ABSTRACT

BRINGING LIGHT TO BELOW GROUND PATTERNS: ARBUSCULAR MYCORRHIZAE FUNGI DIVERSITY ALONG AN ELEVATION GRADIENT IN SOUTHERN CALIFORNIA

By

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August 2015

Necessary for the diversity and survival of most terrestrial plants, arbuscular mycorrhizae (AMF) are fungi that form mutualistic symbiotic relationships with approximately 90 percent of terrestrial plant families. While the biodiversity and abundance of plants and animals have received much attention, these patterns for the belowground organisms on which they rely, such as AMF, remain poorly understood. While studies have found indications that AMF are fundamental to ecosystem structure and function, relatively few of these studies have been conducted *in situ*. In their ability to accommodate the complexity found in natural ecosystems, *in situ* studies may be vital in providing information relevant to the restoration and conservation of ecosystems. This thesis sought to explore *in situ* how AMF diversity and root colonization changed across ecosystems along an elevation gradient in Southern California. The findings indicate that certain soil parameters may be especially influential and that intra-species competition may play a role in AMF root colonization.

BRINGING LIGHT TO BELOW GROUND PATTERNS: ARBUSCULAR MYCORRHIZAE FUNGI DIVERSITY ALONG AN ELEVATION GRADIENT IN SOUTHERN CALIFORNIA

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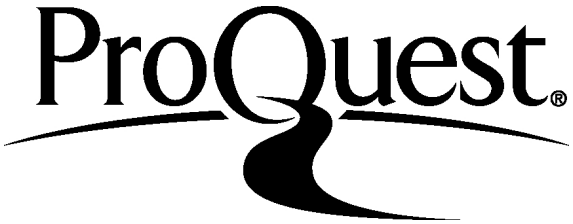

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CHAPTER 1

INTRODUCTION

Biodiversity is essential to the maintenance of the variety of ecosystems that support life. Ecosystems regulate climatic processes, remove CO_2 from the air, filter and purify water, buffer against flooding, recycle nutrients and break down wastes, provide fertile soil, and supply natural resources such as food and wood. Loss of biodiversity due to the destruction of habitat, over exploitation for human use, and global climate change, severely threaten these processes. To protect and restore the ecosystems upon which we rely, it is imperative that we improve our knowledge of biodiversity and the abundance of species and ecosystems and our understanding of the mechanisms responsible for their diversity. Necessary for the diversity and survival of most terrestrial plants, arbuscular mycorrhizae (AMF) are fungi that form mutualistic symbiotic relationships with over 80 percent of terrestrial plant families (Allen 1991; Gai et al. 2012). While biodiversity and the abundance of plants and animals have received much attention, these patterns for the belowground organisms on which they rely, such as AMF, remain poorly understood (Bryant et al. 2008; Sundqvist et al 2011; Gai et al. 2012).

AMF are crucial components of terrestrial ecosystems, assisting plants in the uptake of water and limiting nutrients, such as phosphorous and nitrogen, and providing defense against pathogens (Smith and Read 1997; Koide and Mosse 2004; Whipps 2004; Pozo and Azcon-Aguilar 2007). Indeed, studies have found indications that AMF are

fundamental to ecosystem structure and function (Allen 1991; Cripps and Eddington 2005; Yao et al. 2008). Furthermore, AMF may be especially critical to plant success and survival in harsh abiotic conditions, such as arid environments or nutrient poor soils (Gardes and Dahlberg 1996; Cripps and Eddington 2005; Yao et al. 2008).

Despite their significant ecological role, much remains unknown about AMF patterns of abundance and diversity with relation to climate, elevation, and aboveground associations (Gai et al. 2012). Furthermore, studies reveal conflicting results, especially between those studies conducted in controlled settings *versus* those that have been conducted *in situ* (in natural surroundings) (Allen 1991; Bever et al. 2001; Yao et al. 2008). The complexity of the relationships between AMF, host plants, and their environment is extremely difficult, if not impossible to replicate in controlled settings. It is these complex relationships that are vital to the perseverance of ecosystems (Allen 1991; Bever et al. 2001; Sturmer, Sturmer, and Pasqualini 2013).

The conservation, and perhaps more drastically, the ecological restoration of ecosystems requires the consideration of all components of an ecosystem. Ecosystems include a myriad of process and often a countless variety of species that are essential to their proper function. While conservation can be viewed as the more passive of the two, recent findings have demonstrated that just preventing development and minimizing human disturbance are often not enough to conserve an ecosystem, especially those located in areas near high population centers or those that receive large numbers of tourists. In locations, such as Southern California, where many ecosystems have been highly impacted and/or modified, restoration is becoming a critical component of protecting the environment and biodiversity. Ecological restoration involves intentional

human intervention in the renewing and restoring of damaged, degraded, or destroyed ecosystems (Society for Ecological Restoration International Science and Policy Working Group 2004). California sage scrub and California native wetlands have been reduced to less than 10 percent of their former extent (Westman 1981). Furthermore, the pressures placed upon chaparral and mixed oak and pine forests produce a picture that is not much less grim. In order to protect and restore these ecosystems, an understanding of all their components, including AMF, is imperative.

One technique to explore differences in ecosystem and species abundance and diversity *in situ*, is to conduct studies along a gradient in elevation. Elevation gradients have been used to explore how changes in temperature and associated climatic variables influence ecological processes since the foundation of biogeography (Humboldt 1814; Bryant et al. 2008; Gai at al. 2012). Steep elevation gradients result in dramatic changes in precipitation, temperature, and other abiotic factors, which are key determinants in the distribution of species and ecosystems. Unlike laboratory and other controlled studies, where there is the ability to isolate and control for various elements, studies along elevation gradients may make it difficult to desegregate factors. However, laboratory and controlled studies cannot replicate the complexity of *in situ* relationships, and may result in overlooked or inadequately understood variables and associations. Studies done *in situ* also have the benefit of potentially providing and/or narrowing the direction for future laboratory research by eliminating or elucidating various factors, and also provide higher external validity (Allen 1991).

The lack of studies examining AMF *in situ* and across environments limits our understanding of ecological communities and ultimately our ability to restore and protect

these communities. Countless dollars and hours of effort are spent restoring and protecting ecosystems. As our knowledge of the complexity of ecosystems increases, it is becoming more and more apparent that restoration and conservation efforts need to be dynamic and holistic, taking into consideration the multiple facets of ecosystems. Thus, this research hopes to help shed light on important belowground phenomena in an effort to inform and potentially aid restoration and conservation efforts, as well as to establish a baseline upon which future research can expand.

Specifically, in this study a mega-transect (a transect spanning across several miles from mountain base to subalpine zone) was established in Southern California across an elevation gradient from 600 meters to 3,100 meters to determine 1) whether AMF occur across the elevation gradient, 2) how the diversity of AMF spores changes across the elevation gradient, and 3) how the percent of host plant root colonization by AMF changes across the elevation gradient. I hypothesized that AMF do indeed occur across the elevation gradient, that spore diversity would echo the diversity of the above ground vegetation, and that the percent of the root colonized would be greater at lower and higher elevations, reflecting the "harshness" of these environments. These hypotheses reflect the literature and findings that AMF may be an integral part of aboveground ecosystem structure and that AMF are especially critical in environments where nutrients are less prevalent and growing conditions are more "harsh."

Although AMF may be vital components of terrestrial ecosystems, until recently, our understanding of these organisms has remained relatively impoverished. As we seek to protect and restore the ecosystems essential to biodiversity and the ecosystem services we are dependent upon, a more complete understanding of AMF and their contributions

to the function and structure of ecosystems will prove indispensible. The following chapter succinctly summarizes some of the existing knowledge of the ecology and contributions of AMF and highlights areas where further research is sorely needed.

CHAPTER 2

EXISTING RESEARCH AND UNDERSTANDING

This chapter briefly reviews the existing knowledge on AMF ecology and the contributions of AMF to ecosystem function and structure. It begins with a discussion of the importance of intact, healthy ecosystems and how this includes not just the aboveground, more obvious part of an ecosystem, but also to the less visible belowground components. Next, the chapter considers the ecology of AMF and succinctly summarizes some of the existing research available on AMF contributions to ecosystems. Finally, the chapter concludes by discussing the significance of *in situ* studies and the advantage of using an elevation gradient to study phenomena across a variety of ecosystems.

Biodiversity, Ecosystems, and the Importance of Belowground Ecology

Intact, healthy ecosystems are critical to both biodiversity and proper ecosystem function. While much attention has been paid to aboveground diversity of ecosystems, such as the mammals and plants present, less attention has been paid to the belowground diversity of ecosystems. This is due partially to the limited visibility of these organisms, as they are not only small and often invisible to the naked eye, but it is also due to the fact that they occur in a medium less familiar to us, the soil.

As David Carle (2010) noted in his text *Introduction to Earth, Soil, and Land in California*, "Living soil" is the "fundamental substrate of our terrestrial environment"

(pp. xiv). Living soil is composed of broken down inorganic and organic materials, which include an incredibly diverse compilation of bacteria, worms, insects, fungi, and other organisms (Carle 2010). Healthy terrestrial systems are dependent upon healthy living soil. Soil is a crucial link in a variety of terrestrial ecosystem cycles. It is where the nutrients essential to the health and growth of plants are found. And while the nutrients may be present, they are not always readily available to plants. Often, a necessary component in the uptake of these nutrients is a symbiotic relationship between plants and fungi. Plants, as well as whole ecosystems, rely on a variety of fungi and other microorganisms to maintain healthy and productive functions. Thus, it is critical that light be brought to the biodiversity and health of belowground organisms in discussions of conservation and restoration.

Mycorrhizae Ecology

While there are many types of mycorrhizae, two are especially abundant and widespread. Ecto- and endo-mycorrhizae occur in most terrestrial ecosystems, together forming associations with over 90 percent of plant families (Allen 1991; Kendrick 2000; Bever et al. 2001; Moore, Robinson, and Trinci 2011). AMF comprise the largest, most abundant subtype of endo-mycorrhizae. Both ecto- and endo-mycorrhizae have an external hyphal matrix that extends into the soil to find and take up nutrients, as well as an exchange surface between the fungus and plant (Allen 1991). However, they also differ in many ways. Most notably, endo-mycorrhizae hyphae penetrate the root cortical cell walls; however, the hyphae do not penetrate the cell membrane. Ectomycorrhizae hyphae do not penetrate cortical cell walls. Another visible and more easily discernible difference is the sporophores, more commonly known as the “mushrooms,” produced by

ectomycorrhizal species. Whichever mychorrizae a plant associates with, the plasmalemma (the semi-permeable membrane enclosing the cytoplasm of a cell) surface area of a mycorrhizal plant is greatly increased when compared to a nonmycorrhizal plant, thus dramatically increasing the surface area for the absorption of nutrients (Allen 1991). Arbuscular mycorrhizae may have been the earliest of the mycorrhizae later evolving into more complex mycorrhizae, such as ectomycorrhizae and the mycorrhizae associated with orchids (Allen 1991; Moore, Robinson, and Trinci 2011). The only consistently non-mycothrophic plants are annual, "weedy" plants (especially adapted to invasion of disturbed fertile habitats) and even many of these plants form facultative symbiosis with mycorrhizae (Allen 1991; Vogelsang and Bever 2009; Lekberg et al. 2013).

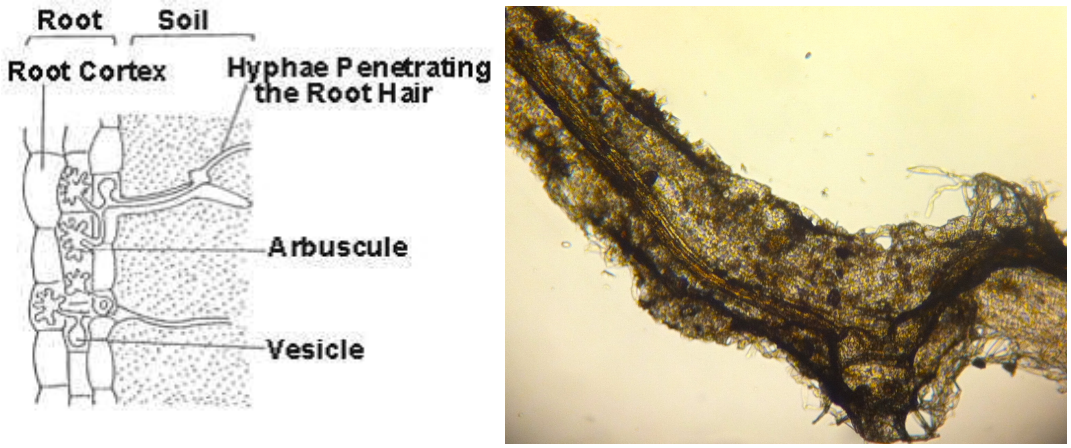

FIGURE 1. Parts of a mycorrhizal symbiosis (Brundett 2008) and (right) infected portion of root (100x).

Necessary to the success and survival of the majority of terrestrial plants, AMF are Glomeromycete fungi, inhabiting the soil and plant roots (Figure 1). Studies suggest that the symbiosis between AMF and plants was essential for the colonization of

terrestrial habitats over 400 million years ago (Allen 1991; Kendrick 2000; Bever et al. 2001). While early plants could photosynthesize they had yet to develop extensive root systems (Allen 1991; Kendrick 2000; Moore, Robinson, and Trinci 2011). Furthermore, terrestrial soils lacked nutrient rich organic matter. AMF with their fine filamentous hyphae (root-like structures), were well adapted for exploring the soil and obtaining nutrients and water. However, the fungi required the carbon-compounds plants readily produced (Kendrick 2000; Moore, Robinson, and Trinci 2011). Faced with these harsh conditions and limitations, it was only through a symbiosis with AMF that plants were able to colonize terrestrial habitats. Indeed, studies looking at the fossil record conducted by Gerdemann (1968), Trappe (1987), and Koske et al. (1985) indicate that all of the "primitive" plants had AMF associations. In fact, fossils discovered in mid-Ordovician rocks show distinct Glomermycotan fungi in association with pre-vascular plants dated to approximately 460 million years ago (Moore, Robinson, and Trinci 2011). Furthermore, studies examining plant colonization of sites of recent volcanic disturbance have found that all the early re-colonizing plants were falcultatively mycotrophic species (Allen 1991).

Table 1 summarizes the parts of AMF and the various functions each part is believed to perform. The "fan-shaped" mycelia, with dichotomously branched hyphae radiating out from a main hypha are one of the most notable features, often visible to the naked eye when looking closely (Allen 1991). Intraradical hyphae penetrate plant roots and extraradical hyphae extend into the soil with absorbing hyphae initiating from a main, "runner" hypha. Absorbing hyphae can extend up to 7 cm from "runners," however, the most intensive hyphal development remains relatively close to roots (Allen

1991). Extraradical hyphae absorb soil resources, initiate new colonization sites, and participate in spore formation while intraradical hyphae connect vesicules and arbuscules. Vesicules are believed to accumulate and store lipids. Arbuscules are responsible for the transfer of water, carbon, and nutrients. Spores form singularly or in loose or tight masses called sporocarps. Spores, often the most stress-tolerant and mobile structures of the fungus, are responsible for reproduction and are important in initiating new infections. Less common and less understood structures are auxiliary cells. These form only in genera that do not form vesicles. They are found in the soil or media, outside of the cortical cell and are often in clusters that are spiny, knobby, or smooth in appearance (Allen 1991).

TABLE 1. Summary of AMF Parts and Functions

Structure	Appearance	Location	Function
Arbuscules	Complex, branched, tree-like	Inside host cortical cells	Transfer water, carbon, and nutrients
Vesicles	Globular, round, elliptical	Inside or between cortical cells	Accumulate lipids
Inraradical AMF hyphae	Rope-like, can form coils and loops	Inside or between cortical cells	Connect arbuscules and vesicules
Extraradical AMF hyphae	Varies with function, rope-like	In media, outside of cortical cell	Absorb soil resources, initiate new colonization sites, participate in spore formation
Auxiliary cells	Clusters, spiny, knobby, or smooth	In media, outside of cortical cell	Unknown, found only in genera that do not form vesicles
Spores	Varies with Family/species, singularly or in clusters	In media	Reproduction

AMF occur in a broad range of environments, are geographically widespread, and have continued to co-evolve with plants, forming symbioses with approximately 80 percent of terrestrial species (Allen 1991; Bever et al. 2001; Cripps and Eddington 2005). Active primarily in the top 20 cm of soil, they are among the most abundant microorganisms (Allen 1991). Some estimate that there are approximately 20,000 kilometers of AMF hyphae per square meter (Moore, Robinson, and Trinci 2011). Furthermore, with only 230 species described, it is believed most remain undiscovered and undescribed (Sturmer, Sturmer, and Pasqualini 2013). In fact in 2001, AMF were recognized as a distinct phylum within the Fungal Kingdom (Koide and Mosse 2004). Ten genera of AMF are currently recognized: *Archaeospora, Geosiphon, Acaulaspora, Entrphospora, Diversispora, Pacispora, Gigaspora, Scutellaspora, Glomus, Paraglomus*. While it is difficult to delineate what constitutes a species, AMF are currently separated into "species" by morphotypes. It has also been difficult to develop phylogenies based on multi-gene sequencing due to the contamination of samples by other micro-organisms (Moore, Robinson, and Trinci 2011). That being said, molecular markers are indicating a great deal of diversity, further demonstrating there may be far more than the 230 species currently recognized (Moore, Robinson, and Trinci 2011; Sturmer, Sturmer, and Pasqualini 2013). Moreover, when Bever et al. (2001) conducted one of the first studies extensively aimed at examining AMF diversity, their study identified twelve previously undescribed species in a study site comprising only one hectare. At that time just 145 species had been described, meaning 85 new species (and counting) have been identified in a little over a decade.

Existing Research on Arbuscular Mycorrhizal Fungi

Research on AMF has remained relatively sparse for a variety of reasons. The most obvious is due to the location of the organisms below ground, further hampered by the fact they are difficult to distinguish with the naked eye (Kendrick 2000; Bever et al. 2001). Additionally, the phenomena of mutualisms have traditionally been met with skepticism, and when these relationships were observed they were thought to be atypical (Allen 1991). For example, ecologist M. Williamson in a 1972 paper considered mutualisms an oddity, and R. M. May (1974, 1981) later argued they were "mathematically unstable." These beliefs stem largely from an historical emphasis in ecological studies on competition between species and the division of limited resources. However, findings now suggest that pairs or groups of organisms may facilitate each other's survival through adaptations that allow the partitioning of resources or by "trading" resources or services.

An ecological debate from the early 1900s between Fredrick Clements (1916) and Henry Gleason (1917) exemplifies the complexity of beliefs surrounding species associations, let alone mutualisms. Clements (1916) viewed ecosystems holistically and his ideas developed into the "super-organism" concept, where each species, and the interactions between species, play an important role in the structure and function of an ecosystem. Gleason countered Clements by arguing for the individualistic, or open community concept, in which community composition was more random. He suggested that associations between species would be less predictable and that a species' individual requirements determined its distribution. In Gleason's model, associations of plants are not viewed to be highly organized or dependent upon each other. Neither concept has

been found to be completely accurate, nor have they been found to be mutually exclusive. However, Clements's (1916) model supports the idea that interactions between species are crucial for ecosystem structure. Thus, coupled with findings from other studies involving AMF, reconsideration of Clements's model supports the need for further research into species associations, mutualisms, and the importance of AMF to ecosystem structure and function.

Furthermore, AMF were thought to be functionally redundant, providing the same services to plants and filling the same niches, therefore resulting in a limited need for diversification (Bever et al. 2001). However, as Bever et al. found in their 2001 study, not only are AMF ecologically diverse with different species active in different seasons and aiding plants in different ways, there is considerable genetic diversity within species.

While it is recognized that AMF assist plants with the uptake of nutrients and water, the intricacies of the relationships between AMF, host plants, and other environmental factors are still only vaguely understood (Allen 1991; Berver et al. 2001; Vogelsang and Bever, 2009; Oehl et al. 2010). The micronutrients nitrogen and phosphorous have received the most attention in AMF studies (Allen 1991; Miransari 2011; Smith et al. 2011). For example, Smith et al. (2011) demonstrates that AMF plants are able to access limited stores of phosphorous more effectively than non-mycorrhizal plants. Miransari (2011) notes that, while it is commonly understood that AMF facilitate nitrogen uptake along with the nitrogen-fixing bacteria Rhizobium, the precise role of AMF is still poorly understood. Furthermore, recent findings suggest very specific host plant and AMF associations (Yao et al. 2008; Vogelsang and Bever 2009; Martinez-Garcia and Pugnaire 2011). For example, Martinez-Garcia and Pugnaire (2011) found

that AMF communities differed among plant species. These, as well as results from other studies, suggest that AMF contribute to plant community dynamics and populations and thus to whole ecosystem structure and function (Allen 1991; Cripps and Eddington 2005; Yao et al. 2008; Vogelsang and Bever 2009).

Until recently, much of the knowledge on AMF was gathered through studies done in relatively controlled settings either in greenhouses or agricultural fields focusing on how different soils and soil parameters affect AMF inoculation (potential of AMF to form symbiosis with plants) and plant health and growth rates (Allen 1991; Koide and Mosse 2004; Oehl et al. 2010). In his extensive text *The Ecology of Mycorrihzae*, Allen (1991) acknowledged the need for a more thorough understanding of AMF in their natural environments. Since then, a growing body of research has been conducted *in situ* with a number of findings conflicting with those found in controlled settings (Bever et al. 2001; Yao et al. 2008; Vogelsang and Bever 2009). Community interactions appear to be critical to AMF abundance, diversity, and inoculum potential. Abiotic factors and inter-species competition also influence the extent and type of exchanges between AMF and plants (Allen 1991; Bever et al. 2001; Yao et al. 2008; Martinez-Garcia and Pugnaire 2011). Controlled settings are unable to replicate and account for the complexity found in natural settings, thus potentially missing crucial interactions (Allen 1991; Bever et al. 2001; Sturmer, Strumer, and Pasqualini 2013).

Many studies of AMF conducted *in situ* have occurred in specific environments, such as those done in alpine environments by Cripps and Eddington (2005), and Sundqvist et al. (2011) or in sandy environments by Sturmer, Strumer, and Pasqualini (2013), Yang, Chen, and Li (2008), and Shi et al. (2007), or tropical environments by

Yao et al. (2008). Findings from these and additional studies suggest that AMF are critical to plant establishment and survival in harsh environments. However, little research has examined AMF diversity across environments. Exceptions include Fisher and Fulé (2004), Bryant et al. (2008), and Gai et al. (2012) to be discussed further below. Furthermore, many studies tend to be "plant based" with AMF root colonization, not AMF themselves, being the primary focus.

Why Elevation Gradients

One technique to explore differences in ecosystem and species abundance and diversity *in situ* is to conduct studies along a gradient in elevation. Humboldt (1814) recognized elevation gradients as useful for exploring how changing environmental and climatic factors influence diversity as early as 1814. Gradients in elevation provide researchers with significant changes in environmental factors in relatively short distances (Figure 2). Moreover, continuous gradients provide ecotones, transition areas between habitat types, where communities meet and integrate (Risser 1995). Ecotones offer unique opportunities to examine how the composition, diversity, and abundance of species are affected by changing habitats (Risser 1995). Studies along elevation gradients have also gained attention as useful in exploring the potential effects of climate change on species distribution, diversity, and abundance (Erschbamer et al. 2009).

D.J. Read (1983) developed a classification of mycorrhizae based upon elevation or latitude and nitrogen and phosphorus availability. His assertion was that as latitude and elevation increases soils change and thus mycorrhizal associations would change correspondingly. He hypothesized that mycorrhizal associations at lower elevations and latitudes tend to be AMF while at higher elevations and latitudes associations tend to be

ecto-mycorrhizal, based upon his belief that soil changes dictated the type of mycorrhizae available. However, studies are indicating the AMF are found in virtually every habitat, at both high latitudes and high altitudes. Allen et al. (1987) and Bryant at al. (2008) found AMF in alpine ecosystems and many studies, including Treseder and Cross (2006), Sundqvist et al. (2011), and Gai et al. (2012), have found AMF north of the Arctic Circle.

Bryant et al. (2008) and Gai et al. (2012) both conducted studies across ecosystems along elevation gradients in Colorado and Tibet, respectively. Their specific purposes and methods for acquiring and processing data differed. Bryant et al. (2008) contrasted plant diversity and general soil microorganism diversity, thus including a broad range of organisms in their assessment and not focusing on AMF and their symbiotic relationship with plants. They used well-established sampling and taxonomic methods for assessing plant diversity and DNA extraction to assess microbial diversity. Alternatively, Gai et al. (2012) looked purely at AMF diversity, soil parameters, and elevation; however, they disregarded aboveground plant diversity. Their methods for soil assessment and root collection and AMF processing and identification were used as a model for my own methods. Fisher and Fulé (2004) also conducted a study along an elevation gradient at a site in Arizona. Their study focused primarily on understory plant diversity in relation to canopy diversity and AMF inoculation potential. Their study was extensive and was conducted over a period of three years, revisiting established plots every year. However, soil samples to determine inoculation potential were collected during only one year of their study.

The discrepancy between *in situ* and controlled studies and the potential of the importance of AMF for both conservation and restoration efforts necessitate the need for

more *in situ* studies targeting AMF specifically. If, as many of the studies mentioned above suggest, AMF are not only critical to plant survival and success, but also exhibit significant diversity and proclivities for specific host species: then continued research across environments targeting a variety of species should help elucidate these complex relationships and thus potentially provide more robust data to inform conservation and restoration efforts. Informed by the methods previously mentioned, this thesis seeks to do just that by examining the PRC and diversity of AMF along an elevation gradient in Southern California.

CHAPTER 3

METHODOLOGY

Study Site

Southern California is designated one of the twenty-four biodiversity hotspots on Earth in recognition that its distinctive climate, location, and other physical features support a unique variety of species and ecosystems (Myers et al. 2000). Located along the southwestern coast of the United States of America, Southern California experiences a Mediterranean climate, with warm dry summers and short mild winters (Figure 2). Along with this unique climate, occurring in only four other locations throughout the world, isolating mountain ranges and an ocean boundary surround the area (Bakker 1984). This isolation and climate results in a unique mix of species, including several endemic to the area. These species form distinctive ecosystems, many of which are especially adapted to the fires that frequent the area and the harsh, dry Southern California summers. A growing human population, the destruction of habitat, and global climate change all pose serious threats to Southern California's distinctive habitats and the biodiversity they support.

The San Jacinto Mountains are located east of Los Angeles in Riverside County. The thirty-mile range reaches elevations above 3,000 meters (10,000 feet), with the highest peak, Mount San Jacinto, attaining an elevation of 3,302 meters (10, 834 feet). It is home to over 200 species of plants, 165 species of birds, 18 species of reptiles, and

1000 invertebrates (University of California Riverside (UCR) 2010). As part of the San Jacinto National Forest and the University of California Natural Reserve System, the U.S. Forest Service and the University of California Riverside manage and protect the area. The range is the northernmost of the Peninsular Ranges and forms a natural boundary between the coast and the Colorado Desert. This range of habitat types, from coastal to subalpine to desert, provides for extraordinary biodiversity (Bakker 1984).

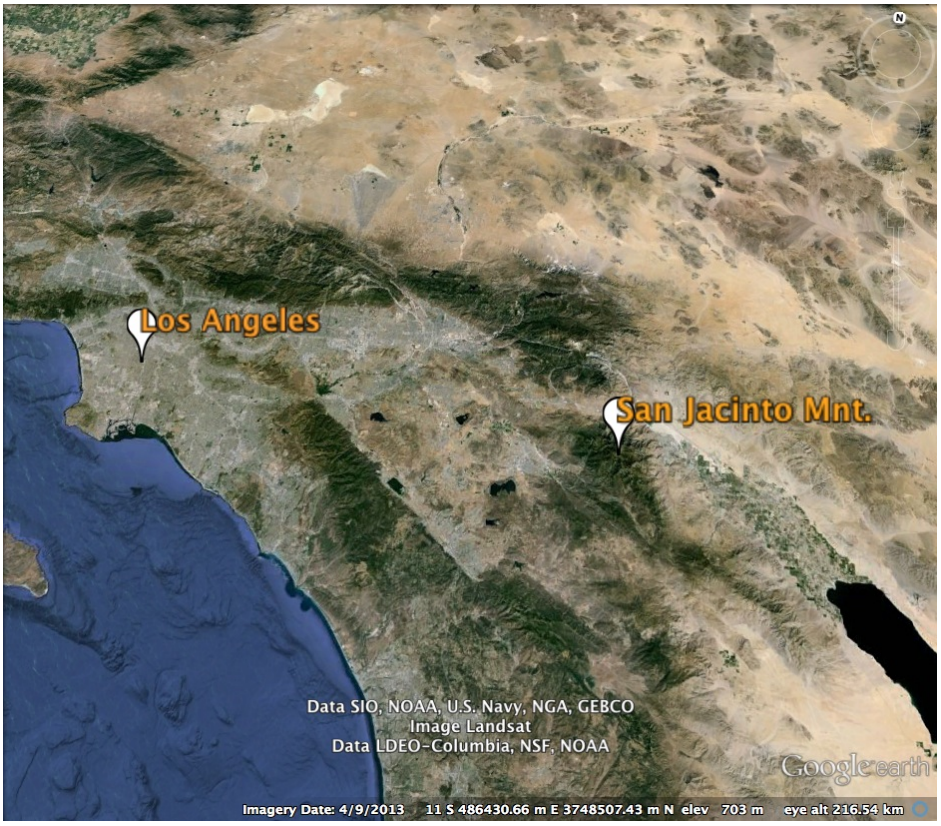

FIGURE 2. The San Jacinto Mountains.

To the west of the range is the rapidly growing Inland Empire, to the east is the San Jacinto Valley, also increasing in population and development. All of the surrounding development is putting pressure on native habitats. While managers are working to implement protection measures, such as invasive species eradication and

removal of non-native disturbance regimes, the loss of native habitats continues (UCR 2010). Furthermore, despite a growing body of research, gaps in knowledge exist as to the extent and the drivers of the biodiversity found within the area.

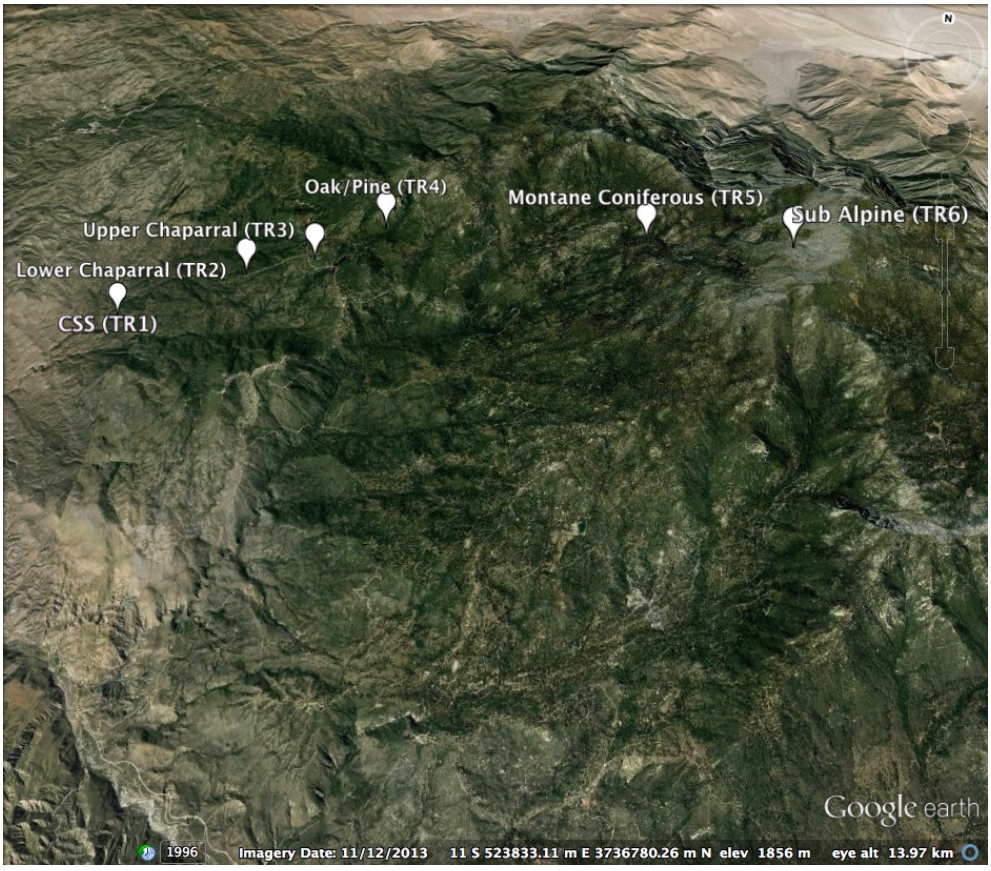

FIGURE 3. Sub-transects along the mega-transect delineated by associated vegetation.

Field Collection

To explore the relationship among AMF, elevation, and above ground biodiversity, a mega-transect was established spanning from the base of San Jacinto Mountain to above 2,400 meters (8,000 feet) (subalpine zone). Using remote imaging

and some local knowledge, six sampling sites were chosen along the established transect and entered into a Garmin e-Trex 10 GPS unit. The six sub-transects were each at a different elevation and represented the gradient of vegetation types found as elevation changes (Figure 3). The vegetation types represented, from lowest elevation to highest, are: interior California sage scrub (CSS) (approximately 600 meters), lower chaparral (approximately 1,200 meters), upper chaparral (approximately 1,600 meters), oak/pine forest (approximately 2,100 meters), montane coniferous forest (approximately 2,600 meters), and subalpine (approximately 3,100 meters). Figure 4 shows a typical elevation gradient in Southern California and the species that tend to be associated with various elevations.

Upon arrival, general observations of each site, such as canopy cover, were recorded. Five 1 meter by 1 meter quadrats were placed at equal intervals (every 5 meters) along a 30 meter sub-transect within each study site, similar to the technique used by Bryant et al. in their 2008 study. Two soil samples were collected from each quadrat along a diagonal using a handheld soil corer (Figure 5). Along the other diagonal, a small section of roots was collected from each species that intersected the diagonal. Samples were immediately placed into a soft-sided cooler with ice packs and transferred to a freezer upon return to the California State University Long Beach Geography and Environmental Science and Lab. Additionally, vascular plants within each quadrat were identified to species and the percent cover of each species was recorded similar to the assessment conducted by Fisher and Fulé (2004).

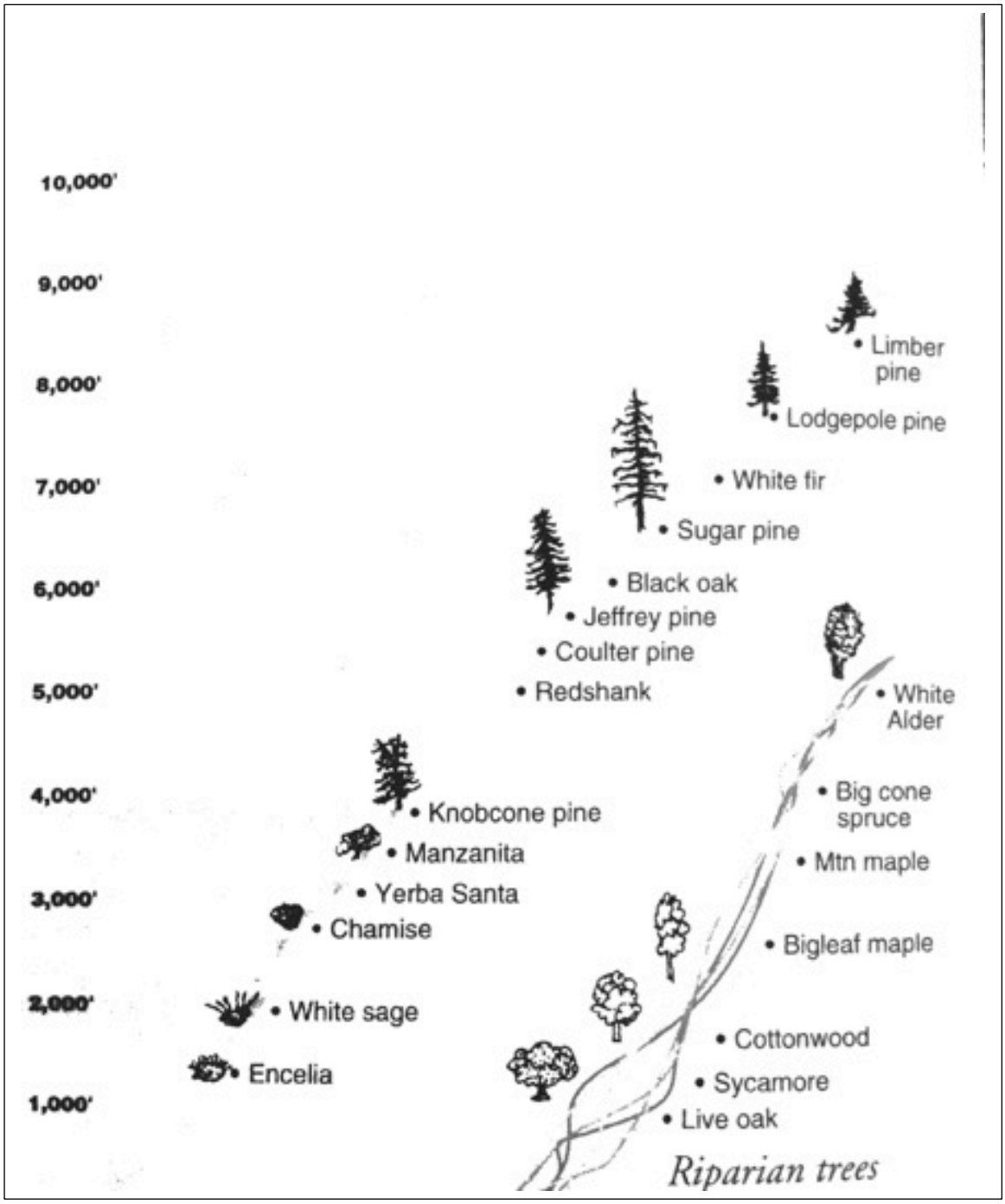

FIGURE 4. Typical Southern California elevation gradient with associated species (Havert and Gray 1996).

FIGURE 5. Field collection of soil samples and root balls (Photograph 1: Emily Feliciano; Photograph 2: Mystyn Mills).

Laboratory Processing

Roots and soil were brought back to California State University Long Beach's Geography and Environmental Science Laboratory. Following methods previously established (Brundrett 2008; Gai et al. 2012; Sturmer, Strumer, and Pasqualini 2013), roots were carefully washed, placed loosely into biopsy cassettes, treated with 10 percent KOH (potassium hydroxide), and placed in an autoclave for processing. KOH clears the roots of cytoplasm, making it possible to see the hyphae (symbiotic structures), vesicles, and arbuscules of AM fungi if they are present. Typical autoclave runtimes for my samples were between 45 and 60 minutes. After removing the samples from the autoclave they were rinsed gently with distilled water and a solution of 2 percent hydrochloric acid diluted with distilled water to acidify the roots for staining. They were then stained with a solution of 50 percent India ink and vinegar. After 20 to 30 minutes

in the solution, the roots were then examined under a microscope to assess presence and percent root colonized (PRC).

PRC was determined using the Subjective Visual Estimation Technique. While a subjective assessment, relying on the researcher's estimate of the percent of the root colonized by AMF hyphae, this method has been shown to give quite reliable results with a calculated standard of error between 2 percent and 5 percent (Giovannett et al. 1994; Utobo et al. 2011). It is also noted for its time-efficiency and for its appropriateness when examining samples from natural settings (Brundett 2008; Utobo et al. 2011).

To assess the alpha diversity of spores present (number of species present), soil samples were wet-sieved and centrifuged to separate spores from soil and other debris. Following a mix of the methods described on the International Culture Collection of (vesicular) Mycorrhizal Fungi website and from Johnson (1999), 300 grams of soil were selected from each quadrat's soil samples. They were mixed with water and wet-sieved through a top sieve of 500 micrometers to capture roots and debris with a 35 micrometer sieve placed beneath to capture spores and other debris. The material on the bottom sieve was collected in a beaker and transferred into centrifuge tubes. The tubes were then filled with 20/60 percent sucrose/water mixture (the sucrose creates a density gradient). The tubes were centrifuged in a tabletop centrifuge for 20 minutes. After 20 minutes, soil and other particulates formed a "pellet" precipitate at the bottom of the tube, while less-dense spores and fine-grained detritus were suspended in the sucrose. This sucrose supernatant, minus the "pellet," was then quickly poured through a LaMotte 9m soil filter paper, divided into quarters with a grid and fitted carefully into a sieve with edges turned up (Figure 6). The paper caught the suspended spores and detritus while allowing the

sucrose solution to slowly pass through, allowing for much easier identification and sorting than attempting the process while spores were still suspended in the solution. I gently swirled the solution to be sure spores were distributed evenly across the filter paper. Using the roll of a single die, a quarter of the filter paper was selected for the spore diversity assessment. Spores were carefully assessed by morphology and total number of morphological species present was determined. Identification of both spores and hyphae was aided by the use of the online resource the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM), as detailed in Gai et al. (2012) and Sturmer, Sturmer, and Pasqualini (2013). While minimum. maximum, and mean spore diversity counts are reported for samples from all quadrats at all sub-transects, analysis utilized the maximum spore diversity counts within each sub-transects as this represented the maximum potential diversity for the sub-transect. Using the maximum potential diversity helped to offset the likely possibility that some species were missed during collection and/or through laboratory processing, as opposed to the less likely possibility of a species over-count.

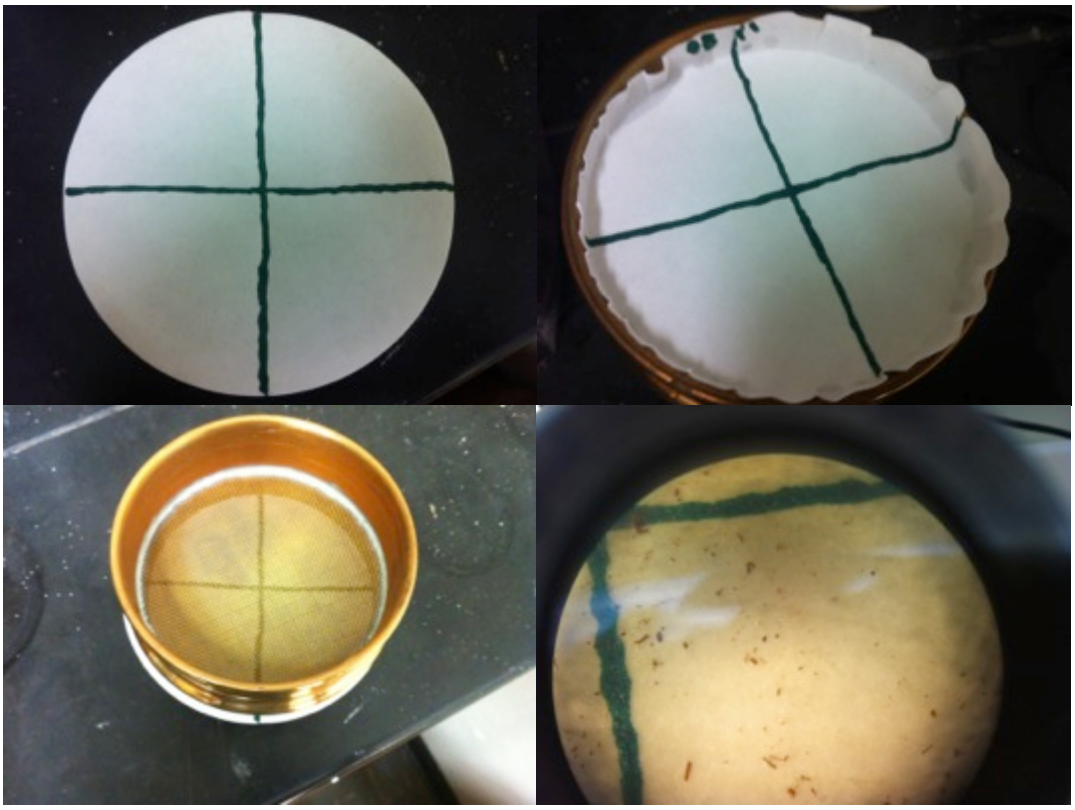

FIGURE 6. Spore extraction using LaMotte filter paper and sieves (Photos: Mystyn Mills).

The soil parameters nitrogen, pH, potassium, and phosphorous (Gai et al. 2012) were also measured using a LaMotte soil analysis kit. After determining that variation across sub-transects was minimal, I used the roll of a single die to select soil samples randomly from a quadrat from each of the six sub-transects. Samples were analyzed following the LaMotte kit's detailed procedures.

Statistics

To determine the significance of associations between variables, due to the small number of samples (n of six) and curved nature of the data, pair-wise comparisons were performed using second-degree polynomial linear regressions. The data collected during

this study displayed a "hump-like" distribution that a linear regression would be unable to depict. Thus, the single bend curve provided by a second-degree polynomial regression was deemed more appropriate for depicting the relationships among my data. A principal components analysis was also performed to examine which combinations of variables appear to explain the largest amount of variation. An alpha of 0.10 was determined to be appropriate in light of the exploratory nature of this study. The risk of a Type II error, failing to detect potential associations, was determined to be more detrimental than the risk of a type I error. The exploratory nature and the use of an alpha of 0.10 offset the power disadvantages of the small sample size of this study, as small sample sizes can detect significant relations if there are strong effect sizes, although there may be weakness in detecting more subtle relations.

CHAPTER 4

RESULTS

This chapter presents the results for vegetation, PRC, spore diversity and soil parameters along the San Jacinto elevation gradient spanning 600 meters to approximately 3,100 meters. Overall vegetation, PRC, spore diversity and soil parameters all varied across the elevation gradient. The total number of plant species per sub-transect conforms to the classic "hump" shape, with more diversity found at middle elevations (seven species) and lower numbers for total species present found at the lower (five species) and higher elevations (four species). Additionally, the lowest and highest elevations both had more open canopies and were less densely vegetated than the middle elevations. PRC was greater at lower and higher elevation. Spore diversity was similar to vegetation diversity, displaying a "hump-like" distribution with higher counts of morphological species at middle elevations. Soil parameters also varied across the elevation gradient. Nitrogen and phosphorous generally decrease as elevation increases while pH and potassium, especially, displayed higher readings at middle elevations and lower readings at lower and higher elevations. Following is a detailed summary of results for vegetation, soil parameters, PRC, and spore diversity.

Vegetation and Soil Parameters By Sub-Transect

TABLE 2. Summary of aboveground species and soil parameters by sub-transect.

Transect	Quadrat	Elevation (m)	# Of Species Aboveground	N lb./ac.	K lb./ac.	P lb./ac.	pH
1	A	600	5	60.00	120.00	25.00	6.50
2	E	1250	5	60.00	120.00	25.00	6.00
3	B	1600	7	40.00	170.00	10.00	6.00
4	D	2100	7	20.00	160.00	10.00	7.00
5	A	2600	4	10.00	75.00	10.00	6.00
6	F	3100	4	20.00	75.00	7.50	50.5

While the soil tests and determination of percent coverage of vegetation were qualitative assessments based upon personal interpretation and could have introduced some bias, I alone performed all assessments negating variation between assessors and establishing consistency. Sub-transect one was established near the base of San Jacinto mountain at 33º 54’ 26” and 116º 52’ 22” with an elevation of approximately 600 meters. The vegetation at this site consists of interior California sage scrub mixed with native and invasive grasses with an open canopy. Five species were present along sub-transect one. Table 2 summarizes the soil parameters nitrogen, phosphorous, potassium and pH for each sub-transect. Sub-transect one had 60 pounds of nitrogen per acre, 120 pounds of potassium, and 25 pounds of phosphorous with a pH of 6.5.

Sub-transect two, located at 33º 51’ 05” and 116º 49’ 44” at an elevation of approximately 1,250 meters was composed of lower chaparral species with a somewhat closed canopy. This site displayed evidence of a recent burn; however, extensive

regrowth has occurred. Along sub-transect two five different species were represented. The soil parameters for sub-transect two were 60 pounds of nitrogen per acre, potassium remained at 120 pounds per acre, and at 25 pounds per acre for phosphorous with a pH of 6. There was no change from sub-transect one to sub-transect two in above ground species diversity and nitrogen, potassium, and phosphorous pounds per acre.

At sub-transect three, 33º48’ 20” and 116º 46’ 53” at approximately 1,600 meters, the species present were characteristic of upper chaparral. The vegetation was dense with a closed canopy. Number of species present increased to seven. At sub-transect three there were 40 pounds of nitrogen per acre, 170 pounds of potassium per acre and 10 pounds of phosphorous per acre with a pH 6. Nitrogen and phosphorous decreased from sub-transect two while potassium increased and pH remained the same.

Sub-transect four, with an elevation of approximately 2,100 meters and a latitude of 33º 48’ 52” and longitude of 116º 45’ 00”, consisted mainly of a mix of oak and pine vegetation type. The canopy was somewhat closed. Seven species were represented. Sub-transect four had 20 pounds of nitrogen per acre, 160 pounds of potassium per acre, and 10 pounds of phosphorous with a pH of 7. Nitrogen, potassium, and phosphorous decreased from sub-transect three while pH increased.

Sub-transect five, at approximately 2,600 meters and located at 33º 80’ 10” and 116º 70’ 25”, was composed of the lodge pole pine vegetation type with a closed canopy. The number of species represented dropped to four. Snow was present when samples were collected. At sub-transect five, nitrogen decreased to 10 pounds per acre, potassium decreased to 75, phosphorous held at 10 pounds per acre while pH decreased to 6.

Sub-transect six was located at 33º 81' 22" and 116º 68' 34" with an elevation of approximately 3,100 meters. This site, located above the subalpine zone, had an open canopy with little undergrowth and scattered scrub/shrub and herbaceous growth. Only four species were represented along the transect. At the final sub-transect, the soil parameters were 20 pounds of nitrogen per acre, a slight increase from sub-transect five. Potassium held at 75 pound per acre. Phosphorous dropped to less than 10 pounds per acre and pH dropped to 5.5.

Overall vegetation and soil parameters both varied across the elevation gradient. Total number of plant species per sub-transect conforms to the classic "hump" shape, with more diversity found at middle elevations (seven species) and lower numbers for total species present found at the lower (five species) and higher elevations (four species). Additionally, the lowest and highest elevations both had more open canopies and were less densely vegetated than the middle elevations. Soil parameters also varied across sub-transects. Nitrogen and phosphorous generally decrease as elevation increases. Potassium and pH had lower readings at higher and lower elevations and higher readings at the middle elevations.

Percent Root Colonized

PRC was determined using the qualitative Subjective Visual Estimation Technique, and due to its qualitative nature, bias may have been introduced. However, once again, I was the only one to perform assessments, negating inter-assessor variation and establishing consistency. For each sub-transect, minimum, maximum and mean PRC is reported, as there were six sampling quadrats per sub-transect. Table 3 summarizes the findings for PRC across all six sub-transects. Sub-transect one had a high PRC of 70

percent and a low PRC at 65 percent with a mean across quadrats of 64.17 percent. For sub-transect two, the highest PRC was 60 percent and the lowest PRC was 50 percent, with a mean across quadrats of 53.33 percent, slightly lower than sub-transect one. The highest PRC for sub-transect three was 50 percent and the lowest PRC was 40 percent. The mean across quadrats for sub-transect three was 44.17 percent, 9.16 percent lower

TABLE 3. Summary of PRC and spore diversity per quadrat at each sub-transect.

Tran 1 600 meters Mean PRC: 64.17	Quad	PRC	Spore Div	Tran 2 1,250 meters Mean PRC: 53.33	Quad	PRC	Spore Div
	A	70	9		A	50	11
	B	65	8		B	55	10
	C	60	8		C	50	9
	D	65	9		D	55	9
	E	65	9		E	50	11
	F	60	8		F	60	9
Tran 3 1,600 meters Mean PRC: 44.17	Quad	PRC	Spore Div	Tran 4 2,100 meters Mean PRC: 45.83	Quad	PRC	Spore Div
	A	50	11		A	40	11
	B	45	13		B	45	11
	C	40	11		C	50	12
	D	50	15		D	45	11
	E	40	12		E	50	12
	F	40	12		F	45	13
Tran 5 2,600 meters Mean PRC: 54.17	Quad	PRC	Spore Div	Tran 6 3,100 meters Mean PRC: 58.33	Quad	PRC	Spore Div
	A	50	10		A	50	12
	B	50	13		B	55	12
	C	55	10		C	60	10
	D	60	9		D	65	11
	E	55	10		E	65	12
	F	55	12		F	55	10

than sub-transect two. Sub-transect four had a high PRC of 50 percent and a low PRC of 40 percent, with a mean of 45.83 percent, a slight increase from sub-transect three. The high PRC for sub-transect five was 60 percent, the low PRC was 50 percent, and the mean across quadrats was 54.17 percent, higher than sub-transect four by 8.34 percent. Sub-transect six had a high PRC of 65 percent, a low PRC of 50 percent, and a mean of 58.33 percent, showing a slight increase in mean PRC from sub-transect five to sub-transect six.

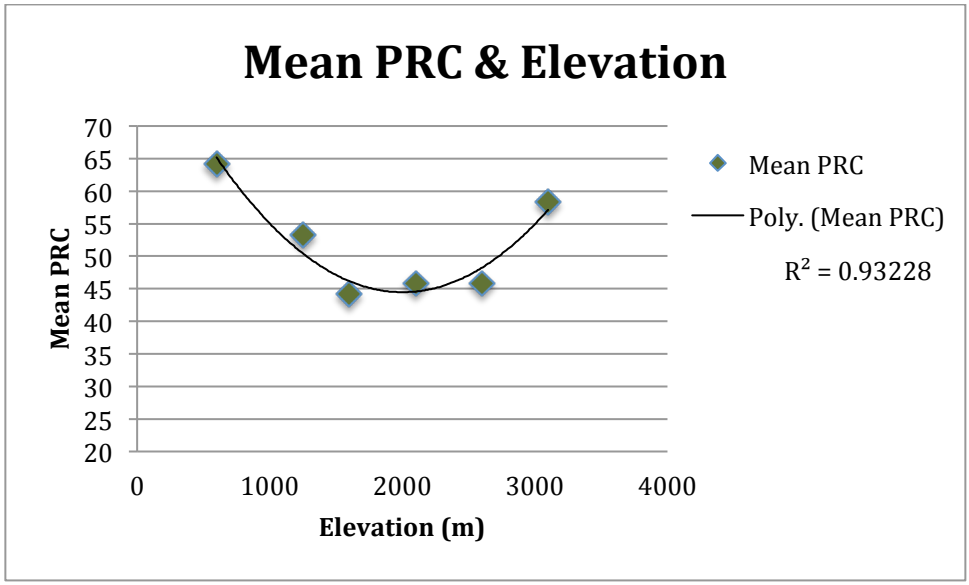

FIGURE 7. Mean PRC along the elevation gradient.

Figure 7 and Table 5 (Appendix) summarizes the results from a second-degree polynomial regression of mean sub-transect PRC and elevation. With an R^2 of 0.932 and a P-value of 0.018, the change in mean PRC correlated strongly with elevation. Mean PRC displayed a slight trough at the middle elevations (see Figure 7). Mean PRC appeared to decrease until sub-transect three located at approximately 1,600 meters. At

sub-transect four, located at approximately 2,100 meters, mean PRC began to increase and continued to increase at the higher elevations. Figure 8 and Table 5 (Appendix) summarizes second-degree polynomial regression results for change in mean PRC and change in vegetation. With an R^2 of 0.578 and a P-value of 0.274, change in mean PRC with elevation had no significant correlation with change in vegetation with elevation.

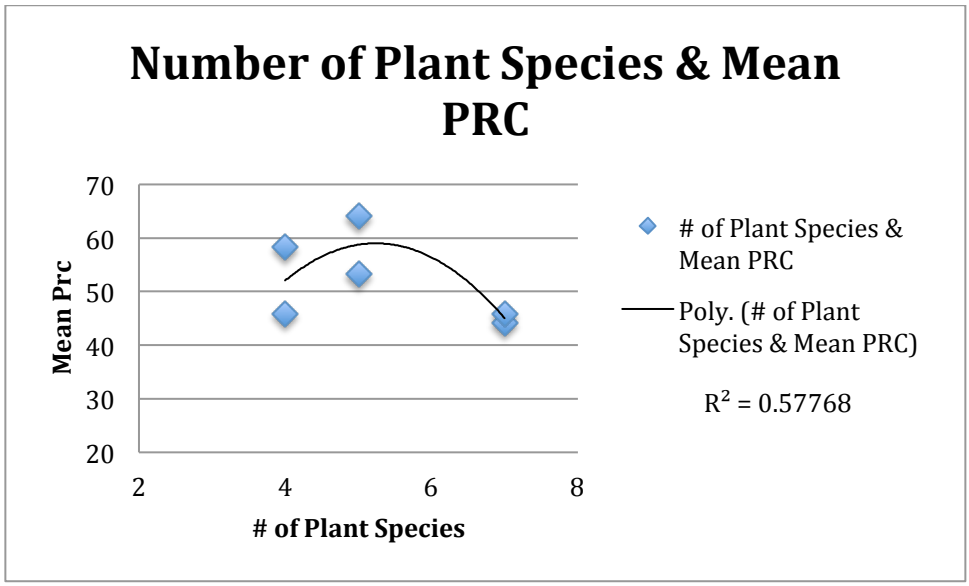

FIGURE 8. Mean PRC and diversity of vegetation.

Figures 9 through 12 and Table 5 (Appendix) show the results of second-degree polynomial regressions of mean PRC and the soil parameters nitrogen, potassium, phosphorous, and pH. There appears to be little correlation between mean PRC and nitrogen, with an R^2 of 0.340 and a P-value of 0.536, and mean PRC and pH with an R^2 of 0.024 and a P-value of 0.964. There is also no significant correlation of mean PRC with potassium with an R^2 of 0.563 and a P-value of 0.288. Mean PRC and phosphorous appear to be strongly correlated with an R^2 of 0.815 and a P-value of 0.080.

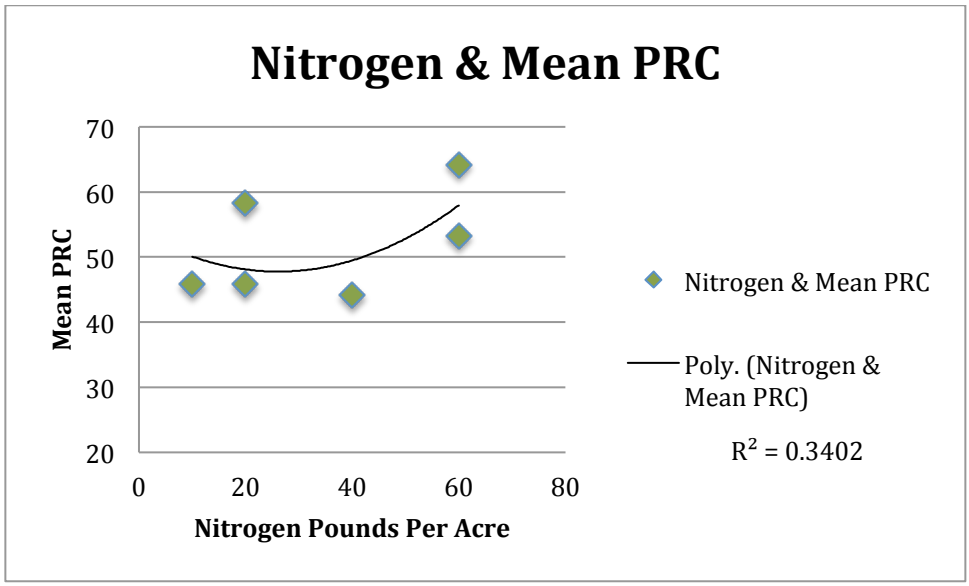

FIGURE 9. Mean PRC and nitrogen.

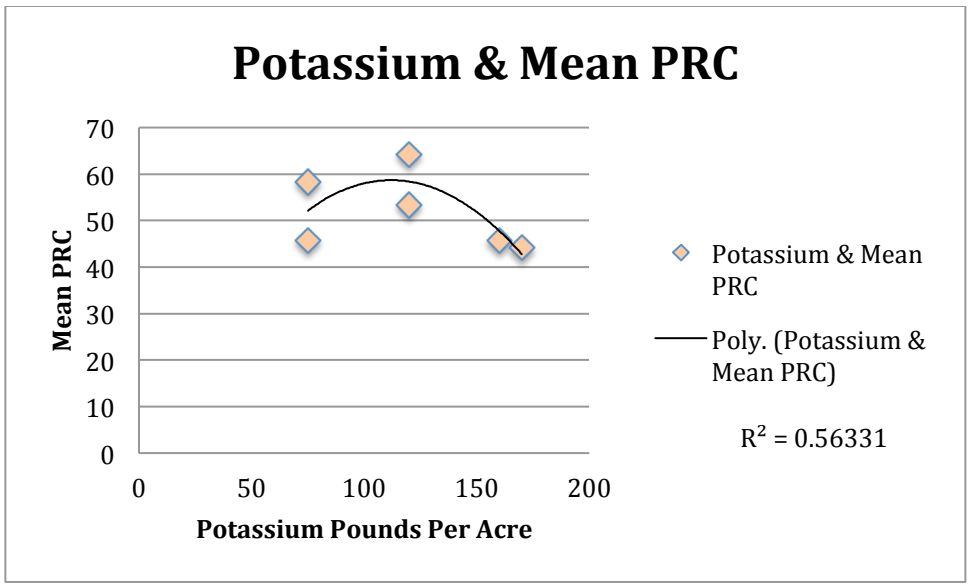

FIGURE 10. Mean PRC and potassium.

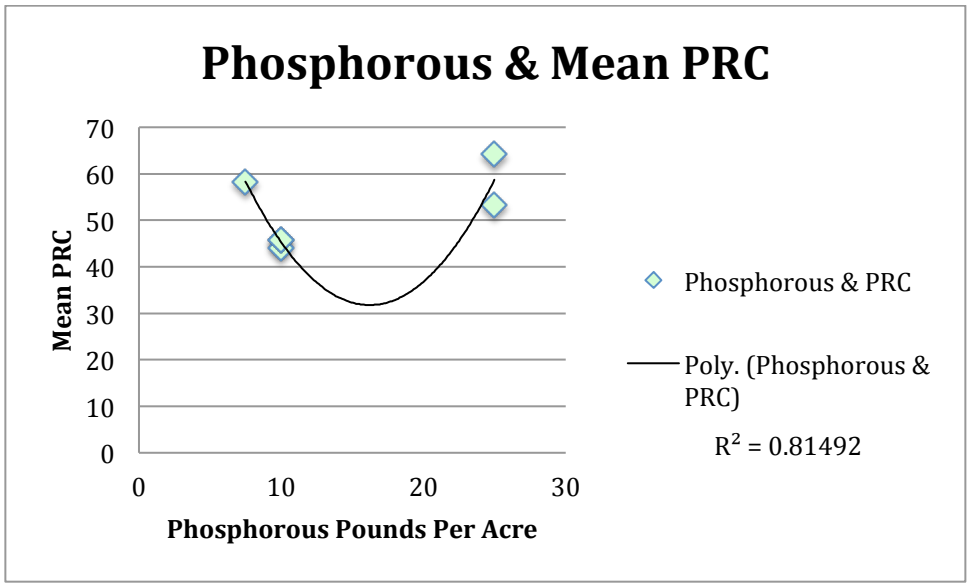

FIGURE 11. Mean PRC and phosphorous.

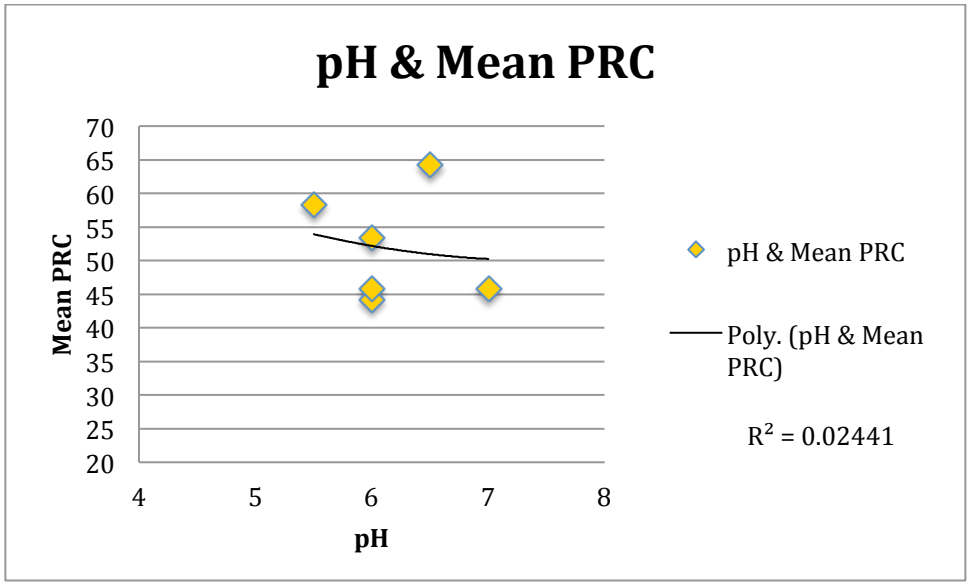

FIGURE 12. Mean PRC and pH.

Figure 13 and Table 5 (Appendix) summarize the results of a second-degree polynomial regression of change in mean PRC and max spore diversity along the elevation gradient. They appear to have a strong correlation with an R^2 of 0.79547 and a P-value of 0.084. Maximum spore diversity appears to decrease as PRC increases.

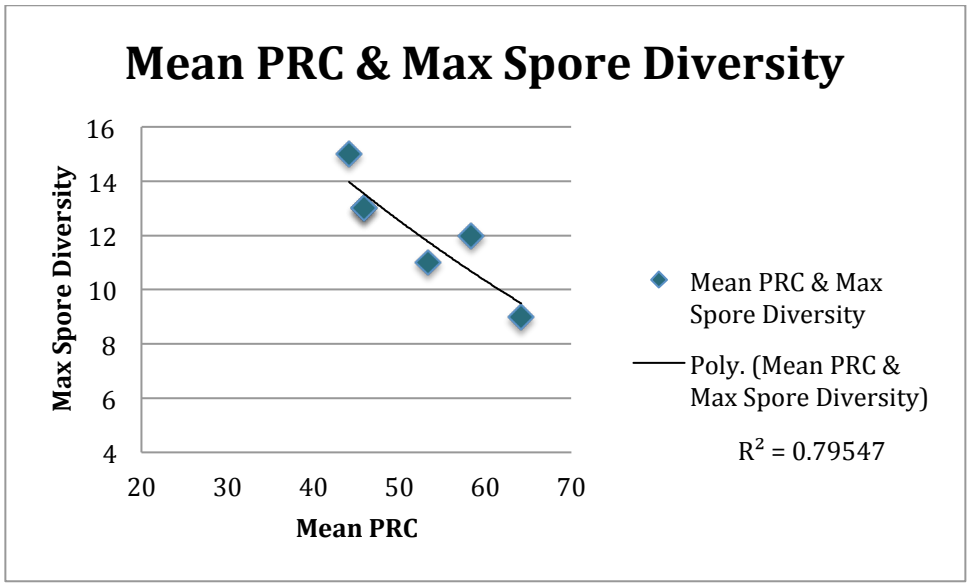

FIGURE 13. Mean PRC and max spore diversity.

AMF Spores

Table 3 summarizes the spore diversity found across the elevation gradient. At sub-transect one quadrat F had the high diversity of 9 and quadrats B and C with the lowest diversity of 8, with the high of 9 selected to represent the potential of the sub-transect. Sub-transect two had quadrats A and E with a high diversity of 11 and quadrats C and D with a low diversity of 9. The high quadrat for sub-transect three was quadrat D, with a diversity of 15 and the low quadrats A and C, with a diversity of 11. For sub-transect four, quadrat F with a diversity of 13 was the high and quadrat A, B, and D with a diversity of 11, were the low. Sub-transect five had quadrat B with a high diversity of 13 and quadrat D with a low diversity of 9. At sub-transect six quadrats A, B, and E had a high diversity of 12 while quadrats C and F both had the low diversity of 10.

Using the quadrat with the highest diversity counts to represent the highest potential for each transect, Figure 14 and Table 5 (Appendix) summarize the results of a

second-degree polynomial regression of spore diversity and elevation. An R^2 of 0.074 and a P-value of 0.130 suggests no significant correlation between spore diversity and elevation. Spore diversity appears to increase from low to middle elevations before beginning to decrease again at higher elevations. Sub-transect one has the lowest potential diversity with a diversity count of 9, sub-transect three has the highest potential diversity with a diversity count of 15. The highest elevation at sub-transect six (3,100 meters), has a potential diversity of 12, lower than the middle elevations but still higher than the two lower elevations, sub-transect one (600 meters, diversity count of 9) and sub-transect two (1,250 meters, diversity count of 11).

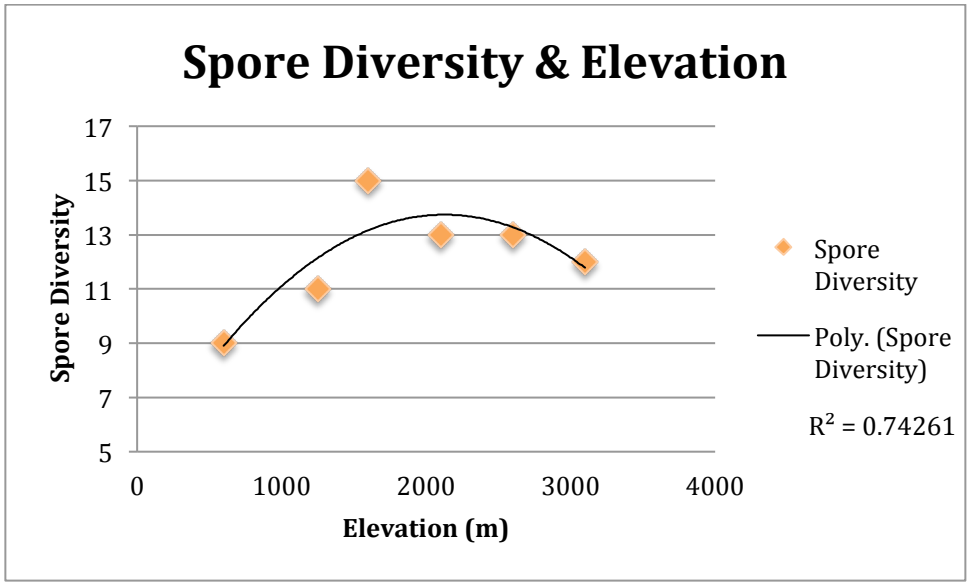

FIGURE 14. Spore diversity along the elevation mega-transect.

The second-degree polynomial regression results for max spore diversity and number of plant species can be seen in Figure 15 and Table 5 (Appendix). With an R^2 of 0.784 and a P-value of 0.100 max spore diversity and number of plant species appears to be weakly correlated. Spore diversity does, like vegetation diversity, display the

classically humped pattern, with higher diversity counts at middle elevations. However, spore diversity is more varied than the diversity of vegetation. Additionally, while vegetation diversity is lowest at the higher elevations, spore diversity is lowest at lower elevations.

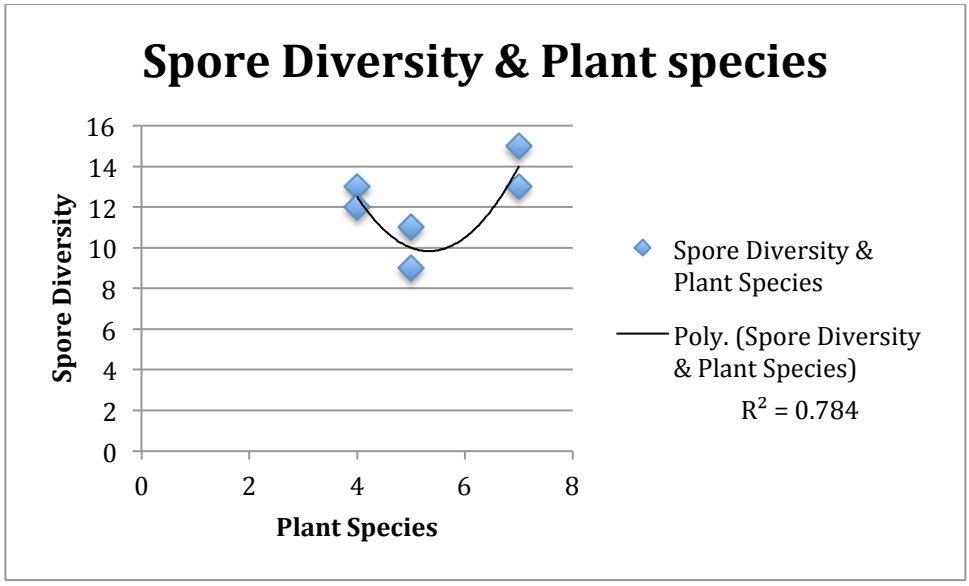

FIGURE 15. Spore diversity and number of plant species.

Figures 16 through 19 and Table 5 (Appendix) show the results of second-degree polynomial regressions of max spore diversity and nitrogen, potassium, phosphorous, and pH. There seems to be no significant correlation between spore diversity and nitrogen along the elevation gradient with an R^2 of 0.652 and a P-value of 0.205. With an R^2 of 0.0366 and a P-value 0.946, the correlation between pH and max spore diversity is not significant. Phosphorous also appears to be have no significant correlation with spore diversity with an R^2 of 0.776 and a P-value of 0.106. Potassium appears to be strongly correlated with max spore diversity, with an R^2 of 0.876 and a P-value of 0.043.

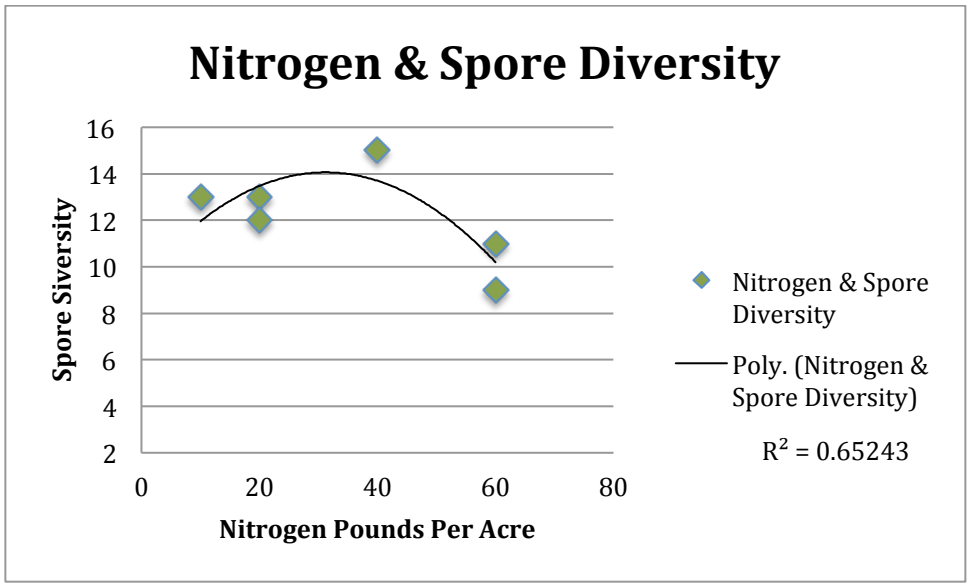

FIGURE 16. Nitrogen and spore diversity.

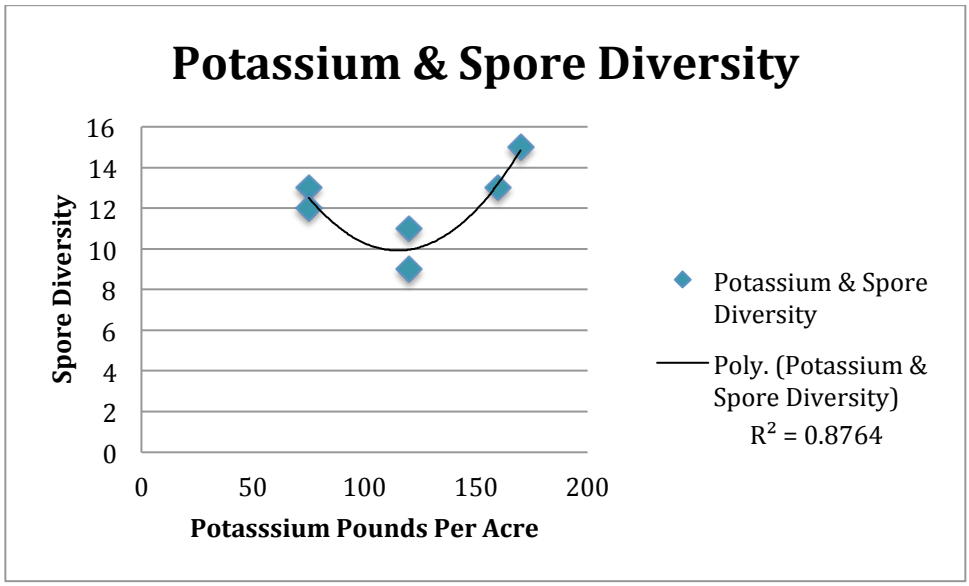

FIGURE 17. Potassium and spore diversity.

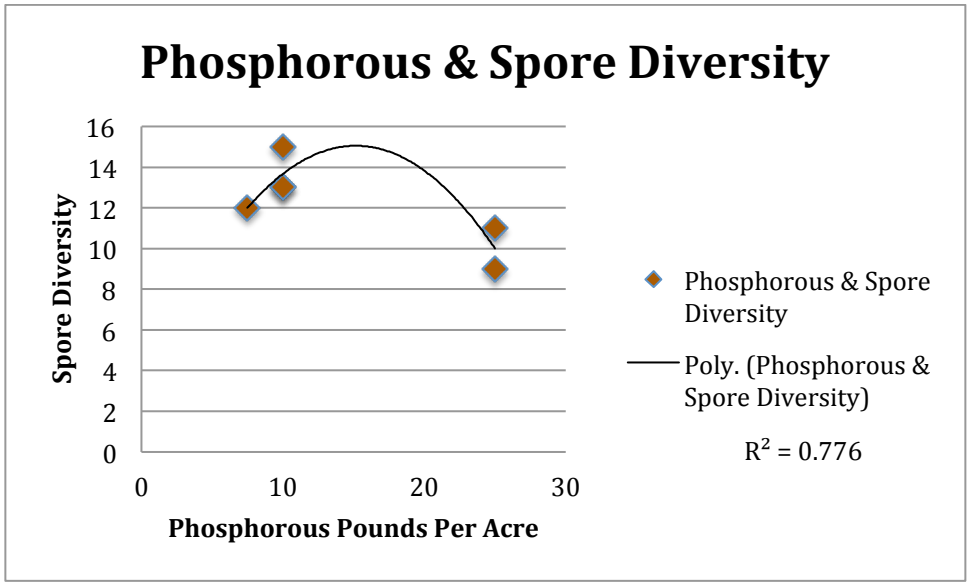

FIGURE 18. Phosphorous and spore diversity.

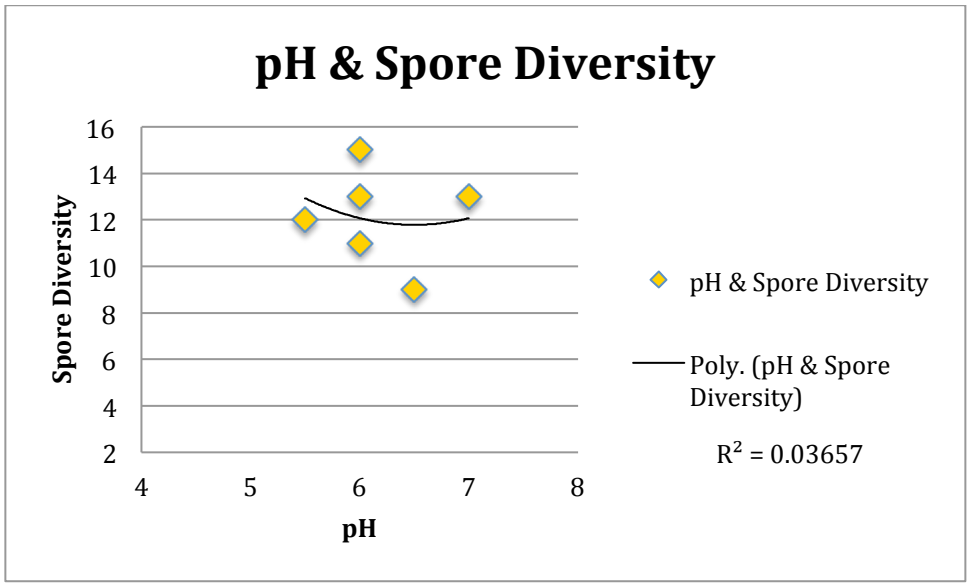

FIGURE 19. pH and spore diversity.

TABLE 4. Principal Component Analysis: Rotated Component Matrix

	Component 1	Component 2	Component 3
Elevation (m)	-0.900	-0.312	-0.233
N lb./ac.	0.966	0.217	-0.120
K lb./ac.	0.169	0.931	0.302
P lb./ac.	0.958	-0.110	0.107
pH	0.137	0.313	0.940
# Plant species	-0.028	0.926	0.348
Mean PRC	0.643	-0.579	-0.068
Max Spore Div.	-0.781	0.584	-0.191

Total Variance explained: Rotation Sums of Squared Loadings

Component	Eigenvalue	% Variance	Cumulative Var
1	3.753	41.700	41.700
2	2.754	30.603	72.303
3	2.099	23.323	95.626

Extraction Method: Principal Component Analysis.
Rotation Method: Quartimax with Kaiser Normalization.[a]
a. Rotation converged in 5 iterations

The results of the PCA can be seen in Table 4. The first component, accounts for 41.700 percent of the variance with an Eigenvalue of 3.753. This component could be viewed as the Below Ground Component, as it comprises primarily below ground factors. Elevation loads heavily on this component in the negative, as does maximum spore diversity. Nitrogen and phosphorous also load heavily but in the positive direction, together with PRC. The positive pole of this component groups together the root-AMF symbiosis and two limiting micro-nutrients, nitrogen and phosphorous. It is well established that AMF colonization facilitates the uptake of phosphorous (Smith et al. 2011). While root uptake of nitrogen is commonly understood to be facilitated by nitrogen-fixing Rhizobium bacteria, the role of AMF in enhancing nitrogen uptake is only recently being addressed (Miransari 2011). Component 2 explains 30.603 percent of the variance with an Eigenvalue of 2.754. This component could be called the Above

Ground/Potassium Component, as number of plant species loads heavily upon it in the positive at 0.926. Potassium also loads heavily on this component at 0.931. The final component accounts for 23.323 percent of the variance with an Eigenvalue of 2.099. This component could be called the pH Component, as pH is the only factor that loads on it at 0.940, its isolation on the third component expressing its irrelevance to understanding either aboveground or belowground diversity in the study area. Together, these three components account for 95.626 percent of the variance.

CHAPTER 5

DISCUSSION

AMF did indeed occur across the elevation gradient (600 meters to 3,100 meters) of San Jacinto Mountain in Southern California. AMF diversity and PRC also varied across the gradient. While my hypothesis that PRC would be greater at lower and higher elevations, reflecting the "harshness" of these environments and that AMF diversity would echo the "humped" diversity of vegetation across the gradient was confirmed, my analysis revealed a complexity of potentially responsible factors. Although PRC was strongly correlated with elevation, spore diversity was not. The diversity of above ground species does appear to play a moderate role in AMF diversity. However, it has no impact on PRC. Specific soil parameters appear to also have strong associations with certain biodiversity measures. Potassium proved significantly associated with maximum spore diversity and with aboveground plant diversity, while phosphorous was significantly related to mean PRC. Neither nitrogen, nor pH had any connection with mean PRC, maximum spore diversity, or aboveground plant diversity. Many studies, including those conducted by Allen (1991) and Bever et al. (2001), have found strong relationships between AMF and soil parameters. This mixed association with soil parameters also reflects some of the findings of studies performed in more controlled environments (Allen 1991; Simard et al. 2012). Additionally, there seems to be interplay between the diversity of AMF and PRC, where PRC appears to increase as spore

diversity decreases. This correlation hints at possible competition between AMF species for root colonization.

This chapter will discuss the results of my study on AMF along on elevation gradient in comparison with existing research and theory. Additionally, the chapter will include a discussion of findings of particular interest and possible avenues for further, or more focused, research.

Elevation and Aboveground Diversity

While Read (1983) suggested that as elevation increases, AMF associations would taper off or disappear, my findings indicate just the opposite. As Read suggested, AMF associations were strong at lower elevations; however, PRC increased again at higher elevations. Furthermore, spore diversity was in fact found to be higher at the upper elevations than it had been at the lowest (though spore diversity did peak in the middle elevations). It is, however, important to note the sub-transect at the lowest elevation consisted primarily of non-native species and sub-transect two displayed evidence of a burn (a natural disturbance regime within the chaparral ecosystem) although with considerable regrowth and mature plants. These findings, especially the trough-like pattern of PRC with elevation, may reflect the findings of Cripps and Eddington (2005), Sundqvist et al. (2011), Sturmer, Sturmer, and Pasqualini (2013), Yang, Chen, and Li (2008), and Shi (2007), that AMF are critical to plant establishment in more harsh environments, such as those found at the desert-like base of San Jacinto and at the sub-alpine zone.

Several studies along elevation gradients thus far have found that microbial communities vary across the gradient. How the communities varied, however, has been

inconsistent. This may partially reflect the communities assessed by the studies, such as Bryant et al. (2008) and Margesin et al. (2009) who found conflicting results in their studies of multiple microorganisms across elevations. Bryant et al. (2008) found a decrease in diversity with elevation, whereas Margesin and Miteva found an increase. However, like Bryant (2008), Gai et al.'s (2012) study that focused on AMF across an elevation gradient in Tibet found that both AMF and PRC decreased with elevation. My study contrasted with all three of these studies, finding instead that spore diversity neither increased nor decreased with elevation and instead displayed a hump-like distribution, similar to that of vegetation and reminiscent of the humped or bell-like curves of species abundance and environmental factors with an optimum zone and zones of increasing stress on either side of it. Perhaps these inconsistences suggest that despite my finding of what appears to be a strong correlation between PRC and elevation and of no correlation between spore diversity and elevation, that factors acting at the scale of an elevation gradient are too varied and "messy" to help elucidate belowground mechanisms. Although studies along elevation gradients provide the opportunity to sample from multiple environments in a relatively short distance, smaller scale studies within these environments might reveal fewer inconsistencies. There may also be other factors, such as geology, disturbance, and rainfall, for which I did not account, that may have some explanatory power. In juxtaposition with the inconsistencies with other *in situ* studies and these considerations, the findings of my study imply a need for more thorough and strategic studies of AMF diversity and PRC in relation to plant diversity and across a variety of environments.

Soil Parameters

Bever et al. estimated in their 2001 study of a one hectare agricultural field that as much as 30 percent of AMF distribution could be explained by soil parameters, such as nitrogen, phosphorous, and potassium. Although the mechanisms of nutrient transfer between AMF and plants are still poorly understood, many previous and subsequent studies have sought to elucidate the relationships between AMF and soil parameters (Allen 1991; Miransari 2011; Smith et al. 2011; Simard et al. 2012). Although the focus of this thesis was AMF diversity and PRC along a gradient and with relation to above ground diversity, the importance of soil parameters with regard to AMF and plant ecology was not overlooked. Along the elevation gradient in Southern California, some soil parameters were indeed found to correlate with PRC and the diversity of spores present. However, PRC and spore diversity appeared to have differing relationships with the soil parameters tested.

PRC

As mentioned above, PRC appeared to have no correlation with pH levels. More interestingly, the correlation between PRC and nitrogen was statistically non-existent. It is believed that AMF are responsible for aiding plants and nitrogen fixing bacteria, although the detailed role of AMF in this process is still poorly understood. Some have even hypothesized that AMF may enhance nitrogen within soil, although this is believed to be highly variable (Allen 1991). The small sample size of this study may be have influenced my findings. Conversely, other factors, such as lack of nitrogen fixing bacteria, the presence of ammonium as opposed to nitrogen (Allen 1991), or the influence

of other limiting micronutrients might be factors. It should also be noted that levels of nitrogen were higher at lower elevations where non-natives were more prevalent and evidence of a recent fire was present, both disturbances that have been found to impact PRC (Yao et al. 2008; Vogelsang and Bever 2009; Leckberg 2013).

As with nitrogen, the relationships between phosphorous and AMF has received considerable attention in previous studies (Allen 1991). Not only have AMF been found to aid plants in the uptake of phosphorous, there are indications that networks of AMF hyphae may be partially responsible for the distribution of phosphorous throughout a system, both by creating zones of depletion and zones of fertility and by affecting overall amounts of phosphorous in the system over time (Allen 1991). PRC appeared to have a trough-like relationship with phosphorous, where roots were more heavily colonized when phosphorous pounds per acre were low, less colonized at mid-levels of phosphorous and most heavily colonized when there were more pounds of phosphorous per acre. This could reflect the findings of Allen (1991) where AMF are believed to be involved in the development of "islands of fertility" around plants in arid regions (i.e. the harsh conditions of the subalpine region). Again, consideration of the conditions at lower elevations must be taken into account, as both non-natives and recent fire have been shown to adversely affect PRC (Yao et al 2008; Vogelsang and Bever 2009).

Potassium has received less attention in AMF studies, with estimates placing AMF contribution to 10 percent of plant uptake of potassium (Marschner and Dell 1994; Garcia and Zimmermann 2014). My study found no statistical relationship between potassium and PRC, perhaps indicating why there may be paucity of studies.

Spore Diversity

As previously stated, the relation of nitrogen and phosphorous to AMF maybe the most well understood facet of AMF ecology, albeit there are still many inconsistencies and unknowns in the findings (Allen 1991, Bever et al. 2001, Simard 2012). For example, Bever et al. (2001) found that some species were negatively associated with the presence of phosphorous while others were positively associated. Additionally, how AMF networks allocate these nutrients is still weakly understood (Miransari 2011). My findings suggested that there was no relationship between spore diversity and pH, spore diversity and nitrogen, and spore diversity and aboveground plant diversity along the San Jacinto elevation gradient. While the relationship found between spore diversity and phosphorous just missed statistical significance, this may be reflective of my small sample size. Conversely, it may indicate that, as Bever et al. (2001) suggests, specific species may be positively and negatively associated with phosphorus, where some species become more prevalent with higher concentrations of phosphorous while the reverse is true for other species. Without further investigation, perhaps looking at spore diversity and phosphorous at various scales, overtime, and/or in a more controlled setting, it is difficult to discern the intricacies of the nature of this relationship.

There appears to be a strong relationship between potassium and spore diversity. As mentioned previously, there is a considerable lack of studies on potassium and AMF and the relationship between AMF and this micronutrient may be the least understood (Marschner and Dell 1994; Garcia and Zimmermann 2014). My findings indicate that this lack may be detrimental to our understanding of AMF diversity. Garcia and

Zimmermann (2014) found that AMF improve plant absorption of potassium, especially when potassium is a limiting resource in the environment. Their findings, along with my own, indicate that the relationship between AMF and potassium may be more important than previously believed and that more in-depth, strategic studies of the relationship between potassium and AMF have the potential shed light on the subtleties of AMF contribution to plant growth and survival success.

As with PRC, the conditions found at sub-transect one and two must be taken into consideration with regard to spore diversity. However, it should also be noted that these conditions are not unusual, as fire is a natural disturbance regime associated with the area and invasive species are now, and will continue to be, part of the composition of ecosystems in Southern California. Although the impact of disturbances such as fire (found at the second sub-transect) on spore diversity is poorly understood, the majority of studies have found that the impacts of non-natives, especially grasses, such as those found at sub-transect one, have been shown to negatively impact spore diversity (Allen 1991; Vogelsang and Bever 2009). A notable exception is work done by Leckberg et al. (2013), where severe invasions of knapweed (*Centaurea stoebe*) and leafy spurge (Euphorbia esula) increased fungal abundance and diversity. However, their findings did not hold for cheat grass (*Bromus tectorum*). It is important to take into consideration that these factors may also be exerting an influence on spore diversity, as well as on PRC.

AMF Diversity and Percent Root Colonized

While AMF were historically thought to be functionally redundant, providing the same services to plants and filling the same niches, therefore resulting in a limited need for diversification, Bever et al. found, in their 2001 study, that AMF are not only

ecologically diverse with different species active in different seasons and aiding plants in different ways, there is considerable genetic diversity within species. This suggests that competition among species has resulted in diversification. Furthermore, studies finding inter-species competition also influence the extent and type of exchanges between AMF and plants (Allen 1991; Bever 2001; Yao et al. 2008; Martinez-Garcia and Pugnaire 2011). The strong relationship between PRC and spore diversity found during this study also might hint at how competition may be shaping belowground relationships. It appears that spore diversity and PRC are inversely related, with an increase in spore diversity corresponding to a decrease in the portion of roots colonized by AMF hyphae. This may indicate that as intra-species competition intensifies, the amount of hyphae infecting roots is reduced. The reduction could be due to AMF energies directed elsewhere (e.g., reproduction, increased external hyphae propagation due to increased competition for resources, etc.), to AMF and plant host species symbiosis becoming more specialized/specific, or to direct competition between AMF species resulting in the exclusion of species from colonization once one species has inoculated a host. Studies have found that AMF can provide protection to host plants by preventing infection from parasitic fungi and nematodes (Allen 1991; Kendrick 2000; Bever et al. 2001; Brundrett 2008; Simard 2012). Is it possible that AMF may also use these protection techniques to ward off inoculation by other AMF species? This inverse relationship between PRC and spore diversity found during my study and the possible implications it suggests certainly warrant further investigation.

CHAPTER 6

CONCLUSION

Some limitations of this study included the sample size, the duration of the study, and the limited region over which the study was conducted. Partially due to time constraints and partially due to the exploratory nature of this study, sampling was conducted during only one season, included only six elevation sub-transects on a western mountain slope, and comprised a limited number of samples. This study is, therefore, underpowered and findings of no significant difference could result from insufficient sample size or from an actual lack of association. Other factors that warrant more investigation are the relationship of rainfall, disturbance, and fire to AMF, as well as competition among AMF species and AMF other microorganisms.

In light of these limitations and the findings of this study, it is highly recommended that future studies be conducted in a variety of different locations, throughout the various seasons, that consider factors not explored herein (e.g., rainfall, fire, other disturb aces) and over a longer period of time. As previous studies have indicated (Allen 1991; Bever et al. 2001; Simard 2012), AMF activity and diversity varies throughout the year and over multiple years; thus, conducting studies that revisit sites through the seasons and over multiple years also have the potential to give us a much more detailed understanding of AMF ecology. Moreover, future comparisons of multiple mega-transects would have the potential to reveal if findings are consistent

across regions. For example, it would be interesting to compare the coastal side of a mountain range to its leeward side. Additionally, larger sample sizes and an increased number of sub-transects along a mega-transect would provide a more robust dataset and possibly indicate relationships more distinctly.

Despite the limitations of this study, it has managed to illuminate several interesting phenomena regarding AMF in Southern California. This study demonstrated that not only do AMF occur at higher elevations, they occurred consistently across the elevation gradient with an increase of activity (PRC) at higher and lower elevations, conforming with suggestions in previous studies, that AMF may be especially important to host plants in harsh environments. This thesis also corroborated the findings of other studies with regard to the importance certain soil parameters to the distribution of AMF and PRC, notably phosphorous and PRC, as well as potassium and diversity of AMF. Furthermore, the findings of this study illuminated several areas that could use more in-depth investigation. The finding of the relationship between potassium and diversity of AMF especially calls for further investigation, as previous studies are limited. The inverse relationship between PRC and spore diversity is also particularly interesting as it suggests the possible role of intra-species competition. Consequently, this study has the potential to serve as a baseline to help direct and inform future studies to further illuminate the complex ecology of AMF and the fungi's contribution to ecosystems structures and functions.

The relationships between AMF, vegetation patterns, and changes in elevation, as well as other environmental parameters are important to conservation and restoration efforts. Studies indicate that the distribution of AMF and the spatial relationships of these

fungi have a significant impact on above ground vegetation (Allen 1991; Bever 2001; Simard 2012), which is implied in this thesis' findings. To increase the success of conservation, and especially restoration efforts, a more thorough understanding of these complex relationships is necessary. As studies of AMF *in situ* become more numerous, their results have the potential to inform conservation and restoration practices greatly by bringing to light the diverse and multifarious nature of these fungi and their relationship to ecosystem structure and function. This study provided a glimpse of how the spore diversity and PRC of AMF change along an elevation gradient in Southern California with relation to above ground diversity and common soil parameters, and brought to light several areas requiring future investigation.

APPENDIX

TABLES

TABLE 5. Results of Second-Degree Polynomial Regressions

Mean PRC & #SPP			
a_1	-4.515	47.305	-64.900
S.E. a_1	2.993	33.430	88.931
R^2	0.578	6.789	
F	2.052	3.000	
$SS_{regression}$	189.119	138.256	
P	0.274		
Mean PRC & N			
a_1	0.009	-0.464	53.825
S.E. a_1	0.015	1.123	16.151
R^2	0.340	8.485	
F	0.773	3.000	
$SS_{regression}$	111.373	216.002	
p	0.536		
Mean PRC & K			
a_1	-0.005	1.062	-0.827
S.E. a_1	0.003	0.687	38.453
R^2	0.564	6.903	
F	1.935	3.000	
$SS_{regression}$	184.414	142.960	
P	0.288		
Mean PRC & P			
a_1	0.350	-11.341	123.717
S.E. a_1	0.124	4.246	28.786
R^2	0.815	4.494	
F	6.605	3.000	
$SS_{regression}$	266.784	60.590	
P	0.080		

TABLE 5. Continued

Mean PRC & pH			
a_1	1.025	-15.269	106.902
S.E. a_1	18.013	226.459	708.587
R^2	0.024	10.318	
F	0.038	3.000	
$SS_{regression}$	7.993	319.382	
p	0.964		
Max Spore & #SPP			
a_1	0.193	-1.584	0.000
S.E. a_1	0.073	0.625	0.000
R^2	0.784	1.225	
F	5.444	3.000	
$SS_{regression}$	16.333	4.500	
p	0.100		
Max Spore & N			
a_1	-0.004	0.244	8.331
S.E. a_1	0.001	0.089	1.280
R^2	0.652	0.672	
F	8.918	3.000	
$SS_{regression}$	8.061	1.356	
p	0.205		
Max Spore & K			
a_1	0.001	-0.250	23.552
S.E. a_1	0.000	0.061	3.392
R^2	0.876	0.609	
F	11.201	3.000	
$SS_{regression}$	8.304	1.112	
p	0.043		
Max Spore & P			
a_1	-0.018	0.469	8.664
S.E. a_1	0.024	0.824	5.586
R^2	0.776	0.872	
F	4.691	3.000	
$SS_{regression}$	7.135	2.282	
p	0.106		

TABLE 5. Continued

Max Spore & pH			
a_1	2.278	-28.889	101.704
S.E. a_1	2.782	34.971	109.423
R^2	0.037	1.593	
F	0.355	3.000	
$SS_{regression}$	1.800	7.616	
p	0.946		
Max Spore & Mean PRC			
a_1	-0.003	0.138	10.462
S.E. a_1	0.012	1.277	33.732
R^2	0.806	1.092	
F	2.449	3.000	
$SS_{regression}$	5.839	3.577	
P	0.084		

TABLE 6. Transect Data

Transect	1	2	3	4	5	6
Quadrat	A	E	B	D	A	F
Elevation (m)	600	1250	1600	2100	2600	3100
N lb./ac.	60.00	60.00	40.00	20.00	10.00	20.00
K lb./ac.	120.00	120.00	170.00	160.00	75.00	75.00
P lb./ac.	25.00	25.00	10.00	10.00	10.00	7.50
pH * 10	65.00	60.00	60.00	70.00	60.00	55.00
pH	6.50	6.00	6.00	7.00	6.00	5.50
# Spp	5.00	5.00	7.00	7.00	4.00	4.00
Mean PRC	64.17	53.33	44.17	45.83	45.83	58.33
Mean Spore Div	8.50	9.83	12.33	11.67	10.67	11.17
Max Spore Div	9.00	11.00	15.00	13.00	13.00	12.00

REFERENCES

REFERENCES

Alguacil, M.M., E. Lumini, A. Roldan, J.R. Salinas-Garcia, P. Bonfante, and V. Bianciotto. 2008. The impact of tillage practices on arbuscular mycorhizal fungal diversity in subtropical soils. *Ecological Applications* 18: 527-536.

Allen, M. F. 1991. *The ecology of mycorrhizae*. Cambridge: Cambridge University Press.

Allen, E.B., J.C. Chambers, K.F. Coonor, M.F. Allen, and R.W. Brown. 1987. Natural reestablishment of mycoorhizae in disturbed alpine ecosystems. *Artic Alpine Research* 19:11-20.

Bakker, E. 1984. *An island called California*. Oakland: University of California Press.

Bever, J. D., P.A. Schultz, A. Pringle, and J.B. Morton. 2001. Arbuscular mycorrhizal fungi: More diverse than meets the eye, and the ecological tale of why. *Bioscience* (51) 11: 923-933.

Brundett, M.C. 2008. Mycorrhizal Associations: The Web Resource. http://mycorrhizas info/info.html (last accessed 10 March 2013).

Bryant, J.A., C. Lamanna, H. Morlon, T. Kerkhoff, B. J. Enquist, and J.L. Green. 2008. Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. *Proceedings of the National Academy of Sciences* 105: 11505-11511.

Carle, D. 2010. *Introduction to earth, soil, and land in California*. Oakland: University of California Press.

Clements, F. 1916. *Plant succession: An analysis of the development of vegetation.* Washington: Carnegie Institute in Washington.

Cripps, C. L., and L.H. Eddington. 2005. Distribution of mycorrhizal types among alpine vascular plant families on the Beartooth Plateau, Rocky Mountains, U.S.A., in reference to large-scale patterns in arctic-alpine habitats. *Arctic, Antarctic, and Alpine Research* 37 (2): 177-188.

Erschbamer, B., T. Kiebacher, M. Mallaun, and P. Unterluggauer. 2009. Short-term signals of climate change along an altitudinal gradient in the South Alps. *Plant Ecology* 202: 79-89.

Fisher, M. A. and P.Z. Fulé. 2004. Changes in forest vegetation and arbuscular mycorrhizae along a steep elevation gradient in Arizona, *Forest Ecology and Management* 200 (25): 293-311.

Gai, J.P, H. Tian, F.Y. Yang, P. Christie, X.I. Li, and J.N. Klironomos. 2012. Arbuscular mycorrizal fungal diversity along a Tibetan elevation gradient. *Pedobiologia* 55: 145-151.

Garcia, K., and S.D. Zimmermann. 2014. The role of mycorrhizal associations in plant potassium nutrition. *Frontiers in Plant Science* 5: 337-338. doi:10.3389/fpls.20 14.00337

Gardes, M., and A. Dahlberg. 1996. Mycorrhizal diversity in arctic and alpine tundra: an open question. *New Phytologist* 133: 147-157.

Gerdemann, J. W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. *Annual Review of Phytopathology* 6: 397-418.

Gleason, H. A. 1917. The Structure and development of the plant association. *Bulletin of the Torrey Botanical Club* 43: 463-481.

Havert, B., and G. Gray. 1996. *Nature guide to the mountains of southern California by car and by foot*. Baldwin Park: Earth Trails Publications.

Hendricks, David M. 1985. *Arizona soils*. Tucson: University of Arizona, Centennial Publication of the College of Agriculture.

Humboldt, A. 1814. *Personal narrative of travels of the equinocial regions of the New Continent during years 1799-1804.* Vol. 1. London: Longman, Hurst, Rees, Orme, and Brown.

Johnson, N.C., T.E. O'Dell, and C.S. Bledsoe. 1999. Methods for ecological studies of mycorrhizae. In *Standard soil methods for long-term ecological research.* Ed. G.P. Robertson, D.C. Coleman, C.S. Bledsoe, and P. Sollin 378-406. Oxford: Oxford University Press.

Kendrick, B. 2000. *The fifth kingdom*. 3rd ed. Cambridge: Focus Publishing.

Koide, R.T., and B. Mosse. 2004. A history of research on arbuscular mycorrhiza. *Mycorrhiza*. 14: 145-163.

Koske, R.E., C.F. Friese, P.D. Olexia, and R. I. Hauke. 1985. Vesicular-arbuscular mycorrhizas. *Transactions of the British Mycological Society* 85: 350-353.

Lekberg, Y., S.M. Gibbons, S. Rosendahl, and P.W. Ramsey. 2013. Severe plant invasions can increase fungal abundance and diversity. *International Society for Microbial Ecology* 7: 1424-1433.

May, R. M. 1974. *Stability and complexity in model ecosystems*. Princeton: Princeton University Press.

May, R.M. 1981. Models for two interacting populations. *Theoretical Ecology, Principles and applications.* ed. R. M. May, pp.78-104. Sunderland: Sinauer Associates.

Margesin, R., M. Jud, D. Tscherko, and F. Schinner. 2009. Microbial communities and activities in alpine and subalpine soil. *Microbiology Ecology* 55: 705-713.

Marschner, H. and B. Dell. 1994. Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil* 159 (1): 89-102.

Martínez-García, L.B. and F.I. Pugnaire. 2011. Arbuscular mycorrhizal fungi host preference and site effects in two plant species in a semiarid environment, *Applied Soil Ecology* 48 (3): 313-317.

Miransari, M. 2011. Arbuscular mycorrhizal fungi and nitrogen uptake. *Archives of Microbiology* 193 (2): 77-81. doi: 10.1007/s00203-010-0657-6.

Moore, D., G. D. Robinson, and A.P. Trinci. 2011. *21st Century guidebook to fungi. Cambridge:* Cambridge University Press.

Myers, N., R.A. Mittermeier, C.G. Mittermeier, G.A. Da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403 (6772): 853-858. http://www.ncbi.nlm.nih.gov/pubmed/10706275 (last accessed 9 March 2013).

Oehl, F., E. Laczko, A. Bogenrieder, K. Stahr, R. Bösch, M. Heijden, and E. Sieverding. 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biology and Biochemistry* 42 (5): 724-738.

Pozo, M.J., and C. Azcon-Aguilar. 2007. Unraveling mycorrhiza-induced resistance. *Current Opinion in Plant Biology* 10: 393-398.

Read, DJ. 1983. The biology of mycorrhiza in the Ericales. *Canadian Journal of Botany*, 61: 985-1004.

Risser, P.G. 1995. The status of the science examining ecotones. *BioScience* 45 (5): 318-325.

Shi Z.Y., L.Y. Zhang, X.L. Li, G. Feng, C.Y. Tian, and P. Christie. 2007. Diversity of arbuscular mycorrhizal fungi associated with desert ephemerals in plant communities of Junggar Basin, northwest China. *Applied Soil Ecology* 35 (1): 10-20.

Simard, S. W., K.J. Beiler, M.A. Bingham, J.R. Deslippe, L.J. Phillip, and F.P. Teste. 2012. Mycorrhizal networks: Mechanisms, ecology and modeling. *Fungal Biology Reviews* 26: 39-60.

Smith, S. E., I. Jakobsen, M. Gronlund, and F.A. Smith. 2011. Roles of arbuscular mycorrhizas in plant phosphorous nutrition: Interactions between pathways of phosphorous uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorous acquisition. *Plant Physiology* 156 (3): 1050-1057.

Smith, S. E., and D.J. Read. 1997. *Mycorrhizal symbiosis*. 2nd ed. London: Academic Press.

Society for Ecological Restoration International Science & Policy Working Group. 2004. The SER international Primer on ecological restoration. Tucson: Society for Ecological Restoration International.

Sturmer, S. L., R. Sturmer, and D. Pasqualini. 2013. Taxonomic diversity and community structure of arbuscular mycorrhizal fungi in three maritime sand dunes in Santa Catarina State, south Brazil. *Fungal Ecology* 6: 27-36.

Sundqvist, M. K., R. Giesler, B.J. Graae, H. Wallander, E. Fogelberg, and D.A. Wardle. 2011. Interactive effects of vegetation type and elevation on aboveground and belowground properties in a subarctic tundra. *Oikos* 120 (1):128-142.

Trappe, J.M. 1987. Phologentic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint. In *Ecophysiology of VA mycorrhizal plants.* ed. G.R. Safir 5-25. Boca Raton: CRC Press.

Treseder, K. K. and A. Cross. 2006. Global distributions of arbuscular mycorrhizal fungi. *Ecosystems* 9: 305-316.

University of California Riverside. 2010. James San Jacinto Mountain Reserve. http:// james.ucnrs.org/field-station/ (last accessed 10 March 2013).

Vogelsang, K. M., and J.D. Bever. 2009. Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. *Ecology* 90 (2): 399-407.

Westman, W.E. 1981. Diversity relations and succession in Californian coastal sage scrub. *Ecology* 62 (1): 170-184.

Whipps, J.M. 2004. Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Canadian Journal of Botany* 82: 1198-1227.

Williamson, M. 1972. *The Analysis of Biological Populations*. London: Academic Press.

Yang, Y., Y. Chen, and W. Li. 2008. Arbuscular mycorrhizal fungi infection in desert riparian forest and its environmental implications: A case study in the lower reach of Tarim River. *Progress in Natural Science* 18 (10): 983-991.

Yao, Q., H. Zhu, Y. Hu, and L. Li. 2008. Differential influence of native and introduced arbuscular mycorrhizal fungi on growth of dominant and subordinate plants. *Plant Ecology* 196 (2): 261-268.