GARRY OAKS: AN EVALUTATION OF MYCORRHIZAL INOCULATION AND PLANT COMMUNITY IMPACTS ON SURVIVORSHIP AND GROWTH OF SEEDLINGS ON JOINT BASE LEWIS-MCCHORD

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ABSTRACT

Garry oaks: An evaluation of mycorrhizal inoculation and plant community impacts on survivorship and growth of seedlings on Joint Base Lewis-McChord

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Prairies and oak savannas in the Willamette Valley-Puget Trough-Georgia Basin (WPG) are one of the most endangered ecosystems in North America. In the south Puget lowlands, native prairie cover has declined by approximately 97%. Historically, these open areas were maintained by Native Americans' frequent burning. However, fire suppression, habitat fragmentation, development, species invasion and native species decline have contributed to their increasing rarity in the WPG ecoregion. A keystone species that exists in the ecotone between open prairies and woodlands is Garry oak (*Quercus garryana*). The restoration of Garry oak is essential for the protection of prairies and associated habitat for numerous plant and animal species, but survival is often poor. Vital to oak survival is the development of mycorrhizas (a symbiotic relationship between plants and fungi), yet the need to apply this in nurseries is often overlooked. For this project, 1,000 Garry oak seedlings were planted in six sites in different training areas within Joint Base Lewis-McChord during the fall and winter of 2019. Half were inoculated during greenhouse production with commercial mycorrhizae and half were not. Survival, height and trunk diameter were measured one year after planting and site quality, proximity to established oak stands and inoculation treatment were recorded to determine if artificial inoculation or site characteristics significantly influence survival and growth rates. Results showed inoculation did not have a statistically significant effect on survivorship or growth, although average survivorship was higher in inoculated seedlings. Site quality proved to have a positive aboveground growth was on average higher in uninoculated seedlings. Through this study, restorationists can determine with greater accuracy whether artificial inoculation is worth the added expense in these areas and attain greater levels of understanding regarding the role symbiotic fungi play in Garry oak survival.

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Literature Review

Essential to the comprehension of this thesis project is attaining a comprehensive review of the scholarship focusing on prairie/oak ecosystems, Garry oaks and mycorrhizae. My literature review provides frameworks for continued study of Garry oaks and synthesizes foundational research necessary to understand the variables responsible for Garry oak seedling survival in six different planting sites on Joint Base Lewis-McChord (JBLM) and unpacks the symbiotic relationship between mycorrhizal fungi and Garry oaks in nursery and field settings. This review will begin with a brief overview of the geologic and cultural history of prairies and oak savannas in the WPG ecoregion, followed by a brief introduction of the benefits and challenges of native plant reintroductions. The third section of this literature review will describe Garry oak species status, environmental history, and treatments to improve survivability and growth. The fourth section will narrow in focus to unpack the history of mycorrhizal research, mycorrhizal types and important studies which establish the underpinnings that support my research and highlight gaps in knowledge. The conclusions drawn from my research could benefit future restoration managers and conservationists by contributing to our collective knowledge of variables that affect Garry oak seedling establishment and the effects of mycorrhizal inoculation on survivability and growth. Also, my research provides a potential first step in a longitudinal study. The data in this study will provide future researchers with a baseline when analyzing the variables associated with long-term Garry oak survival and growth, and the potential effects of mycorrhizal inoculation as a treatment variable. Garry oaks take decades to mature and continued study is required to draw strong conclusions with greater statistical significance.

Environmental History of Prairie/Oak Ecosystems

Ecoregions provide important frameworks for researching, managing and monitoring ecosystems and their various components (Floberg et al., 2004). A vital step toward grasping the variables responsible for oak seedling survival and growth rates is attaining historical context regarding the habitats they once occupied and the forces responsible for their decline. The oak savannas and prairies of the Willamette Valley-Puget Sound-Georgia Basin (WPG) ecoregion extend 600 km from Vancouver Island in the north to Willamette Valley in the south and include parts of British Columbia, Washington, and Oregon. The WPG forms a long, narrow band of valley lowlands and inland coastal waters nestled along the craggy peaks of the Cascades and adjoined coastal mountain ranges of British Columbia, Washington and Oregon (Floberg et al., 2004). J. Harlen Bretz (a scholar and geologist) was among the first to hypothesize the forces responsible for forming this large ecoregion and the prairies within it, although the term WPG was not yet in existence. His book, *Glaciation of the Puget Sound Region,* goes into incredible detail when describing the glaciers responsible for topographic changes and subsequent warming, eruption of the Lake Missoula ice dam, and the glacial outwash that resulted in favorable soil conditions for the emergence of prairies, especially in the south Puget lowland sub-region (Thysell & Carey, 2001). A historical factor heavily responsible for the geography of the Puget Trough was the Frasier Glaciation event, its maximum extent occurring roughly 15,000-13,000 years ago (Kruckeberg, 1995). Part of the Frasier Glaciation, the Cordilleran ice sheet, encompassed the Vashon glacier which extended to the south, occupying what is now Washington, Idaho and part of Montana. Part of the Vashon ice sheet known as the Puget Lobe, was forced between The Olympic Mountains and the Cascade Mountains 15,000-13,500 years ago in what is now the Olympia area. The Puget Lobe's glacial retreat left deposits and glacial till which have contributed to the course, cobbled, well-drained soils currently present in these landscapes. Additionally, massive volumes of meltwater helped shaped the region and have left behind a legacy of soil scouring and erosion, depressions with poor drainage, and a degradation of soil nutrients (Frederica & Hamman, 2016). The Puget Lobe persisted at its maximum extent for around 300 years, a relatively short amount of time in glacial timescales. Large bodies of water were held back by the Puget Lobe, with periodic flooding occurring at the ice sheet edges. These floods created somewhat narrow channels in which large depositions of gravel were left (Kruckeberg, 1995).

The WPG contains a wide spectrum of various hydrologic and soil conditions (and in turn high species richness and diversity), a direct result of the changes that occurred over the last 20,000 years. Knowledge of the environmental history of the area is crucial in the management and restorations of landscapes (Whitlock, 1992). Vegetation and habitat types in the WPG were initially described by David Douglas, who explored the region in the early 1800's (Dunwiddie & Bakker, 2011). Not until 1973 was the first summary of WPG prairies created by Franklin and Dyrness (1973). The authors highlight how glacial outwash provided favorable substrate conditions for prairie and oak woodlands to thrive in the lower Puget Sound (Whitlock, 1992). Numerous localized vegetation studies were conducted after Douglas' explorations, but in 1997 Chappel and Crawford created extensive vegetation catalogs (Crawford & Hall, 1997). These catalogs are used as baseline reference points for determining current vegetation ranges and predicting future assemblage changes.

Prairie landscapes formed during the early Holocene (11,000-7,250 YBP) as a result of warm and dry environmental conditions following the last glacial period and subsequent outwash described by Bretz. 5,000 to 6,000 years ago, a wetter, cooler climate developed. Evidence of this climatic shift is provided in the increased abundance of trees, such as western red cedar (*Thuja plicata*) and Pacific yew (*Taxus brevifolia*), which flourish in cooler, wetter conditions. Storm & Shebitz (2006) support these findings by showing a reduction in charcoal inputs in the soil during this period. However, prairie and oak savannas continued to flourish in the area until European contact in the 1800's (Boyd, 1999).

Although debates exist regarding the extent to which natural wildfires and anthropogenic burning played in shaping the vegetation mosaics of WPG prairie and oak savannas, it is ubiquitously accepted that the use of frequent low intensity fires played an essential ecological role and held great cultural importance for indigenous tribes (Storm & Shebitz, 2006). Pellatt & Gedalof (2014) utilize a multi-disciplinary approach to analyze vegetation changes in Garry oak ecosystems from the early Holocene to present to discuss the impacts of global anthropogenic ecosystem degradation. Through the lenses of historical ecology, paleoecology, and bioclimatic modelling, the authors distill how indigenous land management practices were essential in maintaining Garry oak ecosystems, and knowledge of these practices is important for current and future ecosystem restoration management plans. Pollen and charcoal analysis show that, despite a cooler, wetter climate 3,800 years ago, Garry oak savannas persisted due to cultural burning, plant resource harvesting and wildfire (Walsh, 2008). The authors write that Garry oak ecosystems are endangered due to the absence of fire. As a result of European colonization and the decimation of indigenous peoples, almost all WPG oak ecosystems experienced altered fire regimes in less than 100 years.

Indigenous prescribed burning events had a multitude of ecological and cultural benefits. Prescribed burning allowed indigenous people to manage large swaths of open land and support specific species and habitats necessary for survival. Conducting periodic burns had a multitude of benefits, including: limiting the risk of wildfires, allowing for open travel corridors, supporting greater grazing and line of sight for game, maintaining berry grounds and maximizing plant production, like camas and strawberries (Storm & Shebitz, 2006). The Traditional Ecological Knowledge (TEK) required to effectively manage these landscapes and utilize fire as an ecological tool is incredibly nuanced, and only recently has there been greater acknowledgment of these processes, and a concerted effort to reintroduce fire into some of these ecosystems (Dunwiddie & Bakker, 2011). The importance of fire is highlighted in the languages of indigenous people in the area. For instance, the Upper Chehalis people use about 20 different words to describe environments maintained by fire (Storm & Shebitz, 2006). This is the case on JBLM prairies, where designated fire crews conduct seasonal burns in a coordinated process to mimic historic fire regimes, manage and prepare restoration sites, limit invasive species and create open training areas.

European settlers prioritized prairies and oak ecosystems as locations to homestead, because of the flat topography and open space they provided. With homesteading came the introduction of grazing animals which continued to degrade Garry oak habitats through the introduction of non-native plant species, such as Scotch broom (*Cytisus scoparius)* (Thysell & Carey, 2001;). Furthermore, European settlers, agricultural development, conifer encroachment and non-native species invasion have contributed to the rapid decline of Garry oak in conjunction with prairie and oak savanna degradation. The largest remaining oak stands in the Puget Sound Area (PSA) are on Joint Base Lewis McChord (JBLM). Following the spread of settler colonialism and systematic displacement of tribes, prairie and oak savannas have become increasingly rare and are now one of the most highly endangered ecosystems in North America (Boyd, 1999). Among the first to discuss the decline of prairie and oak savannas was Giles

(1970), who acknowledged decline of these open spaces due to the encroachment of Douglas-fir, Grand fir (*abies grandis*) and Shore Pine (*pinus contorta)* Garry oak ecosystems used to cover 111,000 hectares in the Puget Sound; a mere 3% of that exists today. The Puget lowlands had some of the largest prairie and oak savannas in the WPG, but now make up a mere 9% of their original acreage. In addition, approximately 2-3% of remaining prairie and oak savannas are dominated by native plant species, as shown in Figure 2 (Dunwiddie & Bakker, 2011).

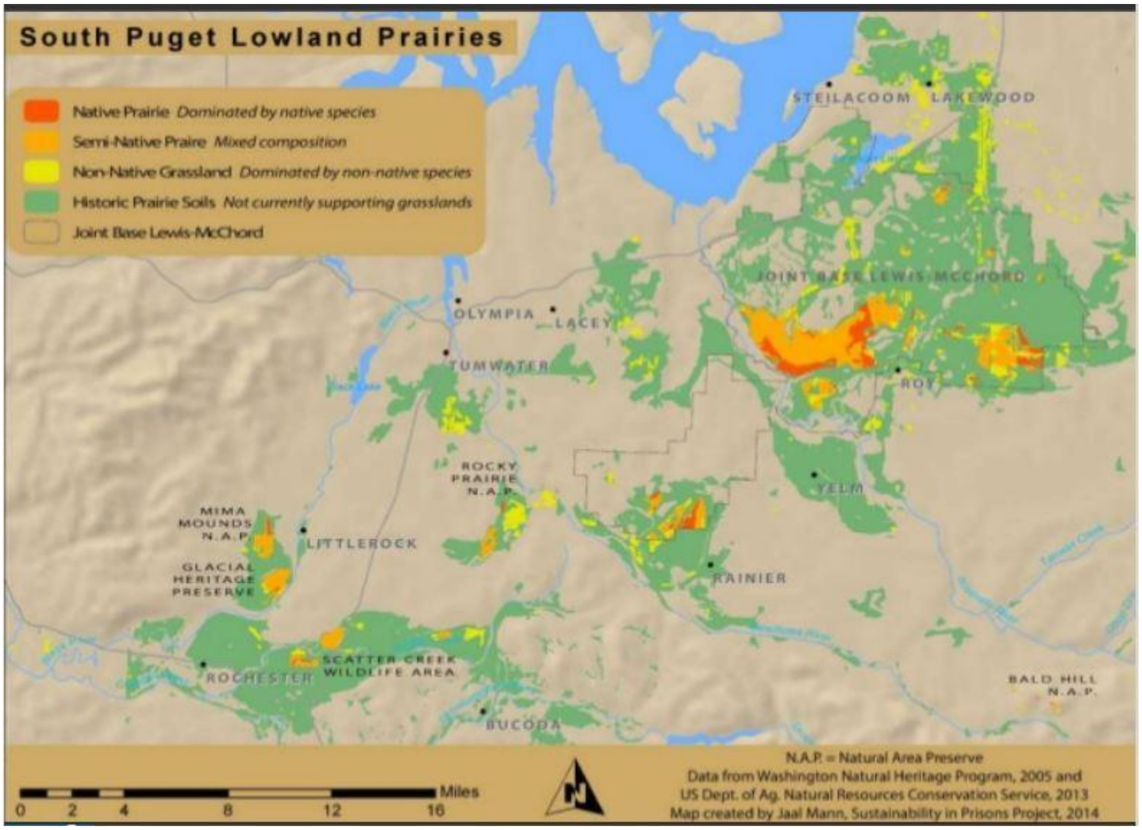

Figure 1: Historic native prairie and grassland areas, non-native grasslands and semi-native prairies in the south Puget Sound.

Such losses in prairie extent and biodiversity have contributed to lower ecosystem functionality, due to the reduction in species interactions between fragmented prairie and oak savanna ecosystems. Kearns et al. (1998) discuss the negative effects of habitat fragmentation and population decline among host/pollinator interactions, coining the term “endangered mutualism”. The authors argue that the fate of many plants depends on mutualistic interactions and the “…web of organisms that affect both plant and pollinator” (Kearns et al., 1998, p. 297). This research helped pioneer the concept of co-extinction and has led to a plethora of research on the topic. Rezende et al. (2007) analyze the interactions between plants and animal seed dispersers, highlighting the importance of these interactions in developing Earth’s biodiversity. The authors conclude because of these phylogenic relationships, simulated extinction events lead to coextinction of related species.

Conservation and restoration efforts have increased over time and regional collaborations have occurred at extraordinary levels, as highlighted in the 24 papers published in a special issue of *Northwest Science,* the journal of the Northwest Scientific Association, and the creation of the Cascadia Prairie-Oak Partnership (CPOP) (Dunwiddie & Bakker, 2011). Many of the papers embedded in this special issue acknowledge the importance and challenges involved in reintroducing native and endangered plant species to prevent extinction and improve ecosystem functionality in prairie ecosystems.

Reintroduction of Native Plants

Reintroducing native plant species to disturbed sites is vital for the restoration of degraded ecosystems and is a standard technique in restoration ecology (Drayton & Primack, 2012; Maunder, 1992; Stanley et al., 2011). However, the re-establishment of rare and native plant species has been largely unsuccessful, and the results of reintroduction efforts are rarely reported (Godefroid et al., 2011). Furthermore, long term monitoring of reintroductions is generally lacking, which inhibits collective conclusions of current and future viability assessments (Maunder, 1992). Godefroid (2011) conducted a meta-analysis of 249 plant reintroductions to determine how successful they have been and what factors lead to the most successful reintroductions. The authors discovered that out of 249 plant species reintroductions around the world, survival rates were low (52%), with individual experiment success rates declining over time. The analysis of numerous variables that influence plant reintroduction outcomes shows that improvements could be seen if greater attention is paid to species biology, increases in the number of plants being introduced (specifically seedlings rather than seeds) and consistent long-term monitoring. This thesis project is the foundation for continued long term monitoring of the analyzed Garry oak planting sites through its easily replicable study, clear results and visualization of vegetation changes using GIS software. The sample size (n = 941) of JBLM seedlings is larger than any Garry oak seedling studies I could find. The oak planting report produced by restoration experts detailing the overview of the JBLM oak plantings acknowledges the need for post planting treatments, and their benefits in increasing survival and long-term establishment (Killingsworth, "Oak Planting Report 2020."). This is reflected in the implementation of inoculation, mulch rings, protective plastic shelter tubes, and artificial mycorrhizal inoculation. The evidence supporting these treatments are included in this literature review.

Garry Oak: Species Status, Restoration Challenges

Garry oak (also known as Oregon white oak or Oregon oak) was named in the early 1800's by botanist David Douglas as an homage to his friend Nicholas Garry (Renninger, n.d.). Garry oak acorns were a food source for the Salish people, after soaking to leach bitter tannins. The bark was an ingredient used in the Saanich' four bark medicine used to treat tuberculosis and other sicknesses (Storm & Shebitz, 2006)**.** Garry oaks are the only native oak species in the Pacific Northwest, historically existing in prairie, wetland, and conifer-dominated ecosystems throughout the PSA and greater WPG ecoregion, primarily populating areas with relatively low rainfall (Bakker et al., 2012). The geographic range of Garry oaks extends from southwest British Columbia to central California (Kanne, 2019). In Washington State, they grow primarily on the west side of the Cascades although they are the most drought-tolerant tree species in the Pacific Northwest (Clements et al., 2011). Garry oaks are shade intolerant, broadleaved deciduous hardwood trees that can grow twenty meters high or more and have trunk diameters of up to 100 cm (Gould et al., 2011). In the last century, Garry oak woodlands have become increasingly fragmented in conjunction with prairie ecosystems, subjected to land use changes, the suppression of fire, invasion of non-native plants and increases in browser populations (MacDougall et al., 2010).

Garry oak savannas are unique among PSA landscapes and provide ecological value through their support of associated native flora and fauna in three main ways. First, oak acorns are an essential food source for numerous mammals. Second, oaks act as homes for reptiles, birds, mammals, amphibians, and many plant species. Third, if actively managed and allowed to establish, oaks can shade out invasive species, allowing shade-tolerant natives to grow, and open prairie and oak savannas to persist (Devine et al., 2007; Dunwiddie & Bakker, 2011; Southworth et al., 2009). Often, species richness is higher in areas dominated by oaks than adjacent conifer forests (Thysell & Carey, 2001). Moreover, Garry oaks are essential for maintaining ecosystem resilience and species diversity in prairie ecosystems. Their populations must be enhanced and protected through active management if prairie ecosystems are going to persist (Gould et al., 2011).

Although increasing efforts to restore Garry oak savannas and woodlands have occurred, since European settlement oak recruitment in the PNW has decreased dramatically and regeneration is low (Devine et al., 2007). Also, there is little information regarding Garry oak growth rates and survival (Gould et al., 2011). MacDougall et al., (2010) discuss the factors that influence Garry oak recruitment failure. Although their study extent is in the Quamichan Garry Oak preserve in the Cowichan Valley on Vancouver Island in British Columbia, similarities exist between their studied prairies and those on JBLM in the high levels of disturbance, invasive species encroachment, historical suppression of fire, habitat loss and recent resurgence of prescribed burning practices. The study results show herbivory killed or damaged 100% of seedlings that were unprotected during the winter. Further, the damage done by herbivory reduced growth rates, leaf production, and made seedlings more vulnerable to insect attacks. Competition from other plants such as Douglas fir can also decrease growth and survivability of Garry oaks (Devine et al., 2007).

The major factors influencing oak regeneration include acorn production and acorn predation. Peter and Harrington (2002) discuss the importance of Garry oak acorns as a fall and winter food source for the federally endangered Western gray squirrel (*Sciurus griseus*) who’s remaining populations on the west side of the Cascades in Washington state exist specifically on JBLM. In addition, the extirpation of oaks in these areas is a primary reason why Western gray squirrel populations have declined in Washington State (Ryan & Carey, n.d.). Furthermore, mammal mycophagy and seed dispersal are essential for expanding oak woodlands, and as food is limited for mammals due to low acorn production, the ability to disperse seeds and regenerate oaks is severely limited (J. L. Frank et al., n.d.). Understory community composition and area openness are important, with more open area and recent burning positively influencing acorn production numbers (Peter & Harrington, 2002). The oak plantings in my research study are sites with active prescribed burning regimes. In addition, some amount of conifer removal occurred in the planting sites because the military is federally mandated to provide suitable habitat for protected species under the Endangered Species Act (ESA) (*Environmental Protection at JBLM – Basewatch*, n.d.). Conifer removal has been shown to be an effective management tool to increase oak seedling survivability by limiting competition and increasing UV exposure (Clements et al., 2011). Tactics to improve survivability of Garry oaks are important for enhancing populations of obligate species and improving ecosystem functionality and resilience. Due to continued prairie fragmentation by agricultural and urban development of Garry oak ecosystems in conjunction with low regeneration rates, planting oaks is a vital restoration method (Devine et al., 2007). Pre and post planting treatments are also essential to increase survivability, growth, and establishment of Garry oaks.

Garry Oak Treatments

Garry oaks experience high recruitment failure and require a combination of treatments and active management to establish long term (Stanley et al., 2011). Devine et al. (2007) highlight the lack of research focusing on techniques to increase survivability of Garry oaks in sites where they once existed, and challenges involved in restoration. The authors discuss treatments implemented in their study to increase survivability and seedling growth in planting sites containing almost identical soil conditions (somewhat excessively drained with slopes less than 2%) and in relative proximity to my research sites, providing support for my study design treatment methods. The authors hypothesize that the growth and survival of Garry oak seedlings can be increased by controlling vegetation, installing tree shelters, fertilizing, and irrigating. Although irrigation and fertilization were not applied to the JBLM seedlings after they left the nursery, tree shelters and vegetation controls were used. Devine et al. (2007) found that solid tree shelters (which were also used in the JBLM seedlings) significantly increased annual growth of seedlings compared to seedlings in mesh or no shelter. Additionally, this effect increased over the four-year study period. Gould et al. (2011) point out that Garry oak growth is highly influenced by the amount of competition around each tree, which inhibits their ability to capture limited resources necessary for photosynthesis and nutrient uptake. An experiment by Bakker et al. (2012) analyzes variables responsible for survival and growth rates of Garry oaks in a semiarid arid East Cascades site. The utilization of plastic mulch, tree shelters, and first-year irrigation were tested with 1-3 year old seedlings to measure seedling performance. Results show most seedling mortality was due to low stock quality and happened early in the first season. Plastic mulch had the strongest positive association with growth. Tree shelters were also beneficial, but with a lower association strength. This study shows the use of post-planting treatments catered to local site conditions are vital for restoring Garry oak stands in an economically feasible and ecologically responsible way. Conclusions from these studies build upon the idea that there is no single treatment to ensure successful restorations, but a combination of methods must be implemented and monitored to allow for the highest chance of reestablishment. Garry oak restoration requires active management due to the ecological forces working against them. On JBLM land, restoring oaks can be obstructed by other management activities and military training exercises in addition to biotic stressors.

Mycorrhizal Symbiosis

The word mycorrhiza originates from the Greek ‘mukès’ meaning fungus, and ‘rhiza’ meaning root. These ‘fungus-roots’ are specialists in an enormous population of microorganisms that populate most ecosystems within the rhizosphere and are the primary force responsible for nutrient uptake by terrestrial plants. Mycorrhizas are unique among other microorganisms in their abilities to use and move nutrients within the soil, their dependence on the host plant for organic carbon, and their recognizable and fairly consistent structures (Smith & Read, 1997). Molecular data shows that mycorrhizal evolution split away from other organisms sometime during the Proterozoic eon, and most likely existed long before the colonization of land plants (Smith & Read, 1997). Brundrett, (2002) points out that the first bryophytes (non-vascular flowerless plants) contained vesicular-arbuscular mycorrhizas (VAM) 400-500 million years ago, evolving concurrently with land plants. The authors discuss how plant roots formed from rhizomes to establish better conditions for mycorrhizae to flourish and allow plants to benefit from greater access to nutrients and water.

Mycorrhizas form symbiotic relationships with approximately 90 percent of all plant species and are divided into seven different types, according to Smith & Read (1997). The existence of mycorrhizas has long been acknowledged by academics. De Bary, (1887) was one of the first to note the existence of plant/fungi parasitism, but it is only in recent decades that the incredible variance in the function, structure and development of mycorrhizal symbiosis has been acknowledged. Only in the last few decades, with the increasing prevalence of DNA sequencing and molecular analysis, that postulation has been confirmed regarding the roles mycorrhizal fungi play in nutrient cycling, plant species diversity, soil characteristics and ecological health (Cairney, 2000). Furthermore, the field of mycorrhizal research is expanding rapidly, leading to changes in terminology, taxonomy, and knowledge of function. However, knowledge gaps exist in relation to the effects of mycorrhizal symbiosis on plant species composition and competition (Pande et al., 2007). To increase efficiency and relevance, only two types of mycorrhizal fungi will be discussed in this literature review. Arbuscular-mycorrhizal fungi (AMF) and Ectomycorrhizal mycorrhizal fungi (EMF). These two types of mycorrhizae form with Garry oaks at all life stages, however EMF tend to become more present later in the lifecycle (Holste et al., 2017). Figure 1 provides a visualization of AMF (right) and EMF (left). Valentine et al., 2009) discovered 28 ectomycorrhizal morphotypes from 31 difference fruiting fungal bodies around one oak site in Jackson County, Oregon.

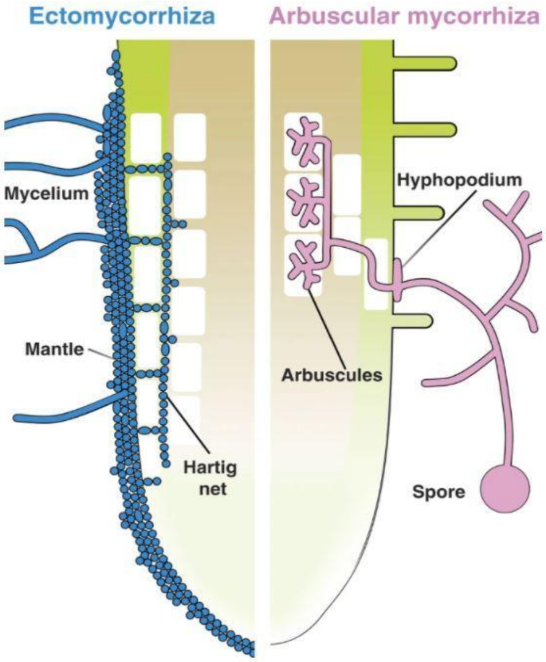

Figure 2: AMF (pink) and EMF (blue) colonize roots differently. Source: (Bonfante & Genre, 2010).

Arbuscular Mycorrhizal Fungi

AMF are the most common type of mycorrhizal fungi, associating with 80% of land plant families (Smith & Read, 1997). Also, AMF are responsible for up to 80% of their hosts Phosphorus uptake and 25% of their Nitrogen uptake (Holste et al., 2017). AMF species exist in most terrestrial ecosystems and are the most prevalent type in grassland and tropical ecosystems (Turrini & Giovannetti, 2012; Brundrett, 2002; Stürmer, 2012). Recently, AMF have been placed in a separate phylum (Glomeromycota) a result of DNA analysis unveiling differences previously undiscovered. Recent molecular evidence highlights the vast genetic variability within what was once a single taxa, and the quickly evolving nature of mycorrhizal research and taxonomy (Smith

& Read, 2010). Öpik et al., (2010) have summarized the available Glomeromycota DNA data available and discovered their geographic distribution may be limited, although data incongruities and gaps are discussed. This assertion provides evidence to support studying AMF distributions and their ecological effects, as collective knowledge on the subject is evolving rapidly.

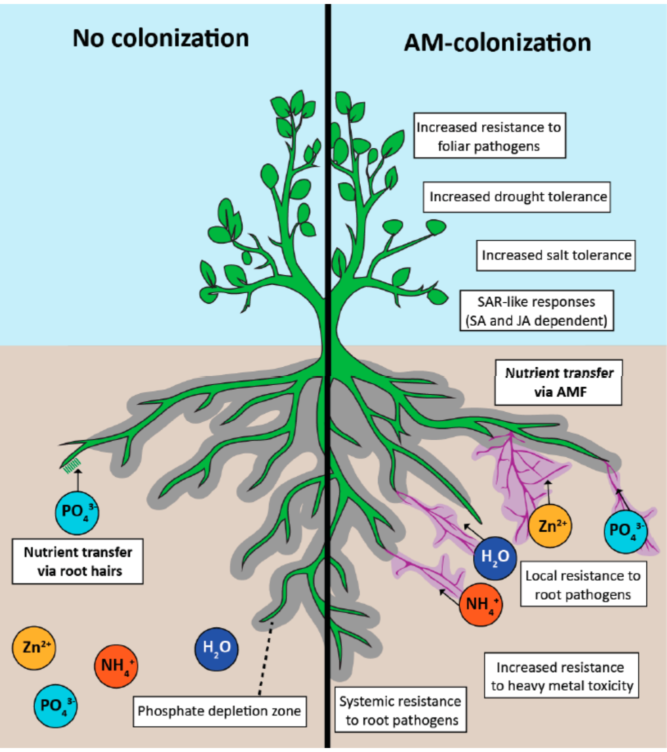

Figure 3: AMF root colonization and associated positive effects. Source: (Jacott et al., 2017).

AMF are incredibly significant organisms, responsible for connecting plants and soil by affecting plant nutrition, species diversity and growth rates (Smith & Read, 2010; Southworth et al., 2009; Turrini & Giovannetti, 2012). Other types of mycorrhizal fungi are for the most part only able to partner with certain plant families and are highly host selective. AMF are able to partner with a wide variety of autotrophs which explains their prevalence in most terrestrial ecosystems (Smith & Read, 2010; Stürmer, 2012; Turrini & Giovannetti, 2012). Plants with associated AMF have higher growth rates, greater mineral uptake and are more resistant to biological stressors than non-mycorrhizal plants (Turrini & Giovannetti, 2012). AMF form masses of threadlike hyphae that extend into the soil with branching tendrils, which help prevent erosion. As Figure 1 depicts, AMF morphology differs from other mycorrhizas in that AMF hyphae penetrate the host roots between the cortical cells, creating arbuscules which transfer nutrients bidirectionally (Smith & Read, 2010). Richardson et al., (2000) discuss how AMF are cosmopolitan in their distribution, meaning that most invading plant species can also form AMF associations. It is largely unknown whether this leads to a competitive advantage over native plants. However, species invasions are increasing in occurrence partially because they contain a wider array of mycorrhizal fungi (Richardson et al., 2000). (Bauer et al., 2018) assert that levels of disturbance play a significant role in AMF fungi species diversity and abundance, which in turn have direct consequences for plant community succession and trophic dynamics. The authors found that in areas with anthropogenic disturbance, late successional fungi became less prevalent because they aren't able to adapt as easily as early successional (weedy) fungal species. Further, early successional fungal species did not promote the growth and establishment of late successional plants. When AM fungal compositions are altered due to succession of disturbance, such changes persist for extended periods of time (Koziol et al., 2018). Bauer et al (2018) highlight the importance of using native AMF from local late successional environments to improve ecosystem function and increase restoration success. They also discuss the relationship between commercial mycorrhizal fungi and early successional fungi. Results indicate most commercial mycorrhizal inoculants are sold without reference to their origin, have eight to ten times higher propagules per gram than what is found naturally and include a small number of different species that are common in highly disturbed, early successional soils.

Middleton (2015) found that some commercial fungi inhibit late successional growth and don't provide the same ecological benefits (improving species richness and increasing native plant cover) than native AMF species. Moreover, it is due to the negative potential outcomes of introducing nonnative AMF in restoration sites that researchers advocate for the utilization of native, locally adapted, late successional AMF species.

Although mycorrhizal inoculation is thought to be primarily positive in restoration settings, AMF relationships exist on a vast spectrum, from parasitic to mutualistic (Smith & Read, 2010). Klironomos, (2003) utilize 64 species with one AMF inoculant (*Glomus etunicatum*) and found a high variance in growth rates compared to non-inoculated plants. The direction (- 49% to + 46%) of growth rates and the magnitude of each response differed among the host plants. The authors indicate AMF interactions exist on a spectrum from mutualism to parasitism; greater understanding is required to comprehend the nuances within AMF host interactions. In a second experiment within the same study, plants were grown with home AMF, foreign AMF and foreign plants grown with home AMF. Home plant and AMF samples were taken from a Long-Term Mycorrhiza Research Site, foreign plants originated in other locations, and foreign AMF were collected from other grassland ecosystems or purchased from the international culture collection of vesicular arbuscular mycorrhizal fungi. Results show a wide spectrum of growth responses, depending on the combination of AMF species and plant species. No consistent associations (either positive or negative) among any of the tested plant and AMF species were found, although mycorrhizal species sensitivity was significantly less when foreign AMF or plants were used. Additionally, it was found that exotic AMF communities support less diverse responses than native AMF. This evidence points to the locally adapted nature of AMF and plant populations, indicating plants select for AMF that positively benefit them most. In a restoration setting, this study supports adding local and diverse AMF species to promote ecosystem diversity, but calls into question whether providing a wide range of AMF inoculants (some of which are foreign) results in positive growth and survival of Garry oak seedlings. (Klironomos, 2003). *Glomus etunicatum* was used as one of the AMF species in the JBLM Garry oak seedling artificial inoculant blend, but I have not found any studies in which *Glomus etunicatum* associates with Garry oak.

Ectomycorrhizal Fungi

EMF associate with roughly 2% of land plants and are predominantly found in association with woody perennial plants, although some plant species like oaks have a propensity to associate predominantly with EMF and possibly AMF (Smith & Read, 2010, Southworth et al., 2009). Although less prevalent than AMF, EMF can produce up to seven times more hyphae than AMF (Kolari & Sarjala, 1995). EMF are morphologically unique in that they exist mostly on the exterior of the host root, as opposed to penetrating the host cells. If penetration occurs from the hartig net or hyphael sheath by EMF fungi it is referred to as ectendomycorrhiza (Smith & Read, 2010). As Figure 1 visualizes, EMF are composed of three main structures: intraradical hyphae which forms the hartig net, the extraradical hyphae that branch out into the soil, and the mantle which creates the root tip sheath (Rosling et al., 2003). Frank et al., (2008) discovered more than 40 species of EMF species associating with Garry oaks at a 25-ha site at Whetstone Savanna Preserve In Jackson County, Oregon. These various fungus roots take up nutrients (predominantly nitrogen and phosphorous) and transfer them to the host root in exchange for carbon. EMF are able to uptake organic N through decomposition, whereas AMF are dependent on the bacterial mineralization of organic N (Smith & Read, 2010). Although various studies show that oak seedlings planted in close proximity to mature oaks have higher rates of mycorrhizal infection and survival (Dickie et al., 2002, 2007; Southworth et al., 2009), it is unclear whether root colonization increased growth rates (Southworth et al., 2009). Holste et al., (2017) examined the effects of AMF and EMF inoculation on nutrient uptake and growth of two trees (*Eucalyptus grandis* and *Quercus costaricensis*) grown in a greenhouse and treated with either AMF or EMF. The authors acknowledged the need to study early seedling growth and mycorrhizal interactions with species that can host EMF and AMF in order to see differences. Further, unveiling the differences in associations is vital in comprehending various forest ecosystem functions. Results from Hoste et al (2017) study found a variety of effects (positive and negative) of mycorrhizal fungal type on plant growth and tissue nutrient content. This study supports the idea that mycorrhizal associations exist on a nuanced spectrum ranging from parasitic to mutualistic and often dependent on the various environmental conditions in which reside or are used (Klironomos, 2003; Richardson et al., 2000).

This literature review establishes the necessary context and historical foundation supporting my research and study design. With this research I aim to contribute to the collective understanding of Garry Oak seedling survival and long-term establishment by analyzing the variables responsible for survival/growth rates of seedlings throughout six planting sites on JBLM. In addition, I want to aid in restoration efforts by adding to scholarship regarding mycorrhizal inoculation, species complexity and fungal community variance between artificial inoculation and natural communities.

Introduction

A question recently presented to me provoked substantial concern in my mind. In response to the submission of my literature review outline, my professor commented, “Why are we conserving habitats (any habitats) in the first place? Humans are responsible for their destruction and yet we work hard to preserve fragments of them. What’s up with that?” (Kathleen Saul). I was forced to confront the reality of the looming larger picture. Why should we care about conserving and restoring prairies and oak savannas in the South Puget Sound if we don’t care about conserving and restoring ecosystems in the first place? Here is my response.

To maintain high ecological function and ecosystem resilience (and in turn our ability to survive on this planet) we must support biodiversity. As David Attenborough once said, “It is that range of biodiversity that we must care for – the whole thing – rather than one or two stars” (Gronau, 2007). Although the outwash prairies of the south Puget lowlands don’t have enigmatic megafauna lumbering across their landscapes, they contain highly sensitive wildlife, complex fungal communities and gorgeously rare species of plants, butterflies and birds which support an abundance of beauty and preserve ecological health. Prairie and oak savannas in the Willamette Valley-Puget Trough-Georgia Basin (WPG) have endured extreme levels of degradation and are now highly endangered in North America (Stanley et al., 2011) Historically, Native Americans’ frequent burnings maintained the vegetative structures and rich diversity of these habitats (Boyd, 1999). However, fire suppression, habitat fragmentation, land use changes, species invasion and native species decline have contributed to their increasing rarity in the WPG ecoregion and the south Puget lowlands specifically (Dunwiddie & Bakker, 2011). It has never been more important to understand the ecological nuances within these habitats to implement the best restoration methods possible. Although restoration objectives are often established utilizing metrics like the Floristic Quality Assessment (FQA) and other above ground indicators, understanding the microbial underpinnings beneath the soil is a vital yet often overlooked variable. To restore prairies and oak savannas through the planting of oaks, it is essential to understand the mutualistic relationships between oaks and soil fungi (known as mycorrhizae).

To glean greater understanding of Garry oak survivorship and plant community impacts, the following research questions are posed. Does mycorrhizal inoculation have a statistically significant effect on survivorship and growth? Does artificial inoculation benefit the oak seedlings even if the specific species used are not known to have a direct association with Garry oaks? Does site quality have a measurable effect on seedling growth and survivorship?

Materials and Methods

The design for this study was derived from conversations with Dennis Buckingham and reading through the JBLM Fish and Wildlife's Oak Planting Report. The Oak Planting Report outlines a concerted effort to restore South Puget Sound (SPS) prairies by enhancing habitats for federally listed endangered species, as required under the Endangered Species Act and the existing Endangered Species Management Plans (ESMP). In this case, the Taylor's checkerspot butterfly, streaed horned lark and Mazama pocket gopher are the ESA listed species, although numerous other plant and animal species are experiencing population declines in SPS prairies and are listed as either endangered or threatened by Washington State Department of Fish and Wildlife. Most of these stat and federeally-listed species require open prairies without dense understories to thrive (USFW, 2013).

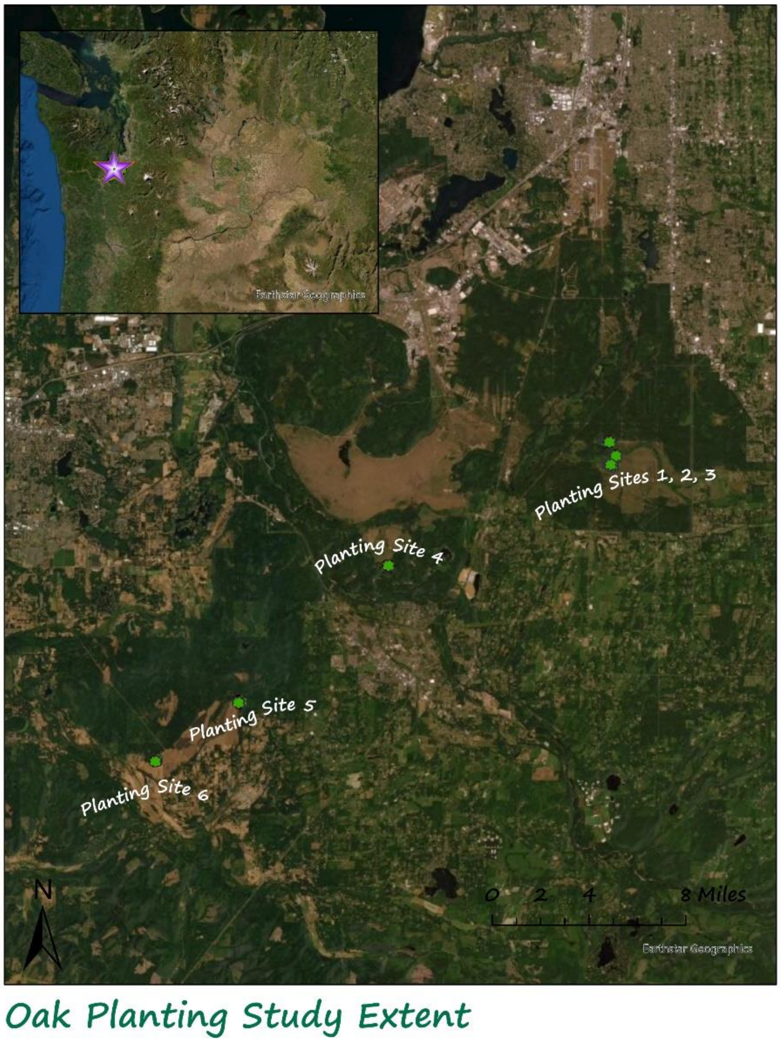

Figure 4: Map of six planting sites on Joint Base Lewis-McChord.

Site Description and Selection

JBLM is located in the South Puget Sound (SPS) lowlands in Thurston and Pierce counties (47°05'13.4"N 122°29'34.5"W). The prairies existing in this region are the largest remnant prairies remaining in western Washington. JBLM was established in 1917, and the prairies containing the six oak planting sites are within this active military base. The geographic region encompassing JBLM is known as the Puget Trough. All of the planting sites exist within well-drained gravelly channels, which contributed to the unique soil types and, in turn, the plant communities that thrive there. The soil in which the planting sites reside are all part of the Spanaway series, geomorphologically positioned as outwash terraces. Classified as sandy loam with very little slope, the Spanaway soil series is well suited for the growth of oaks as they thrive in well-draining, somewhat low nutrient soils (Devine & Harrington, 2005). JBLM receives average annual precipitation of 80-90 cm, the majority of which falls between October and April. Temperature, topography, soil type and disturbance all are relatively similar amongst all planting study sites. Differences include each planting site's floristic quality assessment, size, and relative location on JBLM. Planting sites are scattered across JBLM. Sites 1, 2 and 3 are all under 1km from each other, whereas the distance between the two farthest sites (1 and 6) is 37.01km (Figure 1). All the study sites under analysis exist within prairie edges, exept the Upper Weir planting site (Figure 4) which is an oak savannah restoration site.

Sites were selected by restoration experts at JBLM. Variables which affected site selection included the prevalence of historical oak occurrence and distance to existing oak stands. Oak seedlings are vulnerable to fire, herbivory and competition. As ecological prescribed burning and invasive removal treatments occur at various times across all the planting sites, coordination with prescribed fire managers and low exotic species loads (especially Scotch broom (*Cytisus scoparius*) were determined to be vital to oak establishment. As a result, areas with higher native species abundance and richness were selected because oak planting sites can't be harrowed or seeded, which inhibits other potential restoration activities. Consultation with prescribed fire managers was conducted to minimize the risk of fire on seedlings, however, a burn did occur in Planting Site 6, killing over 100 seedlings.

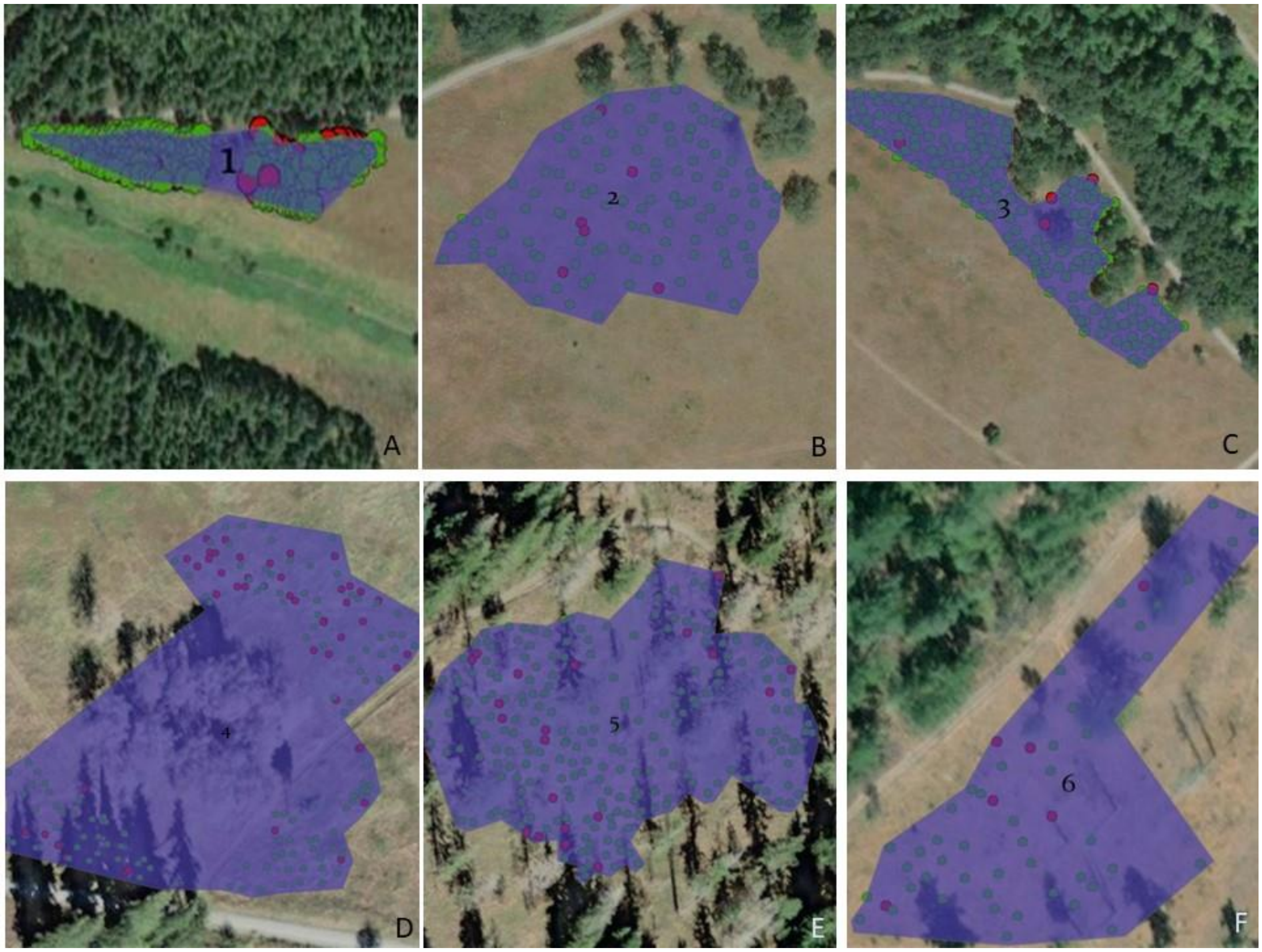

Figure 5: TA 13 Planting Sites 1,2,3 (A, B, C), TA 18 Butler Butte Planting Site 4 (D), TA 20 Upper Weir Planting Site 5 (E), TA 23 South Weir Planting Site 6 (F).

Seedling Growth and Inoculation

Acorns of Garry oak were sown November 2018 at the Sustainability in Prisons Project (SPP) Nursery. SPP is a collaboration between Washington Department of Corrections (WDOC) and The Evergreen State College (TESC), which connects incarcerated people to the fields of science, conservation and sustainability in an effort to reduce the environmental and social costs of prisons (LeRoy et al., 2012.). On June 18th, 2019 , 1,000 seedlings were inoculated with a commercial, water soluble suspended powder formulation of arbuscular mycorrhizal and ectomycorrhizal fungi called Mycoapply Soluble MAXX™. Nine species of endomycorrhizae (*Glomus intraradices, Glomus mosseae, Glomus aggregatum, Glomus etunicatum, Glomus deserticola, Glomus clarum, Glomus monosporum, Paraglomus brasilianum, Gigaspora margarita*) and ten species of ectomycorrhizae (*Rhizopogon villosulus, Rhizopogon luteolus, Rhizopogon amylopogon, Rhizopogon fulvigleba, Pisolithus tinctorius, Scleroderma cepa, Scleroderma citrinum, Suillus granulatus, Laccaria bicolor, Laccaria laccata*) make up the inoculation mix used. Two pounds of the solution were mixed in 75 gallons of water and agitated for 15 minutes, then again every 15 minutes during the 60-minute hand drenching period. The seedlings were grown in 2.5 liter Stuewe treepot™ pots and were irrigated three days after this initial drenching, then every two to three days for the remainder of the growing season. Seedlings were grown in a soil made from 60% decomposed fir bark, 20% peat, and 20% pumice by volume. White zip ties were used to indicate inoculation and black zip ties indentified uninoculated seedlings.

Research Methods

Half of the inoculated and half of the uninoculated seedlings were out-planted into six sites within four different training areas (TA) on JBLM (Figure 5). TA 13 includes three planting sites: North Creek - 190 trees Figure 5a), Shuey Savannah - 117 trees (Figure 5b), and Steele's Sanctuary - 198 trees (Figure 5c). TA 18 has Butler Butte planting with 151 trees (Figure 5d). TA 20 in the Upper Weir contains the largest planting of 249 trees (Figure 5e). Lastly, TA 23 has the South Weir planting site, with 151 trees (Figure 5f). This study is a paired treatment design, with inoculation representing the treatment variable, blocked by planting site.

Utilization of geographic information software (GIS), ArcMap, was vital to develop planting polygons, and later to collect survival and growth data. In an effort to mimic oak savannah habitats, roughly 109 trees were planted per acre (Killingsworth, n.d.). Within each polygon, oak planting density was determined using a combination of habitat type analysis and anecdotal survival rate observations. Crews of volunteers and JBLM Fish and Wildlife employees navigated to the predetermined planting polygons, and utilizing a PTO driven posthole digger and seven crews, planted trees in each drilled hole and wrapped them with blue Protex tree shelters. These protective tubes help stimulate vertical growth by increasing carbon dioxide levels, relative humidity, and temperature within the tube. Acting as a greenhouse, they also protect the seedlings from browsing pressures and are photodegradable. Zip ties were used to secure the tree tubes to a bamboo stake which was driven into the ground. White zip ties again indicated inoculated seedlings, while black signified uninoculated seedlings. Mulch was then evenly spread in a ring around the seedling with a 12-inch buffer between the base of the tree and the ring. This gap was implemented to prevent damage to the seedling during prescribed burning, although this didn’t work in the South Weir planting site, where 120 out of the original 150 trees were consumed by fire.

Data Collection

Data were collected using handheld tablets with Esri’s Collector application. Each tree was geolocated and given a unique ID before measurements were taken. Beginning in September, 2020, height (cm) was measured with a standard tape measure from the base of the seedling to its tallest point. Trunk diameter measurements (mm) were taken 10cm from the ground with calipers, and survival data were collected for all 941 trees by visually inspecting each seedling. JBLM Fish and Wildlife interns helped in the collection process, and were integral to collecting data over a limited period of time. The survival and growth data were then loaded into Arcpro where multiple maps analyzing soil properties, topography and vegetation across the planting sites were developed. Additionally, a multitude of Arcpro functions were used to visualize the entire study extent and create 3D web maps to show differences in height and survival status of inoculated and uninoculated seedlings.

It is well established in the literature that oak seedlings planted within the root zone of established oaks can access mycorrhizal networks, which can increase survivorship (Dickie et al., 2002). In an effort to see if any associations existed between growth and proximity to established oaks, ten seedlings were randomly selected from each planting site (5 inoculated, 5 uninoculated) and the distance to nearest adult oak was measured for each one.

Floristic Quality Assessment/Index

An important factor to consider when analyzing the growth and survival of seedlings is the vegetative makeup of the sites in which they are planted. To add robustness to this study and provide more context for describing tree growth and survival rates by planting site, an assessment of the vegetative quality of each site was conducted. A Floristic Quality Assessment (FQA) was utilized to analyze the ecological integrity and habitat quality of each planting site (Bauer et al., 2018). Each species identified was given a numerical value based on a coefficient of conservatism (C-score) that is on a scale of 0-10. C scores measure the relative conservatism of a species. A species with a low C-score has a higher tolerance to disturbance, a generalist in their habitat selection. Species with high C-scores are well adapted for specific habitats and less tolerant of disturbance (Bauer et al., 2018). Each species C score was calculated using the universal FQA calculator (*Universal FQA Calculator*, n.d.). In this case, all native plants had various C scores, while all nonnative plants were given a value of ‘0’ as they have no conservation value. In addition to an FQA, A modified Floristic Quality Index (FQI) was calculated for each site to provide a single quantitative measure to compare against the other variables. Study plots were walked by botanist Adam Martin and I, dominant species were listed and given a score from 1-5 based on abundance - 1 indicating greater rarity and 5 being more common. For each species, ordinal rank was turned into a percent and multipled by it's corresponding C-score. The sum of these scores for all native species in each site was then dived by 100, and that corresponding number was multiplied by 10. This modified FQI is based on Equation 2 in the United States Geological Survey's *Floristic Quality Index: An Assessment Tool for Restoration Projects and Monitoring Sites in Coastal Louisiana*; (USGS, 2011), which uses percent cover as a substitute for the total number of species in a site.

Statistical Methods

The data collected in Arc Collector were transferred to Arcpro, a desktop mapping application within Esri's program suite. After data were cleaned and organized, the FQI values were imported into Rstudio for statistical analysis. A generalized mixed effects model was used to include multiple distributions from both inoculated and uninoculated data and predict the rates of survival. These methods were employed because the data could not meet the assumptions of a normal distribution. For these reasons, a generalized linear model was used for survival and linear models were used for remaining variables to see the probabilities of how likely these data represent a normal distribution given an arbitrary number of trials. A reduced sample size of 60 oaks (n=60) was used to analyze the effects that distance to nearest established oak had on height and trunk diameter using the measurement tool in Arc GIS pro. A Poisson distribution was implemented to analyze the rates of survival, height, and trunk diameter based on distance to nearest established oak and each site's FQI score for the independent variables.

Results

Inoculation Effects on Survival

First year survival of all oak seedlings (inoculated and uninoculated) was high amongst all sites (Table 1). Mean survival rates of inoculated and uninoculated seedlings were 92.5% (n=484) and 85.52% (n=450), respectively. This difference in mean percent survival between inoculated and uninoculated were not statistically significant (p = 0.70). Predicted survival rates were similar to the raw data with inoculated survival at (91.6%) and uninoculated survival (87.9%), but with relatively high levels of uncertainty (Figure 6). Although results from the predictive model 'm_surv' did not contain statistically significant p-values for inoculation (p = 0.77; Appendix A, Table 1), it is apparent that inoculation had some positive measurable effect on predicted survivability (Figure 7).

Table 1: Survival rates of inoculated and uninoculated seedlings.

Planting Site	**Number of Planted Oaks**	**Inoculated Survival Rate (%)**	**Uninoculated Survival Rate (%)**
1	178	83.30%	79.30%
2	114	97.60%	90.00%
3	183	96.60%	98.00%
4	167	92.50%	68.20%
5	235	92.00%	91.20%
6	52	93.00%	86.40%
Total	**934**	**92.50%**	**85.52%**

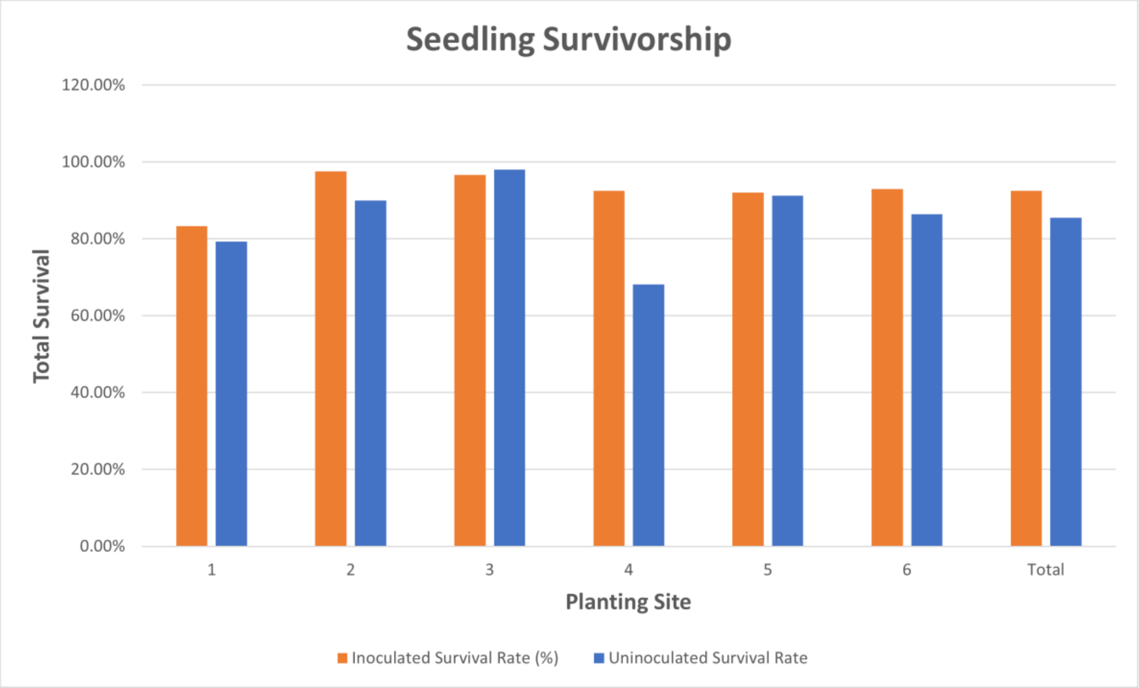

Figure 6: Survival rates of inoculated and uninoculated seedlings across the six planting sites.

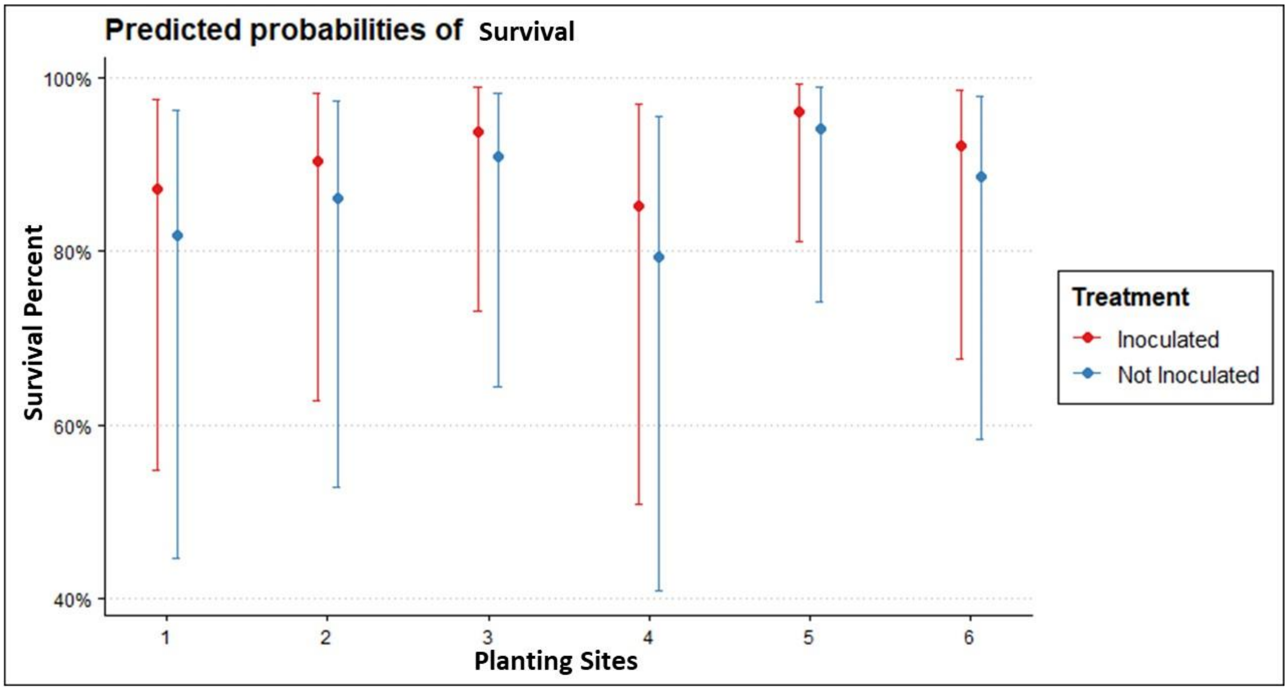

Figure 7: Predicted survival statistics for each planting site given an arbitrary number of trials.

Aboveground Growth

Above ground growth (height and trunk diameter) was greater among uninoculated seedlings across most planting sites than inoculated seedlings (Appendix C, Figure 1). Across all sites, mean heights for inoculated seedlings were 24.7 cm and 25.8 cm, respectively. Trunk diameter means were 4.36mm for inoculated and 4.48mm for uninoculated seedlings. The predictive linear model outputs for height (m_ht) showed high levels of uncertainty, especially when looking at confidence intervals (Appendix A, Table 3). The inoculation parameter provides an example of a weak treatment effect on seedling height (β = 1.12 | LCL = 0.59 | UCL = 2.73|).

Distance to Nearest Established Oak

Results indicate oak seedlings growing farther away from established oaks were taller than those growing closer to oaks (Figure 16). However, as with the other models, there is high levels of statistical uncertainty, as depicted in the large confidence intervals and high p-values in the m_dis model (Appendix A, Table 5).

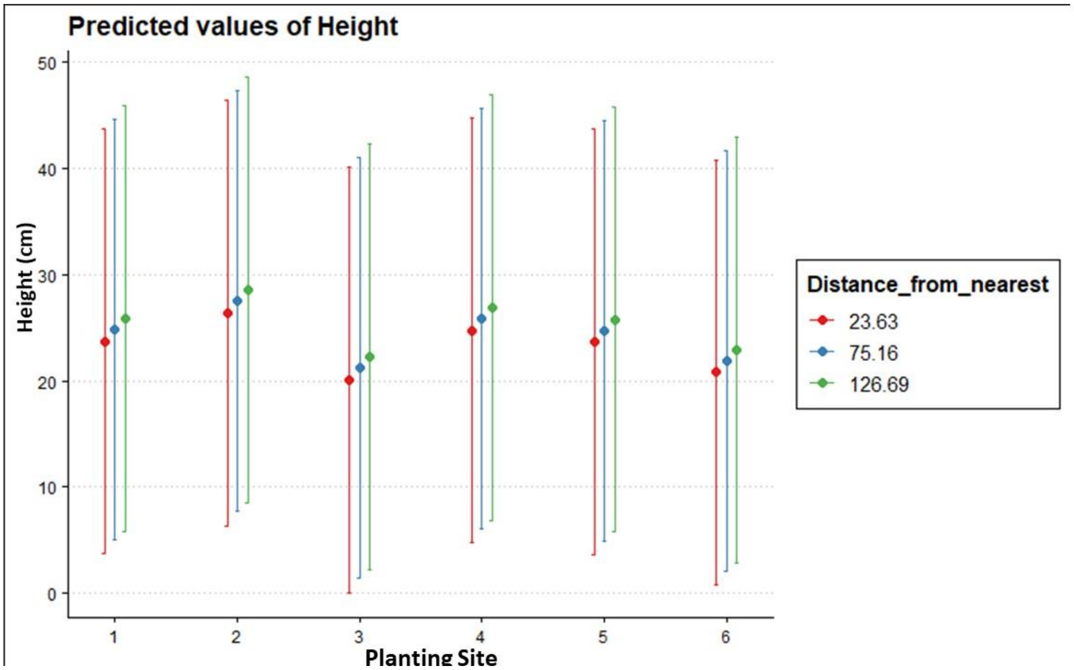

Figure 8: Average height by planting site with three buckets representing average distance from established oaks.

FQI

Floristic quality varied across all six planting sites. Site two represented the highest quality site (78.1), and site 5 was the lowest (23.2) (Table 1; Figure 9). Also, site two had the highest rates of survival (Figure 6) Although site two had the highest average survivorship for inoculated seedlings, site 5 did not have the lowest average survivorship (Table 1). Essential to FQI calculation was attaining abundance scores for native and non native species in each site (Appendix B, Table 1). There was some shared native species amongst the planting sites, but much more ubiquity in the non-native species present (Appendix B, Table 1).

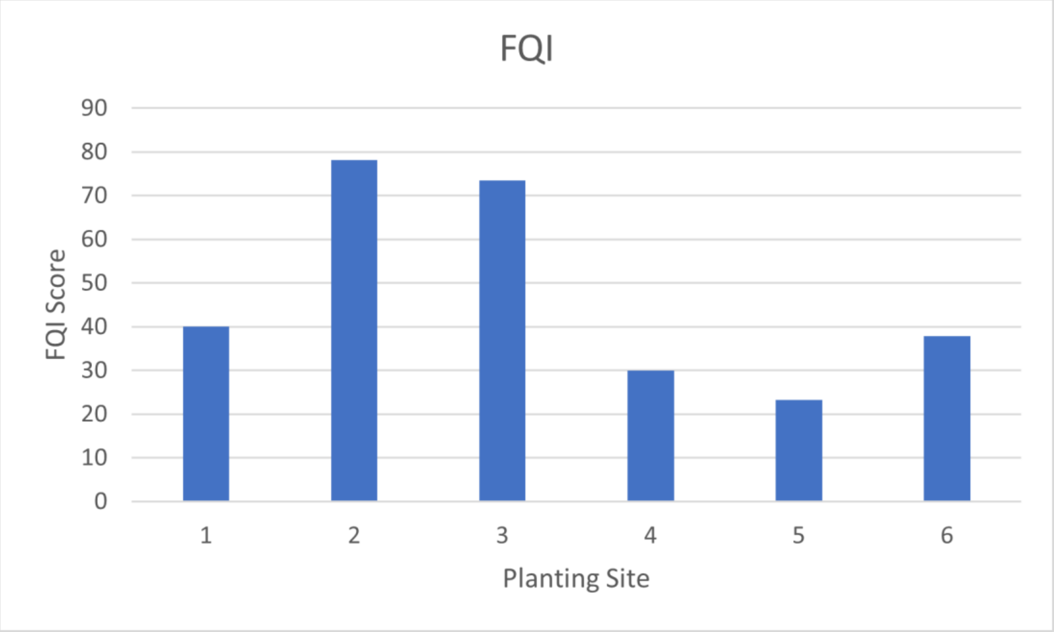

Figure 9: FQI score by planting site.

I used a generalized linear model to measure the interaction between oak survival and FQI. Results showed that FQI had a stronger effect on survivability than inoculation (β3.4574| LCL 6.79 | UCL 0.02) and provided the only statistically significant result (p = 0.02). This is reflected in the fixed effects reported for the m_surv predictive model (Appendix A, Table 1). Figure 10 visualizes the positive association: as site quality increases, survivorship increases across all sites.

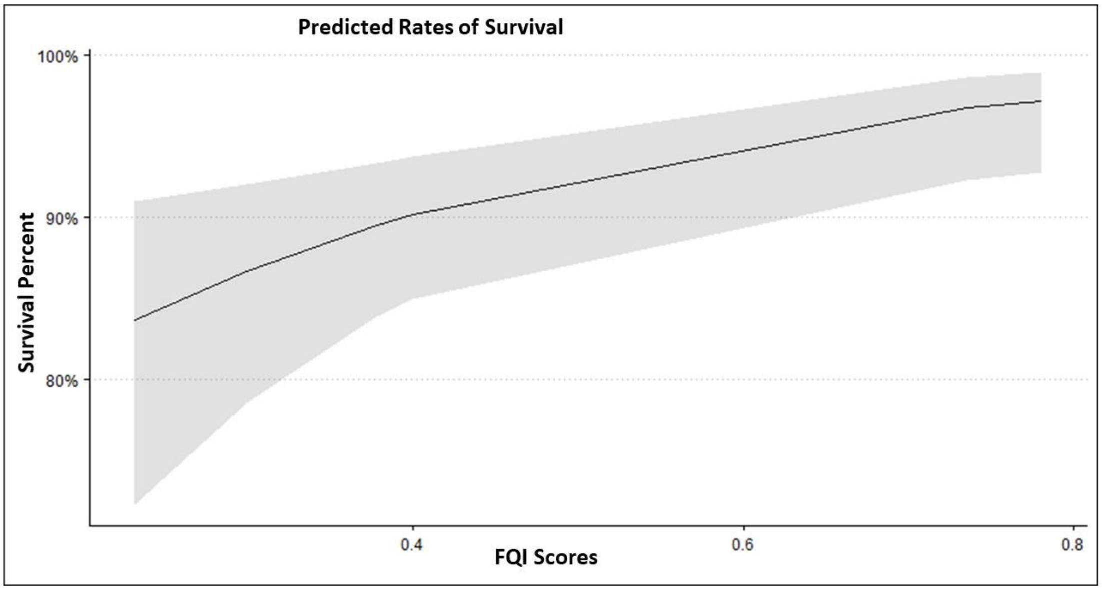

Figure 10: Oak seedling survival relationship with FQI across all sites.

Discussion

Through this thesis project I aimed to determine if inoculation of Garry oak seedlings affected early survivorship and growth in six planting sites in SPS lowland prairies. One of the problems facing Garry oak conservation is our lack of knowledge surrounding the nuanced role mycorrhizae play in plant survivability, plant community impacts and long-term establishment of oaks in SPS lowland prairies, and how to effectively administer the right inoculum to increase the odds of successful reintroductions. However, enormous progress has occurred in identifying mycorrhizal species and developing methodologies needed to measure species specific responses to inoculation. Some recent work has started to investigate which species colonize the roots of oaks at various life stages, and how this affects their long-term survival and growth. Southworth et. al (2009) showed that the number of mycorrhizal root tips of *Tuber* and *Laccaria spp.* was a stronger predictor of growth than initial seedling basal area in nursery-grown Garry oak seedlings, suggesting that these organisms play incredibly important roles in early growth and survivorship. (Devine et al., 2009) discuss the benefits of mycorrhizal inoculation on container-grown garry oaks and their results indicate the most effective methods for increasing growth and root development of oaks is a combination of air-pruning and ectomycorrhizal inoculation. Jacott et al. (2017) produced a metanalysis siting multiple studies which detail the positive and negative effects of AMF inoculation, showing increases and decreases in biomass of AMF inoculated hosts depending on the ratio of host carbon provisions to photosynthate (a product of photosynthesis) (Dickie et al., 2002). More research is needed to determine appropriate methodologies for artificially inoculating oak seedlings with beneficial mycorrhizae to improve survival and growth.

An important factor potentially responsible for lower growth rates in inoculated seedlings is the use of commercial mycorrhizal inocula. Koziol et al. (2018) argue that many commercial inocula are similar to early successional fungi that associate with weedy invasive species because they are easier to harvest. Also, species present in many commercial inoculum blends are aggressive and can outcompete native fungi due to higher propagules per gram and ability to colonize soils quickly after disturbance. Furthermore, some commercial inocula blends can inhibit late successional plant growth and have been shown to inhibit species richness and native plant cover, which can negatively impact long term restoration goals (Middleton et al., 2015).

While effects of mycorrhizal inoculation on Garry oak survival and growth were not statistically significant, some interesting associations emerged from the data in the forms of FQI association and growth rate within and among the six planting sites. This thesis project aids in prairie/oak restoration and management by bolstering support for strategically planting oaks in higher quality sites, and inoculating seedlings with mycorrhizal species that have been proven to associate with oaks. Early successional, weedy mycorrhizal species may inhibit oak growth and help competing invasive species, rendering artificial inoculation as detrimental. In addition, these results point toward the need to further oak seedling survivability research with a specific focus on AMF and EMF species specific interactions combined with long-term monitoring analysis. This research project in partnership with Dennis Buckingham and the JBLM Fish and Wildlife internship program provides a unique opportunity to continue monitoring the survivorship and growth of the oaks over time, something that is largely missing in current restoration activities.

Inoculation

Inoculation did not significantly affect survivability of Garry oak seedlings in their first year of field growth. Although survival rates of inoculated seedlings were higher than uninoculated seedlings across all sites, there are high levels of uncertainty (reflected in the negative random effect intercepts and high confidence intervals. Looking below ground to analyze which mycorrhizal species colonized the seedling roots, or whether inoculum sourced by nearby trees or residence soil species are utilized could add to the robustness of this study, but it is a larger undertaking given the resources required to harvest and identify mycorrhizal species. Pande et al, (2007) found that oak seedlings grown with the ectomycorrhizal fungal *Russula* species, but less growth with *Amanita* species.The long-term effects of inoculation as a treatment may prove to be statistically and biologically significant over time with the addition of multiple years of growth data and with soil and root colonization analysis. Although Garry oaks seem to associate with a wide variety of EMF species (Valentine et al., 2009), more research is needed to describe these various species and understand where in the mutualistic-to-parasitic spectrum they fall. In addition, out of the 18 species of AMF and EMF that made up the commercial inoculum used in this study, none of the species in the commercial mix have been found to positively associate with oaks in nature. Different species of mycorrhizae become activated (and in turn beneficial, or at times parasitic to the host) at different life stages and in response to various environmental and ecological factors (Ronsheim, 2012), so the full effects of inoculation are not fully known unless continued analysis and monitoring occurs. Koziol & Bever, (2017) found that soil AM fungal inoculation didn’t initially affect above ground growth, but heavily contributed to the productivity of late successional plants.

An initial soil analysis of fungal communities at the planting sites was never conducted. It is fair to assume that due to the high levels of historic and current disturbances (prairie encroachment by Douglas-fir, grazing, conversion to agriculture, nonnative species invasions, herbicide treatments, military activities), in addition to the suppression of fire in the six planting sites, the soil microbial communities have been altered over time. The inoculant used could potentially be introducing nonnative species of AMF and EMF into the soil. As a result, the inoculant could be negatively impacting the survival and growth of oak seedlings. Further, the artificial inocula can become mutualistic with competing weedy species and not accurately represent historical fungal communities (Klironomos, 2003). Mycorrhizal inoculation should be species specific to benefit the host oaks and represent native fungal communities so as not to parasitize the host or benefit invasive species which are already highly prevalent across the prairies sites (Middleton et al., 2015). AMF and EMF interactions are nuanced and vary depending on species interactions and soil conditions. Fully characterizing is the microbial communities associated with Garry oaks, and determining which mycorrhizal species result in the greatest benefit to the host will advance conservation and restoration of this ecosystem.

Aboveground Growth

An unexpected finding in this research was that mean heights and trunk diameters of uninoculated seedling were greater than inoculated seedlings across all six planting sites. Reasons for these results are unknown, but numerous potential variables may be at work simultaneously. Literature regarding Garry oaks show there is high variability in growth rates, and that root morphology is a strong predictor of growth and survivorship of oak seedlings (Devine & Harrington, 2005). Gould et al, (2008) studied oak mortality and growth and found that tree size and competitive status strongly influenced the predicted 5-year mortality probability. Further, seedlings dry mass was greater when oaks were grown separately from pines, as opposed to when they were grown in a mixture. It is possible that the seedlings planted on JBLM experienced various growth rates (regardless of inoculation) due to differences in competition, soil moisture, disturbances, or mycorrhizal species associations (Clements et al., 2011). Because no pre-planting soil analyses were done on the planting sites, it is impossible to know what abiotic or biotic conditions, particularly driven by the microbial species present helped or hindered the oak seedlings without further analysis. Due to the high invasive species populations in all six of the planting sites, it is fair to assume high populations of early successional, weedy fungi were present which could have a negative impact on oak survivorship and growth (Koziol et al., 2018). Longitudinal research is needed to attain greater understanding regarding the full effects of inoculation and the plant community impacts of artificial inoculation and competitive outcomes of the oak seedlings after more growing seasons have occurred.

FQI

Results from this study support the idea that site quality is an important factor in the success of restoration efforts. Navarro-Cerrillo et al., (2014) provides ample evidence to support the importance of site quality in tree survivorship. The authors found that site quality and planting date had the greatest impact on survival rates after one growing season. In this study, FQI had a statistically significant effect on oak seedling survival in the first field growing season, which outweighs the importance of inoculation from a survivorship perspective. However, invasive species like Scot's broom (*Cytisus scoparius*) and colonial bentgrass (*Agrostis capillaris*) continue to flourish in all of the planting sites except planting site 6, which was burned months after seedlings were planted. Continued active management of these sites is required to keep FQI scores high, and in turn increase the odds of reestablishing oak communities in these areas. Also, plantings took months to complete, and as planting date is a vital factor in survivorship it may have impacted the results of this study.

Limitations

Garry oaks are a slow growing deciduous tree and take years to establish and mature, with much of their growth energy directed towards root establishment during the first few years. A limiting factor in this study is that it evaluates survival and growth data for two-year old seedlings, which is a small snapshot in their lives. Any statistically significant variable associations drawn from this research must acknowledge the short timeframe and can't be conclusive without additional research. However, this study provides a starting point for a longitudinal study. As the seedlings continue to grow and mature a researcher could easily replicate my study and potentially draw stronger conclusions given more time, and certain species of mycorrhizae may benefit and activate later in the trees' lifecycle. The lack soil samples analyzing abiotic variables and microbial interactions forced assumptions to be made about growth and survivorship responses. Evaluating root colonization of planted trees in the future could help to determine if certain species of mycorrhizae activate and benefit the trees later in their life cycles.

Conclusion

Prairies and oak savannas in the Willamette Valley-Puget Trough-Georgia Basin (WPG) Ecoregion are one of the most endangered ecosystems in North America. In the south Puget lowlands, native prairie cover has declined by approximately 97% (Crawford & Hall, 1997). Historically, these open areas were maintained by indigenous utilization of fire. However, fire suppression due to habitat fragmentation, development, species invasion and native species decline due to settler colonialism have contributed to their increasing rarity in the WPG Ecoregion, and SPS specifically (Dunwiddie & Bakker, 2011). A species that maintains high ecological and cultural importance is Garry oak (*Quercus garryana*). The restoration of Garry oak is essential for the protection of prairies and associated habitats they support, but survival is often poor (Clements et al., 2011). Vital to oak survival is the development of mycorrhizas, yet historically the need to apply this in nurseries is often overlooked (Southworth et al., 2009) . Although the results of this study contained high levels of statistical uncertainty, results indicate floristic site quality is the most significant predictor of first year seedling survival, and above ground growth is initially negatively impacted by inoculation with commercial inoculum. Building upon these data with repeated monitoring, land managers can determine with greater confidence whether artificial inoculation is worth the added expense and effort in similar conditions. Additionally, they can attain greater levels of understanding regarding the role symbiotic fungi play in Garry oak survival and long-term establishment with the utilization of this thesis as a starting point in a longitudinal study.

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Appendices

Appendix A:

Table 1: Fixed effects of m_surv, showing slope, confidence levels and p-values for inoculation treatment, FQI and inoculation x FQI interaction.

Fixed Effects for m_surv				
Parameter	**Value (β)**	**LCL**	**UCL**	**P-value**
Intercept	0.8306391	-0.73249	2.362808	0.2348
Inoculation	-0.2137	-1.32152	0.891856	0.7006
FQI	3.4574	0.368116	6.785313	0.0202
Inoculation:FQI	-0.4257	-3.25057	2.375316	0.7607

Table 2: Random Effect intercepts for all models across all sites.

Random Effects Intercepts				
Site	**Survival Model (m_surv)**	**Height Model (m_ht)**	**Trunk Diameter Model (m_dia)**	**Distance from Nearest Oak (m_dis)**
1	-0.48544178	-1.9336488	0.001047028	0.02842692
2	-0.1568826	1.8877502	0.108627185	5.58298649
3	0.32013904	-0.693055	-0.107959601	-1.43847696
4	-0.64070202	-3.9213019	-0.032811337	0.34685688
5	0.77801698	5.3143905	0.051400619	-1.34280742
6	0.05985833	-0.6541351	-0.017140517	-3.17698589

Table 3: Shows fixed effects for seedling height model.

Fixed Effects for m_ht				
Parameter	**Value (β)**	**LCL**	**UCL**	P-value
Intercept	15.7632	20.88577	26.3653	0.00001
Not Inoculated	0.6525	-0.59528	2.733701	0.186195
FQI	12.5756	-1.32467	18.34954	0.10475

Table 4: Fixed effects for trunk diameter model.

Fixed Effects for m_dia				
Parameter	**Value (β)**	**LCL**	**UCL**	**P-value**
Intercept	3.864	2.925499	4.7928	0.00001
Not Inoculated	0.2563	0.03595	0.467013	0.019628
FQI	0.9464	-0.83963	2.749301	0.317607

Table 5: Fixed effects for distance to nearest oak predictive model.

Fixed Effects for m_dis (height~ Treatment + Distance from nearest oak + FQI				
Parameter	Value (β)	LCL	UCL	P-value
Intercept	17.24349	7.040911	27.34062	0.003504
Inoculation	1.0429	-3.97515	6.018207	0.682562
Distance from Nearest Oak	0.02073	-0.04234	0.074156	0.494149
FQI	11.66397	-4.96866	28.46119	0.216961

Appendix B:

Table 1: FQA Species abundance scores and C-scores.

FQA Species List Abundance Score by Planting Site							
Species List							
Native Species	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**	**Species C-Score**
Agrostis capilaris	4	5	4	5	5		0
Agrostis pallens		2				3	4
Agrostis stolonifera		3	3			3	0
Aira caryophyllea					2		0
Aira praecox					3		0
Amelanchier alnifolia						1	4
Apocynum androsaemifolium					2		4
Arbutus menziesii					1		3
Arctostaphylos uva					2		4
Arrhenatherum elatius	3	2		4			0
Bromus sterilis	3					2	0
Camassia quamash		4	4	3	1	2	3
Carex Inops	3	2	2			3	4
Cerastium arvense			1				4
Claytonia rubra						1	3
Cytisus Scoparius	3	4	4	3	3	3	0
Dactylis glomerata						1	0
Danthonia californica					1		4
Elymus repens	2			3			0
Eriophyllum lanatum		2					4
Festuca roemeri		4	3				5
Fritillaria affinis			1				6
Holcus lanatus				1	4	3	0
Hypericum perforatum	1				2	2	0
Hypocurous Radicatta	4	3	3	3	3	4	0
Lepidium campestre		2					0
Lomatium utriculatum		2	3				5
Lotus micranthus			1		1		4
Luecanthemum vulgare		2			2		0
Luzula campestris		1			1	2	0
Mahonia aquifolium				2	1		3
Native Species total	3	16	21	8	11	12	83

Oemleria cerasiformis				1			3
Plantago lanceolata		2	2		2		0
Poa Compressa	2						0
Pseudotsuga menziesii					2	2	1
Ranunculus occidentalis			3				4
Rubus ursinus				2			3
Rumex acetosella				2	2	2	0
Solidago simplex			1				5
Symphoricarpos albus			2				3
Teesdalia nudicaulis						2	0
Trifolium subterraneum	2					1	0
Vicia sativa				3	1	1	0
Vulpia bromoides		3			1	2	0
Non-native species Total	24	27	16	24	31	28	0

Appendix C:

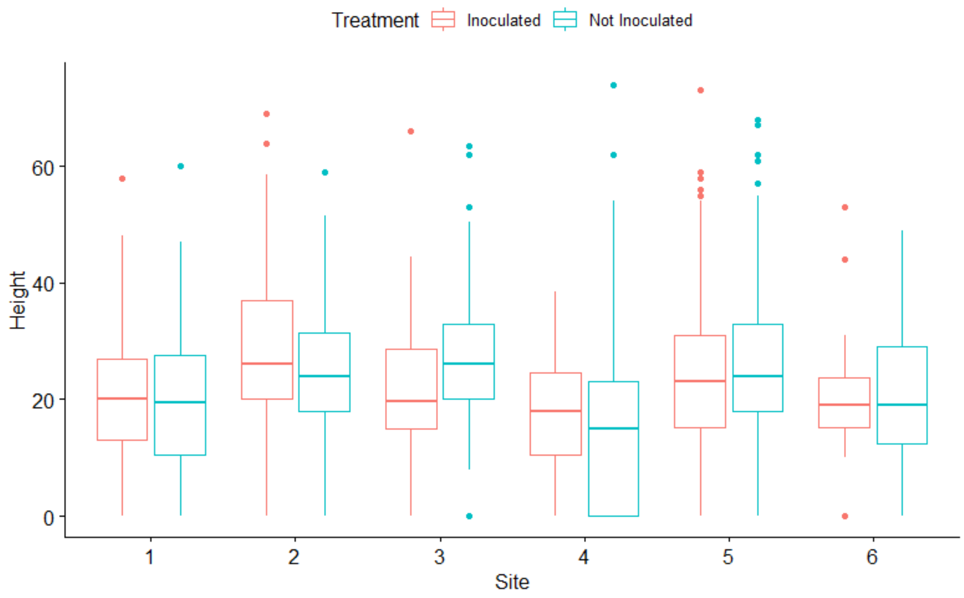

Figure 1: Average seedling Height by planting site for inoculated (orange) and uninoculated (blue).

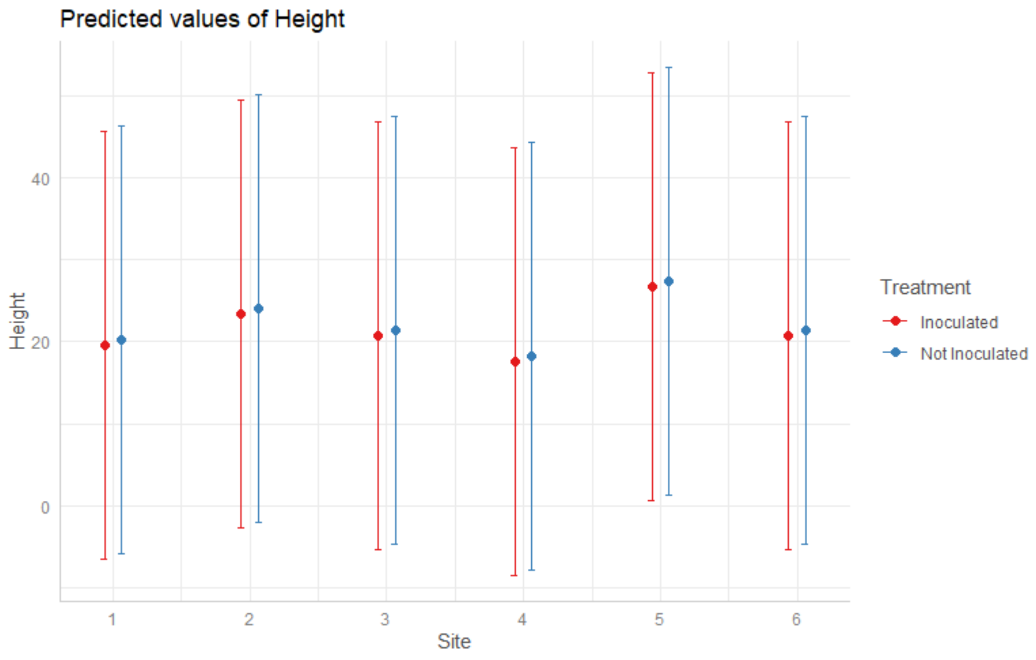

Figure 2: Predicted height values for inoculated and not inoculated seedlings grouped by site.

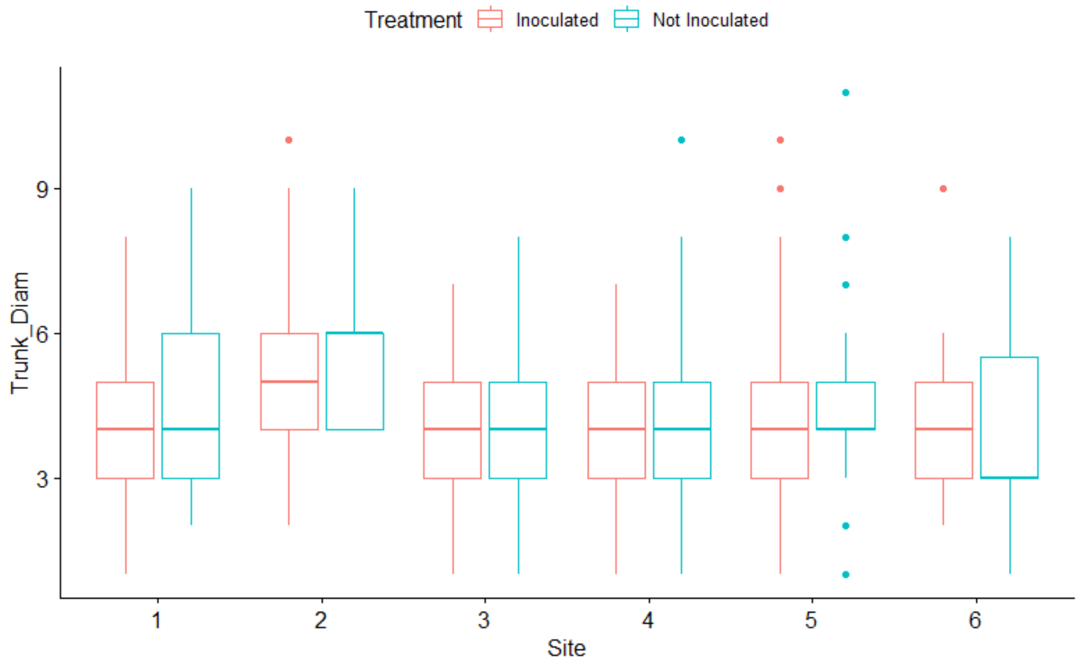

Figure 38: Trunk diameter means for inoculated (orange) and uninoculated seedlings (blue) grouped by site.

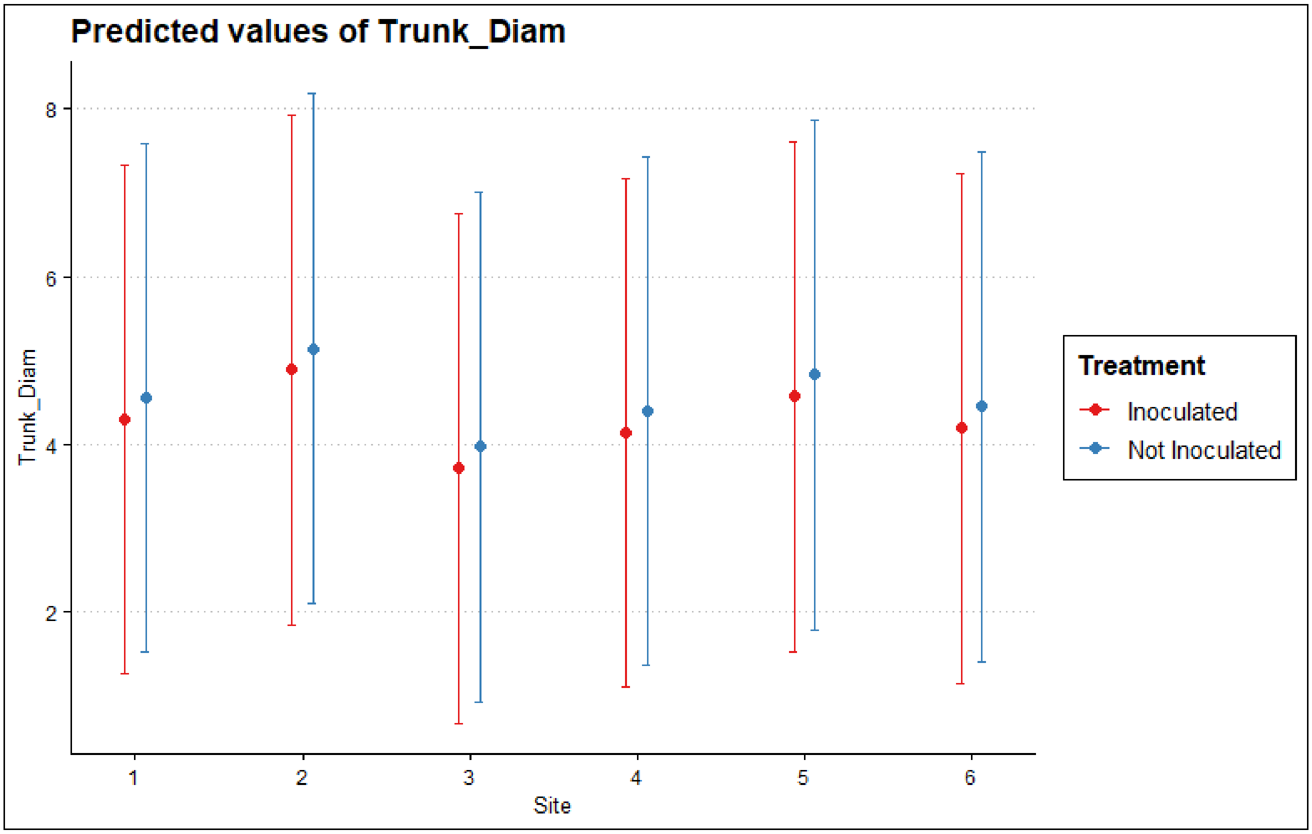

Figure 4: Predicted Trunk diameter means for inoculated and uninoculated seedlings.