UNIVERSITY OF WISCONSIN-LA CROSSE
Graduate Studies

ENDOPHYTIC FUNGAL DIVERSITY IN WHITEBARK PINE: LINKS BETWEEN SPECIES ASSEMBLAGE, BIOGEOGRAPHY, AND BLISTER RUST OCCURRENCE

A Chapter Style Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science

Dylan J. Baldassari

College of Science and Health
Biology

January 2022

ENDOPHYTIC FUNGAL DIVERSITY IN WHITEBARK PINE:

LINKS BETWEEN SPECIES ASSEMBLAGE, BIOGEOGRAPHY,

AND BLISTER RUST OCCURRENCE

By Dylan J. Baldassari

We recommend acceptance of this thesis in partial fulfillment of the candidate's requirements for the degree of Master of Science in Biology.

The candidate has completed the oral defense of the thesis.

Tom Volk, Ph.D. Date
Thesis Committee Co-Chairperson

Todd Osmundson, Ph.D. Date
Thesis Committee Co-Chairperson
On behalf of the committee members named below:

Meredith Thomsen, Ph.D.

Cathy Cripps, Ph.D.

Thesis accepted

Meredith Thomsen, Ph.D. Date
Dean of Graduate & Extended Learning

ABSTRACT

Baldassari, D.J. Endophytic fungal diversity in whitebark pine: links between species assemblage, biogeography, and blister rust occurrence. MS in Biology, January 2022, 141 pp. (T. Volk)

Whitebark pine is a threatened, keystone and foundational species in the northern Rocky Mountains that occurs in a narrow elevational zone and has experienced 40-90% population declines. These declines are due to an invasive fungal disease (white pine blister rust), mountain pine beetle, shifting fire regimes, and climate change. In this study I examined the foliar endophytic fungal communities of whitebark pine across three environmental gradients: mountain range isolation, disease stage, and elevational driven ecotype. Additionally, I examined the host specificity of these communities to another co-dominant subalpine conifer. Our results show that community composition similarity (beta diversity) was significantly driven by study site, disease stage, and ecotype. I found that species richness was greatest at the lowest elevations sampled in this study and that community composition varied significantly between the lowest elevation sites and the highest elevation sites. Results of the study suggest that these communities are highly host specific, and that the loss of whitebark pine will likely lead to the loss of its foliar fungal symbionts. Additionally, species' occurrence patterns and diversity trends of these communities suggest that elevational driven ecotype is a greater driver of community assemblage than mountain range isolation or disease infection state.

ACKNOWLEDGMENTS

There are many individuals who I consider essential to the success and completion of this project, and to all of whom I am very grateful. I am grateful for my committee, Tom Volk, Todd Osmundson, Meredith Thomsen, and Cathy Cripps. Thank you, Tom, for giving me the opportunity to work on a master's under your guidance, catalyzing a passion for all things fungi, and for continued encouragement and support. Thank you, Todd, for the many lessons in genetics, ecology, and life; without your insights and knowledge this work would look far different. Thank you, Meredith, for your support and advice throughout, as well as instruction in field ecology, writing, and presentation. Thank you, Cathy, without whom I would likely still be in the Rocky Mountains moving boulders to access whitebark pine mycorrhizae; your knowledge of whitebark pine, Rocky Mountain ecosystems, and fungi in general has been invaluable. Furthermore, your feedback during the editing process was impressive and sets a high bar for me going forward. Prior to this project I had the honor of working with and sharing many conversations with Roger Ruess, Barrett Klein, and Calvin Heslop; thank you all for instruction, advice, and your contagious passion for science.

There are many others who contributed significantly to this project whom I would like to thank. In the field, Chance Noffsinger and Holly Kritter sacrificed their time and energy to assist in sampling, thank you both. Thank you to Liz Dominguez who secured additional funding to sequence Engelmann spruce endophytes and for being a valuable lab mate during the DNA extraction process. Thank you, Ehren Moler, for the timely advice and information regarding DNA extraction and PCR protocols. I would also like to thank Annie Schauster and Thomas Roehl, whose knowledge of macrofungi far

surpasses mine, and from whom I learned much. Thank you to Jeff Henderson and Caleb Kasper, who added color to my life as a graduate student via constant, successful requests to hunt, fish, and forage. Thank you to my brothers Dugan and Dustan, and my uncle Jim for laying the groundwork in natural history and for dragging me into the woods during my youth, leaving me cold, wet, and alone to ruminate on the natural world. Thank you to my parents who encouraged me to pursue an education in the natural sciences and who have been supportive throughout the many ups and downs that compose a life well lived. Thank you to my grandmother Diane, who I know would have loved to see me graduate and who encouraged and supported me in pursuing higher education. I would also like to thank the generous institutions that funded this work, including the UW-La Crosse Graduate Studies Research Service Education and Leadership Program, the Oregon Mycological Society Scholarship, and the Sonoma County Mycological Association Scholarship.

TABLE OF CONTENTS

PAGE

LIST OF FIGURES

FIGURE PAGE

LIST OF APPENDICES

CHAPTER I

WHITEBARK PINE ECOLOGY, ENDOPHYTES, AND PATHOGENS

Introduction

The Whitebark Pine Ecosystem

Whitebark pine (*Pinus albicaulis*) is both a keystone and a foundational species at high elevations in the northern Rocky Mountains (McCaughey & Schmidt, 2001; Resler & Tomback, 2008). Since whitebark pine is more tolerant of treeline conditions than other species, it has been proposed that whitebark is particularly important for establishing and expanding subalpine forests (Resler, 2004). The establishment of a single whitebark pine creates a microclimate that mitigates the harshness of wind and temperature fluctuations found in these areas (Resler, 2004). This microclimate fosters establishment of a variety of alpine forbs and subalpine forbs, shrubs, and trees, catalyzing a feedback loop that allows many other plant species as well as birds and mammals to establish and reside. Additionally, whitebark pine effects hydrological processes at the landscape scale; by redistributing snowpack, acting as a snow trap, and providing shade, whitebark pine extends snowmelt discharge and stream flow over the summer (Tomback *et al*., 2016). It is for these reasons that whitebark pine is considered a foundational species, as it plays an imperative role in structuring the plant communities of the subalpine.

Whitebark pine functions as a keystone species in part by producing large nuts that are dense in calories and protein, serving as a key food source for a wide variety of birds and mammals (Lanner & Gilbert, 1994; Tomback & Kendall, 2001; McKinney & Fiedler, 2010). Grizzly bears (*Ursus arctos horribilis*) in particular rely on whitebark pine nuts as a food source; higher consumption of nuts by female grizzlies produces larger, healthier litters, and high-yield years exhibit reduced human altercations (Mattson & Jonkel, 1990; Mattson, 2000). Grizzly bears can gain up to 2.27 kg per day from eating whitebark pine nuts. Black bears (*Ursus americana*), red squirrels (*Tamiasciurus hudsonicus*), Marriam's turkey (*Meleagris gallopavo*), and chipmunks (*Eutamias* sp.), among other species of mammals and birds, also depend on the calories provided from whitebark pine nuts (Mattson & Jonkel, 1990; Mattson & Reinhart, 1994; McCaughey & Schmidt, 2001; Hutchins & Lanner, 1982; Tomback & Kendall, 2001).

The indehiscent cones of whitebark pine produce wingless seeds, physiologically evolved for dispersal via animals as opposed to wind or other mechanical adaptations (Tomback & Linhart, 1990). However, dispersal of seeds does not occur via their consumption but rather by the caching (underground storage) of small quantities for later consumption by animals (Tomback, 1982). Inevitably, many of these caches are forgotten and germinate to become the next spring's seedling cohort (Hutchins & Lanner, 1982). A single species of bird, the Clark's nutcracker (*Nucifraga columbiana*), takes part in the annual fall act of caching seeds and therefore has a disproportionate impact on the ecology and distribution of whitebark pine (Hutchins & Lanner, 1982; Tomback, 1982).

The widespread and profound role that whitebark pine plays in the high elevation ecosystems of the Rocky Mountain west cannot be understated; the loss of this species

will likely have a cascade of effects on Rocky Mountain food webs. Yet, the loss of whitebark pine is a situation we currently face. Whitebark pine is threatened by the invasive white pine blister rust (WPBR; *Cronartium ribicola)*, habitat fragmentation, shifting fire regimes, competitive habitat exclusion driven by climate change, and the mountain pine beetle (*Dendroctonus ponderosae*; Kendall & Keane, 2001; Gibson *et al*., 2008; Resler & Tomback, 2008; Pansing *et al*., 2020). The byproduct of these threats has manifested as range-wide population declines, with over 90% whitebark pine decline in some areas (Kendall & Arno, 1990; Keane & Arno, 1993; Achuff *et al*, 2010). As a result, whitebark pine has recently been listed as threatened species under the Endangered Species Act in the United States and has been designated as an endangered species by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) with "a high risk of extirpation from Canada" (Achuff *et al*, 2010; Fish and Wildlife Service, 2020).

Cronartium ribicola, the most destructive force affecting whitebark pine populations, infects the tree by growing through the needle stomata, into the branch and vascular system where it remains, parasitizing the tree until its lifecycle is complete. Fungal endophytes are also found within whitebark pine needles, given the close spatial interaction they may provide a first line of defense against blister rust (Ganley & Newcombe, 2006; Bullington, 2017). Thus, understanding the interaction between the two types of fungi is an important area of study as we seek to mitigate the impacts of blister rust in Rocky Mountain forests.

The plant microbiome has been identified as an important component of plant health and success (Berendsen *et al*., 2012; Turner *et al*., 2013). A key fungal group of the plant microbiome, called foliar endophytes, has been found in the leaves of all plants

studied to date (Arnold *et al*., 2000; Ganley & Newcombe, 2006; Rodriguez *et al*., 2009). These foliar fungal endophytes are similar to the human skin microflora and sometimes have profound roles in plant disease/predator interactions; these fungi can produce chemicals toxic to herbivores and pathogens, increase stress tolerance, and prime the plant host against infection (Kimmons *et al*., 1990; Rowan & Latch, 1994; Clarke *et al*., 2006). Despite the importance of the plant microbiome, the dynamic interaction between fungal endophytes and white pine blister rust is not well understood in whitebark pine. Furthermore, dispersal limitation and primary drivers of community structuring of fungal endophytes and other eukaryotic microbes is still up for debate in many systems. Due to whitebark pine being limited to high elevations, a patchy island-like distribution is formed; allowing us to examine questions of dispersal limitations and community ecology in fungi through the lens of island biogeographic theory.

By sampling across gradients of elevation, habitat isolation, and disease (white pine blister rust) prevalence, I addressed questions related to the roles of habitat, dispersal limitation and ecological interactions in shaping these fungal communities; the biocontrol potential of endophytic fungi against white pine blister rust, and the factors that affect endophytic community assemblage under pathogen antagonism. Additionally, I examined whitebark pine's dynamic role at the treeline ecotone, where it pioneers treeline advance into the alpine zone, tying in questions regarding climate change and the role it plays in structuring new plant and fungal communities in the highest elevation habitats suitable for whitebark pine.

The Clark's Nutcracker

The Clark's nutcracker (*Nucifraga columbiana*), a nucivorous bird from the family Corvidae (Fig. 1), specializes in eating and caching pine nuts (Tomback, 1982). In the late summer and early fall, the Clark's nutcracker carries seeds in its sublingual pouch, caching them throughout the subalpine and treeline ecotone at distances up to 29 kilometers from the original source of the seeds (Lorenz & Sullivan, 2009). Unrecovered seeds become next year's seedling cohort, explaining the ubiquity of this coevolved cooperation in whitebark pine forests (Tomback, 1982). Tomback & Linhart (1990) proposed that nutcrackers select for larger seeds, which is likely the cause of whitebark pine seeds averaging 175 milligrams. Other subalpine pines, such as limber pine, foxtail, bristlecone, western white and lodgepole pine, produce substantially smaller edible nuts: 93 mg, 27 mg, 25 mg, 16 mg, and 4 mg respectively (Tomback & Linhart, 1990). Although the Clark's nutcracker can carry up to 100 seeds in its sublingual pouch, on average only 7-14 seeds are stored in a Clark's nutcracker cache (Tomback, 1978; Hutchins & Lanner, 1982). Emerging seedlings grow in a tight vicinity for their entire lives; their trunks often fuse above- or belowground, causing genetically distinct

Fig. 1 Clark's nutcracker removing the nuts from the cone of a whitebark pine.

individuals to appear as a single tree (Furnier *et al.*, 1987; Rogers *et al*., 1999).

Population Genetic Structure of Whitebark Pine

The annual barter of food for seed dispersal via the Clark's nutcracker may be a central mechanism structuring whitebark pine population genetic structure at a regional scale (Richardson *et al*., 2002). Biparentally inherited allozymes, commonly used to evaluate population genetic structure prior to the advent of high-throughput DNA sequencing technologies, have suggested both pollen- and seed-mediated gene flow resulting in low genetic diversity between populations compared to other pine species (Yandell, 1992; Jorgensen & Hamrick, 1997; Bruederle *et al*., 1998). Seed-mediated gene flow is likely the result of the collecting and caching habits of the Clark's nutcracker. In collecting seeds from whitebark pine, a Clark's nutcracker will make numerous stops at whitebark pine communities varying in distance. Before caching, these seeds are stored and mixed in its sublingual pouch.

The late Pleistocene glaciers (glacial maximum occurring 18,000-20,000 years ago) likely altered genetic diversity in whitebark pine, displacing northern populations southward and lower in elevation; however, most of this displacement likely did not affect populations in the US Rocky Mountains (Thompson, 1990; Thompson & Anderson, 2000; Richardson *et al*., 2002). Macrofossils of whitebark pine found in Yellowstone National Park clearly suggest that the species was present during the Wisconsin glaciation (the last of the Pleistocene glaciation occurring 11,000-75,000 years ago; Richardson *et al*., 2002). The maximum extent of glaciation (the Laurentide ice sheet) only reached parts of northern Montana, which may be in part why the US Rocky Mountain populations were preserved. Therefore, Rocky Mountain populations in the

southern portions of whitebark pine's range (e.g., central and southern Montana), as well as populations in the southern Pacific Northwest are likely remnants of a much larger subalpine forest (Thompson, 1990).

The specialist fungal symbionts that associate with whitebark pine, both above and below ground, likely share parallel journeys of bottlenecking followed by expansion. As glaciers receded, whitebark pine likely re-colonized the northern Montana and Canadian Rockies (Richardson *et al*., 2002). It is still unknown how whitebark pine genetic diversity on more isolated mountain ranges located on the outskirts of the main Rocky Mountain corridor have been affected by isolation and glaciation. It is possible that inhospitable habitats for whitebark pine (i.e., low elevation plains and montane slopes) represent geographic barriers to dispersal, influencing patterns of genetic diversity in these isolated populations compared to populations connected to larger, more contiguous woodlands found within the main corridor of the Rocky Mountains. The two most isolated populations of whitebark pine sampled in this study (Bridger Mountains and Crazy Mountains) vary in their range to other populations. Whitebark pine suitable habitat in the Bridger range is separated by approximately 7.7 miles from suitable whitebark pine habitat in the Gallatin range to the south. Whitebark pines of Crazy Mountains are separated from the Bridger population by approximately 29.56 miles to the southwest, from the Absaroka population to the south by approximately 27.98 miles, and from the Castle Mountain population to the northwest by 21.92 miles. These populations are not within the maximum range of Clarks nutcracker seed dispersal (18 miles), and it seems likely that gene flow would be met with greater levels of resistance as compared to more contiguous populations.

White Pine Blister Rust

Cronartium ribicola is an invasive fungal pathogen native to eastern Asia that causes the disease white pine blister rust. All nine species of endemic North American white pines (five-needle pines) are susceptible to this pathogen (Hoff & McDonald, 1980; Kinloch, 2003; Tomback & Achuff, 2010), although resistance varies both between and within species (Kinloch & Dupper, 2002). Introduced to the eastern United States around 1898, blister rust was brought in on nursery stock (Maloy, 1997; Kinloch, 2003). Subsequent introductions of the disease occurred on both the east and the west coast throughout the early 1900s (Kinloch, 2003). By the 1950s blister rust had spread to every major commercial white pine region in the United States (Maloy, 2001). Remote outbreaks have occurred as far as 600 miles from the nearest source populations (Hawksworth, 1990).

Blister rust has a complex life cycle involving five spore stages and three known alternate hosts (Kinloch, 2003). Yellow-orange aeciospores are produced on the branches, stems, and trunks of pine trees in mid to late summer; these thick-walled spores are resistant to drying and capable of traveling long distances where they then infect alternate hosts (Maloy, 2001; Kinloch, 2003). *Ribes* spp. (currants, Grossulariaceae) and *Pedicularis* spp. (lousewort, Orobanchaceae) were the first alternate host species identified for blister rust; more recently, *Castilleja* spp. (Fig. 2; Indian paintbrush, Orobanchaceae) has been identified as a viable alternate host (Maloy, 2001; Kinloch, 2003; McDonald *et al*., 2006). Urediniospores develop on the underside of the alternate host's leaves (Fig. 3), and these spores continue to form and spread to alternate hosts in the vicinity until teliospores mature in the late summer (Kinloch, 2003). In spring,

basidiospores are produced from teliospores on the alternate host leaves. These basidiospores can land on living pine needles, infiltrate by growing germ tubes through the needle stomata, enter the vascular system, and establish a mycelium (Patton & Johnson, 1970; Liu *et al*., 2015). Occasionally, the basidiospore inoculum will enter directly into the trunk or branches via wounds. Branch swelling, followed by bark cracking, are initial symptoms of the blister rust disease; this is the genesis of the blister rust canker that will go on to produce aeciospores (Patton & Johnson, 1970).

Basidiospores are generally effective for short distance dispersal (approximately 300 meters), but under particular environmental conditions (cool moist air), vulnerable basidiospores are able to avoid desiccation and damage by sunlight (Maloy, 2001; Kinloch, 2003). Under these conditions, basidiospores can travel much greater distances, infecting large swaths of five-needle white pine forests; years where these conditions are present can serve as "wave years," whereby infection rate and intensity experience a short-term boom (Kinloch, 2003). Continued climate warming may decrease the incidence of wave years, creating warmer, drier conditions that destroy the fragile basidiospores (Kinloch, 2003).

Fig. 2 Non-infected *Castilleja* sp. growing in western Montana.

The presence of wave years due to seasonal climate conditions has had a major impact on the success of blister rust mitigation programs. Basidiospores appeared to be a logical weak link in the blister rust life cycle since they are produced on the alternate host and are more vulnerable to desiccation. Forest managers have attempted to exterminate the alternate host species (particularly *Ribes* spp.) in infected forests (Maloy, 2001; Kinloch, 2003). These efforts are costly, time consuming, and ultimately failed to reduce blister rust outbreaks. The presence of blister rust in forests lacking alternate host species directly correlated to the advent of wave years and was the first sign that the eradication strategy may not be completely effective (Kinloch, 2003). After 70 years of local *Ribes* spp. eradication, disease incidence was reduced from 9% in untreated areas to 4% in *Ribes* eradicated areas (Ostrofky *et al*., 1988). While reducing *Ribes* populations may be an effective local strategy for commercial white pine plantations, managing natural populations with this approach is viewed as less feasible (Schoettle & Sniezko, 2007). Other mitigation strategies of varying efficacy have also been employed in an attempt to decrease white pine forest devastation: reducing pest populations, increasing host vigor, breeding for resistance, and forest age class diversification. A combination of these strategies

Fig. 3 Underside of an infected *Ribes* leaf. Urediniospore development appears as yellow/orange spotting.

may be useful in sustaining white pine ecosystem function and sustainability (Schoettle & Sniezko, 2007).

Insects such as the mountain pine beetle are another key factor determining the resilience and health of white pine forests. Although the mountain pine beetle primarily affects lodgepole pine, ponderosa, limber, and numerous other pine species, whitebark pine can be affected when outbreaks are severe (Reid, 1962; Amman, 1982; Cerezke, 1995; Safranyik *et al*., 2010). Historically, beetle outbreaks have followed a cyclical boom-bust pattern (Kurz *et al*., 2008). However, recent outbreaks have been approximately an order of magnitude greater in area and severity compared to those seen in the past (Kurz *et al.,* 2008). Climate change has been implicated in providing optimal conditions during the mountain beetle's reproductive stages and by allowing northward expansion (Kurz *et al*., 2008). Ultimately, these outbreaks affect white pine forests in two distinct ways: 1) by decreasing vigor in reproductive age white pines, which may make individual trees more susceptible to blister rust and 2) by killing trees, thereby decreasing forest genetic variation for evolutionary adaptation to other stress factors.

It is unclear whether increasing host vigor necessarily reduces the severity of infection caused by WPBR, but it has been shown to improve resilience of the host to environmental conditions during critical establishment years and to damage caused by other agents such as the mountain pine beetle (Gottschalk *et al*., 1998; Schoettle & Sniezko, 2007; Lonergan *et al*., 2014). Seedlings planted in the Greater Yellowstone Ecosystem showed increased survival under certain planting strategies; for example, inoculating with mycorrhizal fungi, planting in burn sites (that lack bear grass), and planting near microtopographical features such as large logs and rocks showed positive

results (Lonergan *et al*., 2014). A combination of these planting strategies may be effective for regenerating forests, although the long-term success on forest sustainability is unclear (Lonergan *et al*., 2014).

Outplanting of rust-resistant seedling stock is considered to be a focal restoration approach, whereby seeds from resistant parental pines are collected and cultivated in gardens for future planting in natural ecosystems (Sniezko, 2006). These disease-resistant trees are selected from highly infected forests, containing 80-90% mortality of white pines, by caging the cone-bearing stems in mid-summer (Schoettle & Sniezko, 2007). This caging strategy sequesters seeds away from nucivores (animals that feed on nuts), allowing for collection in the early fall. The success of this strategy is limited by three major factors; 1) local adaptations from parental trees are not necessarily advantageous for all regions, 2) progeny of resistant parental trees may not carry resistant genotypes, and 3) seed collection, seed transfer, artificial growing, and seedling planting are collectively a laborious process (Schoettle & Sniezko, 2007). Furthermore, each step in the process yields another opportunity for seedling stress or death. This proactive intervention may not pay dividends for 30-50 years until white pines reach reproductive age (McCaughey & Schmidt, 1990). However, due to the dramatic nature of white pine decline, positive intervention in any form is necessary for future forest sustainability.

Forest age class diversification is another proactive strategy suggested to reduce the loss of ecosystem function displayed when a region is hit by WPBR (Schoettle & Sniezko, 2007). This strategy attempts to regenerate resistant seedlings in white pine forests prior to a major disturbance event (WPBR infestation, forest fire, or beetle outbreak), allowing the loss of mature seed-producing pines over time that sustain forest

function while promoting the growth of immature seedlings that will eventually fill the functional niche (Schoettle & Sniezko 2007).

White pine blister rust prevalence has been examined in the krummholz ecotone in the same ecoregion (Montana Rocky Mountains) where I collected samples for my thesis research. Resler & Tomback (2008) found that 33.7% of whitebark pines sampled within their study were infected with blister rust. Previous studies hypothesized that WPBR prevalence may be lower at the highest elevations of white pine habitat due to dry, cold conditions that could promote desiccation of basidiospores (Campbell & Antos, 2000). However, Resler & Tomback's (2008) results disagree with this hypothesis, finding rates of WPBR prevalence in krummholz ecotypes similar to non-krummholz ecotypes. WPBR prevalence was also found to be correlated with the proximal distance of nearby trees. Whitebark pines that were a part of "multi-tree islands" (groupings of trees with overlapping branches), had more cankers per tree compared to solitary whitebark pines (Resler & Tomback, 2008). This study also found that whitebark pine was the primary initial colonizer of "tree-islands" and facilitated the growth of other conifers and forbs. The implications of high WPBR prevalence at these elevations may have important ramifications for subalpine vegetation expansion.

Whitebark Pine Habitat and Climate Change

At high elevations, whitebark pine thrives, having carved out a niche over the millennia as the dominant subalpine tree (Arno & Hoff, 1990). Thriving at high elevations is a defining characteristic of whitebark pines, as they are well known for being the highest elevation tree species in the northern Rocky Mountains, leading them to also commonly be called creeping pines. This common name describes the action of

whitebark pines establishing at increasingly higher elevations, a phenomenon known as treeline advance or treeline creep (Holtmeier & Broll, 2007). The upper elevational limits of the subalpine forest are known as the treeline ecotone, representing a transition zone between subalpine plant communities and alpine vegetation (Körner, 1998; Holtmeier, 2009). This distinct vegetational boundary can be seen both at high elevations and northern latitudes and is thought to be controlled by a variety of local and regional factors, including temperature and duration of growing season, quantity of winter snowpack, presence of geomorphic features (providing microclimate shelter), oxygen availability, CO_2 concentration, nitrogen deposition, and precipitation (Crawford *et al*., 1992; Grace *et al*., 2002; Resler *et al*., 2005; Holtmeier & Broll, 2007). Ecologists have noted a shift in the treeline via an expansion of subalpine forests into the previously uninhabitable alpine zone. In a meta-analysis of a global dataset with records dating back to 1900 AD, treeline advancement was recorded at 52% of sites with only 1% of sites showing recession and the others showing no change (Harsch *et al*., 2009). Treelines are generally recognized as having three forms: diffuse, abrupt, and krummholz. Diffuse treelines are defined by decreasing tree density with increasing elevation or latitude. Abrupt treelines have a homogenous density of canopy right up to treeline. Krummholz treelines may be diffuse or abrupt but are distinguished by trees exhibiting shrublike vertically stunted growth with branches growing close to the ground. It has been hypothesized that treelines displaying the diffuse form, like those of the Rocky Mountains, are more likely to advance than those displaying an abrupt form (Harsch *et al*., 2009).

The factors affecting spatiotemporal dynamics at treeline covary and are often nonlinear; however globally treeline is considered to be an important and sensitive bioindicator of historical and recent climate patterns (Kullman, 1998; Grace *et al*., 2002). Temperature has been implicated as the primary driver of treeline advancement (Kupfer & Cairns, 1996; Holtmeier & Broll, 2005). At temperatures between 5–20 °C, temperature appears to be the greatest limiter of growth and reproduction (Grace *et al*., 2002). At temperatures outside of this range, other factors such as length of photoperiod and winter snowpack accumulation can be the greatest limiters of growth and reproduction.

As the climate warms, habitat availability is increased at high elevations and the northern edge of the range (Elsen & Tingley, 2015). Inversely, at lower elevations and at the southern edge of the range, suitable habitat is diminished. Warmer climate at lower elevations favors regeneration of other tree species that outcompete whitebark pine. Unfortunately for whitebark pine, altitudinal shifts in habitat availability result in net loss since inherently the base of the mountain has more surface area than its skyward counterpart. Habitat displacement for whitebark pine is synonymous with habitat displacement for any of its host-specific symbionts. Currently, ecological niche modeling suggests that climate warming will have significant negative impacts on the distribution of whitebark pine; however, these models may underestimate the capacity for whitebark to respond to changing climate patterns. Paleoecological data (pollen dating) spanning 15,000 yr BP interpolated with past climate data suggest that whitebark pine may be more tolerant to higher summer temperatures and fire disturbance than current models

predict (Iglesias *et al.* 2015). However, these studies may be flawed due to inability in differentiating whitebark pine pollen from limber pine pollen.

Shifting climate patterns and rising global temperatures have dovetailed with decades of fire suppression, resulting in increased frequency and intensity of forest fires across the Rocky Mountain west (Higuera *et al*., 2015; Riley & Loehman, 2016). The results of these ecosystem shifts may have both positive and negative effects on whitebark pine populations (Hansen *et al*., 2016). Intense fires clear out all species, resetting ecosystems to a fire-scarred void and giving an advantage to whitebark pines that have bird dispersed seeds which have been buried and can thus survive the fire; the species also thrives in post-fire soils (Keane *et al*., 2012). Spruce and subalpine fir (wind disseminated seeds) take longer to invade post-fire ecosystems but will often outcompete whitebark pine overtime, climaxing in spruce/fir dominated seral stands (Campbell & Antos, 2003). The fire-tolerant whitebark pine is able to survive moderate-severity fires, while competitor populations, spruce and subalpine fir, are reduced during mid-intensity fires (Morgan *et al*., 1994; Larson & Kipfmueller, 2012). Ultimately, whitebark pine populations may be favored by trending fire regimes; however, with seed source populations declining, natural regeneration may not be able to maintain pace with the influx of increased fire-scarred landscapes.

Krummholz Whitebark Pine

At the highest elevations suitable for whitebark pine, a distinct morphology, known as krummholz, is found to be predominant if not universal. Characterized by a windswept, shrublike, vertically stunted growth, and branches growing close to the ground, krummholz form marks the upper edge of the subalpine (Arno & Hoff, 1990; Tomback, 1986). In krummholz habitat, the threat of mountain pine beetle may be less severe due to increased brood time and lower fecundity (Amman, 1982). Brood production and abundance has been found to be negatively associated with increasing elevation. Recently, it has been suggested that the krummholz growth form developed at high elevations is immune to mountain pine beetle attack (Logan *et al*., 2010). However, as the climate warms, upright growth is sometimes assumed by formerly krummholz whitebark pines. This dichotomy illustrates how climate change has multiple impacts on high elevation ecosystem dynamics.

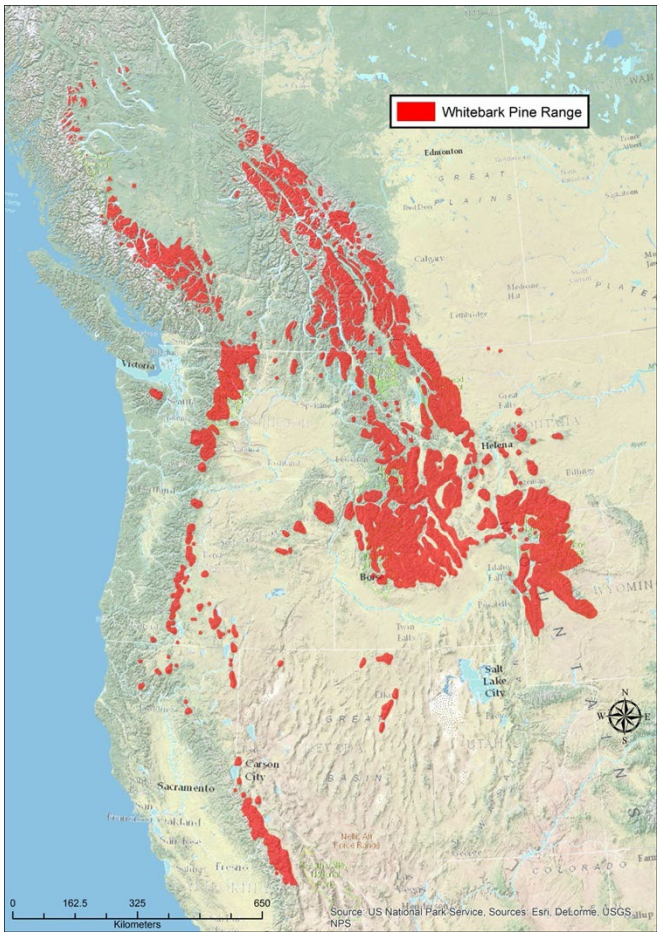

Fig. 4 Map of continental distribution of whitebark pine (source: Whitebark Pine Ecosystem Foundation). Being limited to high elevations across its entire range, whitebark pine displays an archipelago-like pattern of growth at the continental scale.

Island Biogeography of High Elevation Habitat Islands

Island biogeographic theory has been used to explain the uneven distribution of species richness based on level of isolation (distance to source populations) and the size of the island. Equilibrium theory of island biogeography (McArthur & Wilson, 1967) proposes that an island's biodiversity is controlled by the dynamic balance between

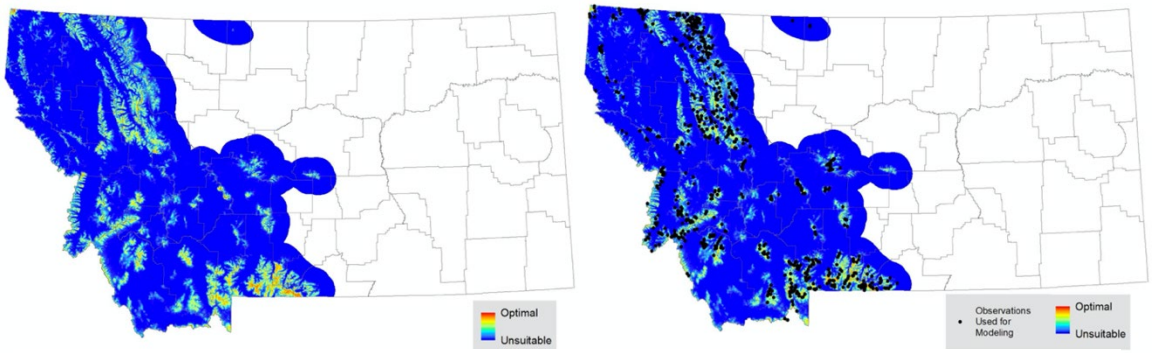

Fig. 5 Map of continuous habitat suitability model for whitebark pine in the Montana Rocky Mountains (left; Montana National Heritage Program, 2018). Suitable habitat for whitebark displays a patchy distribution of habitat islands driven primarily by elevation. (Right) Map of continuous habitat suitability model for whitebark pine in the Montana Rocky Mountains overlaid with 647 whitebark pine observations (Montana National Heritage Program, 2018).

origination and extinction rates. Dispersal, or rather dispersal limitation, is a principal area of study in ecology that can also be examined through the lens of island biogeographic theory. The dispersal limitations of organisms affect many ecological patterns such as origination-extinction rates, species richness, niche competition, and gene flow (concomitant with genetic diversity). Using island biogeographic theory for terrestrial habitat islands requires more consideration of confounding factors; that is to say, not all levels of isolation are created equal. Instead, terrestrial habitat islands exist on a spectrum of habitat continuity; in the case of true islands (land outcroppings arising from a body of water), there is sharp and distinct break in habitat between islands that is completely uninhabitable by non-aviary terrestrial organisms under normal conditions.

For whitebark pine, seed carrying birds may act as a vector for endophytic fungal dispersal.

The biogeography of the whitebark pine is unusual compared to many other conifers; it can be found in a large distribution of mountain ranges but is limited to the subalpine (Arno & Hoff, 1990; McCaughey & Schmidt, 2001). Other pines are also widely distributed, and many are restricted to particular elevation zones (e.g., subalpine fir, limber pine, bristlecone pine, lodgepole pine, limber pine, and Colorado spruce). However, naturally occurring whitebark pines only grow at high elevations; as a result, a patchy distribution of habitat islands has developed (Figs 4, 5). This same biogeographic ecology can be found in the sky-islands of Arizona or in a variety of island archipelagoes. The Arizona sky islands are a series of isolated mountain ranges separated by varying distances, hosting incredible diversity that dynamically shifts in distribution between mountain ranges over time. In the case of the Arizona sky-islands, habitat breaks are fairly distinct; although some species are capable of living or surviving in the deserts that separate the mountain islands, the deserts provide a dispersal barrier for many species. In the case of the subalpine Rocky Mountain habitat islands, habitat continuity – which can be considered analogous to island size – varies for different species since what constitutes suitable habitat depends on each species' range of environmental tolerance and ecological interactions (e.g., presence or absence of competitors, prey, and symbionts).

Island systems have been used to demonstrate the effects of habitat size and isolation on biodiversity for macro-organisms for many years (MacArthur & Wilson 1967; Hill *et al*., 1996; Wilcox, 2001). Recently, studies using modern molecular methods have illuminated these patterns for microorganisms, disputing the Baas-Becking

hypothesis that, “everything is everywhere but the environment selects” (Baas-Becking, 1934; Andrews *et al*., 1987; Peay *et al*., 2007; Sato *et al*., 2015; Li *et al*., 2020). However, there is still some debate as to whether all microorganisms display strong biogeographic patterns, to what degree microbial communities are controlled by the selecting environment, and to what degree dispersal ability limits community assemblage (Staley *et al*., 1997; Finlay, 2002; Fenchel & Finlay, 2003; Hedlund *et al*., 2003). While some argue that those studies using morphological species recognition concepts may be flawed for microorganisms that have few morphological features, this highlights the need for molecular based approaches (Fenchel & Finlay, 2003; Taylor *et al*., 2006). Studies examining eukaryotic soil microbes have contributed mounting evidence that this group of microorganisms exhibits strong biogeographic patterns (Peay *et al*., 2010; Sato *et al*., 2015). However, another study examining root associated fungal community assemblage (*Phialocephala fortinii* s.l.– and *Acephala applanata* species complex) found no biogeographical pattern (Queloz *et al*., 2011a,b).

There is also some debate regarding the effects of spatial isolation on community structure of eukaryotic foliar endophytes (Saikkonen, 2007). At fine spatial scales, Higgins *et al*. (2014) found that foliar endophytic community similarity decayed strongly over a scale of hundreds of meters, finding patterns consistent with dispersal limitation. At the regional scale, some studies have found similar endophytic communities across the range of the host plant species (Amazonian Palm and Coastal Redwood), although these studies are limited to the plants surveyed (Rollinger & Langenheim, 1993; Rodrigues, 1994). The endophytic level of host specificity was not examined in these studies; however, one may hypothesize that lower levels of habitat continuity and greater distance

between individuals would result in more associations with generalist species. For example, coastal redwood has relatively high habitat continuity, while Amazonian palm has high levels of habitat continuity despite high distance between individuals. A study on island archipelagoes in Finland found that the frequency of the most common endophytes correlated with distance to the source population (mainland) and increased with island size (Helander *et al*., 2007). In the Finish Island archipelago study, distance to mainland explained 29-35% of the variation found in the frequency of endophytic fungi, while island size explained 32-35% of variation (Helander *et al*., 2007). The distance of these islands from the mainland ranged from 0.4-18.1 km, and island size ranged from 0.03-0.99 km^2. This study may represent the most elegant system for studying fungal dispersal, although it lacks the ecological variables (e.g., reservoir populations occupying intermediate landscapes) accompanying most heterogeneously distributed populations. The conflicting results of these studies may be due to the varying levels of habitat continuity between sampled sites.

Martiny *et al*. (2006) proposed that, in general, the intimate associations between macroorganisms and microorganisms may be largely responsible for the patterns seen in microbial diversity. For whitebark pine in the Rocky Mountains, growing only in the subalpine and upper montane ecosystems, non-suitable habitat at low elevations can be classified as a fairly distinct geographic barrier. Thus, whitebark pine-specific microorganisms are limited to whitebark pine forests, while generalist endophytes would have greater host distributions. If this proved to be true, we would likely find an increased frequency of generalist endophytes at isolated sites, since those endophytes may be less susceptible to habitat fragmentation. Although little is currently known about

host specificity in conifer endophytes, a much greater understanding could be gained by simply comparing the endophytes that have been found in numerous studies. To my knowledge, a meta-analysis of this nature has not been performed; regional variation in climate and surroundings would likely play some role in confounding the confidence gleaned from any meta-analysis.

As a result of a better understanding of dispersal limitation and habitat barriers, there has been a surge of wildlife management efforts centered around preserving and re-establishing wildlife corridors, migration routes, and generally contiguous habitat that allows for population dispersal and gene flow. Management centered around charismatic megafauna has largely left fungi out of this conversation, while plants have received considerable attention especially in the context of recently shifting climate regimes. For example, large intense fires may be altering successional patterns in the boreal forest, mainly driven by dispersal limitation in evergreen conifers (Tautenhahn *et al*., 2016). Tree expansion is sometimes both seed and establishment limited, such as is the case with treeline recruitment of black spruce (*Picea mariana*) and Tamarack (*Larix laricina*; Crofts & Brown, 2020). In some conifer-dominated stands, deciduous trees (beech, lime, hornbean, elm, ash) have been found to be mainly limited by poor dispersal (Götmark *et al*., 2005). Conifer communities have been viewed through the lens of island biogeographic theory, finding results more consistent with stochastic (e.g., passive sampling) and deterministic processes (e.g., assembly rules; dispersal, abiotic environment, and biotic interactions; Burns *et al*., 2010). Ultimately, there appears to be some contention regarding the relative importance of habitat size (niche diversity is not always linked to island size) and competition (total abundances) in structuring plant

communities (Hortal *et al*., 2009). Modeling plant communities has reached high levels of complexity; forging theories of island biogeography, niche diversity, and dispersal limitation is likely the best bet to resolve these models. Fungal ecology has yet to reach these levels of model resolution.

Fungi are often viewed from the perspective of the Baas-Becking Hypothesis (1943) ("everything is everywhere, but the environment selects") by the layman; studies on fungal biogeography may prove that fungi are an important consideration for conservation organizations. A renewed interest in fungal biogeography over the last 15 years has largely disproved the Baas-Becking hypothesis and has shown that microbial species are not ubiquitous in suitable habitat. More recent studies have shown that isolation, habitat size, and dispersal limitation are important determinants of species richness; however, the scale and confidence to which they can be applied in fungal conservation is not well agreed upon (Hedlund *et al*., 2003; Helander *et al*., 2007; Peay *et al*., 2010).

Whitebark Pine Mycobiome

While many species rely upon whitebark pine, their level of dependence varies; host-specific fungal species can be classified as highly specific in their relationship to their host plants (Bruns *et al*., 2002). However, many species of fungi appear to be generalists, forming associations with many other species of host plants (Moora *et al*., 2011). Therefore, the level of isolation applied to whitebark pine-associated fungal endophytes is likely fungal species-specific; currently the host specificity of whitebark pine endophytes is unknown. Interestingly, the family to which whitebark pine belongs, Pinaceae, forms mycorrhizal associations with suilloid fungi; suilloid fungi are restricted

to forming connections with plants in the family Pinaceae (Bruns *et al*., 2002). Naturally, one may wonder if some endophytic guilds have similar restrictions. Specialist symbionts of whitebark pine may offer the best study organisms for ecologists seeking to understand aspects of dispersal, genetic variation, and gene flow. While generalist symbionts of whitebark pine may shed light on these same principles, deciphering these patterns may be more difficult; however, the study of generalists can yield interesting insights regarding endophytic networks, host-switching, and horizontal transmission.

Microbes associate with plants in a variety of functional relationships; these microbes make up the plant microbiome, the vast majority of which are bacterial or fungal. Generally, fungal relationships appear to play a disproportionately critical role for the success and health of the plants they associate with (White *et al*., 1992; Clarke *et al*., 2006; Koh & Hik, 2007; Hubbard *et al*., 2013). One way to divide these major groups of relationships is to discern the plant tissues these microbes associate with (root, vascular, bark, or leaf). Belowground plant-associated mutualistic fungi include mycorrhizae or root endophytes; these fungi may play an important role in whitebark pine seedling survival (Cripps & Grimme, 2011). All pines, including whitebark, exhibit mycorrhizal relationships in nature; these associations are believed to be obligate; i.e., necessary for survival of both symbionts (Smith & Read, 2008).

Endophytes

Endophytes are most commonly defined as microorganisms inhabiting asymptomatic (lacking conspicuous infection) plant tissues, occurring within roots, stems, bark or leaves. Fungal endophytes represent a highly diverse, polyphyletic group that occur in all plant species studied to date (Arnold *et al*., 2000; Ganley & Newcombe,

2006; Rodriguez *et al*., 2009). In recent years, a large number of studies has been published on these diverse, ubiquitous and highly cryptic microfungi (Arnold, 2007). This body of literature has illuminated many aspects of endophytic fungi such as life histories, production of novel secondary metabolites, evolutionary origins, and their often profound ecological importance.

Endophytes appear to play an important role in plant survival. These microorganisms are capable of inducing increased drought tolerance in plants, reducing herbivory of host tissue, and providing various forms of resistance against fungal pathogens (Kimmons *et al*., 1990; Rowan & Latch, 1994; Clarke *et al*., 2006). Foliar endophytes may enter plants through stomata or intercellular spaces (horizontal transmission), or via penetration of the embryo within the host seeds (vertical transmission). Some endophytes can directly defend host plants against insect herbivores by producing toxic chemical compounds, as is the case with the fungal metabolite peramine produced by the ryegrass hosts *Epichloë*/*Neotyphodium* (Tanaka *et al*., 2005). Additionally, endophytes have been reported to deter mammalian herbivores, such as with the toxic fungal alkaloids (e.g., ergot alkaloid, lysergic acid amide) from *Claviceps* sp. that deter grazers of sleepygrass (Faeth *et al*., 2006). Endophytes can deter fungal pathogens either directly (e.g., metabolite production, competitive habitat exclusion, and mycoparasitism) or indirectly (e.g., by inducing host resistance mechanisms) (Aneja *et al*., 2005; Bailey *et al*., 2006; Garbelotto *et al*., 2019). Competitive habitat exclusion is a form of direct inhibition whereby the fungal endophyte spatially excludes pathogens from growing. Mycoparasitism has been shown in *Theobroma cacao* to reduce or eliminate pathogens *in vitro* (Bailey *et al*., 2008).

Fungal endophytes can stimulate a host plant's immune system, triggering a pathogenic defense response. The effects are often long-lived and create systemic acquired resistance (SAR) that primes the plant against future pathogenic fungal or insect attacks (Ganley *et al*., 2008; Gao *et al*., 2010). Ganley *et al.* (2008) demonstrated that fungal endophytes in *Pinus monticola* (Western white pine) induce a resistance response that mediates the effects of the white pine blister rust pathogen (Ganley *et al.*, 2008). Endophyte-inoculated seedlings survived longer, more often, and showed less disease severity than endophyte-free seedlings. This study highlights the potential for endophytic biocontrols in other *Pinus* species, such as the whitebark pine.

Fungal endophytes of coniferous trees have also been shown to produce a wide variety of metabolites that antagonize pathogens, delay needle senescence, or decrease herbivory (McMullin *et al*., 2018). *Rhabdocline parkeri* appears to mitigate infestation of gall midges on Douglas fir, and *in vitro* studies using the extracts of this fungus reduced growth and increased mortality of eastern budworm (Carroll, 1986; Miller, 1986; Miller *et al*., 2002). *Hendersonia pinicola* produces a potent antifungal compound (Richardson *et al*., 2015), and it was speculated that this compound may have been the primary driver reducing infection of *Lophodermella concolor* in a study by Darker (1967) on jack pine. Minter (1981) suggested that *Lophodermium conigenum* is a primary excluder of the needle cast pathogen *Lophodermium seditioum* in Scots pine. Fungal endophytes of spruce and pine have been shown to produce rugulosin (antiinsectan), macrolides (weakly alkaline antibiotics), griseofulvin (antifungal), racemic sesquiterpenoids (antifungal), chlorinated dihydrobenzofurans (antifungal), and xanthenes (antifungal; Sumarah *et al*., 2011; Richardson *et al*., 2014; McMullin *et al*., 2015; Richardson *et al*., 2015; Sumarah

et al., 2015). *Lophodermium nitens* is responsible for production of racemic sesquiterpenoids as well as macrolides including pyrenphorol (Sumarah *et al*., 2011; McMullin *et al*., 2015). Pyrenphorol has been shown to reduce the growth *C. ribicola* in culture (Sumarah *et al*., 2015) and griseofulvin produced by *Xylaria* sp. has been shown to reduce the growth of rusts (Richardson *et al*., 2014). These studies collectively provide support for the further study of whitebark pine needle endophytes, suggesting the potential of whitebark pine needle endophytes to be utilized in active restoration strategies and conservation programs for whitebark pine.

Endophytes - Phylogenetics

Taxonomically, the vast majority of fungal endophytes are classified in the fungal phylum Ascomycota, with two major subgroups being recognized; clavicipitaceous endophytes (C-endophytes) and non-clavicipitaceous endophytes (NC-endophytes) (Rodriguez *et al*., 2009). This delineation casts a broad light on taxonomy, habitability of plant host, and functional ecology. C-endophytes generally infect grasses, have a narrow host range and biodiversity, and disperse either vertically or horizontally (Rodriguez *et al*., 2009). To my knowledge, C-endophytes have not been found in conifer species. NC-endophytes commonly inhabit a range of non-vascular plants, ferns and allies, angiosperms, and conifers (Rodriguez *et al*., 2009). C-endophytes have been the focus of much study in recent years due to their significance in agricultural systems, while many aspects of NC-endophytes have been overlooked. As a result, the diversity, dispersal, ecology, and functional role of NC-endophytes still remains a frontier in many plant systems.

Endophytes in Whitebark Pine

To date, two studies have been published investigating the foliar endophytic communities of whitebark pine, both in the context of white pine blister rust (Bullington *et al*., 2018; Moler & Aho, 2018). In a low-elevation common garden study, whitebark pine seedlings experimentally inoculated with *Cronartium ribicola* exhibited endophytic community shifts post-inoculation (Bullington *et al*., 2018). In total, 1,348 operational taxonomic units (OTUs) were recovered from 141 whitebark pine seedlings, highlighting the hyperdiversity found in fungal endophytes (Bullington *et al*., 2018). However, no single OTU was recovered from all seedlings (Bullington *et al*., 2018). Endophytic communities may be most highly correlated with parental genotypes, as endophytic communities appear to differ based on the geographical origin of the seedling's parents (Bullington *et al*., 2018). Operational taxonomic units closely resembling *Lophodermium* and *Paraphoma* spp. were associated with decreased disease severity in experimentally inoculated trees (Bullington *et al*., 2018). Additionally, *Metarhizium anisopliae* was found in 62.4% of seedlings and was correlated with decreased needle spotting and branch cankers (Bullington *et al*., 2018). Species richness decreased after *C. ribicola* inoculation regardless of parental genetic resistance to WPBR; however, susceptible seedlings experienced a greater decrease in richness than resistant seedlings (Bullington *et al*., 2018). It should be noted that the experimental garden was not located near naturally-occurring whitebark pines and the researchers conducting the study classified the surrounding habitat as "highly different" from whitebark pine habitat (Bullington *et al*., 2018). Also, trees have been shown to exhibit endophyte community succession as a tree ages (Oono *et al*., 2015). Therefore, seedling endophyte communities from the

experimental garden study on seedlings may differ significantly from those of mature trees in a naturally occurring high-elevation ecosystem.

An ecological study performed on whitebark pine needle endophytic communities took a different approach to understanding fungal community composition, focusing more heavily on abiotic factors such as aspect, elevation, latitude and longitude; and biotic factors such as proximal alternative plant hosts, host tree DBH, duff class, and presence or absence of cankers on the host tree (Moler & Aho, 2018). Results from these two studies vary on a number of fronts, indicating that the factors affecting endophyte community composition are still not well understood. Most notably, presence or absence of cankers on host trees wasn't found to be significantly correlated with endophytic community composition in the field study (Moler & Aho, 2018). However, it should be noted that only 16 of the 96 (14.6%) trees sampled in this study showed presence of WPBR cankers, whereas 100 of the 141 (70.9%) trees sampled by Bullington *et al*. (2018) showed canker development. Although the total number of trees sampled was relatively similar (96 compared to 141), the extent of genetic information acquired after quality filtering (3,401,416 sequences compared to 1,631,451 sequences) and the number of unique OTUs recovered (383 compared to 1,348) differed substantially (Bullington *et al*., 2018; Moler & Aho, 2018). The stark difference in recovered OTUs may be due to the exceptionally wide WBP genetic variation used by Bullington *et al*. (2018), age class of the trees sampled (mature trees vs. seedlings), or a non-linear relationship between OTU richness and number of trees sampled. OTU delineation may have also led to differences in richness, though both studies clustered OTUs at 97% similarity. Other factors such as study site characteristics and thoroughness of sequencing would suggest

inverse results; intuitively, it would seem that natural whitebark pine habitat and increased DNA sampling would result in the discovery of a greater number of unique OTUs. Mountain aspect, host DBH, duff class, UTM northing, and presence/absence of alternate host may prove to be important factors influencing endophytic community composition (Moler & Aho, 2018).

Congruent with past studies examining the endophytic communities of conifers, Ascomycota dominated endophyte sequence libraries from both WBP studies (Bullington *et al*., 2018; Ganley & Newcombe, 2006; Moler & Aho, 2018). Leotiomycetes was the most abundant class, Rhytismatales and Helotiales the most abundant orders, and *Lophophacidium dooksii* (100% of sample libraries), *Caloplaca lenae* (99%), *Physcia magnussonii* (99%), *Lirula exigua* (85%), *Coccomyces multangularis* (81%), and *Coccomyces dentatus* (81%) were the most abundant species found by Moler and Aho (2018). Interestingly, *Caloplaca lenae* (saxicolous lichen, generally occurring on rocks) and *Physcia magnussonii* (foliose lichen) were both detected in 99% of sample libraries (Moler & Aho, 2018). This may indicate a flaw in their surface sterilization procedures or previously undescribed niches for these organisms.

An additional study performed on whitebark pine needle endophytes' have not been published in a scientific journal but rather presented at the Mycological Society of America's annual conference in 2016 (Noffsinger & Cripps, 2016). This study compared endophytes in green and red senescent needles of whitebark pine at treeline from four whitebark pine forests in southwest Montana. Using culture-based methods, the authors found endophytes consistent with other whitebark pine needle endophyte studies (e.g., *Lophodermium* sp., *Pseudoplectania* sp., *Sydowia polyspora*). Although the sample size

was likely too small to detect shifts in community assemblage before and after senescence and endophytic species were highly variable among sites and trees. However, they did find *Sydowia polyspora* (a pathogen associated with needle necrosis) in senescent needles at every site, suggesting that this fungus plays an important in role removing dead or parasitized needles (Noffsinger & Cripps, 2016).

Objectives

The primary aims of this thesis research are to identify endophytic assemblages associated with whitebark pines under gradients of elevation (Objective 1), disease (Objective 2), and isolation/connectedness (Objective 3). By sampling across gradients of elevation, disease (white pine blister rust) prevalence, and habitat isolation, I am addressing questions related to the roles of habitat, ecological interactions, and dispersal limitation in shaping these fungal communities; the biocontrol potential of endophytic fungi against white pine blister rust, and the factors that affect pathogen dynamics and their role in driving whitebark pine population declines. Additionally, field surveys of advancing treeline plant communities were collected in an aim to understand the effects of climate change in shaping the advancing treeline ecotone.

Objective 1: Identify endophytic fungal species associating with natural populations of lower elevation whitebark pine, established krummholz whitebark pine, and pioneering krummholz.

Research Question 1.1: Do fungal communities differ between ecotypes?

Research Question 1.2: Do the naturally occurring fungal communities sampled in this study (Western Montana) differ from those found in the Pacific Northwest (PNW) studies (both naturally occurring and garden plantings)?

Endophytic fungal communities in WBP needles have been characterized in two studies, one of which examined endophytic communities of whitebark seedlings before and after WPBR inoculation in an experimental garden (near the Oregon coast) at low elevations with the nearest natural population found in the Cascade Mountain range (Bullington *et al*., 2018). The other study examined endophytic communities of naturally growing whitebark pines in a small geographic area of the southern Cascade Mountain range (Moler & Aho, 2018). Both studies used cultureless techniques to examine endophytic communities. To date, no study has examined the community assemblages of whitebark pine in the treeline ecotone (i.e., krummholz whitebark pine) using next-gen sequencing. However, a study on Lone Mountain in southwest Montana compared high elevation whitebark pine endophytes in senesced and green needles using culture based methods (Noffsinger & Cripps, 2016).

Using cultureless methods and next-gen DNA sequencing, I identified and characterized endophytic communities of natural whitebark pine populations found in the northern Rocky Mountains of Montana. Additionally, communities of endophytes were compared between ecotypes. Since these stands generally exist along a gradient driven by mountain elevation, continuity of whitebark stands varies between sites. Some sites have a distinct separation between krummholz and lower elevation stands while some lower elevation sites slowly transition into krummholz form. It seems reasonable that krummholz ecotypes would have a lower species richness than lower elevation stands due to the climatic extremes experienced by the krummholz ecotype, the smaller habitat area of the krummholz ecotype, and the generally younger age class of trees found in the krummholz ecotype. Certain species of fungi residing in islands of krummholz whitebark

pine may confer unique benefits and resistance against wind or drought not found in lower elevation stands due to the high exposure at these locations.

Objective 2: Characterize the endophytic communities of WBP associated with colonization levels of white pine blister rust in krummholz and lower elevation stands.

Research Question 2.1: Do elevation (both across site variation in elevation and ecotype level classification of elevation) and disease class have interactive effects on endophyte community assemblage?

Research Question 2.2: Does indicator species analysis reveal endophytes that are associated with specific classes of disease infection severity?

Research Question 2.3: Does indicator species analysis reveal endophytes that are associated with healthy trees in highly infected stands?

The trends observed in the two previous studies are somewhat in conflict regarding the effect of WPBR on community assemblage: Moler & Aho (2018) found an insignificant pattern associated with the presence or absence of WPBR cankers, while Bullington *et al.* (2018) found significant community shifts after inoculation with WPBR. It is my goal to draw more definitive conclusions on pathogen-endophyte dynamics by centering my sampling strategy around trees that exhibit WPBR symptoms. Consequently, I sampled trees from each site that varied across a spectrum of WPBR infection severity. “Canopy kill” (i.e., needle dieoff and branch death) was used to separate infection severity into discrete classes (modified from Resler & Tomback, 2008). Out of 10 trees selected from each site, three whitebark pine trees were selected that were completely healthy (showing no signs of infection), two that had a low-severity WPBR infection (1-24% canopy kill), two that had a mid-severity infection (25-74%

canopy kill), and three that had a high-severity infection (75-99% canopy kill). Canopy kill was used as a determining factor for severity; however, trees were primarily determined to be infected by their presence or absence of cankers and/or branch swelling. Lack of symptoms does not necessarily confirm lack of infection; in asymptomatic cases, however, we would expect minimal to no effect on endophytic communities given the early stage of infection. On the other end of the infection spectrum, highly infected tree needles may be susceptible to opportunistic endophytes and could challenge the hypothesized patterns of diversity.

Level of tree infection severity may not be a significant source of endophytic community variance between trees within an ecoregion. However, if WPBR does act as a significant determinant of endophytic community variance, I may find differences in species diversity or unique community assemblages tied to levels of WPBR severity. Unique endophytic communities of WPBR-free whitebark pines may illuminate what species of fungi are able to confer resistance to the blister rust pathogen and which endophytic species drop out first when a tree gets infected. Deciphering co-occurrence and a causal relationship may make it difficult to reach a definitive conclusion. I used indicator species analysis to identify endophytes that are associated specifically with a particular disease class. Taxa that are present in uninfected needles may confer WPBR resistance; alternatively, they may be susceptible to competition from the pathogen. These possible outcomes could suggest avenues for further testing in subsequent *in vitro* competition studies or *in vivo* common garden studies.

Since abiotic variables such as moisture (relative humidity and precipitation), wind, and temperature have been determined as range-limiting factors for WPBR, I may

find differences in the prevalence of the WPBR between krummholz stands and lower elevation stands. One may predict that krummholz stands have a lower frequency of infection due to lower tree density and the possibility of basidiospore desiccation and maybe even a lower infection severity (due to spore load). A single study has, however, been published examining the occurrence of WPBR in krummholz stands, finding that infection frequency closely matches that of surveys performed on lower elevation forests (Resler & Tomback, 2008). To date, no study has compared the frequency of infection relative to the nearest lower elevation stand simultaneously. Since stand genetics and abiotic factors (soil type, climate, etc.) will be most similar between the stand types found on the same mountain, this strategy should offer further insights into the role that the environment plays on WPBR limitation.

Objective 3: Examine species diversity in the context of habitat area and distance to metapopulations.

Research Question 3.1: Does community similarity decay (decrease) as distance between metapopulations increases?

Research Question 3.2: Does the size of the contiguous whitebark pine habitat surrounding the site regulate the abundance of endophytes found within whitebark pine community assemblages?

There is some debate as to the degree that dispersal limitation affects microbial communities. Species-area estimates reflect the impact that habitat size has on the diversity of an ecosystem. Classically, island biogeographic theory has defined this as a principle of mammalian and plant ecology, while other mainstream hypotheses for the ecology of microbes, such as the Baas-Becking hypothesis (1934), have disputed the

effect of habitat size and isolation on microbial community diversity. By sampling mountain ranges that vary both in habitat size and isolation I have the opportunity to examine the links between biogeography and endophytic community diversity. Defining source populations in this study is more easily grasped by intertwining Levins's theory on metapopulations with island biogeographic theory (Levins, 1969). In other words, all mountain ranges act as source populations to some degree, and the persistence of populations is regulated by local as well as regional populations.

The high elevation mountain ranges selected in this study represent habitat islands for whitebark pine endophytes and are some of the most isolated ranges found in the Rocky Mountains. If no differences are found between sites varying in isolation, then dispersal limitation may not be biologically relevant for Rocky Mountain endophytic fungi. However, if species-distance relationships are strong, this would show the importance of habitat continuity and migration corridors even for fungi. Furthermore, it would add another disincentive for habitat fragmentation and the potential loss of biodiversity that can be caused by historically larger and more intense forest fires.

Objective 4: Characterize prevalence and intensity of WPBR in the krummholz ecotone.

Research Question 4.1: Is WPBR intensity and prevalence reduced at higher elevations?

To date, only one study has assessed the prevalence and intensity of white pine blister rust in the krummholz ecotone (Resler & Tomback, 2008). Given the ecological value of whitebark pines at high-elevations, understanding the health of these populations is important. Whitebark pines were found to be the primary initiator of tree-island

generation in the krummholz ecotone, displaying their value in structuring vegetative patterns in these communities. Establishment of tree-islands creates sheltered microclimates where less cold- and wind-tolerant species can then establish. These plant communities go on to stabilize the soil, preventing erosion, thereby influencing geochemical processes that stretch down the mountainside (Resler & Tomback, 2008).

Range-wide declines in whitebark pine populations may vary across ecotypes; factors such as relative humidity, temperature, and wind can have substantial impacts on the success and infection rate of WPBR. Kearns & Jacobi (2007) found that WPBR incidence in limber pine was negatively correlated with elevation. Resler & Tomback (2008) found that 33.7% of the whitebark pines in the krummholz ecotone had at least one potential or certain WPBR canker. Active or inactive cankers were found on 24.3% of all whitebark pines examined (Resler & Tomback, 2008). These data report similar values to what has been reported for WPBR incidence at lower elevations (Kearns & Jacobi, 2007). More data are needed to accurately assess the role that elevation plays in the formation of WPBR infection.

Objective 5: Characterize the endophytic fungal communities of Engelmann spruce and compare to whitebark pine.

Engelmann spruce is often codominant with whitebark pine in the northern Montana Rocky Mountains at the upper subalpine. Engelman spruce is well positioned to replace whitebark pine in these forests if whitebark pines populations continue to decline. Despite their prevalence in the subalpine, endophytic fungal communities of Engelmann spruce have not been examined and thus our characterization of these communities represents novel territory. In this study, we characterize novel spruce endophyte

communities and compare them to whitebark pine endophytes sharing the same ecosystem.

CHAPTER II

ENDOPHYTIC FUNGAL DIVERSITY IN WHITEBARK PINE: LINKS BETWEEN SPECIES ASSMBLAGE, BIOGEOGRAPHY, AND BLISTER RUST OCCURRENCE

Introduction

Whitebark pine (*Pinus albicaulis*) is both a keystone and a foundational species at high elevations in the northern Rocky Mountains (McCaughey & Schmidt, 2001; Resler & Tomback, 2008). Whitebark pine functions as a keystone species in part by producing large nuts that are dense in fat calories and protein, serving as a key food source for a wide variety of birds and mammals (Lanner & Gilbert, 1994; Tomback & Kendall, 2001; McKinney & Fiedler, 2010). Since whitebark pine is more tolerant of treeline conditions than other species, it has been proposed that whitebark is particularly important for establishing and expanding subalpine forests (Resler, 2004). The establishment of a whitebark pine creates a microclimate that mitigates the harshness of wind and temperature fluctuations found in these areas (Resler, 2004). This microclimate fosters establishment of a variety of alpine and subalpine forbs and trees, catalyzing a feedback loop that allows for many other species to establish and reside, including birds and mammals. Additionally, whitebark pine affects hydrological processes at both the local and landscape scale; by redistributing snowpack, acting as a snow trap, and providing

shade whitebark pine extends snowmelt discharge and stream flow over the summer (Keane *et al*., 2012; Tomback *et al*., 2016). It is for these reasons that whitebark pine is considered a foundational species, as it plays an imperative role in structuring the plant communities of the subalpine.

Whitebark pine is threatened by the invasive white pine blister rust (WPBR; *Cronartium ribicola)*, habitat fragmentation, shifting fire regimes, competitive habitat exclusion driven by climate change, and the mountain pine beetle (*Dendroctonus ponderosae*; Kendall & Keane, 2001; Gibson *et al*., 2008; Resler & Tomback, 2008; Pansing *et al*., 2020). These threats have caused range-wide population declines, with over 90% reduction in some areas and more dead standing whitebark pines than living ones (Kendall & Arno, 1990; Keane & Arno, 1993; Achuff *et al*, 2010; Goeking & Izlar, 2018). As a result, whitebark pine was recently listed as a threatened species under the United States Endangered Species Act and designated as an endangered species by the Committee on the Status of Endangered Wildlife in Canada with a high risk of extirpation in that country (Achuff *et al*, 2010; Fish and Wildlife Service, 2020). Mitigation of whitebark pine loss has been a conservation and forestry focus since the mid-1900s, yielding marginal improvements in regeneration and lowering the rate of decline (Kendall & Keane, 2001; Keane *et al*., 2012; Goeking & Izlar, 2018). Achieving greater success in supporting the persistence of these critical five-needle pine ecosystems will likely require novel approaches in combination with targeted forest management goals and regeneration efforts.

One such novel approach is to manipulate the plant microbiome, also known as endophytic organisms. Endophytism is most commonly defined as microorganisms

inhabiting asymptomatic plant tissues (i.e., lacking conspicuous infection), occurring within roots, stems, bark or leaves. Fungal endophytes represent a highly diverse, polyphyletic group that occur in all plant species that have been examined (Arnold *et al*., 2000; Ganley & Newcombe, 2006; Rodriguez *et al*., 2009). These microorganisms can induce increased drought tolerance in plants, reduce herbivory of host tissue, and provide various forms of resistance against fungal pathogens (Kimmons *et al*., 1990; Rowan & Latch, 1994; Clarke *et al*., 2006). Endophytes can deter fungal pathogens either directly (e.g., metabolite production, competitive habitat exclusion, and mycoparasitism) or indirectly (e.g., by inducing host resistance mechanisms) (Aneja *et al*., 2005; Bailey *et al*., 2006). Because *C. ribicola* infects the tree by growing through the needle stomata before migrating to the plant's vascular system, it may experience a close spatial interaction with needle endophytes during this critical first stage of infection. Research suggesting that needle endophytes may provide a first line of defense against blister rust make this interaction an imperative area of study (Ganley & Newcombe, 2006; Bullington, 2017).

Ganley *et al*. (2008) demonstrated that fungal endophytes in *Pinus monticola* (Western white pine) induce a systemic acquired resistance response that mediates the effects of the WPBR pathogen (Ganley *et al*., 2008). Fungal endophytes of coniferous trees have also been shown to produce a wide variety of metabolites that antagonize pathogens, delay needle senescence, or decrease herbivory (Sumarah *et al*., 2011; Richardson *et al*., 2014; McMullin *et al*., 2018). In whitebark pine, infection by *C. ribicola* is associated with foliar endophytic community shifts (Bullington *et al*., 2018; Moler & Aho, 2018). Taxa most closely resembling *Lophodermium*, *Paraphoma*, and

Metarhizium have been correlated with decreased disease severity (needle spotting and branch cankers) (Bullington *et al*., 2018).

In addition to the importance of whitebark pine and its fungal endophytes, the geographical distribution of this system provides another rationale for further study. Whitebark pine is restricted to high elevation habitats, resulting in a patchy distribution of "tree islands." This system therefore provides an opportunity to examine biogeographic questions such as the association of endophytic fungal communities with habitat isolation, species-area relationships, and dispersal capacity. One such lens through which this system can be viewed is that of island biogeographic theory. Island biogeographic theory has been used to explain the uneven distribution of species richness based on level of isolation (distance to source populations) and the size of the island (McArthur & Wilson, 1967). The dispersal limitations of organisms affect many ecological patterns such as origination-extinction rates, species richness, niche competition, and gene flow (concomitant with genetic diversity). Recently, studies using molecular methods have illuminated these patterns for microorganisms, disputing the Baas-Becking hypothesis that, "everything is everywhere but the environment selects" (Baas-Becking, 1934; Andrews *et al*., 1987; Peay *et al*. 2007; Sato *et al*., 2015; Li *et al*., 2020). However, there is still some debate as to whether all microorganisms display strong biogeographic patterns, to what degree microbial communities are controlled by the selecting environment, the extent of their host specificity, and at what scale dispersal ability limits community assemblage (Staley *et al*., 1997; Finlay, 2002; Fenchel & Finlay, 2003; Hedlund *et al*., 2003; Queloz *et al*., 2011a,b).

As well as being a dominant subalpine tree, whitebark pine pioneers the elevational advance of the treeline ecotone. At the highest elevations suitable for whitebark pine, a distinct morphology known as krummholz is predominant if not universal. Characterized by a windswept, vertically stunted shrublike growth and branches growing close to the ground, krummholz form marks the upper edge of the subalpine (Tomback, 1986; Arno & Hoff, 1990). Globally treeline is an important and sensitive bioindicator of historical and recent climate patterns (Kullman, 1998; Grace *et al*. 2002). In a meta-analysis of a global dataset with records dating back to 1900 CE, treeline advancement was recorded at 52% of sites with only 1% of sites showing recession and the others showing no change (Harsch *et al*., 2009). Currently, ecological niche modeling suggests that climate warming will have significant negative impacts on the distribution of whitebark pine; however, these models may underestimate the capacity for whitebark to respond to changing climate patterns. Given that endophytes confer advantages to their hosts under drought stress, understanding their role in treeline advancing plant communities may lead to novel insights pertinent to global change ecology.

By sampling across gradients of elevation, habitat size/isolation, and disease (white pine blister rust) severity, we addressed questions related to the roles of habitat, elevation, dispersal limitation, disease state, and tree health in shaping these fungal communities. Additionally, we examined whitebark pine's dynamic role at the treeline ecotone, where it pioneers treeline advance into the alpine zone, tying in questions regarding climate change and the role it plays in structuring new plant and fungal communities in the highest suitable whitebark pine habitats. Finally, we compared host

specificity of fungal endophytic communities between whitebark pine and Engelmann spruce, which are co-dominant and competing species in the subalpine (Tomback *et al*., 2014). Other conifers may harbor whitebark pine endophytes, understanding dimensions of host specificity is therefore necessary to infer geographic drivers of community composition. Furthermore, whitebark pine endophytes may be of conservation concern if they are highly specialized to a single host species.

Methods

Study Area

The study sites are located in the Rocky Mountains, Montana, USA at 3,000 – 3,120 m.a.s.l. (meters above sea level) in krummholz WBP stands and at 2,621 – 2,743 m.a.s.l. in the nearest (relative to the krummholz stand) lower elevation WBP stands. Whitebark pine (*Pinus albicaulis*), subalpine fir (*Abies lasiocarpa*), limber pine (*Pinus flexilis*), lodgepole pine (*Pinus contorta* var. *latifolia*), and Engelmann spruce (*Picea engelmannii*) compose the subalpine forest zone, beginning at about 2,600 m.a.s.l. Whitebark pine can occur in monotypic stands, although mixed stands are more common. The climate in the Montana Rocky Mountains is cold, dry, and windy, with precipitation differing between elevations and mountain range locations. The study sites, from east to west, were: Beartooth Plateau (northeast of the Greater Yellowstone Ecosystem), Crazy Mountains, Sacajawea Saddle (Bridger Range), Mount Cowen (Absaroka Range), and St. Mary's Peak (Bitterroot Range; Fig. 6).

The climate in the high elevation krummholz ecotone is colder, drier, and windier than in subalpine stands. Krummholz stands are characterized by trees having a windswept, stunted morphology, needle flagging on the side of the predominant wind,

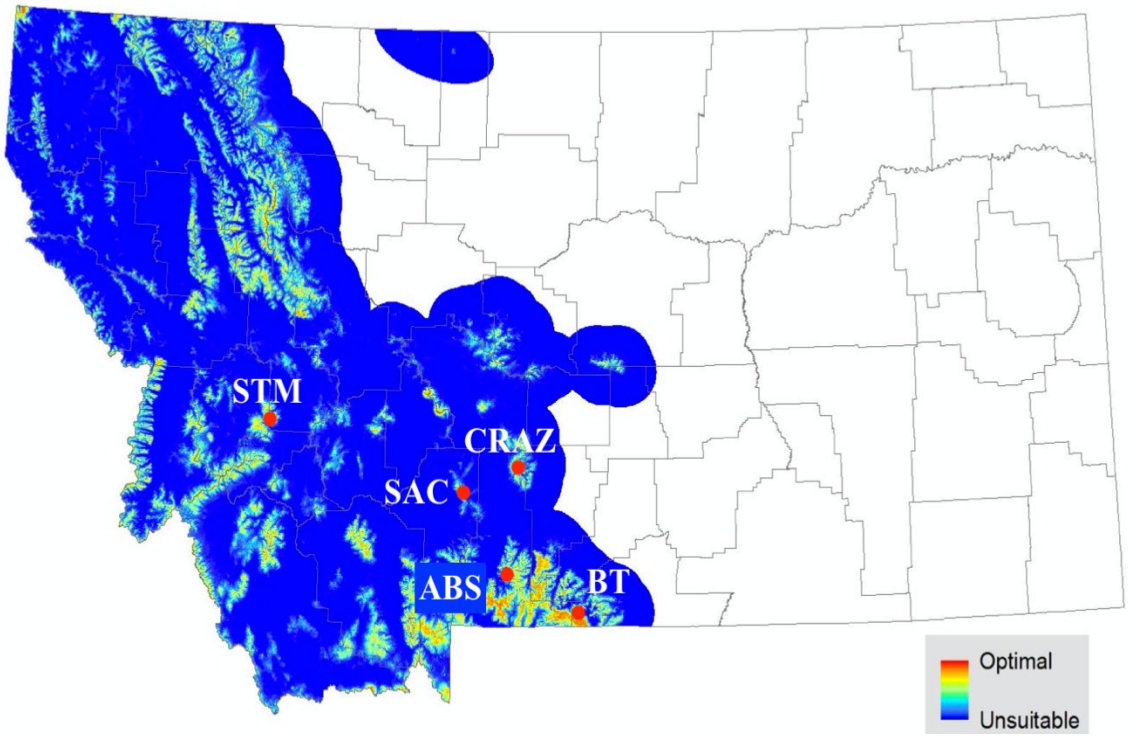

Fig. 6 Map of continuous habitat suitability model for whitebark pine in the Montana Rocky Mountains with sites (red dots) and abbreviations. Sites listed from east to west, were: Beartooth Plateau (BT), Crazy Mountains (CRAZ), Sacajawea Saddle (SAC), Mount Cowen (ABS), and St. Mary's Peak (STM; modified from Montana National Heritage Program, 2018).

Krummholz stands were divided into two distinct ecotypes: pioneering krummholz and established krummholz. Pioneering krummholz stands represent the "treeline advance," where seedlings and young trees are being recruited at higher elevations. Established krummholz stands represent the forest's previous altitudinal limit, where density, tree age, and recruitment has been occurring for at least the last 100 years (Harsch *et al*., 2009). Trees occupying the established krummholz ecotype are often mature but still possess the suite of morphological features attributed to krummholz trees. Established krummholz was sampled in the highest elevation mature krummholz stands

on the mountain, while lower elevation sampling took place in the nearest adjacent mature whitebark pine stands.

Site Characterization

Site characterization and needle collection took place within 48 hours of each other at the Beartooth Plateau (northeast of the Greater Yellowstone Ecosystem), Crazy Mountains, Sacajawea Saddle (Bridger Range), Mount Cowen (Absaroka Range), and St. Mary's Peak (Bitterroot Range; Fig. 6) in early August 2019. Each site contained three distinct ecotypes: pioneering krummholz, established krummholz, and lower elevation (Fig. 7). Plot centers were selected to be representative of the surrounding forest in terms of species composition and density, WPBR infection frequency, and dead tree density. We characterized established krummholz and lower elevation stands for tree species composition and density, WPBR presence, canopy kill, and presence of dead trees within a 60 m^2 plot. For all trees within the plot, WPBR presence was recorded as either P (potential; symptoms such as branch swelling but no past or present sporulation), I (inactive; old canker that sporulated, leaving a scar on dead and

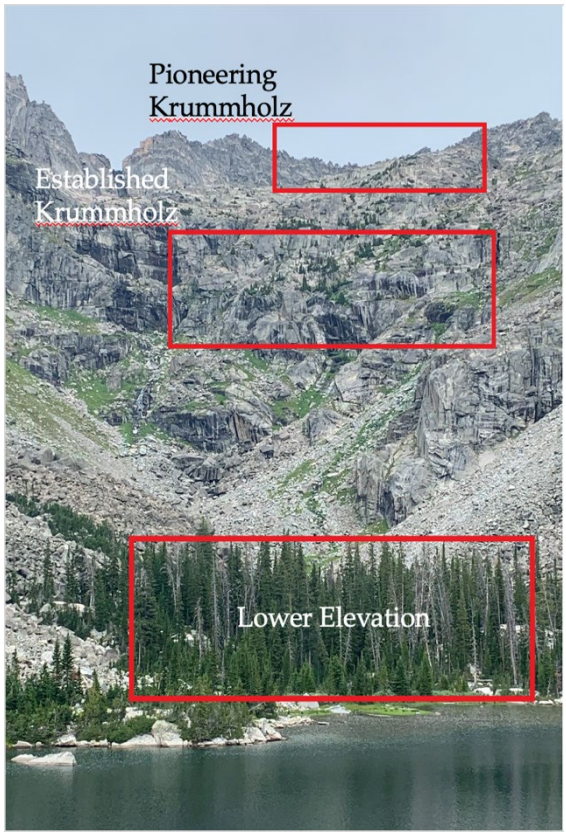

Fig. 7 Ecotype characterization took place along an elevational gradient from highest to lowest elevation: pioneering krummholz, established krummholz, and lower elevation.

cracked bark along with dead local foliage), A (active; current year's aecia present), IA (inactive and active cankers present), or N (none; no signs or symptoms of WPBR). Canopy kill was classified for each tree as 1 (none), 2 (1-24%), 3 (25-49%), 4 (50-74%), 5 (75-100%) (modified from Resler & Tomback, 2008). Presence of alternate host species of WPBR (*Ribes* spp., *Pedicularis* spp., and *Castelleja* spp.*)* was recorded as being (1) in the plot, (2) absent from the plot but within 50 meters of the plot perimeter, or (3) absent from both the plot and 50 meters from the plot perimeter. Additionally, within the plot, we recorded all dead trees, the aspect of the plot, and GPS coordinates, and photographed every tree. Examples of field sampling data sheets are located in Appendix A.

Due to low tree density in the pioneering krummholz ecotone, we did not establish plots. Instead, we sampled the 10 highest elevation whitebark pine and Engelmann spruce descending down the mountain (Fig. 8). Additionally, individual trees were assessed for WPBR presence (as described above), canopy kill, and number and species of trees inhabiting the same microhabitat; to qualify as sharing a microhabitat, trees

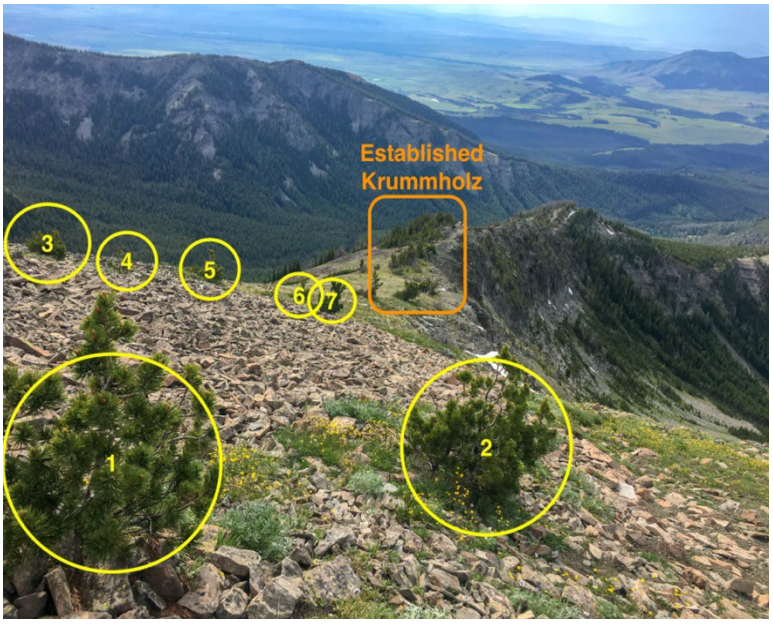

Fig. 8 Photograph of pioneering krummholz and established krummholz ecotypes depicting elevational descending sampling scheme.

needed to either have overlapping foliage or share a sheltered microsite (rock, log, vegetation, terrace riser, or a small topographical depression). Microhabitat was classified in size for each tree as 1 (solitary tree), 2 (two trees), 3 (three trees), 4 (10> trees >4 trees), 5 (15> trees >10 trees), and 6 (15< trees). Microhabitat was classified in terms of tree species composition as W (whitebark pine), S (Engelmann spruce), and D (subalpine fir); when combinations of species occurred, they were recorded (e.g., whitebark pine, Engelmann spruce, and subalpine fir would be recorded as WSD). Microhabitats were assessed as a potential control for variation in endophytic community assemblages. We summarized plant community data at sites to illuminate any obvious differences that may drive differences in fungal community composition (Appendix D, Table 1).

Tree Selection

Sixty trees were selected for needle collection at each site (20 from each of the three ecotypes, except for the St. Mary's Peak site where only 40 trees were sampled due to lack of pioneering krummholz), with the methods for selecting the trees varying based on ecotype. In the pioneering krummholz ecotype, the 10 highest elevation whitebark pine and spruce were sampled. In the lower elevation krummholz ecotype and the subalpine ecotype, trees were selected strategically to cover a spectrum of WPBR severity. In the pioneering krummholz ecotype, trees are sparse and thus selecting across a WPBR gradient was not an option at most sites. However, in ideal cases, three whitebark pine trees were selected that were completely healthy, two that had a low-severity WPBR infection (1-24% canopy kill), two that had a mid-severity infection (25-74% canopy kill), and three that had a high-severity infection (75-99% canopy kill).

Engelmann spruce individuals were selected that were healthy but were otherwise not subjected any further considerations since they are not hosts of WPBR.

Needle Collection

On each tree selected, 10 mid-branch needle fascicles from the lower canopy (0-3 meters in height) were collected (Ganley & Newcombe, 2006; Larkin *et al*., 2012). To avoid bias toward young or old needles and to optimize occurrence of endophytes, second- and third-year needle fascicles were selected (Johnson & Whitney, 1992). For Engelmann spruce we collected 10 branches approximately 3-5 inches in length (containing first, second-, and third-year needles) from the lower canopy to mirror whitebark collection.

In order to preferentially compare communities of endophytic rather than saprotrophic and pathogenic fungi across samples, needles and branches appearing green and healthy were preferentially collected against those showing blemishes, scarring, full or partial senescence, or evidence of pathogens (Bullington, 2017; Moler & Aho, 2018). An individual whitebark pine needle fascicle or Engelmann spruce branch was collected at equally spaced positions around the tree (approximately every 36°), resulting in 10 fascicles or 10 branches collected per tree (Ganley & Newcombe, 2006). The 10 needle fascicles or branches were stored in a Ziploc bag, labeled with site and tree number, then immediately placed on ice in a cooler. Needles and branches remained in the cooler for a maximum of 48 hours before being transferred to a -20°C lab freezer at Montana State University (MSU). One final transfer of needles and branches took place after all sites had been sampled: all samples were frozen for 48 hours in a -80°C freezer, and samples were expeditiously placed on ice in a cooler. The cooler was then transported via vehicle

to the University of Wisconsin-La Crosse (UWL). All samples were then transferred into the -20°C freezer at UWL within 22 hours of being removed from the freezer at MSU.

Needle Processing & DNA Extraction

For whitebark pine, two needles were selected from each of the 10 fascicles collected from an individual tree for surface sterilization and DNA extraction (20 total needles selected). For spruce, two needles were selected from each of the 10 branches collected from an individual tree. For both species, healthy green second and third year needles were preferentially selected. All needles were surface sterilized in 70% EtOH for 1 minute, 6% NaOCl for 5 minutes, and 70% EtOH for 1 minute (Larkin *et al*., 2012). Samples were air dried on ethanol and flame sterilized microscope slides. To verify successful surface sterilization, a subset of two randomly selected needles from each site were streaked across the surface of a petri dish and monitored for fungal growth in the following weeks; no growth was found at any point (Arnold *et al*., 2003). Tissue was then freeze-dried using liquid nitrogen, macerated to a fine powder and homogenized using a sterile mortar and pestle (Bullington & Larkin, 2015; Moler & Aho, 2018), then stored at -20°C in 1.5 mL microfuge tubes until DNA extraction was performed.

Genomic DNA from whitebark pine needle tissue was extracted using the Nucleospin® Plant II kit (Machery-Nagel). 45 mg of homogenized needle tissue was added to a separate 1.5 mL microfuge tube and DNA was extracted following kit instructions; PL2 extraction buffer outperformed PL1 in test runs and was therefore used for all whitebark pine extractions. Negative controls were run from extraction through PCR amplification by treating tissue-free (empty) extraction tubes to the exact suite of procedures as used for the sample tubes. Genomic DNA from spruce needles was

extracted using Qiagen DNEasy Plant Mini kits (Carlsbad, CA, USA). Successful DNA extraction was confirmed using either the Qubit dsDNA HS (high sensitivity) Assay Kit (Invitrogen, Waltham, MA, USA) or a NanoDrop 2000 Spectrophotometer (Thermo Scientific™). Because whitebark pine is a very difficult substrate from which to obtain suitable amounts of DNA with low concentrations of inhibitors, we tested numerous extractions methods and PCR protocols on these recalcitrant plant tissues; a full recounting of unsuccessful methods can be found in Appendix B.

Amplification, Sequencing & Sequence Analysis

Extracted fungal genomic DNA from whitebark pine needles was amplified from three portions of the nuclear ribosomal DNA repeat using a doubly dual indexed two-step library preparation process (Glenn *et al*., 2019a,b). The following primers were used as the core for Adapterama locus-specific fusion primers that include an index sequence and a universal primer sequence. The nuclear ribosomal internal transcribed spacer 5' portion (ITS1) was amplified using the primer pair ITS1F/ITS2 as the core; the internal transcribed spacer 3' portion (ITS2) was amplified using the primer pair fITS7/ITS4 as the core (Ihrmark *et al*., 2012); and the 5' portion of the nuclear ribosomal large subunit (LSU) was amplified using the primer pair LROR/LR21 as the core (R. Vilgalys lab, Duke University). The 140 whitebark pine samples were randomly divided into three sets/plates before amplification. The 110 spruce samples were amplified in a similar manner to what is described below, with some variation due to differences in amplification success between species for methods tested in a pilot study. Methods for spruce endophyte amplification can be found in Appendix C. For each locus, two PCR amplifications were conducted: a primary PCR to amplify the specific locus and add a

pair of index sequences that are combinatorially unique for each sample within the set (PCR plate); and, following pooling of each set, a secondary PCR to add Illumina-compatible i5 and i7 adapters and a second pair of unique dual index sequences that identify each set. Primary PCR reactions were carried out in 25 µL reaction volumes containing 3 µL of template, 2.5 µL 2x Dream Taq™ DNA polymerase buffer containing $MgCl_2$ (Thermo Scientific™), 2.5 µL of 2.5 mM dNTP mix, 0.2 µL Taq (5U/µL), 14 µL H_2O and 1.4 µL of each 10 µM forward and reverse fusion primer. Each reaction was performed on a T100™ Thermal Cycler (BIO-RAD) under the following conditions: 2 min at 95 °C followed by 40 cycles of 60 s at 95°C, 30 s at 50°C, 60 s at 72°C, and a final extension step at 72°C for 5 min. Amplification of the target region was verified and scored (1-10 intensity scale) on a 1.2% agarose gel using a 100bp ladder. Failed amplifications were re-run under the same conditions and constituted a new sixth set/pool. Samples from each set were then pooled together using the intensity to include approximately the same amount of amplification product from each sample. A 100 µL volume of each primary PCR pool was then cleaned and size-selected using a two-step magnetic bead (Axygen® AxyPrep Mag) cleanup protocol using a 0.5x bead-to-sample (v:v) ratio in the first cleanup, followed by a 0.7x ratio in the second cleanup to select for a fragment size range of approximately 330-700 base pairs. DNA was eluted in 40 µL of Tris-low EDTA, effectively concentrating pooled PCR products approximately twofold (Jian, 2017).

The secondary PCR (ligation of plate specific barcodes and Illumina adapters) was carried out for each pooled set for each of the three loci in 25 µL reaction volumes containing 10 µL of pooled and bead-cleaned PCR product, 2.5 µL 2x Dream Taq™

DNA polymerase buffer containing $MgCl_2$ (Thermo Scientific™), 3 µL of 2.5mM dNTP mix, 0.2 µL Taq (5 U/ µL), 4.3 µL H_2O and 2.5 µL of each 5 µM forward and reverse primer. Each reaction was performed on a T100™ Thermal Cycler (BIO-RAD) under the following conditions: 5 min at 95 °C followed by 6 cycles of 30 s at 94°C, 30 s at 60°C, 30 s at 72°C, and a final extension step at 72°C for 5 min. PCR reactions were then cleaned in a one-step magnetic bead (Axygen® AxyPrep Mag) cleanup using a 1.8x (v:v) bead-to-sample ratio to remove primers and other DNA fragments less than ~125 bp in length. DNA was eluted in 30 µL of Tris-low EDTA buffer and then quantified using a Qubit dsDNA BS (broad spectrum) Assay Kit (Invitrogen, Waltham, MA, USA). A subset of eluted PCR product was verified for appropriate band length on 1.2% agarose gel using a 100bp ladder for comparison. PCR products were then pooled in equimolar concentrations to produce the final pooled, doubly dual-indexed library. This pool was then concentrated using a SpeedVac™ vacuum concentrator (Thermo Scientific) for approximately two hours until a final concentration of 17 ng/µL was reached in a final volume of 40 µL. Amplicon libraries were sequenced using 2 x 250 paired-end Illumina MiSeq sequencing at the University of Wisconsin Biotechnology Center DNA Sequencing Facility (Madison, WI).

Demultiplexing of pools based on the outer (set-specific) index pair was conducted by the sequencing facility using bcl2fastq (Illumina). Within-set demultiplexing based on the inner (sample-within-set) index pair was conducted using Mr. Demuxy (https://github.com/lefeverde/Mr_Demuxy). Trimming and quality filtering was conducted using Trimmomatic v. 0.38 (Bolger *et al*., 2014). AMPtk v. 1.5.4 (Palmer *et al*., 2018) was used to concatenate sequences, cluster operational taxonomic units

(OTUs) at > 97% sequence similarity using UPARSE, filter the OTU table to remove singletons and apply a 0.6% index bleed filter (Oono *et al*., 2020), and assign taxonomy using the hybrid taxonomy algorithm. OTUs identified at taxonomic ranks higher than the genus level by AMPtk were searched against the National Center for Biotechnology Information (NCBI) database, which in many cases revealed greater taxonomic resolution; the BIOM file produced by AMPtk was modified to include this additional information prior to further analyses.

Statistical Analyses

Analyses of endophyte alpha and beta diversity were conducted using QIIME2 (Bolyen *et al*., 2019). For all community analyses, we rarified sequencing depth to 500 sequences per tree. Trees with fewer than 500 sequences were removed from further analysis, resulting in a total of 49 whitebark pine and 64 spruce trees. Because sequence read counts are poorly correlated with actual abundance (Amend *et al*., 2010), analyses were conducted using presence-absence metrics only. The QIIME2 diversity core-metrics function was used to calculate an OTU richness (observed features) metric for each sample and calculate a Jaccard distance matrix. QIIME2 was used to generate data visualizations including principal coordinate analyses (PCoA) ordination plots, box and whisker plots, rarefaction curves, and taxonomic bar plots. Whitebark pine was separated from spruce for the majority of these tests except where otherwise stated.

Significance tests for alpha diversity (within-sample OTU richness) were conducted using Kruskal-Wallis tests to examine differences in richness between whitebark pine across sites, ecotypes, blister rust presence, microhabitat size and composition, and aspect. To examine the contribution of host species, we also conducted

these tests with the combined whitebark pine and spruce dataset for all factors excluding blister rust presence and canopy kill (since spruce is not affected by WBPR).

Beta (between sample) diversity was examined using principal coordinate analysis (PCoA) ordination plots to visualize community differences as a factor of host species, aspect, blister rust presence, canopy kill, ecotype and study site based on the Jaccard distance matrix. A multivariate test for significance of beta diversity was conducted using the ADONIS function in QIIME2, with study site, ecotype, canopy kill, blister rust presence, microhabitat size, and microhabitat composition as main effects. Based on the results of this test and in order to determine which specific contrasts showed significant differences in beta diversity, PERMANOVA (permutational multivariate analysis of variance) analyses were conducted with 999 permutations to test for endophyte community dissimilarity among 1) site, 2) ecotype, 3) microhabitat size, and 4) microhabitat composition (Anderson, 2001; Oksanen *et al*., 2020).

Plant community characteristics were compared between sites to determine additional factors that could influence differences between sampling sites. All statistical analyses were conducted using R v3.4.1 (R core team, 2020). We used a one-way analysis of means to compare tree density between sites and a Kruskal-Wallis test to compare tree density among ecotype where density was assessed within plots (lower elevation and established krummholz). We conducted ANOVAs and Tukey's HSD tests to assess the effects of site, ecotype, and blister rust presence on canopy kill. Kruskal-Wallis tests were also used to test whether canopy kill varied between sites, ecotypes, and levels of blister rust presence. We utilized Kruskal-Wallis tests because it is a better fit

for non-parametric data. Tukey's HSD was fit from ANOVAs to determine which ecotypes varied and by how much.

We ran an indicator species analysis using the R package "indicspecies" to determine whether any specific OTUs were significantly associated with individual or groups of sites, ecotypes, blister rust presence levels or canopy kill classes (R version 3.4.1). Analyses used the *multipatt* function to calculate Pearson's phi coefficient of association, corrected for unequal numbers of sites per group (*r.g.* function), using 999 permutations of the OTU presence-absence matrix.

Results

Plant Community Characteristics

We found no significant differences in plant density between sites (one-way ANOVA, $P = 0.715$) and ecotypes for lower elevation and established krummholz (Wilcoxon Rank Sum, $P = 0.256$). Canopy kill was significantly associated with the level of blister rust presence (Kruskal-Wallis rank sum test, $P < 0.001$). Blister rust presence, site, and their interactive effect both had a significant effect on average canopy kill (ANOVA, $P < 0.001$ for all), while ecotype was not found to be significant factor. Trees with both active and inactive cankers had higher average canopy kill than either active or inactive blister rust alone (Tukey's HSD, $P < 0.001$ for both).

Sequencing Depth, OTU recovery, and Rarefaction Curves

After index bleed filtering and removal of OTUs found in negative controls, 302 OTUs remained that were subjected to our tests on alpha diversity, beta diversity, and species occurrence (indicator species analysis). We did not detect ITS2 OTUs matching *C. ribicola* in any of the samples despite obvious infection of sampled and nearby trees.

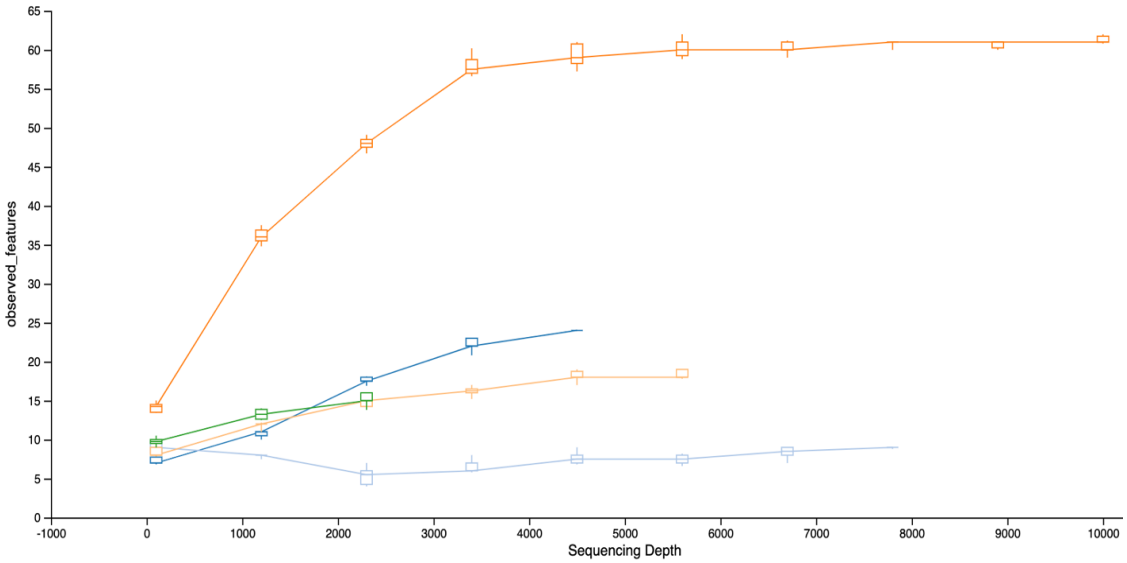

Fig. 9 Rarefaction curve for whitebark samples with OTUs (observed features) on the y-axis and sequencing depth on the x-axis. Absaroka Range (navy blue), Beartooth Plateau (light blue), St. Mary's Peak (green), Sacajawea Saddle (yellow), Crazy Mountains (orange).

Alpha diversity

OTU alpha diversity in whitebark pine samples differed between sites when all groups were compared (Kruskal-Wallis, P= 0.0147). Pairwise comparisons of alpha diversity showed significantly greater diversity at the Crazy Mountains than at multiple sites: Beartooth Plateau (P = 0.009), Absaroka Range (P = 0.006), and Sacajawea Saddle

(P = 0.005; Fig. 10). At the ecotype level, alpha diversity differed significantly between pioneering krummholz and lower elevation ecotypes (Kruskal-Wallis, P = 0.007); alpha diversity was highest in the lower elevation sites and lowest in pioneering krummholz sites. In regard to blister rust presence level, whitebark pines without outward signs of infection had significantly less diversity than trees with inactive cankers (KW test, P = 0.001; Fig. 11). Alpha diversity did not significantly differ between samples based on microhabitat size classes, microhabitat composition types, or aspect. Whitebark pine and spruce showed significant differences (P = 0.009), with whitebark pine showing greater alpha diversity than spruce (Fig. 12).

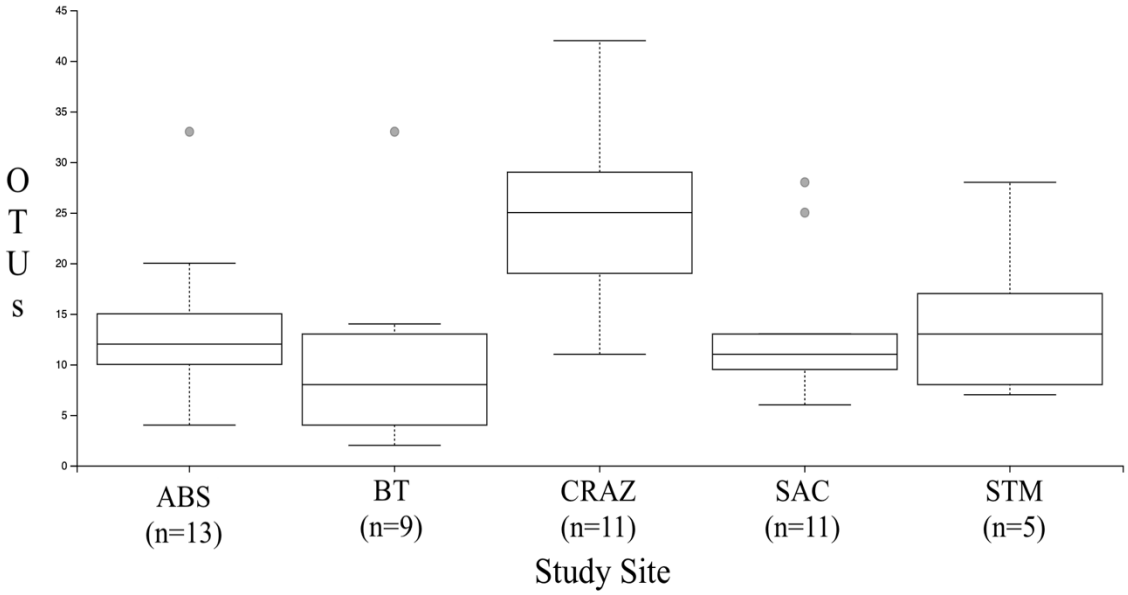

Fig. 10 Box and whisker plot of number of alpha diversity (OTUs) in each study site: ABS (Absaroka Range), BT (Beartooth Plateau), CRAZ (Crazy Mountains), SAC (Sacajawea Saddle), STM (St. Mary's Peak). Pairwise comparisons of alpha diversity showed significant differences between the Crazy Mountains and multiple sites: Beartooth Plateau (P = 0.009), Absaroka Range (P = 0.006), and Sacajawea Saddle.

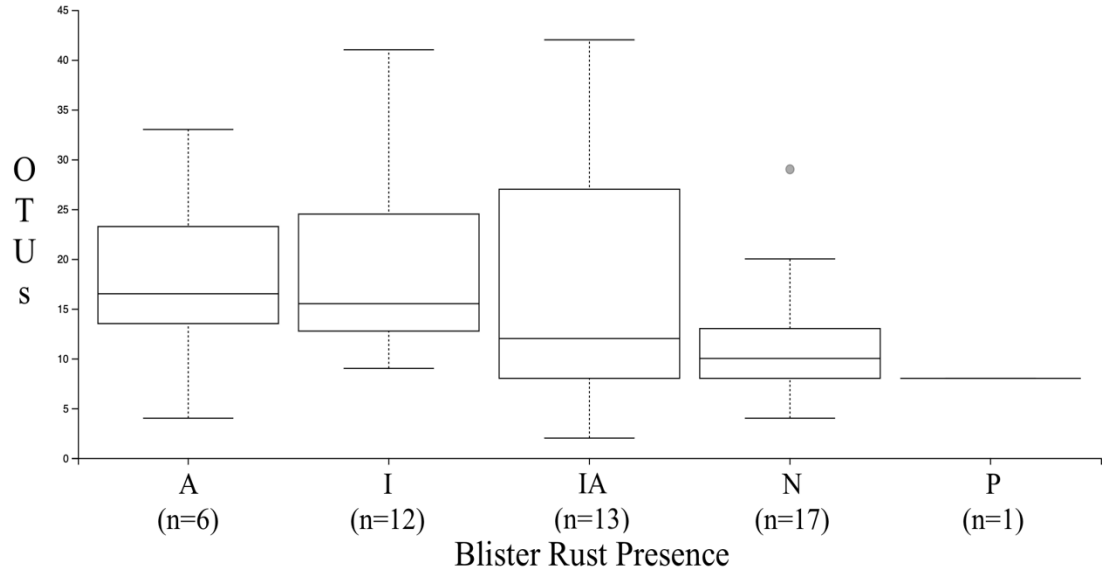

Fig. 11 Box and whisker plot of alpha diversity (OTUs) divided into WPBR infection stage: A (active), I (inactive), IA (inactive and active), N (none or no infection), P (potential). Non-infected trees had significantly lower diversity than trees with inactive infection.

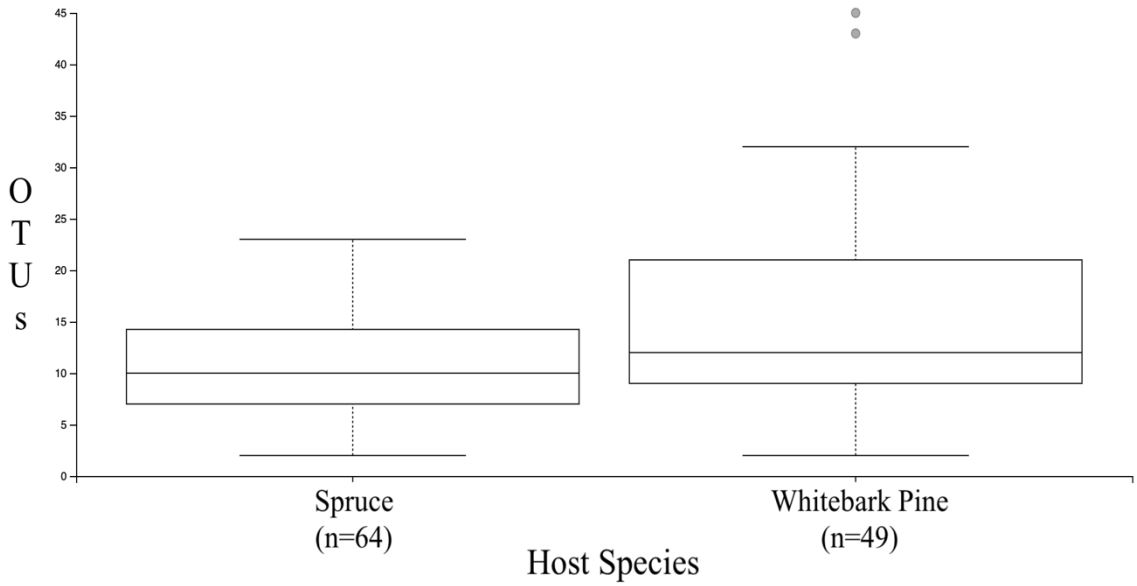

Fig. 12 Box and whisker plot comparing alpha diversity between host tree species (whitebark pine and spruce). Results of Kruskal-Wallis test showed significantly greater alpha diversity in whitebark pine (P=0.009).

Beta Diversity

Beta diversity showed significant dissimilarity between study sites (Adonis, R^2 = 0.128, P = 0.001), ecotypes (Adonis, R^2 = 0.067, P = 0.001), microhabitat sizes (Adonis, R^2 = 0.105, P = 0.039), microhabitat compositions (Adonis, R^2 = 0.187, P = 0.015), and blister rust presence groups (PERMANOVA, n = 49, P = 0.031). These differences appeared largest when comparing groups to uninfected trees (no active signs of infection) and past infection (inactive cankers; Appendix E; Figs 13, 14). Pairwise comparisons showed significant differences in beta diversity between trees with active infection vs no infection (PERMANOVA, P=0.049), and inactive infection vs. no infection (0.003). Beta diversity dissimilarity also varied significantly between ecotypes (PERMANOVA, P = 0.001) and between sites (PERMANOVA, P = 0.001). In pairwise comparisons of beta diversity all sites varied significantly from one another except for Absaroka sites vs. Beartooth Plateau sites (PERMANOVA, P=0.072), and Beartooth Plateau sites vs. St. Mary's Peak sites (PERMANOVA, P=0.630). All ecotype pairwise comparisons of beta diversity showed significant differences (PERMANOVA, P<0.05).

A three-dimensional PCoA ordination plot of whitebark pine and spruce samples combined explained a cumulative 33.295% of variance in taxonomic composition, with 19.61%, 8.674%, and 5.011% of the variance explained by principal coordinates (PC) 1, 2, and 3, respectively (Fig. 15). Taxonomic composition of fungal communities among Engelmann spruce samples was more similar than among whitebark pine samples, as can be seen by the tighter clustering of the spruce samples. It should be noted that infected whitebark pine trees were included in this PCoA, and therefore taxonomic variation in whitebark endophytes may be due to disease state.

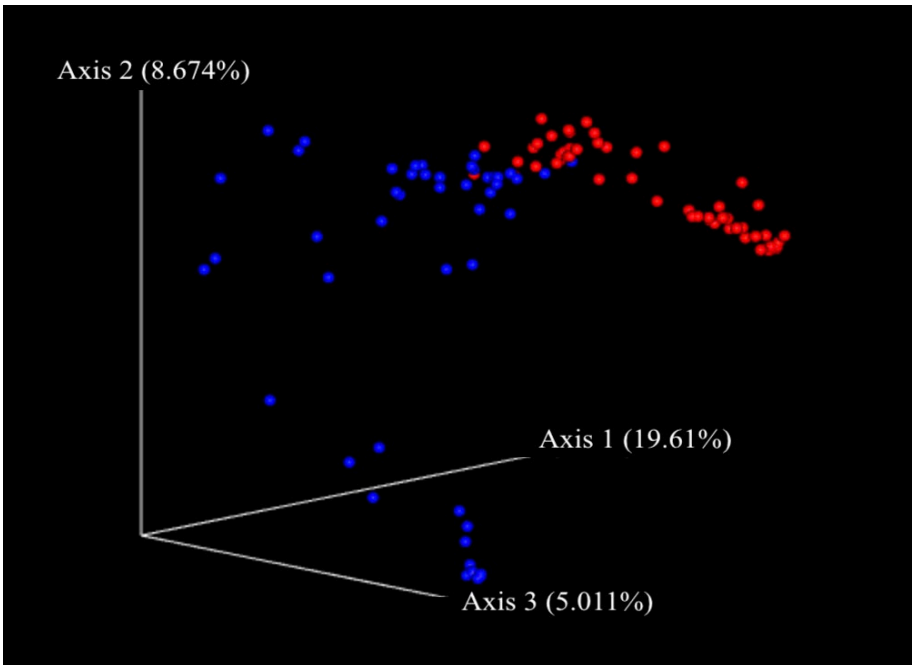

Fig. 15 PCoA of whitebark pine (blue) and spruce (red) samples combined explained a cumulative 33.295% of variance in taxonomic composition (bray-curtis), with 19.61%, 8.674%, and 5.011% being explained by principal coordinate (PC) 1, 2, and 3, respectively

Indicator Species Analysis

Indicator species analysis revealed significant associations of OTUs with multiple variables (sites, ecotypes, blister rust presence, and canopy kill class) for whitebark pine

samples containing a total of 302 OTUs. A multiple level pattern analysis to detect OTUs that are differentially associated with specific sites or groups of sites revealed 28 OTUs that were differentially associated with a single site, 13 OTUs that were associated with two sites, and 6 OTUs that were associated with three sites. OTUs most closely matching unidentified *Phaeomoniellales* and *Neocatenulostroma microsporum* were found to differentially occur in the Absaroka site. OTUs most closely matching *Lecanora laxa*, *Hypogymnia physodes*, *Flammulina velutipes*, unidentified *Ascomycota* (2), *Lecanoromycetes*, *Chaetothyriales*, *Penicillium*, and *Tremella* were found to differentially occur in the Crazy Mountains sites. OTUs most closely matching *Cyclaneusma minus*, *Wettsteinina dryadis*, *Elasticomyces elasticus*, unidentified *Leotiomycetes* (3), and *Helotiales* (3) were found to differentially occur in the Sacajawea site. OTUs most closely matching *Stachybotrys chartarum*, unidentified *Sordariomycetes*, *Sarcinomyces*, *Bulgariaceae*, *Rhytismataceae*, *Pleurotus*, and *Humicola* were found to differentially occur in the St. Mary's Peak sites. OTUs most closely matching unidentified *Helotiales* were found to differentially occur at both the Absaroka and Crazy Mountain sites. OTUs most closely matching unidentified *Ascomycota* were found to differentially occur at both Absaroka and Sacajawea Saddle sites. OTUs most closely matching unidentified *Ascomycota*, *Capnodiales*, *Taphrina*, and *Tremella* were found to differentially occur at both the Beartooth Plateau and Crazy Mountain sites. OTUs most closely matching *Lophodermium nitens* and unidentified *Rhytismataceae* were found to differentially occur at both the Beartooth Plateau and St. Mary's Peak sites. OTUs most closely matching unidentified *Capnodiales* were found to differentially occur at both the Crazy Mountains and Sacajawea sites. OTUs most closely

matching unidentified *Ascomycota*, *Sporormiaceae*, *Phaeomoniella*, and *Perusta* were found to differentially occur at both the Crazy Mountains and St. Mary's Peak sites. OTUs most closely matching unidentified *Ascomycota* and *Pseudeurotium* were found to differentially occur at Absaroka, Crazy Mountains, and Sacajawea sites. OTUs most closely matching *Apiotrichum porosum*, unidentified *Ascomycota*, and *Dothideomycetes* were found to differentially occur at Beartooth Plateau, Crazy Mountains, and St. Mary's Peak sites.

A multiple level pattern analysis revealed 51 OTUs that occurred significantly within a single ecotype, and seven OTUs that were associated with two ecotypes. Of the 7 OTUs that occurred significantly at two ecotypes, five of those were shared between lower elevation and established krummholz sites, and two of those were shared between established krummholz and pioneering krummholz. There were no OTUs that were found to differentially occur at both lower elevation and pioneering krummholz sites. Since 51 OTUs were found to differentially occur at this level of data aggregation, we only listed OTUs occurring with a significance level of 0.001. OTUs most closely matching *Lophodermium fissuratum*, unidentified *Ascomycota* (3), *Dothideomycetes*, *Rhytismataceae*, *Phaeomoniellaceae*, *Phaeomoniella*, *Mycosphaerella*, and *Taphrina* were found to differentially occur at lower elevation sites. OTUs most closely matching unidentified *Rhytismataceae* were found to differentially occur at established krummholz sites. OTUs most closely matching *Melanodiplodia tianschanica* were found to differentially occur at pioneering krummholz sites. OTUs most closely matching *Lophodermium nitens* were found to differentially occur at both lower elevation and

established krummholz sites. OTUs most closely matching *Pseudeurotium* were found to differentially occur at both established krummholz and pioneering krummholz sites.

In terms of blister rust presence level only six OTUs within a single stage of blister rust infection were found to have a significant level of occurrence. However, that stage of infection (potential) only had a single sample and therefore broke assumptions required for indicator species analysis. Thereby, we found that among no stage of blister rust presence had significant occurrence of any OTUs. When testing canopy kill classes for significant occurrence of OTUs we found that six OTUs were significantly associated with individual canopy kill classes and that two were significantly associated with two canopy kill classes. Unidentified OTUs most closely matching *Ascomycota* and *Helotiales* were found significantly associated with canopy kill class 3, *Sarcinomyces crustaceus* was significantly associated with class 4, and *Flammulina velutipes*, *Ganoderma applanatum*, and *Bulleromyces albus* were significantly associated with canopy kill class 5. Unidentified OTUs most closely matching *Lapidomyces* were associated with canopy class 1 and 5, and *Mycosphaerellaceae* was associated with classes 4 and 5.

Discussion

Overall, we recovered 302 OTUs from whitebark pine across all samples (a full list of OTUs recovered from whitebark pine can be found in Appendix F). We found less diversity than Bullington *et al*. (2018) who recovered 1,348 OTUs, and Moler & Aho (2018) who recovered 2,737 OTUs, though there are some notable differences in OTU filtering and study design. In Moler & Aho's 2018 study, 75% of OTUs were singletons and 85% of OTUs were either singletons or doubletons; they removed singletons for

community analysis but not doubletons, leaving them with 684 OTUs. In our study we removed singletons. Bullington *et al.* (2018) did not state if singletons were removed; they performed a robust filtering strategy that may have allowed them to confidently keep singleton OTUs. Moler & Aho (2018) sequenced 96 samples of whitebark pine and normalized to a depth of 2,646 unique sequences per tree. Bullington *et al*. (2018) analyzed community data on 127 whitebark pine individuals and rarefied to a depth of 900 sequences per sample.

There are some notable study design differences between our study and the previous two whitebark pine studies, namely location and tree age. Bullington *et al.* (2018) sampled whitebark pine seedlings, while we primarily sampled mature trees, though deciphering tree age in the subalpine and krummholz is difficult because conditions facilitate extremely slow growth. Trees in the krummholz ecotone appearing rather young based on height and basal diameter can actually be quite old. Research on fungal diversity in relation to tree age is limited, though one study found a strong negative trend in diversity from *Citrus sinensis* trees ranging from two to five years old (Juybari *et al*., 2019). In contrast, Ganley & Newcombe (2006) found a negative correlation in diversity with increasing tree age, but they note that their sample size for recorded tree age was quite low.

Additionally, both of these previous studies occurred in the Pacific Northwest (PNW), the climate of which varies considerably from the Montana Rocky Mountain subalpine. Oita *et al*. (2021b) found that richness decreased linearly with temperature seasonality and precipitation seasonality, both of which are greater in the subalpine Rocky Mountains of Montana than the PNW. Additionally, species diversity of fungal

endophytes appears to peak in the tropics and decrease with increasing latitude, suggesting that temperature plays a key role in endophytic diversity (Arnold & Lutzoni, 2007). Furthermore, Pinophyta-associated endophyte diversity has been shown to increase with increasing growing season length (U'ren *et al*., 2012). Considering this previous work, we would expect the endophytes of whitebark pine found in the Rocky Mountains to be less species rich. However, we do still have concerns around our sampling depth and sequencing sufficiency.

Diversity Differences Among Sites and Ecotypes

We hypothesized that sites with greater local populations of whitebark pine (Beartooth Plateau, St. Mary's Peak, Absaroka Range) would have higher alpha diversity and sites closer to one another would have lower beta diversity (dissimilarity). We also hypothesized that more isolated sites (Crazy Mountains and Sacajawea Saddle) would have the lowest alpha diversity and the greatest beta diversity. Our results did not match our hypothesized pattern in terms of alpha diversity. Instead, we found in pairwise comparisons that alpha diversity of whitebark pine fungal communities was significantly higher in the Crazy Mountains (our most isolated mountain range; containing the second smallest population of whitebark among sites) than in the Beartooth Plateau (large local population of whitebark pine), Absaroka Range (large local population of whitebark pine), and Sacajawea Saddle (semi-isolated; smallest population of whitebark among sites). Beta diversity was found to be significantly different between sites; pairwise comparisons were not tested for beta diversity. Greater alpha diversity in the more restricted Crazy Mountains suggests that the number of potentially uniquely assembled source populations may play a large role in overall diversity at this site (Fig. 20). Given

that community similarity was significantly different among sites we have reason to believe that surrounding mountain ranges of similar size and whitebark habitat would share this quality. The Crazy Mountains may be the recipient of four unique source populations, resulting in greater overall diversity. St. Mary's peak, our second most diverse site, is likely the recipient of at least three fungal endophyte source populations. Data on proximal whitebark pine populations in Idaho have not been assessed in this study for possibility of more source populations to the west. In addition, this study lacks diversity data on alternative source populations for the Crazy Mountains and still doesn't sufficiently explain the lack of overall lack of diversity found at the Beartooth Plateau, though we suspect that local factors may be at play.

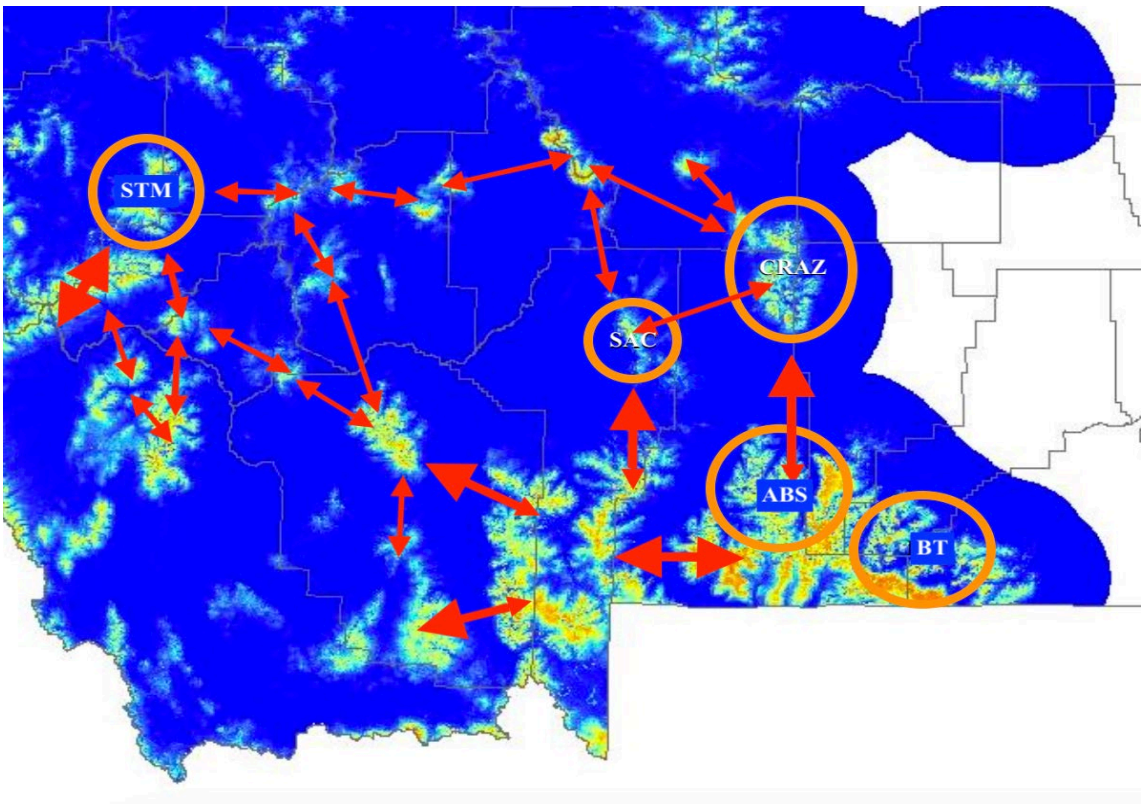

Fig. 20 Map of suitable whitebark pine habitat in the Montana Rocky Mountains with study sites designated by orange circles and potential source populations designated by red arrows. Magnitude of source population (size of habitat) correlates with arrow size (modified from Montana National Heritage Program, 2018).

Considering that each mature whitebark pine holds thousands or tens of thousands of needles and that each needle represents a niche capable of containing multiple endophytes, even these relatively small, isolated mountain ranges contain millions of niches for endophytes to occupy. It may be the case that the habitat size (at these scales) has relatively little effect on overall diversity, and instead the number of unique source populations and the degree of dispersal limitation of endophytes plays the greatest role in overall endophyte community diversity. Alternatively, it is possible that field sampling and/or DNA sequencing depth was insufficient to accurately capture the alpha diversity within these ecosystems at these scales. Though we sampled 150 whitebark pine trees in the field, we were left with 49 individuals that fit our parameters after sequence processing and quality control with a minimum rarefaction depth set to 500 sequences (per tree). OTU discovery (observed features) seems to level off around a sequencing depth of 3,500 sequences per sample at the Crazy Mountains, while leveling off occurs around 1000 sequences for the Beartooth Plateau, Sacajawea Saddle, and St. Mary's Peak sites (Fig. 7). Given that OTU accumulation seems to level off at higher values, we may not have reached a sufficient sequencing depth to make decisive conclusions on OTU richness at sites.

We examined alpha diversity in the context of ecotype, which captures habitat characteristics and elevation in a single variable. Alpha diversity was greater at lower elevation sites than in the pioneering krummholz sites; this finding confirms results from other studies that identify elevation as a primary driver of endophytic community structure (Hashizume *et al*., 2008; Siddique & Unterseher, 2016). Bowman & Arnold (2018) found that fungal endophyte diversity was greatest at mid-to-high elevations, but

abundance and composition did not differ along an elevational gradient. An elevational effect on community structure was also visible at the level of individual taxa; we found a differential occurrence of OTUs at each ecotype and at groups of ecotypes following an elevational driven pattern

Tree age may confound our elevational findings that are tied into age; Martín-García *et al*. (2011) found that fungal endophyte species richness was higher in young stands than old stands. We did not measure tree age in our study because tree coring required for dendrochronology seemed unethical in a threatened species. While tree age may have varied in this study, leaf (needle) age was considered and only second and third year needles were selected; therefore, time to accumulate endophytes in leaves would have been constant across all variables. Host density could influence endophyte community diversity; in the pioneering krummholz ecotype density was not recorded within plots because individual trees were highly spatially distributed.

Host Specificity

In this study we examined the diversity of endophyte communities from two conifer species sharing the same habitat. This is the first-time foliar endophyte communities of whitebark pine have ever been examined in depth, using culture-less methods, in-situ in the Rocky Mountains and this is the first study to examine the eukaryotic foliar endophytes of Engelmann spruce. We saw a strong influence of host species on endophyte community alpha and beta diversity, suggesting that whitebark pine endophyte communities are more diverse. PCoA plots further confirmed these tests, showing strong clustering around host tree species. Other studies have confirmed host species as being a driver of endophytic fungal community structure, though the level of

importance seems to vary or is unclear (Arnold & Lutzoni, 2007; Vincent *et al*., 2016; Apigo & Oono, 2018). In contrast, Higgins *et al*. (2014) found no evidence of host specificity in tropical grasses, and Cannon & Simmons (2002) found no evidence of host-specificity in a study of 12 tropical trees; this may indicate that host specificity varies across systems. Host specificity of fungal endophytes appears to be particularly understudied among conifers; our study provides a foundation for understanding host specificity in conifer species in the Rocky Mountains.

With dramatic losses of whitebark pine populations occurring across North America, we hypothesized that co-occurring conifer species may be able to sustain reservoirs of whitebark pine foliar fungal symbionts. We examined communities in Engelmann spruce, the second most abundant conifer in our study area (behind whitebark pine) and a pioneer of the treeline advance. Our results suggest that community dissimilarity is too great for Engelmann spruce to act as a reservoir of fungal symbionts for whitebark pines. Other conifers may be viable fungal refugia for whitebark and other species such as subalpine fir, limber pine, and lodgepole pine should be tested. If a similar pattern is found, that whitebark pine endophytes are tightly linked to their host species, then we would expect to see the diminishment of whitebark pine lead to extinction and/or greater occurrence of local extirpation of their symbionts across the Mountain West.

Endophytic Diversity Correlates with Blister Rust Infection Stage

We found that the effect of blister rust presence (stage of infection) had significant effects on both alpha and beta diversity and that, specifically, trees with inactive cankers harbored more diversity than trees with no infection. Noffsinger &

Cripps (2016) found that red senescent needles had greater alpha diversity than green living needles but did not examine the role of blister rust. Bullington *et al*. (2018) found that blister rust inoculation decreased overall richness and abundance of multiple taxonomic groups; however, Moler & Aho (2018) did not find evidence of community differences in trees lacking or showing WPBR cankers. These differences could be due to differences in study design; Bullington *et al*. (2018) experimentally inoculated seedlings in a common garden with a non-native surrounding plant community, while Moler & Aho (2018) sampled natural populations of whitebark pine but in the Cascade Range.

We hypothesized that diversity would be greatest in trees with no infection, then as infection stage progressed to active, diversity would decrease in response to production of antifungal compounds in the needles and remain in a state of decreased diversity after infection had become inactive. However, there was no detectable difference in alpha diversity between non-infected trees and actively infected trees. We speculate that diversity increased post-infection because of opportunistic fungi leveraging the weakened the state of the tree to their advantage. The effect of plant-stress on endophyte community diversity seems to be unclear; lack of foliar nutrients or high levels of toxic (antifungal) compounds can have conflicting outcomes on fungal endophyte diversity (Oono *et al*., 2020). High levels of toxic compounds could reduce dominance of individual species and therefore lead to greater overall diversity.

Microhabitat characteristics did not appear to have any significant effect on alpha diversity. In other words, trees being in extremely close proximity (overlapping branches) and the types of tree species in that proximity had no effect on alpha diversity. However, beta diversity was impacted by both the size of the microhabitat (sometimes referred to as

tree islands) and the composition of trees of within those microhabitats. This result suggests that variation in tree species composition and the amount of those trees present influence microbiome assemblages. This may also suggest that dispersal is happening at fine scales, though it does not exclude long range dispersal abilities. Alternatively, or contemporaneously, the effects of wind-reduction, solar radiation, and snow accumulation patterns (which differ in grouped trees vs. solitary trees) may play a role in tree species foliar endophyte assemblage (Gomes, 2018; Petrini, 1991).

Indicator Species Analysis

Alpha diversity measures (OTU richness) failed to indicate a discernable biogeographic pattern, while other studies at finer scales have found relationships between isolation and OTU richness in both mycorrhizae (Peay *et al*., 2007, Glassman *et al*., 2017) and endophytes (Helander *et al*., 2007). While our alpha diversity findings don't align with traditional island biogeographic predictions, our findings on community dissimilarity between sites suggests that more refined models may be applicable.

We found significant associations of specific OTUs to sites and differences in beta diversity among sites, which is in line with studies finding that fungal endophytes exhibit endemism (Sokolski *et al*., 2007). We found that more OTUs were associated with single than multiple sites, again supporting the idea of fungal endemism. These differences could be driven by dispersal capacity, local adaptation, or historical contingency (Helander *et al*, 2007; Fukami, 2015; Rúa *et al*., 2016).

Other studies have concluded that biogeography structures endophytic fungal communities without drawing strong conclusions on proximity of sites (Hoffman & Arnold, 2008; U'ren *et al*., 2012). However, we found no biogeographic pattern in terms

of proximity that suggests that sites sharing significant occurrence of OTUs were more closely located to one another. For example, in our study, the Crazy Mountains (our most isolated site) shared significant OTU occurrence with the Beartooth Plateau (to the south-east), St. Mary's Peak (far to the west), and Absaroka sites (to the south), but not to the closest site to the west (Sacajawea Saddle).

OTU occurrence did follow a linear trend in terms of ecotype, with lower elevation and established krummholz sharing significant occurrence of species, and established krummholz and pioneering krummholz sharing significant occurrence of species. Each ecotype also had reasonably large numbers of OTUs that were specifically associated with that single ecotype. This result suggests that ecotype structures community assemblage along an elevational gradient, similar to the findings of other studies (Bowman & Arnold, 2018).

Stage of blister rust infection did not yield any meaningful patterns in the association of OTUs. This result seems to contradict Bullington *et al.* (2018) findings that suggest significant community shifts take place after blister rust infection. Indicator species analysis as a function of canopy kill (which is significantly associated with the stage of blister rust infection) did suggest that specific OTUs are associated with the level of needle dieoff. In other words, specific OTUs are associated with the health of overall whitebark pine foliage, which is considered a proxy for overall tree health. Oddly, OTUs matched to *Flammulina velutipes*, *Ganoderma applanatum*, and *Bulleromyces albus* were all associated with canopy kill class 5 (51-75%); all of these species are Basidiomycota and are not commonly thought of as endophytes. *Ganoderma applanatum* and *Flammulina velutipes* are generally thought to be wood decay fungi and are prominently

found throughout North American forests. Given that *Flammulina velutipes* is a species of study in our lab, we are wary of these results, despite its lack of occurrence in our ITS2 negative controls. Further analysis on ITS1 and LSU data should illuminate whether this species is a contaminant or if high-elevation forests are a previously undescribed niche.

Blister Rust in the Krummholz Ecotone

It had been previously hypothesized that blister rust would be incapable of infecting krummholz whitebark pine due to basidiospore desiccation in extreme environments. A study by Resler & Tomback (2008) showed that blister rust occurrence in the krummholz ecotone was on par with rates of infection found in other lower elevation studies. Our study confirmed Resler & Tomback's findings and added additional support, as we compared blister rust prevalence in krummholz and lower elevation forests located on the same mountain. We found that WPBR prevalence in the lower elevation sites averaged 21.4%, while krummholz sites (combined) averaged 32.0%. We found that blister rust prevalence did not vary significantly between ecotypes, suggesting that krummholz whitebark pine forests are just as susceptible to population declines driven by white pine blister rust as lower elevation forests. Blister rust prevalence is a reasonable proxy for tree mortality given that initial infection and/or subsequent infection always leads to the death of the tree.

Occurrence of Endophytic Lichen

Our study found five lichen OTUs most closely matching *Lecanora laxa*, *Hypogymnia physodes*, *Elasticomyces elasticus*, unidentified *Lecanormycetes*, and *Chaetothyriales* that differentially occurred among our variables tested in our indicator species analysis. Additionally, we found OTU's most closely matching *Strictis radiata*,

Hypogymnia physodes, *Melanohalea exasperatula*, *Candelaria concolor*, unidentified *Phaeococcomyces*, and *Ostrapales* (2). This is not the first survey of endophytic fungal assemblages in whitebark pine to uncover lichens. Moler & Aho (2018) found reads most closely aligning to *Caloplaca lenae* and *Physcia magnussonii* in 99% of sample libraries, and *Coccomyces multangularis* in 81% of sample libraries. Moler & Aho (2018) explained this discovery as being a) the result of aerial spore rain of lichen propagules (Marshall, 1996; Favero-Longo *et al*., 2014), and b) failure to remove lichen propagules via surface sterilization procedures.

We found no obvious signs of lichens on the needles that we used for DNA extraction prior to surface sterilization of needle tissue, though this does preclude the possibility that lichens were present on surface tissue. Given the lack of free-standing water in the subalpine and the generally low moisture in this environment, we tentatively postulate that the lichens found in this study are inhabiting a previously undescribed endophytic niche, wherein these lichens persist in the ecosystem until finding an algal partner. More supporting data is needed to confirm the validity of this hypothesis.

Potential Sampling Biases

There are two notable potential sampling biases in this study. The first potential bias is the different methods used extract fungal genomic DNA from whitebark pine and Engelman spruce samples. For all whitebark pine needles we extracted fungal genomic DNA using Nucleospin® Plant II kit (Machery-Nagel) using 45 mg of homogenized needle tissue. For all Engelmann spruce samples we extracted fungal genomic DNA using Qiagen DNEasy Plant Mini kits (Carlsbad, CA, USA) using 45 mg of homogenized needle tissue.

Both the Nucleospin® Plant II kit (Machery-Nagel) and the Qiagen DNEasy Plant Mini kit are designed for plant DNA extractions containing high levels of PCR inhibitors; however, their chemistry may differ. Differences in chemistry, extraction efficiency, and inhibitor removal may make different fungal OTUs more amenable to extraction and PCR amplification. In general, we found that Nucleospin® Plant II kit extracted purer DNA from whitebark pine needles at much higher concentrations (10-80 ng/µL) than the Qiagen DNEasy Plant Mini kits (1.5-10 ng/µL). Pipan *et al*. (2018) compared the efficacy of these kits, finding greater average DNA yield using the Nucleospin® Plant II kit (PL2; 138.7 ng/µL) than the Qiagen DNEasy Plant Mini kit (19.5 ng/µL); as well as slightly greater PCR efficacy using the Nucleospin® Plant II kit. However, they suggest that DNA purity was greater in Qiagen DNEasy Plant Mini kit extractions than in Nucleospin® Plant II kit extractions, as evidenced by the presence of smears in gel electrophoresis tests (Pipan *et al*., 2018). They postulate that the greater smearing found in Nucleospin® Plant II kit extractions could be due to degradation of DNA or the presence of RNA fragments.

We submitted 213 total samples, comprised of 136 whitebark pine samples and 77 spruce samples. After quality control and filtering (as described in the methods section), we were left with 49 whitebark pine samples and 64 Engelmann spruce samples. Furthermore, sequencing depth was greater in spruce samples. Despite the greater number of usable samples and greater sequencing in Engelmann spruce, we found whitebark pine communities to be overall more diverse. It isn't clear why or if the differences in extraction protocols led to this result. At a broader level, it isn't clear why sequencing on the Illumina MiSeq sequencing platform didn't result in higher sample

preservation despite solid DNA purity levels, confirmation of DNA amplification at multiple checkpoints, and PEG magnetic bead cleaning at multiple steps.

The second notable bias in our study permeates many foliar fungal endophyte studies and is often considered an inherent issue with studying this group of organisms. In our study, we did not culture fungi directly from foliar tissue; instead, we directly amplified fungal genomic DNA directly from needle tissue using NGS. Comparative studies examining the different outcomes of culture vs culture-less methodologies using the same tissues have generally found greater overall richness and greater relative abundance of particular endophyte taxa using NGS (U'ren *et al*., 2014; U'ren *et al*., 2019; Oita *et al*., 2021a). Many endophytic fungi are unculturable; on the other hand, many fungal loci are more easily amplified with commonly used primer sequences (U'ren *et al*., 2014). While we likely missed fungal taxa that would have been found using culture-based methods, we assessed the advantages and disadvantages of both methods during study design and determined that the cultureless NGS approach was a better route for a system.

Conclusions and Future Research

Diminishing populations of whitebark pine and its patchy island-like distribution (driven by its necessity to inhabit a narrow elevational zone) initially drew our attention to this study system. We recognized a need to catalogue and understand the fungal symbionts of the threatened, keystone whitebark pine and aimed at teasing out the drivers of foliar fungal endophytic community assembly in this system. We found that elevational driven ecotype, disease state, and study site locality drove community assembly in this group of fungi. While we did not find any fungal endophytes associated

with healthy trees in highly infected forests, we recognize that other studies have found promising results suggesting the efficacious use of fungal biocontrol's against white pine blister rust; we recommend that this possibility continues to be explored as the implications are potentially quite large (Ganley *et al*., 2008; Bullington *et al*., 2018).

Foliar fungal endophyte is ecology is a rapidly growing field, studies have been published examining a variety of ecological gradients in numerous systems; the degree to which these gradients drive community assembly appears to be linked to the system and not necessarily ubiquitously applicable. We found that there was a particularly large knowledge gap in Rocky Mountain and conifer systems, while tropical and grass endophyte systems had gleaned the most attention. Our study lays a solid foundation for continued exploration into Rocky Mountain conifer endophyte ecology, finding ecotype (a proxy for elevation) and host tree species to be strong drivers of community assembly. Additional research on subalpine fir, lodge pole pine, and limber pine foliar fungal endophytes could increase resolution significantly and should be considered high-quality candidates for future work.

We attempted to assess the role of fungal endophyte dispersal limitation at a large scale in this study by applying a quasi-island biogeographic lens. Using this framework, we sampled from mountain ranges varying in habitat size and distance to metapopulations. Our results paint a complicated picture, overall diversity was not easily explained by these biogeographic variations, while beta diversity and significant occurrence of particular taxa suggest that there are geographic mechanisms structuring communities of foliar fungal endophytes in whitebark pine. Research of this group of fungi at these scales should continue to be examined with the recommendation that

sampling is robust and that the system is carefully chosen. Fungal endophyte ecology remains at the forefront of unexplored fields with huge implications relevant to microbial ecology, biodiversity, evolution, and ecosystem function.

REFERENCES

Amend AS, Seifert KA, Bruns TD. 2010. Quantifying microbial communities with 454 pyrosequencing: does read abundance count? *Molecular Ecology* **19**: 5555–5565.

Amman GD. 1982. Characteristics of mountain pine beetles reared in four pine hosts. *Environmental Entomology* **11**: 590–593.

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**: 32–46.

Andrews JH, Kinkel LL, Berbee FM, Nordheim EV. 1987. Fungi, leaves, and the theory of island biogeography. *Microbial Ecology* **14**: 277–290.

Aneja M, Gianfagna TJ, Hebbar PK. 2005. *Trichoderma harzianum* produces nonanoic acid, an inhibitor of spore germination and mycelial growth of two cacao pathogens. *Physiological and Molecular Plant Pathology*. **67**: 304–307.

Apigo A, Oono R. 2018. Dimensions of Host Specificity in Foliar Fungal Endophytes. In: Pirttilä AM, Frank AC, eds. *Endophytes of Forest Trees*. Cham, CH: Springer, 15–42.

Arno SF, Hoff RJ. 1990. Whitebark pine *Pinus albicaulis* Engelm. In: Burns RM, Honkala BH, technical coordinators. *Silvics of North America.* Vol. 1, Conifers, Agriculture Handbook 654. Washington, DC: U.S.D.A., Forest Service, 268–279.

Arnold AE. **2007**. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. *Fungal Biology Reviews* **21**: 51–66.

Arnold AE, Lutzoni F. **2007.** Diversity and Host Range of Foliar Fungal Endophytes: Are Tropical Leaves Biodiversity Hotspots? *Ecology* **88**: 541–549.

Arnold AE, Maynard Z, Gilbert GS, Coley PD, Kursar TA. **2000**. Are tropical fungal endophytes hyperdiverse? *Ecology Letters* **3**: 267–274.

Achuff PL, Wilson B, Committee on the Status of Endangered Wildlife in Canada. **2010**. *COSEWIC assessment and status report on the whitebark pine, Pinus albicaulis, in Canada.* Ottawa: Committee on the Status of Endangered Wildlife in Canada.

Bailey BA, Bae H, Strem MD, Crozier J, Thomas SE, Samuels GJ, Vinyard BT, Holmes KA. **2008**. Antibiosis, mycoparasitism, and colonization success for endophytic Trichoderma isolates with biological control potential in Theobroma cacao. *Biological Control* **46**: 24–35.

Bailey BA, Bae H, Strem MD, Roberts DP, Thomas SE, Crozier J, Samuels GJ, Choi I-Y, Holmes KA. **2006**. Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four Trichoderma species. *Planta* **224**: 1449–1464.

Baas-Becking, LGM. 1934. *Geobiologie of inleiding tot de milieukunde*. W.P. Van Stockum & Zoon, The Hague, the Netherlands.

Berendsen RL, Pieterse CMJ, Bakker PAHM. **2012**. The rhizosphere microbiome and plant health. *Trends in Plant Science* **17**: 478–486.

Bolger AM, Lohse M, Usadel B. **2014**. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**: 2114–2120.

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, *et al.* 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* **37**: 852–857.

Bowman EA, Arnold AE. **2018**. Distributions of ectomycorrhizal and foliar endophytic fungal communities associated with *Pinus ponderosa* along a spatially constrained elevation gradient. *American Journal of Botany* **105**: 687–699.

Bruederle LP, Tomback DF, Kelly KK, Hardwick RC. **1998**. Population genetic structure in a bird-dispersed pine, *Pinus albicaulis* (Pinaceae). *Canadian Journal of Botany* **76**: 83–90.

Bruns TD, Bidartondo MI, Taylor DL. **2002**. Host specificity in ectomycorrhizal communities: What do the exceptions tell us? *Integrative and Comparative Biology* **42**: 352–359.

Bullington L. **2017**. *Molecular diversity of foliar fungal endophytes in relation to defense strategies and disease in whitebark pine*. Graduate Student Theses, Dissertations, & Professional Papers. University of Montana, Missoula, MT, USA.

Bullington LS, Larkin BG. **2015**. Using direct amplification and next-generation sequencing technology to explore foliar endophyte communities in experimentally inoculated western white pines. *Fungal Ecology* **17**: 170–178.

Bullington LS, Lekberg Y, Sniezko R, Larkin B. **2018**. The influence of genetics, defensive chemistry and the fungal microbiome on disease outcome in whitebark pine trees. *Molecular Plant Pathology* **19**: 1847–1858.

Burns KC, Berg J, Bialynicka-Birula A, Kratchmer S, Shortt K. 2010. Tree diversity on islands: assembly rules, passive sampling and the theory of island biogeography. *Journal of Biogeography* **37**: 1876–1883.

Cannon PF, Simmons CM. **2002**. Diversity and host preference of leaf endophytic fungi in the Iwokrama Forest Reserve, Guyana. *Mycologia* **94**: 210–220.

Campbell EM, Antos JA. 2000. Distribution and severity of white pine blister rust and mountain pine beetle on whitebark pine in British Columbia. *Canadian Journal of Forest Research* **30**: 1051–1059.

Campbell EM, Antos JA. 2003. Postfire succession in Pinus *albicaulis—Abies lasiocarpa* forests of southern British Columbia. *Canadian Journal of Botany* **81**: 383–397.

Carroll GC. 1986. The biology of endophytism in plants with particular reference to woody perennials. In: Fokkema NJ, Van Den Heuvel J, eds. *Microbiology of the Phylloplane*. Cambridge, England: Cambridge University Press, 205–222.

Cerezke HF. **1995**. Egg gallery, brood production, and adult characteristics of mountain pine beetle, *dendroctonus ponderosae* hopkins (coleoptera: scolytidae), in three pine hosts. *The Canadian Entomologist* **127**: 955–965.

Clarke BB, White JF, Hurley RH, Torres MS, Sun S, Huff DR. **2006**. Endophyte-mediated suppression of dollar spot disease in fine fescues. *Plant Disease* **90**: 994–998.

Crawford RMM. **1992**. Oxygen Availability as an Ecological Limit to Plant Distribution. In: Begon M, Fitter AH, eds. *Advances in Ecological Research*. Academic Press, 93–185.

Cripps CL, Grimme E. **2011**. Inoculation and successful colonization of whitebark pine seedlings with native mycorrhizal fungi under greenhouse conditions. In: Keane RE, Tomback DF, Murray MP, Smith CM, eds. *The future of high-elevation, five-needle white pines in Western North America: Proceedings of the High Five Symposium*. Missoula, MT. Proceedings RMRS-P-63. Fort Collins, CO: USDA, Forest Service, Rocky Mountain Research Station, 312–322.

Crofts AL, Brown CD. **2020**. The importance of biotic filtering on boreal conifer recruitment at alpine treeline. *Ecography* **43**: 914–929.

Darker GD. **1967**. A revision of the genera of the Hypodermataceae. *Canadian Journal of Botany* **45**: 1399–1444.

Elsen PR, Tingley MW. 2015. Global mountain topography and the fate of montane species under climate change. *National Climate Change* **5**: 772–776.

Faeth SH, Gardner DR, Hayes CJ, Jani A, Wittlinger SK, Jones TA. **2006**. Temporal and spatial variation in alkaloid levels in *Achnatherum robustum*, a native grass infected with the endophyte *Neotyphodium*. *Journal of Chemical Ecology* **32**: 307.

Favero-Longo SE, Sandrone S, Matteucci E, Appolonia L, Piervittori R. **2014**. Spores of lichen-forming fungi in the mycoaerosol and their relationships with climate factors. *Science of The Total Environment* **466–467**: 26–33.

Fenchel T, Finlay BJ. **2003**. Is microbial diversity fundamentally different from biodiversity of larger animals and plants? *European Journal of Protistology* **39**: 486–490.

Finlay BJ. **2002**. Global dispersal of free-living microbial eukaryote species. *Science* **296**: 1061–1063.

Fish and Wildlife Service, Interior. 2020. endangered and threatened wildlife and plants; threatened species status for *Pinus albicaulis* (whitebark pine) with section 4(d) rule. *Federal Register* **85**: 77408–77424.

Fukami T. **2015**. Historical contingency in community assembly: Integrating niches, species pools, and priority effects. *Annual Review of Ecology, Evolution, and Systematics* **46**: 1–23.

Furnier GR, Knowles P, Clyde MA, Dancik BP. **1987**. Effects of avian seed dispersal on the genetic structure of whitebark pine populations. *Evolution* **41**: 607–612.

Ganley RJ, Newcombe G. **2006**. Fungal endophytes in seeds and needles of *Pinus monticola*. *Mycological Research* **110**: 318–327.

Ganley RJ, Sniezko RA, Newcombe G. **2008**. Endophyte-mediated resistance against white pine blister rust in *Pinus monticola*. *Forest Ecology and Management* **255**: 2751–2760.

Gao F, Dai C, Liu X. **2010**. Mechanisms of fungal endophytes in plant protection against pathogens. *African Journal of Microbiology Research* **4**: 1346–1351.

Garbelotto M, Lowell N, Chen IY, Osmundson TW. **2019**. Evidence for inhibition of a fungal biocontrol agent by a plant microbiome. *Journal of Plant Pathology* **101**: 457–466.

Gibson K, Skov K, Kegley S, Jorgensen C, Smith S, Witcosky J. **2008**. Mountain pine beetle impacts in high-elevation five-needle pines: Current trends and challenges. USDA, Forest Service, *Forest Health Protection*. Missoula, Montana, USA.

Glenn TC, Nilsen RA, Kieran TJ, Sanders JG, Bayona-Vásquez NJ, Finger JW, Pierson TW, Bentley KE, Hoffberg SL, Louha S, *et al.* 2019a. Adapterama I: universal stubs and primers for 384 unique dual-indexed or 147,456 combinatorially-indexed Illumina libraries (iTru & iNext). *PeerJ* **7**: e7755.

Glenn TC, Pierson TW, Bayona-Vásquez NJ, Kieran TJ, Hoffberg SL, Iv JCT, Lefever DE, Finger JW, Gao B, Bian X, *et al.* 2019b. Adapterama II: universal amplicon sequencing on Illumina platforms (TaggiMatrix). *PeerJ* **7**: e7786.

Goeking SA, Izlar DK. **2018**. *Pinus albicaulis* Engelm. (whitebark pine) in mixed-species stands throughout its US range: broad-scale indicators of extent and recent decline. *Forests* **9**: 131.

Gomes T, Pereira JA, Benhadi J, Lino-Neto T, Baptista P. 2018. Endophytic and epiphytic phyllosphere fungal communities are shaped by different environmental factors in a Mediterranean ecosystem. *Microbial Ecology* **76**: 668–679.

Götmark F, Fridman J, Kempe G, Norden B. **2005**. Broadleaved tree species in conifer-dominated forestry: Regeneration and limitation of saplings in southern Sweden. *Forest Ecology and Management* **214**: 142–157.

Gottschalk KW, Colbert JJ, Feicht DL. **1998**. Tree mortality risk of oak due to gypsy moth. *European Journal of Forest Pathology* **28**: 121–132.

Grace J, Berninger F, Nagy L. **2002**. Impacts of climate change on the tree line. *Annals of Botany* **90**: 537–544.

Hansen A, Ireland K, Legg K, Keane R, Barge E, Jenkins M, Pillet M. **2016**. Complex challenges of maintaining whitebark pine in Greater Yellowstone under climate change: A call for innovative research, management, and policy approaches. *Forests* **7(3)**: 1–54.

Harsch MA, Hulme PE, McGlone MS, Duncan RP. **2009**. Are treelines advancing? A global meta-analysis of treeline response to climate warming. *Ecology Letters* **12**: 1040–1049.

Hashizume Y, Sahashi N, Fukuda K. **2008**. The influence of altitude on endophytic mycobiota in *Quercus acuta* leaves collected in two areas 1000 km apart. *Forest Pathology* **38**: 218–226.

Hawksworth FG. **1990**. White pine blister rust in southern New Mexico. *Plant Disease* **74**: 938.

Hedlund K, Regina IS, Putten WHV der, Lepš J, Díaz T, Korthals GW, Lavorel S, Brown VK, Gormsen D, Mortimer SR, *et al.* 2003. Plant species diversity, plant biomass and responses of the soil community on abandoned land across Europe: idiosyncracy or above-belowground time lags. *Oikos* **103**: 45–58.

Helander M, Ahlholm J, Sieber TN, Hinneri S, Saikkonen K. **2007**. Fragmented environment affects birch leaf endophytes. *New Phytologist* **175**: 547–553.

Higgins KL, Arnold AE, Coley PD, Kursar TA. **2014**. Communities of fungal endophytes in tropical forest grasses: highly diverse host- and habitat generalists characterized by strong spatial structure. *Fungal Ecology* **8**: 1–11.

Higuera PE, Abatzoglou JT, Littell JS, Morgan P. **2015**. The changing strength and nature of fire-climate relationships in the Northern Rocky Mountains, U.S.A., 1902-2008. *PLOS ONE* **10**: e0127563.

Hill JK, Thomas CD, Lewis OT. **1996**. Effects of habitat patch size and isolation on dispersal by *hesperia comma* butterflies: implications for metapopulation structure. *Journal of Animal Ecology* **65**: 725–735.

Hoff RJ, McDonald GI. **1980**. Resistance to *Cronartium ribicola* in *Pinus monticola*: reduced needle-spot frequency. *Canadian Journal of Botany* **58**: 574–577.

Hoffman MT, Arnold AE. **2008**. Geographic locality and host identity shape fungal endophyte communities in cupressaceous trees. *Mycological Research* **112**: 331–344.

Holtmeier F-K. **2009**. Definitions, terminology. In: Holtmeier F-K, eds. *Mountain Timberlines: Ecology, Patchiness, and Dynamics*. Berlin, Germany: Springer-Verlag, 11–27.

Holtmeier F-K, Broll G. **2005**. Sensitivity and response of northern hemisphere altitudinal and polar treelines to environmental change at landscape and local scales. *Global Ecology and Biogeography* **14**: 395–410.

Holtmeier FK, Broll GE. **2007**. Treeline advance - driving processes and adverse factors. *Landscape Online* **1**: 1–33.

Hortal J, Triantis KA, Meiri S, Thébault E, Sfenthourakis S. **2009**. Island species richness increases with habitat diversity. *The American Naturalist* **174**: E205–E217.

Hubbard M, Germida JJ, Vujanovic V. **2013**. Fungal endophytes enhance wheat heat and drought tolerance in terms of grain yield and second-generation seed viability. *Journal of Applied Microbiology* **116**: 109–122.

Hutchins HE, Lanner RM. **1982**. The central role of Clark's nutcracker in the dispersal and establishment of whitebark pine. *Oecologia* **55**: 192–201.

Iglesias V, Krause TR, Whitlock C. **2015**. Complex response of white pines to past environmental variability increases understanding of future vulnerability. *PLOS ONE* **10**: e0124439.

Ihrmark K, Bödeker ITM, Cruz-Martinez K, Friberg H, Kubartova A, Schenck J, Strid Y, Stenlid J, Brandström-Durling M, Clemmensen KE, et al. 2012. New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology* **82**: 666–677.

Jian L. **2017**. Extended ability of the Axygen® AxyPrep MAG PCR clean-up kit for effective dna fragment selection.

Johnson JA, Whitney NJ. **1992**. Isolation of fungal endophytes from black spruce (*Picea mariana*) dormant buds and needles from New Brunswick, Canada. *Canadian Journal of Botany* **70**: 1754–1757.

Jorgensen SM, Hamrick JL. **1997**. Biogeography and population genetics of whitebark pine, *Pinus albicaulis*. *Canadian Journal of Forest Research* **27**: 1574–1585.

Juybari HZ, Tajick Ghanbary MA, Rahimian H, Karimi K, Arzanlou M. **2019**. Seasonal, tissue and age influences on frequency and biodiversity of endophytic fungi of *Citrus sinensis* in Iran. *Forest Pathology* **49**: e12559.

Keane RE, Arno SF. **1993**. Rapid decline of whitebark pine in Western Montana: Evidence from 20-year remeasurements. *Western Journal of Applied Forestry* **8**: 44–47.

Keane RE, Tomback DF, Aubry CA, Bower AD, Campbell EM, Cripps CL, Jenkins MB, Mahalovich MF, Manning M, McKinney ST, *et al.* 2012. *A range-wide restoration strategy for whitebark pine (Pinus albicaulis)*. Fort. Collins, CO, USA: U.S.D.A., Forest Service, Rocky Mountain Research Station

Kendall KC & Arno SF. 1990. Whitebark pine an important but endangered wildlife resource. In: Schmidt WC, McDonald KJ, compilers. *Proceedings of the Symposium: Whitebark Pine Ecosystems: Ecology and Management of a High Mountain Resource*. Bozeman, MT. General Technical Report. INT-270. Ogden, UT, USA: U.S.D.A., Forest Service, Intermountain Research Station: 264–274.

Kendall KC & Keane RE. 2001. Whitebark pine decline: infection, mortality, and population trends. In: Tomback DF, Arno SF, Keane RE, eds. *Whitebark Pine Communities: Ecology and Restoration.* Washington, D.C: Island Press, 221–242.

Kearns HSJ, Jacobi WR. **2007**. The distribution and incidence of white pine blister rust in central and southeastern Wyoming and northern Colorado. *Canadian Journal of Forest Research* **37**: 462–472.

Kimmons CA, Gwinn KD, Bernard EC. **1990**. Nematode reproduction on endophyte-infected and endophyte-free tall fescue. *Plant Disease* **74**: 757–761.

Kinloch BB. **2003**. White pine blister rust in North America: Past and prognosis. *Phytopathology* **93**: 1044–1047.

Kinloch BB, Dupper GE. **2002**. Genetic specificity in the white pine-blister rust pathosystem. *Phytopathology* **92**: 278–280.

Koh S, Hik DS. **2007**. Herbivory mediates grass–endophyte relationships. *Ecology* **88**: 2752–2757.

Körner C. 1998. A re-assessment of high elevation treeline positions and their explanation. *Oecologia* **115**: 445–459.

Kullman L. **1998**. Tree-limits and montane forests in the Swedish Scandes: Sensitive biomonitors of climate change and variability. *Ambio* **27**: 312–321.

Kupfer JA, Cairns DM. **1996**. The suitability of montane ecotones as indicators of global climatic change. *Progress in Physical Geography: Earth and Environment* **20**: 253–272.

Kurz WA, Dymond CC, Stinson G, Rampley GJ, Neilson ET, Carroll AL, Ebata T, Safranyik L. **2008**. Mountain pine beetle and forest carbon feedback to climate change. *Nature* **452**: 987–990.

Lanner RM, Gilbert BK. 1994. Nutritive value of whitebark pines seeds, and the question of their variable dormancy. In: Schmidt WC, Holtmeier FK, compilers. *Proceedings—International Workshop on Subalpine Stones Pines and Their Environment: The Status of Our Knowledge.* General Technical Report. Ogden, UT, USA: U.S.D.A., Forest Service, Intermountain Research Station: 206–211.

Larkin BG, Hunt LS, Ramsey PW. **2012**. Foliar nutrients shape fungal endophyte communities in western white pine (*Pinus monticola*) with implications for white-tailed deer herbivory. *Fungal Ecology* **5**: 252–260.

Larson ER, Kipfmueller KF. 2012. Ecological disaster or the limits of observation? Reconciling modern declines with the long-term dynamics of whitebark pine communities. *Geographic Compass* **6/4**, 189–214.

Levins R. **1969**. Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bulletin of the Entomological Society of America* **15**: 237–240.

Li S, Wang P, Chen Y, Wilson MC, Yang X, Ma C, Lu J, Chen X, Wu J, Shu W, *et al.* 2020. Island biogeography of soil bacteria and fungi: similar patterns, but different mechanisms. *The ISME Journal* **14**: 1886–1896.

Liu J-J, Sturrock RN, Sniezko RA, Williams H, Benton R, Zamany A. **2015**. Transcriptome analysis of the white pine blister rust pathogen *Cronartium ribicola*: de novo assembly, expression profiling, and identification of candidate effectors. *BMC Genomics* **16**: 678.

Logan JA, Macfarlane WW, Willcox L. **2010**. Whitebark pine vulnerability to climate-driven mountain pine beetle disturbance in the Greater Yellowstone Ecosystem. *Ecological Applications* **20**: 895–902.

Lonergan ER, Cripps CL, Smith CM. **2014**. Influence of site conditions, shelter objects, and ectomycorrhizal inoculation on the early survival of whitebark pine seedlings planted in Waterton Lakes National Park. *Forest Science* **60**: 603–612.

Lorenz TJ, Sullivan KA. **2009**. Seasonal differences in space use by clark's nutcrackers in the Cascade Range. *The Condor* **111**: 326–340.

MacArthur RH, Wilson EO. 1967. *The Theory of Island Biogeography*. Princeton, New Jersey, USA: Princeton University Press.

Maloy OC. **1997**. White pine blister rust control in North America: a case history. *Annual Review of Phytopathology* **35**: 87–109.

Maloy OC. **2001**. White pine blister rust. *Plant Health Progress* **2**: 10.

Marshall WA. **1996**. Aerial dispersal of lichen soredia in the maritime Antarctic. *New Phytologist* **134**: 523–530.

Martín-García J, Espiga E, Pando V, Diez J. **2011**. Factors influencing endophytic communities in poplar plantations. *Silva Fennica* **45(2)**: 169–180.

Martiny JBH, Bohannan BJM, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Krumins JA, Kuske CR, *et al.* 2006. Microbial biogeography: putting microorganisms on the map. *Nature Reviews Microbiology* **4**: 102–112.

Mattson DJ. **2000**. *Causes and consequences of dietary differences among Yellowstone grizzly bears (Ursus arctos).* Ph.D. Dissertation. University of Idaho, Moscow, ID, USA.

Mattson DJ, Jonkel CJ. 1990. Stone pines and bears. In: Schmidt WC, McDonald KJ, compilers. *Proceedings—Symposium on Whitebark Pine Ecosystems: Ecology and Management of a High-Mountain Resource.* General Technical Report INT-270. Ogden, UT, USA: U.S.D.A., Forest Service, Intermountain Research Station: 223–236.

Mattson DJ, Reinhart DP. **1997**. Excavation of red squirrel middens by grizzly bears in the whitebark pine zone. *Journal of Applied Ecology* **34**: 926–940.

McCaughey WW, Schmidt WC. 1990. Autecology of whitebark pine. In: Schmidt WC, McDonald KJ, compilers. *Proceedings—Symposium on Whitebark Pine Ecosystems: Ecology and Management of a High-Mountain Resource.* General Technical Report INT-270. Ogden, UT, USA: U.S.D.A., Forest Service, Intermountain Research Station: 85–96.

McCaughey WW, Schmidt WC. 2001. Taxonomy, distribution, and history. In: Tomback DF, Arno SF, Keane RE, eds. *Whitebark Pine Communities: Ecology and Restoration*. Washington, D.C.: Island Press. 29–40.

McDonald GI, Richardson BA, Zambino PJ, Klopfenstein NB, Kim M-S. **2006**. *Pedicularis* and *Castilleja* are natural hosts of *Cronartium ribicola* in North America: a first report. *Forest Pathology* **36**: 73–82.

McKinney ST, Fiedler CE. **2010**. Tree squirrel habitat selection and predispersal seed predation in a declining subalpine conifer. *Oecologia* **162**: 697–707.

McMullin DR, Green BD, Miller JD. **2015**. Antifungal sesquiterpenoids and macrolides from an endophytic *Lophodermium* species of *Pinus strobus*. *Phytochemistry Letters* **14**: 148–152.

McMullin DR, Nguyen HDT, Daly GJ, Menard BS, Miller JD. **2018**. Detection of foliar endophytes and their metabolites in *Picea* and *Pinus* seedling needles. *Fungal Ecology* **31**: 1–8.

Miller JD. **1986**. Toxic metabolites of epiphytic and endophytic fungi of conifer needles. In: Fokkema NJ, Van den Huevel J, eds. *Microbiology of the phyllosphere*. Cambridge, England: Cambridge University Press. 221-231.

Miller JD, Mackenzie S, Foto M, Adams GW, Findlay JA. **2002**. Needles of white spruce inoculated with rugulosin-producing endophytes contain rugulosin reducing spruce budworm growth rate. *Mycological Research* **106**: 471–479.

Minter DW. **1981**. Possible biological control of *Lophodermium seditiosum*. Millar CS, eds. *University Forest Department: Current Research on Conifer Needle Diseases*. Aberdeen, Scotland: Aberdeen University Press. 67-74.

Mohatt KR, Cripps CL, Lavin M. **2008**. Ectomycorrhizal fungi of whitebark pine (a tree in peril) revealed by sporocarps and molecular analysis of mycorrhizae from treeline forests in the Greater Yellowstone Ecosystem. *Botany* **86**: 14–25.

Moler ERV, Aho K. **2018**. Whitebark pine foliar fungal endophyte communities in the southern Cascade Range, USA: Host mycobiomes and white pine blister rust. *Fungal Ecology* **33**: 104–114.

Montana National Heritage Program. 2018. Whitebark Pine (*Pinus albicaulis*) predicted suitable habitat modeling. In: Burkholder B, Pipp A, evaluators. *Montana Natural Heritage Program*, Helena, MT, USA.

Moora M, Berger S, Davison J, Öpik M, Bommarco R, Bruelheide H, Kühn I, Kunin WE, Metsis M, Rortais A, *et al.* 2011. Alien plants associate with widespread

generalist arbuscular mycorrhizal fungal taxa: evidence from a continental-scale study using massively parallel 454 sequencing. *Journal of Biogeography* **38**: 1305–1317.

Morgan P, Bunting S, Keane RE, Arno S. 1994. Fire ecology of whitebark pine (*Pinus albicaulis*) forests in the Rocky Mountains, USA. In: Schmidt WC, Holtmeier F-K, compilers. *Proceedings—International workshop on Subalpine Stone Pines and Their Environment: The Status of our Knowledge*. General Technical Report INT-309. St. Moritz, Switzerland: U.S.D.A., Forest Service:136–142.

Nekola JC, White PS. **1999**. The distance decay of similarity in biogeography and ecology. *Journal of Biogeography* **26**: 867–878.

Noffsinger C, Cripps CL. 2016. *Diversity of endophytic fungi from green and senescent red needles of whitebark pine (Pinus albicaulis) at treeline in Montana*. Poster, Mycological Society of America annual meeting, Berkeley, CA, USA.

Oita S, Carey J, Kline I, Ibáñez A, Yang N, Hom EFY, Carbone I, U'Ren JM, Arnold AE. **2021a**. Methodological approaches frame insights into endophyte richness and community composition. *Microbial Ecology* **82**: 21–34.

Oita S, Ibáñez A, Lutzoni F, Miadlikowska J, Geml J, Lewis LA, Hom EFY, Carbone I, U'Ren JM, Arnold AE. **2021b**. Climate and seasonality drive the richness and composition of tropical fungal endophytes at a landscape scale. *Communications Biology* **4**: 1–11.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, *et al.* 2020. *Vegan: Community Ecology Package*. R package version 2.5-7.

Oono R, Black D, Slessarev E, Sickler B, Strom A, Apigo A. **2020**. Species diversity of fungal endophytes across a stress gradient for plants. *New Phytologist* **228**: 210–225.

Oono R, Lefèvre E, Simha A, Lutzoni F. **2015**. A comparison of the community diversity of foliar fungal endophytes between seedling and adult loblolly pines (*Pinus taeda*). *Fungal Biology* **119**: 917–928.

Osmundson TW, Eyre CA, Hayden KM, Dhillon J, Garbelotto MM. **2013**. Back to basics: an evaluation of NaOH and alternative rapid DNA extraction protocols for DNA barcoding, genotyping, and disease diagnostics from fungal and oomycete samples. *Molecular Ecology Resources* **13**: 66–74.

Ostrofsky WD, Rumpf T, Struble D, Bradbury R. **1988**. Incidence of white pine blister rust in Maine after 70 years of a *Ribes* eradication program. *Plant Disease* **72**: 967–970.

Palmer JM, Jusino MA, Banik MT, Lindner DL. **2018**. Non-biological synthetic spike-in controls and the AMPtk software pipeline improve mycobiome data. *PeerJ* **6**: e4925.

Pansing ER, Tomback DF, Wunder MB. **2020**. Climate-altered fire regimes may increase extirpation risk in an upper subalpine conifer species of management concern. *Ecosphere* **11**: e03220.

Patton RF, Johnson DW. **1970**. Mode of penetration of needles of Eastern White Pine by Cronartium ribicola. *Phytopathology* **60**: 977–982.

Peay KG, Bruns TD, Kennedy PG, Bergemann SE, Garbelotto M. **2007**. A strong species–area relationship for eukaryotic soil microbes: island size matters for ectomycorrhizal fungi. *Ecology Letters* **10**: 470–480.

Peay KG, Garbelotto M, Bruns TD. **2010**. Evidence of dispersal limitation in soil microorganisms: Isolation reduces species richness on mycorrhizal tree islands. *Ecology* **91**: 3631–3640.

Petrini O. 1991. Fungal endophytes of tree leaves. In: Andrews JH, Hirano SS, eds. *Contemporary Bioscience. Microbial Ecology of Leaves*. New York, NY, USA: Springer New York: 179–197.

Pipan B, Zupančič M, Blatnik E, Dolničar P, Meglič V. **2018**. Comparison of six genomic DNA extraction methods for molecular downstream applications of apple tree (*Malus X domestica*). *Cogent Food & Agriculture* **4**:1.

Queloz V, Grünig CR, Berndt R, Kowalski T, Sieber TN, Holdenrieder O. **2011a**. Cryptic speciation in *Hymenoscyphus albidus*. *Forest Pathology* **41**: 133–142.

Queloz V, Sieber TN, Holdenrieder O, McDonald BA, Grünig CR. **2011b**. No biogeographical pattern for a root-associated fungal species complex. *Global Ecology and Biogeography* **20**: 160–169.

R Core Team. 2020. R: A language and environment for statistical computing. *R Foundation for Statistical Computing*. Vienna, Austria. URL https://www.R-project.org/.

Reid RW. **1962**. Biology of the mountain pine beetle, *Dendroctonus monticolae Hopkins*, in the east Kootenay region of British Columbia I. Life cycle, brood development, and flight periods. *The Canadian Entomologist* **94**: 531–538.

Resler LM. **2004**. Conifer establishment sites on a periglacial landscape, Glacier National Park, Montana. *Texas State University-San Marcos. Ph.D. dissertation*.

Resler LM, Butler DR, Malanson GP. **2005**. Topographic shelter and conifer establishment and mortality in an alpine environment, Glacier National Park, Montana. *Physical Geography* **26**: 112–125.

Resler LM, Tomback DF. **2008**. Blister rust prevalence in krummholz whitebark pine: implications for treeline dynamics, northern Rocky Mountains, Montana, U.S.A. *Arctic, Antarctic, and Alpine Research* **40**: 161–170.

Richardson BA, Brunsfeld SJ, Klopfenstein NB. **2002**. DNA from bird-dispersed seed and wind-disseminated pollen provides insights into postglacial colonization and population genetic structure of whitebark pine (*Pinus albicaulis*). *Molecular Ecology* **11**: 215–227.

Richardson SN, Nsiama TK, Walker AK, McMullin DR, Miller JD. **2015**. Antimicrobial dihydrobenzofurans and xanthenes from a foliar endophyte of *Pinus strobus*. *Phytochemistry* **117**: 436–443.

Richardson SN, Walker AK, Nsiama TK, McFarlane J, Sumarah MW, Ibrahim A, Miller JD. **2014**. Griseofulvin-producing *Xylaria* endophytes of *Pinus*

strobus and *Vaccinium angustifolium*: evidence for a conifer-understory species endophyte ecology. *Fungal Ecology* **11**: 107–113.

Riley KL, Loehman RA. **2016**. Mid-21st-century climate changes increase predicted fire occurrence and fire season length, Northern Rocky Mountains, United States. *Ecosphere* **7**: e01543.

Rodrigues KF. **1994**. The foliar fungal endophytes of the Amazonian palm *Euterpe oleracea*. *Mycologia* **86**: 376–385.

Rodriguez RJ, Jr JFW, Arnold AE, Redman RS. **2009**. Fungal endophytes: diversity and functional roles. *New Phytologist* **182**: 314–330.

Rogers DL, Millar CI, Westfall RD. **1999**. Fine-scale genetic structure of whitebark pine (*Pinus albicaulis*): Associations with watershed and growth form. *Evolution* **53**: 74–90.

Rollinger JL, Langenheim JH. **1993**. geographic survey of fungal endophyte community composition in leaves of coastal redwood. *Mycologia* **85**: 149–156.

Rúa MA, Antoninka A, Antunes PM, Chaudhary VB, Gehring C, Lamit LJ, Piculell BJ, Bever JD, Zabinski C, Meadow JF, *et al.* 2016. Home-field advantage? evidence of local adaptation among plants, soil, and arbuscular mycorrhizal fungi through meta-analysis. *BMC Evolutionary Biology* **16**: 122.

Rowan DD, Latch GCM. 1994. Utilization of endophyte-infected perennial ryegrasses for increased insect resistance. In: Bacon CW, White JF, eds. *Biotechnology of Endophytic Fungi of Grasses*. Boca Raton, FL, USA. CRC Press: 169-183.

Safranyik L, Carroll AL, Régnière J, Langor DW, Riel WG, Shore TL, Peter B, Cooke BJ, Nealis VG, Taylor SW. **2010**. Potential for range expansion of mountain pine beetle into the boreal forest of North America. *The Canadian Entomologist* **142**: 415–442.

Saikkonen K. **2007**. Forest structure and fungal endophytes. *Fungal Biology Reviews* **21**: 67–74.

Sato H, Tanabe AS, Toju H. **2015**. Contrasting diversity and host association of ectomycorrhizal basidiomycetes versus root-associated ascomycetes in a dipterocarp rainforest. *PLOS ONE* **10**: e0125550.

Schoettle AW, Sniezko RA. **2007**. Proactive intervention to sustain high-elevation pine ecosystems threatened by white pine blister rust. *Journal of Forest Research* **12**: 327–336.

Siddique AB, Unterseher M. **2016**. A cost-effective and efficient strategy for Illumina sequencing of fungal communities: A case study of beech endophytes identified elevation as main explanatory factor for diversity and community composition. *Fungal Ecology* **20**: 175–185.

Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis*. Cambridge, UK: Academic Press.

Sniezko RA. **2006**. Resistance breeding against nonnative pathogens in forest trees — current successes in North America. *Canadian Journal of Plant Pathology* **28**: S270–S279.

Sokolski SS, Bernier-Cardou MB-C, Piché YP, Bérubé JABA. **2007**. Black spruce (*Picea mariana*) foliage hosts numerous and potentially endemic fungal endophytes. *Canadian Journal of Forest Research* **37**: 1737–1747.

Staley JT. **1997**. Biodiversity: are microbial species threatened? *Current Opinion in Biotechnology* **8**: 340–345.

Steiner JJ, Poklemba CJ, Fjellstrom RG, Elliott LF. **1995**. A rapid one-tube genomic DNA extraction process for PCR and RAPD analyses. *Nucleic Acids Research* **23**: 2569–2570.

Sumarah MW, Kesting JR, Sørensen D, Miller JD. **2011**. Antifungal metabolites from fungal endophytes of *Pinus strobus*. *Phytochemistry* **72**: 1833–1837.

Sumarah MW, Walker AK, Seifert KA, Todorov A, Miller JD. **2015**. Screening of fungal endophytes isolated from eastern white pine needles. In: Jetter R, ed. *Recent Advances in Phytochemistry. The Formation, Structure and Activity of Phytochemicals*. Cham, CH: Springer International Publishing, 195–206.

Tanaka A, Tapper BA, Popay A, Parker EJ, Scott B. **2005**. A symbiosis expressed non-ribosomal peptide synthetase from a mutualistic fungal endophyte of perennial ryegrass confers protection to the symbiotum from insect herbivory. *Molecular Microbiology* **57**: 1036–1050.

Tautenhahn S, Lichstein JW, Jung M, Kattge J, Bohlman SA, Heilmeier H, Prokushkin A, Kahl A, Wirth C. **2016**. Dispersal limitation drives successional pathways in Central Siberian forests under current and intensified fire regimes. *Global Change Biology* **22**: 2178–2197.

Taylor JW, Turner E, Townsend JP, Dettman JR, Jacobson D. **2006**. Eukaryotic microbes, species recognition and the geographic limits of species: examples from the kingdom Fungi. *Philosophical Transactions of the Royal Society B: Biological Sciences* **361**: 1947–1963.

Thompson, RS. 1990. Late Quaternary vegetation and climate in the Great Basin. In: *Packrat Middens: the Last 40,000 Years of Biotic Change*, Betancourt JL, Van Devender TR, Martin PS, eds. Tucson, AZ, USA: University of Arizona Press, 200 –239.

Thompson RS, Anderson KH. **2000**. Biomes of western North America at 18,000, 6000 and 0 14C yr bp reconstructed from pollen and packrat midden data. *Journal of Biogeography* **27**: 555–584.

Tomback DF. 1978. Foraging strategies of Clark's Nutcracker. *Living Bird*. **16**: 123–161.

Tomback DF. **1982**. Dispersal of whitebark pine seeds by Clark's Nutcracker: a mutualism hypothesis. *Journal of Animal Ecology* **51**: 451–467.

Tomback DF. **1986**. Post-fire regeneration of krummholz whitebark pine: a consequence of nutcracker seed caching. *Madroño* **33**: 100–110.

Tomback DF, Achuff P. **2010**. Blister rust and western forest biodiversity: ecology, values and outlook for white pines. *Forest Pathology* **40**: 186–225.

Tomback DF, Chipman KG, Resler LM, Smith-McKenna EK, Smith CM. **2014**. Relative abundance and functional role of whitebark pine at treeline in the northern Rocky Mountains. *Arctic, Antarctic, and Alpine Research* **46**: 407–418.

Tomback DF, Linhart YB. **1990**. The evolution of bird-dispersed pines. *Evolutionary Ecology* **4**: 185–219.

Tomback D F, Kendall KC. 2001. Biodiversity losses: the downward spiral. In Tomback DF, Arno SF, Keane RE, eds. *Whitebark Pine Communities: Ecology and Restoration.* Washington, D.C: Island Press, 243–262.

Tomback DF, Resler LM, Keane RE, Pansing ER, Andrade AJ, Wagner AC. **2016**. Community structure, biodiversity, and ecosystem services in treeline whitebark pine communities: potential impacts from a non-native pathogen. *Forests* **7**: 21.

Turner TR, James EK, Poole PS. **2013**. The plant microbiome. *Genome Biology* **14**: 209.

U'Ren JM, Lutzoni F, Miadlikowska J, Laetsch AD, Arnold AE. **2012**. Host and geographic structure of endophytic and endolichenic fungi at a continental scale. *American Journal of Botany* **99**: 898–914.

U'Ren JM, Lutzoni F, Miadlikowska J, Zimmerman NB, Carbone I, May G, Arnold AE. **2019**. Host availability drives distributions of fungal endophytes in the imperilled boreal realm. *Nature Ecology & Evolution* **3**: 1430–1437.

U'Ren JM, Riddle JM, Monacell JT, Carbone I, Miadlikowska J, Arnold AE. **2014**. Tissue storage and primer selection influence pyrosequencing-based inferences of diversity and community composition of endolichenic and endophytic fungi. *Molecular Ecology Resources* **14**: 1032–1048.

Vincent JB, Weiblen GD, May G. **2016**. Host associations and beta diversity of fungal endophyte communities in New Guinea rainforest trees. *Molecular Ecology* **25**: 825–841.

Wang H, Qi M, Cutler AJ. **1993**. A simple method of preparing plant samples for PCR. *Nucleic Acids Research* **21**: 4153–4154.

White RH, Engelke MC, Morton SJ, Johnson-Cicalese JM, Ruemmele BA. **1992**. Acremonium Endophyte Effects on Tall Fescue Drought Tolerance. Acremonium endophyte effects on tall fescue drought tolerance. *Crop Science* **32**: 1392–1396.

Wilcox C. **2001**. Habitat size and isolation affect colonization of seasonal wetlands by predatory aquatic insects. *Israel Journal of Zoology* **47**: 459–476.

Yandell UG. 1992. *An allozyme analysis of whitebark pine (Pinus albicaulis Engl.)*. MSc Thesis, University of Nevada, Reno, NV, USA.

APPENDIX A

FIELD DATA SHEETS

Study Site: ***Ecotype:*** ***Tree Data***

Tree Species :Tally	*Date:*	*Miscellaneous Notes*
*WBP**		
L		
*LP**		
*S**		
SAF		

Tree #	Species	Aspect	BD	BR Pres.	Can. K.	Is. Size & Comp.	Photo ID	N.C.	GPS	Form
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
30										

Study Site: ***Ecotype:*** ***Tree Data***

Tree Species :Tally		*Date:*	*Miscellaneous Notes*
*WBP**			
L			
*LP**			
*S**			
SAF			

Tree #	Species	Aspect	BD	BR Pres.	Can. K.	Is. Size & Comp.	Photo ID	N.C.	GPS	Form
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
30										

APPENDIX B

UNSUCCESSFUL MOLECULAR METHODS

We spent a considerable amount of time optimizing extraction methods and PCR protocols. Future research working with similar plant material would benefit the field if researchers described not only their successful methods, but also some of the unsuccessful methods they tested. In this appendix we describe a variety of the methods and protocols we tested that did not result in a successful outcome.

We tested a variety of DNA extraction methods in combination with a variety of PCR conditions and designs. Surface sterilized, ground, and homogenized whitebark pine needles were the primary material used in these tests, however, Engelmann spruce needles were also tested using several of these methods. We tested bead beating of needles in place of grinding needles under liquid nitrogen, and generally obtained lower DNA yield and purity values. We began with testing the most expeditious and simple extraction methods, and then progressively moved toward more laborious, robust methods. The extraction methods and kits we tested include: NaOH (Wang *et al*. 1993; Osmundson *et al*. 2013), rapid one step extraction (ROSE; Steiner *et al*., 1995; Osmundson *et al*., 2013), ROSE extraction with phenol chloroform cleanup (with and without EtOH precipitation), single and double phenol chloroform extraction (with and without an EtOH precipitation), modified CTAB extraction protocol of Bullington & Larkin (2015), Qiagen GENECLEAN kit, Qiagen DNeasy Plant Mini Kit, and Mobio Plant Power Soil Kit. We tested various amounts of tissue in each kit, ranging from 25-250 mg of ground needle tissue. We also tested varying amounts of elution solution to balance inhibitor removal with rate of DNA recovery. Lastly, we tested PEG magnetic bead cleanups to bind DNA and remove PCR inhibitors.

Additionally, we tested the products of these extractions under numerous PCR designs and variations in reagent amounts. We tested semi-nested PCR design, straight reamplification, touch-up and touch-down PCRs (in preamplification and Illumina tag addition PCR round 1), and non-nested designs. We generally found that PCR designs with greater than 45-50 cycles (combined total of internal and external PCRs) produced PCR artefacts. We tested various reagent amounts of template DNA, taq polymerase, BSA, dNTPs, and primers. DNA template amounts tested ranged from 7µL of undiluted DNA extract down to 1µL of 1:100 dilution. We also tested Hot start taq and standard taq polymerases. Our goal was to improve efficiency without compromising specificity. Many of the methods and kits listed here were tested in a combinatorial fashion, though not every extraction product was subjected to every combination of PCR design. Based on DNA yield and purity values, we made decisions on whether to continue testing or redo the extraction with a new method. What is described in the methods section and Appendix C are the only methods that we found consistently successful for working with these recalcitrant tissues.

APPENDIX C

SPRUCE MOLECULAR METHODS

Extracted fungal genomic DNA from spruce needles was amplified at three loci using a dual indexed three-step library preparation process following a semi-nested PCR design (). The external target region for ITS was amplified using ITS1F/ITS4 and for LSU was amplified using the primer pair LROR/LR7. External thermal cycler conditions for ITS1 included an initial denaturation step of 85 sec at 95°C; 6 cycles of: 35 sec at 95°C, 55 sec at 57.5°C, 75 sec at 72°C; 6 cycles of: 35 sec at 95°C, 55 sec at 57.5°C, 2 min at 72°C; and a final 72°C elongation step of 10 min. External thermal cycler conditions for LSU included an initial denaturation step of 85 sec at 95°C; 6 cycles of: 35 sec at 95°C, 55 sec at 57.5°C, 75 sec at 72°C; 6 cycles of: 35 sec at 95°C, 55 sec at 57.5°C, 2 min at 72°C; and a final 72°C elongation step of 10 min. The external PCR reactions were carried out in 25 µL in reaction volumes containing 1 µL of template, 2.5 µL 2x Dream Taq™ DNA polymerase (Thermo Scientific™), 2.5 µL of 2.5mM dNTP, 0.2 µL Taq, 16.8 µL H2O and 1 µL of each forward and reverse primer. Each reaction was performed on a T100™ Thermal Cycler (BIO RAD).

The internal ITS1, ITS2, and LSU amplicons were generated directly from the external PCR. Internal thermal cycler conditions for ITS1 and ITS2 included an initial denaturation step of 2 min at 95°C; 13 touchdown cycles of: 60 sec at 94°C, 60 sec starting at 63°C and decreasing 1°C for each of the final 12 steps of the touchdown cycle, 2 min at 72°C; 12 cycles of: 60 sec at 94°C, 60 sec at 52°C, 2 min at 72°C; and a final 72°C elongation step of 10 min. Internal thermal cycler conditions for LSU included an initial denaturation step of 2 min at 94°C, followed by 35 cycles of: 60 sec at 94°C, 45 sec at 50°C, 60 sec at 72°C; and a final elongation step of 7 min at 72°C. The internal PCR reactions were carried out in 25 µL in reaction volumes containing 1 µL of

template, 2.5 µL 2x Dream Taq™ DNA polymerase (Thermo Scientific™), 2.5 µL of 2.5mM dNTP, 0.2 µL Taq, 16.8 µL H2O and 1 µL of each forward and reverse primer. Each reaction was performed on a T100™ Thermal Cycler (BIO RAD).

All needles were surface sterilized using an EtOH - NaClO - EtOH protocol to remove any contaminants on the outer surface of the needles prior to DNA extraction. To verify successful surface sterilization, a subset of two randomly selected needle fascicles from each site were streaked across the surface of a petri dish and monitored for fungal growth in the following weeks. Two needles from each of the 10 fascicles of a sampled tree were pooled. Tissue was then freeze-dried using liquid nitrogen, macerated to a fine powder and homogenized with a mortar and pestle (Bullington & Larkin, 2015; Moler & Aho, 2018); a subset (~50 mg) was then used for fungal DNA extraction.

Genomic extraction from ground needle tissue followed the modified CTAB extraction protocol of Bullington & Larkin (2015). The CTAB lysis buffer was made using 0.5 M Tris-HCL, 5 M sodium chloride, 10% CTAB buffer, 0.5 M EDTA (pH 8), PVPP (polyvinylpolypyrrolidone), 2-beta-mercaptoethanol, proteinase K, and DI water. 900 mL of the CTAB lysis buffer was then added to each tissue sample and vortexed for 10-15s. Three, 10-minute freeze-thaw cycles were applied to samples using a 65°C heating block and -20°C freezer. Immediately following the final freeze-thaw cycle, samples were incubated in the 65°C water bath with periodic agitation for 10 min. Two rounds of phenol-chloroform extraction were employed on the samples using 25:24:1 phenol : chloroform : isoamyl alcohol to remove proteins and purify DNA.

A concentrated salt precipitation step was then utilized using 3 M sodium acetate (pH 5.5) and an equal volume isopropanol. 5 µL of RNAse A was then added to the

samples and incubated at room temperature for two hours. Samples were then centrifuged at 10,000 G for 10 min in an effort to concentrate DNA. Each DNA pellet was then was washed with 70% EtOH, suspended in 30mL of 1X Tris-EDTA buffer, and stored at -20°C. DNA was then amplified using a two-step PCR protocol. Step one amplified the ITS2 region using a mix of forward (fl ITS7) and reverse (ITS4) primers. A second round of PCR included a heterogeneity spacer region (0-6 nucleotides) between target primers and 22-bp Fluidigm universal tags, CS1 and CS2). PCR step one used 1µL of template and 20 pmol of each primer in 1xGoTaqVR Green Master Mix (Green GoTaqVR Reaction Buffer, 200 µM dATP, 200 µM, 200 µM GTP, 200 µM dCTP, 200 µM dTTP and 1.5 mM $MgCl_2$; Promega, Madison, WI, USA). Cycling conditions were 3 min at 94°C, 35 cycles of 60s at 95°C, 40s at 57°C, 40s at 70°C, 7 min at 68°C, and storage at 4°C (Bullington *et al*., 2018). PCR products were confirmed using 1.5% agarose gel electrophoresis with a 100-bp ladder. The second PCR reaction utilized 1 µL of template and 20 pmol of each primer in 1xGoTaqVR Green Master Mix (Promega), with cycling conditions of 95°C for 1 min, 10 cycles of 95°C for 30s, 60 °C for 30s, 68°C for 1 min, and 68°C for 5 min. PCR products were then checked for quality again via gel electrophoresis. Amplicon libraries were sequenced using 2 x 300 paired-end reads on an Illumina MiSeq sequencing platform at the University of Wisconsin Biotechnology Center (Madison, WI).

APPENDIX D

PLANT COMMUNITY SUMMARY TABLE

Table 1. Site characteristics and tree species composition at each site listed as percent community composition of *Pinus albicaulis* (PA), *Abies lasiocarpa* (AL), *Picea engelmannii* (PE), and *Pinus flexilis* (PF). Tree density was calculated based on plot size and trees occurring within plot. Canopy kill was recorded for each tree as 1 (none), 2 (1-24%), 3 (25-49%), 4 (50-74%), 5 (75-100%) (Modified from Resler & Tomback, 2008).

Site	Elevation (ft)	Tree Density	Tree Diversity (Shannon)	Community Composition (%)				Dead WBP (%)	(%) WBP with BR Symptoms	Mean Canopy Kill (class)	
				PA:	AL:	PE:	PF:			w/ BR:	w/o BR:
ABS – PK	10,220-10,380		0	100	0	0	0	6.7	35.7	3	1.9
ABS – EK	9,900	0.261	1.009	44.7	36.2	19.2	0	16	48.0	3.2	1.8
ABS – LE	8,640	0.535	0.540	11.7	84.4	3.9	0	0	0	-	1.8
BT – PK	10,040-9,960		0.122	2.6	0	97.4	0	0	0	-	1
BT – EK	9,790	0.622	0.743	37.5	1.8	60.7	0	0	23.8	2.8	1.25
BT – LE	9,250	0.489	0.642	72.7	9.1	18.2	0	30.4	50.0	3.1	1
CRAZ – PK	9,440-9,530		0	100	0	0	0	0	16.6	2.5	2
CRAZ – EK	9,400	0.689	0.687	41.9	58	0	0	10.3	46.2	3.25	1.8
CRAZ – LE	8,760	0.261	0.385	83.0	17	0	0	27.8	30.1	3.5	1.7
SAC – PK	9,350-9,480		0	100	0	0	0	0	30.3	2.6	2
SAC – EK	9,320	0.678	1.180	24.6	50.8	16.4	8.2	11.8	46.7	2.4	1.5
SAC – LE	9,140	0.922	0.644	68.7	31.3	0	0	3.4	15.8	2.6	1.9
STM – EK	8,960	0.752	0.277	90.3	8.1	1.6	0	11.1	41.1	3.1	1.9
STM – LE	8,580	0.611	0.098	98.2	0.91	0.9	0	6.1	11.1	3.5	1.9

APPENDIX E

QIIME OUTPUTS – GRAPHS AND FIGURES

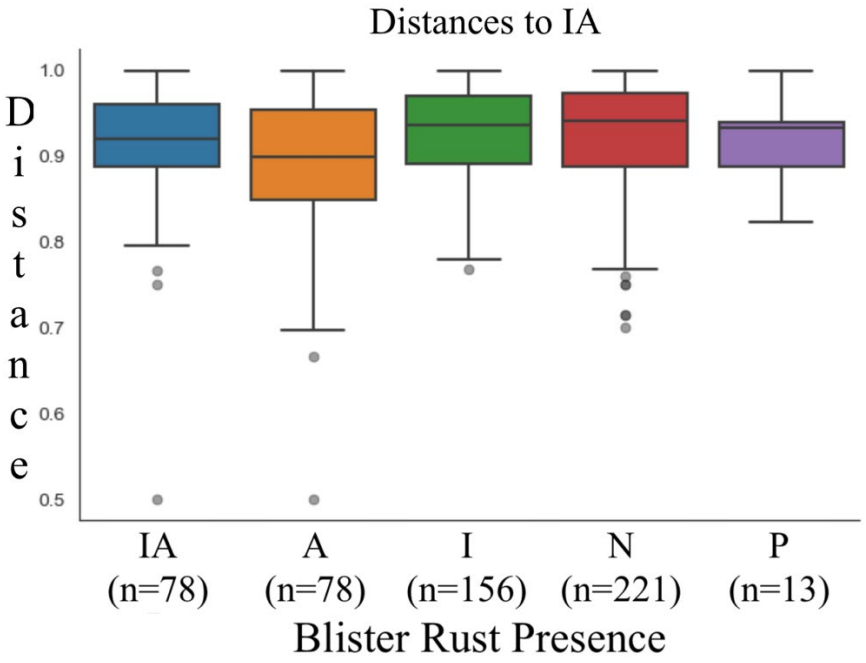

Fig. 13 Beta diversity distance (dissimilarity) of communities to trees with active and inactive blister rust infection.

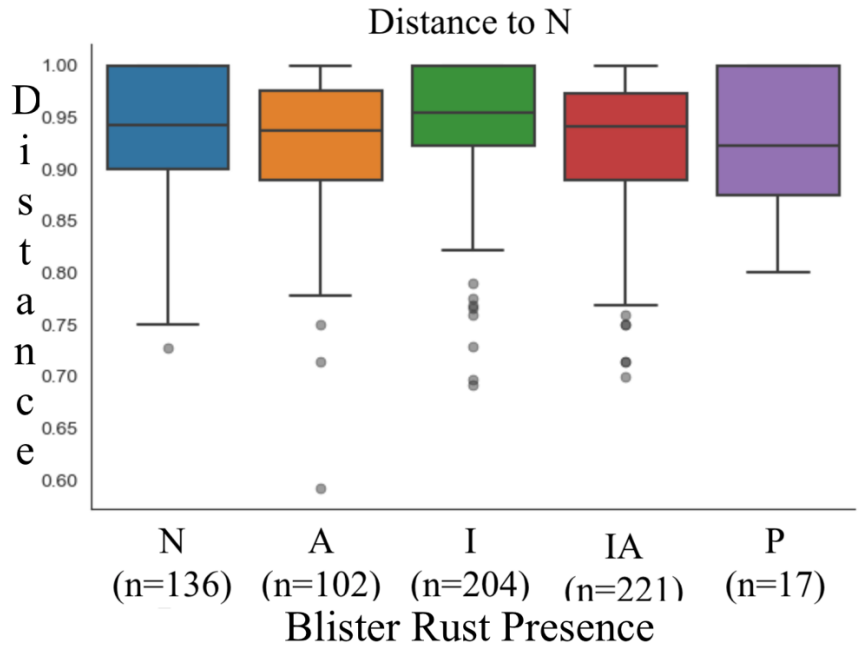

Fig. 14 Beta diversity distance (dissimilarity) of communities to trees lacking blister rust infection.

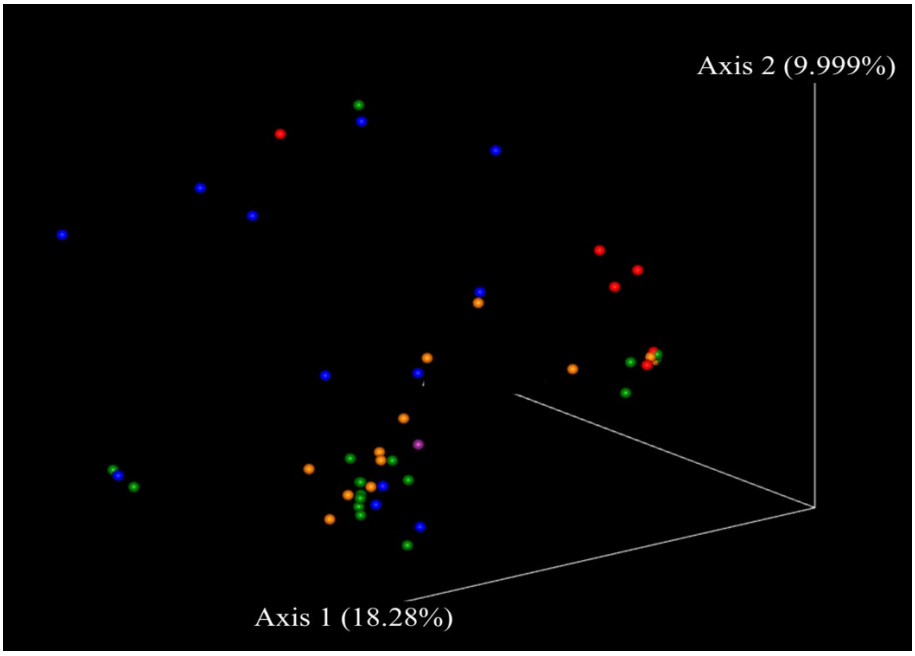

Fig. 16 PCoA plot of beta diversity with blister rust presence colorized: active infection (red), inactive infection (blue), inactive and active infection (orange), no infection (green), and potential infection in whitebark pine trees.

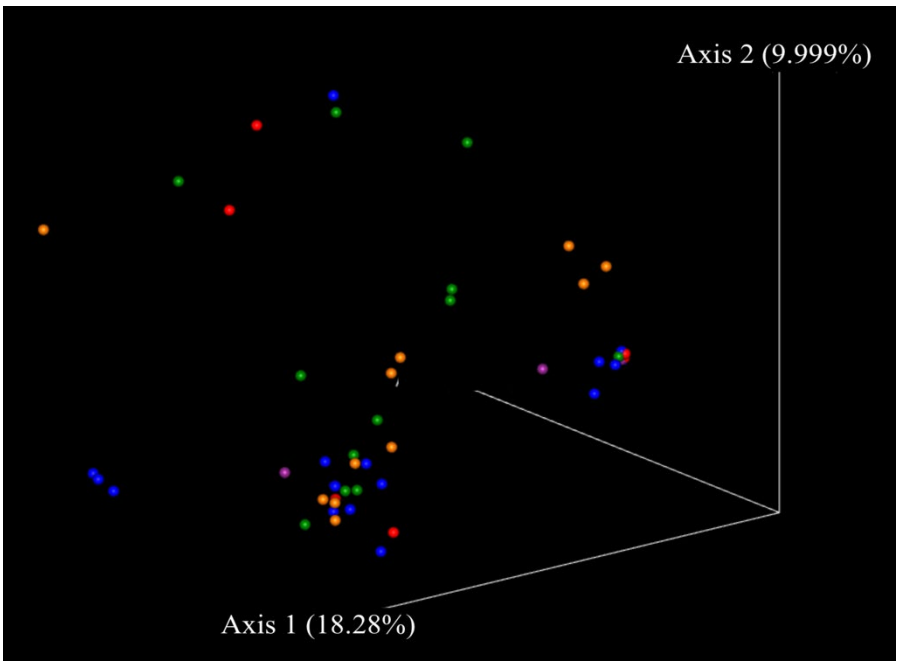

Fig. 17 PCoA plot of beta diversity with canopy kill classes colorized: class 1 (red, no canopy kill), class 2 (blue, 1-5% canopy kill), class 3 (orange, 6-25% canopy kill), class 4 (green, 26-50% canopy kill), class 5 (purple, 51-75% canopy kill) in whitebark pine trees.

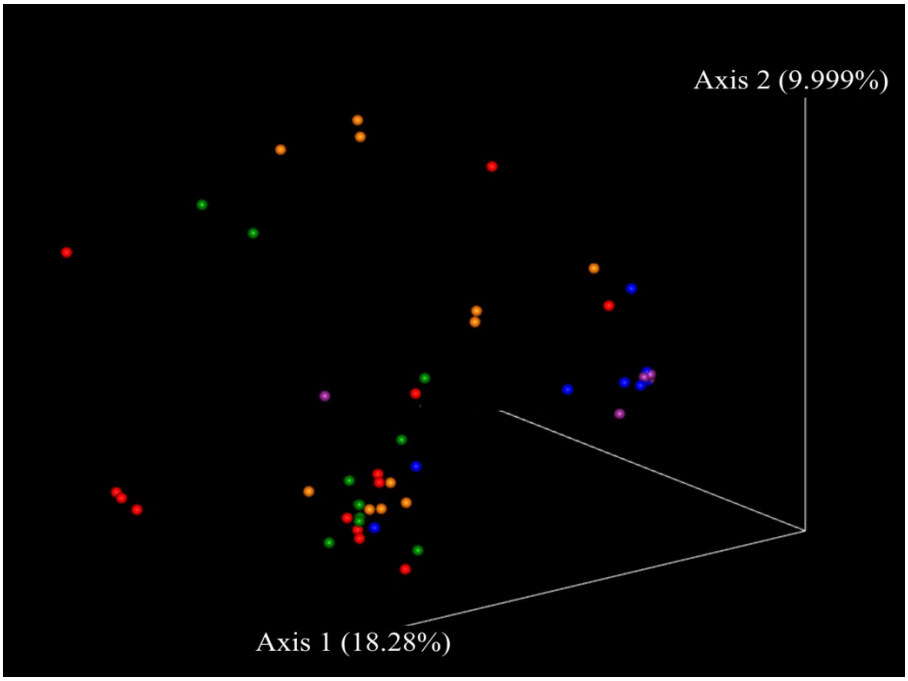

Fig. 18 PCoA plot of beta diversity with study sites colorized: Absoraka Range (red), Beartooth Plateau (blue), Crazy Mountains (orange), Sacajawea Saddle (green), St. Mary's Peak (purple) in whitebark pine trees.

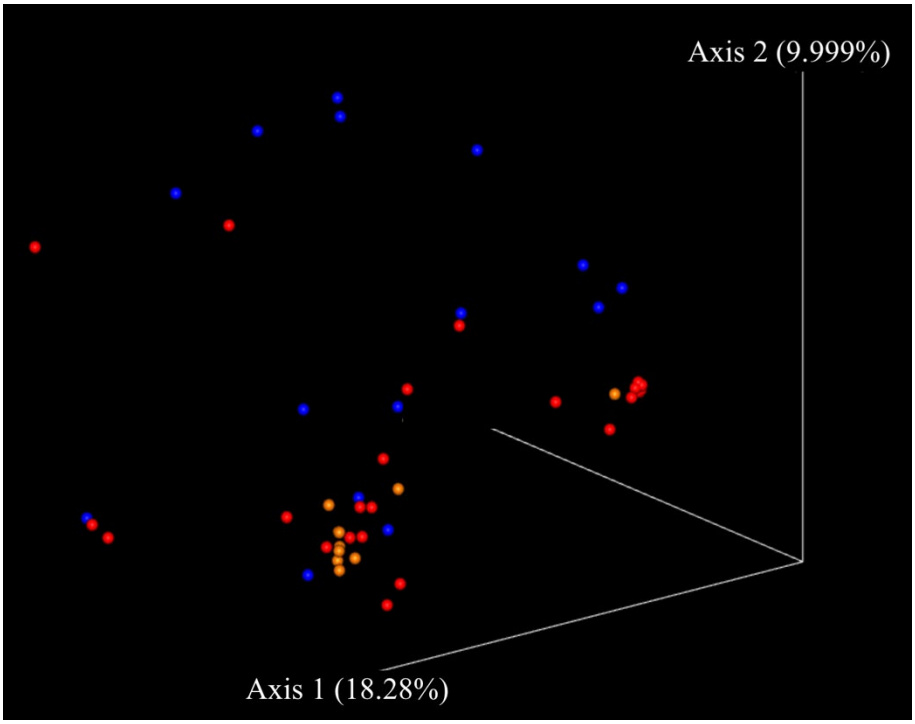

Fig. 19 PCoA plot of beta diversity with ecotypes colorized: established krummholz (red), lower elevation (blue), pioneering krummholz (orange) in whitebark pine trees.

APPENDIX F

OTU TAXONOMY TABLE

Table 2. OTU taxonomy table for whitebark pine.

OTU	Phylum	Class	Order	Family	Genus	Species
3	Ascomycota	Leotiomycetes	Helotiales			
5	Ascomycota	Leotiomycetes	Helotiales	Vibrisseaceae		
10	Ascomycota	Dothideomycetes	Venturiales			
12	Ascomycota	Leotiomycetes	Rhytismatales	Rhytismataceae	Lophodermium	Lophodermium nitens
13	Ascomycota	Dothideomycetes	Pleosporales	Melanommataceae	Herpotrichia	
14	Ascomycota	Dothideomycetes	Capnodiales		Phaeotheca	Phaeotheca fissurella
21	Ascomycota	Dothideomycetes	Pleosporales	Leptosphaeriaceae	Subplenodomus	Subplenodomus galicola
22	Ascomycota	Leotiomycetes	Helotiales	Vibrisseaceae		
25	Ascomycota	Leotiomycetes	Helotiales			
26	Ascomycota	Lecanoromycetes	Ostropales			
27	Ascomycota	Leotiomycetes	Thelebolales	Pseudeurotiaceae	Pseudeurotium	
28	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae		
29	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Heterosphaeria	Heterosphaeria patella
30	Ascomycota	Eurotiomycetes	Chaetothyriales			
31	Ascomycota	Leotiomycetes	Rhytismatales	Rhytismataceae	Lophodermium	Lophodermium fissuratum
32	Basidiomycota	Microbotryomycetes	Kriegeriales	Kriegeriaceae	Phenoliferia	Phenoliferia psychrophila
34	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Tetracladium	
35	Ascomycota	Leotiomycetes	Helotiales			
37	Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	Athelia	Athelia acrospora
39	Ascomycota					
40	Ascomycota	Leotiomycetes	Helotiales			
41	Basidiomycota	Agaricomycetes	Atheliales			
42	Ascomycota	Leotiomycetes				
43	Ascomycota	Eurotiomycetes	Phaeomoniellales	Phaeomoniellaceae	Phaeomoniella	
44	Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	Coprinopsis	Coprinopsis atramentaria
45	Ascomycota	Leotiomycetes				
48	Ascomycota	Leotiomycetes	Helotiales	Vibrisseaceae		
51	Ascomycota					
54	Ascomycota	Dothideomycetes	Dothideales	Dothioraceae	Perusta	
55	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae		
58	Basidiomycota	Agaricomycetes	Cantharellales			

59	Ascomycota	Lecanoromycetes	Ostropales			
60	Ascomycota	Leotiomycetes	Rhytismatales	Rhytismataceae	Bifusella	Bifusella linearis
62	Ascomycota	Dothideomycetes	Capnodiales			
63	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	Meristemomyces	Meristemomyces frigidus
67	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Comoclathris	Comoclathris arrhenatheri
68	Ascomycota	Sordariomycetes	Xylariales	Amphisphaeriaceae		
70	Ascomycota	Leotiomycetes	Rhytismatales	Rhytismataceae		
71	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	Lapidomyces	
72	Ascomycota	Dothideomycetes	Tubeufiales	Tubeufiaceae	Helicoma	
73	Ascomycota	Eurotiomycetes	Chaetothyriales			
74	Ascomycota	Leotiomycetes	Helotiales			
75	Ascomycota	Leotiomycetes	Helotiales	Vibrisseaceae	Phialocephala	
76	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae		
77	Ascomycota	Leotiomycetes	Phacidiales	Bulgariaceae		
79						
83	Basidiomycota	Agaricomycetes	Atheliales			
86	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae		
87	Ascomycota	Sordariomycetes				
88	Ascomycota	Dothideomycetes	Pleosporales			
90	Ascomycota	Leotiomycetes	Leotiales	Mniaeciaceae	Epithamnolia	Epithamnolia xanthoriae
92	Ascomycota	Dothideomycetes	Pleosporales			
93	Ascomycota					
94	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Comoclathris	
95	Ascomycota	Dothideomycetes	Mytilinidiales	Mytilinidiaceae	Lophium	Lophium arboricola
96	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	
98	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Filobasidium	
99						
100	Ascomycota	Sordariomycetes	Diaporthales	Valsaceae	Leucostoma	Leucostoma persoonii
101	Ascomycota	Dothideomycetes	Pleosporales		Thyrostroma	Thyrostroma compactum
102	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Lenzites	Lenzites betulina
104	Ascomycota	Dothideomycetes	Venturiales	Venturiaceae	Venturia	
105	Ascomycota	Eurotiomycetes	Phaeomoniellales			
106	Basidiomycota	Malasseziomycetes	Malasseziales	Malasseziaceae	Malassezia	Malassezia globosa
107	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Sporormiella	Sporormiella intermedia
109	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	Lachnellula	Lachnellula suecica
110	Ascomycota	Dothideomycetes	Capnodiales	Extremaceae	Extremus	Extremus antarcticus
111	Ascomycota	Dothideomycetes	Mytilinidiales	Mytilinidiaceae	Mytilinidion	

112	Ascomycota	Dothideomycetes	Pleosporales		Wettsteinina	Wettsteinina dryadis
113	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	Cistella	
115	Basidiomycota	Tremellomycetes	Cystofilobasidiales	Mrakiaceae	Mrakia	Mrakia cryoconiti
117	Ascomycota	Dothideomycetes	Pleosporales	Melanommataceae	Melanodiplodia	Melanodiplodia tianschanica
121	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae		
122	Ascomycota					
123	Ascomycota					
124	Ascomycota	Dothideomycetes				
125	Basidiomycota	Tremellomycetes	Tremellales			
130	Ascomycota	Dothideomycetes	Capnodiales			
131	Ascomycota	Sordariomycetes	Hypocreales			
132	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Penicillium	
134	Ascomycota	Dothideomycetes	Capnodiales			
136	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Trametes	Trametes pubescens
137	Ascomycota	Taphrinomycetes	Taphrinales	Taphrinaceae	Taphrina	
138	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Naganishia	
139	Ascomycota	Leotiomycetes	Helotiales	Vibrisseaceae		
140	Ascomycota					
141	Basidiomycota	Tremellomycetes	Trichosporonales	Trichosporonaceae	Cutaneotrichosporon	Cutaneotrichosporon jirovecii
143	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Talaromyces	Talaromyces rugulosus
144	Ascomycota	Leotiomycetes	Helotiales			
145	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	Vishniacozyma victoriae
147	Ascomycota	Eurotiomycetes				
148	Ascomycota	Leotiomycetes	Helotiales	Mollisiaceae	Phialocephala	
149	Ascomycota	Leotiomycetes	Helotiales			
152	Ascomycota	Dothideomycetes				
153	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Tremella	Tremella encephala
154	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Mycosphaerella	
155	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Juncaceicola	Juncaceicola oreochloae
156	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	Elasticomyces	Elasticomyces elasticus
157	Basidiomycota	Tremellomycetes				
159	Ascomycota	Lecanoromycetes				
160	Ascomycota					
162	Ascomycota	Leotiomycetes	Helotiales			
163	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	Chaetomium	
165	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia	Preussia dubia
166	Ascomycota					

168	Ascomycota	Dothideomycetes	Capnodiales	Cladosporiaceae	Toxicocladosporium	Toxicocladosporium rubrigenum
170	Ascomycota	Dothideomycetes	Tubeufiales	Tubeufiaceae	Helicoma	Helicoma isiola
171	Ascomycota	Dothideomycetes	Capnodiales			
173	Ascomycota	Leotiomycetes	Helotiales			
174	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Mycosymbioces	
175	Ascomycota	Dothideomycetes	Dothideales	Dothioraceae	Perusta	Perusta inaequalis
176	Ascomycota					
178						
180	Basidiomycota	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	Rhodotorula	Rhodotorula mucilaginosa
181	Ascomycota	Dothideomycetes	Pleosporales	Melanommataceae	Herpotrichia	
182						
184	Ascomycota	Sordariomycetes	Diaporthales	Cryphonectriaceae	Celoporthe	Celoporthe tibouchinae
185	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Phaeosphaeria	
186	Basidiomycota	Agaricomycetes	Russulales	Hericiaceae	Hericium	Hericium yumthangense
187	Ascomycota	Dothideomycetes	Capnodiales	Dissoconiaceae	Dissoconium	Dissoconium eucalypti
189	Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae		
190	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae		
191	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	Resupinatus	
196	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	Brunnipila	Brunnipila calyculiformis
197	Ascomycota	Eurotiomycetes	Phaeomoniellales	Phaeomoniellaceae		
199	Ascomycota	Dothideomycetes	Capnodiales	Cladosporiaceae	Verrucocladosporium	Verrucocladosporium visseri
200	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Hannaella	Hannaella sinensis
201	Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	Piskurozyma	
204	Ascomycota	Leotiomycetes				
205	Ascomycota					
206	Ascomycota	Lecanoromycetes	Ostropales	Stictidaceae	Stictis	Stictis radiata
207	Basidiomycota	Tremellomycetes	Trichosporonales	Trichosporonaceae	Trichosporon	Trichosporon asahii
208	Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	Dermea	Dermea viburni
209	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Cerioporus	Cerioporus squamosus
211	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae		
213	Ascomycota	Lecanoromycetes	Ostropales	Stictidaceae	Stictis	Stictis radiata
214	Ascomycota	Dothideomycetes	Capnodiales		Arthrocatena	Arthrocatena tenebrio
216	Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	Mollisia	Mollisia cinerea
221	Ascomycota	Leotiomycetes	Helotiales			
222	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	Elasticomyces	Elasticomyces elasticus
224	Ascomycota	Eurotiomycetes	Chaetothyriales		Sarcinomyces	
225	Ascomycota	Lecanoromycetes	Lecanorales	Parmeliaceae	Hypogymnia	Hypogymnia physodes

226	Ascomycota	Leotiomycetes	Helotiales			
232	Ascomycota	Saccharomycetes	Saccharomycetales	Phaffomycetaceae	Cyberlindnera	Cyberlindnera jadinii
234	Ascomycota	Sordariomycetes	Xylariales			
235	Basidiomycota	Agaricomycetes	Polyporales	Irpicaceae		
237	Ascomycota	Sordariomycetes	Glomerellales	Plectosphaerellaceae	Musicillium	Musicillium theobromae
239	Ascomycota					
241	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	Rosellinia	
242	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Parastagonospora	
246	Basidiomycota	Microbotryomycetes	Leucosporidiales	Leucosporidiaceae	Leucosporidium	
247	Ascomycota	Dothideomycetes	Myriangiales	Gobabebomyces		
248	Ascomycota	Leotiomycetes	Helotiales			
253	Ascomycota	Leotiomycetes	Helotiales			
255	Basidiomycota	Tremellomycetes	Trichosporonales	Trichosporonaceae	Apiotrichum	Apiotrichum porosum
256	Ascomycota	Saccharomycetes	Saccharomycetales		Diutina	Diutina catenulata
257	Basidiomycota	Agaricomycetes	Hymenochaetales		Trichaptum	Trichaptum biforme
258	Basidiomycota	Tremellomycetes	Tremellales			
259	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Pragmopora	Pragmopora amphibola
260	Ascomycota	Leotiomycetes	Helotiales			
261						
263	Ascomycota	Leotiomycetes				
264	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Juncaceicola	
266	Ascomycota	Leotiomycetes	Helotiales			
270	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae		
272	Ascomycota					
273	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	Mycena	
275	Ascomycota					
276	Basidiomycota	Agaricomycetes	Polyporales	Ganodermataceae	Ganoderma	Ganoderma applanatum
278	Ascomycota	Dothideomycetes	Capnodiales		Phaeotheca	Phaeotheca fissurella
281	Basidiomycota	Tremellomycetes	Cystofilobasidiales	Mrakiaceae	Tausonia	Tausonia pullulans
282	Basidiomycota	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	Rhodosporidiobolus	Rhodosporidiobolus colostri
284	Ascomycota	Leotiomycetes	Helotiales	Cenangiaceae	Cenangium	
285	Basidiomycota	Agaricomycetes	Russulales	Stereaceae	Stereum	
286	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Claussenomyces	
287						
288	Ascomycota	Leotiomycetes				
290	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Tremella	
291	Ascomycota	Dothideomycetes	Dothideales		Selenophoma	Selenophoma linicola

292	Ascomycota	Sordariomycetes	Hypocreales			
293	Ascomycota	Lecanoromycetes	Lecanorales	Parmeliaceae	Melanohalea	Melanohalea exasperatula
294	Basidiomycota	Agaricomycetes	Agaricales	Physalacriaceae	Flammulina	Flammulina velutipes
296	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Bulleromyces	Bulleromyces albus
299	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae		
301	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Dentocorticium	Dentocorticium portoricense
302	Ascomycota	Sordariomycetes	Xylariales		Hansfordia	Hansfordia pulvinata
304	Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	Chlorophyllum	Chlorophyllum molybdites
305	Ascomycota	Leotiomycetes	Helotiales	Leotiaceae	Alatospora	
306	Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	Entoloma	
307	Ascomycota	Dothideomycetes	Dothideales			
309	Ascomycota	Leotiomycetes	Helotiales			
310	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Acrodontium	Acrodontium simplex
312	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Tremella	Tremella subalpina
314	Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	Mollisia	
317	Ascomycota	Leotiomycetes	Thelebolales	Pseudeurotiaceae	Pseudeurotium	Pseudeurotium desertorum
318	Ascomycota	Lecanoromycetes	Candelariales	Candelariaceae	Candelaria	Candelaria concolor
319	Ascomycota	Dothideomycetes	Botryosphaeriales			
320	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	Tricharina	Tricharina praecox
321	Ascomycota					
323	Ascomycota	Sordariomycetes	Diaporthales	Gnomoniaceae		
327	Ascomycota					
328	Ascomycota	Leotiomycetes	Helotiales			
329	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	Pseudopithomyces	
333	Basidiomycota	Agaricomycetes	Polyporales	Grifolaceae	Grifola	Grifola frondosa
335	Ascomycota	Leotiomycetes	Rhytismatales	Rhytismataceae	Bifusella	Bifusella linearis
338	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Sporormiella	
342	Ascomycota	Dothideomycetes	Mytilinidiales	Mytilinidiaceae	Lophium	Lophium mytilinum
343	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	Neocatenulostroma	Neocatenulostroma microsporum
344	Ascomycota	Dothideomycetes	Dothideales	Dothioraceae	Perusta	
346	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Aspergillus	Aspergillus penicillioides
347	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia	Preussia lignicola
348	Ascomycota	Dothideomycetes	Capnodiales		Hispidoconidioma	Hispidoconidioma alpinum
349						
351	Ascomycota	Leotiomycetes	Helotiales		Cyclaneusma	Cyclaneusma minus
352	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Dioszegia	Dioszegia zsoltii var zsoltii
353	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Passalora	Passalora fulva

359	Ascomycota	Eurotiomycetes	Chaetothyriales	Trichomeriaceae	Bradymyces	
361	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae		
362	Ascomycota	Eurotiomycetes	Chaetothyriales		Sarcinomyces	Sarcinomyces crustaceus
363	Ascomycota	Leotiomycetes	Helotiales	Vibrisseaceae		
364						
367	Ascomycota	Saccharomycetes	Saccharomycetales			
369	Ascomycota	Dothideomycetes	Tubeufiales	Tubeufiaceae	Helicoma	
370	Basidiomycota	Agaricomycetes	Hymenochaetales	Schizoporaceae	Xylodon	Xylodon flaviporus
372	Ascomycota	Leotiomycetes	Helotiales			
375	Ascomycota	Leotiomycetes				
376	Ascomycota	Leotiomycetes	Helotiales			
378						
379	Ascomycota	Dothideomycetes	Venturiales	Sympoventuriaceae	Troposporella	Troposporella monospora
380	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Sporormiella	Sporormiella minima
381	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	Pseudotaeniolina	Pseudotaeniolina globosa
382	Ascomycota	Orbiliomycetes	Orbiliales			
384	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	Humicola	
385	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Dematiopleospora	Dematiopleospora salsolae
386	Basidiomycota	Agaricomycetes	Agaricales	Schizophyllaceae	Schizophyllum	
390	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	Poronia	Poronia punctata
396	Ascomycota	Arthoniomycetes				
402	Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	Entoloma	
403	Ascomycota	Leotiomycetes	Rhytismatales	Rhytismataceae		
406	Ascomycota	Dothideomycetes	Mycosphaerellales	Mycosphaerellaceae		
408	Basidiomycota	Agaricomycetes	Atheliales			
409	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	Tremateia	
411	Basidiomycota	Agaricomycetes	Polyporales	Meruliaceae	Mycoacia	Mycoacia fuscoatra
418	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Passalora	Passalora fulva
419	Ascomycota	Lecanoromycetes	Lecanorales	Lecanoraceae	Lecanora	Lecanora laxa
421	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Passalora	Passalora fulva
426	Ascomycota	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	Purpureocillium	Purpureocillium lilacinum
432	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Tremella	
433	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Tympanis	Tympanis abietina
434	Ascomycota	Leotiomycetes	Helotiales			
437	Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	Gymnopus	Gymnopus luxurians
448	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Talaromyces	
449	Basidiomycota	Agaricomycetes	Polyporales	Meruliaceae	Phlebia	Phlebia lividina

452	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	Meristemomyces	Meristemomyces frigidus
453	Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	Cryptosporiopsis	
454	Ascomycota	Leotiomycetes	Helotiales	Sclerotiniaceae		
456	Ascomycota	Leotiomycetes				
459	Basidiomycota	Agaricomycetes	Agaricales	Pleurotaceae	Pleurs	Pleurs cornucopiae
462	Ascomycota	Leotiomycetes	Rhytismatales	Rhytismataceae	Bifusella	Bifusella linearis
463	Basidiomycota	Agaricomycetes	Agaricales	Pleurotaceae	Pleurs	
464						
465	Ascomycota	Leotiomycetes	Helotiales			
470	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	Capnocheirides	
473	Basidiomycota	Malasseziomycetes	Malasseziales	Malasseziaceae		
474	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Hannaella	Hannaella surugaensis
475	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	Mycena	Mycena leptophylla
478	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Passalora	Passalora fulva
481						
483	Ascomycota					
484	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	Cistella	
485						
486	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	
487	Olpidiomycota					
490	Ascomycota	Leotiomycetes	Helotiales			
504	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	
505	Ascomycota					
508	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Neosetophoma	Neosetophoma samararum
511	Ascomycota	Leotiomycetes	Helotiales	Vibrisseaceae		
513	Ascomycota					
518	Ascomycota	Dothideomycetes				
519	Ascomycota	Arthoniomycetes	Lichenostigmatales	Phaeococcomycetaceae	Phaeococcomyces	
523	Basidiomycota	Agaricomycetes	Polyporales			
524	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	Melanocarpus	Melanocarpus albomyces
527	Ascomycota	Leotiomycetes				
528	Ascomycota	Leotiomycetes	Helotiales			
529	Ascomycota	Leotiomycetes	Rhytismatales	Rhytismataceae		
531	Ascomycota					
532						
534	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Passalora	Passalora fulva
538	Basidiomycota	Agaricomycetes	Polyporales	Steccherinaceae	Ceriporiopsis	Ceriporiopsis gilvescens

543	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Exophiala	Exophiala xenobiotica
555	Basidiomycota	Microbotryomycetes		Chrysozymaceae	Pseudohyphozyma	
556	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae		
557	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	Coniochaeta	
559	Ascomycota	Dothideomycetes				
560	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae		
561	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Passalora	Passalora fulva
562	Ascomycota	Dothideomycetes	Capnodiales			
565	Ascomycota	Leotiomycetes	Helotiales			
566	Ascomycota	Sordariomycetes	Hypocreales	Stachybotryaceae	Stachybotrys	Stachybotrys chartarum