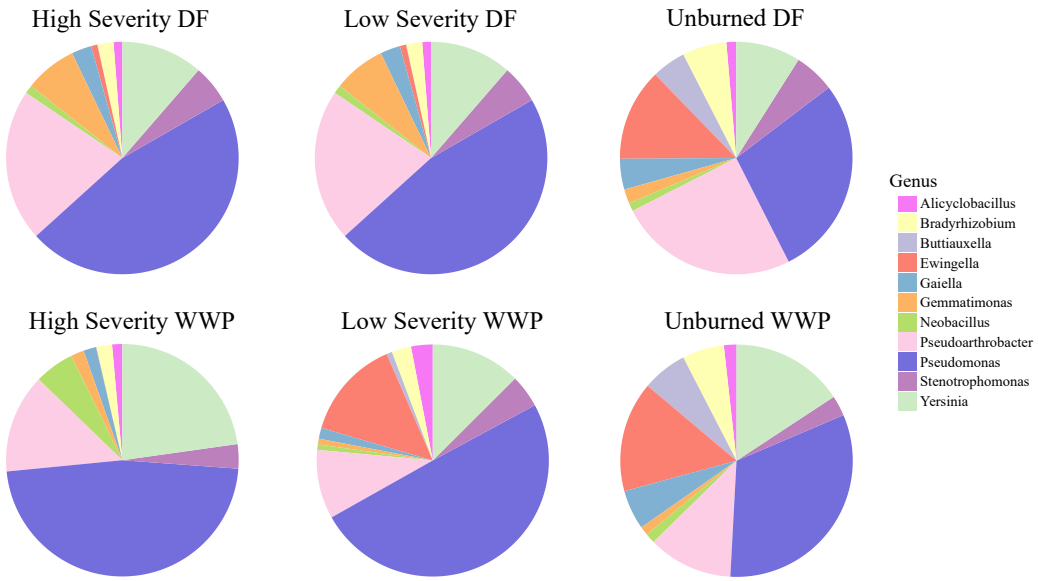
Supplemental Figure 2.1. Rarefaction curves for bacterial (A) and fungal (B) metagenome data.



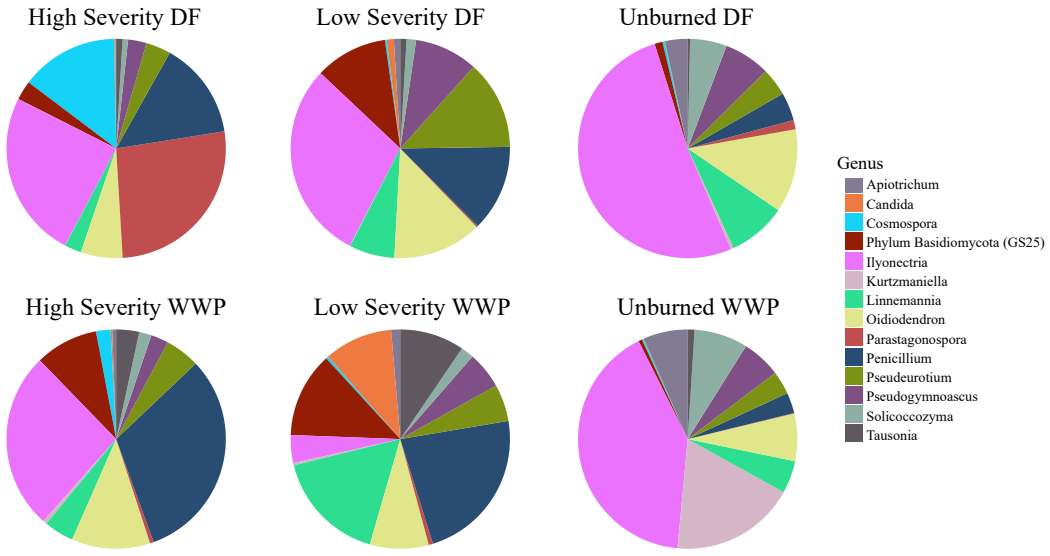
Supplemental Figure 2.2. Principle coordinate analysis (PCoA) plots for bacterial (A, C) and fungal (B, D) communities separated by burn severity (A, B) and BurnSpecies (C, D). HighD, LowD, and UnburnedD = high-severity burn, low-severity burn, and unburned Douglas-fir (*Pseudotsuga menziesii*), respectively, HighW, LowW, and UnburnedW = high-severity burn, low-severity burn, and unburned western white pine (*Pinus monticola*), respectively.



Supplemental Figure 2.3. Non-metric multi-dimensional scaling (NMDS) plots for bacterial (A) and fungal (B) communities grouped by *Armillaria* species association.



Supplemental Figure 2.4. Pie charts depicting bacterial genera present in at least 1% relative abundance in communities separated by BurnSpecies. DF = Douglas-fir (*Pseudotsuga menziesii*) host, WWP = western white pine (*Pinus monticola*) host.



Supplemental Figure 2.5. Pie charts depicting fungal genera present in at least 5% relative abundance in communities separated by BurnSpecies. DF = Douglas-fir (*Pseudotsuga menziesii*) host, WWP = western white pine (*Pinus monticola*) host.

Supplemental Table 2.1. Full list of soil characteristics and those removed following Pearson's correlation analysis.

Characteristic Included in Variable selection	**Correlated Variable(s) Removed** and Direction of Correlation (+ or -)
Soil pH	Ward buffer pH (+), hydrogen (H) saturation (-), Calcium (Ca) saturation (+)
Soluble salts (mmhos/cm)	Sulfur (S) ppm (+), Ca ppm (+), Mg ppm (+)
Organic matter loss on ignition (LOI)	
Kg nitrogen (N) per hectare	Nitrate N ppm (+)
Potassium (K) parts per million (ppm)	
Zinc (Zn) ppm	
Iron (Fe) ppm	
Manganese (Mn) ppm	
Copper (Cu) ppm	
Sodium (Na) ppm	S ppm (+)
Cation exchange capacity (CEC)	
K saturation	
Magnesium (Mg) saturation	Ward buffer pH (+), H saturation (-)
Na saturation	
Mehlich-3 phosphorus (P) ppm	

Supplemental Table 2.2. Bonferroni adjusted *p*-values for pairwise comparisons of bacterial and fungal communities grouped by burn severity.

Community	Burn Severity Comparison	Bonferroni-adjusted *P*-value
Bacteria	High vs. Low	0.2748
Bacteria	High vs. Unburned	0.0003
Bacteria	Low vs. Unburned	0.0027
Fungi	High vs. Low	0.4077
Fungi	High vs. Unburned	0.003
Fungi	Low vs. Unburned	0.0021

Supplemental Table 2.3. Bacterial genera from metabarcoding analysis proliferating under each burn severity level.

Genus	Abundant in High	Abundant in Low	Abundant in Unburned	Consistent in High	Consistent in Low	Consistent in Unburned
Achromobacter		X				
Acidibacter		X	X			
Acidiferrimicrobium		X	X			
Acidiferrobacter			X			
Acidiphilium		X	X			
Acidipila			X			X
Acidisoma			X			
Acidobacterium		X			X	
Acidocella			X			
Acidopila			X			
Acidovorax	X			X		
Actinoallomurus	X	X				
Actinocorallia			X			X
Actinomadura	X	X			X	
Actinomarinicola		X	X			
actinomycete		X				
Actinomycetospora		X	X			
Actinoplanes		X	X			X
Adhaeribacter	X	X	X	X		
Aeromicrobium	X			X		
Aeromonas	X	X				
Aetherobacter			X			
Afifella		X	X			
Afipia		X	X			
Aggregatilinea			X			
Agrococcus	X	X		X		
Agromyces		X	X			
Alicyclobacillus		X	X		X	
Alkaliphilus			X			
Altererythrobacter			X			X
Amaricoccus		X	X			
Ammoniphilus			X			X
Amorphus	X		X			
Amycolatopsis			X			
Anoxybacillus		X			X	
Aquicella	X					
Arboricoccus		X			X	
Archangium		X	X			
Arenimicrobium			X			
Arenivirga			X			X
Aridibacter			X			
Armatimonas		X	X			
Arthrobacter	X		X			X
Aureimonas	X					
Azohydromonas		X	X			
Bacillaceae			X			X
Bauldia			X			
Belnapia		X				
Blastococcus			X			
Bosea		X	X			
Bradyrhizobium			X			
Brevitalea			X			
Brevundimonas	X					
Bryobacter	X			X		
Buttiauxella		X	X			
Caedibacter			X			
CandidatusKoribacter		X	X			
CandidatusNitrososphaera		X	X			
Carnobacterium	X	X				
Caulobacter	X			X		
Cedecea			X			X
Cellvibrio		X				
Cerasicoccus		X	X			
Cereibacter		X	X			
Chelatococcus			X			X
Chiayiivirga			X			X
Chitinophaga	X			X		
Chloronema			X			
Chloroplast	X			X		
Chryseolinea		X	X			
Chryseotalea	X	X				
CL500-29_marine_group			X			X
Clostridium_sensu_stricto_1			X			
Clostridium_sensu_stricto_13			X			
Compostibacillus			X			
Conexibacter			X			
Corynebacterium	X	X				
Couchioplanes			X			X
Coxiella	X	X		X		

Cryobacterium	X	X				
Cryocola		X	X		X	
Curtobacterium	X	X				
Cystobacter		X	X			
Cytobacillus	X			X		
Cytophaga			X			
Daejeonella	X			X		
Deinococcus			X			
Desertimonas			X			
Desulforudis			X			X
Devosia		X				
Dictyobacter		X				
Domibacillus	X	X	X			
Dongia			X			
Edaphobacter			X			X
Effusibacillus	X					
Embleya	X					
Enhygromyxa		X	X			
Ensifer			X			X
Enterovirga	X					
env.OPS_17		X	X			
Erwinia	X					
Ewingella		X	X			
Exiguobacterium	X	X			X	
Ferruginibacter			X			X
Fictibacillus	X			X		
Fimbriimonas			X			X
Flavihumibacter			X			
Flavisolibacter			X			X
Flavitalea			X			X
Friedmanniella			X			
Frondihabitans		X	X			
Fulvimonas		X	X			
Gaiella			X			
Geminicoccus			X			
Geobacillus	X			X		
Geobacter		X	X			
Geodermatophilus	X	X	X			X
Geomonas			X			X
Gracilibacillus			X			
Granulicella		X	X			
Hafnia-Obesumbacterium			X			X
Haliangium		X	X			
Halocella			X			
Hamadaea		X	X			
Herbiconiux	X					
Hydrogenophaga	X			X		
Hyphomicrobium		X	X			
Iamia		X	X			
Ilumatobacter		X	X			
Jatrophihabitans		X	X			
Jiangella			X			
Kaistia	X	X				
Kallotenue		X	X			
Kibdelosporangium			X			X
Kineosporia			X			
Knoellia		X	X			
Kofleria			X			X
Kouleothrix			X			
Kuenenia			X			X
Labilithrix		X	X			
Labrys		X	X			
Laceyella	X	X				
Lacipirellula			X			
Lacunisphaera	X					
Lautropia		X	X			
Lechevalieria	X		X			
Legionella			X			
Leifsonia			X			X
Lentzea	X					
Leptolyngbya_EcFYyyy-00			X			X
Limnochorda		X	X			
Litorilinea		X	X			
Longimicrobium		X	X			
Longispora			X			X
Luteitalea			X			X
Lysobacter		X	X			
M55-D21	X					
Magnetospira	X	X				
Marinicrinis			X			
Melghirimyces		X	X			

Melioribacter	X		X			
Mesobacillus	X					
Mesorhizobium			X			
Methylibium			X			
Methylobacterium			X			
Methylobacterium-Methylorubrum			X			
Methylocapsa			X			
Methylocella	X			X		
Methylophilus	X			X		
Methylovirgula		X	X			X
Microbacterium			X			
Microlunatus		X	X			
Micromonospora	X					
Microterricola			X			
Microvirga		X	X			
Minicystis			X			X
Modestobacter			X			X
Mucilaginibacter	X	X				
Muricoccus			X			
Mycetocola	X	X		X		
Mycoavidus		X				
Mycobacterium			X			
Nakamurella		X	X			
Nannocystis		X	X		X	
Neochlamydia	X					
Niabella		X	X			
Niastella	X					
Nitrolancea		X	X			
Nitrososphaera			X			
Nitrospira			X			
Nocardioides	X		X	X		
Nonomuraea	X		X	X		
Nordella			X			
Nostoc_PCC-73102			X			X
Nostocoida		X	X			
Novibacillus		X	X			
Noviherbaspirillum	X	X		X		
Novosphingobium		X	X			
Oerskovia	X	X		X		
Ohtaekwangia			X			X
Oligoflexus			X			
Omnitrophales	X					
Opitutus		X			X	
Oscillochloris		X	X			
Paenactinomyces			X			
Paenarthrobacter	X	X				
Paenibacillus		X	X			
Paeniglutamicibacter	X	X		X		
Paenisporosarcina	X		X			
Pajaroellobacter		X			X	
Pantoea	X	X				
Parablastomonas	X			X		
Paraburkholderia		X	X			
Parachlamydia		X	X			
Paracoccus			X			
Parafrigoribacterium		X	X			
Parageobacillus	X					
Pararhizobium		X				
Parviterribacter		X	X			
Patulibacter	X					
Pedobacter	X	X				
Phycicoccus	X	X				
Planctomicrobium			X			
planctomycete			X			X
Planifilum		X	X			
Planococcus	X					
Planomicrobium	X	X		X		
Plantactinospora	X		X			
Plantibacter		X			X	
Plesiocystis		X	X			
Polycladomyces	X	X		X		
Pontibacter			X			
possible_genus_04			X			
Povalibacter		X	X			X
Propionibacterium	X					
Pseudarthrobacter			X			X
Pseudogracilibacillus			X			
Pseudolabrys		X	X			X
Pseudolysobacter			X			
Pseudomonas	X	X				
Pseudonocardia		X	X			

Pseudorhizobium	X			X		
Pseudoxanthomonas		X			X	
Psychrobacillus		X	X			
Pusillimonas	X	X				
Racemicystis		X	X			
Ramlibacter		X	X			
Rathayibacter	X	X				
Reyranella		X	X			
Rhizobacter			X			
Rhizomicrobium	X					
Rhizorhabdus	X		X			
Rhodoblastus		X			X	
Rhodococcus	X					
Rhodoferax			X			X
Rhodoplanes		X	X		X	
Rhodopseudomonas			X			X
Rickettsiella		X	X			
Roseiarcus		X	X			
Roseococcus	X	X			X	
Rubellimicrobium			X			
Rubrivivax		X	X			
Rubrobacter		X	X			
Saccharothrix			X			
Salinibacterium	X	X				
Sandaracinus		X	X			
Sanguibacter	X	X				
Schlesneria			X			X
Schnuerera			X			
Segetibacter	X			X		
Shimazuella	X	X	X		X	
Silvibacterium			X			X
Singulisphaera	X					
Skermanella			X			X
Solirubrobacter			X			
Sorangium			X			
Sphaerobacter		X	X			
Sphingobium	X	X		X		
Sphingopyxis	X	X				
Sphingosinicella			X			X
Sporacetigenium		X	X			
Sporosarcina			X			X
Stenotrophobacter		X	X			
Stenotrophobium		X			X	
Stenotrophomonas		X				
Steroidobacter			X			
Sterolibacterium			X			X
Streptacidiphilus			X			X
Streptomyces	X	X		X		
Subtercola		X			X	
Sulfurifustis			X			
Symbiobacterium		X			X	
Syntrophaceticus			X			X
Tardiphaga			X			X
Tatlockia			X			
Tepidimicrobium			X			
Terrimonas		X	X			
Thermoactinomyces	X	X				
Thermoclostridium			X			
Thermogutta			X			
Trabulsiella			X			X
Trinickia			X			
Tumebacillus		X	X		X	
Tundrisphaera		X	X			
Turicibacter		X	X			
Tychonema_CCAP_1459-11B			X			X
Uliginosibacterium		X				
Undibacterium			X			X
Verrucomicrobium			X			
Verticiella		X			X	
Vicinamibacter			X			
Virgisporangium	X	X	X			
Vulgatibacter			X			
Woodsholea			X			
Xenophilus		X				
Xylophilus			X			
Yersinia	X	X				
Zavarzinella		X	X			

Supplemental Table 2.4. Fungal genera from metabarcoding analysis proliferating under each burn severity level.

Genus	Abundant in High	Abundant in Low	Abundant in Unburned	Consistent in High	Consistent in Low	Consistent in Unburned
Absidia	X	X				
Akanthomyces	X					
Amanita			X			
Amphinema			X			
Amplistromataceae_gen_Incertae_sedis			X			X
Amyloathelia			X			
Anthracobia	X					
Apiotrichum			X			X
Articulospora			X			X
Atheliales_gen_Incertae_sedis			X			X
Auxarthron	X					
Basidioascus	X	X				
Beauveria	X		X			
Brahmaculus			X			
Calloriaceae_gen_Incertae_sedis			X			
Calyptrozyma	X			X		
Candida	X	X				
Capronia		X				
Cenococcum			X			
Cephaliophora			X			
Cephalothecaceae_gen_Incertae_sedis			X			
Cercophora			X			X
Chaetomium		X			X	
Chaetosphaeria	X					
Chalara			X			
Chalastospora	X			X		
Chloridium			X			X
Chrysozymaceae_gen_Incertae_sedis	X	X				
Chytridiomycota_gen_Incertae_sedis	X			X		
Cladophialophora		X	X			
Cladorrhinum		X	X			
Cladosporium	X					
Clavaria			X			
Clavulina			X			X
Colacogloea	X					
Coniophora		X			X	
Cordycipitaceae_gen_Incertae_sedis			X			
Cosmospora	X			X		
Cryptococcus	X	X				
Cryptosporiopsis	X		X			
Curvibasidium	X	X				
Cystobasidium	X					
Cystofilobasidium			X			
Dactylonectria		X	X			
Devriesia			X			
Diplogelasinospora		X			X	
Endogonomycetes_gen_Incertae_sedis	X					
Entoloma		X	X			
Epibryon			X			
Filobasidium	X					
Fungi_gen_Incertae_sedis		X				
Fusicolla	X					
Geoglossum			X			
Geomyces			X			
Glutinomyces			X			X
Goffeauzyma			X			X
Gorgomyces			X			X
GS11_gen_Incertae_sedis		X			X	
GS25_ord_Incertae_sedis_gen_Incertae_sedis	X	X				
Gymnostellatospora			X			
Halokirschsteiniothelia	X					
Hamamotoa	X					
Haptocillium		X	X			
Harpochytriaceae_gen_Incertae_sedis	X	X				
Herpotrichia		X			X	
Herpotrichiellaceae_gen_Incertae_sedis		X				
Heteroconium			X			
Hirsutella			X			
Hormiactis		X			X	
Hormonema	X			X		
Humicola	X		X			
Hyalorbilia		X				
Hyaloscypha			X			
Hygrocybe		X				
Hygrophorus		X			X	
Ilyonectria			X			X
Immersiella			X			
Infundichalara			X			X
Inocybe			X			X
Jugulospora	X					

Kotlabaea	X		X	X		
Krasilnikovozyma			X			
Kurtzmaniella			X			X
Laccaria			X			X
Lachnellula			X			X
Lasionectriopsis		X				
Lecanicillium		X	X			
Leptobacillium		X	X			
Leptodontidium			X			X
Leucosporidium	X					
Lipomyces	X			X		
Lophiotrema		X				
Lyophyllum	X	X				
Magnaporthaceae_gen_Incertae_sedis		X			X	
Magnaporthiopsis		X	X			
Malassezia	X	X				
Meliniomyces			X			
Metschnikowiaceae_gen_Incertae_sedis			X			X
Microascaceae_gen_Incertae_sedis		X			X	
Microdochium		X	X			
Monoblepharidales_gen_Incertae_sedis	X					
Mrakia	X					
Mycena			X			X
Mycoarthris			X			X
Mycopappus	X		X			
Mytilinidiales_gen_Incertae_sedis	X			X		
Myxomphalia	X	X				
Myxotrichum			X			X
Naganishia	X					
Nectria	X		X			
Neoascochyta			X			X
Neosetophoma	X					
Neurospora		X				
Ochrocladosporium	X			X		
Oliveonia			X			
Onygenales_gen_Incertae_sedis	X					
Paracremonium		X				
Paragibellulopsis	X			X		
Paramyrothecium	X		X			
Parastagonospora	X					
Paratritirachium			X			
Penicillago		X				
Penicillium	X					
Perilachnea		X				
Pezizella			X			
Phacidium			X			X
Phaeosphaeria			X			
Phenoliferia	X			X		
Phialea			X			X
Piloderma			X			
Pithoascus	X		X			
Plicaria	X			X		
Podospora	X					
Polyozellus			X			X
Polyphilus			X			
Preussia	X			X		
Psathyrella		X				
Pseudocoleophoma			X			
Pseudodictyosporium			X			X
Pseudogymnoascus			X			
Pseudohyphozyma	X	X				
Pulvinula	X					
Purpureocillium			X			
Pyrenopeziza			X			
Ramicandelaber	X					
Rhizosphaera		X				
Rhodosporidiobolus	X					
Rhodotorula	X			X		
Sabuloglossum		X			X	
Saccharomycetales_gen_Incertae_sedis			X			
Saitoella	X			X		
Sakaguchia			X			X
Samsoniella			X			X
Sarocladium	X			X		
Schizothecium	X					
Sclerococcales_gen_Incertae_sedis			X			
Scleroderma			X			X
Scopulariopsis		X	X			
Sebacina			X			
Serendipita		X				
Sesquicillium			X			

Sistotrema			X			X
Slooffia		X				
Smaragdiniseta			X			X
Solicoccozyma			X			X
Spirosphaera		X			X	
Spizellomycetales_gen_Incertae_sedis	X			X		
Sporidiobolales_gen_Incertae_sedis			X			
Sporormiaceae_gen_Incertae_sedis		X			X	
Stagonosporopsis	X			X		
Striaticonidium			X			
Subramaniula		X	X			
Sugiyamaella			X			X
Suillus		X				
Sympodiella		X	X			
Tausonia		X				
Tetragoniomycetaceae_gen_Incertae_sedis			X			X
Tolypocladium			X			
Tomentella			X			X
Torula	X			X		
Trichoglossum	X					
Triscelophorus		X			X	
Tritirachiaceae_gen_Incertae_sedis	X					
Truncocolumella			X			
Tympanidaceae_gen_Incertae_sedis		X			X	
Tyrannosorus			X			X
Varicellaria			X			X
Venustampulla		X			X	
Vexillomyces		X				
Wilcoxina		X				
Xenoacremonium	X			X		
Xenochalara		X	X			
Xenopolyscytalum			X			

Supplemental Table 2.5. Bacterial genera from metabarcoding analysis proliferating in association with *Armillaria* species.

Genus	Abundant in ALT	Abundant in SOL	Abundant in None	ALT consistent	SOL consistent	None consistent
Acidiferrimicrobium		X	X			
Acidipila	X					
Acidisoma		X			X	
Acidithrix			X			X
Acidobacterium			X			
Acrocarpospora			X			
Actinoallomurus		X	X			
Actinocorallia	X					
Adhaeribacter			X			
Aeromonas			X			X
Afipia		X	X			X
Agrococcus		X				
Agromyces			X			
Alicyclobacillus			X			
Allosphingosinicella	X					
Aminobacter		X			X	
Ammoniphilus	X			X		
Anoxybacillus	X			X		
Aquicella		X	X			
Arenimicrobium			X			
Arenivirga	X	X				
Armatimonas	X					
Arthrobacter		X				
Bacillaceae			X			
Bdellovibrio		X				
Belnapia			X			
Brevibacillus	X					
Brevundimonas		X				
Burkholderia		X				
Buttiauxella	X		X	X		
CandidatusKoribacter			X			X
Catellatospora	X					
Catenulispora	X	X	X		X	
Cedecea			X			X
Cellulomonas		X			X	
Cellvibrio			X			X
Chelatococcus	X			X		
Chitinophaga		X			X	
Chloroplast		X				
Chryseobacterium		X			X	
Chryseotalea			X			
Corallococcus	X					
Coxiella		X				
Craurococcus-Caldovatus		X				
Cryobacterium			X			
Cryocola			X			
Cupriavidus		X			X	
Curtobacterium			X			
Daejeonella		X			X	
Deinococcus		X				
Domibacillus	X		X			
Dongia	X		X			
Edaphobacter		X				
Edaphobaculum			X			
Effusibacillus	X			X		
Enterovirga			X			
env.OPS_17			X			
Erwinia		X	X			
Erythrobacter		X				
Exiguobacterium	X					
Fimbriimonas		X				
Flavihumibacter			X			
Flavisolibacter			X			X
Frondihabitans			X			
Fulvimonas			X			
Geobacillus		X	X			
Geodermatophilus	X		X			
Geomonas	X			X		
Gorillibacterium			X			
Hafnia-Obesumbacterium		X			X	
Hydrogenophaga		X			X	
Jiangella		X				
Kaistia		X	X			
Kibdelosporangium		X	X		X	
Kitasatospora			X			X

Labilithrix		X	X			
Laceyella		X	X			
Lacibacterium		X			X	
Lacunisphaera		X				
Leifsonia	X					
Lentzea		X	X		X	
Longimicrobium			X			
Longispora	X	X				
Luteimonas		X			X	
Lysinimonas	X	X				
M55-D21		X				
Massilia		X			X	
Methylocella		X			X	
Methylophilus			X			X
Methylotenera		X			X	
Microlunatus			X			
Minicystis			X			
Mucilaginibacter		X	X			X
Muricoccus		X				
Mycetocola		X			X	
Nakamurella			X			
Nannocystis			X			
Neochlamydia		X	X			
Nitrolancea	X		X			
Nonomuraea		X				
Nostoc_PCC-73102		X				
Noviherbaspirillum		X				
Novosphingobium	X		X			
NS11-12_marine_group		X				
Oerskovia		X	X			
Oscillochloris	X			X		
Paenarthrobacter		X			X	
Paeniglutamicibacter	X	X				
Paenisporosarcina	X	X				
Pantoea		X			X	
Parachlamydia			X			
Paracoccus		X	X			
Pedobacter			X			
Planctopirus	X	X				
Plantactinospora		X				
Plantibacter			X			
Polaromonas		X			X	
Polycladomyces		X	X			
Povalibacter			X			X
Promicromonospora	X					
Propionibacterium		X			X	
Prosthecobacter			X			
Pseudolabrys		X				
Pseudopelobacter			X			X
Pseudorhizobium		X	X			
Pseudoxanthomonas			X			
Pusillimonas			X			
Racemicystis			X			X
Rhizobacter		X	X		X	
Rhizomicrobium		X				
Rhodoblastus			X			X
Rhodoplanes			X			X
Roseococcus			X			X
Sanguibacter		X				
Sediminibacterium		X				
Segetibacter		X	X			
Shimazuella	X					
Silvibacterium			X			X
Solirubrobacter		X	X			
Sphaerobacter			X			
Sphingopyxis		X				
Sporacetigenium			X			
Stenotrophobacter	X		X			
Stenotrophobium			X			X
Streptacidiphilus		X				
Subtercola	X		X			
Thermanaerothrix		X	X			
Trabulsiella	X			X		
Tundrisphaera			X			
Verticiella			X			X
Virgisporangium	X	X	X			

Supplemental Table 2.6. Fungal genera from metabarcoding analysis proliferating in association with *Armillaria* species.

Genus	Abundant in ALT	Abundant in SOL	Abundant in None	ALT consistent	SOL consistent	None consistent
Acidomelania	X			X		
Agaricostilbales_gen_Incertae_sedis			X			
Aphelidiomycetes_gen_Incertae_sedis	X		X			
Articulospora	X					
Atheliales_gen_Incertae_sedis		X			X	
Auxarthron		X	X			
Balsamia	X			X		
Berkeleyomyces		X			X	
Brachyphoris			X			
Branch06_gen_Incertae_sedis	X			X		
Cephaliophora		X				
Cercophora		X			X	
Chaetomium	X		X	X		
Chaetosphaeria	X			X		
Chalastospora		X			X	
Chytridiomycota_gen_Incertae_sedis		X			X	
Clavispora		X			X	
Clavulina		X				
Colacogloea			X			
Collarina	X			X		
Coniophora	X			X		
Coniosporium		X				
Cyphellophora			X			
Cystolepiota	X			X		
Diplogelasinospora	X		X			
Dothiorella	X					
Endogonomycetes_gen_Incertae_sedis	X		X			
Enterocarpus	X			X		
Entoloma	X		X			
Epibryon	X			X		
Fellozyma			X			X
Flavocillium		X			X	
Fungi_gen_Incertae_sedis			X			X
Geomyces		X	X			
Geopyxis			X			
Gorgomyces	X					
GS31_gen_Incertae_sedis		X			X	
Halokirschsteiniothelia		X			X	
Hamamotoa			X			
Hamatocanthoscyphaceae_gen_Incertae_sedis			X			X
Hapsidospora			X			X
Haptocillium			X			X
Heydenia			X			
Hirsutella			X			
Hormiactis			X			
Hyalorbilia			X			
Hypocreales_gen_Incertae_sedis			X			
Hysterangium	X		X			
Infundichalara	X		X	X		
Jugulospora		X			X	
Keithomyces			X			
Laburnicola			X			
Lasionectriopsis	X		X			
Lecanicillium			X			
Lecythophora		X	X			
Leptobacillium		X	X			
Lophiotrema			X			
Lophium			X			
Lyophyllum	X					
Macrophomina			X			
Magnaporthaceae_gen_Incertae_sedis			X			X
Meliniomyces		X			X	
Membranomyces	X			X		
Monosporascus			X			
Myxomphalia			X			X
Neoascochyta	X	X				
Neonectria			X			
Neurospora			X			
Ochroconis			X			X
Oliveonia	X					
Orbiliales_gen_Incertae_sedis			X			
Paragibellulopsis		X	X		X	
Paramyrothecium	X	X				

Periconia	X	X				
Perilachnea			X			
Piskurozyma			X			
Pithoascus		X			X	
Pseudodictyosporium	X			X		
Pseudohyphozyma			X			X
Pseudopyrenochaeta	X					
Pyrenopeziza	X					
Rasamsonia			X			
Rhizophlyctis			X			
Rhizopycnis		X			X	
Rhizosphaera			X			
Rubellisphaeria			X			
Saitoella		X			X	
Samsoniella			X			
Saprochaete		X				
Sebacina		X			X	
Siepmannia		X	X			
Sistotrema	X					
Slooffia			X			
Smaragdiniseta	X					
Sporormiaceae_gen_Incertae_sedis			X			X
Striaticonidium	X		X			
Stylonectria	X			X		
Subramaniula	X		X			
Suillus	X		X			
Sympodiella			X			
Synnemapestaloides			X			
Talaromyces			X			
Tricharina			X			
Truncocolumella	X	X		X		
Tympanidaceae_gen_Incertae_sedis			X			X
Varicosporium	X			X		
Xenochalara			X			
Yurkovia			X			X
Zygorhynchus			X			

Supplemental Table 2.7. Bacterial and fungal genera isolated from soils associated with each burn severity level.

Genus	Bacterial (B) or Fungal (F)	isolated from high	isolated from low	isolated from unburned
Absidia	F		X	
Achromobacter	B	X	X	
Acremonium	F	X		
Alternaria	F	X	X	
Ambomucor	F		X	X
Arthrobacter	B	X		X
Aspergillus	F	X		
Bacillus	B	X	X	X
Burkholderia	B			X
Caballeronia	B			X
Chitinophaga	B		X	
Chryseobacterium	B	X	X	X
Cladosporium	F	X		X
Collimonas	B	X		X
Cosmospora	F	X		
Devosia	B	X		
Fictibacillus	B		X	
Flavobacterium	B	X	X	X
Fusarium	F		X	X
Herminiimonas	B		X	
Humicolopsis	F		X	
Lecythophora	F			X
Linnemannia	F		X	
Lysobacter	B	X	X	X
Massilia	B	X	X	
Microbacterium	B		X	
Microvirga	B	X		
Mortierella	F	X	X	X
Mucilaginibacter	B		X	
Mucor	F			X
Oidiodendron	F	X		X
Paenibacillus	B	X	X	X
Paraburkholderia	B			X
Paraphoma	F	X		
Pedobacter	B	X		
Penicillium	F	X	X	X
Phyllobacterium	B		X	X
Polaromonas	B	X		
Pseudarthrobacter	B	X		
Pseudomonas	B	X	X	X
Raoultella	B			X
Rhodococcus	B			X
Sagenomella	F	X	X	X
Serratia	B	X		
Sphingobium	B	X		
Sphingomonas	B		X	
Sphingopyxis	B	X	X	
Sphingosinicella	B	X		
Stenotrophomonas	B	X		
Streptomyces	B	X	X	X
Talaromyces	F	X	X	
Trichoderma	F	X	X	X
Umbelopsis	F	X	X	X
Variovorax	B	X	X	
Venturia	F		X	

Supplemental Table 2.8. *Armillaria* species rhizomorph and mycelial fan identification.

Armillaria Isolate ID	Tree Host #	Tree Host Species	Sample Type	Collection date	Species ID	GenBank Accession Number
SJNF#019MF	3	DF	Mycelial fan	October 2022	*A. solidipes*	PP694565
SJNF#020MF	4	DF	Mycelial fan	October 2022	*A. solidipes*	PP694548
SJNF#020R	4	DF	Rhizomorph	June 2023	*A. altimontana*	PP694577
SJNF#021MF	6	WWP	Mycelial fan	October 2022	*A. solidipes*	PP694550
SJNF#022MF	8	DF	Mycelial fan	October 2022	*A. altimontana*	PP694578
SJNF#022R	8	DF	Rhizomorph	October 2022	*A. altimontana*	PP694579
SJNF#022R-2	8	DF	Rhizomorph	June 2023	*A. altimontana*	PP694574
SJNF#023R	14	DF	Rhizomorph	October 2022	*A. altimontana*	PP694580
SJNF#024R	16	WWP	Rhizomorph	October 2022	*A. sinapina*	PP694568
SJNF#025R	20	WWP	Rhizomorph	October 2022	*A. sinapina*	PP694569
SJNF#026R	24	DF	Rhizomorph	October 2022	*A. altimontana*	PP694572
SJNF#026R-2	24	DF	Rhizomorph	June 2023	*A. altimontana*	PP694576
SJNF#027R	26	WWP	Rhizomorph	October 2022	*A. altimontana*	PP694581
SJNF#027R-2	26	WWP	Rhizomorph	June 2023	*A. altimontana*	PP694575
SJNF#028MF	5	WWP	Mycelial fan	June 2023	*A. solidipes*	PP694549
SJNF#028R	5	WWP	Rhizomorph	June 2023	*A. solidipes*	PP694544
SJNF#029MF	7	WWP	Mycelial fan	June 2023	*A. solidipes*	PP694547
SJNF#029R	7	WWP	Rhizomorph	June 2023	*A. altimontana*	PP694573
SJNF#030R	15	DF	Rhizomorph	June 2023	*A. solidipes*	PP694566
SJNF#031R	29	DF	Rhizomorph	June 2023	*A. altimontana*	PP694571
SJNF#032MF	10	DF	Mycelial fan	June 2023	*A. solidipes*	PP694545
SJNF#033MF	27	DF	Mycelial fan	June 2023	*A. solidipes*	PP694543

Supplemental Table 2.9. GenBank accession numbers for bacterial isolates.

Seq_ID	Burn Severity	Organism	GenBank Accession Number
T10C12	high	*Massilia aurea*	PP583378
T10C3	high	*Paenibacillus alginolyticus*	PP583375
T10C6	high	*Flavobacterium* sp.	PP583376
T10C8	high	*Bacillus* sp.	PP583377
T1C11	high	*Pseudomonas* sp.	PP558967
T1C12	high	*Pseudomonas baetica*	PP558968
T1C13	high	*Pseudomonas* sp.	PP583270
T1C15	high	*Pseudomonas* sp.	PP583271
T1C16	high	*Sphingobium aromaticiconvertens*	PP583272
T1C17	high	*Stenotrophomonas rhizophila*	PP583273
T1C18	high	*Pseudomonas* sp.	PP583274
T1C1R	high	*Bacillus* sp.	PP583268
T1C2	high	*Bacillus mojavensis*	PP558962
T1C20	high	*Serratia* sp.	PP583275
T1C22	high	*Bacillus* sp.	PP583276
T1C23	high	*Pseudomonas baetica*	PP583277
T1C3	high	*Bacillus* sp.	PP558963
T1C4	high	*Pseudomonas* sp.	PP558964
T1C6F	high	*Pseudarthrobacter* sp.	PP583269
T1C8	high	*Sphingomonas* sp.	PP558965
T1C9	high	*Flavobacterium* sp.	PP558966
T2C1	high	*Bacillus* sp.	PP583278
T2C14	high	*Microvirga aerophila*	PP583284
T2C15	high	*Variovorax ginsengisoli*	PP583285
T2C16	high	*Sphingobacteriaceae bacterium*	PP583286
T2C2	high	*Bacillus* sp.	PP583279
T2C20	high	*Bacillus* sp.	PP583287
T2C21	high	*Bacillus* sp.	PP583288
T2C24	high	*Bacillus* sp.	PP559175
T2C26	high	*Flavobacterium hibernum*	PP583289
T2C27	high	*Polaromonas ginsengisoli*	PP583290
T2C29	high	*Bacillus* sp.	PP583291
T2C3	high	*Pseudomonas yamanorum*	PP583280
T2C30	high	*Bacillus* sp.	PP583292
T2C4	high	*Bacillus* sp.	PP583281
T2C6	high	*Bacillus* sp.	PP583282
T2C9	high	*Streptomyces mirabilis*	PP583283
T3C1	high	*Sphingosinicella* sp.	PP583293
T3C10	high	*Bacillus* sp.	PP583301
T3C11	high	*Streptomyces violascens*	PP583302
T3C12	high	*Bacillus halotolerans*	PP583303
T3C13	high	*Bacillus* sp.	PP583304
T3C14	high	*Bacillus* sp.	PP583305
T3C15	high	*Streptomyces mirabilis*	PP583306
T3C16	high	*Pedobacter borealis*	PP583307
T3C17	high	*Sphingosinicella* sp.	PP583308

T3C18	high	*Bacillus* sp.	PP583309
T3C2	high	*Devosia* sp.	PP559177
T3C20	high	*Pedobacter roseus*	PP583310
T3C3	high	*Chryseobacterium* sp.	PP583294
T3C4	high	*Serratia* sp.	PP583295
T3C5	high	*Pseudomonas* sp.	PP583296
T3C6	high	*Streptomyces violascens*	PP583297
T3C7	high	*Sphingopyxis* sp.	PP583298
T3C8	high	*Bacillus luciferensis*	PP583299
T3C9	high	*Bacillus* sp.	PP583300
T4C1	high	*Bacillus* sp.	PP583311
T4C10	high	*Pseudomonas* sp.	PP583319
T4C11	high	*Pseudomonas* sp.	PP583320
T4C12	high	*Pseudomonas* sp.	PP583321
T4C14	high	*Pseudomonas baetica*	PP583322
T4C15	high	*Pseudomonas* sp.	PP583323
T4C2	high	*Pseudomonas* sp.	PP583312
T4C3	high	*Pseudomonas* sp.	PP583313
T4C5	high	*Pseudomonas* sp.	PP583314
T4C6	high	*Pseudomonas* sp.	PP583315
T4C7	high	*Arthrobacter* sp.	PP583316
T4C8	high	*Pseudomonas* sp.	PP583317
T4C9	high	*Pedobacter* sp.	PP583318
T5C10	high	*Achromobacter deleyi*	PP583329
T5C11	high	*Lysobacter* sp.	PP583330
T5C12	high	*Bacillus luciferensis*	PP583331
T5C13	high	*Flavobacterium* sp.	PP583332
T5C14	high	*Pedobacter roseus*	PP583333
T5C16	high	*Bacillus* sp.	PP583334
T5C17	high	*Bacillus* sp.	PP583335
T5C19	high	*Bacillus thuringiensis*	PP583336
T5C2	high	*Bacillus* sp.	PP583324
T5C21	high	*Bacillus* sp.	PP583337
T5C23	high	*Bacillus* sp.	PP559176
T5C27	high	*Bacillus* sp.	PP583338
T5C3	high	*Bacillus* sp.	PP583325
T5C4	high	*Bacillus* sp.	PP583326
T5C7	high	*Oxalobacteraceae bacterium*	PP583327
T5C8	high	*Pseudomonas koreensis*	PP583328
T6C1	high	*Flavobacterium* sp.	PP583339
T6C10	high	*Flavobacterium* sp.	PP583345
T6C11	high	*Polaromonas aquatica*	PP583346
T6C12	high	*Pseudomonas* sp.	PP583347
T6C13	high	*Lysobacter capsici*	PP583348
T6C2	high	*Bacillus luciferensis*	PP583340
T6C3	high	*Pseudomonas* sp.	PP583341
T6C4	high	*Lysobacter capsici*	PP583342
T6C5	high	*Collimonas arenae*	PP583343

T6C9	high	*Bacillus* sp.	PP583344
T7C10	high	*Pedobacter borealis*	PP583356
T7C12	high	*Bacillus* sp.	PP583357
T7C17	high	*Bacillus* sp.	PP583358
T7C18	high	*Bacillus* sp.	PP559178
T7C19	high	*Bacillus* sp.	PP583359
T7C21	high	*Pedobacter* sp.	PP583360
T7C3	high	*Pseudomonas baetica*	PP583349
T7C4	high	*Paenibacillus* sp.	PP583350
T7C5	high	*Pedobacter* sp.	PP583351
T7C6	high	*Pseudomonas* sp.	PP583352
T7C7	high	*Lysobacter* sp.	PP583353
T7C8	high	*Flavobacterium* sp.	PP583354
T7C9	high	*Lysobacter* sp.	PP583355
T8C10	high	*Bacillus* sp.	PP583365
T8C11	high	*Stenotrophomonas rhizophila*	PP583366
T8C15	high	*Pseudomonas baetica*	PP583367
T8C4	high	*Pedobacter* sp.	PP583361
T8C6	high	*Pseudomonas koreensis*	PP583362
T8C7	high	*Pseudomonas baetica*	PP583363
T8C9	high	*Pseudomonas baetica*	PP583364
T9C10	high	*Pedobacter panaciterrae*	PP583373
T9C12	high	*Bacillus* sp.	PP583374
T9C2	high	*Pedobacter* sp.	PP583368
T9C4	high	*Bacillus* sp.	PP583369
T9C5	high	*Pseudomonas frederiksbergensis*	PP583370
T9C7	high	*Flavobacterium* sp.	PP583371
T9C9	high	*Pedobacter* sp.	PP583372
T11C10	low	*Bacillus* sp.	PP583383
T11C12	low	*Rhizobiaceae bacterium*	PP583384
T11C15	low	*Bacillus* sp.	PP583385
T11C18	low	*Sphingopyxis bauzanensis*	PP583386
T11C19	low	*Mucilaginibacter formosus*	PP583387
T11C2	low	*Pseudomonas* sp.	PP583379
T11C4	low	*Pseudomonas* sp.	PP583380
T11C6	low	*Pseudomonas* sp.	PP583381
T11C9	low	*Bacillus* sp.	PP583382
T12C1	low	*Pseudomonas* sp.	PP583388
T12C11	low	*Bacillus* sp.	PP583395
T12C15	low	*Bacillus* sp.	PP583396
T12C2	low	*Massilia litorea*	PP583389
T12C3	low	*Streptomyces decoyicus*	PP583390
T12C5	low	*Bacillus* sp.	PP583391
T12C7	low	*Bacillus* sp.	PP583392
T12C8	low	*Bacillus* sp.	PP583393
T12C9	low	*Bacillus* sp.	PP583394
T17C1	low	*Bacillus* sp.	PP583426
T17C11	low	*Pseudomonas frederiksbergensis*	PP583435

T17C13	low	*Pseudomonas frederiksbergensis*	PP583436
T17C14	low	*Pseudomonas frederiksbergensis*	PP583437
T17C15	low	*Pseudomonas baetica*	PP583438
T17C16	low	*Pseudomonas yamanorum*	PP583439
T17C17	low	*Bacillus* sp.	PP583440
T17C18	low	*Pseudomonas helmanticensis*	PP583441
T17C2	low	*Bacillus* sp.	PP583427
T17C3	low	*Sphingomonas* sp.	PP583428
T17C4	low	*Massilia litorea*	PP583429
T17C5	low	*Microbacterium oxydans*	PP583430
T17C6	low	*Mucilaginibacter* sp.	PP583431
T17C7	low	*Pseudomonas frederiksbergensis*	PP583432
T17C8	low	*Flavobacterium* sp.	PP583433
T17C9	low	*Pseudomonas baetica*	PP583434
T18C1	low	*Bacillus* sp.	PP583442
T18C2	low	*Bacillus* sp.	PP583443
T18C3	low	*Bacillus* sp.	PP583444
T19C1	low	*Chryseobacterium* sp.	PP583445
T19C10	low	*Chryseobacterium* sp.	PP583449
T19C26	low	*Bacillus* sp.	PP583450
T19C4	low	*Phyllobacterium myrsinacearum*	PP583446
T19C8	low	*Pseudomonas* sp.	PP583447
T19C9	low	*Achromobacter* sp.	PP583448
T21C1	low	*Flavobacterium* sp.	PP583459
T21C2	low	*Pseudomonas* sp.	PP583460
T21C3	low	*Bacillus luciferensis*	PP583461
T21C4	low	*Pseudomonas* sp.	PP583462
T21C5	low	*Flavobacterium saccharophilum*	PP583463
T21C6	low	*Flavobacterium pectinovorum*	PP583464
T22C1	low	*Variovorax ginsengisoli*	PP583465
T22C10	low	*Bacillus* sp.	PP583473
T22C11	low	*Fictibacillus barbaricus*	PP583474
T22C12	low	*Paenibacillus nebraskensis*	PP583475
T22C13	low	*Bacillus* sp.	PP583476
T22C2	low	*Bacillus* sp.	PP583466
T22C4	low	*Sphingomonas* sp.	PP583467
T22C5	low	*Pseudomonas* sp.	PP583468
T22C6	low	*Chryseobacterium* sp.	PP583469
T22C7	low	*Bacillus* sp.	PP583470
T22C8	low	*Bacillus luciferensis*	PP583471
T22C9	low	*Pseudomonas baetica*	PP583472
T23C1	low	*Bacillus* sp.	PP583477
T23C10	low	*Flavobacterium araucananum*	PP583482
T23C11	low	*Bacillus* sp.	PP583483
T23C12	low	*Chitinophaga* sp.	PP583484
T23C4	low	*Pseudomonas* sp.	PP583478
T23C5	low	*Bacillus* sp.	PP583479
T23C7	low	*Pseudomonas* sp.	PP583480

T23C9	low	*Pseudomonas* sp.	PP583481
T24C10	low	*Flavobacterium saccharophilum*	PP583491
T24C11	low	*Pseudomonas* sp.	PP583492
T24C13	low	*Herminiimonas arsenitoxidans*	PP583493
T24C14	low	*Pseudomonas* sp.	PP583494
T24C2	low	*Bacillus* sp.	PP583485
T24C3	low	*Pseudomonas* sp.	PP583486
T24C4	low	*Bacillus* sp.	PP583487
T24C5	low	*Bacillus* sp.	PP583488
T24C7	low	*Herminiimonas arsenitoxidans*	PP583489
T24C8	low	*Bacillus luciferensis*	PP583490
T29C4	low	*Lysobacter* sp.	PP583526
T29C5	low	*Pseudomonas baetica*	PP583527
T29C6	low	*Lysobacter* sp.	PP583528
T29C7	low	*Bacillus* sp.	PP583529
T29C8	low	*Bacillus* sp.	PP583530
T13C1	unburned	*Bacillus luciferensis*	PP583397
T13C4	unburned	*Chryseobacterium vrystaatense*	PP583398
T13C5	unburned	*Pseudomonas* sp.	PP583399
T13C6	unburned	*Pseudomonas* sp.	PP583400
T14C1	unburned	*Pseudomonas* sp.	PP583401
T14C11	unburned	*Bacillus subtilis*	PP583405
T14C4	unburned	*Bacillus* sp.	PP583402
T14C6	unburned	*Pseudomonas* sp.	PP583403
T14C9	unburned	*Caballeronia udeis*	PP583404
T15C1	unburned	*Pseudomonas laurylsulfativorans*	PP583406
T15C10	unburned	*Pseudomonas* sp.	PP583412
T15C11	unburned	*Pseudomonas* sp.	PP583413
T15C12	unburned	*Bacillus* sp.	PP583414
T15C2	unburned	*Arthrobacter* sp.	PP583407
T15C4	unburned	*Arthrobacter* sp.	PP583408
T15C5	unburned	*Bacillus* sp.	PP583409
T15C7	unburned	*Collimonas arenae*	PP583410
T15C8	unburned	*Pseudomonas moorei*	PP583411
T16C1	unburned	*Pseudomonas* sp.	PP583415
T16C10	unburned	*Bacillus* sp.	PP559179
T16C12	unburned	*Bacillus* sp.	PP583422
T16C15	unburned	*Bacillus* sp.	PP583423
T16C16	unburned	*Bacillus* sp.	PP583424
T16C17	unburned	*Bacillus* sp.	PP583425
T16C3	unburned	*Bacillus* sp.	PP583416
T16C4	unburned	*Paraburkholderia* sp.	PP583417
T16C5	unburned	*Bacillus* sp.	PP583418
T16C6	unburned	*Bacillus halotolerans*	PP583419
T16C8	unburned	*Burkholderia* sp.	PP583420
T16C9	unburned	*Bacillus* sp.	PP583421
T20C1	unburned	*Lysobacter* sp.	PP583451
T20C10	unburned	*Flavobacterium* sp.	PP583457

T20C11	unburned	*Pseudomonas* sp.	PP583458
T20C4	unburned	*Bacillus* sp.	PP583452
T20C5	unburned	*Arthrobacter methylotrophus*	PP583453
T20C7	unburned	*Bacillus* sp.	PP583454
T20C8	unburned	*Arthrobacter methylotrophus*	PP583455
T20C9	unburned	*Bacillus* sp.	PP583456
T25C1	unburned	*Pseudomonas* sp.	PP583495
T25C10	unburned	*Bacillus* sp.	PP583500
T25C11	unburned	*Pseudomonas helmanticensis*	PP583501
T25C12	unburned	*Bacillus* sp.	PP583502
T25C2	unburned	*Bacillus luciferensis*	PP583496
T25C4	unburned	*Bacillus* sp.	PP583497
T25C6	unburned	*Rhodococcus erythropolis*	PP583498
T25C9	unburned	*Bacillus* sp.	PP583499
T26C10	unburned	*Pseudomonas* sp.	PP583509
T26C11	unburned	*Pseudomonas* sp.	PP583510
T26C2	unburned	*Bacillus* sp.	PP583503
T26C5	unburned	*Bacillus* sp.	PP583504
T26C6	unburned	*Bacillus* sp.	PP583505
T26C7	unburned	*Pseudomonas* sp.	PP583506
T26C8	unburned	*Flavobacterium* sp.	PP583507
T26C9	unburned	*Pseudomonas* sp.	PP583508
T27C2	unburned	*Collimonas arenae*	PP583511
T27C4	unburned	*Bacillus* sp.	PP583512
T27C5	unburned	*Collimonas arenae*	PP583513
T27C6	unburned	*Pseudomonas* sp.	PP583514
T27C7	unburned	*Pseudomonas* sp.	PP583515
T27C8	unburned	*Pseudomonas* sp.	PP583516
T27C9	unburned	*Bacillus luciferensis*	PP583517
T28C1	unburned	*Phyllobacterium* sp.	PP583518
T28C10	unburned	*Bacillus mycoides*	PP583525
T28C2	unburned	*Bacillus* sp.	PP583519
T28C4	unburned	*Chryseobacterium* sp.	PP583520
T28C5	unburned	*Bacillus* sp.	PP583521
T28C6	unburned	*Bacillus* sp.	PP583522
T28C7	unburned	*Bacillus mycoides*	PP583523
T28C8	unburned	*Pseudomonas baetica*	PP583524
T30C1	unburned	*Chryseobacterium* sp.	PP583531
T30C10	unburned	*Lysobacter capsici*	PP566969
T30C3	unburned	*Paenibacillus nebraskensis*	PP583532
T30C4	unburned	*Bacillus* sp.	PP583533
T30C5	unburned	*Chryseobacterium* sp.	PP583534
T30C6	unburned	*Chryseobacterium* sp.	PP583535
T30C7	unburned	*Bacillus* sp.	PP566967
T30C8	unburned	*Raoultella planticola*	PP566968

Supplemental Table 2.10. GenBank accession numbers for fungal isolates.

Seq_ID	Burn Severity	Organism	GenBank Accession Number
PFB1	high	*Penicillium murcianum*	PP562365
PFB5	high	*Umbelopsis vinacea*	PP562367
T10C19	high	*Cosmospora* sp.	PP573578
T10C20	high	*Cladosporium* sp.	PP573579
T10C21	high	*Cladosporium* sp.	PP573580
T10C23	high	*Penicillium glabrum*	PP573581
T10C26	high	*Mortierella minutissima*	PP573582
T1C25	high	*Aspergillus* sp.	PP573512
T1C26	high	*Penicillium* sp.	PP573513
T1C27	high	*Penicillium* sp.	PP573514
T1C28	high	*Fungal* sp.	PP573515
T1C29	high	*Trichoderma harzianum*	PP573516
T1C30b	high	*Penicillium* sp.	PP573517
T1C30c	high	*Penicillium* sp.	PP573518
T1C32	high	*Mortierella alpina*	PP573519
T1C33	high	*Mortierella alpina*	PP573520
T1C34	high	*Penicillium* sp.	PP573521
T1C35	high	*Penicillium* sp.	PP573522
T1C36	high	*Penicillium* sp.	PP573523
T1C37	high	*Penicillium* sp.	PP573524
T2C12	high	*Penicillium* sp.	PP573525
T2C32	high	*Sagenomella* sp.	PP573526
T2C33	high	*Penicillium* sp.	PP573527
T2C34	high	*Sagenomella* sp.	PP573528
T2C35	high	*Penicillium* sp.	PP573529
T2C36	high	*Penicillium* sp.	PP573530
T2C37	high	*Oidiodendron chlamydosporicum*	PP573531
T2C40	high	*Mortierella* sp.	PP573532
T2C42	high	*Penicillium* sp.	PP573533
T2C43	high	*Penicillium* sp.	PP573534
T2C44	high	*Umbelopsis* sp.	PP573535
T2C45	high	*Umbelopsis* sp.	PP573536
T2C47	high	*Mortierella alpina*	PP573537
T2C49	high	*Penicillium* sp.	PP573538
T2C50	high	*Penicillium* sp.	PP573539
T2C51	high	*Penicillium arenicola*	PP573540
T3C21	high	*Talaromyces* sp.	PP573541
T3C22	high	*Penicillium* sp.	PP573542
T3C23	high	*Penicillium* sp.	PP573543
T3C26	high	*Mortierellales* sp.	PP573544
T3C27	high	*Mortierellaceae* sp.	PP573545
T3C28	high	*Acremonium* sp.	PP573546
T4C16	high	*Talaromyces* sp.	PP573547
T4C17	high	*Fungal* sp.	PP573548
T4C20	high	*Penicillium* sp.	PP573549
T4C22	high	*Penicillium glabrum*	PP573550
T4C24	high	*Aspergillus niger*	PP573551
T4C25	high	*Penicillium* sp.	PP573552

T4C26	high	*Paraphoma fimeti*	PP573553
T4C27	high	*Mortierella alpina*	PP573554
T4C28	high	*Penicillium* sp.	PP573555
T4C29	high	*Penicillium jensenii*	PP573556
T4C30	high	*Cladosporium* sp.	PP573557
T4C31	high	*Mortierella alpina*	PP573558
T4C32	high	*Penicillium* sp.	PP573559
T4C33	high	*Penicillium* sp.	PP573560
T5C28	high	*Penicillium arenicola*	PP573561
T5C29	high	*Umbelopsis* sp.	PP573562
T5C31	high	*Penicillium* sp.	PP573563
T5C32	high	*Mortierella alpina*	PP573564
T5C35	high	*Penicillium* sp.	PP573565
T6C15	high	*Mortierella minutissima*	PP573566
T6C16	high	*Penicillium arenicola*	PP573567
T6C17	high	*Mortierellaceae* sp.	PP573568
T6C18	high	*Penicillium rubens*	PP573569
T7C1	high	*Penicillium* sp.	PP573570
T7C22	high	*Alternaria* sp.	PP573571
T7C24	high	*Penicillium glabrum*	PP573572
T7C25	high	*Umbelopsis nana*	PP573573
T8C17	high	*Umbelopsis nana*	PP573574
T8C20	high	*Mortierella* sp.	PP573575
T9C21	high	*Umbelopsis nana*	PP573577
T9C6	high	*Mortierella minutissima*	PP573576
PFB2	low	*Penicillium rubens*	PP562366
PFB4	low	*Trichoderma paraviridescens*	PP600952
T11C21	low	*Penicillium quercetorum*	PP573583
T11C22	low	*Penicillium* sp.	PP573584
T11C23	low	*Penicillium* sp.	PP573585
T11C24	low	*Penicillium arenicola*	PP573586
T11C25	low	*Absidia* sp.	PP573587
T11C27	low	*Penicillium arenicola*	PP573588
T11C28	low	*Penicillium* sp.	PP573589
T11C29	low	*Penicillium* sp.	PP573590
T11C30	low	*Penicillium* sp.	PP573591
T11C31	low	*Penicillium arenicola*	PP573592
T11C32	low	*Mortierella* sp.	PP573593
T11C33	low	*Linnemannia amoeboidea*	PP573594
T11C34	low	*Penicillium* sp.	PP573595
T12C17	low	*Penicillium* sp.	PP573596
T12C18	low	*Penicillium* sp.	PP573597
T12C19	low	*Penicillium* sp.	PP573598
T12C20	low	*Penicillium* sp.	PP573599
T12C22	low	*Penicillium* sp.	PP573600
T12C24	low	*Mortierellaceae* sp.	PP573601
T12C25	low	*Umbelopsis* sp.	PP573602
T12C26	low	*Penicillium arenicola*	PP573603
T12C28	low	*Penicillium* sp.	PP573604
T12C30	low	*Mortierella alpina*	PP573605

T17C19	low	*Penicillium* sp.	PP573640
T17C20	low	*Penicillium* sp.	PP573641
T17C21	low	*Penicillium* sp.	PP573642
T17C22	low	*Penicillium* sp.	PP573643
T17C23	low	*Penicillium* sp.	PP573644
T17C24	low	*Trichoderma* sp.	PP573645
T17C25	low	*Mortierella alpina*	PP573646
T17C26	low	*Mortierella minutissima*	PP573647
T17C27	low	*Penicillium* sp.	PP573648
T17C28	low	*Penicillium* sp.	PP573649
T18C10	low	*Penicillium* sp.	PP573653
T18C11	low	*Penicillium arenicola*	PP573654
T18C13	low	*Penicillium* sp.	PP573655
T18C14	low	*Penicillium arenicola*	PP573656
T18C15	low	*Penicillium* sp.	PP573657
T18C17	low	*Mortierella minutissima*	PP573658
T18C18	low	*Penicillium kojigenum*	PP573659
T18C19	low	*Penicillium swiecickii*	PP573660
T18C7	low	*Penicillium swiecickii*	PP573650
T18C8	low	*Absidia frigida*	PP573651
T18C9	low	*Umbelopsis vinacea*	PP573652
T19C12	low	*Talaromyces* sp.	PP573661
T19C13	low	*Venturia hystrioides*	PP573662
T19C14	low	*Talaromyces acaricola*	PP573663
T19C15	low	*Talaromyces rugulosus*	PP573664
T19C18	low	*Penicillium* sp.	PP573665
T19C20	low	*Penicillium* sp.	PP573666
T19C22	low	*Umbelopsis* sp.	PP573667
T19C23	low	*Penicillium canescens*	PP573668
T19C24	low	*Mortierella minutissima*	PP573669
T21C12	low	*Penicillium arenicola*	PP573676
T21C13	low	*Umbelopsis* sp.	PP573677
T21C14	low	*Penicillium swiecickii*	PP573678
T21C15	low	*Penicillium swiecickii*	PP573679
T21C16	low	*Umbelopsis nana*	PP573680
T21C17	low	*Penicillium swiecickii*	PP573681
T21C18	low	*Mortierellales* sp.	PP573682
T21C19	low	*Penicillium* sp.	PP573683
T22C14	low	*Penicillium* sp.	PP573684
T22C15	low	*Penicillium* sp.	PP573685
T22C18	low	*Penicillium* sp.	PP573686
T22C19	low	*Penicillium* sp.	PP573687
T22C21	low	*Umbelopsis* sp.	PP573688
T22C22	low	*Mortierella alpina*	PP573689
T23C13	low	*Fusarium oxysporum*	PP573690
T23C14	low	*Penicillium* sp.	PP573691
T23C15	low	*Penicillium arenicola*	PP573692
T23C16	low	*Penicillium* sp.	PP573693
T23C18	low	*Mortierella minutissima*	PP573694
T23C19	low	*Sagenomella* sp.	PP573695

T23C20	low	*Umbelopsis* sp.	PP573696
T23C21	low	*Penicillium arenicola*	PP573697
T23C22	low	*Umbelopsis nana*	PP573698
T23C23	low	*Mortierella* sp.	PP573699
T23C26	low	*Penicillium* sp.	PP573700
T24C15	low	*Penicillium* sp.	PP573701
T24C16	low	*Trichoderma minutisporum*	PP573702
T24C17	low	*Trichoderma minutisporum*	PP573703
T24C18	low	*Penicillium* sp.	PP573704
T24C19	low	*Umbelopsis* sp.	PP573705
T24C20	low	*Eurotiales* sp.	PP573706
T24C21	low	*Mortierella alpina*	PP573707
T29C10	low	*Humicolopsis cephalosporioides*	PP573739
T29C11	low	*Fungal* sp.	PP573740
T29C12	low	*Penicillium arenicola*	PP573741
T29C14	low	*Eurotiales* sp.	PP573742
T29C15	low	*Alternaria* sp.	PP573743
T29C16	low	*Ambomucor seriatoinflatus*	PP573744
T29C17	low	*Umbelopsis* sp.	PP573745
T29C18	low	*Penicillium* sp.	PP573746
T29C19	low	*Penicillium arenicola*	PP573747
T29C20	low	*Umbelopsis nana*	PP573748
T29C21	low	*Penicillium* sp.	PP573749
T29C22	low	*Mortierella elongata*	PP573750
PFB3	unburned	*Umbelopsis* sp.	PP562368
T13C18	unburned	*Penicillium nodositatum*	PP573606
T13C19	unburned	*Oidiodendron* sp.	PP573607
T13C21	unburned	*Sagenomella griseoviridis*	PP573608
T13C22	unburned	*Sagenomella griseoviridis*	PP573609
T13C24	unburned	*Penicillium* sp.	PP573610
T13C26	unburned	*Penicillium arenicola*	PP573611
T13C27	unburned	*Penicillium arenicola*	PP573612
T13C28	unburned	*Fungal* sp.	PP573613
T13C30	unburned	*Mortierella parvispora*	PP573614
T13C32	unburned	*Penicillium* sp.	PP573615
T13C33	unburned	*Mortierellaceae* sp.	PP573616
T14C14	unburned	*Cladosporium* sp.	PP573617
T14C15	unburned	*Mortierellaceae* sp.	PP573618
T14C16	unburned	*Penicillium swiecickii*	PP573619
T14C19	unburned	*Penicillium* sp.	PP573620
T14C20	unburned	*Umbelopsis* sp.	PP573621
T14C21	unburned	*Penicillium arenicola*	PP573622
T14C22	unburned	*Penicillium arenicola*	PP573623
T14C23	unburned	*Umbelopsis* sp.	PP573624
T14C25	unburned	*Mortierella alpina*	PP573625
T15C16	unburned	*Ambomucor seriatoinflatus*	PP573626
T15C17	unburned	*Umbelopsis* sp.	PP573627
T15C19	unburned	*Umbelopsis* sp.	PP573628
T15C20	unburned	*Penicillium* sp.	PP573629
T15C21	unburned	*Mortierella* sp.	PP573630

T16C22	unburned	*Mortierella* sp.	PP573631
T16C26	unburned	*Fusarium* sp.	PP573632
T16C27	unburned	*Sagenomella* sp.	PP573633
T16C29	unburned	*Umbelopsis* sp.	PP573634
T16C30	unburned	*Penicillium* sp.	PP573635
T16C32	unburned	*Umbelopsis* sp.	PP573636
T16C33	unburned	*Mortierella alpina*	PP573637
T16C34	unburned	*Penicillium* sp.	PP573638
T16C35	unburned	*Mortierellales* sp.	PP573639
T20C14	unburned	*Trichoderma* sp.	PP573670
T20C14_2	unburned	*Trichoderma paraviridescens*	PP600951
T20C16	unburned	*Mucor hiemalis*	PP573671
T20C17	unburned	*Eurotiales* sp.	PP573672
T20C18	unburned	*Mortierella minutissima*	PP573673
T20C19	unburned	*Mortierella minutissima*	PP573674
T20C20	unburned	*Trichoderma oblongisporum*	PP573675
T25C17	unburned	*Mortierella parvispora*	PP573708
T25C19	unburned	*Umbelopsis vinacea*	PP573709
T25C20	unburned	*Mortierella verticillata*	PP573710
T25C22	unburned	*Mortierella alpina*	PP573711
T25C23	unburned	*Penicillium swiecickii*	PP573712
T26C14	unburned	*Mortierella minutissima*	PP573713
T26C15	unburned	*Lecythophora mutabilis*	PP573714
T26C16	unburned	*Penicillium* sp.	PP573715
T26C17	unburned	*Penicillium* sp.	PP573716
T26C18	unburned	*Mortierella cystojenkinii*	PP573717
T26C20	unburned	*Umbelopsis* sp.	PP573718
T26C21	unburned	*Penicillium* sp.	PP573719
T26C22	unburned	*Mortierella minutissima*	PP573720
T27C15	unburned	*Penicillium* sp.	PP573721
T27C16	unburned	*Penicillium* sp.	PP573722
T27C17	unburned	*Eurotiales* sp.	PP573723
T27C18	unburned	*Penicillium* sp.	PP573724
T27C19	unburned	*Penicillium arenicola*	PP573725
T27C20	unburned	*Sagenomella* sp.	PP573726
T27C21	unburned	*Sagenomella griseoviridis*	PP573727
T27C22	unburned	*Penicillium* sp.	PP573728
T27C23	unburned	*Mortierella verticillata*	PP573729
T27C24	unburned	*Umbelopsis dimorpha*	PP573730
T27C25	unburned	*Penicillium* sp.	PP573731
T28C21	unburned	*Penicillium* sp.	PP573732
T28C22	unburned	*Penicillium* sp.	PP573733
T28C23	unburned	*Umbelopsis* sp.	PP573734
T28C24	unburned	*Umbelopsis* sp.	PP573735
T28C25	unburned	*Eurotiales* sp.	PP573736
T28C26	unburned	*Penicillium swiecickii*	PP573737
T28C27	unburned	*Mortierellales* sp.	PP573738
T30C12	unburned	*Mucoromycota* sp.	PP566970

Supplemental Table 3.1. Dual culture confrontation test measurements between *Armillaria solidipes* and putative bacterial biological control agents.

Biocontrol	Replicate	Day	Armillaria control (cm^2)	Biocontrol control (cm^2)	Test Armillaria (cm^2)	Test Biocontrol (cm^2)	AIP	Day	Armillaria control (cm^2)	Biocontrol control (cm^2)	Test Armillaria (cm^2)	Test Biocontrol (cm^2)	AIP
Bacillus sp. PP583282	1	28	14.971	3.31	9.887	1.338	35.4423768	56	19.98	4.219	15.943	1.433	20.85615475
	2	28	17.517	2.944	9.413	1.264	38.5373817	56	23.299	4.212	16.154	1.262	19.80871378
	3	28	13.457	n/a	8.886	0.418	41.9784525	56	17.154	n/a	11.265	0.401	44.07856635
Bacillus sp. PP559176	1	28	14.971	12.565	9.27	0.873	39.4711068	56	19.98	11.805	14.908	0.815	25.99407608
	2	28	17.517	5.632	15.308	n/a	0.04570682	56	23.299	6.575	22.646	n/a	-12.41871163
	3	28	13.457	n/a	7.688	1.386	49.8008488	56	17.154	n/a	13.73	1.357	31.84187447
Bacillus sp. PP559175	1	28	11.645	2.653	3.406	1.239	75.6951547	56	18.117	3.401	5.58	1.168	71.49036906
	2	28	13.897	3.262	4.674	0.953	66.6468447	56	21.204	3.781	8.664	1.044	55.73343325
	3	28	16.499	n/a	4.31	1.073	69.2443091	56	19.396	n/a	6.781	1.178	65.35415638
Bacillus sp. PP583357	1	28	7.35	6.635	1.773	1.91	82.8480217	56	17.95	7.298	1.685	1.858	89.79303382
	2	28	13.002	5.892	2.412	1.814	76.6663442	56	17.578	7.668	3.228	2.022	80.44623927
	3	28	10.659	n/a	4.085	0.939	60.4817645	56	13.997	n/a	8.153	0.826	50.61282181
Bacillus sp. PP559178	1	28	7.35	12.199	1.82	1.857	82.3933443	56	17.95	12.871	1.922	1.761	89.33266027
	2	28	13.002	6.483	2.751	2.003	73.3868627	56	17.578	7.989	3.693	1.971	77.62948006
	3	28	10.659	n/a	1.709	2.292	83.4671568	56	13.997	n/a	1.968	2.264	88.07874811
Bacillus sp. PP559179	1	28	14.18	6.874	2.773	3.045	74.5852809	56	22.075	8.146	3.274	2.914	80.20795551
	2	28	8.99	5.012	3.142	2.697	71.2033727	56	13.6	7.21	4.322	2.842	73.8725668
	3	28	9.563	n/a	2.26	3.519	79.2869581	56	13.951	n/a	2.55	3.598	84.58469351
Caballeronia udeis PP583404	1	28	15.983	4.31	3.384	2.585	78.8618902	56	21.769	5.955	5.739	3.067	74.22874848
	2	28	15.22	2.529	3.767	1.711	76.4694859	56	20.58	6.409	6.4	1.806	71.26049665
	3	28	16.824	n/a	3.484	1.358	78.2372416	56	24.458	n/a	5.646	1.376	74.64636939
Collimonas arenae PP583343	1	28	15.826	5.897	11.016	n/a	34.1883065	56	21.623	6.729	20.487	0.858	7.73838117
	2	28	18.836	6.778	9.424	0.931	43.6992194	56	25.202	7.973	19.924	0.971	10.27380809
	3	28	15.554	n/a	12.134	0.512	27.5091604	56	19.791	n/a	21.81	0.534	1.780353068
Devosia sp. PP559177	1	28	15.983	3.335	11.237	1.467	29.8082329	56	21.769	8.451	16.56	1.319	25.63653509
	2	28	15.22	6.491	11.003	1.74	31.2699107	56	20.58	8.572	19.429	2.452	12.75315461
	3	28	16.824	n/a	10.725	1.107	33.0064339	56	24.458	n/a	14.049	1.224	36.91229961
Lysobacter capsici PP583342	1	28	15.826	3.755	13.948	n/a	16.6719771	56	21.623	3.883	26.699	n/a	-20.23688003
	2	28	18.836	7.611	14.203	n/a	15.1485582	56	25.202	6.653	28.964	n/a	-30.43713222
	3	28	15.554	n/a	13.703	n/a	18.135654	56	19.791	n/a	29.016	n/a	-30.6713102
Pseudomonas sp. PP583271	1	28	8.263	3.393	5.224	2.967	41.9534057	56	18.377	3.277	11.589	2.871	30.69747045
	2	28	9.776	1.885	7.236	2.369	19.5970221	56	16.031	1.933	16.115	3.154	3.631869556
	3	28	8.96	n/a	7.359	3.287	18.2303048	56	15.759	n/a	15.529	3.063	7.136165208
Pseudomonas sp. PP583347	1	28	14.18	1.753	13.011	n/a	-19.246632	56	22.075	2.713	21.403	n/a	-29.38580583
	2	28	8.99	1.492	12.237	n/a	-12.152873	56	13.6	2.772	17.995	n/a	-8.783702092
	3	28	9.563	n/a	13.509	n/a	-23.810833	56	13.951	n/a	21.329	n/a	-28.93845968

Supplemental Table 3.2. Dual culture confrontation test measurements between *Armillaria solidipes* and putative fungal biological control agents.

Biocontrol	Replicate	Day	Armillaria (cm^2)	Biocontrol (cm^2)	BCI	Day	Armillaria (cm^2)	Biocontrol (cm^2)	BCI	Day	Armillaria (cm^2)	Biocontrol (cm^2)	Overlap	BCI	Day	Armillaria (cm^2)	Biocontrol (cm^2)	BCI
Mortierella elongata PP573750	1	2	8.216	8.834	51.81	5	9.054	28.838	76.11	7	9.212	32.341		77.83	14	9.919	43.073	81.28
	2	2	6.068	10.597	63.59	5	6.933	29.445	80.94	7	6.939	34.122		83.10	14	7.767	43.402	84.82
Penicillium murcianum PP562365	1	2	10.019	1.404	12.29	5	11.08	4.777	30.13	7	11.459	7.076		38.18	14	12.708	12.893	50.36
	2	2	10.884	1.642	13.11	5	12.137	4.577	27.38	7	12.247	6.545		34.83	14	13.188	10.154	43.50
	3	2	10.669	1.777	14.28	5	11.578	4.486	27.93	7	11.64	6.382		35.41	14	12.464	9.585	43.47
Penicillium rubens PP562366	1	2	11.526	2.488	17.75	5	13.04	9.297	41.62	7	14.168	11.898		45.65	14	16.362	16.616	50.39
	2	2	10.131	2.787	21.57	5	10.238	17.128	62.59	7	11.058	18.982		63.19	14	12.194	21.704	64.03
	3	2	9.254	3.433	27.06	5	9.401	10.057	51.69	7	9.952	12.903		56.46	14	11.266	15.432	57.80
Trichoderma harzianum PP573516	1	2	7.303	15.299	67.69	5	7.77	48.187	86.11	7	7.537	46.243		85.99	14	7.997	54.208	100.00
	2	2	7.692	15.676	67.08	5	8.043	44.921	84.81	7	8.13	45.164		84.74	14	8.463	54.906	100.00
	3	2	8.883	15.735	63.92	5	8.923	43.379	82.94	7	9.051	44.128		82.98	14	9.486	55.38	100.00
Trichoderma oblongisporium PP573675	1	2	10.154	11.219	52.49	5	10.592	36.67	77.59	7	10.208	42.811	2.3	84.41	14	10.674	55.016	100.00
	2	2	13.398	9.776	42.19	5	14.307	24.751	63.37	7	14.169	26.594	0	65.24	14	15.121	33.995	69.21
	3	2	10.455	9.684	48.09	5	11.446	32.783	74.12	7	11.372	40.744	1.518	80.52	14	12.09	54.928	100.00
Trichoderma paraviridescens PP600952	1	2	14.728	11.541	43.93	5	15.789	33.074	67.69	7	14.681	38.578	0.499	73.12	14	16.744	56.691	100.00
	2	2	10.796	10.674	49.72	5	11.636	36.372	75.76	7	11.105	41.342	0.641	79.80	14	13.192	55.594	100.00
	3	2	11.116	10.749	49.16	5	12.026	36.674	75.31	7	11.99	40.741	0.499	78.00	14	12.658	54.717	100.00
Trichoderma paraviridescens PP600951	1	2	14.222	9.747	40.67	5	15.151	34.14	69.26	7	14.414	39.072	2.597	76.78	14	15.913	55.71	100.00
	2	2	12.781	9.959	43.80	5	13.312	33.279	71.43	7	13.766	39.077	2.102	77.01	14	14.441	56.425	100.00
	3	2	13.216	11	45.42	5	13.407	35.398	72.53	7	13.671	38.98	2.444	77.64	14	14.459	56.186	100.00
Umbelopsis sp. PP573536	1	2	15.573	1.572	9.17	5	15.977	4.063	20.27	7	15.958	6.424		28.70	14	18.579	12.865	40.91
	2	2	10.588	1.681	13.70	5	12.034	3.671	23.37	7	12.794	5.864		31.43	14	15.949	10.941	40.69
	3	2	15.154	1.576	9.42	5	15.649	4.479	22.25	7	15.804	7.196		31.29	14	18.931	15.404	44.86
Umbelopsis sp. PP562368	1	2	10.765	2.058	16.05	5	11.02	6.313	36.42	7	11.344	9.968		46.77	14	13.937	21.569	60.75
	2	2	13.775	1.958	12.45	5	15.434	4.857	23.94	7	15.392	7.246		32.01	14	19.932	15.604	43.91
	3	2	12.791	2.086	14.02	5	14.284	4.087	22.25	7	14.831	6.516		30.52	14	19.812	13.95	41.32
Umbelopsis vinacea PP562367	1	2	11.221	2.613	18.89	5	13.691	8.915	39.44	7	13.255	13.986		51.34	14	14.649	23.078	61.17
	2	2	8.834	2.564	22.50	5	9.289	9.793	51.32	7	9.453	14.148		59.95	14	10.807	21.683	66.74
	3	2	9.288	2.61	21.94	5	10.803	7.724	41.69	7	10.681	12.039		52.99	14	11.971	19.977	62.53