

## **Addition of Arbuscular Mycorrhizal Fungi to Seeds at Alta Vicente in Palos Verdes Peninsula**

Ahn E, Chiang J, Chui C, Varner M, Vaughn S, Wittenberg S

### **Abstract**

Coastal sage scrub (CSS) is a habitat characterized by drought-resistant shrubs found in Mediterranean-type coastal climates such as Southern California. Many of these habitats are threatened due to increased urban development, historic grazing and extensive farming, and thus the Palos Verdes Peninsula Land Conservancy (PVPLC) has begun to make efforts to repopulate the native species communities. Studies have shown that Arbuscular Mycorrhizal Fungi (AMF) displays positive effect to native species by extending the root surface, hence increasing the uptake of limiting resources, especially phosphorus and water. This prediction was tested by an experimental addition of AMF to the seed mix of a CSS restoration site located in Alta Vicente, Palos Verdes Peninsula. Limited field germination of the native seed mix occurred in the first spring, following a winter of above average rainfall and events of lower than average temperatures. Subsequent growing seasons may result in higher native germination, at which time the effect of AMF addition can be re-assessed. The application of AMF did not have an effect on non-native plants, except for sow thistle (*Sonchus oleraceus*), a non-native annual herb. Sow thistle abundance and cover were lower ( $p < 0.05$ ,  $n = 20$ ) with the addition of AMF.

## **Introduction**

Natural ecosystems are often threatened by the invasion of exotic and non-native plant species which have the ability to prevent the growth of native plant species of that particular ecosystem. CSS is one vegetative community found in Mediterranean climates that has been an important point of interest due to its fragmentation along the California coast (PVPLC 2011). CSS provides important habitat to endemic species. For example, the endangered Palos Verdes Blue butterfly (*Glaucopsyche lygdamus palosverdesensis*) rely solely on the CSS habitat throughout their life cycles. Males have been observed leaving their small patch of CSS only occasionally and females have never been observed leaving their home patch (Lipman 1999). This behavior speaks to the importance of restoring CSS habitat. Fragmentation of CSS habitat has been due to increased disturbance (PVPLC 2007) and consequent settlement of invasive plants such as mustards and grasses (PVPLC 2007). Currently, over 30% of the ecosystem is covered with non-native species, putting the native vegetation in danger of extinction (Bowler 2000). Different methods aimed at reducing the pervasion of invasive exotic plant species have been proposed by restoration ecologists across the world including alleopathy, herbivory, nitrogen and physical alterations, and competition limiters (St. John 1999).

A method commonly now used in CSS restoration is to add AMF into the soil to increase the competitive abilities and enhance the longevity of native plants. Much of the AMF network in Southern California that formed naturally in the past has been destroyed because of human land exploitation. As a result, competitive advantage may be given to non-mycorrhizal or facultative exotics since many disturbed landscapes that were formerly CSS have been colonized by non-native plant communities.

CSS is an ecologically important habitat for a variety of species besides the Palos Verdes Blue Butterfly, including the endangered Cactus Wren (*Campylorhynchus brunneicapillus*) (U.S. Fish and Wildlife Services 2011). Fortunately, with proper re-vegetation techniques in addition to the re-establishment of mycorrhizal networks, native species can potentially out-compete these invasive grasses and return to a similar historic ecological composition. Returning sites to their former ecological compositions may take multiple seasons, because the presence or absence of AMF can change entire community compositions and succession patterns (Janos 1980) causing large-scale shifts from non-mycorrhizal communities in nutrient rich soils to native mycorrhizal communities in nutrient poor soils. Therefore, AMF determines which community type will have a competitive advantage and therefore dominate. This dominance is hard to change as AMF presence pushes the succession to native, mutualistic species, and the lack of AMF relationships causes the AMF to dissipate as they are no longer receiving the benefits of fixated carbon from host plants. In either situation, more than one growing season is required since there is very little chance of the non-dominate community to suddenly become dominate as they are inherently outcompeted by the specific physical conditions.

Thus, our study focuses on the efforts of the Palos Verdes Peninsula Land Conservancy (PVPLC) whose goal is to conserve and restore several open space preserves scattered across the Palos Verdes Peninsula. Their acquired lands were originally composed of rich and diverse coastal sage scrub communities, but now have been replaced in many areas by dominant annual grasses and forbs. Additionally, the previously fertile soil has become severely degraded due to extensive agricultural practices that restoration techniques such as traditional weeding are costly, labor

intensive, time consuming and generally ineffective in the long-term. Therefore, we studied the effectiveness of integrating AMF as an agent to help the re-colonization of native plant communities across an experimental site. The following literature review explores the practical aspects of implementing AMF with respect to developing resistance to invasive and exotic plant species as well as restoring the former coastal sage scrub habitat.

### Arbuscular Mycorrhizal Fungi

Mycorrhiza is the symbiotic relationship between fungi and roots of vascular plants (Harrison 2005) and is found in connection with over 80% of terrestrial plants (Rinaudo *et al.* 2010). By looking at fossil records, we gather that this symbiosis evolved around 400 million years ago and played an important role for the initial colonization of terrestrial plants as well as the evolution of vascular plants (Harrison 2005). The fungi colonize the roots of host plants as endomycorrhiza or ectomycorrhiza. AMF, the focus of this paper, colonize intracellularly by invaginating the cell membrane of root plant cells (Harrison 2005). Mycorrhizal fungi are ubiquitous among the ecosystems that we are studying, with the exception of barren lands due to the low vegetation cover (Allen *et al.* 2003; Greipsson and El-Mayas 1999; Harnett and Wilson 2002; Jayachandran and Fisher 2008).

Though this association is generally mutualistic, parasitism has also been observed in these relationships (Francis and Read 1994; Smith *et al.* 1998). Mutualism is a term in which both species benefit, but their dependence on the relationship varies (Goodwin 1992). Facultative plant species can survive without mycorrhizae while lower fitness may be observed, but obligate plant species require it to survive and reproduce at all. AMF are obligate mycotrophs as the host plants are their only source of carbon, whereas host plants can be facultative or obligate mycotrophs, depending on species relationships (Smith *et al.* 1998). AMF can benefit host plants mainly by increasing their micro and macro nutrient uptake because they increase the root surface area for absorption (Goodwin 1992). Furthermore, AMF can aid host plants by increasing their resistance to disease and toxicity (Chaudhary and Griswold 2001; Greipsson and El-Mayas 1999; Hartnett and Wilson 2002) as well as increasing their chances of colonizing nutrient poor soils (Rinaudo *et al.* 2010; Smith *et al.* 1998; White *et al.* 2008).

Incorporating mycorrhizal fungi in ecosystem restorations have proven useful, namely by increasing plant diversity and ridding lands of invasive exotic species (St. John 1998). The aim to inoculating the soil with AMF is to establish an extended root system that can stabilize the soil and provide structural support for late succession plants (Riefner *et al.* 1998). Furthermore, it minimizes erosion by providing continuous vegetative cover (Estuan *et al.* 2007). AMF can also affect species diversity in an ecosystem (Hartnett and Wilson 2002; Allen *et al.* 2003); in the beginning of the restoration process, the habitat is usually dominated by a few species of weeds, but after the addition of AMF, plant species diversify thus allowing more native plants to establish (Jordan *et al.* 2000). However, on the other hand, AMF can also lower species diversity in the long run by allowing stronger obligate mycotrophs to excel and outcompete other plants (Smith *et al.* 1998). Through this process, AMF can alter plant species composition, favoring obligate mycotrophs over facultative or non-mycotrophs. As previously mentioned, native plants are usually obligate mycotrophs while weeds are not,

hence, the addition of AMF can help take out invasive, exotic species (Greipsson and El-Mayas 1999; Hartnett and Wilson 2002). By giving an edge to obligate native mycotrophs and increasing competition for non-mycotrophic invasive exotics, AMF can also influence the rate of succession in these ecosystems (Allen *et al.* 2003; Estauin *et al.* 2007; Riefner *et al.* 1998).

In California, AMF inoculation at restoration sites has helped resist invasive exotic such as Russian thistle (*Salsola tragus*) (Chaudhary and Griswold 2001) and black mustard (*Brassica nigra*) (Riefner *et al.* 1998) as demonstrated at San Onfre state beach where researchers attempted to restore a sage scrub habitat (Riefner *et al.* 1998). The short-term solution included adding organic mulch on the surface of the soil to temporarily take up the nutrients, which prevented weedy species from re-invading. This organic material then decomposed and released the nutrients back into the soil for future plant growth. The long-term approach, though, was to implement a mycorrhizal network that connects to plants' roots (same species and different plant species) and have continuous growing vegetation cover over the site. To successfully implement a mycorrhizal network, (Riefner *et al.* 1998) the researchers used land imprinting because of its low cost and effectiveness if the imprints are of good quality. Imprinting over dead organic mulch and placing commercial inoculum with native seeds in the root zone sets the basis of establishing a mycorrhizal network (St. John 1998; Riefner *et al.* 1998). The seeds they chose were local, mycorrhizal net-builders, such as California brome (*Bromus carinatus*), golden yarrow (*Eriophyllum confertiflorum*), bush sunflower (*Encelia californica*) and coast goldenbush (*Isocoma menziesii*), that germinates and grows rapidly (St. John 1998). With the right host plants, it creates a strong mycorrhizal network that allows plants to take up all the nutrients in the soil, further decreasing the chance of weedy species re-invading and/or increasing the competition for weedy seeds already in place in the soil (Riefner *et al.* 1998). The San Onfre beach site before restoration was a field of continuous black mustard cover, but now consists of mainly native bunchgrass (*Festuca idahoensis*), coastal sage shrub, California buckwheat (*Eriogonum fasciculatum*) and many other native species. (Chaudhary and Griswold 2001; Riefner *et al.* 1998).

### Environmental Variance and Its Effect on AMF

#### *Pre-site Conditions and Season Length*

Disturbance causes suppression of mycorrhizal fungi and without the presence of host plants, it will eventually die out (Chaudhary and Griswold 2001; Goodwin 1992; Jayachandran and Fisher 2008). The level and intensity of disturbances (e.g. tree fall or wildfire) can affect the abundance and diversity of fungal species, which will eventually affect plant growth (Allen *et al.* 2003). Species composition can be altered such that large spored fungi are found more abundantly in undisturbed sites. Ecosystems with high-intensity or frequent disturbances are categorized by low density and diversity of fungal spores (Jayachandran and Fisher 2008), as well as little to no vegetative cover (Chaudhary and Griswold 2001; Smith *et al.* 1998). Moreover, AMF spores are dispersed by the wind and subsequently trapped by vegetation (Goodwin 1992) so the level of recruitment will also decrease with decreasing vegetation cover. During ecosystem restoration, natural reestablishment of AMF in disturbed sites is a slow process (Chaudhary and Griswold 2001); therefore, plant diversity will suffer in the first

few years because plants require a minimal AMF level (Allen *et al.* 2003). Following this, early and late successional fungi will have variable effects on plant growth, with respect to different plant species at different successional stages (Allen *et al.* 2003). Therefore, it is recommended to add AMF early in the restoration process to elude these problems (Chaudhary and Griswold 2001; Vátovec and Huerd 2005). This encourages reestablishment of mycorrhizal plants early on, generally native species, and provides a disadvantage to nonmycorrhizal plants, usually non-native (St. John 1999; Goodwin 1992).

Natives plants are already more difficult to grow in short amounts of time and thus the presence of weeds greatly increases their growing time—it could take longer than 3 years if conditions are not optimal for native growth, and factors such as sunshine, rainfall, and anthropogenic disturbance could elongate the growth time as well (Skousen and Venable 2007). These variables and any deviations from the customary microclimate will likely exacerbate the capability of native plant growth and lengthen the timespan for growth to occur (Skousen and Venable 2007). Although two to three years is an average time for native plant growth to occur during restoration, this could be extended due to inefficient removal of weeds and uncharacteristic microclimate, so appropriate management practices are essential for future restoration success (Skousen and Venable 2007).

### *AMF and Plant Species Correlation*

Especially relevant to the practical aspect in restoration ecology, there are major distinctions between native and invasive plant species, all of which have variable responses to different AMF inocula. Exotic plant species tend to be annual crops with roots that grow over 1 cm per day and have a lifespan of weeks to months while native species are often perennial crops with roots that grow less than 1 mm per day and live for several years. Though the roots of perennial plants are more developed than those of annuals allowing greater structural support and protection from drought, they also require much more nutrients to fulfill their production costs (Brundrett 2002). These differences enable fast-growing weedy plants to invade and thrive in ecosystems that they are foreign to but, fortunately, most of them do not form mycorrhizal relationships. Unlike these highly adaptable exotic species, the functioning of newly introduced AMF and associated native plant communities varies depending on the environment (Ji *et al.* 2010). The movement of AMF inocula into non-native environments causes changes in its own spore and taxonomic compositions and often results in less infectivity of new host plant species. Conversely, the mycorrhizal dependency of plants on native AMF inocula increases phosphorus uptake and plant colonization compared to the same plant species incorporating foreign AMF inocula in their roots (Yao *et al.* 2007). Thus, even though AMF are not entirely host-specific, the tendency of native plant species to work best with and obtain the most benefits from other native AMF species suggests some degree of plant-AMF specificity and that their mutual development in local habitats affect the resulting plant communities. Overall, there have been many case studies where mycorrhizal associations benefit native plants and help resist invasive exotic species (Goodwin 1992; Hartnett and Wilson 2002; Jayachandran and Fisher 2008; Pringle and Bever 2008). These associations differ with every plant family, varying in degree and dependency (Goodwin 1992; Hartnett and Wilson 2002). As mentioned before, obligate mycotrophs are very dependent on these mycorrhizal associations, and without them, their chance of establishment is greatly reduced. Facultative and non-mycotrophs can survive without this association, and often, the presence of AMF with mycorrhizal plants will increase their competition and reduce their fitness (Goodwin 1992; St. John 1999). The degree to which mycorrhiza can help host plants is dependent on the plant's life history and its root structure (Hartnett and Wilson 2002). Furthermore, there is high fungal specificity with plant species, so certain species of fungi can benefit some more than others (Allen *et al.* 2003; Jayachandran and Fisher 2008; Pringle and Bever 2008; Smith *et al.* 1998).

There are several types of AMF inocula used in ecosystem restoration, and they differ in their compatibility with plant species as well as fungal diversity. Natural inoculum comes in the form of topsoil and it usually consists of numerous species of fungi (Jayachandran and Fisher 2008). Commercial inoculum can be bought in large amounts and it is more practical when there is a limited time or lack of storage space, but usually contains only one type of fungi (Chaudhary and Griswold 2001). Lab-produced inocula are produced from specifications of local inoculum (White *et al.* 2008), but it cannot be used if there is a large demand because of the cost ineffectiveness. Some experiments show that natural inoculum, especially local topsoil, contain the greatest number of fungal species, therefore diluting the problem of host specificity (Chaudhary and Griswold 2001; Jayachandran and Fisher 2008); however, some experiments found

that there were no difference between the three different types of AMF inoculum, all produce a varying level of positive results (Rinaudo *et al.* 2010; White *et al.* 2008).

### *Rainfall Patterns*

Coastal sage scrub which is composed of vegetation from both chaparral and desert climates requires very low levels of precipitation (Padgett *et al.* 2000). Increased rainfall or addition of irrigation systems can lead to fleeting successes but does not allow the proper drought resistant adaptations necessary for species to be successful in the long-term (Poole and Miller 1975). Increase in non-native growth occurs in ecosystems with this increase in water as the drought-adapted natives are outcompeted by non-native species.

Beauchamp *et al.* (2006) illustrated a similar situation of the effect on AMF in flooded environment. The paper discussed wetlands, a low oxygen level environment which is similar to water-logged soil after heavy rainfall. AMF are aerobes and they need oxygen for respiration (Beauchamp, Stromberg and Stutz 2006). Despite its disadvantage in anoxic condition, some species that require less oxygen manage to colonize wetland plant species and acquire oxygen directly through plant roots (Cornwell, Bedford and Chapin 2001) but AMF is less seen in flooded condition like wetland. However, since California has seasonal heavy rainfall, AMF may help maintain species richness by colonizing dicotyledonous plant roots and help acquire phosphorus during drier seasons ((Beauchamp, Stromberg and Stutz 2006).

In respect to our studies, the southern California coast received higher than average rainfall throughout our experiment, which likely affected the growth of the non-native species by enabling them to utilize the abundance of water to their advantage (Poole and Miller 1975). Since they are inherently rapid-growing species, the non-native weeds are able to outcompete the native species by using the water as an instrumental growth tool (Padgett *et al.* 1999). This increased and unforeseen precipitation present during the duration of our habitat restoration project proves a larger than expected influence on our frequency and aerial percent cover recorded in the field. The large increase in water available had the potential to discount any help that the AMF might have had if the resources provided for the species were limited and the AMF was able to extend natives' roots in order to obtain these thought-to-be limited resources (Padgett *et al.* 2000).

Thus, climate and rainfall patterns had to be considered in our study. These factors, as well as other natural variability such as presence of herbivores and micro-scale environmental occurrences may have played a significant role in the low numbers of native plant recruitment and success. Given that herbivores in Mediterranean climates tend to eat plants that are wetter, the native plants growth could have suffered due to herbivory in tandem with the excessive amount of rainfall this growing season in this scenario (Padgett *et al.* 2000). In drier years, herbivory may not pose such a problem, but this topic must be studied as a separate variable in order to delineate this as truth.

### Management Practices

There are multiple ways to add AMF inocula, all of which aim to successfully establish a mycorrhizal network in the soil (Riefner *et al.* 1998). Seeds are generally applied simultaneously with AMF inoculum to ensure their proximity (White *et al.* 2008). One of the earlier methods used to establish fungi involved using inoculated container plants which were also planted on the site (Chaudhary and Griswold 2001). Modern techniques, though, directly inoculate sites, which include hand dug trenches, land imprinting, broadcasting and hydroseeding. These techniques vary in efficiency as well as the area it can inoculate. Broadcasting involves overlaying the AMF inoculums over the soil and this is a viable option when the landscape is not suitable for the use of machines and equipments (White *et al.* 2008). Hydroseeding is the process of hydraulically spraying a mixture of seeds, fertilizers, mulches and AMF inoculums across the restoration site, (Chaudhary and Griswold 2001) which is actually very efficient and allows treatment to large areas (Estaun *et al.* 2007). However, these two methods are surface soil treatments which expose the inoculum to factors such as weathering and grazing that will increase mortality of the seedlings. Although hand dug trenches place the seedlings and AMF inoculum into the soil, a heavy rainstorm could melt the soil into a crusted pile (Riefner *et al.* 1998; White *et al.* 2008). The most successful technique is land imprinting as it is cost effective and establishes a strong mycorrhizal network in a short time (St. John 1999). The imprinter is heavy roller that places the AMF inoculum into the soil and presses the seeds into small imprint holes that can store water which will infiltrate through the lower layers. It also puts the seeds in close proximity to the soil (with the inoculum) which allows for faster germination (Riefner *et al.* 1998). While it cannot cover as large an area as hydroseeding, it is a relatively efficient use of currently available equipment.

### Seed Viability

When storing seeds for either a long or short period of time, the viability of the seeds is very important to consider especially when using them for a specific purpose such as vegetation restoration. A seed's viability is its likelihood of germinating and growing into an adult plant once it is planted, and every species of plant has a different natural longevity depending on what kind of conditions it has been evolved to withstand (Ellis and Roberts 1980). The problem is that each seed's viability is difficult to predict so whether the seed will be able to germinate or not after storage is also usually unknown (Ellis and Roberts 1980).

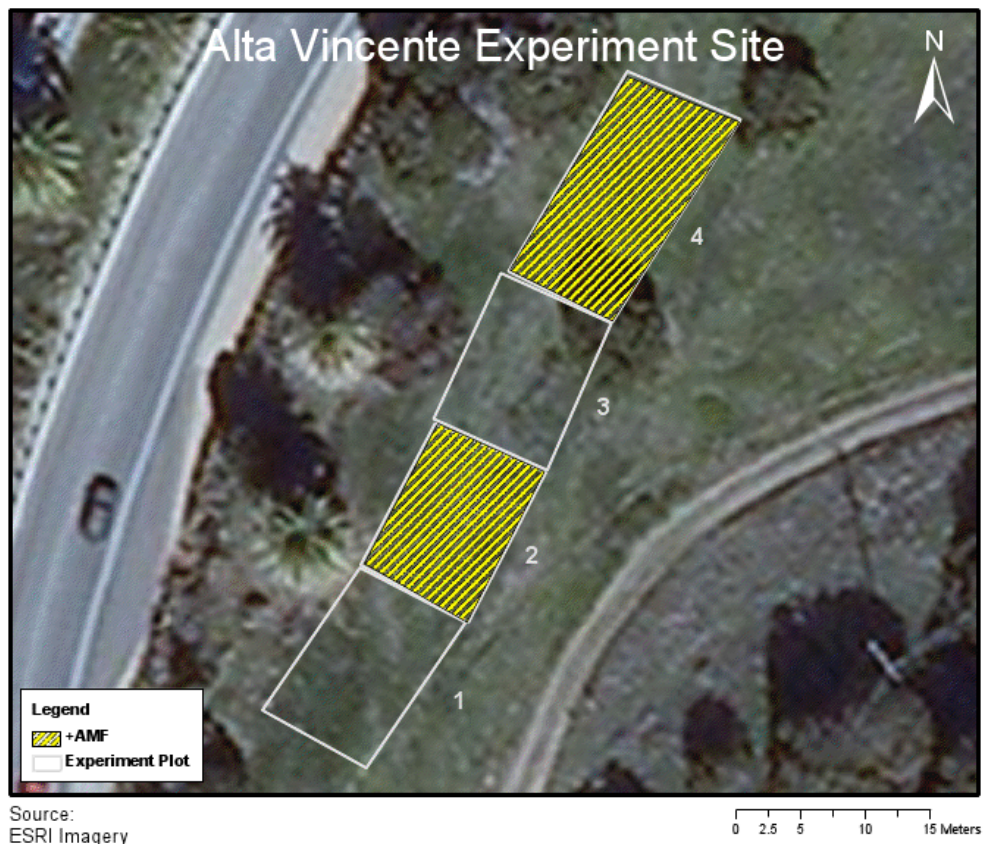


## Methodology

### Experimental Design

We conducted our AMF experiment at Alta Vicente, which is located on the south-west corner of the Palos Verdes peninsula in Los Angeles County, California. The experimental site has a dimension of 9.14m x 57.30m (30 ft x 41ft). The area is further divided into 4 plots; plots 1, 2 and 3 have a dimension 9.14m x 12.50m (30 ft x 41 ft).

Plot 4 has a dimension of 9.45m x 18.90m (31 ft x 61 ft 11 in) (Figure 1). For consistency in sampling methods, we measured plot 4 to 9.45m x 13m (31 ft x 42 ft 7.8 in). Each plot was separated with orange flags and white string. We have a total of 2 true replicates (n=2) for the experiment, which is common for agricultural experiments. Plots 1 and 3 are our control plots with no AMF inoculation (-AMF). Plots 2 and 4 have AMF inoculation and are the manipulative treatment (+AMF). There are 10 feet buffer zones between each plot that we did not collect data from to minimize errors in the experiment results. See Table A-5 in Appendix A for seed mix composition.



**Figure 1.** Diagram of Alta Vicente Field Experiment Layout.

### PVPLC Management

The PVPLC weed-wacked Alta Vicente the last week of December and then installed the AMF/seed mix at the site during the first week of January 2011. In the nursery, the PVPLC waters the plants on an as needed basis.

### Pseudoreplication:

We collected 10 randomly selected samples from each plot using 0.5m x 0.5m quadrats. Using string, the quadrats were further divided into 4 X 4 segments giving a total of 16 0.125m X 0.125m squares within each. This allowed for easier estimation and increased accuracy of percent cover within each pseudoreplicate. A total of 20 -AMF and 20 +AMF samples were collected yielding 20 pseudoreplicates (n=20). Although the number of true replicates in this experiment were few, the number of pseudoreplicates is efficient enough to have a strong and representative result as it is difficult to set up a large number of true replicates in agricultural and restoration experiments as there are scale and practical limitations (Hurlbert 1984). We can treat the pseudoreplicates as true replicates because we are assuming heterogeneous landscape across our experimental site, therefore establishing variable environmental conditions and significant differences between samples (Graham 2010).

### Field Sampling Methods

We collected our data on the afternoon of Friday April 15, 2011. We took note of both aerial cover and frequency described below. We then input our data into prepared spreadsheets to be analyzed.

#### I. Aerial cover:

At each pseudoreplicate, we observed and estimated the percent of vegetative cover of the occurring native and non-native plant species within each quadrat as well as accounting for bare ground. The percentage cover was determined by looking down over each pseudoreplicate and estimating the percent cover of each plant species that fell within the top layer of vegetation. Plant cover that fell below the top layer of vegetation was not accounted for in aerial cover in order to keep aerial percent cover to 100%.

#### II. Frequency:

Plant frequency was calculated by directly looking at and counting the stem or shoot, which was erected directly out of the soil, of each plant that fell completely within each quadrat that was placed at each pseudoreplicate. In order for a plant to be counted for frequency within each sample, the entire stem of the plant had to fall within the boundaries of each quadrat.

### Seed bank Frequency Sampling:

After the soil was seeded at Alta Vicente, we collected 40 soil samples in order to get a better idea of what seeds, from the mix and in the natural seed bank, are housed in the soil. We collected one sample from each pseudoreplicate, and cultivated them in a nursery. This allowed seeds that were in the soil to germinate and grow revealing the potential species of plants that we might see growing at our field site. We were also able

to compare and contrast our field results with our seed bank results to expose possible differences in species composition.

In order to see what kind of germination could actually occur with our seed mix, a seed bank maintained at a separate location—the nursery—with different management practices from our own experimental site. Nurseries present optimal growing conditions for plants and serve as a location for plant propagation. In the Palos Verdes Peninsula Land Conservancy, the nursery is monitored meticulously with biweekly watering and protection from herbivory. Our seed bank, which comprises samples adjacent to our real experimental plots, was set up in late January after the Alta Vicente project began so the observed growth is lesser in quantity and age so almost all the germinated plants we identified were seedlings. It is located on a military base with limited access in the San Pedro region, 10 miles east of our experimental site, which we are able to check up on because we are from an academic institution.

Each pseudoreplicate had a corresponding seed bank sample that was taken during the third week of March. The samples were collected on either left and right or top and bottom sides and 2cm away from the quadrat. To ensure consistency in volume of the seed bank samples, we used bucket augers to extract the soil. Each sample was 8 cm deep and 8 cm wide, and they were transported to the nursery the next day. The samples then were transferred from plastic bags into 25.4cm x 25.4cm (10in x 10in) trays that were lined with a thin layer of barren soil. The nursery staff looked after the seed bank and watered whenever the soil was dry. Two trays were filled with just the barren soil and served as controls for the seed bank samples. Every Friday morning we observed what was growing in the seed bank sample trays. Once seedlings were identified, they were recorded and removed from the tray. The final data from the seed bank was taken on the morning of Friday April 22, 2011.

#### Statistical Analysis Methodology

We applied natural log transformation [ $1+\ln(x)$ ] to achieve normal distribution with the frequency data, when applicable, so that we could run a T-test and generate P-values. We also performed Mann-Whitney U Tests with untransformed data on cover, individual species and native vs. non-native for both +AMF and -AMF treatments.

## **Results**

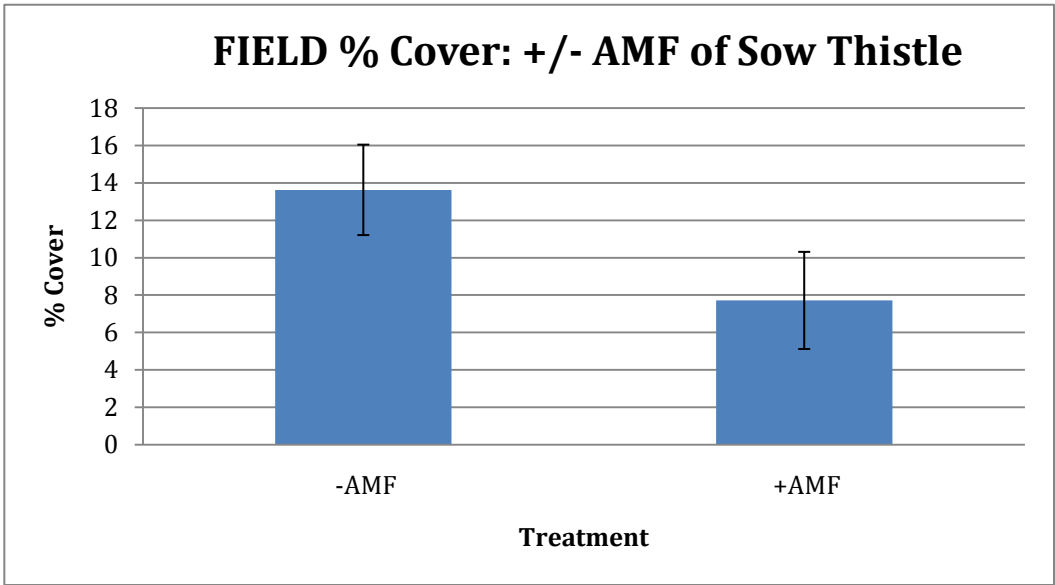
Prior to our field experiment, Palos Verdes Peninsula Land Conservancy performed the site preparation. The Alta Vicente site was portioned into four sections of similar area. The site experienced heavy rains at the end of the fall season into early winter. Grasses and other non-natives with quick germination were then removed by weed-whacking. The litter was raked into piles, but not removed from the study site. The seed mix was then applied the first week of January.

Native species were not common in our field experiment with only lupine (*Lupinus succulentus*) and cholla (*Cylindropuntia fulgida*) occurring in our treatment and control plots. However, cholla cannot be accounted for as it was planted prior the experiment hence it was not considered in this analysis. Lupine occurred infrequently, with 15 total seedlings from -AMF treatment plots and 40 total seedlings from +AMF treatment plots. Lupine's occurrence was not limited to our experimental plots as it was observed growing naturally elsewhere in Alta Vicente, hence its presence and germination may not be due to the seed mix applied from the land conservancy, but rather from the natural, preexisting seed bank of the site. In the field, the % aerial cover was higher than the frequency count in both +/-AMF treatment plots, making lupine more abundant than common (See Appendix, Tables A-1 and A-2). Due to the low germination from the seed mix applied and infrequent occurrences of natives in the samples, it proves difficult to evaluate the effect of the treatment.

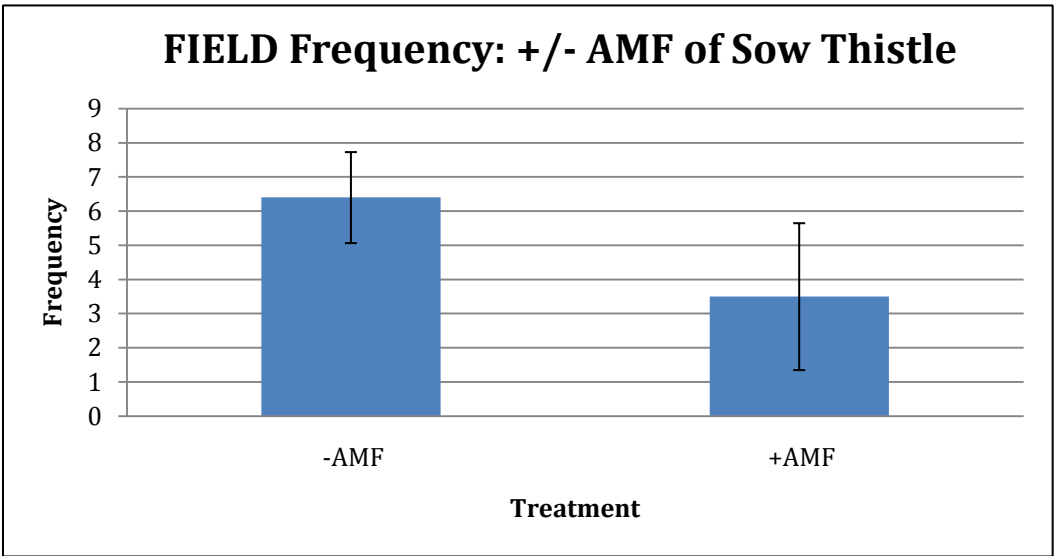
Though certain factors such as temperature and moisture content in storage conditions have shown to have strong correlations to seed viability, the most practical method to determine the viability of the seeds in our mix is to send in a sample to a lab where it can be grown in controlled conditions, and then see the resulting germination (Ellis and Roberts 1980). After this, we can deduce which seeds simply were not viable and relate it back to our collected data. Since we could not incorporate this test in our study though, it could be a possible explanation for the lack of native seed growth at our experimental site and also an improvement for future studies on restoration projects involving AMF.

There were more native species germinating in seed bank samples grown in the nursery, but they also occurred in low frequency. The species that occurred were lupine, California brittlebush (*Encelia californica*), and cliff buckwheat (*Eriogonum parvifolium*). The latter two were not seen in the field plots. In the +AMF treatment plot, both cliff buckwheat and lupine had a frequency of 1 and in the -AMF treatment plots, California brittlebrush had a frequency of 2, Cliff buckwheat had a frequency of 5, and lupine had a frequency of 1. These values were the actual counted values for all 20 samples. There were no significant differences between the two treatments due to the low germination rates of the native species.

There was no significant difference of the bristly ox tongue (*Picris echinoides*) in any part of our experiment, but there was a significant difference between field treatments ( $p < 0.05$ ,  $n = 20$ ) for both % aerial cover and frequency of sow thistle (Figure 2 and 3). There was less sow thistle germination in the +AMF treatment plots and those that did germinate had a lower overall cover.



**Figure 2:** Sow Thistle Field % Aerial Cover +/- AMF



**Figure 3:** Sow thistle Field Frequency +/- AMF

## **Discussion**

The lack of germination of the native restoration seed mix may be due to a variety of factors, including the remaining leaf litter from the mechanical mowing or weed-wacking of the site that was not removed. This lack of litter removal may have decreased soil-seed contact when the restoration seed mix was applied. Second, the seed mix was applied in January which is late for the growing season. It would have been more effective to sew the seeds into the soil in October at the beginning of the first rains that occur in the fall and early winter. Third, the seed mix should have been tested for seed viability to ensure that the seeds are actually equipped for germination, yet the same seed mix was used in the PVPLC Nursery so this may not have been a dilemma. Fourth, the study may have benefited if there were an increase in seed bulk rates so that there would be enough seeds to ensure high target germination rates in the restoration area at Alta Vicente.

There was some native germination observed in the seed bank although it was infrequent, and it is not definite whether this is from the applied seed mix or whether it is from the latent seed bank from the Alta Vicente site since PVPLC added the seed mix sample before we collected our sample. The nursery may provide more optimal growing conditions than the Alta Vicente site, including better soil aeration and watering practices compared to the field, which has erratic rainfall and was not irrigated. The better growing conditions in the nursery may have been responsible for the nursery's higher species diversity as well. This means that regular watering and shading had a beneficial effect on the plants that came up in the nursery. Also, the nursery seedbank pulled soil from the depth of 0-28 cm, which was then spread out and given a chance to see sunlight and receive more water than if it had stayed deeper within the soil. This may have allowed for some latent native seeds' germination in the nursery. The top 3 species that germinated in the field (Bristly ox-tongue, Sow thistle and Lupine) was not the most abundant species in the seed mix, which were *Artemisia californica*, *Lotus scoparius* and *Eriogonum parvifolium* (by weight).

Another reason native seeds applied had a low germination rate was because the seeds may not be viable. Furthermore, there are different successional stages for AMF, where it is characterized by low native diversity in the beginning but increases over the years (Allen *et al.* 2003). Changes in AMF species compositions affects their relationship with different native plants, syncing with different plant successional stages. Therefore when the AMF network grows stronger and more complex, it can increase the chances of mycorrhizal plant germination and establishment. Hence, we may see increased germination and cover of native mycorrhizal plants with a longer study time relative to the AMF and plant successional stages.

In relation, failure of native species to successfully outcompete non-natives could also be attributed to the fact that they have not yet had adequate time to grow. Since our study ended in the spring, many of the seeds may not have yet reached their optimal germination time, and will possibly thrive during the following summer months when the weather is warmer and the climate is drier. In a previous study done on increasing recruitment of native plant species, cover by seeded natives was close to zero at all experimental sites until after the third growing season (Skousen and Venable 2007). It also illustrated how native plants mandate a much longer timespan to successfully grow because approximately three years for the native species to comprise 25% of the site.

Although native species' growth will be much slower and more deliberate, they do have the growth capacity to eventually displace non-natives over a longer period of time (in the range of six to seven years). This particular study also found that partial or complete removal of established non-natives species was necessary and vital to the future development and success of the slower-growing native species (Skousen and Venable 2007) and that failure to remove weeds on a consistent basis will decrease native growth and its ability to flourish. Any weeds that reside in the area will outcompete natives at any level of growth, and even seedlings of weeds can interfere with the success of natives, thus the removal of weeds is critical to a more successful outcome for native plant growth (Skousen and Venable 2007).

The above average rainfall seen along the peninsula during the duration of our experiment may have another factor that influenced our field results. As previously mentioned in Beauchamp, Stromberg and Stutz's study, heavy water additions can result in a decrease of oxygen in the soil and increase in phosphorus. AMF requires oxygen to be present in the soil to survive the reduction of oxygen alone could have proved detrimental to AMF growth. And when soil are fertile, or have high levels of nutrients such as phosphorus, AMF produces less of a benefit to mycorrhizal plants (Janos 1980; Cornwell *et al.* 2001). Beyond simply being unnecessary, Reynolds *et al.* (2005) discusses the shift of AMF to a parasitic organism in such conditions, which may be the mechanism behind the observed decrease in sow thistle in the +AMF treatment plot. With the above average rainfall in winter 2010/2011, they may have been an increase in soil nutrients and decrease of drought conditions. In such conditions, AMF associations are no longer as beneficial for mycorrhizal plants, allowing fast settling and fast growing non-natives to outcompete CSS native species.

Monocotyledoneous plants are mostly less mycorrhizal than dicotyledoneous plants. The extensive aerenchyma in monocotyledoneous plants give rise to effective transportation of oxygen to roots. Addition of oxygen to flooded environment resulted from heavy rainfall promotes mineralization of phosphorus in soil (Beauchamp, Stromberg and Stutz, 2006). Increase in phosphorus concentrations leads to less AMF colonization in monocotyledoneous plant roots (Cornwell, Bedford and Chapin, 2001). Dicotyledoneous plants, which are usually phosphorus limited, have an advantage of associating with AMF in flooded environments since AMF increases phosphorus availability in soil, but likewise suffer a disadvantage in anoxic and water-logged soil (resulting from heavy rainfall or poor watering practices) because reduced oxygen levels available to AMF hinders their colonization (Beauchamp, Stromberg and Stutz, 2006).

This information provides insight for the PVPLC which should act to implement non-native removal practices on a regular basis in order to effectively increase the cover of the natives. Previous studies have elucidated that having any existing plant cover hinders the potential growth of native species, and that natives flourish under conditions where litter and bareground constitute most of the growing site because competition with non-natives is not a disturbing variable (Skousen and Venable 2007). They have also shown that rainfall conditions and the lengths of growing seasons are also important variables that may hinder expected and desired results.

### Management Practices

PVPLC should adopt management practices that include raking up the litter after weed-whacking prior to seeding because the leftover litter decreases the seed-soil contact and prevents the seed from embedding into the soil hence lowering its germination rate. Furthermore, the seed mix should be added in early October coinciding with the last fall/first winter rains rather than seeding in late December/early January. Lastly, the seed mix should be tested for its viability to ensure high germination rates under optimal conditions.

Researchers should also delineate management practices to best serve the goals of the Palos Verdes Peninsula Land Conservancy, including weed-wacking prior to installing the AMF/seed mix into the soil. The weed-whacking practice is harsh and can create further disturbance that can render the research methods ineffective because it can mechanically wound the soil (Cornell 1999). PVPLC did not yet implement a regular irrigation schedule which could potentially lead to more abundance of native plants in the first season to extend the growing season. If a season presents very little rain, these irrigation practices may be needed, yet if substantial or excessive rainfall occurs, these practices may not be as necessary. Given the fact that this year yielded an extremely uncharacteristically large volume of rainfall, there was little need for additional irrigation, but it must be noted that the rainfall or cold temperatures may have dampened the success of native plant growth. It may take an additional year or two in order to produce significant amounts of native vegetation. As stated in the discussion, excessive rainfall has been shown to actually increase the weedy species' competitive advantage over natives, causing native plants to suffer in growth due to its inherently slower germination and growth rates.

### AMF Interactions

More research on the interactions between specific host plants and AMF should be employed to garner a better understanding of particular mycotrophic relationships. Since there are a variety of mycotrophic relationships, including facultative and obligate, and an immense number of native plant species, there are potentially hundreds of different combinations of ideal interactions which are not yet known. This knowledge could transform the land conservancy's research success and enable scientists to redirect their focus to the right kind of interaction for each native species. Very little research on individual host/fungal interactions has been done, and this information is invaluable to the field of restoration biology and the future success of native plant conscription.



## Works Cited

- Allen E.B., Allen M.F., Egerton-Warburton L., Corkidi L., Gomez-Pompa A., 2003. Impacts of early- and late seral mycorrhizae during restoration in seasonal tropical forest, Mexico. *Ecol App* 13:1701-1717.
- Allen M.F., Allen E.B., Gomez-Pompa A., 2005. Effects of mycorrhizae and nontarget organisms on restoration of a seasonal tropical forest in Quintana Roo, Mexico: factors limiting tree establishment. *Restor Ecol* 13:325-333.
- Allen, E., Padgett, P., Bytnerowics, A., Minnich, R., 1999. Nitrogen deposition effects on coastal sage vegetation of southern California. *Proceedings of the international symposium on air pollution and climate change effects on forest ecosystems*. 131-139.
- Addy, H.D., Bowsell, E.P., Koide, R.D., 1998. Low temperature acclimation and freezing resistance of extraradical VA mycorrhizal hyphae. *Mycological Research*. 102, 582-586.
- Bary F., Gange A., Crane M., Hagley, K.J., 2010. Fungicide levels in golf putting greens and relations with arbuscular mycorrhizal fungi. *Ecosystems Environ* 31, 328-331.
- Bray S.R., Kitajima K., Sylvia D.M., 2003. Mycorrhizae differentially alter growth, physiology, and competitive ability of an invasive shrub. *Ecological Applications* 13, 565-574.
- Beauchamp, V.B., Stromberg, J.C., Stutz, J.C., 2006. Arbuscular mycorrhizal fungi associated with populus-salix stands in a semiarid riparian ecosystem. *New Phytologist*. 170 (2), 369-379.
- Cornwell, W.K., Bedford, B.L., Chapin, C.T., 2001. Occurrence of arbuscular mycorrhizal fungi in a phosphorus-poor wetland and mycorrhizal response to phosphorus fertilization. *American Journal of Botany*. October 88 (10), 1824-1829.
- Carey E.V., Marler, M.J., Callaway, R.M., 2004. Mycorrhizae transfer carbon from a native grass to an invasive weed: evidence from stable isotopes and physiology. *Plant Ecol* 172, 133-141.
- Chaudhary B., Margot G., 2001. Mycorrhizal fungi – a restoration practitioner’s point of view. *Ecesis*: 1-4.
- Clapp J.P., Young, J.P.W., Merryweather, J.W., Fitter A.H., 1995. Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. *New Phytologist* 130, 259-265.
- California Invasive Plant Council. 2006. Protect California’s natural areas from wildland weeds. *Cal-IPC News*. Summer 14 (2).

- Cornwell, W. K., B. L. Bedford and C. T. Chapin. 2001. Occurrence of Arbuscular Mycorrhizal Fungi in a Phosphorus-Poor Wetland and Mycorrhizal Response to Phosphorus Fertilization. *American Journal of Botany*. October 88 (10): 1824-1829.
- Ellis, R.H. and Roberts, E.H. (1980) Improved equations for the prediction of seed longevity. *Annals of Botany* 45, 13-30.
- Estaun V., Vincente S., Calvet C., Camprubi A., Busquets M. Integration of arbuscular mycorrhiza inoculation in hydroseeding technology: effects on plant growth and inter-species competition. *Land Degrad Develop* 18, 621-630.
- Eliason, S.A., Allen, E.B., 1997. Exotic grass competition in suppressing native shrubland re-establishment. *Restoration Ecology* 5, 245-255.
- Egerton-Warburton, L. M., Allen, E.B., 2000. Shifts in arbuscular mycorrhizal communities along anthropogenic nitrogen deposition gradient. *Ecological Applications*. April 10 (2), 484-496.
- Egerton-Warburton, L. M., Graham, R.C., Allen, E.B., Allen, M.F., 2002. Reconstruction of the historical changes in mycorrhizal fungal communities under anthropogenic nitrogen deposition. *Proceedings: Biological Sciences*. December 268 (1484), 2479-2484.
- Ferguson J., Rathinasabapathi B., 2003. Alleopathy: how plants suppress other plants. *Hort Sci* 19, 1-3.
- Francis, R., Read, D.J., 1994. The contributions of mycorrhizal fungi to the determination of plant community structure. *Plant Soil* 159, 11-25.
- Giasson, P., Karam, A., Jaouich, A., 2008. Arbuscular mycorrhizae and alleviation of soil stresses on plant growth. *Mycorrhiza* 8, 99-134
- Goodwin, J., 1992. The role of mycorrhizal fungi in competitive interactions among native bunchgrasses and alien weeds: a review and synthesis. *Northwest Sci* 66, 251-260.
- Greipsson, S., El-Mayas, H., 1999. Large scale reclamation of barren lands in Iceland by aerial seeding. *Land Degrad Develop* 10, 185-193.
- Gavito, M. E., Olsson, P.A., Rouhier, H., Medina-Peñafiel, A., Jakobsen, I., Bago, A., Azcón-Aguilar, C., 2005. Temperature constraints on the growth and functioning of root organ cultures with arbuscular mycorrhizal fungi. *New Phytologist*. October 168 (1), 179-188.
- Harrisson, M.J., 2005. Signaling in the arbuscular mycorrhizal symbiosis. *Microbiology* 59, 19-47.

Hartnett, D., Wilson, G., 2002. The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. *Plant Soil* 244, 319-331.

Janos, David P. 1980. Mycorrhizae Influence Tropical Succession. *Biotropica* 12: 56-64

Jayachandran, K., Fisher, J., 2008. Arbuscular mycorrhizae and their role in plant restoration in native ecosystem. *Mycorrhiza* 8, 195-209.

Jordan, N.R., Zhang, J., Huerd, S., 2000. Arbuscular-mycorrhizal fungi: potential roles in weed management. *Weed Research* 40, 397-410.

Johnson, N. C., Graham, J.H., Smith, F.A., 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist*. April 135 (4), 575-586.

Kawaguchi, M., Minamisawa, K., 2010. Plant-microbe communications for symbiosis. *Oxford Journals Plant Cell Physiology*. 51 (9), 1377-1380.

Kothari, S.K., Marschner, H., Romheld, V., 1991. Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil. *Plant Soil* 131, 177-185.

Kardol, P., Van der Wal, A., Bezemer, M., Boer, W., Duyts, H., Holtkamp, R., Putten, W., 2008. Restoration of species-rich grasslands on ex-arable land: Seed addition outweighs soil fertility reduction. *Biological Conservation* 141, 2208-2217.

Miller, R.M., 1987. The ecology of vesicular-arbuscular mycorrhizae in grass- and shrublands. In: Allen MF, ed. *Mycorrhizal functioning: an integrative plant-fungal process*. New York: Chapman and Hall 438-467.

McArthur, D. A. J., Knowles, N.R., 1993. Influence of vesicular-arbuscular mycorrhizal fungi on the response of potato to phosphorus deficiency. *Plant Physiology*. January 101 (1), 147-160.

Pendelton, R.L., Pendelton, B.K., 2003. Soil microorganisms affect survival and growth of shrubs grown in competition with cheatgrass. *Ecol Restor* 21, 215-218.

Pringle, A., Bever, J.D., 2008. Analogous effects of arbuscular mycorrhizal fungi in the laboratory and North Carolina field. *New Phytol* 180, 162-175.

Padgett, P., Allen, E., 1999. Differential response to nitrogen fertilization in native shrubs and exotic annuals common to Mediterranean coastal sage scrub of California. *Plant Ecology* 144 93-101.

Padgett, P., Kee, S., and Allen, E. 2000. The Effects of Irrigation on Revegetation of Semi-Arid Coastal Sage Scrub in Southern California. *Environmental Management*. Vol. 26, No. 4, Pg. 427-435.

Palos Verdes Peninsula Land Conservancy. 2007. Habitat Restoration Plan for Alta Vicente Ecological Reserve in the Portuguese Bend Nature Preserve for the Rancho Palos Verdes Natural Community Conservation Plan and Habitat Conservation Plan. Pg 9 –14.

Paulitz, T. C., Menge, J.A., 1984. Is *spizellomyces punctatum* a parasite or saprophyte of vesicular-arbuscular mycorrhizal fungi? *Mycologia*. January-February 76 (1), 99-107.

Reynolds, H. L., Hartley, A.E., Vogelsang, A. M., Bever, J.D., Schultz, P.A., 2005. Arbuscular mycorrhizal fungi do not enhance nitrogen acquisition and growth of old-field perennials under low nitrogen supply in glasshouse culture. *New Phytologist*. September 167 (3), 869-880.

Roberts, K. J., Anderson, R.C., 2001. Effect of garlic mustard [*alliaria petiolata* (Beib. Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. *American Midland Naturalist*. July 146 (1), 146-152.

Riefner, R., Pryor, D., St. John, T., 1998. Restoration at San Onofre state beach, California. *Land and Water* Jul/Aug:15-18.

Rinaudo, V., Barberi, P., Giovannetti, M., Van der Heijden, M., 2010. Mycorrhizal fungi suppress aggressive agricultural weeds. *Plant Soil* 222, 7-20.

Skousen, J.G., Venable, C.L. 2007. Establishing Native Plants on Newly-Constructed and Older-Reclaimed Site Along Highways. *Wiley Interscience* 2-8

Staddon, P. L., Heinemeyer, A. Fitter, A.H., 2002. Mycorrhizas and global environmental change: research at different scales. *Plant and Soil*. 244, 253-261.

Solaiman, Z.M.,P., Blackwell, L.K. Abbott, Storer, P., 2010. Direct and residual effect of biochar application on mycorrhizal root colonisation, growth and nutrition of wheat. *Australian Journal of Soil Research*. 48 (7), 546 -554.

Smith, M.R., Charvat, I., Jacobson, R.L., 1998. Arbuscular mycorrhizae promote establishment of prairie species in a tallgrass prairie restoration. *Can J Bot* 76, 1947-1954.

St. John, T., 1998. Mycorrhizal inoculation in habitat restoration. *Land and Water* Sept/Oct:17-19

St. John, T., 1999. Nitrate immobilization and the mycorrhizal network for control of exotic ruderals. *CalEPPC News* 6, 4-11.

Stejskalova, H., 1989. The role of mycorrhizal infection in weed crop interactions. *Agric Ecosystems Environ* 29, 415-419.

Santos, J.C., Finlay, R.D., Tehler, A., 2006. Molecular analysis of arbuscular mycorrhizal fungi colonising a semi-natural grassland along a fertilisation gradient. *New Phytologist* 172, 159-168.

Schwab, S.M., Menge, J.A., Tinker, P.B., 1991. Regulation of nutrient transfer between host and fungus in vesicular-arbuscular mycorrhizas. *New Phytologist* 117, 387-398.

U.S. Fish and Wildlife Services. 2011. Species Report. [Online] (Updated 15 February 2011) Available at: <http://www.fws.gov/endangered/>

Vatovec, C., Jordan, N., Huerd, S., 2005. Responsiveness of certain agronomic weed species to arbuscular mycorrhizal fungi. *Renewable Agricul and Food Sys* 20, 181-189.

Van Aarle, I. M., Olsson, P.A., Söderström, B., 2002. Arbuscular mycorrhizal fungi respond to the substrate pH of their extraradical mycelium by altered rowth and root colonization. *New Phytologist*. July 155 (1), 173-182.

White, J., Tallaksen, J., Charvat, I., 2008. The effects of arbuscular mycorrhizal fungal inoculation at a roadside prairie restoration site. *Mycologia* 100, 6-11.

## Appendix A

**Table A-1:** Field Percent aerial cover of species by treatment

Species	Native/Non-native	Annual/Perennial	Herb/Shrub	AMF+ Avg. +/- SE	AMF- Avg. +/- SE
Bristly ox tongue	Non-native	Annual	Herb	32.50 +/- 7.56	41.58 +/- 6.53
Sow thistle	Non-native	Annual	Herb	7.72 +/- 2.60	13.63 +/- 2.42
Wild oats	Non-native	Annual	Herb	3.39 +/- 2.65	2.12 +/- 1.01
Lupine	Native	Annual	Herb	11.65 +/- 5.91	4.06 +/- 2.75
Bur clover	Non-native	Annual	Herb	0.25 +/- 0.06	0.25 +/- 0.18
Spring vetch	Non-native	Annual	Herb	0.00 +/- 0.00	0.06 +/- 0.06
Cheeseweed	Non-native	Annual	Herb	0.25 +/- 0.25	0.00 +/- 0.00
Spiny sow thistle	Non-native	Annual	Herb	1.28 +/- 0.95	0.00 +/- 0.00
Rip gut brome	Non-native	Annual	Herb	0.94 +/- 0.70	0.08 +/- 0.08
Pineapple weed	Non-native	Annual	Herb	0.00 +/- 0.00	0.00 +/- 0.00
False Brome	Non-native	Annual	Herb	0.16 +/- 0.16	0.00 +/- 0.00
Coastal Heron's Bill	Non-native	Annual	Herb	0.09 +/- 0.09	0.00 +/- 0.00
Black Mustard	Non-native	Annual	Herb	0.00 +/- 0.00	0.47 +/- 0.47

**Table A-2:** Field frequency of species by treatment

Species	Native/Non-native	Annual/Perennial	Herb/Shrub	AMF+ Avg.+/- SE	AMF- Avg.+/- SE
Bristly ox tongue	Non-native	Annual	Herb	8.60 +/- 4.06	17.50 +/-7.35
Sow thistle	Non-native	Annual	Herb	3.50 +/- 2.15	6.40 +/- 1.33
Wild oats	Non-native	Annual	Herb	0.15 +/- 0.11	0.70 +/- 0.26
Lupine	Native	Annual	Herb	2.00 +/- 0.80	0.75 +/- 0.23
Bur clover	Non-native	Annual	Herb	1.10 +/- 0.48	0.15 +/- 0.11
Spring vetch	Non-native	Annual	Herb	0.05 +/- 0.05	0.00 +/- 0.00
Cheeseweed	Non-native	Annual	Herb	0.05 +/- 0.05	0.00 +/- 0.00
Spiny sow thistle	Non-native	Annual	Herb	0.30 +/- 0.22	0.00 +/- 0.00
Rip gut brome	Non-native	Annual	Herb	1.05 +/- 0.66	0.05 +/- 0.05
Pineapple weed	Non-native	Annual	Herb	0.00 +/- 0.00	0.05 +/- 0.05
False Brome	Non-native	Annual	Herb	0.10 +/- 0.10	0.00 +/- 0.00
Coastal Heron's Bill	Non-native	Annual	Herb	0.10 +/- 0.10	0.00 +/- 0.00
Black Mustard	Non-native	Annual	Herb	0.00 +/- 0.00	0.00 +/- 0.00

**Table A-3: Seed bank frequency of species by treatment**

Species	Native/Non-native	Annual/Perennial	Herb/Shrub	AMF+ Avg.+/- SE	AMF- Avg.+/- SE
Bristly ox tongue	Non-native	Annual	Herb	0.57 +/- 0.25	1.10 +/- 0.49
Sow thistle	Non-native	Annual	Herb	0.00 +/- 0.00	0.00 +/- 0.00
Acacia	Non-native	Perennial	Shrub	0.00 +/- 0.00	0.10 +/- 0.10
California Brittlebrush	Native	Annual	Shrub	0.00 +/- 0.00	0.10 +/- 0.10
Coastal Heron's Bill	Non-native	Annual	Herb	0.00 +/- 0.00	0.00 +/- 0.00
Lupine	Native	Annual	Herb	0.05 +/- 0.05	0.05 +/- 0.05
Sweet clover	Non-native	Annual	Herb	0.00 +/- 0.00	0.00 +/- 0.00
Pineapple weed	Non-native	Annual	Herb	0.00 +/- 0.00	0.00 +/- 0.00
Cliff Buckwheat	Native	Annual	Shrub	0.05 +/- 0.05	0.28 +/- 0.14
Cheeseweed	Non-native	Annual	Herb	0.00 +/- 0.00	0.00 +/- 0.00



**Table A-4:** Seed mix for Alta Vicente

<i>Species</i>	<b>Lbs. Per Acre</b>	<b>Total (Lbs.)</b>	<b>Total (grams)</b>
<i>Artemisia californica</i>	4	16	7257
<i>Encelia californica</i>	2	8	3629
<i>Eriogonum cinereum</i>	3	12	5443
<i>Eriogonum parvifolium</i>	5	20	9072
<i>Eriophyllum confertiflorum</i>	1	4	1814
<i>Gnaphalium bicolor</i>	0.5	2	907
<i>G. californicum</i>	0.5	2	907
<i>G. canascens</i>	1	4	1814
<i>Isocoma menziessi</i>	1	4	1814
<i>Lessingia filaginifolia</i>	1	4	1814
<i>Lotus scoparius</i>	4	16	7257
<i>Lupinus succulentus</i>	3	12	5443
<i>Malacothrix saxatilis</i>	0.5	2	907
<i>Melica imperfecta</i>	1	4	1814
<i>Nassella lepida</i>	1	4	1814
<i>N. pulchra</i>	3	12	5443
<b>Total Lbs./Grams per Acre</b>		<b>126</b>	<b>57152</b>

**Table A-5:** P-values from T-Test and Mann Whitney U-Test

	T-test Non-native	T-test Native	U test Non-native	U test Native
NURSERY	0.7805	0.2244	0.8896	0.3268
FIELD_Frequency	0.04645	0.3962	0.04643	0.821
FIELD_%Cover	0.03563	0.4141	0.1762	0.3268