THESIS

ABIOTIC AND BIOTIC FACTORS INFLUENCING WESTERN UNITED STATES CONIFEROUS FORESTS

Submitted by

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ABSTRACT

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In the next decade, climate models suggest that global temperatures will continue to rise. In the western United States, increases in temperatures and changes in precipitation patterns will escalate the risk of drought conditions. These potentially warmer, drier conditions could induce physiological changes within trees, subsequently increasing stress on coniferous forests that are adapted to cool, wet environments. The abiotic stress accompanied by drought conditions can predispose susceptible hosts to biotic stress of insect and disease populations. In particular, high elevation subalpine fir (*Abies lasiocarpa*) have encountered higher than average mortality rates throughout the western United States in association with abiotic and biotic agents.

Chapter 2 of this thesis investigated the potential drivers of subalpine fir mortality and determined how climatic factors and site and stand characteristics influenced the presence of mortality and biotic agents. The objectives were to identify factors driving subalpine fir mortality in Colorado and included 1) determine abiotic and biotic factors that directly and indirectly affect subalpine fir mortality, 2) determine factors associated with the presence of *D. confusus* or *Armillaria* spp., and 3) determine if climate variables were correlated to subalpine fir mortality or the presence of *D. confusus* and *Armillaria* spp. I hypothesized that sites with a higher density (i.e. basal area, trees per hectare, or canopy closure) would experience greater mortality due to decreased growth rates from competition and that *D. confusus* or *Armillaria* spp. prevalence would be a function of tree stress (i.e. increased density), elevation, slope, and departures from normal precipitation (i.e. drought), and minimum and maximum temperatures.

Stand health monitoring plots found that the most relevant factors to subalpine fir mortality are the presence of *D. confusus* ($p = 0.003$) and the percent subalpine fir on plot ($p = <0.0001$). I identified that stand density ($p = 0.0038$), elevation ($p = 0.0581$), and *Armillaria* spp. ($p = 0.0006$) were the greatest influences on the presence of *D. confusus*, while the largest influences on the presence of *Armillaria* spp. are warmer maximum summer temperatures ($p = 0.0136$) and the presence of *D. confusus* ($p = 0.0289$). Results indicated that increased subalpine fir mortality was attributed to high stand density as a predisposing factor, warming temperatures as an inciting factor, and bark beetles (*Dryocoetes confusus*) and root disease (*Armillaria* spp.) as contributing factors. The combination of predisposing, inciting, and contributing factors suggests that subalpine mortality can be defined as subalpine fir decline. Management strategies used to reduce the impact of subalpine fir decline will need to address ways to improve stand health, while decreasing populations of both, *D. confusus* and *Armillaria* spp. In regards to *Armillaria*, the inability to successfully manage the disease using current techniques highlights the need to find novel management strategies to minimize its impacts. Since this disease is a root pathogen, soil microbes likely influence its growth and survival. Utilizing soil microbial communities as biocontrols may assist in management of *Armillaria*. Field sampling within the Priest River Experimental Forest in northern Idaho provided the opportunity to observe how soil microbial communities are associated with two species of *Armillaria*, *A. solidipes* (primary pathogen) and *A. altimontana* (weak pathogen).

My research objective for Chapter 3 was to identify the soil fungal communities associated with tree health status (healthy, moderate and dead) and each *Armillaria* species, *A. solidipes* and *A. altimontana*, both of which have differing ecological behaviors (virulent pathogen and non-pathogen, respectively) on western white pine. I hypothesized that soil microbial communities associated with virulent *A. solidipes* and non-pathogenic *A. altimontana* would differ in fungal richness and diversity with the latter having a greater richness and diversity due to its beneficial qualities to tree health. While richness and diversity is likely to shift

among tree health with a greater diversity and richness for soils associated with healthy trees due to root exudate production near the rhizosphere. Soil samples were collected alongside western white pine (*Pinus monticola*), while *Armillaria* rhizomorphs were excavated near the roots. The most abundant fungal taxon was *Mortierella* spp., which functions as saprophyte decomposing dead and down wood. No significant differences in fungal diversity or richness were found in soils associated with *Armillaria* species, but, although not significant, there where slight differences between soils associated with moderate and dead trees with a greater diversity and richness in soils with dead trees ($p = 0.18$). Additionally, soil pH was significantly influenced by soil carbon, nitrogen, and organic matter, while moisture significantly influenced soil carbon, nitrogen, and organic matter, acting as indicators to overall health in the stand. Although not significantly different, more Hypocreaceae (*Trichoderma*), a known biocontrol for root pathogens, were found within soils associated with *A. altimontana* and healthy trees. More research is needed to solidify differences, yet these factors give insight into potential beneficial aspects of soil fungal communities in association with *Armillaria* species and tree health.

Changing climates regimes outside of 30-year averages cause increased stress to forests. This stress may predispose trees to a greater abundance biotic agents such as bark beetles and secondary pathogens, such as Armillaria root disease specifically in association with subalpine fir in Colorado. Understanding the role that soil fungal communities play in association to Armillaria root disease and tree health may assist in forest management practices to increase the health of high elevation forests.

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DEDICATION

I would like to dedicate this degree to my grandparents, Robert and Joy Lalande. Throughout my life, I have always appreciated your love and support. No matter where I was, or what I was doing, you wanted to make sure that I was doing well. I cannot thank you both for everything you have done for me: from allowing me to live with you during college to our phone calls after I moved to Colorado. I hope that Marilyn and I can become such great people and maybe one-day be able to follow in your footsteps as the matriarch and patriarch our family. I will always strive to continue to live my life as you did; to dedicate your lives to your family, enjoy your love with your partner, and to live life passionately! I love you both and hope that we can continue to live as you did. To my grandma specifically, I know that you are always watching down on us, I love you and miss you so much.

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CHAPTER 1: INTRODUCTION AND BACKGROUND

1.1 Effects of climate and on forests

By the next century, annual temperatures are predicted to rise between 2 to 4 °C globally (Allen et al., 2010; Bentz et al., 2010). In combination with these higher temperatures, a decrease in the duration and frequency of precipitation will likely lead to widespread drought across the western United States (Seager et al., 2007). These climatic factors interacting with increasing levels of CO_2 will have varying responses with xeric species out-competing mesic species (Allen et al, 2010; Hanson and Weltzin, 2000). The acclimation to minimal precipitation will assist in the ability to persist in occurrence of drought (Allen et al., 2010). Within drought environments, trees can withstand stressors by utilizing physiological and biochemical responses (Allen et al., 2010; Hanson and Weltzin, 2000; Rennenberg et al., 2006). The initial response to warmer temperatures and drought is to close stomata, limiting water loss and reducing the CO_2:O_2 ratio, which induces competition between photorespiration and photosynthesis (Allen et al., 2010; Rennenberg et al., 2006). Release of water eventually occurs as stomates open to uptake additional CO_2 for energy production (Hanson and Weltzin, 2000). In annual, seasonal drought regions, lack of available soil water and high transpiration rates reduces the ability for trees to uptake water through xylem tissue, resulting in cavitation or embolism. Cavitation is caused by air bubbles that form in the xylem, due to low water potential within the plant (Allen et al., 2010; Hanson and Weltzin, 2000). Additionally, lack of precipitation causes reduced soil decomposition, which may result in a greater concentration of immobilized nutrients, causing greater competition between plants for limited resources (Hanson and Weltzin, 2000; Rennenberg et al., 2006).

As drought conditions persist, biochemical defense responses are activated in response to increased stress factors. In particular, isoprenoids are stimulated due to higher temperatures and soil drought (Rennenberg et al., 2006). Isoprenoids are produced to protect trees against

biotic and abiotic stresses, yet they have been found in reduced levels with rising CO_2 levels (Rennenberg et al., 2006). The cyclic production of isoprenoids results in weakened stress responses, making trees susceptible to attack by insects and diseases. Stress, induced by climate change and resulting interacting factors influencing a tree's response, can change stand characteristics and increase risk of forest pests and pathogens (Allen et al., 2010).

1.2 Effects climate may play on forest pests and pathogens

As climate continues to change, there will be direct and indirect relationships between host and pest interactions (Sturrock et al., 2011). Seasonally warmer temperatures have a direct effect on the life cycle of bark beetles (Bentz et al., 2010). Beetles that have a two-year life cycle utilize their extended maturity as a way to coordinate with their environment and withstand harsh temperature extremes; i.e. cold tolerance is observed in spruce beetle (*Dendroctonus rufipennis*) (Bentz et al., 2010). Cryoprotectant responses are enabled during winter to tolerate cold temperatures as beetles protect themselves within the tree by overwintering until spring. The fluctuation of winter temperatures may result in an adaptation to the timing of overwintering (Bentz et al., 2010). Additionally, as summer temperature and the length of frost-free periods increase, it may result in a one-year life cycle, ultimately leading to a substantial population growth as their maturity is expedited due to warmer temperatures (Bentz et al., 2010).

Climate change-induced stress in hosts could indirectly affect beetle populations. Bentz et al., (2010) stated that climate change might influence the growth of fungi that are associated with mountain pine beetles. Climate regimes may indirectly affect beetles by affecting the optimal environment needed for fungal growth (Bentz et al., 2010). The impact of drought also affects the host's ability to defend themselves against infestation, as less beetles are needed to successfully attack a host (Bentz et al., 2010; Rennenberg et al., 2006). However, Huberty and Denno (2004), found that a loss in water content and turgor pressure may prevent phloem feeders from acquiring adequate nitrogen from prolonged drought-stressed trees, contradicting

the view that drought benefits beetles. Although increases in beetle populations do occur in stressed environments, intermittent drought will likely provide adequate levels of nutrients for phloem feeders to thrive, while prolonged drought may adversely affect their ability to reproduce (Huberty and Denno, 2004).

Similar to bark beetles, pathogens will experience direct and indirect effects associated with climate change (Kolb et al. 2016). The direct effects of warmer temperature and less precipitation may change the host and disease range with pathogens adapting quicker to the new environment (Sturrock et al., 2011). Shorter disease life cycles, earlier sporulation, and interactions with insect vectors may alter the spread of pathogens, which will likely increase the role of pathogens as mortality agents in the forest (Sturrock et al., 2011). The indirect effects, as previously stated, relate to host susceptibility. As host ranges expand or contract, the movement of disease will subsequently follow. The increase of drought environments will allow many diseases to adversely affect tree health. Stressed trees will not be able to withstand infections from forest pathogens, facilitating populations to reach epidemic levels (Bentz et al., 2010).

Numerous studies have encompassed the effects that climate may have on root diseases, specifically Armillaria root disease (Klopfenstein et al., 2009; Kubiak et al., 2017). It is hypothesized that as future temperatures increase, the range of *Armillaria gallica* and *Armillaria mellea* may expand as more susceptible hosts become compromised due to drought stress, allowing these weak pathogens to find stressed trees (Kubiak et al., 2017). Klopfenstein et al. (2009) have modeled that as climate changes in the inland western United States, the range of Douglas-fir (*Pseudotsuga menziesii*) may constrict, while *Armillaria solidipes* will likely shift within Douglas-fir's current and previous ranges, inducing a pathogenic response to maladapted hosts.

The type of pests may determine the effects that warmer and drier climates have on insects and diseases. Drought environments may adversely affect primary pathogens, such as rusts and foliar diseases that depend on water to infect or spread. For these diseases, the lack

of moisture may limit the efficacy of the pathogen and prevent its ability to spread (Kolb et al., 2016). Whereas, secondary pathogens and insects, such as root pathogens and wood borers may increase as tree stress is exacerbated by drought conditions (Kolb et al., 2016).

1.3 Association between bark beetles and root diseases

Bark beetles and root disease have a close relationship in forested environments (James and Goheen, 1981). In association with root disease, it is hypothesized that the defenses are compromised in stressed trees making them more susceptibility to infestation. The susceptibility of the host to root disease has a direct relationship with the ability for bark beetles to establish in a tree (Goheen and Hansen, 1993). These relationships have been observed in most of the western states (Ferrell and Smith, 1976; Hertert et al., 1975; Lane and Goheen, 1979) with heightened mortality occurring in the Rocky Mountains (CSFS, 2009; James and Goheen, 1981). In a small sample of 326 trees in southern Colorado, over 80% of conifers infected with root disease (*Heterobasidion occidentale* and *Armillaria* spp.) were also infested with bark beetles, in particular fir engraver on white fir and western balsam bark beetle (WBBB) on subalpine fir (James and Goheen, 1981).

Current mortality levels are documented by aerial pest surveys in Colorado. Yearly damage expanded to a maximum around 140,000 ha in 2008 for the subalpine fir mortality complex, driven in combination by WBBB and Armillaria root disease (CSFS, 2009). The mortality caused by the relationship between bark beetles and root disease coincides with predisposing abiotic factors to cause widespread mortality (McMillin et al., 2003). The relatively dense stands and drought conditions in high elevation forests likely predispose subalpine fir to contributing mortality agents such as WBBB and *Armillaria*. Within the extent of subalpine fir, elevated mortality levels have been witnessed in the last decade in association to these factors, prompting the idea that this mortality may be a decline disease.

1.4 Distribution of WBBB

Western balsam bark beetle (*Dryocoetes confusus*) is an important mortality agent to spruce-fir forests of the subalpine regions in western North America (Garbutt and Vallentgoed, 1992; Negron and Popp, 2009). Infestation occurs throughout the range of its hosts from British Columbia to New Mexico (Negron and Popp, 2009). Subalpine fir (*Abies lasiocarpa*) is the preferred host for WBBB, while infrequent infestations also occurs with other true firs, white spruce (*Picea glauca*), and Engelmann spruce (*Picea engelmannii*) (Garbutt and Vallentgoed, 1992). Endemic populations of the beetle act as sanitizers killing stressed trees, while increased population levels may accumulate in blowdown and eventually infest healthy trees (McMillin et al., 2003; Negron and Popp, 2009).

1.5 Biology of WBBB

Dryocoetes confusus, in association, with a pathogenic fungus (*Ophiostoma dryocoetidis*) and root disease have been the cause for significant loss of subalpine fir over the last two decades (Garbutt and Vallentgoed, 1992; McMillin et al., 2003; Negron and Popp, 2009; USDA-FS, 2011). Infestations occur in high elevation forests, which offer typically cool, wet environments (Reich et al., 2016). The two-year life cycle begins in late spring as beetles emerge from trees in May to June, coinciding with 15°C temperatures (Garbutt and Vallentgoed, 1992; Negron and Popp, 2009). As the pioneer beetle, the adult male is attracted to susceptible trees by means of kairomone, a chemical attractant exuded from the host. The male will bore the nuptial chamber and emit pheromones to attract three or more females (Garbutt and Vallentgoed, 1992; USDA-FS, 2011). Following mating, the females will lay eggs along brood chambers running off the nuptial chamber. The resulting galleries will make a distinct stellate (star) or y-shape, which is used for identification (Garbutt and Vallentgoed, 1992). Adults

overwinter in the tree and lay additional eggs the following spring, later emerging to find another susceptible host (Garbutt and Vallentgoed, 1992) (Figure 1-1).

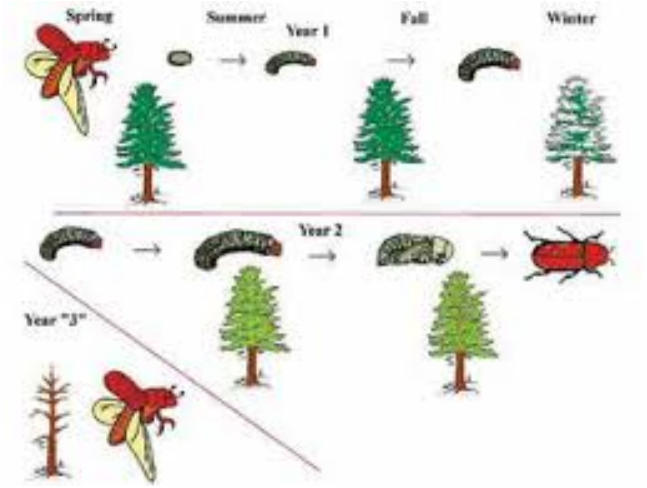

Figure 1-1: Two-year bark beetle life cycle (CSFS, 2014).

Beetles have the greatest impact in larger diameter stands (McMillin et al., 2003). Larvae will continue to feed and grow within the tree during spring and summer. The culmination of maturation occurs with a diapause, starting in the summer, followed by pupation stage through fall and winter to emerge the next spring (Bentz et al., 2010). High temperatures may prevent the diapause stage (Bentz et al., 2010), resulting in a one-year life cycle as adults emerge in the fall (Bentz et al., 2010; Garbutt, 1992).

A symbiotic relationship occurs as the beetle vectors a fungus, *O. dryocoetidis* (Garbutt and Vallentgoed, 1992; Molnar, 1965). The fungus is carried in mycangial pockets on the beetle's thorax and is spread to the host after initial feeding (Molnar, 1965). In British Columbia, Molnar (1965) identified that all beetle attacks had fungal associations, and even an unsuccessful attack by the beetle resulted in fungal inoculation into the host's cambial tissue. Once inside the host vascular system, fungi can kill the tree without the beetle (Garbutt and Vallentgoed, 1992; Molnar, 1965).

Indirect evidence of beetle attack includes pitch flow or frass in conjunction with entry holes. Pitch flow typically occurs when a host successfully withstands the attack and pitches out the beetle. Frass is a combination of boring dust and excrement that results from a successful attack (Garbutt and Vallentgoed, 1992; USDA-FS, 2011). Tree death, following an attack, results in red needles that can remain on a tree for three or more years (USDA-FS, 2011). Direct evidence includes observing the beetle and the stellate egg galleries under the bark

(Garbutt and Vallentgoed, 1992; USDA-FS, 2011). The signs and symptoms on the tree will allow forest managers to identify the beetle, but further understanding the beetle's relationship with root diseases is required to further assist in the management of *D. confusus*.

1.6 Distribution of *Armillaria*

Armillaria spp. are one of the most damaging fungal root pathogens in North America (Baumgartner et al., 2011). It is ubiquitous and can be found in temperate and tropical forests. *Armillaria* can infect hundreds of hosts ranging from trees and woody shrubs to forbs (Williams et al., 1986). *Armillaria ostoyae* (Romagnesi) Herink, now identified as *Armillaria solidipes* Peck, Bull. Torrey Bot. Club (Burdsall and Volk, 2008), is the primary pathogen within coniferous forests associated with *A. altimontana* Brazee, B. Ortiz, Banik, and D.L. Lindner (formerly North American Biological Species X) (Brazee et al., 2012; Ferguson et al. 2003; Kim et al., 2010; Warwell et al., 2019). While *A. solidipes* has a wide range, *A. altimontana* is only found in a small niche in western North America (Brazee et al., 2012). Their co-occurrence has been documented in the inland western United States in mesic, coniferous regions (Brazee et al., 2012; Ferguson et al., 2003). The pathogenicity of *A. altimontana* has not been well studied, yet it is thought to be beneficial to its host (Warwell et al., 2019).

1.7 Biology of *Armillaria* spp.

Armillaria spp. are mostly known as highly virulent pathogens, although they are facultative necrotrophs capable of both pathogenic and saprophytic lifestyles (Baumgartner et al., 2011; Kile et al., 1991). Their ability to actively parasitize their host and persist on dead tissue allows for their continuous spread when susceptible hosts are limited (Kile et al., 1991). The three main signs of infection are mycelial fans (sheets of mycelium under the barks of infected trees), rhizomorphs (aggregations of hyphae with a melanized outer layer either in the soil or under the bark), or basidiomes (above ground fruiting bodies/mushrooms) (Baumgartner

et al., 2011; Morrison et al., 1991). The symptoms associated with infection may include reduced shoot growth (stunting) (Bamugartner et al., 2011), crown dieback (reddening/flagging), or basal resinosis at the base of the tree (Morrison et al., 1991; Williams et al., 1986). These symptoms are typically observed in stressed trees, which may prompt for further investigation to

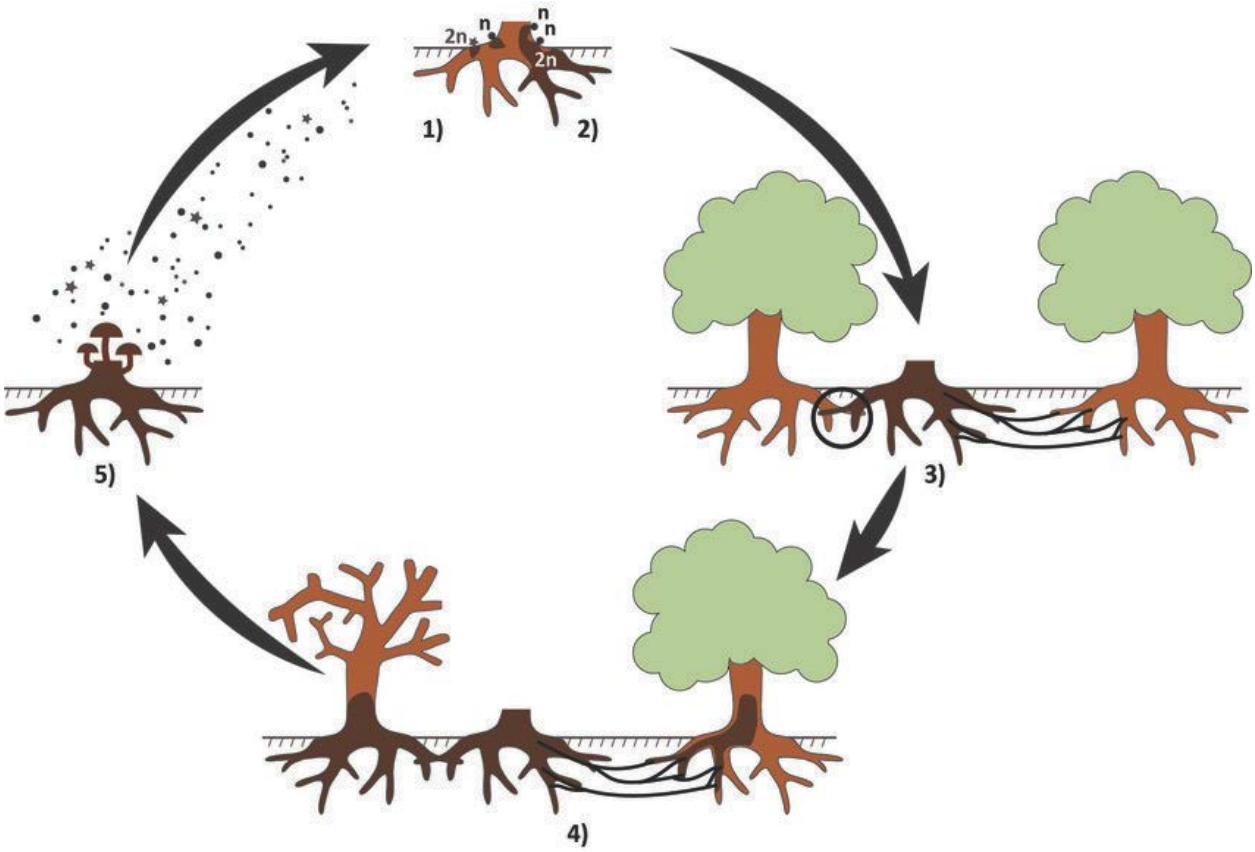

Figure 1-2: Life cycle of Armillaria root disease. 1) Basidiospore germination on dead stump. 2) Formation of diploid mycelium. 3) Infections of roots by rhizomorphs. 4) Spread via root-to root contact. 5) Release of basidiospores from fruiting bodies (Heinzelmann et al., 2019)

determine the main factor causing the symptoms.

The infection process can occur either from contact between a root and a rhizomorph or by contact with an infected root (Redfern and Filip, 1991). Once the rhizomorph contacts the root, the tip penetrates the bark, moving into the cork cells. Hyphae will spread subcortically within the root tissues. Hyphae do not need a wound to enter the root, yet wounds will act as an

infection court for the hyphae to easily enter the host (Garraway et al., 1991; Kile et al., 1991) (Figure 1-2).

As rhizomorphs attempt to penetrate the root, the host will respond to inhibit infection using three types of responses: biochemical, exudation, and meristematic cork and cambium formation (Garraway et al., 1991; Morrison et al., 1991). Biochemical responses release phenols or tannins that inhibit the growth of *Armillaria* in the root, preventing the spread into the host tissues. Exudation of resin forms a physical barrier to the hyphae, preventing it from penetrating the tissues. Cork and cambium formation walls off the infection similar to compartmentalization of decay in trees (CODIT) to further prevent the spread within the root (Morrison et al., 1991). In susceptible hosts, these barriers may not fully prevent the spread of the hyphae into the host tissues, allowing for further spread of the fungus within the host. Once the hyphae infiltrate the tissues, the mycelial fans spread under the bark, increasing the level of decay. Under favorable climatic conditions, i.e. precipitation and warmer temperatures, mycelial fans form clusters at the base of the tree, initiating the establishment of basidiomes (Morrison et al., 1991).

The spread of *Armillaria* occurs from either basidiospores dispersed from clustered mushrooms or by vegetative growth of rhizomorphs under the soil (Morrison et al., 1991; Redfern and Filip, 1991). Dispersal via mushrooms is less prevalent since spores do not have enough nutrients to survive and need to find an infection court (wound) on a susceptible host to germinate (Redfern and Filip, 1991). Free-living rhizomorphs spread at a slow rate, ranging from 0.22 m/year to 1.3 m/year (Ferguson et al., 2003). The ability to find susceptible hosts and spread via root-to-root contact allows for rapid dispersal and a greater chance of encountering available resources from decaying host tissues (Redfern and Filip, 1991). The dispersal method depicts how pathogenic species spread. Since *Armillaria* spp. also acts as a saprophyte the ability to spread via infections is less likely. In the case of *A. altimontana*, the weak pathogen establishes a wide dispersal to find more resources throughout the soil (Redfern and Filip, 1991). This has also been observed at the Priest River Experimental Forest in northern Idaho,

where the two species co-occur with *A. altimontana* occupying greater space than *A. solidipes* (Warwell et al., 2019).

Many management techniques are used to minimize the effects of *Armillaria*. Silvicultural practices are commonly used, which include thinning to increase the vigor of remaining trees, clearcutting to remove all trees, and selective thinning to change species composition to more resistant trees (Kile et al., 1991). Silvicultural techniques successfully remove susceptible hosts to increase growing space, but they may spread the pathogen if the remaining trees are damaged or stressed following management (Kile et al., 1991; Wargo and Harrington, 1991; Williams et al., 1986). To reduce the inoculum load, stump removals following silvicultural practices and soil fumigation are used to minimize the spread. These techniques assist in the management yet are invasive and not cost effective (Baumgartner et al., 2011; Hagel and Shaw, 1991). The inability to successfully manage *Armillaria* opens up the idea to utilize less invasive biocontrols within the soils to suppress the disease.

1.8 Biocontrol of root pathogens

Lack of adequate management techniques for forest root pathogens has prompted the need to fully understand the interactions between the forest soil microbiome and pathogens. The role that soil microbial communities play on the suppression of root pathogens has been well studied (Baumgartner and Warnock, 2006; Chapman and Xiao, 2000; Elad et al., 1979; Fu et al., 2017; Futai et al., 2008; Kope and Fortin, 1989; Mesanza et al., 2016; Trivedi et al., 2017; Xiong et al., 2017). Fungal and bacterial microbes have been identified as a potential biocontrol to combat infection of root pathogens with most studies focusing on potential bacteria for soil suppression. High microbial diversity may provide competition to the pathogens, specifically in response to *Fusarium oxysporum* f. sp. *cubensis,* which may induce an inhibitory response in comparison between biological control agent-amended soil samples and compost control soils (Fu et al., 2017). Beneficial changes in microbial communities included higher levels of

Sphingobium, Gp6, Gp4, Lysobacter, Sphingopyxis, and *Dyadobacter* for bacteria and *Cryptococcus* for fungi were associated with suppressive soil (Fu et al., 2017). Trivedi et al., (2017) identified similar results showing that phyla *Actinobacteria*, *Firmicutes*, and *Acidobacteria* were major predictors to soil suppression of *Fusarium oxysporum* in Australia. Further studies showed that the above phyla and *Verrucomicrobia* might be associated with the inhibition of *Fusarium* wilt disease, suggesting that they may also be useful for the management of other root pathogens (Xiong et al., 2017). Mesanza et al. (2016) utilized bacteria (*Pseudomonas*, *Bacillus*, and *Erwinia*) harvested in the rhizosphere of radiata pine (*Pinus radiata*) to measure the *in vitro* effects on tree root pathogens, *A. mellea* and *Heterobasidion annosum*. Results showed that *P. fluorescens* and *B. simplex* were antagonistic to both root pathogens, while *Erwinia billingae* had a large effect on *H. annosum* but only a small reduction in *A. mellea*. Baumgartner and Warnock (2005) also showed that *Bacillus* and *Pseudomonas* play a role in the inhibition of *A. mellea* isolated from grapevines.

Along with bacteria, fungi may play an integral role in the management of root pathogens using suppressive soils, especially since fungi provide a greater amount of biomass than bacteria within the soil (Lee Taylor and Sinsabaugh, 2014). The most diverse type is ectomycorrhizal (ECM) fungi, which make up 5,000 – 6,000 species within forests (Futai et al., 2008). Ectomycorrhizal fungi function to increase the uptake of nutrients and water and to provide a physical barrier (mantle) to inhibit the infection of pathogens (Futai et al., 2008). To identify what types of ECM are suppressive to pathogens, Hope and Fortin (1989) tested seven ECM as potential inhibitors to 20 phytopathogens made up of Ascomycetes, Basidiomycetes, and imperfect fungi. They documented that *Pisolithus tinctorius* and *Tricholomas pessundatum* were antagonist toward most phytopathogens including root pathogens (i.e. *Armillaria mellea*, *Fusarium oxysporum*, and *Rhizoctonia* spp. and others). Although both exhibited inhibitory qualities, *P. tinctorius* was antagonistic to 85% of the root pathogens, whereas *T. pessundatum* only suppressed 55% (Kope and Fortin, 1989). A study assessing the differences between

natural soils that suppressed *F. oxysporum* and soils that were conducive to the disease, Xiong et al., (2017), identified more *Mortierella*, *Ceratobasidium*, and *Gymnopus* in association to suppressive soil compared to conducive soil. *Trichoderma harzianum,* a common soil fungicide can be used to inhibit the growth of root pathogens (Elad et al., 1979). In a greenhouse and field study, the use of *T. harzianum* wheat bran inoculum to previous infested soil successfully protected crops from *Rhizoctonia solani* and *Sclerotium rolfsii* (Elad et al., 1979). A field study in British Columbia inoculated *Hypholoma fasciculare* (an abundant fungus isolated from soil at the site) on stumps already infected with *Armillaria ostoyae* suggesting that fungi can act as direct competition to root pathogens, inhibiting the spread within soil (Chapman and Xiao, 2000). Two years after the study, one of the sites showed a large reduction in roots infected by *A. ostoyae*, yet more time was needed to determine that *Armillaria* could be eradicated with *H. fasciculare* (Chapman and Xiao, 2000). The use of both bacterial and fungal antagonists, naturally occurring in the soil, may assist in the overall management of root pathogens.

1.9 Conclusion and Hypotheses

As climates change over the coming century, drought environments will exacerbate increased forest susceptibility to insect and disease damages. The likely expansion of bark beetles and root disease induce by changing climates will result in increased mortality across landscapes. This relationship complicates the ability to manage forests, prompting the need to understand the ultimate drivers of mortality agents. The assessment of subalpine fir mortality in Colorado will assist in understanding of how abiotic and biotic factors influence high elevation forests (Chapter 2). Identifying factors driving subalpine fir mortality in Colorado focused the objectives to 1) determine abiotic and biotic factors that directly and indirectly affect subalpine fir mortality, 2) determine factors associated with the presence of *D. confusus* or *Armillaria* spp., and 3) determine if climate variables were correlated to subalpine fir mortality or the presence of *D. confusus* and *Armillaria* spp. I hypothesized that sites with a higher density (i.e. basal area,

trees per hectare, or canopy closure) would experience greater mortality due to decreased growth rates from competition and that *D. confusus* or *Armillaria* spp. prevalence would be a function of tree stress (i.e. increased density), elevation, slope, and departures from normal precipitation (i.e. drought), and minimum and maximum temperatures.

While the evaluation of soil fungal communities associated with Armillaria root disease will assist in providing novel management techniques for root pathogens (Chapter 3). My research objective was to identify the soil fungal communities associated with tree health status (healthy, moderate and dead) and each *Armillaria* species, *A. solidipes* and *A. altimontana*, both of which have differing ecological behaviors (virulent pathogen and non-pathogen, respectively) on western white pine. I hypothesize that soil microbial communities will likely differ in richness and diversity in comparison between the virulent *A. solidipes* and the non-pathogenic *A. altimontana* with the latter having a greater richness and diversity due to its beneficial qualities. While richness and diversity is likely to shift among tree health with a greater diversity and richness for soil associated with healthy trees due to root exudate production near the rhizosphere.

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CHAPTER 2: SUBALPINE FIR DECLINE IN COLORADO IS ASSOCIATED WITH STAND DENSITY, WARMING CLIMATES AND AN INTERACTION AMONG FUNGAL DISEASES AND THE WESTERN BALSAM BARK BEETLE

2.1 Preface

Subalpine fir mortality complex has caused significant damage to high elevation forests within Colorado. For the past two decades the climate of spruce-fir forests have trended towards being warmer and drier, which likely has had a direct effect on subalpine fir mortality. I examined potential links among abiotic (i.e. deviations in temperature and precipitation) and biotic factors (Armillaria root disease [*Armillaria* spp.], Western balsam bark beetle [*Dryocoetes confusus*], and forest structure) with subalpine fir mortality in subalpine fir (*Abies lasiocarpa)* and Engelmann spruce (*Picea engelmannii*) dominated forests of Colorado. The objectives of this study were to determine: (1) Do site and stand characteristics influence subalpine fir mortality? (2) What factors are associated with the presence of *Armillaria spp.* and *D. confusus*? (3) Do warming temperatures and less precipitation influence subalpine fir mortality and/or the presence of biotic agents? My results suggested that the presence of biotic agents (*D. confusus*, *Armillaria* spp., and *O. dryocoetidis*) and stand density influenced subalpine fir mortality, while climatic factors had a direct influence on the presence of biotic agents, thereby only indirectly affecting mortality. In terms of the significance of climate, increasing maximum summer temperatures were found associated with the presence of *Armillaria* spp. While the climatic variables investigated in this study did not significantly influence *D. confusus*, stand density was associated with increasing prevalence of *D. confusus*. My results show that *Armillaria* spp. and *D. confusus* are significantly related to tree decline, but that several other factors can also be associated with mortality, suggesting a complex interaction of factors are likely involved. To identify subalpine fir mortality as a decline disease, I determined that stand density was likely a predisposing factor, drought was the inciting factor, and *D. confusus* and

Armillaria spp. were contributing factors. Understanding factors involved in subalpine fir decline are important for management as this decline continues to threaten Colorado forests.

2.2 Introduction

High elevation forests in Colorado, roughly at 2,400 to 3,800 meters and primarily comprised of spruce and fir, provide benefits to water quantity and quality, outdoor recreation, wood products, and food and shelter for wildlife (CSFS, 2008). The spruce-fir forest type consists mainly of subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) and Engelmann spruce (*Picea engelmannii* Parry ex. Engelm.). As the third largest forest type in public lands of Colorado, encompassing 1.86 million hectares (ha) (CSFS, 2019), these high elevation forests are particularly sensitive to drier and warmer climates because they occupy a particular niche in Colorado's forest within a narrow climate range (Reich et al., 2016).

Background mortality is described as the amount of mortality necessary to sustain existing stand dynamics, resulting from healthy amounts of pests in a stand (Manion, 2003). Background mortality of around 1% naturally occurs within stands, yet it is projected that doubling (2%) this mortality over a span of 20 to 30 years can result in a decrease in >50% of the age diversity and size of trees in a stand (van Mantgem et al., 2009). This background mortality of 18,600 ha over 30 years (1% in Colorado spruce-fir forests), is typically not concerning at a forest level, yet higher rates would prompt for further research into the cause of mortality. Mortality trends documented in annual aerial pest surveys have highlighted varying rates of subalpine fir mortality with an estimated total of 744,000 ha in Colorado from 2008 to 2017. Estimated rates each year have ranged from 139,200 ha in 2008 to 20,200 ha in 2017 (CSFS, 2008-2017). The high level of variation observed from aerial survey data is likely partially attributed to the spatially aggregated nature of the species across the landscape (Garbutt and Vallentgoed, 1992). From aerial surveys, it is estimated that clustered subalpine fir mortality has occurred throughout 40% of spruce-fir forests in Colorado since 2008. A study

conducted in southern British Columbia found that subalpine fir mortality doubled from 16.7% to 31.3% in 1996/7 and 2014, respectively (Maclauchlin, 2016). In the Front Range of Colorado, comparisons of overall mortality levels from 1982-2007 to 2008-2013 in the Arapaho-Roosevelt NF, found increases in subalpine fir mortality over the past 20 years (Smith et al., 2015).

Climate plays a significant role in the structure and distribution of species on a landscape (Habeck, 1987). Spruce-fir forests in high elevation landscapes are restricted which is likely due to a lack of adaptation to high temperatures and low moisture content (Alexander, 1987). Temperatures in north-facing spruce-fir forests tend to be colder and wetter in comparison to pine forests at similar elevations, which usually grow on south and east facing aspects (Graham and Jain, 2005). In more xeric environments, higher temperatures limit the growth potential for spruce-fir in favor of co-occurring pine species (Villalba et al., 1994). As climate conditions transition to warmer and drier, it has been proposed that in spruce-fir forests these conditions will be drivers of increased mortality (Reich et al., 2016; Villalba et al., 1994). Subalpine fir mortality has been linked to drought in Colorado, particularly early-season drought where the potential for mortality increases for a span of 11 years, while late-season drought increases mortality risk for two years (Bigler, 2007). Further, reduced radial growth due to elevated stand densities prior to any drought can often serve as a predisposing factor to mortality events (Bigler, 2007). Periods of drought can decrease growth rates, tree vigor, and increase susceptibility to insects and disease (Furniss and Carolin, 1977). Understanding how effects of climate interact with damaging biotic agents may provide insights into future of how subalpine fir mortality rates will evolve with future weather patterns and help in the develop of new management strategies.

Biotic factors attributed to the subalpine fir mortality complex in Colorado include *Dryocoetes confusus* Swaine (Western balsam bark beetle), Armillaria root disease, and black stain fungi (*Ophiostoma dryocoetidis* (Kendrick & Molnar) De Hoog & Sheffer) (James and Goheen, 1981; McMillin, 2003; Molnar, 1965; Negron and Popp, 2009). A survey of 150 trees in

four national forests in Colorado (Grand Mesa, Rio Grande, San Isabel, and San Juan), found that the majority of subalpine fir mortality occurred in association with Armillaria root disease and bark beetles (James and Goheen, 1981). In separate studies, *A. ostoyae* (Worrall et al., 2004), now identified as *Armillaria solidipes* Peck, Bull. Torrey Bot. Club (Burdsall and Volk, 2008) and *A. sinapina* (Burns et al., 2016) have been identified on subalpine fir within Colorado forests, prompting the use of *Armillaria* spp. in the study. Smith et al. (2015), showed that *D. confusus* was the most significant mortality agent, as it was present in 20% of dead trees from 2011-2013, and likewise Buxton and Maclauchlin (2014) showed that *D. confusus* contributed to 25-53% subalpine fir mortality in each plot. Attacks by *D. confusus* typically occurs in small groups, thus making openings to release shade-tolerant seedlings and providing a scattered mortality structure throughout the landscape (Garbutt and Vallentgoed, 1992). Subalpine fir mortality is prevalent in most western states, and interestingly mortality agents differ from state to state. Additional biotic factors include Balsam wooly adelgid (*Adelgis piceae* Ratzeburg) (ID, MT, OR, WA), *Heterobasidion occidentale* Otrosina and Garbelotto (UT), wood borers (UT), and smaller bark beetles (UT) (USDA-FS 2012, 2013a, 2013b, 2015, 2016, 2017). Comparisons of percent spruce-fir affected by subalpine fir mortality complex between all western states, from 2008 to 2016, shows that Colorado has the greatest percent of mortality, with comparable mortality in Oregon and Wyoming, while all over states display far less mortality. The combination of contributing factors, including warming temperatures, long periods of prolonged drought, and their association with root disease and bark beetle invasion susceptibility has led to landscape-scale subalpine fir mortality events and suggests that this mortality, if driven by these factors, is a decline disease.

Identifying factors driving subalpine fir mortality in Colorado focused the objectives to 1) determine abiotic and biotic factors that directly and indirectly affect subalpine fir mortality, 2) determine factors associated with the presence of *D. confusus* or *Armillaria* spp., and 3) determine if climate variables were correlated to subalpine fir mortality or the presence of *D.*

confusus and *Armillaria* spp. I hypothesized that sites with a higher density (i.e. basal area, trees per hectare, or canopy closure) would experience greater mortality due to decreased growth rates from competition and that *D. confusus* or *Armillaria* spp. prevalence would be a function of tree stress (i.e. increased density), elevation, slope, and departures from normal precipitation (i.e. drought), and minimum and maximum temperatures. Results from this study will be discussed in the context of identifying subalpine fir mortality in Colorado as a decline disease.

2.3 Methods

2.3.1 Study Areas

Study areas were identified using aerial survey and vegetation data for Colorado. GIS layers of the Colorado aerial pest survey maps conducted by Region 2 Forest Health Protection of the USDA Forest Service from 1994 through 2012 were obtained (USDA-FS, 2012). Using ArcGIS (ESRI, 2011), georeferenced data were displayed as distribution maps in which visible patches associated with subalpine fir mortality were delineated. To determine cumulative area of subalpine fir mortality, years ranging from 1994 to 2012 were joined to establish a range of current and past mortality. Presence of spruce-fir forests in Colorado were established using the Colorado Division of Wildlife vegetation types in accordance to Reich et al. (2016). Spruce-fir vegetation layers were merged with subalpine fir mortality to establish areas of interest.

All roads within designated state lands and national forests that occurred within the spruce-fir forest cover type were suitable for surveys. Sampling was conducted in two phases, with the first set of plots established to form a statewide characterization survey and the second set used to perform a stand health monitoring survey. All plots were placed along roads located within designated state land (Colorado State Forest State Park, CSFSP) and eight national forests (Grand Mesa, Pike, Rio Grande, Roosevelt, Routt, San Juan, Uncompahgre, and White River).

2.3.1.1 Statewide characterization survey

The statewide characterization survey was conducted in 2013 to assess the overall health of subalpine fir in Colorado on a large scale and to assist in determining plot locations for the detailed stand health monitoring survey (Figure 2-1). Between May 2013 and September 2013, 1142 plots were established in a subset of random areas of spruce-fir type within the eight national forests, and the Colorado State Forest State Park. The 1,142 plots consisted of fixed area plots [16 m (50 ft.) deep and 30 m (100 ft.) wide] measured on each side of the road every 0.8 km (0.5 mile). Data recorded included location, slope position, aspect, number of crown layers, ocular estimates of percent mortality of each species, and insects and diseases observed within dead or damaged trees.

2.3.1.2 Stand health monitoring plots

The stand health monitoring survey was conducted in 2014 to provide specific observations of coincident forest structure, species composition, topographic variables, and climatic attributes that could be correlated with subalpine fir mortality and determine incidence levels of *D. confusus* and *Armillaria* spp. in subalpine fir stands (Figure 2-1). Potential stand health monitoring plot locations were identified following the statewide characterization survey in 2013. From the statewide characterization plots, 57 locations were randomly chosen within stratified spruce-fir forested areas both with and without detected mortality using ArcGIS.

At each location, three independent stand health plots were established. The plots were spaced 61 m (200ft) apart along the randomly selected side of the road and 61 m (200 ft) into the forest. Data collected at each plot included location, forest type, slope position, aspect, and percent canopy closure of overstory trees using a spherical densitometer. To measure overstory trees, a variable radius plot was established using a metric BAF of 4.592 (20 English BAF) with basal area and trees per hectare values derived from each plot. Individual tree measurements consisted of live or dead status, diameter at breast height (DBH), height, crown base height,

presence of *D. confusus* and *Armillaria* spp. (mortality agents), and any other relevant insects or diseases.

The presence of exit holes and egg/larval galleries associated with dieback on susceptible hosts were used to indicate *D. confusus* presence in a tree, whereas mycelial fans at the base and roots of the tree, and the presence of root rot by sounding the tree with a hammer were used to determine if *Armillaria* spp. was present. The occurrence of *O. dryocoetidis* was identified by removing bark, at beetle exit holes, to witness black staining on the phloem. Another bark beetle, *Dendroctonus rufipennis* (spruce beetle), an associated beetle affecting trees in the spruce-fir forest type was identified by egg/larval galleries under the bark. The galleries of *D. confusus* had one centralized mating chamber with numerous egg galleries (polygamous) branching off, while *D. rufipennis* galleries consisted of one egg gallery (monogamous).

Four regeneration 13.5 m^2 (1/300th acre) circular plots were established in each cardinal direction (N, S, E, W) eight meters from plot center. On these plots, the number of seedlings (DBH < 2.5 cm) and saplings (DBH 2.5 cm – 10 cm) for each species were recorded.

2.3.2 PRISM climate data

Climate data for each plot was obtained from the Oregon State University PRISM Climate Group using the standard PRISM 4 km resolution (PRISM Climate Group, 2004). Maximum summer temperatures were collected for July through September from 1985-2014, and values were averaged over the three-month period. Minimum winter temperatures were collected for November through April from the winters of 1984/85-2013/14, averaging over the six-month period. A longer, six-month, period was selected for the winter months to better estimate the prolonged colder temperatures typically found at higher elevation landscapes. Cumulative annual precipitation data were collected for 30 years ranging from 1985-2014. To represent climate change in these metrics at each site, the five years prior to sampling were

averaged, then subtracted from the 30-year average. This was done to identify recent deviations from historic averages that might be impacting susceptibility to disturbance agents.

2.3.3 Data Analysis

For the statewide characterization plots, overstory mortality was used to determine overall plot mortality. Percent of mortality for statewide characterization plots was estimated for all plots and averaged for each national forest and state land to identify overall mortality from surveyed locations.

Percent of mortality for the 2013 aerial surveys was estimated by taking the sum of the hectares of subalpine fir mortality complex and spruce beetle within each national forest and the state of Colorado. Mortality levels for each national forest was calculated by providing a buffer of 500 m, 1000m, 1500 m, and 2000 m from each characterization plot location to directly compare mortality results. The accumulation of Colorado spruce-fir mortality was clipped using the state boundaries. Spruce-fir vegetation was compiled using land cover raster files to determine the extent of spruce-fir in each national forest and the entire state of Colorado. For statewide characteristics plots, overall percent mortality was calculated using overstory values to emulate aerial pest survey mortality polygons. Average plot mortality consists of plot ocular overstory mortality averaged over each national forest. Comparisons between the statewide characterization plots and aerial surveys were considered at a forest level and a statewide level.

Using the RStudio (RStudio, 2015) interface to R (R Core Team, 2017), a logistic regression was performed to determine correlation between site and forest structure attributes to the three response variables: presence of subalpine fir (SAF) mortality, presence of *D. confusus* and/or *Armillaria* spp. Predictor variables analyzed included biotic agents, site characteristics, forest structure and composition, and climatic measurements. A generalized linear model (glm) was used to correlate the three response variables to 19 predictor variables (Table 2-3) for a full model that included interactions between Δ minimum winter temperature, Δ maximum summer temperature, and Δ annual precipitation. The full model was reduced through

backwards stepwise analysis with the Akaike information criterion (AIC) using the MuMin package (Barton, 2016). This process was conducted to identify the set of predictor variables that minimized the predictive models AIC value. The Hosmer-Lemeshow Lack of Fit test was used to confirm that correct predictors were selected for each model.

2.4 Results

2.4.1.1 Statewide characterization survey data

The statewide characterization plots revealed intermittent spruce-fir mortality throughout the surveyed locations at an estimated level of 4.7% across the range of spruce-fir forests in Colorado (Table 2-1). Mortality occurred on 216 (19%) out of the 1142 totals plots with no mortality occurring on 926 plots (81%). For each national forest, the percent of plots affected by mortality ranged from 10% in Pike NF to 41% in Routt NF.

Table 2-1: Estimates of spruce-fir mortality for statewide characterization plots compared to Colorado aerial pest surveys.

	2013 Statewide Characterization Plots	**2013 Colorado Aerial Pest Survey**				
	Average plot mortality	**500 m**	**1000 m**	**1500 m**	**2000 m**	**Total NF**
Grand Mesa National Forest	4.1%	14%	14%	12%	11%	16.4%
Pike National Forest	0.8%	13%	10%	10%	10%	3.2%
Rio Grande National Forest	4.9%	26%	70%	29%	32%	27.5%
Roosevelt National Forest	6.2%	3%	4%	4%	4%	8.3%
Routt National Forest	10.8%	8%	6%	6%	6%	4.1%
San Juan National Forest	2.6%	1%	2%	2%	2%	14.3%
Uncompahgre National Forest	1.6%	15%	14%	11%	8%	5.9%
White River National Forest	6.2%	10%	10%	8%	7%	7.4%
Average spruce-fir mortality	4.7%	11%	16%	10%	10%	10.9%
Total NF spruce-fir mortality						9.5%
Statewide spruce-fir forests						8.6%

When compared with the Colorado aerial pest survey mortality levels, the highly concentrated statewide characterization surveys typically provide a lower estimated mortality level across each national forest. These lower estimates were consistent for all national forests other than Routt NF, which was almost 7% higher than the aerial survey (Table 2-1). Spruce-fir

mortality for the aerial survey was estimated at 9.5 and 8.6% for Colorado national forests and statewide spruce-fir extent, respectively.

2.4.1.2 Stand health monitoring plot data

Average incidence of mortality (dead trees/total # of trees) for subalpine fir and Engelmann spruce was 37% and 15% throughout all plots. This includes plots with a range from 0% to 100% mortality for both species. A total of 50 (33%) out of the 153 stand health monitoring plots had the occurrence of subalpine fir mortality. Out of the 50 plots, 42 plots (84%) had trees that were infested with *D. confusus*, whereas 22 plots (44%) had trees infected with *Armillaria* spp. All 22 plots with trees infected with *Armillaria* spp. also had trees infested with *D. confusus*. Eight of the 103 plots without subalpine fir mortality had trees infested with *D. confusus* and infected with *Armillaria* spp. This means that 95 of the 153 total plots did not have any mortality or presence of the two biotic agents.

2.4.1.3 Stand health monitoring tree level data

A total of 967 trees were measured on all 153 plots, including subalpine fir, Engelmann spruce, white fir (*Abies concolor* (Gord. & Glend.) Lindl.), lodgepole pine (*Pinus contorta* Dougl. Ex. Loudon), and aspen (*Populus tremuloides* Michx.). Seven hundred sixty-nine out of the 967 trees (80%) measured were either subalpine fir (n = 296) or Engelmann spruce (n = 473). For subalpine fir, 131 trees (44%) were observed to be dead, ranging from 19% to 48% on individual national forests, with exceptions occurring within the Pike NF with 0% and Uncompahgre NF at 100% mortality. On average, 20% of the Engelmann spruce on each national forest were dead, with the majority occurring on Roosevelt (66%) and Routt NF (49%), while no spruce mortality was identified in Pike, Rio Grande, and White River NF.

Across national forests, subalpine fir mortality occurred in all diameter classes (Figure 2-2). Furthermore, of the 131 dead subalpine fir, 56 trees (43%) were only infested with *D. confusus*, 9 trees (7%) were only infected with *Armillaria* spp., while 24 trees (18%) had both biotic agents (Figure 2-3). Engelmann spruce mortality occurred on all diameter classes greater

than 20 cm with no mortality occurring in smaller diameter trees (Figure 3-2). Neither tree diameter nor crown ratio showed a discernable relationship with the presence of *D. confusus* or *Armillaria* spp.

2.4.1.4 Stand heath monitoring plot level climatic data

A diverse range of average temperatures and annual precipitation levels occurred throughout all national forests. The highest maximum summer temperatures and minimum winter temperatures were recorded in the Uncompahgre NF, with averages of 25.6 and -6.7 °C, respectively (Table 2-2). Additionally, the least amount of annual precipitation also occurred within the Uncompahgre NF with 40 cm recorded on average from 1985-2014 (Table 2-2).

Climate departures [Δ minimum winter temperatures (°C), Δ maximum summer temperatures (°C), and Δ annual precipitation (cm)] varied across and within each national forest. All but one plot showed an increase in maximum summer temperatures, including increased average departures for each national forest ranging from 0.2 – 0.5° C, with the greatest increases occurring in Routt NF (Table 2-2). All plots recorded increased minimum winter temperatures throughout the entirety of the study. The average 5-year departures for each national forest ranged from 0.5 – 1.6 °C, with the largest increases in Rio Grande and San Juan NF (Table 2-2). Precipitation deviations fluctuated in comparison to the temperature values with the range of 5-year precipitation departures from -17.5 – 8.7 cm. The largest decrease in precipitation occurred within Rio Grande NF, while Grand Mesa, San Juan, Uncompahgre, and White River NF also displayed a decreased amount of precipitation from the 30-year norm (Table 2-2). Of the 153 plots, 38 (25%) had an increase in precipitation, while 115 (75%) decreased. Furthermore, of the 153 plots, 78 (51%) had at least a 5 cm decrease in precipitation from the 30-year average.

Table 2-2: Climatic factors on stand health monitoring plots averaged within each national forest. The 30-year norm data consist of temperatures from 1984/1985 – 2014, while annual precipitation is from 1985 – 2014. To calculate for 5-year departures the average temperatures and annual precipitation from 2009-2014 were subtracted from 30-year climatic norms.

	30-year norms			5-year departure		
	Max Summer Temps (°C)	**Min Winter Temps (°C)**	**Annual Precip. (cm)**	**Max Summer Temps (°C)**	**Min Summer Temps (°C)**	**Annual Precip. (cm)**
Grand Mesa NF	17.1	-13.4	106.7	0.4	1.1	-6.2
Pike NF	16.7	-12.1	64.2	0.4	1.1	5.0
Rio Grande NF	17.2	-11.3	93.3	0.4	1.6	-17.5
Routt NF	17.8	-12.9	76.0	0.5	0.5	6.9
Roosevelt NF	19.4	-10.4	59.3	0.3	0.7	8.7
San Juan NF	20.4	-9.8	82.1	0.3	1.5	-7.2
Uncompahgre NF	25.6	-6.7	40.0	0.4	0.6	-2.3
White River NF	17.1	-11.2	95.8	0.2	1.1	-3.5

2.4.2 Logistic regression

2.4.2.1 Presence of subalpine fir mortality

The use of interactions between climatic factors in the logistic regression resulted in a lack of significance for site, stand, and climatic variables. This lack of significance prompted the removal of the interactions toward analyzing for each climatic factor individually. Climatic factors were not shown to influence the presence of subalpine fir mortality. Consequently, site and stand characteristics and biotic agents were correlated to the presence of subalpine fir mortality (Table 2-3).

In terms of abiotic influences, although not significant, the cosine of aspect showed a positive correlation with the presence of subalpine fir mortality (Table 2-3), indicating that more northerly sheltered aspects are indicative of mortality events. In terms of forest structure, increased dominance of subalpine fir on a plot was correlated with greater likelihood of subalpine fir mortality. Additionally, a negative relationship with Engelmann spruce sapling was found, indicating that regeneration conditions supporting spruce were less likely in association with subalpine fir mortality. Both the *D. confusus* and *O. dryocoetidis* biotic agents were positively correlated to subalpine fir mortality, while not significant the presence of *Armillaria* spp. was negatively correlated to the presence of subalpine fir mortality (Table 2-3).

Table 2-3: Significant predictor variables for each response variable selected using Akaike information criterion through backwards stepwise logistic regression. These models were reduced from the full model using all 19 predictor variables from the 2014 stand health monitoring plots (n=153).

	Presence of subalpine fir mortality		**Presence of *D. confusus***		**Presence of *Armillaria* spp.**	
	Coefficient	**p-value**	**Coefficient**	**p-value**	**Coefficient**	**p-value**
SAF mortality presence	-	-	7.536	<0.0001*	-	-
D. confusus presence	4.964	0.0003*	-	-	2.060	0.0289*
D. rufipennis presence	-	-	-5.190	0.0028*	-	-
Armillaria spp. presence	-1.997	0.0956	3.401	0.0006*	-	-
O. dryocoetidis presence	2.555	0.0196*	-	-	1.911	0.0506
% SAF	0.069	<0.0001*	-0.042	0.0809	-	-
Elevation (m)	-	-	0.004	0.0581	-	-
Cosine aspect (north vs south)	1.052	0.0742	-	-	-	-
Sine aspect (west vs east)	-	-	-	-	-	-
∆ min. winter temperature (°C)	-	-	-2.678	0.0783	-	-
∆ max. summer temperature (°C)	-	-	-	-	5.422	0.0136*
Δ annual precipitation (cm)	-	-	-	-	-	-
SAF sapling stems	-	-	-	-	-	-
SAF seedling stems	-	-	-	-	-	-
ES sapling stems	-0.008	0.0227*	-	-	-0.003	0.1941
ES seedling stems	-	-	-0.004	0.0109*	-	-
Overall basal area (m^2 ha^{-1})	-	-	0.072	0.0038*	-	-
% slope	0.041	0.1410	-0.048	0.1064	0.038	0.0825
Overall trees per hectare	0.004	0.1143	-	-	-0.002	0.1368
Hosmer-Lemeshow Lack of Fit:		0.9711		0.9213		0.7204

* Significance was based on a p-value < 0.05

2.4.2.2 Presence of D. confusus

Inclusion of *O. dryocoetidis* in the *D. confusus* presence model resulted in a Hosmer-Lemeshow Lack of Fit value of <0.0001, prompting the removal of *O. dryocoetidis* as a predictor variable. The removal of *O. dryocoetidis* increases the Lack of Fit value to 0.9213, indicating that the high correlation between *O. dryocoetidis* and *D. confusus* diminishes the efficacy of the model. The change in minimum winter temperatures had a negative correlation to the presence

of *D. confusus*, demonstrating that areas with warmer minimum winter temperature departures saw a lower presence of *D. confusus*.

In terms of forest attributes, both increases in basal area and the presence of SAF mortality were correlated with increased likelihood of *D. confusus* being present on a plot (Table 2-2). Conversely, as the density of Engelmann spruce seedlings increased on a plot there was a reduced probability of finding *D. confusus*. While no abiotic variables were significant at the $\alpha = 0.05$ level, a weak correlation was found with elevation that indicated greater probability of finding *D. confusus* at higher elevations. Conflicting correlations were found for the biotic agents *D. rufipennis* and *Armillaria* spp. The presence of *Armillaria* spp. increased the probability of finding *D. confusus* on a site, while presence of *D. rufipennis* reduced the likelihood of finding *D. confusus* (Table 2-3).

2.4.2.3 Presence of Armillaria spp.

For abiotic factors, maximum summer temperatures were positively correlated to the presence of *Armillaria* spp. (Table 2-3). Although weaker than the $\alpha = 0.05$ level, increases in a site's percent slope resulted in an increased probability of finding *Armillaria* spp. present. Similar to the other models, increasing density of Engelmann spruce seedlings on a plot reduced the probability of finding *Armillaria* spp. The biotic agents *D. confusus* and *O. dryocoetidis* had positive correlations to the presence of *Armillaria* spp., suggesting that as other biotic agents increased, the probability of *Armillaria* spp. presence subsequently increased (Table 2-3).

2.5 Discussion

2.5.1 Significant predictors

The stand health monitoring plots showed that the most relevant factors to subalpine fir mortality are stand density and the presence of *D. confusus*. I identified that stand density, elevation, and *Armillaria* spp. are the greatest influences of the presence of *D. confusus*, while the largest influences on the presence of *Armillaria* spp. are warmer maximum summer

temperatures and increased slope percentage. My data indicated that both *D. confusus* and *Armillaria* spp. subsequently effect subalpine fir mortality, therefore, I can conclude that an increase in summer temperatures and higher stand densities indirectly influence subalpine fir mortality in Colorado's national forests. Therefore, higher density and increases in summer temperatures may induce stress to trees increasing the likelihood of *Armillaria* spp. and *D. confusus*, subsequently leading to subalpine fir mortality.

2.5.2 Spruce-fir mortality comparisons

At a statewide level, my estimates of subalpine fir mortality of 4.7% showed a closer resemblance to aerial pest surveys from 2013 that estimated 8.7% of spruce-fir forests showed elevated mortality levels. However, when examining estimated mortality levels within an individual forest, correlations were less congruent at any spatial scale. The estimates were on average 6% less than the coincident aerial survey data. The comparison of mortality within the roadside surveys and aerial pest surveys is best described at a statewide level rather than an individual national forest, watershed, or stand level. These differences observed between the aerial survey data and the characterization plots could be due to a combination of over estimation of subalpine fir mortality in the aerial surveys and the highly concentrated nature of the characterization plots. According to Coleman et al. (2018), aerial surveys conducted throughout the Northeastern, Southwestern, and Pacific United States identified that bark beetles were the most prolific cause of damage, yet the error rate was large due to multiple species of bark beetles associated with different tree species. The ability to accurately identify mortality agents is contingent on successfully recognizing tree species during aerial surveys (Coleman et al., 2018), but this can be difficult to accomplish across complex mountainous terrain. Most bark beetles are host specific, therefore the identification of host species can distinguish between numerous bark beetle infestations. Mortality occurring from *D. confusus* and *D. rufipennis* may be difficult to distinguish in a mixed stand as a result of *D. confusus* infesting both *A. lasiocarpa* and *P. engelmannii* within spruce-fir forests. In the statewide

characterization plots, I determined overall mortality of spruce-fir rather than distinguishing between *A. lasiocarpa* and *P. engelmannii*. This should have provided a more balanced comparison between my statewide characterization plots and aerial pest surveys, which likely encompassed numerous bark beetles within mortality polygons. Additionally, the differences in direct comparison between the geographically accessible statewide characterization plots and the aerial survey mortality locations is partially due to inherent error in both sampling techniques. Together the clustered nature of the characterization plots, limited my ability to reliably attribute the mortality agents, while the potential location errors associated with the aerial survey, made it difficult to reliably compare the datasets at a scale less than the entire state. Direct comparisons between aerial surveys and ground assessments can be made by conducting a more thorough and dispersed ground survey, which could establish greater sample area as compared to the expansive aerial surveys, thus eliminating accessibility limitations. Another comparison is to have targeted ground surveys for the aerial survey technician to focus on while identifying damage throughout the entire forest.

2.5.3 Abiotic and biotic factors influencing subalpine fir decline

Previous research suggests climate is among one of the strongest influences on subalpine fir mortality (Bigler, 2007; Reich et al., 2016). While decreasing levels of precipitation is detrimental to all plants, subalpine fir forests have been shown to be more sensitive to drought conditions, likely because they typically occur in cool, wet environments (Reich et al., 2016). Reich et al. (2016) suggested that severely warmer and drier conditions alone where enough to cause subalpine fir mortality. This corresponds within the Uncompahgre NF stand health monitoring plots. The temperature and precipitation data highlighted this area as the warmest and driest and 100% of subalpine fir were dead. Interestingly, my model suggested that the presence of biotic agents (*D. confusus*, *Armillaria* spp., and *O. dryocoetidis*) and stand density influenced subalpine fir mortality, while climatic factors had a greater direct influence on the presence of biotic agents, thereby only indirectly affecting mortality. These results indicate

that subalpine fir mortality is likely driven by biotic factors, with only an indirect linkage to climate, suggesting this mortality represents a decline disease.

In many systems, the risk for beetle expansion from endemic to epidemic levels increases as drought occurs (Bentz et al., 2010; Berryman, 1982). Yet, my data showed that precipitation did not have a significant effect to the presence of *D. confusus*. Although climatic factors promoting drought, were not shown to influence *D. confusus*, factors such as other biotic agents and increased stand density (basal area) were significantly correlated to beetle presence within plots. Because of the highly clustered nature of subalpine fir mortality, the lack of total hectares measured throughout stand health monitoring plots may have influenced the ability to identify if additional climatic variables affected the presence of *D. confusus*. Additionally, the removal of *O. dryocoetidis* from my reduced model resulted in a greater p-value for the Hosmer-Lemeshow Lack of Fit test. This could be due to the direct relationship between *D. confusus* vectoring *O. dryocoetidis* to infected trees (Molnar, 1965; Garbutt and Vallentgoed, 1992).

My data suggests that site factors promoting overall tree stress likely drive the increase in presence of *D. confusus*. This was observed as the probability of finding *D. confusus* steadily rose with increasing basal area. In the data, plots with subalpine fir mortality had on average a 50% greater average basal area (38.4 m^{-2} ha) compared to plots lacking mortality (25.7 m^{-2} ha). Stand density (i.e. basal area) may be an indicator of overall tree vigor within the stand and could affect a tree's ability to withstand invasion by biotic agents. As density increases, growing space decreases, eventually reaching a holding capacity. This threshold can induce competition between trees for resources, subsequently increasing stress (Hyink and Zedaker, 1987). According to McMillin et al. (2003), *D. confusus* has the greatest impact in stands with higher density and areas with larger diameter trees. The increased competition can cause decreased vigor due to a lack of water, nutrients, and space. The added stress due to bark beetles on all stands increases insect populations, resulting in widespread mortality (Bentz et al., 2010). The risk of susceptibility to *D. confusus* infestation results from a relationship among diameter, age,

and radial growth. In British Columbia, beetles favored older, larger subalpine fir with thicker bark to provide more protection to their larvae (Bleiker et al., 2003). However, I did not observe an influence of diameter on infested trees. Instead, my study detected an average of 12.7% mortality for trees between 5 and 30 cm DBH, suggesting that increased stress related to stand-level density may play a greater role in infestation than tree diameter or age.

My results concur with James and Goheen (1981), which showed there is a direct relationship between biotic agents, *D. confusus* and *Armillaria* spp.*,* in subalpine fir forests. Rather than a positive relationship, my study found that subalpine fir mortality was significantly associated with an increased presence of *D. confusus*, but with a decrease in the presence *Armillaria* spp. However, when *Armillaria* spp. was present there was greater likelihood of *D. confusus* being present on a site. Furthermore, *D. confusus* was found on 50 plots (33%), while *Armillaria* spp. was only found on 30 (20%), which could have been caused by sampling limitations in the study. The comparison to my study and James and Goheen showed that we underestimated the presence of *Armillaria* spp. while they may have represented a more accurate level of *Armillaria* within dead trees. The ability to more precisely diagnose *Armillaria* on healthy and dead trees would have most likely enhanced the influence of *Armillaria* to the presence of subalpine fir mortality.

Increasing summer temperatures was correlated to an increased presence of *Armillaria* spp. The relationship between *Armillaria* spp., their hosts and drought have been well documented and summarized (Wargo and Harrington, 1991). However, the correlation between presence of *Armillaria* spp. and warming temperatures is less understood. Nechleba (1927) found that the pathogenic response of *Armillaria* spp. increased on true fir species (*Abies* spp.) during dry seasons, while during wet seasons *Armillaria* spp. persisted as a saprophyte. The impact of drier climates affects the defense mechanisms of trees, causing increased infection in dry sites (Morrison, 1981). Stress induced by drought along with increased temperatures has influenced *Armillaria* spp. in fir forests causing the occurrence of mortality (Falck, 1918, 1923;

Muller, 1921; Nechleba, 1927 in Thomas, 1934). Future drier and warmer conditions will most likely continue as climate change persists in forests, allowing pests to become more prolific (Allen et al. 2010). This in turn could further increase the likelihood that *Armillaria* spp. will become pathogenic rather than saprophytic on sites.

2.5.4 Categorization of decline disease designation

Decline diseases are characterized by a slow progression of mortality, whereas, dieback can be seen after the effects of a solitary event (i.e. drought, defoliation, or disease outbreaks). These dieback events cause mortality within a stand but may convalesce as the outbreak subsides (Mueller-Dombois, 1992). Decline diseases occur when mortality is the result of three features: predisposing, inciting, and contributing factors. Predisposing factors are the result of long-term effects or permanent stress on the landscape, which increases the risk of mortality. Inciting factors are short-term effects that create additive stress, making trees more susceptible to secondary biotic contributing factors. Contributing factors are generally pests or pathogens that preferentially attack stressed or weakened trees within the stand. Although the presence of a predisposing, inciting, and contributing factors are needed for classification as a decline disease, the contributing factor may be what ultimately kills the tree (Manion, 1981; Houston, 1992). The main contributors to subalpine fir decline are stand density and the presence of *D. confusus*, which are likely exacerbated by the presence of *Armillaria* spp. and drought conditions brought on by increased summer temperatures. While climatic factors are thought to be a direct influence on subalpine fir mortality (Reich et al. 2016), my data suggests that climate may have a greater effect on biotic factors, especially *Armillaria* spp., which may indirectly influence subalpine fir decline in Colorado. The increase in maximum summer temperatures increases the presence of *Armillaria* spp., leading to additional tree stress and subsequently increasing the presence of *D. confusus*.

2.5.5 Limitations of study

Although an elaborate field was created study over two years, plots were not established throughout the entirety of each national forest, due to time constraints to complete all surveys within the summer and many spruce-fir forests are located within designated wilderness areas and are thus not accessible for roadside surveys. This limitation affected my ability to perform statewide characterization plots in 2013, subsequently reducing the area available study in the following year's stand health monitoring plots. Additionally, while performing stand health monitoring plots, I encountered difficulty in identifying the presence of *Armillaria* spp. on live trees. Colorado has reduced moisture levels compared to other regions where *Armillaria* spp. are more prevalent, such as Oregon and Washington. These reduced moisture levels may limit the spread of its mycelial network (Cruickshank et al., 1997). Further, the reduced prevalence may be due to my survey design whereby a positive identification of *Armillaria* spp. occurred when a mycelial fan was observed. Mycelia fans are produced during an advanced infection, yet rhizomorph formation could have persisted without symptoms progressing aboveground (Greig, Gregory, and Strouts, 1991; Morrison, 1981). Without observing roots and rhizomorphs, the study likely underestimated the amount of *Armillaria* infection. In areas of the Nelson forest region in British Columbia, two-thirds of sampled plots that appeared disease-free were infected belowground by *Armillaria* spp. (Morrison, 1918). Though not completed in this study, utilizing rhizomorph collections and the presence of resinosis along the lower bole, as field indicators would have enhanced my ability to identify *Armillaria* spp. on more trees.

2.6 Conclusion

The elevated levels of subalpine fir mortality indicated that the combination of abiotic and biotic factors, including climate, stand characteristics, and insects and disease have sanitized maladapted trees within the stand. As climate models project changes to warmer and drier high elevation forests, areas affected by subalpine fir decline will inevitably increase due to an

increased presence of biotic factors. Managing forests, via thinning to increase vigor, to minimize predisposing and inciting factors may help reduce the risk of each contributing factor. This will assist to maintain mortality levels closer to background rates and potentially reduce the spread of subalpine fir decline within spruce-fir forests. Learning from other insects and diseases will provide insight into proper management techniques and ways to mitigate mortality agent's populations. Understanding how the host and pathogen life cycles of subalpine fir, *D. confusus,* and *Armillaria* spp. will respond to climatic changes toward reduced precipitation and increased temperature will allow forest managers to prepare for heightened levels of biotic agents prior to epidemic populations in the future.

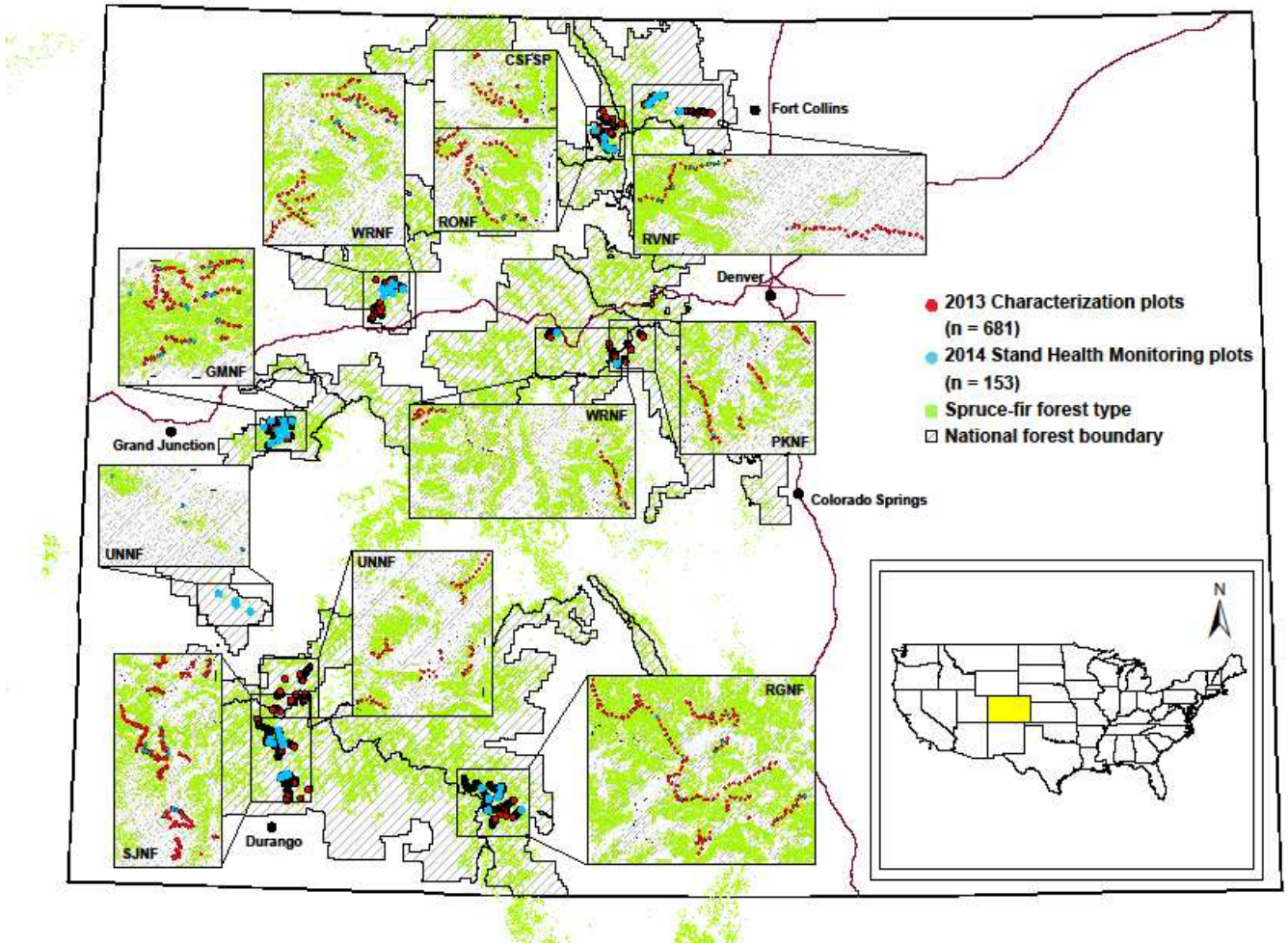

Figure 2-1: Map of all plot locations within eight national forests and state land (dashed with light grey) in Colorado (CSFS*P* = Colorado State Forest State Park, GMNF = Grand Mesa, PKNF = Pike, RGNF = Rio Grande, RONF = Routt, RVNF = Roosevelt, SJNF = San Juan, UNNF = Uncompahgre, & WRNF = White River). Six hundred and eighty-one characterization plots (red circles) were established in 2013 along forest service roads following areas identified as spruce-fir forest type (light green). One hundred and fifty-three stand health monitoring plots stratified by presence of SAF mortality were established in 2014 to determine presence of mortality agents and other stand characteristics that may influence subalpine fir mortality in the Rocky Mountains of Colorado.

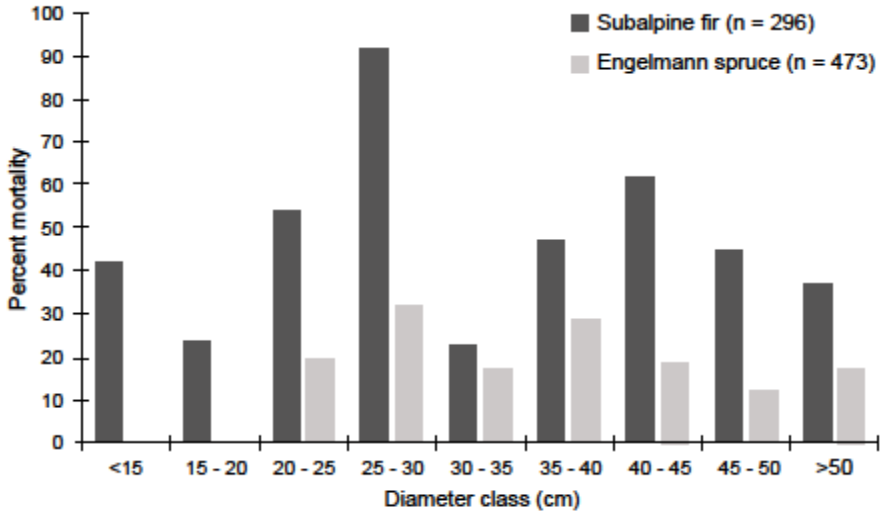

Figure 2-2: Mortality of subalpine fir (dark grey) and Engelmann spruce (light grey) by 5 cm diameter class. Bars represent percent tree mortality from 2014 stand health monitoring plots (n=153) located across eight national forests in Colorado.

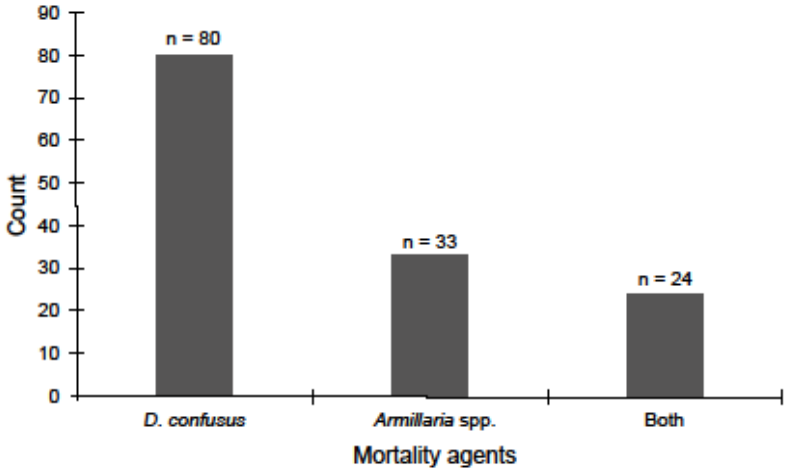

Figure 2-3: A total of 296 subalpine fir in the 2014 stand health monitoring plots were assessed for health status and 131 (44%) identified as dead. Mortality agents were identified on dead subalpine fir; Presence of *Dryocoetes confusus* was determined by larval and egg galleries, while mycelial fans were used to determine presence of *Armillaria* s

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CHAPTER 3: CHANGES IN SOIL FUNGAL COMMUNITIES ASSOCIATED WITH ARMILLARIA ROOT DISEASE ON WESTERN WHITE PINE (*PINUS MONTICOLA*)

3.1 Preface

In forests, soil interactions among *Armillaria* species, fungal communities, and roots may influence tree growth and survival. Two fungal species, *A. solidipes* (highly virulent) and *A. altimontana* (less virulent), frequently co-occur in forests of inland northwestern United States. Understanding fungal communities associated with each *Armillaria* species may provide novel insights for managing Armillaria root disease. Aims of this study were to identify fungal microbes and their community structure in association with *A. altimontana* and *A. solidipes* infected western white pine (*Pinus monticola*) under different health statuses. Results of this field study revealed no significant changes in fungal communities associated with the two *Armillaria* spp., yet slight changes occurred between moderate and dead trees. Although not significant, higher fungal diversity was associated with dead-standing trees and *A. solidipes*. When examining operational taxonomic units (OTUs) within communities, there was an abundance of saprophytic *Mortierella* and numerous ectomycorrhizal fungi associated with all soils. We also found higher levels of Hypocreaceae (*Trichoderma*) species associated with healthy trees and *A. altimontana*. These organisms are known to be important in biocontrol against pathogens in disease-suppressive soils. Additionally, pH is the most significant soils characteristics, as it influences soil carbon, nitrogen, and organic matter. Research suggests that novel approaches could be developed for managing Armillaria root disease by fostering soil conditions to favor fungal communities that suppress Armillaria root disease.

3.2 Introduction

3.2.1 Background of Armillaria in North America

Armillaria spp. are native fungal root pathogens that cause prolific damage to coniferous forests of North America. Currently, ten North American Biological Species (NABS) have been identified, through molecular tools and somatic pairing, occurring on a broad range of hosts throughout the United States (Guillaumin et al., 1991; Kim et al., 2010). Although mostly known as pathogens, *Armillaria* can persist as saprophytes, exhibiting facultative necrotrophic characteristics, as they kill trees and consume dead tissue for nutrition (Baumgartner et al., 2011; Kile et al., 1991). The ability to thrive on live and dead tissue enables *Armillaria* to spread in soil acting as a primary pathogen, impacting tree health, and decomposer increasing soil organic matter (Baumgartner et al., 2011). Once established within a stand, expansion occurs via a vegetative growth of rhizomorphs under the soil (Redfern and Filip, 1991). Growth can occur as root-to-root contact or actively within the soil. Spread can reach up to 1.5 m/year depending on environmental conditions (Ferguson et al., 2003; Redfern and Filip, 1991). Weak pathogens tend to disperse throughout the forest to expand their ability to find nutrients, while pathogenic species spread via root contacts of susceptible hosts, causing infections and mortality (Redfern and Filip, 1991).

Management strategies for *Armillaria* consist of reducing the inoculum load by means of root excavation or soil fumigants, i.e. methyl bromide & carbon disulphide (Hagel and Shaw, 1991). Yet, the feasibility to use methyl bromide may be limited by a potential ban in the United States (Baumgartner et al., 2011). Forest management treatments such as partial cutting are commonly utilized to increase growing space subsequently increasing tree vigor and decreasing susceptibility to most disturbing agents, however it may exacerbate *Armillaria* infection due to added stress form soil compaction and damage to residual trees (Kile et al., 1991; Wargo and Harrington, 1991; Williams et al., 1986). Therefore, clearcutting in disease centers may be a better option to reduce the potential for inoculum to build up on susceptible hosts (Kile et al.,

1991). Changing the species composition by selectively removing susceptible hosts in mixed conifer stands to favor resistant species, has been suggested as the best harvesting management strategy (Kile et al., 1991). Additionally, introducing high intensity fire to the stand may also reduce the inoculum load (Kile et al., 1991), whereas, low intensity fires heat the soils, but may not directly reduce the presence of the disease. However, increased soil temperatures may enhance *Trichoderma* (a known biological control agent for *Armillaria*), consequently reducing infection (Reaves et al., 1990). Due to the complexity of tree damage induced during most silvicultural management strategies, minimizing stress within stands is key to treating *Armillaria* infections (Kile et al., 2019). Soil metagenomics can be used to identify important fungi, bacteria, and archaea associated with tree health that can be utilized to enhance the management of *Armillaria* (Ross-Davis et al., 2015).

3.2.2 Co-occurrence of A. solidipes and A. altimontana

Interactions between *Armillaria* species have been documented between *A. solidipes* Peck [as *A. ostoyae* (Romagnesi) Herink] and *A. altimontana* Brazee, B. Ortiz, Banik, and D.L. Lindner (formerly North American Biological Species X) co-occurring within stands in the inland northwestern United States (Ferguson et al., 2003; Kim et al., 2010; Warwell et al., 2019). *Armillaria solidipes* is known as a primary, virulent pathogen on many North American conifer species (Ferguson et al., 2003). *Armillaria altimontana* has previously been found on dead grand fir (*Abies grandis*) and symptomatic Douglas-fir (*Pseudotsuga menziesii*), yet evidence for pathogenicity has not been documented (Ferguson et al., 2003). Current research at the Priest River Experimental Forest (PREF) suggests that *A. altimontana* may be non-pathogenic due to an increase in diameter, height and percent survival for trees associated with the fungus compared to trees infected with *A. solidipes*. Additionally, *A. altimontana* occupies a larger niche compared to *A. solidipes* indicating that *A. altimontana* may be potentially beneficial to stands in conjunction with a pathogenic species of *Armillaria* (Warwell et al., 2019). Recognizing the

underlying soil factors within host-pathogen interactions and understanding the relationship of soils between high and low virulent root pathogens may enhance the management of *Armillaria*.

3.2.3 Understanding the importance of soil microbial communities

It is hypothesized that soil microbes play an essential part in ecosystem functioning within a forest environment (Baldrian, 2017; Hartmann et al., 2014). Yet, the innate heterogeneity of microbial communities within forest soils provides a challenge in studying the impacts soils may play on forest ecology (Fierer and Jackson, 2006). Understanding those roles may be a key to the future management of forests. Many factors are known to affect the diversity of microbes within the soil, including temperature, moisture, pH, location to rhizosphere, and other biotic factors complicating what ultimately influences community diversity present in a single soil sample (Fierer and Jackson, 2006). The diversity associated with a small amount of soil may result in as many as 2,500 fungal taxa, many of which are unculturable. Utilizing soil metagenomics allows researchers to categorize communities and identifies a higher diversity of microbes compared to culturing (Buee et al., 2009). Understanding the drivers that shape microbial community structure, interactions, and functions through metagenomics and statistical analysis will provide further insight into soil ecological health and management.

The ecosystem services provided by soil microbes enhance many of the functions needed for forests to thrive. The association between mycorrhizae and roots allow for increased water and nutrient uptake to provide essential minerals needed for tree health (Azul et al., 2014; Baldrian, 2017; Leake et al., 2004; Lee Taylor and Sinsabaugh, 2014; Saif and Khan, 1975). Microbes also breakdown litter, maintaining stand health by decomposing organic matter into usable inorganic minerals usable by plants (Cardenas et al., 2015; Chapman and Koch, 2007; Davidson and Janssens, 2006; Robertson and Groffman, 2014; Schloter et al., 2003). In addition, pathogenic soil fungi function as sanitizers of stressed trees, increasing the vigor of residual trees in a stand (Allison and Martiny, 2008; Horwath, 2014; Kile et al., 1991). Whereas

highly virulent soil pathogens can infect healthy tree roots, ultimately degrading the health of stands (Kile at al., 1991). Additionally, the ability to utilize beneficial microbes to inhibit of the growth of root pathogens, which are historically difficult to mitigate, may enhance current management techniques (Kim et al., 2016).

3.2.4 Potential use of biocontrols for root diseases

Bioncontrols have been used to minimize the effects of pathogens within all environments with few studies focusing on forests (Mesanza et al., 2016). Microbial communities can act as an antagonist toward root pathogens by direct competition for nutrients and increase host resistance (Mesanza et al., 2016). Specifically, beneficial fungi can to inhibit the growth of *Armillaria*. *Trichoderma* are well known for their biological control capabilities in association with burned and unburned sites (Reaves et al., 1990). The isolation of *T. citrinoviride* occurred in burn areas, while *T. harzianum* was more abundant in non-burned areas. Both showed signs of inhibition toward *A. ostoyae* (*solidipes*) with *T. cintinoviride* playing a larger antagonistic role, suggesting that the association with fire might assist in the reduction of *Armillaria* inoculum (Reaves et al., 1990). In British Columbia, Chapman et al. (2004) utilized *Hypholoma fasciculare*, a highly abundant fungal saprophyte, to determine *in situ* inhibition to the growth of *A. ostoyae*. Soils in association with *H. fasciculare*, with an adequate supply of wood debris exhibited the capability to reduce mortality, compared to locations without the *Hypholoma* (Chapman et al., 2004). Both studies highlight native, highly abundant soil microbes that can be used in the management of root diseases, specifically *Armillaria*. Proper management of naturally occurring, abundant microbes may improve techniques employed to inhibit root pathogens.

3.2.5 Goals and research objectives

Our research objective was to identify the soil fungal communities associated with tree health status (healthy, moderate and dead) and colonized by *Armillaria* species, *A. solidipes* and *A. altimontana*, both of which have differing ecological behaviors (virulent pathogen and

non-pathogen, respectively) on western white pine. I hypothesize that soil microbial communities will likely differ in richness and diversity in comparison between the virulent *A. solidipes* and the non-pathogenic *A. altimontana* with the latter having a greater richness and diversity due to its beneficial qualities. While richness and diversity is likely to shift among tree health with a greater diversity and richness for soil associated with healthy trees due to root exudate production near the rhizosphere. In better understanding communities associated with each species, we proposed to identify potential biocontrol species that may enhance our ability to develop novel techniques to minimize the effects of *Armillaria* and assist in the management of the disease.

3.3 Methods

3.3.1 Field sampling

The study area was located in the northern panhandle of Idaho at the United States Department of Agriculture-Forest Service Priest River Experimental Forest. The field site was within the Ida Creek study area (elevation at 760 meters), which is a historic western white pine seed provenance plot. In 1971, 2,372 seedlings were planted in a common garden plantation with 1.2 m x 1.2 m spacing between each row and column. Planted seeds were selected from eight national forests in Idaho and Washington, with elevations ranging from 760 to 1,585 meters. In 1987, all 2,076 remaining trees were sampled for diameter at breast height (DBH), height, tree health status and association with *Armillaria* species. *Armillaria* species were identified using methods described in Warwell et al. (2019). Briefly, *Armillaria* were identified using somatic incompatibility pairing tests where an unknown isolate is grown with a reference isolate for each species of *Armillaria*. Unmated pairings were determined by the formation of a pseudosclerotial plate forming between both isolates. Mated pairings were distinguished as compatible due to colorless antagonism of mycelial growth (Figure 3-1) (Warwell et al., 2019). Since 1987, the 2,076 trees had been thinned to increase spacing for the mature trees and to

remove dead trees caused by a combination of *A. solidipes* (a pathogenic species of *Armillaria*), competition, white pine blister rust, and other minor agents of mortality. In 2016, the study area had ~600 trees remaining from the initial planting.

We randomly selected 60 trees for sampling ensuring that half of the trees were historically associated with each of the two species of *Armillaria*. Out of the 30 trees for each species, 15 were historically healthy and 15 were historically dead or dying. If historically dead or dying trees had fallen since the last inventory, adjacent trees were selected that fit similar treatments. Three additional trees were sampled due to needle discoloration and the formation of mycelial fans on the base of the trunk, indicating the presence of *Armillaria*, bringing the total number of trees sampled to 63. Tree health was categorized as healthy, indicating no visible signs or symptoms of disease; moderate, characterized as a qualitative identification of living trees with visible symptoms of decline; and dead.

Sampling was completed in the summer of 2016. Tree measurements included DBH and tree health status. Tree health status measurements were based on total amount of needles, color of foliage, insect and disease presence, and dead/live status. Soil sampling consisted of clearing a 30 cm diameter circle from the duff and litter, in a flat location, one meter from the main stem to minimize damage to the roots. Depth of duff and litter were measured at four cardinal directions within the cleared area for soil sampling. Bulk soil samples were taken for each of the 63 trees using a 15 cm split soil corer hammered into the ground using a compact slide hammer. Each bulk density sampling location was selected to ensure extraction of 15 cm of soil. Orientation to each tree differed, depending on the topography around the tree and its root zone. After each sample, the soil core was taken apart and sanitized using 70% ethanol to remove any contaminant DNA. The soil was placed in quart sized plastic bags, labeled with each unique tree number and homogenized. Two grams of soil were placed in a 15 ml plastic bead tube (Qiagen Powersoil RNA Extaction Kit ®; Germantown, MD), with 5 ml of LifeGuard RNA preservation solution (Qiagen). An additional backup sample for each tree was prepared

following the same protocol. Both tubes were immediately placed in a cooler with ice to preserve the soil RNA and DNA and shipped to Colorado State University. Once in Colorado, the samples were stored in a refrigerator for preservation prior to RNA and DNA extractions. The remaining bulk soil, not collected for DNA extractions, from each tree was sent to the Rocky Mountain Research Station in Moscow, ID for soil characteristics measurements and chemistry calculations.

Armillaria rhizomorphs adjacent to the roots were also collected. Soil around roots were extracted using a “mini Pulaski”. Primary rhizomorph collections occurred on the same side as the soil core while an additional sampling occurred 180° from the core. Rhizomorphs were collected by hand and individually placed in empty 15 ml plastic tubes. Tubes filled with rhizomorphs were placed in a cooler and transported to the Rocky Mountain Research Station in Moscow, ID to isolate pure cultures of mycelium.

3.3.2 Rhizomorph isolation, mycelial DNA extractions and PCR

Rhizomorphs were plated for fungal isolation within seven days of collection. Each rhizomorph was surface sterilized, placed into a sterilized 15 ml test tube, and rinsed with sterile-distilled water to remove the attached soil particles. Rinsed rhizomorphs were soaked in 20% Clorox bleach solution (Sodium Hypchlorite) for 6-10 minutes. After soaking, rhizomorphs were removed from the bleach solution, rinsed with sterile-distilled water, and then soaked in 3% hydrogen peroxide for 6-10 minutes. After removal of the hydrogen peroxide, rhizomorphs were rinsed with sterile distilled water to remove excess solution. After sanitization, rhizomorphs were cut into 1 cm sections and placed in the center of an agar plate of enhanced *Armillaria* media (6 g malt extract, 6 g dextrose, 4 g peptone, 12 g agar, 800 mL DD H_2O). Each plate was incubated at 25°C in the dark until the formation of mycelium. The plates were checked daily to ensure no contamination and re-isolated on *Armillaria* media until a pure culture of *Armillaria* mycelium was obtained. Eighty-seven total rhizomorphs were isolated from 48 total trees with cultures grown for all 87 rhizomorphs.

Once pure cultures were obtained, they were sent to Colorado State University for DNA extractions. For extractions, mycelium was plated onto 0.22 µm pore size MF-Millipore ™ Membrane filters (MilliporeSigma, Burlington, MA) on the enhanced *Armillaria* media. Mycelia from each pure culture were cut into 1 cm^2 sections. Four pieces were evenly placed on the membrane of two plates and grown for 2-3 weeks. DNA of > 50 mg of mycelia were extracted using Zymo DNA extraction kits (Irvine, CA), following manufacturer protocols with a few modifications. To maximize DNA quantity and quality extractions, three 3-mm glass beads were added to a bead tube prior to adding mycelium, and cell lysis was performed in the Thermo Savant FastPrep ® FP120 Cell Homogenizer (Qbiogene, Carlsbad, CA) at 6.0 speed with two 30-second cycles. DNA concentration and quality were quantified using a NanoDrop ™ 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE).

For species identification, DNA was amplified at the translation elongation factor-1α (*tef*1) locus using primers EF-983 and EF-2218 (Rehner and Buckley, 2005). Samples were amplified with a Eppendorf Mastercycler pro Thermal Cycler (Eppendorf, Hamburg, Germany) using the cycle 94 °C for 2:30, 30 cycles of 94 °C for 0:30, 60 °C for 0:30, and 72 °C for 1:30, and ending with 72 °C for 10:00 and maintaining at 4 °C. The resulting PCR product was run on an electrophoresis gel. Successfully amplified PCR products were cleaned using ExoSAP-IT ™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Santa Clara, CA) to remove excess primers and nucleotides. Cleaned PCR products were sent to Eurofins Genomics (Louisville, KY) for Miseq Illumina sequencing in both directions. Forward and reverse DNA sequences were edited and aligned in Geneious R11.1 (https://www.geneious.com). Aligned sequences were referenced to known NABS *Armillaria* spp. for species identification, either *A. solidipes* or *A. altimontana*, and identified by blasting clustered sequences in the National Center for Biotechnology Information Basic Local Alignment Search Tool (BLASTn) (zhang et al., 2000). Sequences corresponding to both *A. altimontana* and *A. solidipes* resulted in 99% identity.

3.3.3 Soil RNA and DNA extraction protocol

DNA from the preserved soil samples was extracted using MoBio Powersoil Total RNA Isolation and DNA Elution Accessory kits (Qiagen®, Carlsbad, CA), following manufacturer protocols. The 15 mL bead tubes with soil were centrifuged for five minutes to allow for the soil and LifeGuard Preservation Solution to separate. The LifeGuard Preservation Solution was pipetted from the tubes and discarded to leave just the soil. The complete MoBio Powersoil instructions were followed, resulting in 100 µL of eluted DNA and RNA for each sample. RNA and DNA qualification and quality were measured using a Nanodrop ™ 2000 spectrophotometer. If the concentration of RNA and DNA were below 10 ng μL^{-1} the sample soil RNA and DNA was extracted again, following the same protocol as above using the additional soil preserved from the Ida Creek study area.

Thirty microliters of the soil DNA were sent to the University of Minnesota Genomics Center for library preparation and sequencing. A total 57 out 63 samples were sent for sequencing; the six remaining samples did not yield sufficient DNA concentration or quality and therefore were excluded. Libraries were prepared for the internal transcribed spacer (ITS2) region to sequence fungal communities. Primers ITS3 (5'-GCATCGATGAAGAACGAGC-3') and ITS4 (5'TCCTCCGCTTATTGATATGC-3') (White et al., 1990) were used to amplify the ITS2 genomic region.

3.3.4 Cleaning DNA sequence data

Files were inputted into the Galaxy Project (Afgan et al., 2018), as fastqsanger files, to clean and analyze the fastq files by trimming primers prior to analysis using Mothur (Schloss et al., 2009). Files were viewed using FastQC (Andrews, 2014) to identify initial quality of samples and to determine protocols for trimming. Within Trimmomatic (Bolger et al., 2014) the parameters for trimming consisted of using HEADCROP to trim the first 50 basepairs (bp) to remove the primers from each fasta file, the SLIDINGWINDOW to view every four bp and remove pairs that fell below a PHRED score of 20, and MINLIN to make the minimum length of the trimmed sequences at least 125 bp. Each fasta file was trimmed using paired end data to

overlap the R1 and R2 files. Sequences were further cleaned following a modified Mothur MiSeq SOP (Kosich et al., 2013), using a perl script prepared by Dr. Zaid Abdo (https://github.com/Abdo-Lab). The protocol was followed with a few exceptions including removal of the initial align.seq setup. Data was classified to the fungal database UNITEv6_sh_dynamic_s (Nilsson et al., 2018). Following the Mothur protocol, the OTU table and taxonomy file were prepared to identify the fungal communities within the soil samples.

3.3.5 Statistical analysis of fungal communities

Using the RStudio (RStudio, 2015) interface to R (R Core Team, 2017), the OTU table and taxonomy file were merged and OTUs with less than 2 reads were removed. Two reads were subtracted from all total OTU abundances. Any negative values were set to zero and then those OTUs with zero abundance were removed from further analyses. After combining the OTU table and taxonomy file, the data reflected the Kingdom, Phylum, Class, Order, Family, Genus, and Species (if available) alongside each unique OTU. A metadata file was uploaded to reflect plot characteristics to each soil sample, including *Armillaria* species and tree health status. The OTU/taxonomy file and metadata table were merged to correlate plot characteristics with fungal data. The initial analysis was calculated at the family level. A rarefaction curve (richness) was established to determine the quality of sequences for each sample using the phyloseq package in R (McMurdie and Holmes, 2013). For all analyses, groups were created to determine the differences between each *Armillaria* spp. (*A. solidipes* and *A. altimontana*) and among tree health status (healthy, moderate, or dead). The relative abundance was determined for the top 17 fungal groups using a stacked bar graph for each group using the metagenomeseq package in R (Paulson et al., nd). Ordination plots were established for each group, using non-metric multidimensional scaling (NMDS) in the Vegan package in R, to determine the dissimilarity of fungal communities (Oksanen et al., 2017). Overlap in an ordination plot determined similarity between fungal communities, while separation described dissimilar microbial communities (Clark, 2017). Diversity and richness were measured, at the

OTU level, using the Shannon and Inverse Simpson indices. The Shannon index is a used to determine diversity utilizing the relationship to richness and rare microbes (Hill et al., 2006; Nagendra, 2002). Inverse Simpson relies on evenness and more dominant microbes to identify diversity (Nagendra, 2002). Richness is described as the amount of individuals identified within a single sample, while evenness explains the relative abundance of the different individuals (Zhang et al., 2012).

To display fungal differences among groups, heat trees were established using the Metacoder package (Foster at al., 2017). Heat trees were developed with all taxon using color and size to determine the abundance and proportion of each OTU for all categories of *Armillaria* spp. and tree health. Heat trees were also produced for fungal communities using each species of *Armillaria* and comparing between the abundance in *A. altimontana* and *A. solidipes*. To determine the differences in fungal communities for tree health status, healthy and dead trees were compared to identify the greatest variability within the samples. Finally, core fungal communities were created for each *Armillaria* spp. and tree health status (within *A. altimontana*) by identifying what OTUs corresponded to each tree species or tree health status. Counts were completed in R to assess the presence of an OTU corresponding to each species of *Armillaria* and tree health status with *A. altimontana* (R Core Team, 2017). Venn diagrams were compiled following the coordination of core and unique fungal communities. Tree health status was not recognized within *A. solidipes* due to low sample size. To identify what soil chemistry properties influenced soil fungal communities, a PERMANOVA analysis was completed using the Vegan package in R. The analysis identified significant predictors by completing a forward stepwise analysis based on the subset of variables that minimized the Akaike Information Criterion (AIC).

3.4 Results

3.4.1 Field sampling and rhizomorph species identification

From the 63 trees sampled, mycelial extractions identified that 44 were associated with *A. altimontana*, 1 with *A. solidipes*, 2 were associated with both *A. altimontana* and *A. solidipes*, 14 did not include rhizomorphs, and 2 were unknown due to inadequate DNA from mycelial extractions. Any tree associated with both species was categorized as *A. solidipes* due to the pathogenic nature of the disease. Thirty-eight trees were healthy, 13 were dead, and 12 were in moderate health (any live tree with crown dieback or symptoms of insect or disease damage).

3.4.2 Next Generation Illumina sequencing soil data analysis

To identify fungal communities in Ida Creek soils, total DNA was extracted from 63 samples and of those 57 were sent for Miseq Illumina sequencing at the ITS2 region. Six samples were not processed due to insufficient DNA quantity or quality. The total number of reads, following trimming, was 4,323,028. Reads were screened using screen.seqs to remove any longer than 275 bp, resulting in the removal of 375,837 from the dataset. The remaining reads were clustered into 387,219 unique sequences. The modified Mothur protocol for the ITS2 data resulted in 6,936 total unique OTUs and a remaining 2,806, following the removal of low coverage OTUs (< 2 sequences) in RStudio. A rarefaction curve was established for all samples to identify if DNA was adequately sequenced. If the curve plateaued, there was sufficient sequencing to reflect high quality fungal communities for each sample (Figure 3-2). Thirty-four (80%) samples plateaued indicating high quality communities, whereas 8 (20%) samples did not plateau, indicating a need to sequence at a greater depth. For final data analyses, 42 samples were used (39 *A. altimontana*, 3 *A. solidipes*, 27 healthy, 6 moderate, and 9 dead).

Samples were grouped either by associated species of *Armillaria* (*A. altimontana* or *A. solidipes*) or tree health status (healthy, moderate, or dead). Non-metric multidimensional scaling (NMDS) ordination plots were used to identify the dissimilarity of fungal communities of each group (Clark, 2017). The two-dimensional NMDS plots identified overlapping between

fungal communities of trees associated with *A. altimontana* and *A. solidipes*, suggesting that *Armillaria* species did not strongly influence soil fungal community composition (Figure 3-3A). Additionally, for tree health, overlaps occurred among all three groups, with fungal taxa of moderately healthy trees were more dissimilar from fungal taxa of dead trees than healthy trees (Figure 3-3B). Using a 3-D NMDS ordination plot, at 90% confidence, overlapping communities between healthy and dead trees and healthy and moderate trees indicated that fungal taxa were similar, yet there was only a slight overlap between moderate and dead trees suggesting a difference in fungal communities.

Fungal communities were also assessed for overall richness and diversity. Richness was analyzed to represent how many species were present in each sample, and Inverse Simpson and Shannon diversity indices were estimated to compare diversity among groups. Fungal species richness was slighter greater in soil associated with *A. solidipes*; however the difference was not statistically significant (Table 3-1). Neither diversity index (Simpson or Shannon) showed significant differences between *Armillaria* species, but once again *A. solidipes* associated samples had a greater diversity. For tree health status, no significant differences were observed in richness or diversity (Table 3-1). However, soil associated with dead trees tended to have the greatest fungal community richness and diversity, while moderately healthy trees had the lowest.

Table 3-1: Richness and diversity indices calculated for *Armillaria* species and tree health status. Values based on samples within each group with standard errors.

	Richness	**Shannon**	**InvSimpson**
A.altimontana	224 ± 11.6	3.26 ± 0.072	13.4 ± 1.01
A.solidipes	133 ± 70.5	2.62 ± 0.434	8.18 ± 6.19
Healthy	229 ± 13.6	3.28 ± 0.085	13.5 ± 1.18
Moderate	179 ± 28.9	3.02 ± 0.181	10.0 ± 2.51
Dead	243 ± 23.6	3.43 ± 0.147	15.9 ± 2.05

The relative abundance of the 17 most abundant fungal taxa are shown in Figure 3-4A. Though not significant, more reads were identified as Atheliaceae, Cortinariaceae, Helotiales, Hypocreaceae (*Trichoderma*), Puccinomycotina, and Rhizopogonaceae in communities associated with *A. altimontana*, whereas more Hypocreales, Inocybaceae, and Leotiomycetes were detected in soil associated with *A. solidipes*. In examining tree health, variation occurred between each health status with greater differences occurring in comparisons between healthy or moderate and dead trees (Figure 3-4B). Greater proportions of Hypocreaceae (*Trichoderma*), Rhizopogonaceae, Trichcomaceae, and Leotimycetes were observed in soils of healthy trees. In soils from moderately healthy trees, a greater proportion of Myxotrichaceae was identified, and Cortinariaceae was more abundant in soils from both healthy and moderate trees. For soils of dead trees, there were greater proportions of Inocybaceae and unclassified fungi.

To understand the variation of OTU abundance among groups, Venn diagrams of the core fungal communities were produced to identify OTUs that were similar between numerous treatments. The diagrams identified which fungal OTUs were associated with both species of *Armillaria* and which were unique to each species (Figure 3-5A). The core fungal community associated with both *A. altimontana* and *A. solidipes* consisted of 521 OTUs. Far surpassing the core community, 2,219 OTUs were unique to *A. altimontana*, whereas only 66 were unique to *A. solidipes*. A Venn diagram was produced to compare all health status categories associated with *A. altimontana* (Figure 3-5B). This analysis was not completed for *A. solidipes* since only three infected trees were identified. A total of 535 fungal OTUs were associated with all tree health status categories (*A. altimontana*), while 1,182 OTUs were unique to healthy trees, 135 OTUs were unique to moderate health trees, and 311 OTUs were unique to dead trees. The core fungal taxa between healthy and dead trees resulted in 389 similar OTUs. For healthy and moderate, 162 OTUs were similar, and for moderate and dead only 66 OTUs were similar. The OTU comparisons between each group concurs with results of the NMDS ordination plots of Figure 3.3, which highlight overlaps or departures in fungal communities.

To further identify differences in fungal taxa associated with each *Armillaria* species and tree health status, the metacoder package in R was used to create heat trees (Foster at al., 2017). The heat trees identified comparisons by calculating the log2 ratio of median proportions and color-coded each difference to establish clear variances in fungal abundance. For the comparison between *A. altimontana* (brown) and *A. solidipes* (blue), the heat maps identified a greater abundance of Agaricomycetes within soils associated with *A. altimontana*, (Figure 3-6). For soils associated with *A. solidipes*, a greater abundance was observed for *Ilyonectria* (Hypocreales), Leotimycetes, and *Mortierella,* which coincides with the stacked bar graphs in Figure 3-4. Tree health associations were created for all pairwise comparisons; healthy/dead, healthy/moderate, and moderate/dead, to characterize fungal communities. The comparison between healthy (brown) and dead (blue) trees, showed that overall Ascomycota were more abundant in soils associated with healthy trees, while Basidiomycota and Zygomycota were more abundant in soils associated with dead trees (Figure 3-7). More specifically, there was greater abundance of Agaricales, *Mortierella*, and *Ilyonectria* (Hypocreales) in soils with dead trees, similar to results observed by the stacked bar graphs. Comparing healthy (blue) and moderate health (brown) trees, more Agaricomycetes and Leotiomycetes were found in soil associated with healthy trees, while more *Mortierella* and *Ilyonectria* (Hypocreales) were detected in soils associated with moderately healthy trees (Figure 3-8). Assessing the differences between moderate (blue) and dead (brown) trees, more Agaricales and Leotiomycetes were identified in soils from moderate trees with no taxa more abundant in dead trees (Figure 3-9). Patterns that arose were that Leotiomycetes were associated with *A. solidipes*, healthy and moderate trees. Additionally, a greater abundance of *Mortierella* was associated with soils of *A.* solidipes and dead trees.

To determine which taxa were significantly different between each *Armillaria* species and tree health status, the (90% confidence) log fold change of OTUs was estimated (Figure 3-10). These analyses recognized that there was significantly greater abundance of *Mucor zonatus*,

Rhizopogon subbadius, Atheliaceae, and unclassified fungi in association with *A. altimontana*. No fungi were found significantly more abundant in association with *A. solidipes*. For healthy versus dead trees, there was a greater abundance of *Mortierella pseudozygospora*, *Penicillium humicoloides*, unclassified Leotiomycetes, and unclassified fungi associated with healthy trees. A greater abundance of *Geminibasidium* spp. and *Penicillium bialowiezense* were observed in dead trees (Figure 3-11). More *Mortierella* spp., *Umbelopsis* spp., Herpotrichiellaceae spp., unclassified Tremellomycetes, and unclassified fungi were associated with healthy trees, whereas for moderate trees, *Archaeorhizomyces* spp. and unclassified Ascomycota were more abundant (Figure 3-12). Comparing moderate and dead trees showed significantly more sequences were identified as *Cladophialophora chaetospira, Leohumicola* spp., unclassified Leotiomycetes, and unclassified fungi in moderate trees, while more *Metarhizium carneum, Mortierella* spp., and unclassified fungi were identified in dead trees (Figure 3-13).

3.4.3 Soil chemistry data analysis

To potentially identify soil characteristics that influenced fungal communities, soil chemistry data was used to help understand potential drivers for each group. Spearman's correlation was used to compare connections between fungal richness and diversity and soil chemical properties (Figure 3-14). Significant positive correlations were observed between fungal richness and diversity, whereas soil nitrogen had a slight positive correlation to Inverse Simpson's sample diversity (Table 3-2). While not correlated with fungal richness or diversity, carbon, nitrogen, organic matter, and moisture were highly correlated with each other (Table 3-2). Although not included within the correlation analyses, a linear model was established to identify if soil chemistry influenced *Armillaria* species and tree health (Table 3-2). Interestingly, since it was not correlated to any soil characteristics, pH was recognized as the most significant factor driving fungal communities with a relatively small range from 5.07 to 6.38 for all samples. Additionally, soil carbon, nitrogen and organic matter had a significant impact on pH levels (Table 3-3). Less influential factors on fungal communities included carbon, organic matter,

nitrogen, and moisture, yet they were removed from the final analysis due to poor to AIC values in the models.

Table 3-2: Determining significance of soil chemistry factors based on linear models to fit ANOVA. Richness and diversity indices were based on all samples combined.

	OM	Carbon	Nitrogen	Shannon	InvSimpson	Richness	Health	Species
Moisture	0.0015*	0.0065*	0.0003*	0.8445	0.5486	0.1334	0.4574	0.0888
Rock	-	-	-	0.2776	0.8394	0.0196*	0.9596	0.9538
Root	-	-	-	0.7795	0.6562	0.9349	0.9185	0.6607
Charcoal	-	-	-	0.9997	0.7136	0.7010	0.6229	0.5899
Other	-	-	-	0.8529	0.6384	0.4837	0.5475	0.4882
pH	0.0012*	<0.0001*	0.0433*	0.6288	0.6797	0.3887	0.7514	0.5480
Organic Matter	-	<0.0001*	<0.0001*	0.5364	0.3592	0.7329	0.3704	0.4167
Carbon	-	-	<0.0001*	0.2156	0.3639	0.3983	0.2374	0.7857
Nitrogen	-	<0.0001*	-	0.1073	0.0803	0.4708	0.1605	0.5875
Shannon	-	-	-	-	-	-	0.2183	0.6646
InvSimpson	-	-	-	-	-	-	0.1905	0.5050
Richness	-	-	-	-	-	-	0.2028	0.7579

* Significance was based on a p-value < 0.05

Table 3-3: Evaluating significance of soil chemistry properties to fungal microbial communities based on permANOVA permutation test.

	F	p-value
PH	3.4289	0.001*
Moisture	1.5348	0.025*
Nitrogen	1.5187	0.035*
Carbon	1.4593	0.015*
Organic Matter	1.3875	0.090
Charcoal	1.3055	0.110
Rock	1.1490	0.185
Other	1.0143	0.385
Root	0.7943	0.880
Species	0.5874	0.970
Health	0.9049	0.770

* Significance was based on a p-value < 0.05

3.5 Discussion

3.5.1 – Significant predictors to fungal communities

The goals of this study were to identify differences in soil fungal communities between trees associated with *A. altimontana* and *A. solidipes* and within healthy, moderately healthy and dead trees. No significant differences were observed in fungal taxa diversity or richness in soils, likely driven by having identified only three trees associated with *A. solidipes.* Comparing

among tree health status (healthy, moderate, and dead), although not significant, there was a clear distinction of fungal taxa associated with moderately healthy trees compared to dead trees, as observed in the 2-D ordination plot (Figure 3.3B). These results suggest that as a trees health declines from moderate to dead, there is an overall increase in richness and diversity of fungal communities. The significant differences, using a 90% log fold change, observed for taxa between moderate and dead trees, found that moderately healthy trees have a greater abundance of *Cladophialophora chaetospira, Leohumicola* spp., and Leotiomycetes, while dead trees have more *Metarhizium carneum* and *Mortierella* spp.

Fungal taxa that act primarily as decomposers and mycorrhizae were associated with trees in all health statuses. *Cladophialophora chateospira* is a common decomposer found in soils, which allows the release of nutrients to be accessible to trees (Badali et al., 2008). *Leohumicola* spp. have a strong relationship with diseased roots known to be associated with trees as they decline in health (Xu et al., 2012). Leotiomycetes are mycorrhizae associated with lower pH soils and are thought to provide additional nutrients to trees in moderate health (Sterkenburg et al., 2015). It is likely that these taxa have relationships that may be beneficial to a tree's health. *Mortierella* spp. were found in high abundance in soils of dead trees, which could be due to their ability to act as saprophytes to decompose dead tissue (Toju and Sato, 2018). In addition, *Mortierella* are the most abundant fungi in all soils. Saprophytic fungi assist in the decomposition of dead tissue by subsequently increasing nutrients for healthy and moderate trees, while providing resources for upcoming seedlings or understory vegetation in association with dead trees.

The soil characteristic with the greatest impact on fungal communities was pH, which is surprising as soil fungi are generally known to be able to withstand a wide range of pH (Rousk et el., 2010). The large influence of soil pH on fungal communities was most likely due to the significant effects carbon, nitrogen, and organic matter. Coniferous forest litter has a high C:N ratio, which constitutes to a slower decomposition of more recalcitrant (higher lignin) litter. Soils

with a higher pH are typically able to breakdown litter more rapidly, which releases more nutrients into the soil and provide an increase to organic matter (Finzi et al., 1998). The pH ranged from 5.07 to 6.36. At this range, there is an increased rate of forest litter decomposition with the higher concentrations of saprophytes within the soil (Finzi et al., 1998). Additionally, moisture significantly affected soil carbon, nitrogen, and organic matter levels. The effects of moisture on soil chemistry manifests through cyclic drying and rewetting of soil. Fierer and Schimel (2002), showed that as soils were subject to drying and rewetting cycles, soil organic matter and carbon were prone to increase, while nitrogen decreased due to increased nitrification and leaching. In combination, soil pH and moisture may have an indirect relationship to fungal diversity and increased tree health.

3.5.2 Functions of fungal taxa associated with Armillaria species

While no overall differences were observed between fungal communities in soils associated with *Armillaria* species, some taxa were more abundant in each category. Seven out of the 17 most abundant taxa were more abundant in soils associated with *A. altimontana* than with *A. solidipes*. Six of these taxa have been shown to increase soil productivity through multiple functions as ectomycorrhizal fungi (Atheliaceae, Cortinariacaeae, Helotiales, and Rhizopogonaceae [Balestrini et al., 2015; Horton et al., 2013; Kipfer et el., 2010; Rudawska et al., 2011]), antagonists (Hypocreaceae [*Trichoderma*] [Reaves et al., 1990]), or mycoparasites (Puccinomycotina [Aime et al., 2014]). More specifically, two identifiable genera were significantly abundant in soils associated with *A. altimontana* compared to *A. solidipes.* Although there is no extensive research regarding *Mucor zonatus*, *Mucorales* may act as an antagonist to pathogens of pea plants *(Pisum sativa*) within the microbial inoculant “Effective Microorganisms” ™ (EMRO, Okinawa, Japan)*,* including *Fusarium*, *Rhizoctonia*, and *Botrytis*, indicating there may be the potential to inhibit *A. solidipes* (Okorski and Majchrzak, 2007). Additionally, *Rhizopogon* spp. may be ectomycorrhizal,, allowing increased uptake of water and nutrients to enhance tree defenses to pathogenic root diseases (Leake et al., 2004). The functions of these

fungi suggest that these fungal communities increase the overall health of the stand, corroborating Warwell et al. (2019), who found that trees associated with *A. altimontana* were larger in both diameter and height than trees not associated with *Armillaria*.

Although not significant, observed differences in fungal taxa within the heat map analyses (Figures 3-6 & 3-7) showed that soils associated with *A. solidipes* and dead trees have a higher abundance of *Mortierella* than soils in conjunction with *A. altimontana* and healthy trees. As stated above, the saprophytic ability of *Mortierella* may assist in the breakdown of dead tissue (Toju and Sato, 2018). This could result in greater mineralization of carbon to build biomass and immobilization of nutrients to facilitate seedling establishment (Allen et al., 1995).

Healthy trees may utilize mycorrhizae to increase the uptake of nutrients, yet mycorrhizae may be outcompeted by saprophytes in conjunction with dead trees to initiate the decomposition of dead roots (Allen et al., 1995). Though not observed in this study because of sample sizes, similar trends could occur in soils associated with *A. altimontana* and *A. solidipes*, with increased mycorrhizae related to *A. altimontana* and increased decomposition with *A. solidipes*. Since neither *Armillaria* species nor tree health may be driving the fungal communities, the ability to understand soil chemistry may be vital to the presence of fungal biocontrols.

3.5.3 Role of soil chemistry in fungal communities

The role of pH as the greatest influence on fungal communities may be convoluted since our analyses did not include soil bacteria. Fungi can live within larger ranges of soil pH compared to bacteria, thus pH has less of an influence on fungi than on bacteria (Rousk et el., 2010). In our samples soil pH ranged from 5.07 to 6.38. This narrow range of pH, likely allows a wide variety of fungi including mycorrhizae and saprotrophs to thrive within the soils, providing a greater diversity and richness within our samples. According to Rousk et al. (2009), the optimum pH for fungal growth is 4.5, which is well below levels observed in our samples. As pH increases there is less fungal growth and greater bacterial growth, therefore as our samples increase from

a pH of 5 to over 6, there is a probability that bacterial communities will have a greater influence than fungal communities (Rousk et al., 2009). Incorporating bacterial community analysis may enhance our ability to see changes related to pH.

Additionally, soil pH was significantly influenced by soil carbon, nitrogen, and organic matter, while moisture significantly influenced soil carbon, nitrogen, and organic matter, acting as indicators to overall health in the stand. Carbon catalyzes soil fungal communities, especially saprophytes to breakdown woody organic material in the soil (Baldrian, 2017). In terms of carbon cycling, as diversity of microbes increase in the soils, the breakdown of organic carbon will result (Morris and Blackwood, 2014). This could indicate that if more carbon is present in the soil, there are likely more highly diverse communities driving that flux in carbon. As labile nutrients are released into the soils, trees are able to uptake these inorganic molecules to improve their structure and withstand stress (Baldrian, 2017; Morris and Blackwood, 2014). This process allows soil properties to directly influence the abundance and diversity of fungal communities (Baldrian, 2017). Soil microbes, specifically mycorrhizae, increase the ability for the soil water and nutrients to be used by plants, sparking photosynthesis (Morris and Blackwood, 2014). Soil moisture allows for an increase in the decomposition of organic matter to release important nutrients (Morris and Blackwood, 2014). The presence of fungi that decompose and assist in the uptake of nutrients could allow for nutrient cycling to actively persist on the site.

3.5.4 Potential fungal microbes as biocontrols

We observed that Hypocreaceae (*Trichoderma*), known biocontrol species, were more abundant in soils associated with *A. altimontana* and healthy trees, compared to soils associated with *A. solidipes* and dead trees. *Trichoderma,* known for its biocontrol properties, has been documented to inhibit the growth of *Armillaria* (Raziq and Fox, 2005; Reaves et al., 1990). Although not significant, likely due to our limited sample size, we found 13 OTUs belonging to *Trichoderma*. Most were found in low numbers within samples associated with *A.*

altimontana, yet the most abundant *Trichoderma* spp. was observed in samples associated with both *A. solidipes* and *A. altimontana*. A closer examination of the three samples associated with *A. solidipes* identified that the lone tree associated only to *A. solidipes* did not have the *Trichoderma* species present. Yet, the two samples with both species of *Armillaria* had a high presence of *Trichoderma*. Albeit a small sample size, this corresponds with the idea that *A. altimontana* may be antagonistic toward *A. solidipes* (Warwell et al., 2019). Overall, 85% (33 trees) of the soils associated with *A. altimontana* had *Trichoderma* within the fungal communities. Further research into a mutualistic relationship between *Trichoderma* and *A. altimontana* may show that the presence of *A. altimontana* may assist in the inhibition of *A. solidipes*.

Utilizing fungal communities to assist in the management of Armillaria root disease may be key to minimizing potential damage to residual trees caused by silvicultural management practices. Beneficial microbes can minimize inoculum loads by inhibiting the pathogen from infecting susceptible hosts (Kile et al., 1991). In this study, a greater diversity of mycorrhizae and saprophytic fungi was observed in association to *A. altimontana* and healthy/moderate trees, demonstrating that mycorrhizae may have a direct influence on hosts within forested environments associated with *Armillaria* species (Balestrini et al., 2015).

3.5.5 Limitations of the study

Selecting trees to sample *Armillaria* species was the greatest limiting factor. The small subsample of *A. solidipes* infected trees is an inherent struggle from taking field samples, as it was difficult to assess *Armillaria* infections under the soil. Sampling each of the remaining ~600 trees may have enhanced our study by increasing the sample size for each species and would have updated the last inventory from 1987. We successfully extracted adequate DNA from 90% of the samples. The ability to collect more than two soil samples for each tree would have allowed for more attempts at extracting quality DNA. As for rhizomorph collections, we only successfully collected rhizomorphs for 78% of the trees. The ability to expand our excavation

methods to collect rhizomorphs could have greatly increased our sample size. This was the largest restriction in our field sampling. Additionally, the utilization of metatranscriptomics could have bolstered our understanding of what fungal microbes are present and their functions within the soils. This could assist in our understanding of what may be occurring as trees transition from healthy to dead and the association between either *Armillaria* species.

3.6 Conclusion

The low sample size of *A. solidipes* greatly decreased our ability to find significant fungal community differences between *Armillaria* species, yet I did observe a slight shift of soil fungi among tree health. Although not significant, microbial community changes were detected as trees transition from moderately healthy to dead. The most abundant fungal microbes associated with all trees were mycorrhizae and saprophytes facilitating to increase the health of trees and decompose dead tissue to release vital nutrients to associative trees. A major finding was that soil properties, specifically pH, carbon, nitrogen, organic matter, and moisture, may indirectly impact the overall abundance of soil fungi.

Since this study did not identify significant suppressive fungal communities, such as *Trichoderma* and *Hypholoma*, although observed at slightly higher levels in association with *A. altimontana* and healthy trees, further research is needed to understand if fungal (or potentially bacterial) communities change in the presence of soil root pathogens and/or though changing tree health status. Additionally population dynamics between *A. altimontana* and *A. solidipes* may provide further knowledge into the relationship between highly virulent and less virulent fungi. Since *A. altimontana* occupies more space at the Ida Creek field site than *A. solidipes*, it may seem that this less virulent species has a competitive advantage over the virulent species at this site. Recognizing how microbes change over time, as infection or inhibition progresses, may give a greater insight into ways to minimize the effects of *Armillaria*. We further propose to use network analysis and machine learning approaches to identify microbial groups with positive

and negative correlations with *Armillaria* species and tree health. This will allow for targeted culturing of beneficial microbes that can be developed as suitable bioinoculants to manage Armillaria root disease.

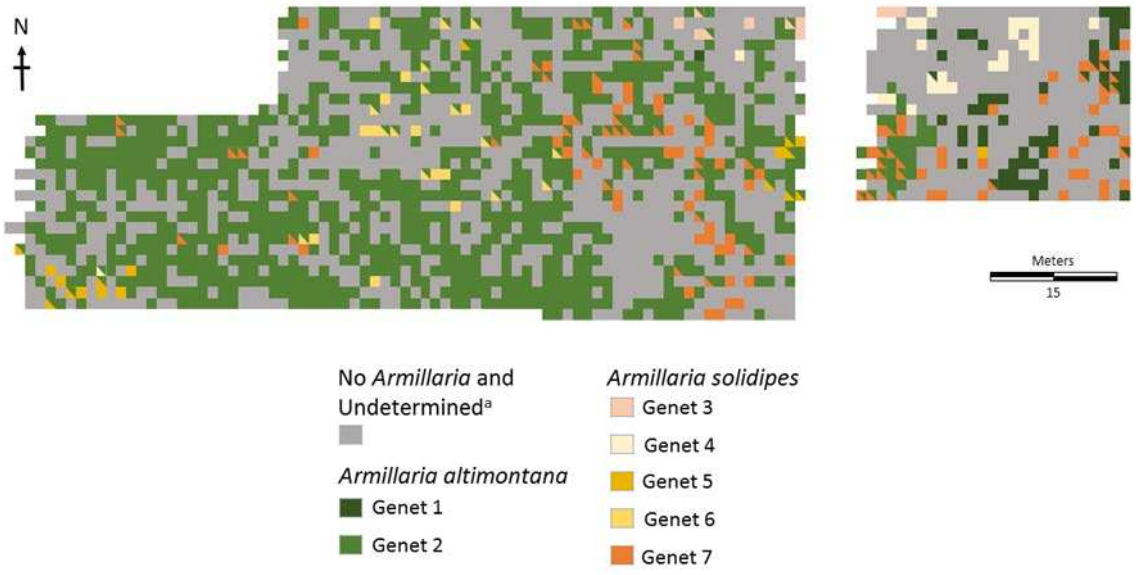

Figure 3-1: *Armillaria* species distribution with Ida Creek field site at the Priest River Experimental Forest (PREF). Pixels represent individual trees with colors representing the association between *Armillaria altimontana* or *A. solidipes* . Split pixels represent trees that were associated with both *A. alitmontana* and *A. solidipes* (Warwell et al., 2019).

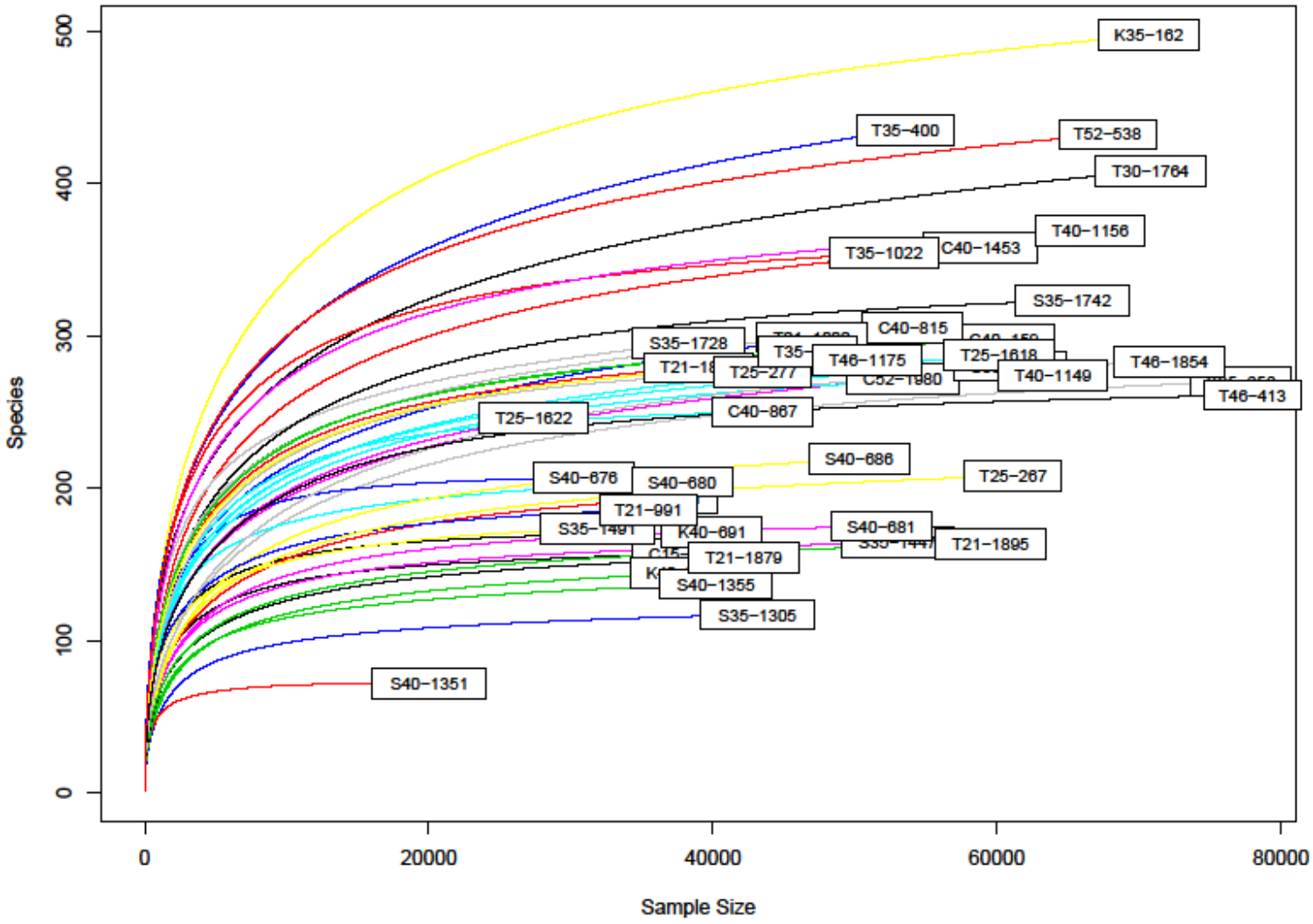

Figure 3-2: Rarefaction curve for all 42 sequenced samples.

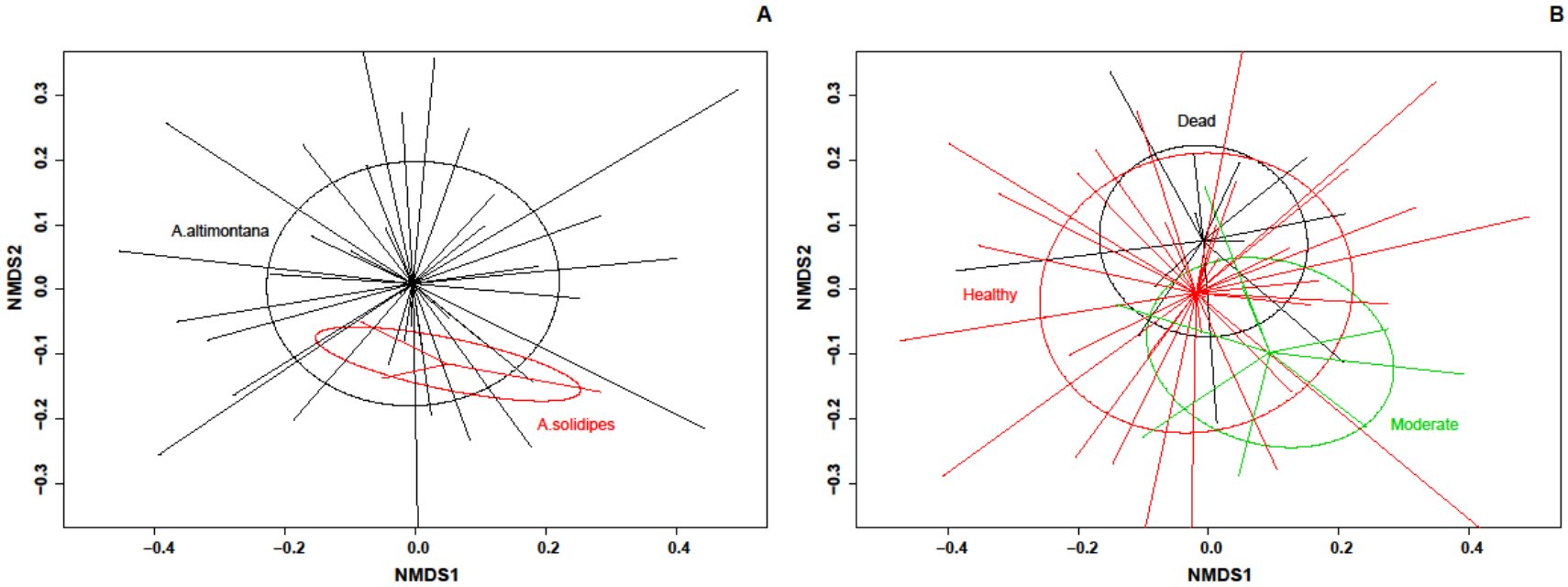

Figure 3-3: Non-metric multidimensional scaling plot to determine dissimilarity of fungal microbial communities associated between: A) *A. altimontana* and *A. solidipes*, B) Tree health status.

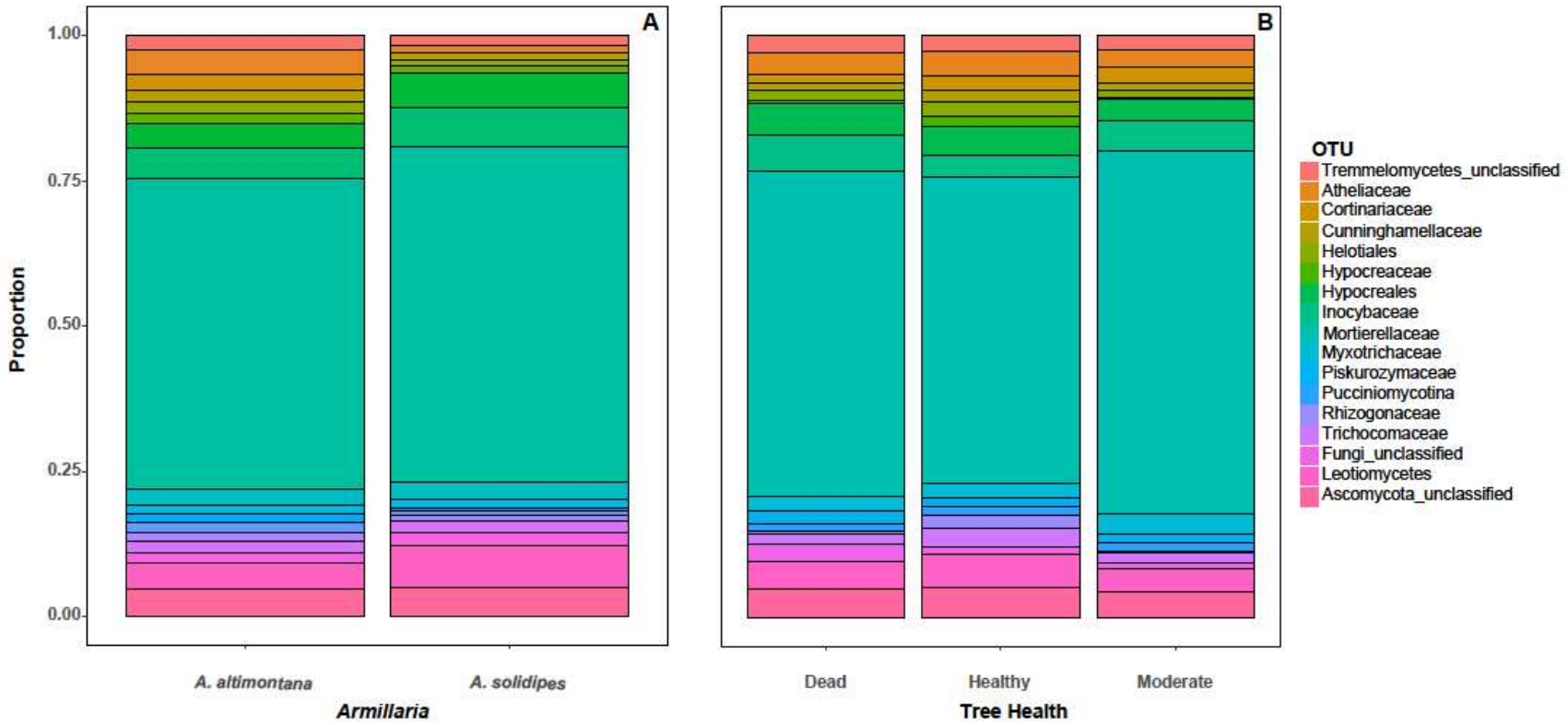

Figure 3-4: Stacked bar graphs of top 17 most abundant fungal taxa for: A) *A. altimontana* and *A. solidipes* and B) tree health status.

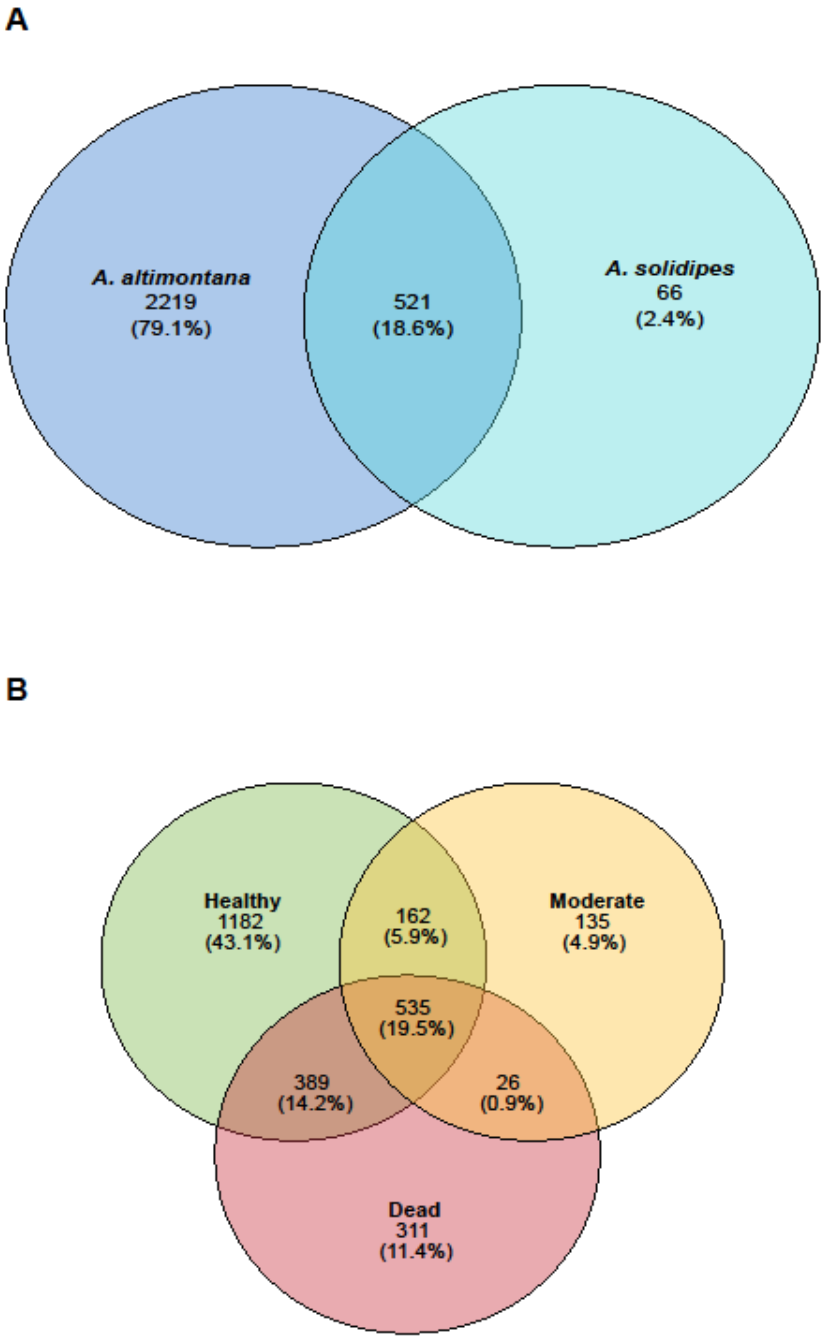

Figure 3-5: A) Microbial communities (OTUs) between *A. altimontana* and *A. solidipes*. Core microbiome encompasses overlap between both species, while unique OTUs occur within each circle. B) Microbial communities associated to tree health status (Healthy, moderate, and dead). Core microbiome encompasses overlap all three groups, while interacting OTUs occur between two groups. Unique OTUs occur within each of the three circles.

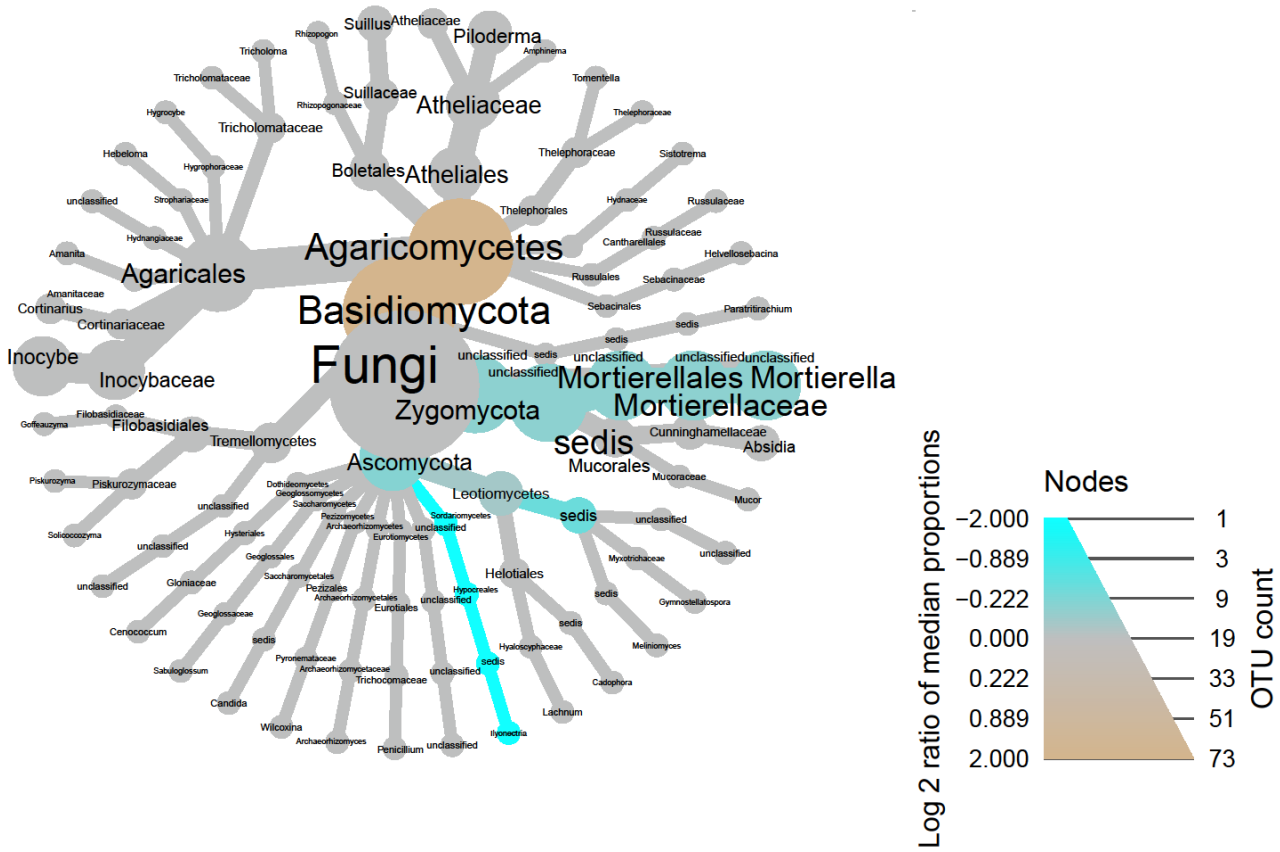

Figure 3-6: Heat tree to compare microbial communities between *A. altimontana* (brown) and *A. solidipes* (blue). Overall abundance is calculated to determine the Log2 ratio of median proportions for each microbe to determine differences.

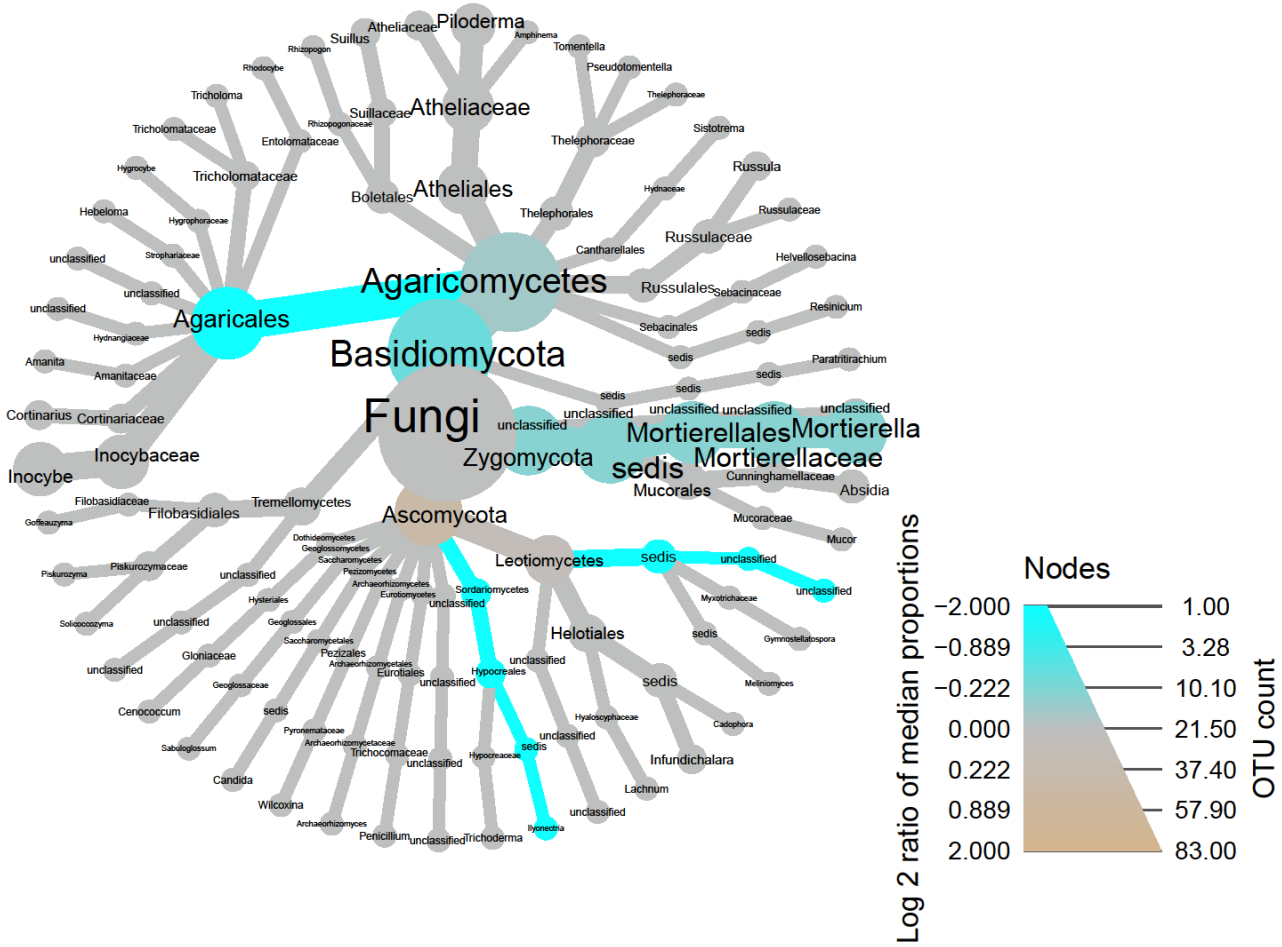

Figure 3-7: Heat tree to compare microbial communities between healthy trees (brown) and dead trees (blue). Overall abundance is calculated to determine the Log2 ratio of median proportions for each microbe to determine differences.

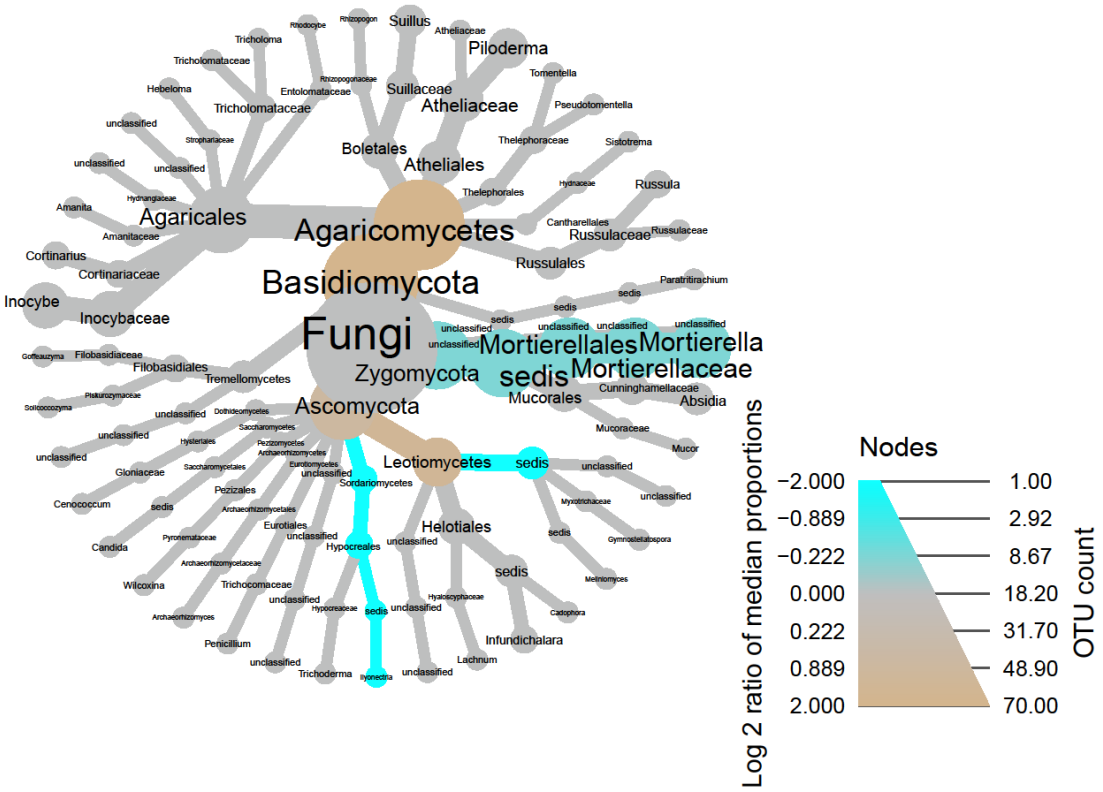

Figure 3-8: Heat tree to compare microbial communities between healthy trees (brown) and moderate trees (blue). Overall abundance is calculated to determine the Log2 ratio of median proportions for each microbe to determine differences.

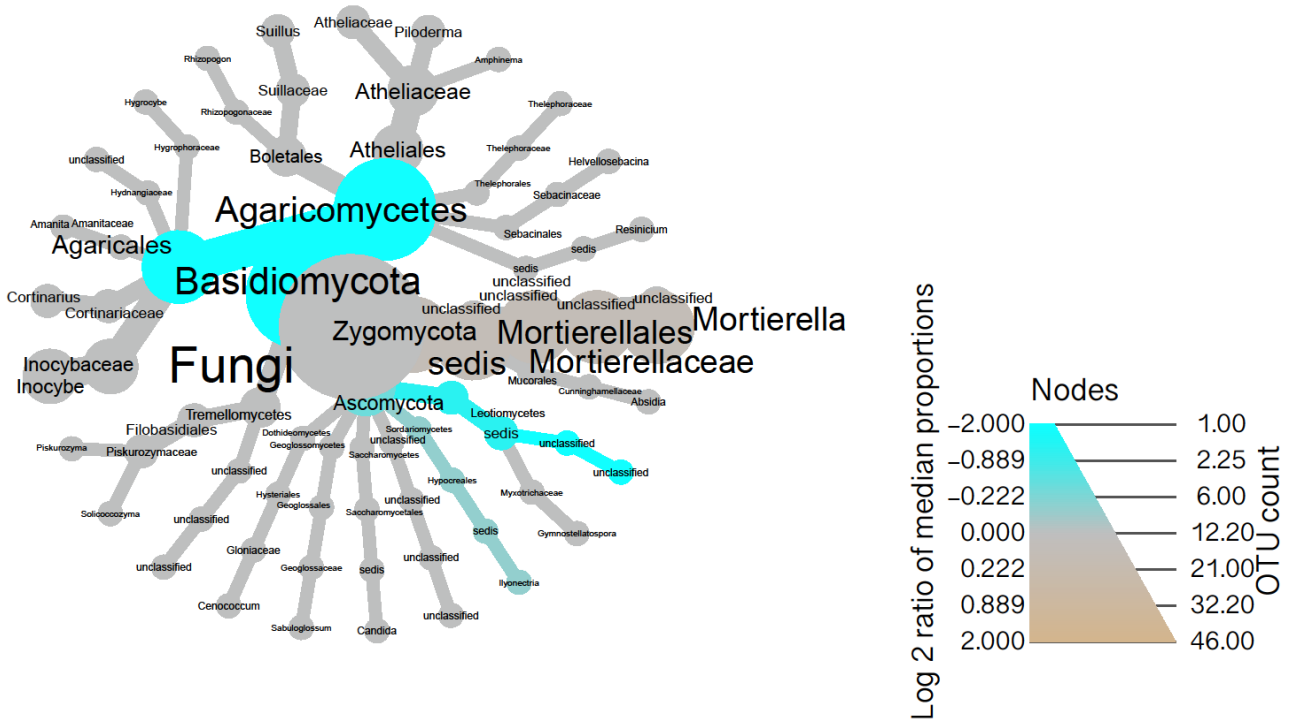

Figure 3-9: Heat tree to compare microbial communities between dead trees (brown) and moderate trees (blue). Overall abundance is calculated to determine the Log2 ratio of median proportions for each microbe to determine differences.

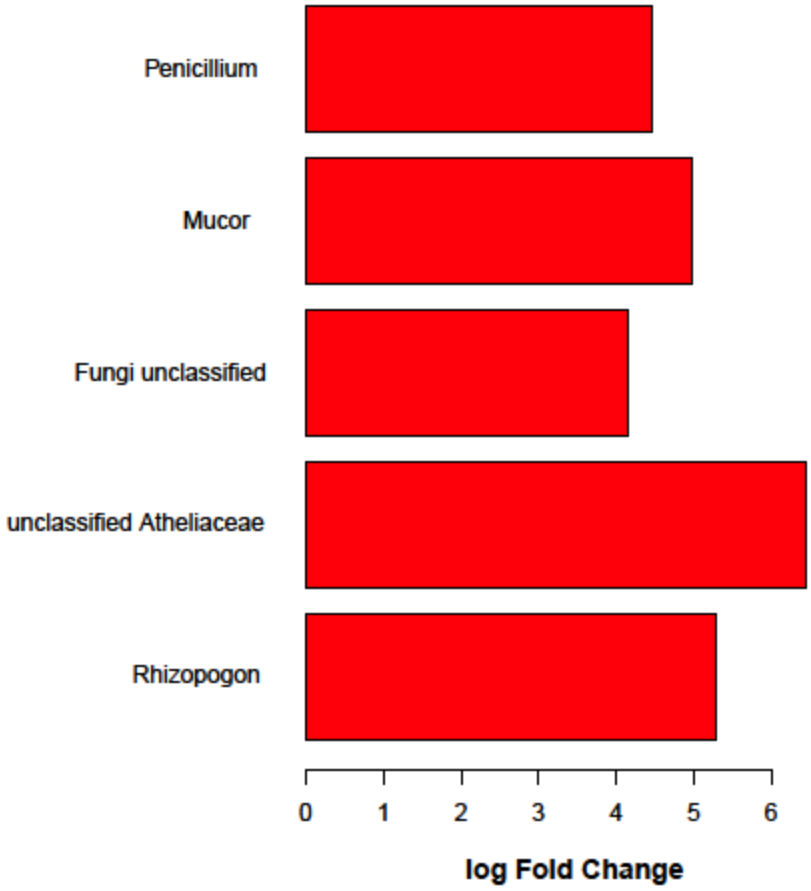

Figure 3-10: Log fold change for unique OTUs in association between *A. altimontana* (red) and *A. solidipes* (no observations). Significance is based on 90% confidence log Fold change between both species of *Armillaria*, with difference displayed for *A. altimontana*.

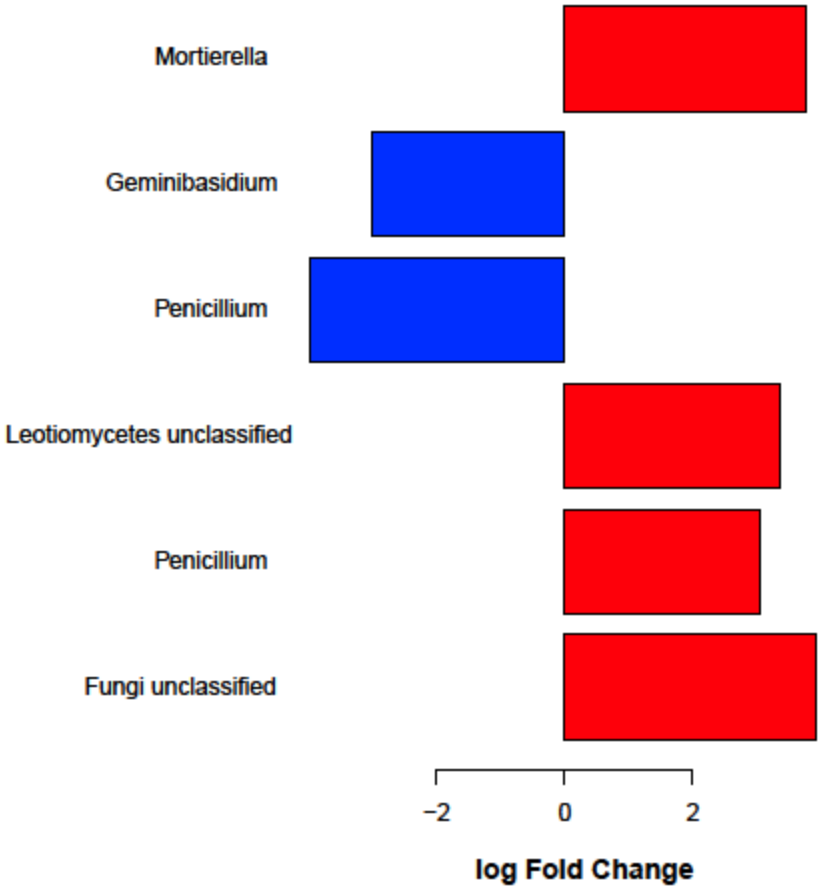

Figure 3-11: Log fold change for unique OTUs in association of tree health: healthy (red) and dead (blue). Significance is based on 90% confidence log Fold change between each status of tree health with differences portrayed for both healthy and dead trees.

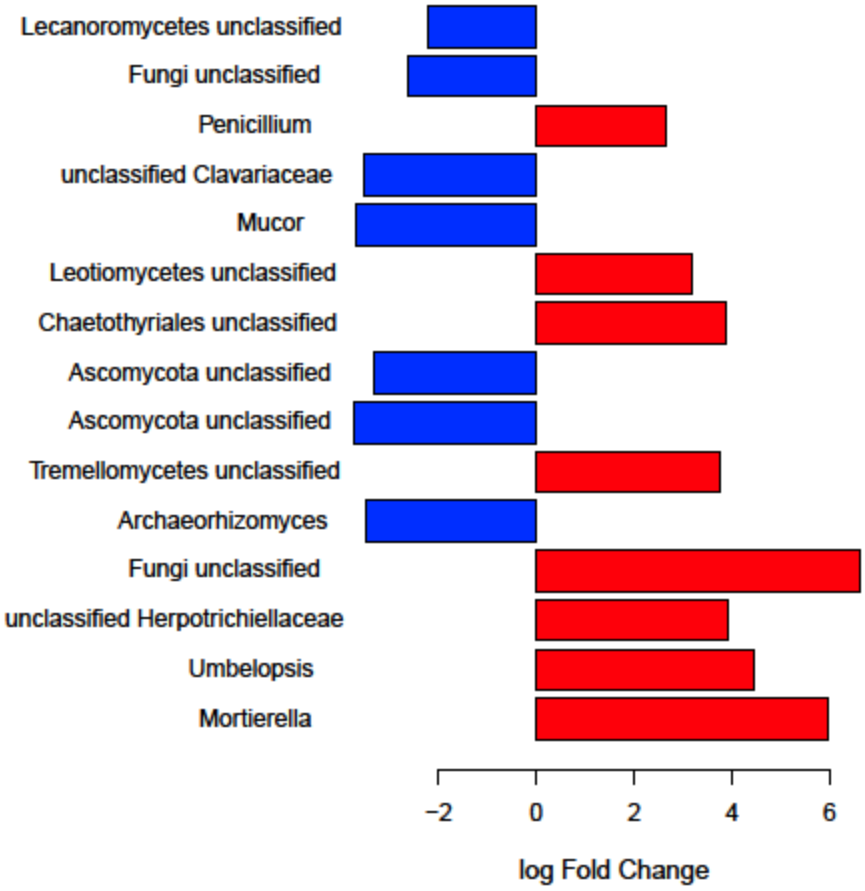

Figure 3-12: Log fold change for unique OTUs in association of tree health: healthy (red) and moderate (blue). Significance is based on 90% confidence log Fold change between each status of tree health with differences portrayed for both healthy and moderate trees.

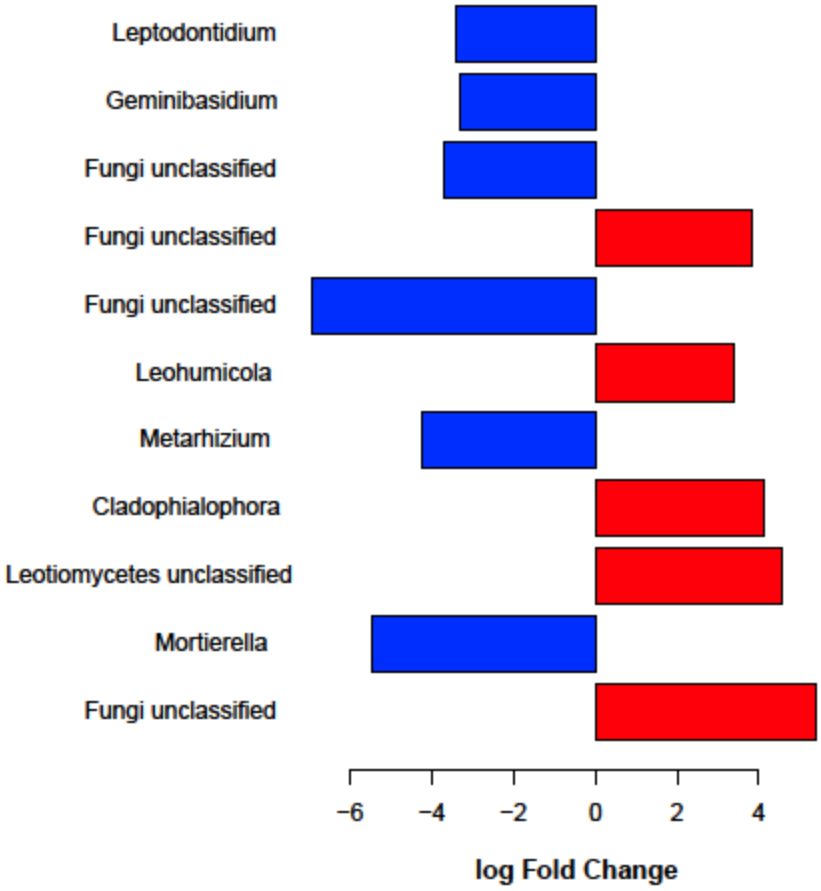

Figure 3-13: Log fold change for unique OTUs in association of tree health: moderate (red) and dead (blue). Significance is based on 90% confidence log Fold change between each status of tree health with differences portrayed for both moderate and dead trees.

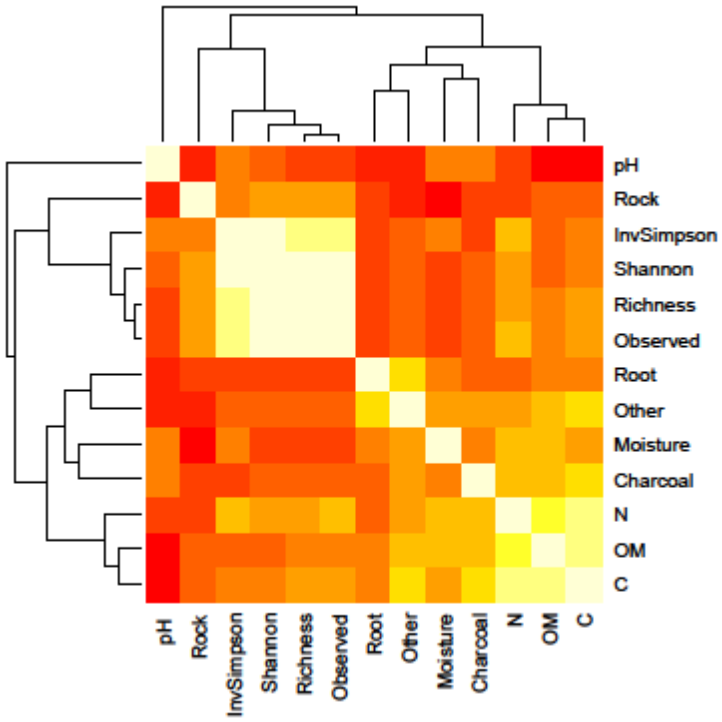

Figure 3-14: Spearman correlation for soil chemistry properties and overall soil microbial richness and diversity indices. Correlations are identified using light colors (white/yellow), while no correlations is presented with dark colors (red/orange). The graph shows that richness, Shannon diversity, and InvSimpson diversity are correlated to each. The only soil property correlated to either richness or diversity is soil nitrogen, which is slightly correlated to the InvSimpson diversity index. For all soil properties, nitrogen, carbon, and organic matter are highly correlated with slight correlations with moisture and charcoal content.

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CHAPTER 4: SUMMARY AND CONCLUSIONS

Coniferous forests within the western United States have endured detrimental effects to overall health due to multiple abiotic and biotic factors, including changing climates, site and stand characteristics, and the presence of insect and disease populations. Climate models indicate that future temperature trends are hypothesized to be warmer and drier, subsequently causing an increase in drought duration and intensity (Allen et al., 2010). In Colorado, average annual temperature anomalies and annual water deficits have increased within the last year thirty years inducing stress to forests (CSFS, 2019; Smith et al., 2015). Climatic effects may induce an increased probability of mortality, specifically within high elevation forest that are frequently located in a cool, wet environment (Reich et al., 2016). Climate may also induce changes to pests and pathogens, whereas drought intensity increases the likelihood for higher plant damage by bark beetles and secondary pathogens within forests (Kolb et al., 2016). Assessing the direct and indirect effects of how abiotic and biotic factors will influence our forests may allow land managers to mitigate impacts within forested areas.

To assess the effects that abiotic and biotic factors had on the presence of subalpine fir mortality in Colorado, roadside surveys and stand health monitoring plots were established. The stand health monitoring plots showed that the most relevant factors to subalpine fir mortality are stand density and the presence of *D. confusus*. I identified that stand density, elevation, and *Armillaria* spp. were the greatest influences on the presence of *D. confusus*, while the largest influences on the presence of *Armillaria* spp. are warmer maximum summer temperatures and increased slope percentage. My data indicated that both *D. confusus* and *Armillaria* spp. subsequently effected subalpine fir mortality, therefore, I can conclude that an increase in summer temperatures and higher stand densities indirectly influence subalpine fir mortality in Colorado's national forests (Figure 4-1). Therefore, higher density and increases in summer

temperatures may have induced stress to trees increasing the likelihood of *Armillaria* spp. and *D. confusus*, subsequently leading to subalpine fir mortality.

Reduced levels of subalpine fir mortality from 122,000 new acres in 2014 to 25,000 new acres in 2018 indicates that the combination of abiotic and biotic factors, including climate, stand characteristics, and insects and disease may have sanitized maladapted trees making stands healthier and less dense. As climate models project changes to even warmer and drier high elevation forests, however, areas affected by subalpine fir decline may increase again due to elevated stress and a greater presence of biotic factors. Managing forests, via thinning to increase vigor, to minimize predisposing and inciting factors may help reduce the risk of each contributing factor.

Due to multiple dispersal methods, Armillaria root disease is difficult to manage in a forested setting. Since fungal biocontrols have been used to assist in the management, my samples were collected with the presence of two species of *Armillaria*, to understand the dynamics of soil fungal communities in association to multiple fungal root diseases. The goals of this study were to identify differences in soil fungal communities between trees associated with *A. altimontana* (non-pathogenic) and *A. solidipes* (highly virulent) and among healthy, moderately healthy and dead trees. No significant differences were observed in fungal taxa diversity or richness in soils, likely caused by low samples sizes (I only identified three trees associated with *A. solidipes*). Comparing among tree health status (healthy, moderate, and dead), although not significant, there was a clear distinction of fungal taxa associated with moderately healthy trees compared to dead trees. These results suggest that as a trees health declines from moderate to dead, there is an overall increase in richness and diversity of fungal communities. Although not significant, Hypocreaceae (*Trichoderma*) was more abundant in soils associated with *A. altimontana* and healthy trees.

Mortierella were the most abundant fungi in soils associated with all treatments, which act as saprophytes to decompose dead tissue (Toju and Sato, 2018). Saprophytic fungi assist in

the decomposition of dead tissue by subsequently increasing nutrients for healthy and moderate trees, while providing resources for other remaining trees, new regeneration, or understory vegetation in association with dead trees. Additionally, mycorrhizae fungi were found within all treatments. Mycorrhizae may assist in the uptakes of nutrients for living trees and may act as a conduit to flush nutrients from declining trees to healthier trees as they die. Therefore, mycorrhizae may act to increase the overall health of the forest even with the presence of dead trees within a stand.

The soil characteristic with the greatest impact on fungal communities was pH, which is surprising since soil fungi are generally known to be able to withstand a wide range of pH (Rousk et el., 2010). The large influence of soil pH on fungal communities is most likely due to the significant effects carbon, nitrogen, and organic matter has on soil pH. Coniferous forest litter has a higher C:N ratio, which constitutes to a slower decomposition of more recalcitrant (higher lignin) litter. Soils with a higher pH are typically able to breakdown litter more rapidly, which releases more nutrients into the soil and provides an increase to organic matter (Finzi et al., 1998). The pH ranged from 5.07 to 6.36. At this range, there is an increased rate of forest litter decomposition with the higher concentrations of saprophytes within the soil (Finzi et al., 1998). Additionally, moisture significantly affected soil carbon, nitrogen, and organic matter levels. In combination, soil pH and moisture may have an indirect relationship to fungal diversity and increased tree health.

The implications of this study infer that there is a potential for suppressive soils with the use of *Trichoderma* spp. due to the greater abundance in soils associated with *A. altimontana* and healthy trees. This may indicate that novel approaches could be developed for managing Armillaria root disease. Additionally, future research observing microbial communities as *Armillaria* spp. establishes in association to trees may provide insight as to how microbes adapt overtime in conjunction with root disease.

The impact of changing climate regimes will inevitably induce stress to forests. This stress will cause further risk of due to biotic agents such as bark beetles and secondary pathogens, such as Armillaria root disease in association with subalpine fir decline. Understanding what microorganisms are present within soil will help determine the primary drivers of overall plant health in association with Armillaria root disease, while connecting these belowground processes with the above ground forest ecology to improve forest management techniques.

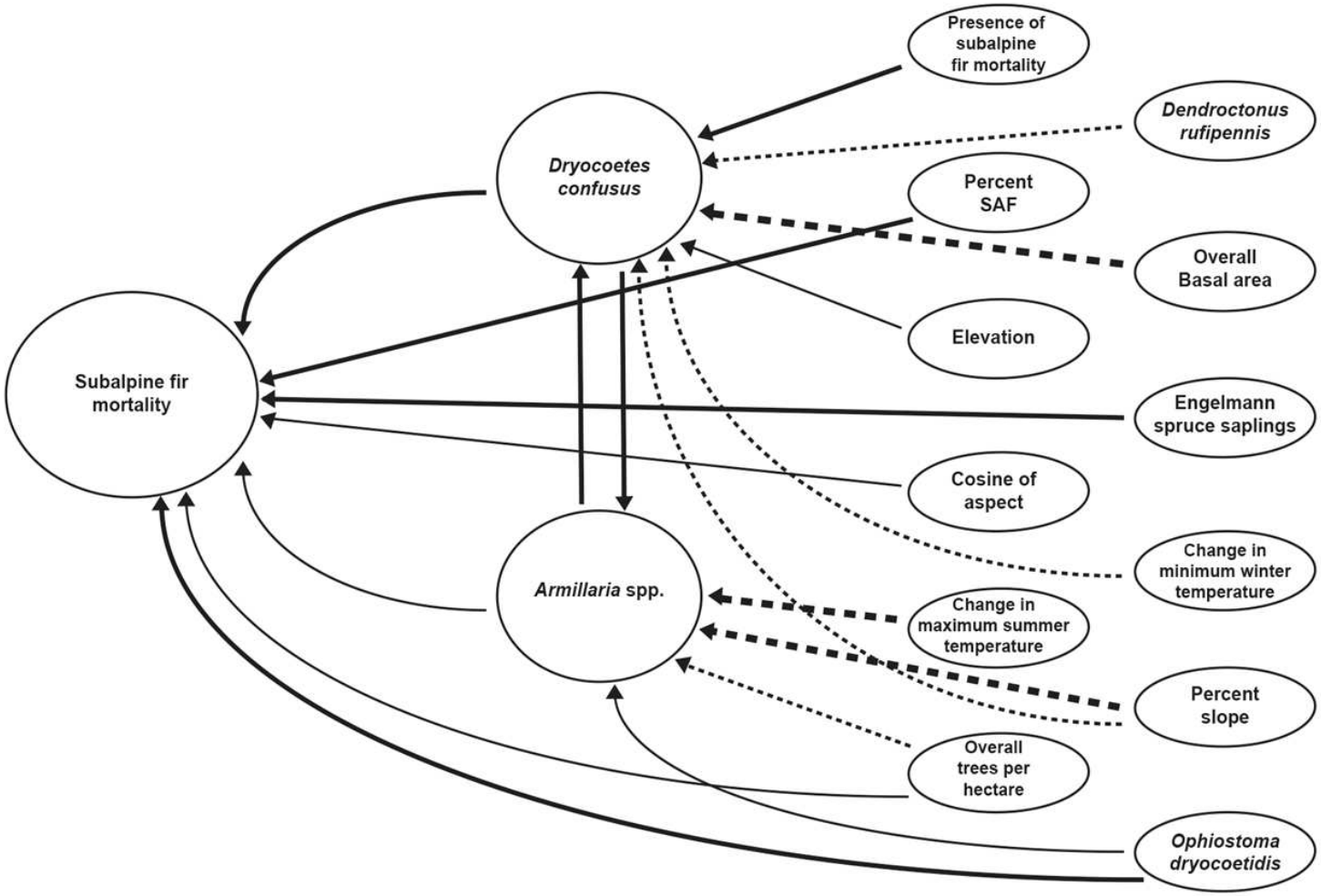

Figure 4-1: A conceptual model of direct and indirect factors of subalpine fir (SAF) mortality based on logistic regressions for the presence of subalpine fir mortality, *Dryocoetes confusus* and *Armillaria* spp. Direct factors are displayed with solid lines, while indirect factors are dashed lines. Significant factors are represented with a thick line with non-significant factors with thin lines. This identifies that biotic factors (*D. confusus, Armillaria* spp., and *O. dryocoetidis*) direct influence SAF mortality, whereas climate (change in max. summer temperatures) and stand density (basal area and trees per hectare) have an indirect influence on SAF mortality.

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