

# The Use of Mycorrhizal Fungi in Erosion Control Applications

Final Report

for

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## **EXECUTIVE SUMMARY**

The construction activity associated with California's road improvement projects often degrades a soil's physical structure along with the biotic functioning of the vegetative and microbial community. As a result, these disturbed soils are easily mobilized during heavy rains. Other road improvement projects result in road cuts characterized by shallow or even non-existent soils and present a special problem for vegetation establishment. Reintroducing lost or missing soil microorganisms is increasingly recognized as an effective strategy for promoting cover of desirable plant species. Arbuscular mycorrhizal (AM) fungi represent a major component of the soil biota thought to be lost as a direct consequence of various road construction activities. AM fungi are soil-dwelling organisms that form obligate symbiotic relationships with plants, and generally improve plant performance by providing greater access to limiting soil nutrients such as phosphorus.

The majority of land plants require association with mycorrhizal fungi to thrive. These fungi are of particular importance in low nutrient soils common along road cuts in southern California. Mycorrhizal fungi are well recognized for their ability to improve a plant's probability of establishment and competitive ability, thereby reducing the soil's exposure to erosive forces such as wind and rain. AM fungi can also directly stabilize soil both through their hyphal network and through the secretion of glue-like chemicals. Commercially available AM inocula, which consist of a single AMF species, are currently applied to some slopes along California roadsides. However, the utility of these products has not been tested against other sources of inoculum with respect to improving plant establishment and stabilizing soil. Other sources of inoculum include

properly salvaged topsoil or cultures that originated from properly salvaged topsoil.

These other sources may be more cost effective to use on a large scale, or the structure of their particular soil communities may offer additional benefits not currently recognized in single species products. In Chapter 9, we report results of a literature survey where we look at some of the issues involved in salvaging and stockpiling topsoil for use as inoculum.

Our research reported in Chapters 2 – 6 tests several interrelated questions. (1) Is inoculation superior to no inoculation? (2) Is inoculation with cultured AM fungi superior to topsoil? (3) Are commercial inocula superior to topsoil? (4) Are multiple species inocula superior to single species inocula? (5) What is the strength of the relationship between native plants, weedy exotic plants, and the AM fungi they associate with? (6) What are the ecosystem consequences of these plant/microbe interactions with respect to soil stabilization? Our experiments suggest that native plants have a stronger relationship with mycorrhizal fungi than weedy exotic plants. In Chapters 2 and 3, we show that exotic plant species are less dependent on mycorrhizal fungi and this may be one reason why they dominate landscapes following disturbance. In contrast, many native species are very dependent on mycorrhizal fungi and perform particularly well with the soil community associated with the less disturbed native sites. The exotic species are also generally inferior hosts for mycorrhizal fungi. The mycorrhizal fungal community is significantly less abundant when associating with exotic host plants than it is when associating with native host plants (Chapters 2 and 3). The net result is that exotic species decrease the density of mycorrhizal fungi and thus easily proliferate in those soils. In contrast, native plant species are better hosts for AM fungi and are known



to perform better with these AM fungal communities. This generates a positive feedback and illustrates the central role of the soil community in the dynamics of the vegetation in southern California. Inoculation with appropriate soil communities could be critical to the ultimate establishment of native vegetation. Moreover, the establishment of both AM fungi and native plants act synergistically to stabilize the soil. In one study, we found that AM fungi, in association with a native host, significantly increases the proportion of water stable soil aggregates (Chapter 4).

The results of our pot experiments set our expectations for our field trials. During the first year, plots inoculated with AM fungi had higher abundances of native plant species, though this effect was only marginally significant (Chapter 5). Unfortunately, this effect disappeared during the second year, which was unusually dry. In our field trials, we also find improved soil aggregate stability due to the addition of live inoculum (Chapter 5). These effects, when considered along with the results of our pot experiment for soil aggregate stability, give us a firm expectation for on-site erosion potential. During the second year of the field trial, we found evidence that inoculation with mycorrhizal fungi can reduce sediment concentration and quantity (Chapter 6).

We also report work related to commercial applications of mycorrhizal fungi. In Chapters 4 – 8, a commercially available mycorrhizal product is featured as one of the treatments. In Chapters 4 – 6, the product is compared to naturally occurring mycorrhizal fungi. In Chapter 7, we test three commercially available soil inocula for their ability to be dispersed through a hydroseeder as well as their actual viability once each product contacts a suitable host plant. In Chapter 8 we investigate a conditioned

seed product, whereby live soil organisms are coated directly onto viable seeds as a way to promote optimal host/microbe contact upon seed dispersal.

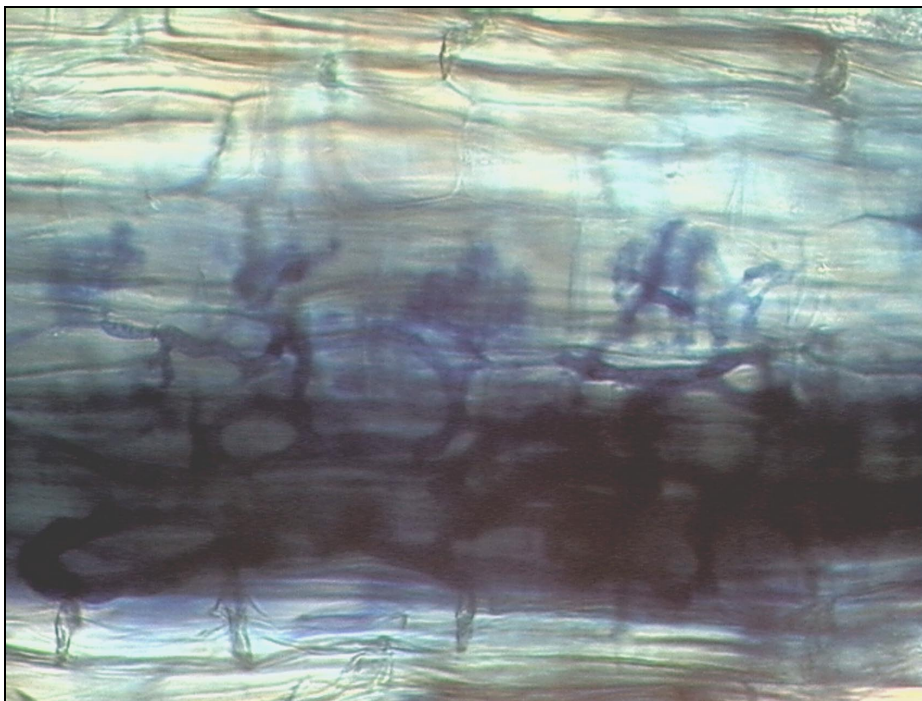
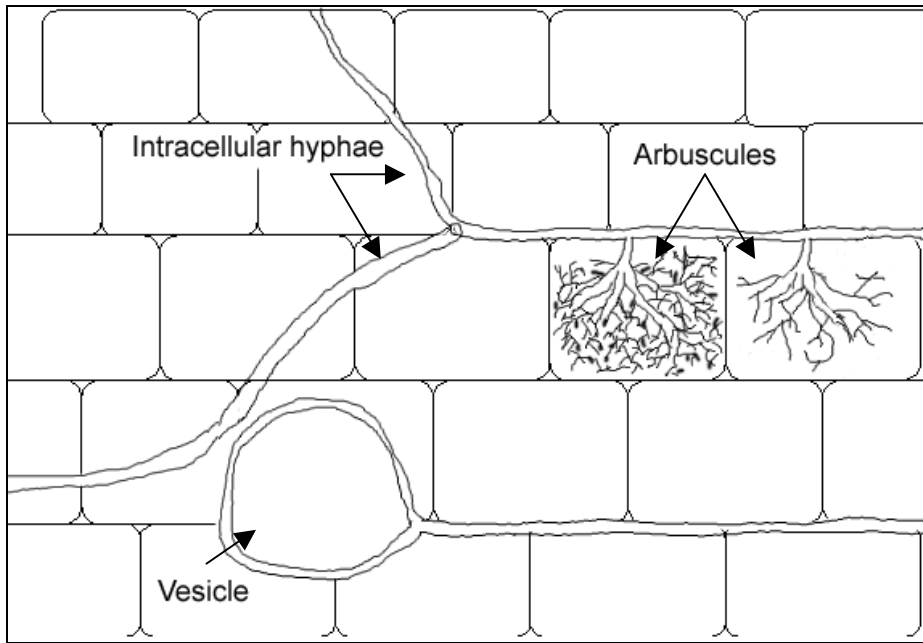
The availability of these products and results we report indicate the increasingly crucial role that mycorrhizal fungi can have in restoration plans, but also suggest caution with respect to selecting commercial products. Specifically, inoculation with the native soil community should speed the establishment of native plant species and improve the stability of the soil. These two important processes should reduce erosion from Caltrans roadcuts. While the greenhouse experiments clearly identify the pivotal role of the soil community in vegetation dynamics and soil stability, the results from our field experiments, while supporting this conclusion, are not as strong. We feel that this is not a surprise given the limited time-frame of the experiments, the saltiness of the experimental location and the poor rain that we had during the early years of the experiment.

It is our hope that this work will improve the ability of Caltrans or its contractors to identify the most effective AM fungal species/communities for use in revegetating degraded landscapes with self-sustaining native species. Not only are these species aesthetically pleasing to California's motorists, but appropriate native species also improve wildlife habitat and help stabilize soils along southern California roadways.

## CHAPTER 1: Introduction

Disturbance such as road construction results in the multifaceted problems of erosion, loss of soil fertility, sedimentation and pollution of waterways, and the loss and fragmentation of critical native habitat. When these problems cannot be avoided, the severity of the negative impacts may be reduced by the rapid re-establishment of native plant communities. However, native communities have proven difficult to re-establish in southern California, with disturbance of soil frequently resulting in the dominance of weedy invasive plant species. These weedy species may be less effective at soil stabilization and require greater maintenance than the locally adapted native communities (Jastrow 1987). In addition, native plant communities provide important habitat for wildlife (O'Connell et al. 1998; Fanolaro and Newman 1998).

Several restoration techniques have proven successful in accelerating the re-establishment of native vegetation and thereby reducing exposure to erosion. These approaches focus on increasing the competitive ability of the native species against the exotic invaders. An approach that holds great promise is the inoculation of plots with a particular group of soil fungi, called arbuscular mycorrhizal fungi or AM fungi (also known as vesicular arbuscular mycorrhizal fungi or VAM). These symbiotic fungi increase plant growth by facilitating plant's uptake of relatively immobile soil minerals. Most native plant species are dependent on these fungi for optimal growth. Growth of many of the pernicious exotic weedy species is not improved, and can even be reduced, by these fungi. In fact, in Chapter 2, we present evidence that this is generally the case in southern California. Inoculation by these fungi has been shown to improve the



**Figure 1.1.** Major mycorrhizal structures consist of hyphae, arbuscules, and vesicles in most species. Arbuscules are the site of nutrient exchange between the fungus and the root. For clarity, the top panel identifies these structures as they are commonly observed in the cortex of colonized plant roots. The bottom panel is a micrograph of arbuscules as they appear in California sagebrush.

competitive ability of the native plant species against the exotic species (e.g. Allen and Allen 1984) and against weedy species in general (Francis and Read 1994). Through these effects, inoculation with AM fungi increases plant density and diversity and shifts plant composition toward less weedy species (Grime et al. 1987; Gange et al. 1990). In field trials within Southern California, inoculation with mycorrhizal fungi has been shown to increase the speed and success rate of native vegetation establishment. For example, inoculation with commercially available mycorrhizal fungi (VAM 80) at San Onofre State Beach successfully suppressed *Brassica nigra*, a pernicious exotic weed of many restorations (Riefner and St. John 1998). By speeding the establishment of vigorous locally adapted vegetation, inoculation with AM fungi can reduce exposure to the forces of erosion.

After the establishment of plant cover, a major determinant of the erodibility of soil is the stability of soil aggregates to wetting. The greater the proportion of the soil held with water stable aggregates, the more resistant soil is to erosion (Brady 1990, Lundekvam and Skoien 1998). Soil particles are formed into water stable aggregates through the combined effects of entanglement by roots and fungal hyphae and the cementing effect of plant and fungal exudates. Mycorrhizal fungi can be particularly important to this process because AM fungal hyphae are abundant in California grasslands and shrublands (Klironomos et al. 1996, Rillig et al. 1999), and because AM fungi secrete a sticky and highly resistant substance (a glycoprotein) known as glomalin. This chemical has been shown to be very important in soil aggregation (Wright and Upadhyaya 1998). Because of these processes, AM fungi have been shown to be at least as important, if not more important to water stable aggregate formation than other soil

components such as plant roots (Thomas et al. 1993, Jastrow et al. 1998) and soil bacteria (Bethlenfalvay et al. 1997, Bethlenfalvay et al. 1999). For example, in an inoculation study in Oregon, pots inoculated with AM fungi had higher percentage of water stable aggregates than uninoculated pots or pots inoculated with rhizosphere bacteria (Bethlenfalvay et al. 1997). Therefore, successful establishment of viable populations of AM fungi can be an important step toward increasing the stability and reducing the erodibility of soils.

The correct matching of plants and mycorrhizal fungi is an important aspect of the successful reestablishment a robust plant community and the successful establishment of AM fungi. AM fungi only grow in association with appropriate host plants and plant species vary in how effective they are as hosts. Many introduced weeds, including *Brassica nigra*, are poor hosts of AM fungi, while many native plant species are good hosts. In Chapter 3, we show that introduced weeds are generally poorer hosts for mycorrhizal fungi than native species. In a study in Illinois, the proportion of the soil in water-stable aggregates increased rapidly after the restoration of native grassland species and this improvement in soil structure was driven by the increased investment by the native plant species in mycorrhizal fungi (Jastrow 1987, Jastrow et al. 1998). Not only was the density of AM fungi higher after re-establishment of native vegetation, but the composition of the AM fungal community shifted as well (Bever et al. 2003). Importantly, the composition of the AM fungal community shifted towards the species that are known to produce larger quantities of the soil glue, glomalin (Wright and Upadhyaya 1999), thereby promoting stable aggregate formation. Therefore, the matching of native plants with their best mycorrhizal fungi can speed the establishment

of robust native vegetation and enhance water-stable aggregate formation, thereby reducing erosion.

Because of the great potential of AM fungi in plant growth enhancement and soil stabilization, we have focused our work on the utility of these fungi in the reducing erosion within Caltrans slopes. With the removal of all vegetation and topsoil during road construction, road cuts are depleted of the entire soil community. Though many components of this soil community besides AM fungi could also be important to plant establishment and soil stabilization, inoculation with AM fungi may be particularly critical given their known ecological importance and their limited dispersal abilities. Their spores are formed within soil and are therefore unlikely to be dispersed by wind. While AM fungi are likely to establish naturally even without inoculation, the presence of AM fungi during the early stages of revegetation can have strong effects on the trajectory of plant establishment (Smith et al. 1998, Bever et al. 2001). We present evidence that this is also true in Southern California.

As a result of these demonstrated benefits, inoculation with AM fungi is currently specified within Caltrans projects. The spores of many AM fungal species are resistant to drying which allows some flexibility in the timing of inoculation. There has also been some success in hydroseeding AM fungal inocula. However, few species of AM fungi are commercially available and therefore Caltrans roadcuts are currently inoculated with single species cultures of AM fungi. The effectiveness of these commercial fungal isolates relative to the diversity of native fungi remains unknown. In our work, we compared the effectiveness of single species commercial inocula, to diverse native inocula and uninoculated controls. We constructed our native AM fungal inocula so that

it also includes many other native soil flora. We also compare inoculation with AM fungal cultures and inoculation with topsoil. With this work, we focus our attention on the influence of the source and diversity of mycorrhizal inocula on plant establishment and soil stabilization.

In this report, we present our findings on how mycorrhizal fungi interact with various host plants, and what these interactions mean for the restoration practitioner interested in revegetating with species native to California. In the course of our three year project, we used literature searches, empirical greenhouse studies, and demonstration field experiments to understand the potential uses of mycorrhizal fungi as a restoration tool.



## **CHAPTER 2: Native plants generally benefit more from mycorrhizal fungi than weedy invasive species: evidence and implications.**

### **INTRODUCTION**

The great success of introduced plant species in California may result from many factors. Globally, both individual species characteristics and habitat properties contribute to successful establishment of naturalized exotic plants. Species characters that contribute to the success of introduced species include high rates of dispersal, self-compatible breeding systems, rapid growth, and high seed production (Baker 1974, Cronk and Fuller 1995, Burke and Grime 1996). Habitat properties that can contribute to the successful establishment of naturalized species include low species diversity (Tilman 1997, Hector et al. 2001) and disturbance by humans (Hobbs and Huenneke 1992, Burke and Grime 1996). In some cases, species characteristics and habitat properties interact to produce highly invaded communities. For example, a flammable European grass (*Bromus tectorum*) introduced into native desert shrub communities in the western United States accelerates historic fire cycles and responds favorably to frequent fires (D'Antonio and Vitousek 1992). As a result, *Bromus tectorum* facilitates its own success by increasing fire frequency and now dominates the rangelands throughout much of the Great Basin region. A similar positive feedback has been suggested in which invasion by one species alters the environment in such a way as to facilitate the subsequent invasion by other introduced species (Simberloff and Von Holle 1999). We suggest that a similar dynamic may be occurring with the introduced plants of California, and that it is mediated by changes in the soil community.

Accumulating evidence indicates that biotic aspects of the environment also contribute to the success of naturalized plant species. Naturalized plants often have

fewer pathogens in their new environment (Mitchell and Power 2003) and have been found to have weaker negative feedbacks with soil organisms (Bever 1994, Klironomos 2002, Van der Putten 2002, Callaway et al. 2004). Interactions with soil mutualists may also influence invasion success (Vitousek et al. 1987, Richardson et al. 2000), however the direction of these results has varied from mycorrhizal fungi facilitating naturalized plants (Marler et al. 1999, Bever 2002, Zabinski et al. 2002), to mycorrhizal fungi inhibiting naturalized plants (Allen et al. 1989, Johnson 1998).

In this study, we test for general differences between native and naturalized plant species with respect to their interactions with mycorrhizal fungi within the flora of southern California. Much of the native vegetation of California has been replaced by species of Eurasian origin following hundreds of years of grazing. It has been observed that native species are slow in re-establishing in naturalized-dominated vegetation, with several authors suggesting that naturalized-dominated landscapes in California represent an alternative equilibrium state (Stromberg and Griffin 1996, Eliason and Allen 1997, Stylinski and Allen 1999). It is possible that the resistance of naturalized-dominated vegetation to re-establishment of native species is mediated through a positive feedback dynamics with respect to the mycorrhizal fungal community. To evaluate this possibility, we compare the mycorrhizal dependence of native and exotic naturalized species using analyses of local floras and tests of responsiveness to inoculation. We also compare the effect of native and exotic naturalized species on mycorrhizal fungal densities. We finally test whether observed differences can contribute to a reinforcing dynamic in which naturalized species have comparatively

greater success with the soil associated with naturalized species than the soil associated with native species.

In this chapter, we show:

- Native species benefit more from mycorrhizal fungi, while exotic species often do not.
- Native species particularly benefit from mycorrhizal fungi associated with other native plants.
- Areas with native plants have greater densities of mycorrhizal fungi.

## **METHODS**

### *Floral surveys*

We surveyed floras of southern California to investigate whether successfully naturalized plant species are generally less dependent than native species on mycorrhizal fungi. We calculated the total number of native and naturalized species present in families that are characteristically non-mycorrhizal (Gerdemann 1968, Newman and Reddell 1987, Tester et al. 1987) from each of seven published floras within southern California (<http://endeavor.des.ucdavis.edu/nps/sbypark.html>, Raven 1963, Little 1977, Lathrop and Thorne 1985, Muns 1988, Hickman 1993, Boyd et al. 1995a, Boyd et al. 1995b, Ross et al. 1996, Bowler and Bramlet 2004). For each flora, plant species designated by origin (native or naturalized) (Hickman 1993) were tallied according to the typical mycorrhizal status of their taxonomic family (mycorrhizal or non-mycorrhizal) (Gerdemann 1968, Newman and Reddell 1987, Tester et al. 1987). We tested for overall differences in the proportion of plant species from mycorrhizal plant families in the

native and naturalized species in the local floras using loglinear analysis in SAS (SAS 1999). Chi-square analyses on species counts were performed on individual local floras in the data set.

#### *Mycorrhizal inoculation experiment (Exp. 1)*

To test for differences in responsiveness of native and naturalized species to inoculation with mycorrhizal fungi, we grew six replicates of ten naturalized and seven native plant species with and without mycorrhizal fungi. Species were chosen with the goal of achieving good representation across common plant families (particularly Poaceae and Asteraceae) and with the constraint of seed availability, but without prior expectations of response to mycorrhizal fungi. The naturalized species included *Avena fatua*, *Brassica nigra*, *Carduus pycnocephalus*, *Melilotus indica*, *Amaranthus retroflexus*, *Rumex crispus*, *Bromus diandrus*, *Lactuca serriola*, *Medicago polymorpha*, and *Lolium multiflorum* and the native species included *Nassella lepida*, *Nassella pulchra*, *Muhlenbergia rigens*, *Hemizonia fasciculata*, *Encelia californica*, *Lotus purshianus*, and *Salvia apiana*. All seed was collected from within Orange County, California. Plants were grown in 500 mL of an autoclaved mixture of sand and soil derived from an area adjacent to the University of California, Irvine Ecological Preserve (UCIEP). The mycorrhizal treatments received ~ 2,000 spores of *Glomus intraradices* (produced by Bionet, Inc., CA). The control treatment received AM fungal-free microbial washings of the spores. Plants were grown in a completely randomized design within a greenhouse. Dried total plant weight was log transformed and analyzed

using analysis of variance in SAS (SAS 1999). Differences in response to mycorrhizal fungi was tested using *a priori* contrasts within the species\*inoculation interaction term. To confirm consistency of the differences in response between the native and naturalized categories we also tested the contrast using species within categories as a random effect.

#### *Soil community inoculation experiment (Exp. 2)*

To evaluate whether native and naturalized species differ in their response to live soil overall and to soil derived from native and naturalized dominated sites, in particular, we grew eight naturalized and six native plant species (selected as in experiment 1) with soil communities derived from native-dominated sites, soil communities derived from naturalized-dominated sites and sterilized soil. The naturalized species included *Rumex crispus*, *Bromus diandrus*, *Lactuca serriola*, *Medicago polymorpha*, *Lolium multiflorum*, *Marrubium vulgare*, *Salsola tragus*, and *Sonchus asper* and the native species included *Lotus purshianus*, *Salvia apiana*, *Nassella pulchra*, *Achillea millefolium*, *Galium angustifolium*, and *Isocoma menziesii*. Each species was replicated five times per treatment. Seeds and inocula were collected from the UCIEP and the Starr Ranch Wildlife Sanctuary in Orange County, California, USA. The two inoculants were created by collecting soil from seven paired locations of native grasslands and adjacent locations dominated by naturalized plant species, within Starr Ranch in March, 1999. These fresh soils were used as starter inoculum in 22 L pots. We filled pots with an autoclaved mixture of local soil and sand, and grew seedlings of eight different native grassland species. The cultures from the native-dominated sites were pooled, as were cultures from sites dominated by naturalized plant species, to produce the inocula for this

experiment. Note that even after culturing the two inoculants with common hosts and in a common environment, the mycorrhizal inoculum potential of the soil derived from native-dominated sites was significantly greater than mycorrhizal inoculum potential from sites dominated by naturalized plant species ( $F_{1, 24} = 5.25$ ,  $p < 0.03$ ) as measured from a inoculum potential assay as described below.

We layered 400 mL of these inocula within 8.8 L of autoclaved local soil that was prepared as in Exp. 1. The sterile treatment received 400 mL of an autoclaved mixture of the two inocula. Seedlings of native and naturalized species were then planted into these 210 pots, which were completely randomized and grown on low benches out of doors. We monitored plant size each month during the growing season by measuring plant height, leaf number, and length and width of the largest leaf. At the end of the growing season, biomass was measured. Total above- and below-ground biomass was used as a measure of performance for all species except the annual species *Bromus diandrus* and *Lotus purshianus*, for which inflorescence mass was used because they had completed their life cycle at harvest. Plant performance was analyzed using analysis of variance followed by *a priori* orthogonal contrasts using SAS (SAS 1999). We specifically tested for differences in the relative performance of the native and naturalized plant species (1) in response to inoculation with living soil communities, and (2) in response to soil communities derived from native sites compared to soil communities derived from naturalized-dominated sites.

*AM inoculum densities in Native and Naturalized dominated sites*

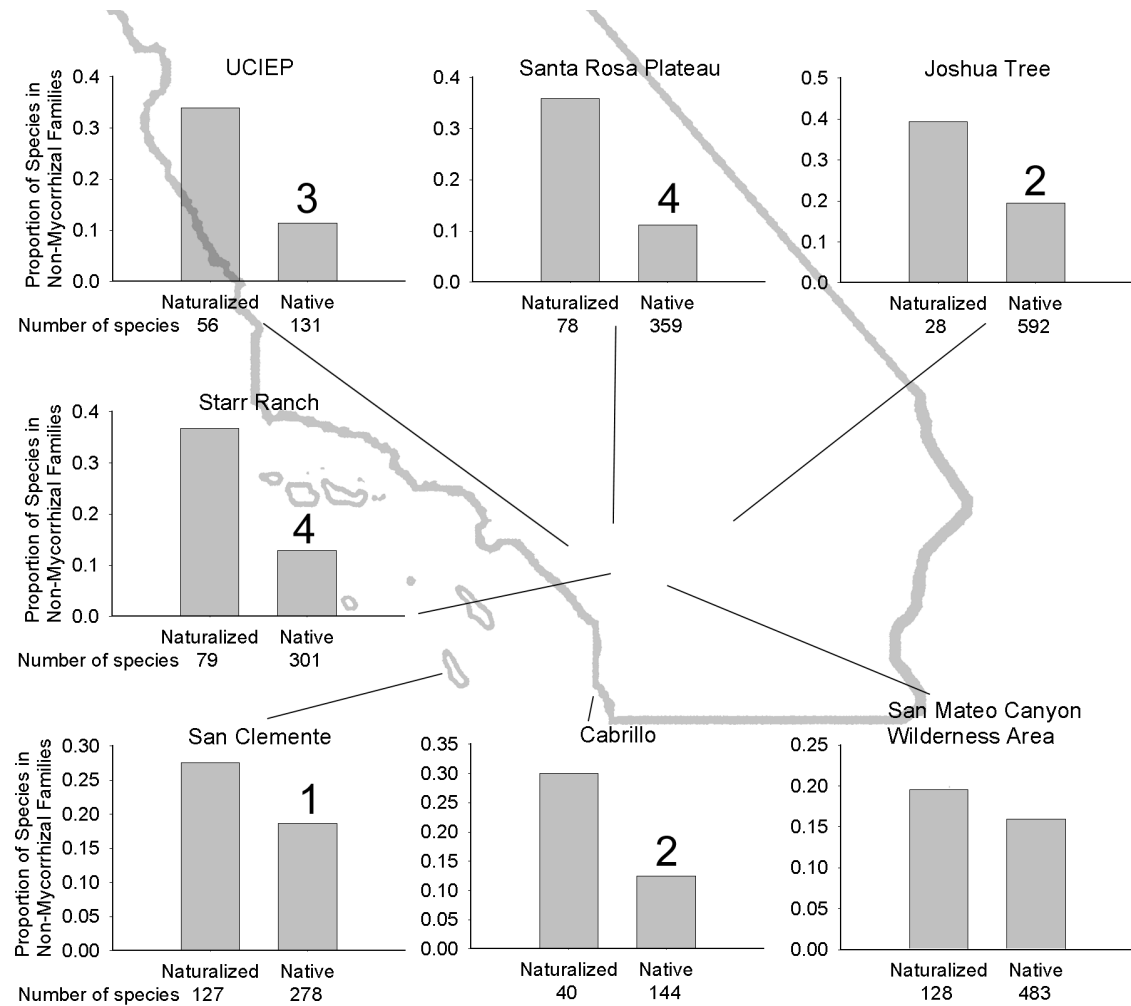
We evaluated whether the mycorrhizal densities differed between areas dominated by natives and areas dominated by naturalized species by evaluating the AM fungal infection potential in five pairs of soil samples from sites dominated by native species and adjacent sites dominated by naturalized species using a common host plant. Sampling locations were paired by slope, exposure, substrate and proximity, but differed in vegetative community (i.e., dominated by naturalized or native species). The goal of our assays was to compare the relative infection potential of multiple inocula. To do this, we compared the infection intensity of a common host plant grown in a common environment with similar dilutions of the inocula. In each assay, 150 mL pots were filled with three dilutions (10, 5 and 1%) of the fresh soils mixed into an autoclaved soil amended with sand. We replicated each treatment five times, seeded all pots with Sudan grass (*Sorghum bicolor*), and watered daily. We harvested each experiment after 3-4 weeks (depending on rates of germination). The roots were washed, and subsamples cleared with 10% potassium hydroxide and stained with trypan blue (Brundrett et al. 1994). Rates of colonization by AM fungi were scored visually at 400X magnification according to standard methods (McGonigle et al. 1990). Data were arcsine square root transformed prior to analyzing with analysis of variance in SAS (SAS 1999). Based on preliminary examination of infection levels in this experiment, we scored and analyzed infection at the highest dilution level.

## RESULTS

### *Floral survey*

In the seven floras tested, we found that a greater proportion of naturalized species than native species occur in families that are characteristically non-mycorrhizal ( $\chi^2 = 63.0$ ,  $p < 0.0001$ ; Fig. 2.1). This difference was significant across all individual floras except the flora of the relatively undisturbed San Mateo Wilderness area in Cleveland National Forest, suggesting the higher incidence of species from non-mycorrhizal families in naturalized floras may relate to disturbance. The consistency of the pattern (Fig. 2.1) suggests that reduced dependence on mycorrhizal fungi may be an important aspect of the success of some naturalized plant species.





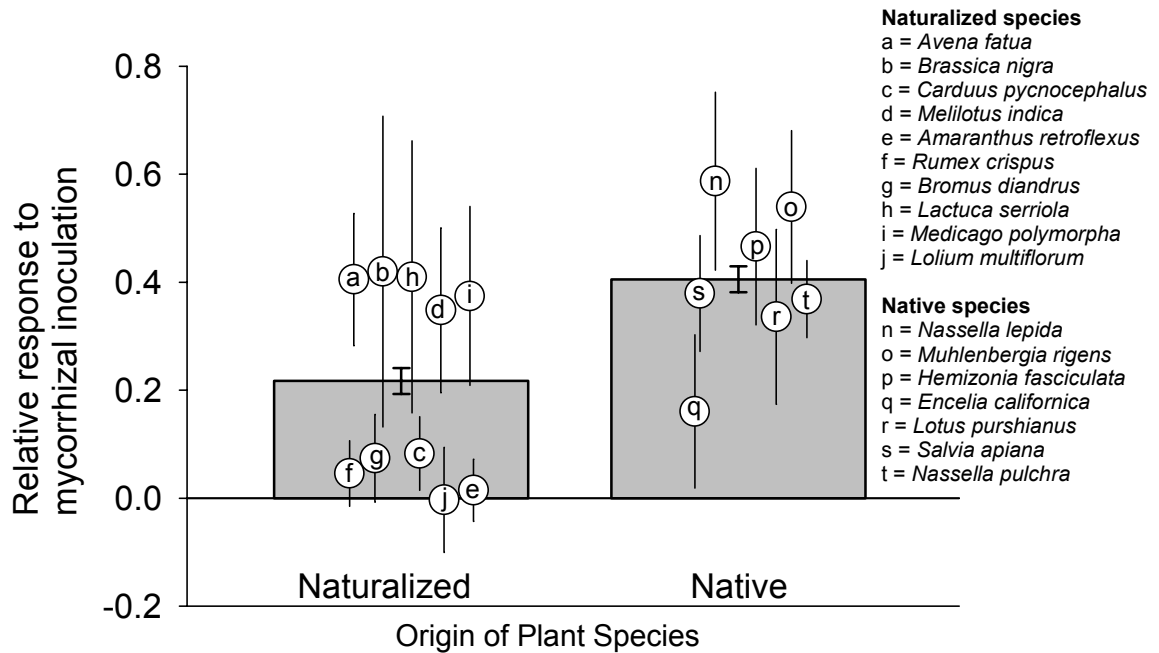
**Figure 2.1.** The proportion of naturalized and native plant species from mycorrhizal and nonmycorrhizal families is presented for seven local floras in southern California. A greater proportion of naturalized species are from plant families that are typically non-mycorrhizal. This difference is significant in all floras except the relatively pristine San Mateo Canyon Wilderness Area of the Cleveland National Forest. Significance of  $p < 0.05$ ,  $0.01$ ,  $0.001$  and  $0.0001$ , are represented by 1, 2, 3, and 4, respectively.

*Mycorrhizal inoculation experiment (Exp. 1)*

In corroboration of the differences observed in the floras, we found that the naturalized plant species, on average, responded less positively to mycorrhizae than the native plant species ( $F_{1,127} = 20.8$ ,  $p < 0.0001$ ; Fig. 2.2). The response to mycorrhizal fungi among naturalized plant species varies substantially (Fig. 2.2), perhaps suggesting multiple avenues to being a successful colonizing species. This variation could also explain observations of particular naturalized plant species being more responsive than individual native plant species (Marler et al. 1999, Bever 2002, Zabinski et al. 2002). Given this variation within the naturalized category, one might question the generality of the difference with native species. Yet, the difference in average responsiveness between native and naturalized plants held true even after removing plants from non-mycorrhizal plant families ( $F_{1,106} = 13.3$ ,  $p < 0.0004$ ) and when testing over the variation between plant species within the naturalized/native categories ( $F_{1,15} = 5.0$ ,  $p < 0.04$ ), indicating that the overall pattern is likely to be independent of the particular native and naturalized plant species chosen.

*Soil community inoculation experiment (Exp. 2)*

We also confirmed this difference between native and naturalized plant species in a second experiment that tested the response of six native plant species and eight naturalized plant species to local fungal cultures. Again, the native plant species generally benefited more from inoculation with the soil cultures than the naturalized plant species ( $F_{1,162} = 6.94$ ;  $p < 0.009$ ; Fig. 2.3a).

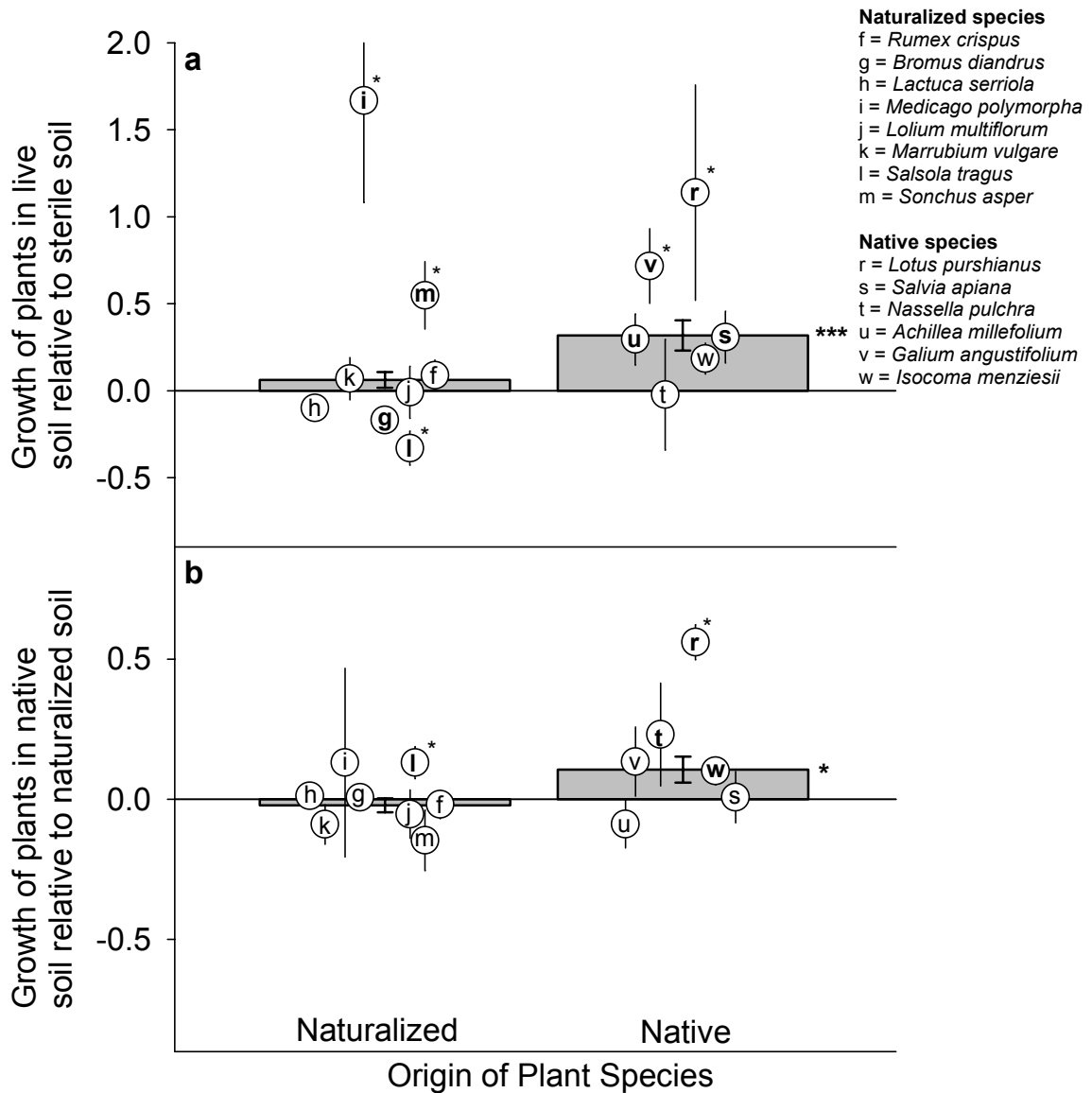


**Figure 2.2.** The difference in biomass between arbuscular mycorrhizal and non-mycorrhizal treatments for 10 naturalized and 7 native plant species. On average (represented by the bars), native plant species benefit significantly more from mycorrhizal inoculation than do naturalized exotic plants.

In this experiment (Fig 2.3a), the naturalized legume, *Medicago polymorpha*, was particularly responsive to inoculation with living soil. This effect likely results from the presence of symbiotic N-fixing bacteria rather than mycorrhizal fungi. Functional nodules did not form in the sterile treatment, but were present with live inocula. Removal of the native and naturalized legumes from the analysis increases the significance of the difference between native and naturalized species.

Native plant species also generally grew better with soil communities derived from sites dominated by native species than from sites dominated by naturalized plant species, while naturalized plant species were not significantly responsive to inoculum

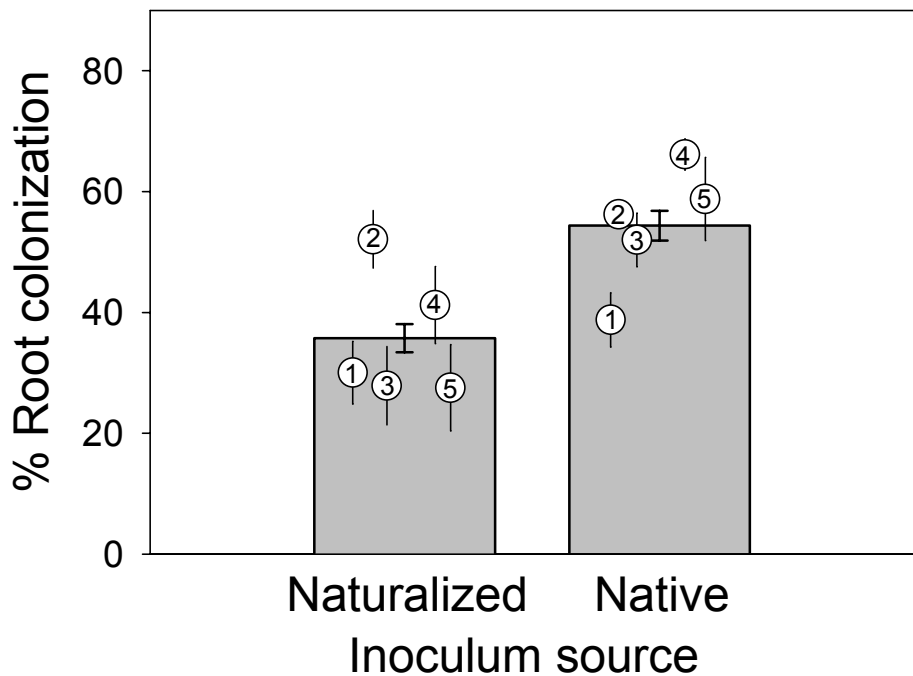
source (Fig. 2.3b,  $F_{1,162} = 5.5$ ,  $p < 0.02$ ). This difference suggests that native plant species may have difficulty establishing in areas dominated by weedy invasive species.



**Figure 2.3.** The relative growth of eight naturalized exotics and six native plant species in response to inoculation with soil communities derived from native and naturalized dominated sites. Panel a presents the relative response to live soil. Native species benefit from inoculation with live soil ( $F_{1,70}=13.42$ ,  $p < 0.0005$ ) while naturalized species do not. Panel b presents the difference in response to soil communities derived from native dominated sites and soil communities derived from sites dominated by naturalized plant species. Native species generally grew better with soil communities derived from sites dominated by native species than with soil communities derived from sites dominated by exotic naturalized species ( $F_{1,70}=4.28$ ,  $p < 0.04$ ), while naturalized species did not show a consistent response.

*AM Inoculum Densities in Native and Naturalized dominated sites*

Areas within UCIEP that were dominated by weedy naturalized plant species had reduced mycorrhizal inoculum potential compared to adjacent native dominated sites (Fig. 2.4). This difference held true when tested over the error variance ( $F_{1,38} = 28.08$ ,  $p < 0.0001$ ) and when tested over the variation between individual sampling sites ( $F_{1,4} = 12.9$ ,  $p < 0.02$ ), suggesting that the result is consistent over the entire preserve.



**Figure 2.4.** Assays of relative inoculum potentials of AM fungi from paired field sites (indicated by matching numbers) dominated by naturalized or native species. Soils from field sites dominated by native plant species have greater AM fungal densities than adjacent sites dominated by naturalized plant species. This differences held true when tested over the error variance ( $F_{1,38} = 28.08$ ,  $p < 0.0001$ ) and when tested over the variation between individual sampling sites ( $F_{1,4} = 12.9$ ,  $p < 0.02$ ), suggesting that the result is consistent regardless of sampling location (indicated by numbers). Bars represent mean % root colonization with  $\pm 1$  SE.

## DISCUSSION

We provide multiple lines of evidence indicating that reduced dependence on the mycorrhizal mutualism is an important aspect of the success of introduced plant species within southern California. First, we find that naturalized plant species are more likely to be from plant families that typically do not associate with mycorrhizal fungi generally have reduced dependence on mycorrhizal fungi compared to the native species (Fig. 2.1). Consistent with this result, we also find that naturalized species generally have reduced growth responses to mycorrhizal fungi than native species. The response to mycorrhizal fungi among naturalized plant species varies substantially (Fig. 2.2), perhaps suggesting multiple avenues to being a successful colonizing species. This variation could also explain observations of particular naturalized plant species being more responsive than individual native plant species (Marler et al. 1999, Bever 2002, Zabinski et al. 2002). Given this variation within the naturalized category, one might question the generality of the differences observed with native species. However, each of the experimental tests of plant growth response were significant when treating species within the native/naturalized category as random effects, indicating the average difference is not dependent on the particular species used in the experiment. This result combined with the results of the analyses of the floras suggests that the reduced dependence on mycorrhizal fungi in naturalized species is general across the flora of southern California. Growth promotion by mycorrhizal fungi has been shown to vary with plant-AM fungal combination (van der Heijden et al. 1998, Bever 2002, Klironomos 2003). The results presented here, however, were consistent across pure mycorrhizal fungal inocula and two different types of soil cultures from southern California (Figs. 2.2, 2.3), suggesting that the result is general across a range of species of mycorrhizal fungi.

Reduced dependence on mycorrhizal fungi can be advantageous to naturalized species under some environmental conditions. Plants with reduced dependence on mycorrhizal fungi have been shown to be superior competitors in areas with low densities of AM fungi (Fitter 1977, Allen and Allen 1984, Grime et al. 1987, Hartnett et al. 1993). The reduced dependence on mycorrhizal fungi can then be viewed as part of a life history strategy that is successful in disturbed environments (Francis and Read 1995). Disturbance is often identified as a factor facilitating the success of naturalized plant species (Hobbs and Huenneke 1992, Stromberg and Griffin 1996). The results of analyses of local floras are consistent with the success of introduced species with low dependence on mycorrhizal fungi being a response to opportunities provided by heavy anthropogenic disturbance, in that the difference in proportion of species from plant families that typically do not associate with mycorrhizal fungi was lowest in wilderness areas that have minimal impacted by humans (Fig. 2.1).

The difference in mycorrhizal dependence of native and naturalized floras could also contribute to the reinforcement of the dominance of naturalized species if the dominance of naturalized species was associated with reduced densities of AM fungi. We observed reduced densities of arbuscular mycorrhizal fungi in areas dominated by naturalized species compared to adjacent areas dominated by native species (Fig. 2.4). The association of low levels of mycorrhizal inoculum with dominance of naturalized plants may be caused by a prior history of soil disturbance. But it is also possible that weedy naturalized plants reduce the densities of mycorrhizal fungi (see evidence of this in the next chapter). In fact, weedy naturalized plant species, which exhibit reduced dependence on AM fungi, would be expected to allocate little of their resources toward maintaining mycorrhizal fungi. In this case, even if the mycorrhizal fungal communities were initially equivalent, the dominance of naturalized plant species would eventually degrade the AM fungal communities relative to sites dominated by native plant species.



Given the greater mycorrhizal responsiveness of native plants and the higher densities of AM fungi in areas dominated by native plants, we predict that native plant species would perform relatively better in association with soil communities derived from sites dominated by native species than from soil communities derived from sites dominated by naturalized species. We observed this exact result in the second growth experiment. Native species grew better when inoculated with soil communities derived from native dominated sites than with soil communities dominated by naturalized species (Fig 2.3b). In this experiment, even though the inocula was grown with common hosts and in a common environment, the densities of arbuscular mycorrhizal fungi in the inocula derived from naturalized plant communities was still reduced relative to the inocula derived from native plant communities. It is therefore likely that the difference in mycorrhizal densities contributed to the difference in relative response of native and naturalized species. It is also possible that other differences in the two types of inocula, including difference in composition of arbuscular mycorrhizal fungi (Bever 2002) or soil pathogens (Klironomos 2002), could contribute to the growth differences. For example, inoculation with native soil communities increased the establishment of native tallgrass prairie species in a restoration, even though there was no difference in mycorrhizal inocula densities (Bever et al. 2003). Regardless of mechanism, the increased response of native species to native inocula is consistent with a soil microbial mechanism of reinforcement of dominance of naturalized species.

Several authors have suggested that highly-invaded vegetation in California is particularly resistant to re-establishment of native species represent an alternative equilibrium state (Stromberg and Griffin 1996, Eliason and Allen 1997, Styliniski and Allen 1999), though this interpretation was called into question by the work of Siemens et al. (2003). Our results suggest that degradation of the mycorrhizal community can facilitate the establishment of newly introduced plant species and reinforce their

dominance. The initial establishment of naturalized species would be facilitated by soil disturbance sufficient to reduce AM fungal densities, thereby giving naturalized species a competitive advantage over native species. With naturalized species successfully established, re-establishment of AM fungi may be slowed, thereby impeding native plant species that exhibit strong dependencies on mycorrhizal fungi. Alternatively, the degradation of the mycorrhizal community may be a secondary event following an initial invasion of naturalized plant species due to other factors such as overgrazing (Wahren et al. 1994). In this case, overgrazing drives the dominance of naturalized plant species, which then results in the degradation of the mycorrhizal community, which subsequently inhibits the re-establishment of native species even under a diminished grazing regime. The net result under either scenario is a reinforcement of the initial dominance of naturalized plant species mediated by changes in the soil community.

This work identifies the soil community as a potential positive feedback switch for community conversion (Wilson and Agnew 1992, Bever et al. 1997, Simberloff and Von Holle 1999) and it highlights one peril of habitat disturbance. Our findings suggest that soil communities may be a critical aspect of ecosystem resistance to invasion. As a result, land management regimes that disrupt soil communities in an effort to control naturalized weeds (such as tilling or mechanical weed extraction) may have the drawback of negatively impacting the mycorrhizal fungal communities. Conversely, inoculation with mycorrhizal fungi may facilitate establishment of native species in sites dominated by naturalized species (Smith et al. 1998, Richter and Stutz 2002). Our results also suggest a mechanism through which the dominance of naturalized plant species could degrade ecosystem function. As mycorrhizal fungi have been shown to be important for several aspects of ecosystem function, including phosphorus cycling and soil aggregate formation and stabilization (Miller and Jastrow 1990, Smith and Read

1997), the dominance of naturalized plant species may have deleterious consequences for ecosystem function through the degradation of the mycorrhizal fungal community.

## **CHAPTER 3: Evidence that naturalized exotic plants in California are poor mycorrhizal hosts**

### **INTRODUCTION**

Naturalized exotic plants that successfully establish and become invasive are a well recognized problem in many native plant communities. Exotic invaders can threaten existing plant community structure (Walker and Vitousek 1991, Bellingham 1998), change ecosystem function (Vitousek and Walker 1989, D'Antonio and Vitousek 1992, Zavaleta 2000), and facilitate a loss of biodiversity (Wilcove et al. 1998). Ongoing investigations have identified many important factors associated with successful invasions, but despite the increasing amount of research attention, it is not always clear what maintains exotic-dominated communities. Consider the patterns often found in the Mediterranean-type ecosystems of California. Of the hundreds of introduced exotic plants in California that possess undesirable invasive properties (CalEPPC 1999), one often finds suites of exotic species maintained for decades apparently as stable communities that resist native species reestablishment (Stromberg and Griffin 1996, Eliason and Allen 1997, Stylinski and Allen 1999). As exotic species increase in abundance, obvious changes to native plant community structure result, but structural changes to soil communities may also be important, especially if soil mutualist viability degrades with the changing plant host community.

Succession may eventually return these exotic-dominated communities to their pre-invaded structure, but successional processes can be unreliable in the arid western United States. In Mediterranean-type ecosystems, for example, native species are not reliably recruited following disturbance (Keeley et al. 1981, O'Leary and Westman 1988). Adequate seed dispersal may be all that limits native species recruitment,

especially if exotic species growing abundantly generate an overwhelming seed supply. Good experimental evidence supports this explanation in Mediterranean-type ecosystems where summer droughts are normal (Cantero et al. 1999, Zobel et al. 2000) and even more generally, a reliable supply of propagules is thought to be a critical proximal component to establish and maintain community structure (Tilman 1997, Levine 2000, Frenot et al. 2001). Under this dispersal limitation hypothesis, native and exotic species do not need distinct ecologies to explain the contemporary patterns of exotic community structure.

When exotic species become established and largely displace the native flora, it is generally assumed, however, that important ecological differences exist among the resident and the invading species. Invading exotic species may exclude native species via competitive superiority. For example, the exotic tree *Acacia saligna* can outcompete the native South African fynbos vegetation for resources (Witkowski 1991) and the competitive success of *Centaurea maculosa*, an invasive exotic thistle to western U.S. range lands, may be mediated by soil community relationships (Marler et al. 1999, Callaway et al. 2001). Examples of competitive superiority are well known, and can cause dramatic changes in community structure. However, competitive superiority does not explain all successful invasions where distinct ecologies are suspected.

Distinct ecologies may initiate environmental changes that effectively promote invader success. That is, increasing exotic abundance may generate positive feedback. For example, the exotic annual *Bromus tectorum* (cheatgrass) has altered the historic fire cycles of intermountain native shrub communities (Knapp 1996). With increasing cheatgrass abundance, rangeland fires have become more frequent, which generally

promotes cheatgrass invasion to the detriment of the native perennials. In the absence of altered disturbance regimes such as fire, cheatgrass abundance would be expected to decline via shrub succession. Soil mutualists are known to contribute to positive feedback, evident in Hawaiian volcanic soils where the invasion of the evergreen tree *Myrica faya* and its actinorhizal mutualist alter nitrogen cycling such that the exotic invader is self-sustained to the detriment of the native forest (Vitousek and Walker 1989, Walker and Vitousek 1991). *Myrica faya* can be viewed as an exotic species with a qualitatively different relationship to soil flora than the displaced native species. Positive feedback between the exotic host plant and the increasingly abundant soil mutualist has facilitated the conversion.

Soil mutualists that decline in abundance may also initiate positive feedback to maintain exotic plants. In systems where native plants have strong mutualistic relationships with soil symbionts such as arbuscular mycorrhizal (AM) fungi, then disturbances that disrupt the mutualism could facilitate the establishment of exotic species. If these exotic hosts are less dependent on the mutualism and invest little in maintaining the soil community structure, then exotic-dominated communities may result. These altered communities would appear stable if the degraded soil community structure prevented successful re-establishment from native species. This degraded mutualist hypothesis may explain patterns of exotic-dominance where distinct ecologies between native and exotic plants are suspected, but cannot be attributed to competitive superiority.

This degraded mutualist hypothesis builds on many assumptions, each of which has empirical support. (1) Soil arbuscular mycorrhizae decline with increasing

disturbance intensity, which is evident in both agricultural and unmanaged systems (Moorman and Reeves 1979, Reeves et al. 1979, Galvez et al. 2001). (2) Colonizing exotic species are often less dependent on mutualists such as AM fungi (Reeves et al. 1979, Allen and Allen 1980, Pendleton and Smith 1983). (3) Plants vary in their AM fungal hosting ability (Newman and Reddell 1987, Trappe 1987). This degraded mutualist hypothesis predicts a pattern of inhibition and growth promotion that can be easily tested, at least conceptually. That is, native plants dependent on AM fungi would be inhibited when grown in killed soil or live soil with a vegetative history of exotic species, and promoted when grown in live soil with a vegetative history of native species. The inhibition and growth promotion would be relative to the performance of exotic plants growing under the same treatments.

In practice, however, this degraded mutualist hypothesis is difficult to test due to confounding abiotic factors inherent in most natural soils. The temporal and spatial heterogeneity of soils is well recognized (Jackson and Caldwell 1993, Cain et al. 1999) and even in the absence of differential nutrient availability, this heterogeneity can affect competitive relationships (Fransen et al. 2001). Moreover, biotic factors like soil pathogens can confound underlying predictions if soil communities feedback negatively on their host communities (Bever 1994, Mills and Bever 1998, Matthews and Clay 2001), and the effects of disturbance itself on the soil community must be considered (Moorman and Reeves 1979, Reeves et al. 1979). Here we describe a mesocosm approach which controlled for many confounding soil factors. After one year of soil training under manipulated native and exotic communities, we tested the feedback

potential of these cultured soils on suitable native and exotic indicator species to learn if vegetative history results in mutualist degradation.

In this chapter, we experimentally demonstrate:

- A native herbaceous plant grows best when grown in soil cultured by a native plant community.
- A weedy naturalized exotic plant grows best when grown in soil cultured by an exotic plant community.
- These growth patterns are related both to vegetative history and the associated mycorrhizal communities.
- A biotic feedback mechanism that can account for vegetation patterns commonly observed in California landscapes.

## **METHODS**

### *Experimental overview*

The experiment involved two phases. In the first phase, we constructed mesocosms that received a common soil community and were cultured with a variety of host plants for one growing season. These mesocosms were plant communities of either native or exotic grassland species grown in large nursery pots. After establishing a vegetative history in each mesocosm, soil from each pot was removed for use as inoculum in a greenhouse growth assay. This assay tested the feedback effects of each cultured soil community on two taxonomically related indicator species; the native grassland forb *Gnaphalium californicum* and the invasive exotic *Carduus*



*pycnocephalus*. The performance of these indicator species allowed a direct test of host community mediated mutualist degradation.

### *Study system*

This experiment commenced in the field over the 1999-2000 growing season at the UCI Arboretum (Irvine, California, USA: 33° 40'N, 117° 51'W, 17 m elevation) and was completed the following year inside a university greenhouse 1.6 km southeast of the arboretum. The mesocosms were modeled after the low elevation coastal grasslands in southern California. Drought conditions prevail each summer with approximately 300 mm of rain occurring during the cool winter months. Winter temperatures range from 7° to 19° C in January to 17° to 28° C in July. The soils are generally fine-textured and vary in depth; we used a local clay soil formed from weathered calcareous sandstone and shale. A diverse mix of perennial and annual grasses and forbs characterize these coastal grasslands (Wester 1981), from which we selected common species to create two pools of grasses and forbs. The native species pool included *Bloomeria crocea* Torr. (common goldenstar: Liliaceae), *Daucus pusillus* Miscx. (yarrow: Apiaceae), *Dichelostemma capitatum* Benth. (blue dicks: Liliaceae), *Gnaphalium californicum* DC. (everlasting: Asteraceae), *Isocoma menziesii* Hook. & Arn. (coast goldenbush: Asteraceae), *Lotus purshianus* Benth. (Spanish lotus: Fabaceae), *Muhlenbergia rigens* (Benth.) A. Hitchc. (deergrass: Poaceae), and *Nassella pulchra* A. Hitchc. (purple needlegrass: Poaceae). The exotic species pool included *Amaranthus retroflexus* L. (pigweed: Amaranthaceae), *Avena fatua* L. (wild oatgrass: Poaceae), *Brassica nigra* (L.) Koch (black mustard: Brassicaceae), *Bromus diandrus* Roth (ripgut brome: Poaceae), *Carduus pycnocephalus*

L. (Italian thistle: Asteraceae), *Cirsium vulgare* L. (bull thistle: Asteraceae), *Melilotus indica* L. (sourclover: Fabaceae), and *Rumex crispus* L. (curly dock: Polygonaceae). We collected soil and seed from the UCI Ecological Preserve and also from a local wildlife sanctuary owned and managed by the Audubon Society (Starr Ranch).

### *Experimental design of mesocosms*

Mesocosms consisted of either native coastal grassland species or naturalized weedy exotic species. Within these two assemblages (native or exotic), we created a richness gradient of communities consisting of 1, 2, 4, 7, or 8 species. Including this gradient allowed species number effects to be distinguished from species composition effects. Each assemblage contained monocultures of all eight species from a given native or exotic pool, and these monocultures were replicated four times for a total of 32 native and 32 exotic species monocultures. In richness treatments 2 and 4, we created eight random combinations from each species pool, with the design constraint that all species must be equally represented. We replicated these eight random combinations to create 16 communities each of two and four species in each assemblage. Richness treatment 7 systematically excluded each species to form eight possible combinations, which were replicated to yield 16 communities. To keep planting density constant at eight individuals in treatment 7, we double-planted one of the species, as determined by a random draw. We used all species in richness treatment 8, and the resulting native and exotic communities were each replicated eight times.

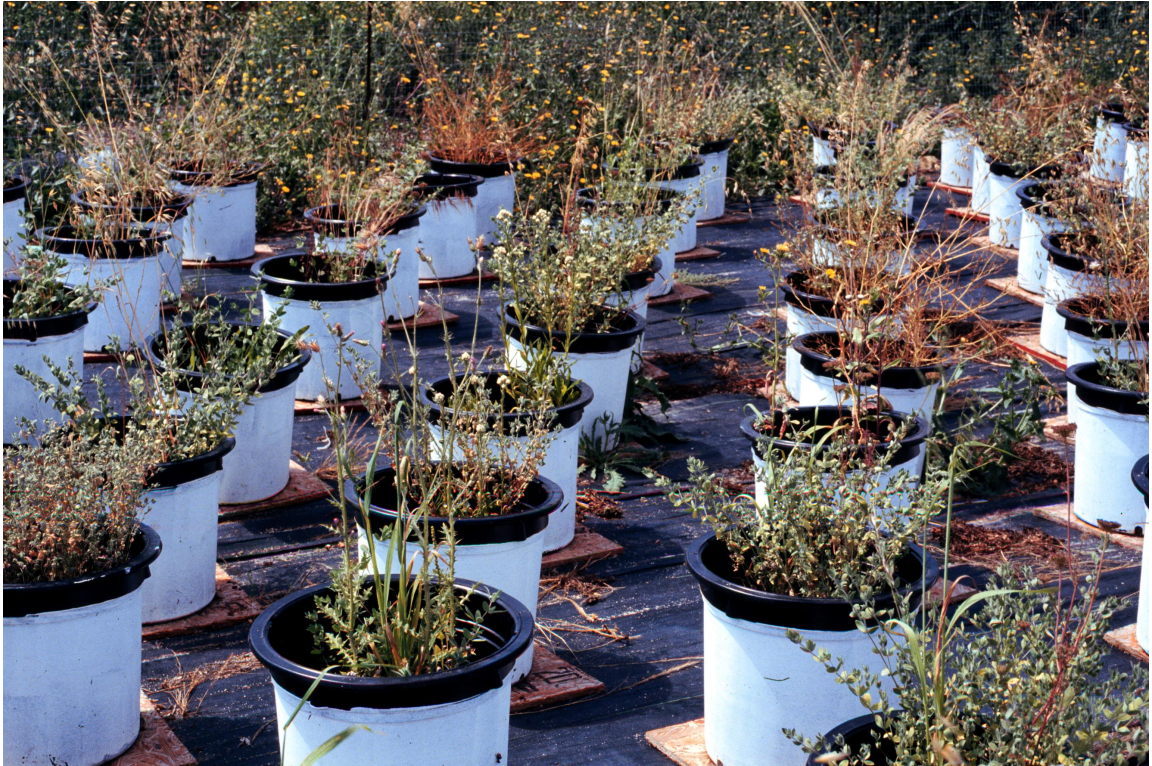
We included eight containers of bare soil to estimate nutrient depletion. These bare soil pots were maintained free of weeds (and thus, potential host plants) throughout

the growing season. Species naturally recruited into an additional set of eight containers maintained for the purpose of generating a treatment that consisted of unmanipulated communities of volunteer exotic and native hosts. These 16 containers received the same inoculum and watering regimes as the other treatments.

### *Mesocosm assembly*

Large plastic nursery containers (36.8 cm diameter × 38.1 cm height; 40 L capacity) were used to house communities of plants and their common soil inoculum. These 192 pots were evenly distributed over a 200 m<sup>2</sup> area nested within a 400 m<sup>2</sup> fenced enclosure. A nylon fabric barrier underneath the array limited weed proliferation in the interspaces. Each of the 192 pots was filled to 75% capacity with a 1:1 homogeneous mixture of washed sand and local soil that had been processed through a 1.3 cm sieve. To remove weeds, the pots of soil were regularly watered for eight weeks to induce seed bank germination and these weeds were promptly removed by hand. This management regime likely reduced the viability of soil mutualists such as AM fungi. To reestablish these soil communities and incorporate species associated with native coastal grasslands, we added 225 mL of live soil to inoculate each mesocosm. This live soil inoculum was then capped by a 4 cm layer of homogenized soil that had been autoclaved

for three hours at 121°C.



**Figure 3.1.** Mesocosm experiment with native and naturalized exotic plant communities to study of the effects of plant community type on mycorrhizal fungi.

Each mesocosm received eight seedlings that were grown in a UCI greenhouse for several weeks in a sterile mix of vermiculite and peat moss. We used a radially symmetrical planting pattern to keep seedlings evenly spaced. The area received 159 mm of rainfall over the growing season, which was well below the annual average precipitation of 312 mm. Thus, pots were hand watered uniformly to supplement rain inputs. A species inventory conducted post-establishment (after 8 weeks) determined surviving density and species richness for each mesocosm, so that these factors could be accounted for in all subsequent analyses.

#### *Soil inoculum preparation*

Live soil obtained from an undisturbed area of Starr Ranch in February 1999 was selected to represent the soil biota of a native perennial coastal grassland. Approximately 500 mL of this soil was used as a starter inoculum grown with a diverse mix of California grassland host species, and cultured in 12 L nursery pots as a way to increase the total volume of potential inoculum. Live soil collected from Starr Ranch in October, 1999 was used to augment the cultured inoculum. Both the cultured and the live soil contained root fragments, fungal hyphae, and other microorganisms ubiquitous in native soils. A separate colonization assay indicated that sufficient live propagules were present in this prepared inoculum (mean root colonization on grain sorghum > 50%). Each mesocosm received 150 mL of cultured soil inoculum and 75 mL of live fresh soil inoculum as indicated previously.

### *Test of mycorrhizal colonization and feedback*

To test the feedback potential of the soil communities cultured under the mesocosm host plants, we removed soil samples from the center of each pot. These soil samples were removed post harvest following the 1999-2000 growing season. Since these soils would later be used as inoculum, it was important to test for differences in macronutrients that may influence growth responses. Each sample was air-dried and subsampled to determine available nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ), phosphorus (P), and potassium (K) by an independent lab (DANR Analytical Laboratories, UC, Davis, Davis, CA). We refrigerated the remaining soil until February, 2001, at which time subsamples were removed for use as post mesocosm inoculum.

### *Mycorrhizal colonization assay*

Investigating mutualist viability required an independent assay of mycorrhizal colonization on a general purpose host. The ubiquity and ecological importance of AM fungi make this group of obligate mutualists good candidates for any feedback effects initiated by manipulated host communities. In a manner similar to the growth assay to test for soil feedback, a set of 192 small seedling cones (3.8 cm diameter  $\times$  21 cm depth; Stuewe & Sons, Inc., Corvallis, Oregon, USA) were filled with autoclaved local soil and sand mixed 1:1 (v:v) previously sieved to exclude aggregates  $> 4.75$  mm. Each cone received 10 mL of post mesocosm inoculum combined with 90 mL of the autoclaved planting mix. An additional set of eight cones received only 100 mL of autoclaved mix and no live inoculum. All cones were seeded with grain sorghum, completely randomized in a UCI greenhouse, and watered daily. After two weeks, all plants

received 10 mL phosphorus-free fertilizer diluted to 300 ppm nitrate (Plant Marvel Laboratories Inc., Chicago Heights, Illinois, USA). All plants were harvested at 24 days, and the roots were washed, cleared with 10% KOH, and stained with trypan blue (Brundrett et al. 1994). The stained roots were randomly subsampled and mounted onto glass slides for inspection under a light microscope. We estimated the mycorrhizal colonization percentage of each sample visually according to methods adapted from McGonigle et al. (1990).

#### *Greenhouse growth assay*

We investigated the feedback potential of soil used in the previous year's mesocosm on two indicator species; the native grassland forb *Gnaphalium californicum* and the weedy exotic *Carduus pycnocephalus*. These species grow abundantly throughout coastal sage/scrub and coastal grassland communities, and they grew reliably in their respective native and exotic mesocosms. These plants both exhibit similar morphologies and biennial life histories, and are known to form mycorrhizae (i.e., they are mycotrophic). Since mycotrophy is common in Mediterranean-type ecosystems characterized by nutrient-poor soils (Bethlenfalvay et al. 1984), both of these species were likely to be good indicators for mutualist effects.

We mixed 50 mL of crushed post mesocosm inoculum processed through a sterilized 2 mm sieve with 150 mL of autoclaved soil:sand mixed 1:1 (v:v) into two sets of 192 seedling cones (5 cm diameter × 18 cm depth; Stuewe & Sons, Inc., Corvallis, Oregon, USA). Each set of 192 cones corresponded directly to the treatments from the previous year's mesocosm system, thus preserving the original replication. Two sets of

five cones filled only with 200 mL of autoclaved soil were added as sterile controls. Seeds of *G. californicum* and *C. pycnocephalus* were randomly assigned to one of the sets, and each set completely randomized across the greenhouse bench. These seeds were covered with 10 mL of autoclaved soil:sand mix, and all 394 cones were gently misted daily to induce germination. Seedlings were thinned to one individual per cone post emergence, and allowed to grow for 12 weeks in a UCI greenhouse under natural light conditions. Since this experiment investigated mutualist viability, low phosphorus growing conditions were necessary so root colonization by AM fungi were not inhibited (Amijee et al. 1989, Demiranda and Harris 1994). Thus in addition to daily watering, all cones received 10 mL of phosphorus-free water soluble fertilizer diluted to 300 ppm nitrate. This solution was applied four times (40 mL total) approximately every fourth week, which corresponded to symptoms of nitrogen deficiency exhibited by the plants. Moreover, fertilizing likely mitigated variations in nutrient availability introduced by the inoculum itself. During this time, both completely randomized arrays were rotated every 10 days to mitigate any block effects due to greenhouse bench position. The roots and shoots of all plants were harvested after 12 weeks, oven dried at 65°C to a constant mass, and weighed.

### *Data analysis*

Soil nutrient differences of the post mesocosm inocula were evaluated for differences by vegetative status (vegetated or bare) and mesocosm type (native or exotic). The minerals  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , P, and K were included as multiple responses in a MANOVA that used community type as the independent class variable. Post hoc



contrasts determined whether the bare soil nutrient controls differed from all vegetated treatments, and also whether nutrients differed among native and exotic communities. All soil nutrient estimates were natural log transformed ( $\ln(1+Y)$ ) to satisfy MANOVA assumptions of equal variances.

The mycorrhizal colonization data were first analyzed to detect overall differences among the five post mesocosm inoculum types of (1) sterile controls; (2) no host plants; (3) exotic hosts only; (4) native hosts only; and (5) naturally recruited host plants. Estimates of percent sorghum root colonization were arcsine transformed ( $Y' = \arcsin \sqrt{Y}$ ) and subjected to ANOVA. Unequal means were evaluated with the post hoc Tukey procedure. A restricted ANCOVA then considered only the effects of manipulated host community richness and host origin, with soil mineral estimates from the mesocosm inoculum as covariates. Backward elimination removed non-significant ( $P > 0.10$ ) covariates from the final model.

Data (total dry biomass) from the greenhouse growth assay of *G. californicum* and *C. pycnocephalus* were first analyzed to detect overall differences among the same five inoculum types used for the mycorrhizal colonization assay. Individual plants failing to germinate or survive more than 3 weeks were excluded; germination for *G. californicum* was low compared to *C. pycnocephalus*, which resulted in 35% fewer observations for *G. californicum*. Additionally, eight inoculum samples of the original 192 mesocosms were excluded from all analyses because the soil communities originated from “dead” mesocosms, i.e., where seedlings were planted but died early in the growing season. The unequal sample sizes in both data sets coupled with the

heteroscedasticity necessitated nonparametric procedures, and so the five groups were compared using the Kruskal-Wallis test.

Separate analyses focused only on the effects of manipulated host community richness and host origin. For these analyses, pots lacking host communities or those consisting of naturally recruited hosts were excluded. This restricted data set exhibits good homoscedasticity, allowing parametric procedures. Thus, two way analyses of covariance on the growth of *G. californicum* and *C. pycnocephalus* used community type (native or exotic) and species richness as main effects, and the results from the mineral analyses on the source inoculum as covariates. Since richness varied independently in the mesocosm, including species richness in this manner tests for any species number effects that are separate from species composition effects. We used backward elimination to systematically exclude soil nutrients and interaction terms from the final model, using  $P > 0.10$  as the exclusion rule.

For *G. californicum*, the final model included the main effects of community type and species richness, along with the interaction term community type x richness, and K as a significant mineral covariate. For *C. pycnocephalus*, only the two main effects remained following the elimination procedure. Finally, we used regression analysis to obtain the slopes of each indicator species as a function of the species richness of the native and exotic community types of host inoculum. All analyses were performed using SAS version 8 (SAS 1999).

## RESULTS

### Post mesocosm soil nutrient differences

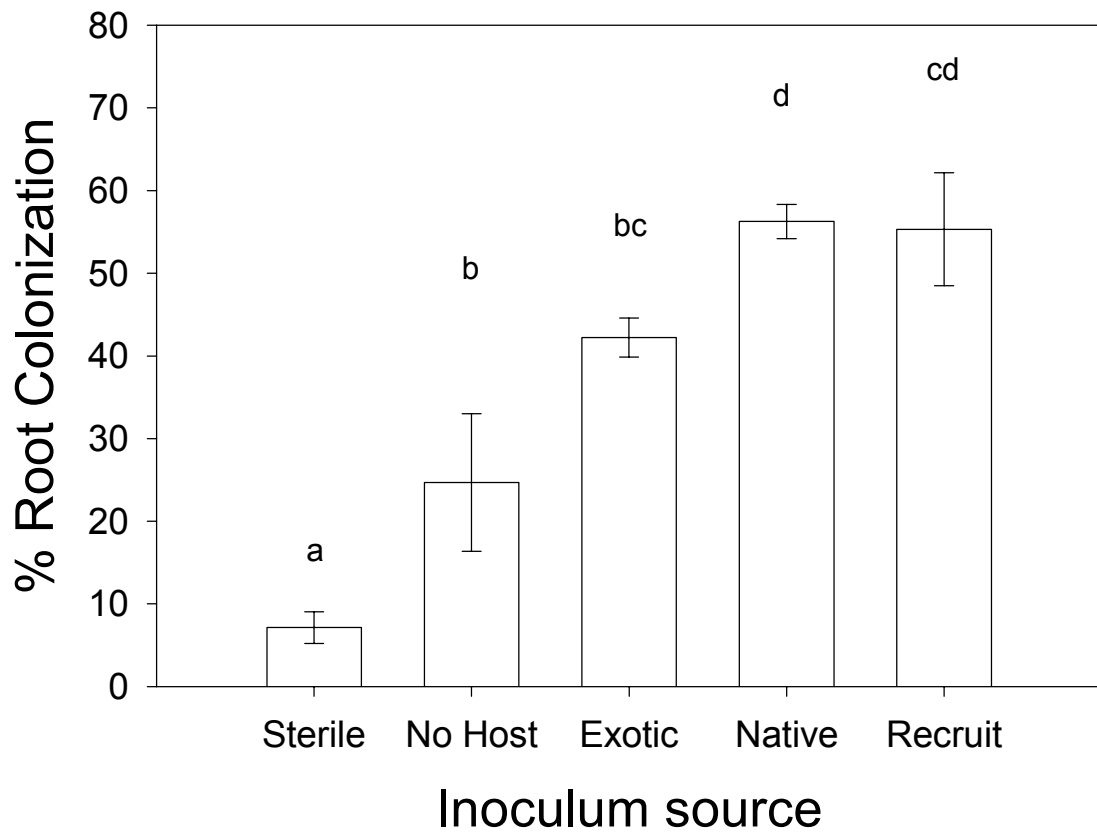
The four community types exhibited significant differences in extractable soil minerals ( $F_{12,537} = 10.80$ ,  $P < 0.0001$ , by MANOVA). The minerals  $\text{NO}_3^-$  and P influenced the overall result; contrast comparisons revealed these two nutrients were significantly higher in the bare soil controls than in vegetated communities ( $\text{NO}_3^-$ :  $F_1 = 151.97$ ,  $P < 0.0001$ ; P:  $F_1 = 28.61$ ,  $P < 0.0001$ ). The minerals  $\text{NH}_4^+$  and K did not differ from the controls ( $\text{NH}_4^+$  and K:  $F_1 = 0.07$ ,  $P = 0.79$ ). The contrast statement between native and exotic host communities revealed significantly higher levels of  $\text{NO}_3^-$  in exotic mesocosms ( $F_1 = 27.46$ ,  $P < 0.0001$ ) along with marginally higher levels of K ( $F_1 = 3.73$ ,  $P = 0.06$ ). No other mineral differences were evident.

### *Mycorrhizal colonization assay*

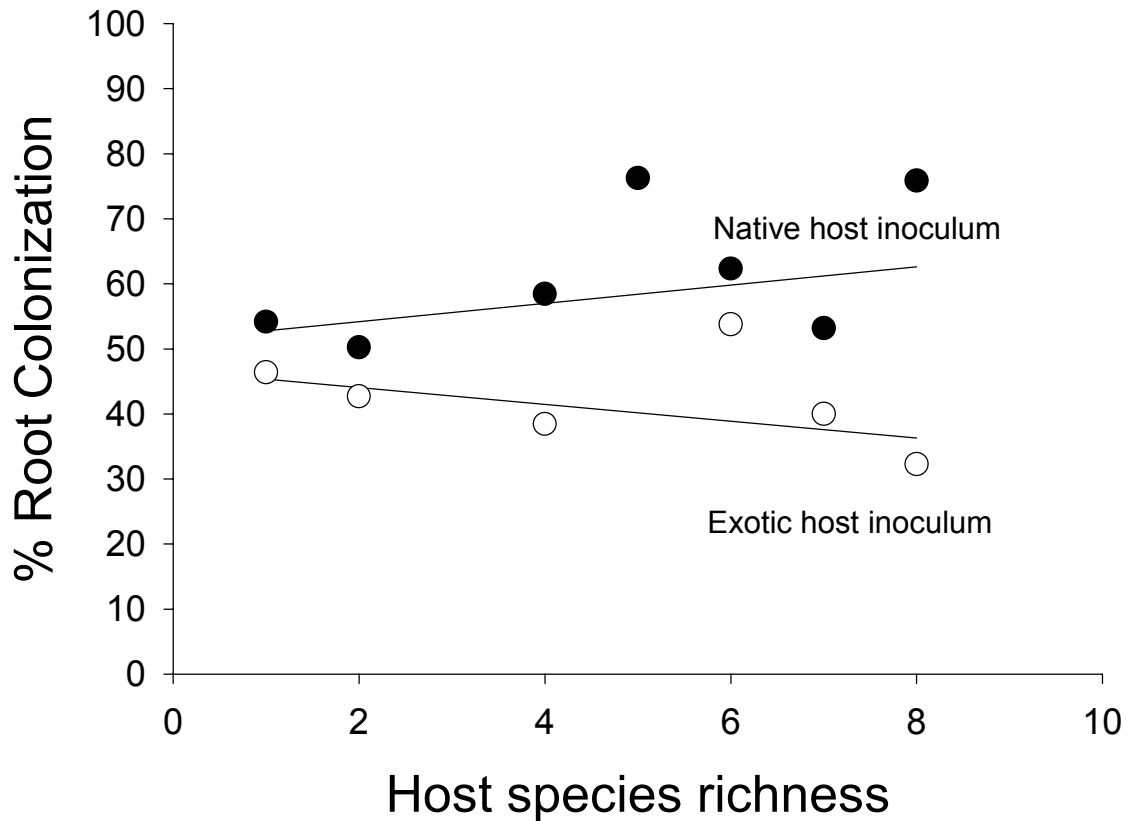
Mean colonization differences ranged from 5.8% (for the sterile controls) to 56.7% for the native host inoculum, which exhibited the highest potential for mycorrhizal colonization. The overall effects of inoculum source differed significantly (Fig.3.2,  $F_{4,187} = 18.11$ ,  $P < 0.0001$ ), with most colonization occurring in the three live soil treatments. The ANCOVA on the restricted data set revealed no effects of species origin or species richness, but a significant interaction between origin and richness (Fig.3.3,  $F_{1,164} = 4.60$ ,  $P = 0.0335$ ). No mineral covariates were significant.

### *Greenhouse growth assay for native indicator*

The most vigorous plants for the native *G. californicum* grew with inoculum from native host communities, and to a lesser extent, the inoculum from exotic and naturally recruited host communities (Fig. 3.4). Of these vigorous plants, the mean biomass improvement of 43% from the exotic inoculum to the native inoculum is of particular interest because it indicates that an important shift occurred in mutualist efficacy due to the exotic hosts. ANCOVA on the restricted data set (comprised only of native and exotic post mesocosm inoculum) indicated no overall host species richness effects (Fig. 3.4,  $F_{1,106} = 1.27$ ,  $P = 0.2626$ ) but significant host community effects ( $F_{1,106} = 9.03$ ,  $P = 0.0033$ ). The interaction of host community origin and species richness was significant ( $F_{1,106} = 4.32$ ,  $P = 0.0401$ ), and the soil mineral K exhibited no significance ( $F_{1,106} = 3.30$ ,  $P = 0.0720$ ). Strong overall growth differences (using the complete data set) were significant (Kruskal-Wallis ANOVA,  $\chi^2_4 = 24.87$ ,  $P < 0.0001$ ) and were most noticeable in the sterile controls and when *G. californicum* grew with inoculum cultured without host plants. This native plant failed to thrive in either of these two treatment groups (Fig. 3.4).



**Figure 3.2.** The mycorrhizal inoculum potential of soil removed from mesocosm pots after 1 year (means  $\pm$  1 SE). This measure of mycorrhizal activity indicates AM fungi differ by community type. *Recruit* and *Native* sources exhibited the highest proportion of colonized roots. Soil from pots lacking host plants and *Exotic* treatments do not differ significantly in their inoculum potential (bars with shared letters do not differ). The pattern suggests community type can affect mutualist effectivity.



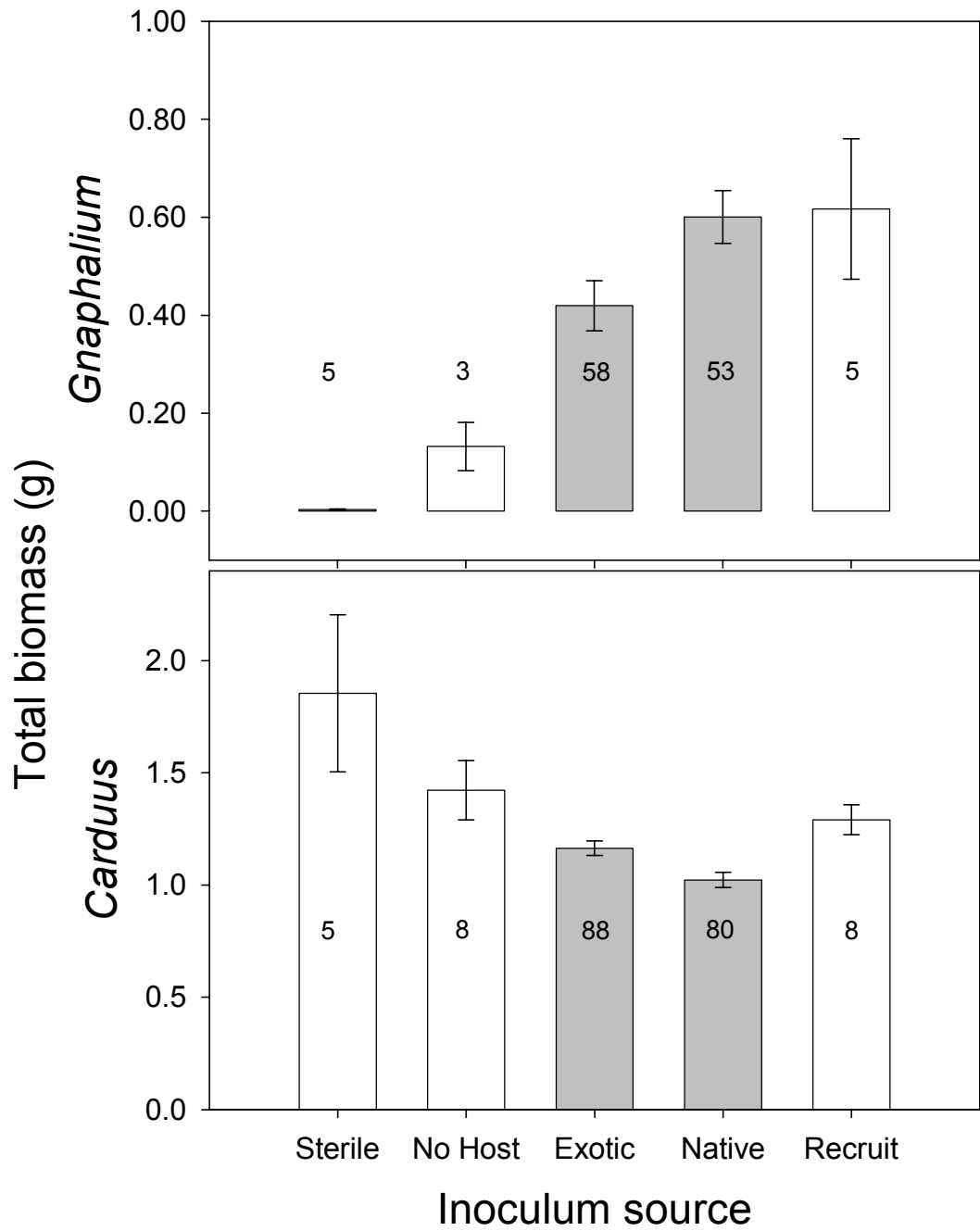
**Figure 3.3.** The inoculum potential of soil removed from mesocosm pots after one year. Native and weedy exotic plants interact differently with AM fungi as species number increases.

#### *Greenhouse growth assay for exotic indicator*

This exotic indicator *C. pycnocephalus* was inhibited most by soil from the native host communities, and mean biomass was nearly 14% greater in the exotic inoculum compared to the native treatment. ANCOVA on the native and exotic post mesocosm inoculum (the restricted data set) revealed significant host species richness effects ( $F_{1,165} = 8.19, P = 0.0048$ ) and host community effects ( $F_{1,165} = 9.35, P = 0.0026$ ). Available soil minerals were not significant biomass predictors. The exotic indicator grew well throughout the experiment, but analysis on the complete data set

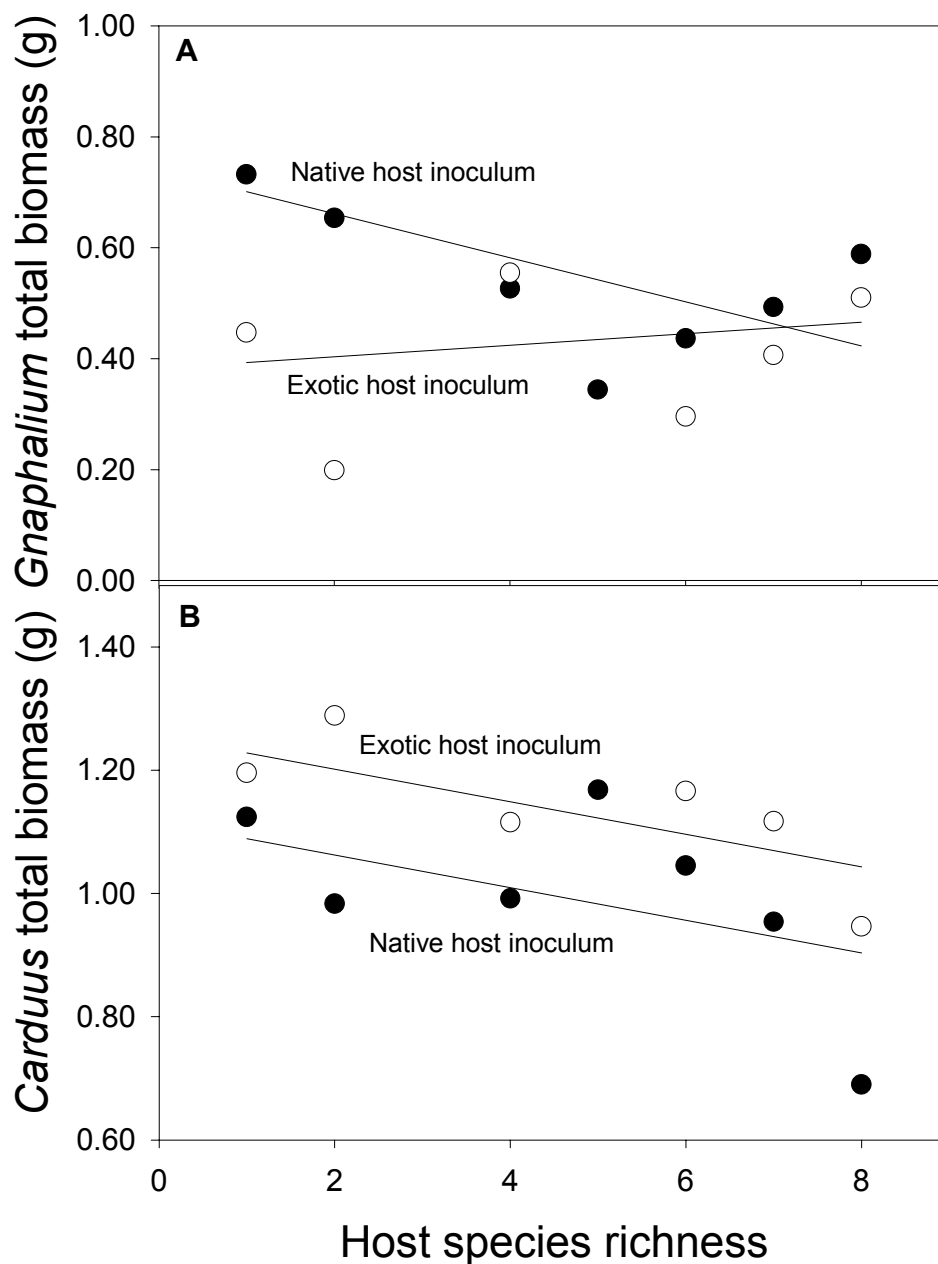
reveals significant differences among the five inoculum types (Kruskal-Wallis ANOVA,  $\chi^2_4 = 26.44$ ,  $P < 0.0001$ ). *C. pycnocephalus* performed best when grown with sterile soil and inoculum cultured without host plants, in sharp contrast to the native *G. californicum*. The overall pattern of growth differs noticeably between the native and exotic indicator species (Fig. 3.4).

In Chapter 2, we report results of a flora survey that describes how non-mycorrhizal exotic plants are disproportionately represented in parts of California. The experimental results reported here in Chapter 3 suggest a more general phenomenon may apply to the naturalized weedy exotics common throughout California's grasslands. The exotic species in this experiment contributed less to the maintenance of their mycorrhizal communities than did the native species. As a consequence, the resulting microbial communities either promoted or inhibited selected indicator species, depending on whether those indicators were native to the flora or introduced from Eurasia. Thus, these results identify a biotic feedback mechanism that can account for the vegetation patterns commonly observed throughout restored, revegetated, or even unmanaged California landscapes with a history of disturbance.



**Figure 3.4.** Evidence for the differential effects of live soil on two indicator species; the native *Gnaphalium californicum* (top panel) and the weedy exotic *Carduus pycnocephalus* (bottom panel). The comparisons between native and exotic host communities (shaded bars) were highly significant for both species. Sample size for each treatment is indicated on or above each bar.





**Figure 3.5.** The productivity of *Gnaphalium californicum*, a native forb, and *Carduus pycnocephalus*, an exotic thistle, when grown in soil cultured by native (filled circles) and exotic (open circles) host communities over a species richness gradient. Linear curves through the means indicate a weak negative relationship for the native plant (panel A) grown with native host inoculum ( $P = 0.0692$ ) and no significant relationship when grown with exotic host inoculum ( $P = 0.6005$ ), however, the overall interaction between host community origin and species richness was significant (see text). The exotic plant (panel B) exhibited improved growth in exotic soil communities relative to its performance with native soil communities. The negative relationships to host species richness differ significantly from zero and have nearly identical slopes. Note that the scale magnitudes and ranges differ between the two vertical axes.

## DISCUSSION

This experiment provides support that soil microbial communities can mediate the success of exotic plant species in southern California. Specifically, exotic host communities appear to have changed the structure of the soil community in a manner that increased the success of the exotic *C. pycnocephalus* and decreased the performance of the native *G. californicum*. Conversely, *G. californicum* grew best with soil microbes from native host communities; the same microbes that most inhibited the exotic *C. pycnocephalus* (Fig. 3.4). These distinct growth patterns are consistent with the performance expected if the community structure of soil mutualists has degraded due to vegetative history.

The component of the soil microbial community most likely mediating the growth responses are the mycorrhizal fungi. This experiment was specifically designed to test for mycorrhizal effects, but the significant differences in inoculum mineral levels allows for the possibility of abiotic rather than biotic growth responses. However, we controlled for abiotic effects by using a small volume of inoculum within a much larger volume of homogenous sterile soil, and further mitigated any nutrient effects with supplemental fertilizer. All mineral predictors lacked significance in the analysis, suggesting an abiotic mechanism is unlikely.

The mycorrhizal colonization assay provides additional evidence that mycorrhizal fungi are a likely biotic mechanism. This measure of mycorrhizal inoculum potential yielded higher root colonization in live versus killed inoculum, with the overall colonization pattern similar to the *G. californicum* growth response pattern. Consistent with expectations if mutualists are being degraded, soil from native host communities

generated the highest levels of colonized roots; an increase of 33% over exotic-induced root colonization (Fig. 3.2). Effects from other soil biota cannot be ruled out in the *G. californicum* and *C. pycnocephalus* assays, but the results from the three assays considered together argue for the more parsimonious mycorrhizal mechanism.

Distinct ecologies between these particular native and exotic species are revealed in part by the significant species richness effects, which occurred as interactions or main effects, depending on the assay (Figs. 3.3, 3.5). The interaction between host community type and host species richness observed in the mycorrhizal colonization assay (Fig. 3.3) is an expected pattern. From the soil mutualist perspective, inoculum potential should vary with host community, possibly due to ecological specificity (McGonigle and Fitter 1990, Ronsheim and Anderson 2001), host preferences (Bever et al. 1996, Zhu et al. 2000), or perhaps host specificity (Eom et al. 2000). In native communities, increasing species richness creates a greater likelihood of including a preferred host, which may account for the improved inoculum potential observed. Conversely, exotic inoculum potential declines with increasing species richness, probably because the likelihood of including unsuitable hosts increases with each additional exotic plant. Host preference effects may also be additive by species, and would thus be another mechanism by which inoculum potential could be increased or degraded. Regardless of whether the actual mechanism is due to inclusion probability or to additive host effects, these patterns suggest exotic and native species can interact differently with common mutualists, and the results are consistent with expectations from the degraded mutualist hypothesis.

What is less clear, however, is the significant interaction between host community type and host species richness observed in *G. californicum*. The inoculum

potential results suggest *G. californicum* performance should also increase with increasing species richness. Yet, performance declined with increasing native species richness in this native indicator, which appears inconsistent with the degraded mutualist hypothesis. Host-specific pathogens may explain this inconsistency. *G. californicum* grew in nearly all species rich communities, but occurred in only 20% of communities with one or two species. Thus, root pathogens specific to *G. californicum* (or related taxa) may have accumulated in high richness treatments, and would be expected to generate negative feedback (Bever 1994, Mills and Bever 1998, Matthews and Clay 2001). Regression analysis using the frequency of *G. californicum* as a predictor does reveal a negative slope, but overall, only weak evidence for negative frequency dependence exists. Increasing exotic species richness had no apparent effect on *G. californicum* performance, although the inoculum potential results suggest a negative relationship will occur. However, the independent relationship is not inconsistent with degraded mutualist viability, if other ecological differences remain between the native and exotic host plants.

Another inconsistency occurs in the exotic indicator, *C. pycnocephalus*, where performance declines with increasing exotic host species richness (Fig. 3.5). A facultative relationship seems evident for this species (Fig. 3.4B), and under the facultative condition, forming the mycorrhizal symbiosis exacts a cost. Thus, *C. pycnocephalus* performance should improve with increasing exotic host species richness to the extent that exotic plants invest less in their mycorrhizae, as expected from the mycorrhizal colonization assay. As with *G. californicum*, host-specific pathogens may be generating negative feedback, which is suggested by the negative slope observed in

the exotic host treatments, but no statistical evidence supports this explanation. The negative slope observed with increasing native species richness is expected, however, if native species increase the inoculum potential. Overall, *C. pycnocephalus* performed best in soil from exotic communities. This observation, along with the strong negative relationship observed with increasing species richness of the native host inoculum, is expected if exotic species have failed to maintain viable soil mutualists.

The ecological differences between *G. californicum* and *C. pycnocephalus* may be due in part to growth trade-offs. The exotic *C. pycnocephalus* grows faster than the native *G. californicum* (Fig. 3.4). The facultative habit of this exotic species may trade-off rapid growth for decreased mycorrhizal investment (Smith and Read 1997). This trade-off may become especially beneficial to the slower growing *G. californicum*, which exhibits an obligate relationship to its AM fungi (Fig. 3.4). The increased mycorrhizal investment by the native not only improves its access to nutrients, but may also inhibit the success of competing species exhibiting less dependence. Janos (1980) has theorized that in nutrient poor soils capable of supporting mycorrhizae, obligate mycotrophs should dominate. Data reported here are consistent with Janos' prediction.

This study demonstrates how a positive feedback mechanism between plants and their soil mutualists can be initiated. The vegetation community can alter the structure of the soil community, and this altered soil community can influence host performance. In natural communities characterized by high mycorrhizal dependency, such as the Mediterranean-type ecosystems of California (Bethlenfalvay et al. 1984), disturbance induced introductions of exotic species less dependent on soil mutualists can fail to support those same mutualists. This degraded mutualist community can facilitate

additional exotic invasions while also limiting native species performance. These dynamics could maintain exotic plant dominance, and produce long term patterns of stability. In Chapter 2, we see evidence for how these dynamics actually contribute to the maintenance of exotic-dominated communities in California. Other mechanisms may also be important. For example, exotic grasses are known to limit native performance by competing for soil water (Eliason and Allen 1997). This suggests native plant communities in Mediterranean-type ecosystems are prone to multiple mechanisms that negatively impact native community structure. Indeed, these mechanisms may interact, but additional work is necessary to investigate this possibility.

## **CHAPTER 4: Native plant-microbe interactions improve ecosystem function: a test from California grasslands comparing native and naturalized exotic plant communities**

### **INTRODUCTION**

What ecosystem consequences result from exotic plants invading into native terrestrial plant communities? At the ecosystem level, researchers have identified multiple plant-induced impacts. These effects include accelerated fire cycles (D'Antonio and Vitousek 1992), local hydrological changes (Gordon 1998, Zavaleta 2000), and altered soil chemistry (Vivrette and Muller 1977, Kloot 1983, Ehrenfeld et al. 2001). Much less is known about how exotic plants interact with soil microbial communities, or the ecosystem consequences of these interactions. Nutrient enrichment is one possible outcome, as observed in some of the volcanic soils in Hawai'i. In this nitrogen-poor system, an exotic evergreen and its nitrogen-fixing mutualist can alter nutrient budgets to the detriment of the native plant community (Vitousek and Walker 1989, Walker and Vitousek 1991). Soil organisms mediate other large ecosystem effects, especially fundamental processes of nutrient cycling (Killham 1994, Vinton and Burke 1995), gas exchange (Hanson et al. 2000) and soil formation (Jenny 1941). At the plant community level, soil microorganisms are known to mediate plant competition (Allen and Allen 1990, Goodwin 1992, Marler et al. 1999) and community structure (van der Heijden et al. 1998). The species diversity, ubiquity, and ecological importance of soil microorganisms argue for understanding the effects of altered soil communities. This chapter considers whether soil aggregation, a property of soils with ecosystem-level effects, exhibits variable stability in response to changes in the soil microbial community. We show that soil microbes associating with native plant communities

increase the stability of soil aggregates more than soil microbes associating with naturalized exotic plants.

Soil particles coalesce into aggregates due to the complex interaction of mineral composition, soil texture, soil organic matter composition, climate regime, and soil biota (Tisdall and Oades 1982, Tisdall 1991, Jastrow et al. 1998, Wright and Upadhyaya 1999). The structural stability of soil aggregates influences important ecosystem processes such as soil erodibility, water infiltration, carbon storage, and nutrient cycling (Bryan 1974, De Ploey and Poesen 1985, Jasper et al. 1991). Soil aggregates between 0.25 mm and 2 mm (macroaggregates) are of particular interest because this size category is most sensitive to biological conditions and land management practices (Tisdall and Oades 1982, Oades 1984, Elliott 1986, Jastrow 1987). Fine roots, fungal hyphae, and a glycoprotein (glomalin) produced by mycorrhizal fungi are the major interacting biological factors creating stable macroaggregates (Tisdall and Oades 1982, Miller and Jastrow 1990, Oades and Waters 1991, Jastrow et al. 1998, Rillig et al. 2002), and factors that alter this stability can potentially have large ecosystem-level consequences.

Plant species introduced into new habitats can change the structure of their adopted plant community, often at multiple levels of biological organization (D'Antonio and Vitousek 1992, Gordon 1998). Altered plant communities often result from exotic plant invasions (Gordon 1998), and in the Mediterranean-type ecosystems of California, exotic-dominated plant communities are indeed structurally distinct from native-dominated grassland and shrub communities (Stromberg and Griffin 1996, Eliason and Allen 1997). Soil communities may also be affected, and within the soil matrix,



microbial communities may associate directly with specific plant traits. Bacterial communities, for example, can be differentially structured by co-occurring plant species (Westover et al. 1997) and even by different genotypes within a species (Neal et al. 1973). Communities of obligate symbionts such as arbuscular mycorrhizal (AM) fungi are well known to vary with plant host attributes (Johnson et al. 1992, Sanders and Fitter 1992, Bever et al. 1996, Eom et al. 2000).

Evidence that invading exotic plants are associated with changes in AM fungal community composition or inoculum potential suggests ecological consequences related to mycorrhizal communities also exist. For example, in previously uninvaded plots located in northern California and Utah, the invasion of exotic Brome grasses resulted in altered AM fungal communities (C. Hawkes, personal communication). Moreover, in Chapters 2 and 3 we report evidence of reduced AM fungal densities associated with exotic species, and a new mechanism by which native species recruitment could be inhibited. Thus, soil stabilization processes mediated by AM fungi may be particularly susceptible to these altered AM fungal communities.

We explored natural patterns of soil aggregate stability and AM fungal density as correlates to native and exotic plant communities in a southern California grassland. We then investigated the processes related to soil aggregate formation and stability by asking two questions. (1) Does soil aggregate stability vary in response to inoculum source? Testing this first question establishes the soil aggregating function of soil microbial communities as a general phenomenon distinct from abiotic soil aggregating processes. (2) Can soil microbial communities associating with native and exotic host plant communities differentially alter the stability of soil aggregates? This second question

follows from the general phenomenon addressed by the first question, but focuses specifically on whether soil aggregate stability relates to an altered plant community.

We addressed these questions with two pot experiments.

In this chapter, we show:

- Soil aggregate stability increases the most when native soil microbes are used to inoculate the soil.
- Native soil microbes are associated with greater mycorrhizal colonization.
- Native-dominated plant communities associating with native soil microbes result in improve soil aggregate formation and stabilization.

## **MATERIALS AND METHODS**

### *Overview of study system*

This investigation used microbial inocula and soil aggregates collected from coastal sage scrub and coastal grassland communities around Orange County, California, U.S.A. Summer droughts characterize this Mediterranean-type ecosystem, with an average of 312 mm of rain distributed only during a few winter months. Temperatures range from 7 to 19 °C in January to 17 to 28 °C in July. Soil for a field survey of soil aggregate stability originated from the University of California, Irvine Ecological Preserve (UCIEP). We collected a clay loam topsoil classified as an Aridic Haploxerert from a construction site adjacent to the UCIEP. We used this clay loam as our growth medium for a field mesocosm and two greenhouse experiments. For the mesocosm experiment, the treatment inoculum originated from native and exotic-dominated plant

communities at Starr Ranch Sanctuary and the UCIEP. For one greenhouse experiment, we used commercially available AM fungal inoculants in addition to live soil.

#### *Field survey of native and exotic plant communities*

Within the 24.3 ha UCIEP, we identified areas where the plant communities were dominated by naturalized exotic species or native species. We stratified five of these sites into exotic-dominated and native-dominated plant community types, and paired these five sites by slope, exposure, substrate and proximity so that they differed only by vegetative community. Soil samples were collected along an elevational gradient from each of the plant community types within each of the five sites for a total of 10 paired samples. This survey occurred in May, 2002 after the cessation of winter rains and field soils were dry. From these samples we assayed the inoculum potential and measured the stability of soil aggregates as described below.

#### *Treatment inocula for greenhouse soil aggregation experiment*

Six of the soil microbial communities used for live inoculum consisted of either non-amended bulk sources (such as topsoil) or were specifically cultured from soil communities that originated as a bulk source. The non-amended bulk sources were as follows: (1) *Glomus intraradices* as Endonet, a commercially available mycorrhizal inoculant in a calcined clay medium (Endonet, Menifee, California); (2) Exotic Bulk, consisting of topsoil from exotic-dominated sites; and (3) Native Bulk, consisting of topsoil from native-dominated sites. These exotic and native sources were pooled from samples collected during UCIEP field surveys during October, 2000 (Exotic N = 6;

Native N = 6). The cultured sources include: (4) Endonet Culture, which originated from the commercial inoculant, but was grown in an autoclaved local soil; (5) Exotic Culture, which originated from exotic-dominated sites; and (6) Native Culture, which originated from native-dominated sites. These field soils were collected from exotic (N = 8) and native-dominated sites (N = 8) within Starr Ranch. Each native field site was paired with an adjacent exotic site that exhibited a similar soil type, and we used these samples as starter inocula to amplify total inoculum volume available for this and other experiments. Native grassland species that characterized the native sites include *Nassella pulchra* A. Hitchc. (purple needlegrass: Poaceae), *Nassella lepida* A. Hitchc. (foothill needlegrass: Poaceae), *Muhlenbergia rigens* (Benth.) A. Hitchc. (deergrass: Poaceae), *Gnaphalium californicum* DC. (everlasting: Asteraceae), *Isocoma menziesii* Hook. & Arn. (coast goldenbush: Asteraceae), *Artemisia californica* Less. (California sagebrush: Asteraceae), *Bloomeria crocea* Torr. (common goldenstar: Liliaceae), *Dodecatheon clevelandii* E. Green (Cleveland's shooting star: Primulaceae), and *Calochortus splendens* Benth. (Mariposa lily: Liliaceae). Exotic grassland species that characterized the exotic sites include *Avena fatua* L. (wild oatgrass: Poaceae), *Bromus diandrus* Roth (ripgut brome: Poaceae), *Bromus hordeaceus* L. (soft brome: Poaceae), *Lolium multiflorum* (Italian ryegrass: Poaceae), and *Carduus pycnocephalus* (Italian thistle: Asteraceae). Cultures began as 500 mL of the starter inoculum in a sterile mix of local soil and sand, and were then grown in pots with native grasses, legumes, composites, garlic, and other suitable host species. Following host senescence, we extracted the root ball and all soil from each pot. These materials were each diced, mixed, and pooled by culture type into their respective groups of Endonet, Exotic, or

Native. A seventh (7) microbial community that lacked AM fungi began as an aqueous solution containing all other inocula. We sieved this solution to exclude particles  $> 38 \mu\text{m}$ . We mixed the resulting filtrate into a carrier medium of autoclaved soil and sand. The eighth (8) treatment used autoclaved soil as a control. These eight inoculum types comprise microbial communities that vary in origin and preparation, and thus are likely to vary in the quality and quantity of soil aggregating microbes such as AM fungi. This design permits the use of a priori contrasts to compare soil aggregate stability by group; i.e., native versus exotic and AM fungi included versus AM fungi excluded.

#### *Greenhouse soil aggregation experiment*

This experiment assessed the effects of the microbial communities in each inoculum on soil aggregation. These microbial communities interacted with the roots of *Artemisia californica*, a native host species common throughout many southern California coastal sage scrub and grassland communities. We germinated seeds of locally collected *A. californica* in a sterile mix of vermiculite and peat moss. After eight weeks, we inoculated the roots of each seedling with 10 mL of one inoculum type for a total of 160 inoculated plants, from which a random subset of 10 seedlings from each of the eight treatment groups were later drawn for a total of 80 experimental units. We allowed four weeks for root colonization to occur prior to drawing the actual experimental units. All seedlings were fertilized with 25 mL phosphorus-free fertilizer (Plant Marvel Laboratories Inc., Chicago Heights, Illinois) diluted to 300 ppm nitrate to encourage AM fungal colonization of inoculated individuals.

The 80 *A. californica* seedlings selected as experimental units were transplanted into seedling cones (6.4 cm x 25 cm ; Stuewe & Sons, Inc., Corvallis, Oregon) filled with a sterile mix of the clay loam soil described above. Prior to use as our planting medium, this soil was sieved to exclude aggregates > 4.75 mm, amended 1:1 (by volume) with sand, and autoclaved at 121 °C. An additional set of 10 filled cones remained free of any inoculum or host plant in order to quantify aggregate stability degradation, but only replicates with a host plant were used for data analysis. All cones were completely randomized and placed in an isolated UCI greenhouse. We followed a 10 day rotation schedule throughout a 25 week growing period to mitigate effects due to bench position. All cones received 50 mL phosphorus-free fertilizer diluted to 300 ppm when plants exhibited symptoms of nutrient deficiency, which occurred approximately every fourth week.

We harvested all plant materials (roots and shoots) after 25 weeks of growth and collected all rhizosphere soil for analysis of soil aggregate stability. All watering ceased 10 days prior to harvest. Under certain conditions, soil moisture is known to be an important covariate to soil aggregate stability, thus prior to harvesting, we measured soil moisture using a TDR soil moisture probe (model CS620, Campbell Scientific Inc., Logan, UT). We then separated roots from their shoots, quantified fresh root mass, and subsampled each root ball for assessing mycorrhizal colonization (see below). The remaining portions of fresh roots and all shoot materials were oven dried at 65 °C and weighed. Soil moisture on air-dried aggregates was also determined gravimetrically in conjunction with wet-sieving procedures.

### *Mesocosm soil aggregation experiment*

We constructed discrete plant communities in nursery pots as part of a larger study on grassland plant/soil interactions (see Chapter 3). These mesocosms consisted of either native coastal grassland species or naturalized exotic species. These two community types each varied in species richness along a gradient of 1, 2, 4, 7, or 8 species. Each mesocosm community type contained monocultures of all eight species from their respective native or exotic species pools, with each monoculture replicated four times. For species richness treatments 2 and 4, we generated random combinations from each native and exotic species pool with the constraint that all species must be equally represented across a given combination. Each combination was replicated twice. We systematically excluded each species from species richness treatment 7 to form eight possible combinations, each of which was replicated twice. We used all species to create species richness treatment 8 and the resulting native and exotic communities were each replicated eight times.

We distributed the mesocosm pots (36.8 cm diameter  $\times$  38.1 cm height; 40 L capacity) evenly over a 200 m<sup>2</sup> area nested within a 400 m<sup>2</sup> fenced enclosure. We filled each pot to 75% capacity with a 1:1 homogeneous mixture of washed sand and the fraction of the clay loam soil described above that passed through a 1.3 cm sieve. Prior to installing the experimental communities, we regularly watered each pot to induce seed germination and exhaust the seed bank. These weeds were promptly removed by hand, and we continued this management regime for eight weeks. To reestablish AM fungi following seed bank exhaustion, we inoculated each pot with 225 mL of live soil that we cultured from Starr Ranch soil samples. These samples originated from an undisturbed

area of Starr Ranch characterized by native perennial coastal grassland species and thought to be representative of the historic biotic community. We capped the inoculum with a 4 cm layer of sterile soil and sand, and then planted eight seedlings into each pot to complete the mesocosms. Pots were hand watered as necessary to supplement natural precipitation.

#### *Assessing mycorrhizal inoculum potential*

Percent mycorrhizal colonization can be used to compare inoculum quality when known fungal structures such as arbuscules and vesicles are counted (Smith and Read 1997). We used percent colonization to estimate the density of the mycorrhizal communities (and thus the inoculum potential) from our field survey samples, our greenhouse soil aggregation experiment, and our mesocosm soil aggregation experiment. For both our field survey and mesocosm soil aggregation experiment, we compared the colonization intensity of a common host plant grown in a common environment with similar dilutions of the inocula. For the field survey, we used 10 mL of the field soil as the inoculum, and for the mesocosm experiment, we used 10 mL of the mesocosm soil as the inoculum. These inocula were mixed with 100 mL of autoclaved field soil amended 1:1 with sand, distributed into 125 mL “Conetainer” pots (Stuewe & Sons, Inc.), and seeded with Sudan grass (*Sorghum bicolor*). For the field survey, we replicated each treatment five times, and for the mesocosm experiment, each Conetainer corresponded directly to one mesocosm, thus preserving the original replication. We harvested each experiment after 3 weeks, washed the roots, cleared subsamples with 10% KOH, and stained with trypan blue (Brundrett et al. 1994). We assessed colonization by AM fungi



visually at 400X magnification according to standard methods (McGonigle et al. 1990). For our greenhouse soil aggregation experiment, we subsampled approximately 0.5 g of fresh root material from each *A. californica* host plant at the end of the experiment, and these roots were cleared, stained, and scored for AM colonization as described above. For this study, we focused specifically on the percent mycorrhizal colonization of the native and exotic bulk topsoil treatments.

#### *Wet-sieving to determine water stable aggregates*

We dry sieved air-dried soil samples from our field samples, our greenhouse soil aggregation experiment, and our mesocosm soil aggregation experiment to capture macroaggregates greater than 1 mm and less than 2 mm. Approximately 8 g of these macroaggregates were loaded onto 7.6 cm diameter sieves (500  $\mu\text{m}$  mesh size) and vapor-wetted in a vaporizing chamber for 45 minutes. After hydration, we agitated all samples in a wet-sieving apparatus for 10 minutes (stroke length 1.3 cm, 35 cycles  $\text{min}^{-1}$ ) as described by Kemper and Rosenau (1986). Receiving cans below the test sieves collected disaggregated soil particles. We used the first agitation cycle to determine the unstable fraction, and a second agitation cycle to separate the water stable fraction from the sand remaining in the sieve. Disaggregation in the second cycle was promoted by adding a 1M solution of NaOH (10 mL) to each receiving can. Both fractions were transferred into stainless steel evaporating pans and oven dried at 110 °C to a constant mass. Estimates of aggregate stability are calculated as a percentage of the mass of stable to unstable particles after excluding the sand:  $Y' = 100 \times (\text{WS} / (\text{WU} + \text{WS}))$ . We repeated these procedures for each sample on sieves with a 250  $\mu\text{m}$  mesh size. For this

smaller mesh sieve, we increased the agitation time to 20 minutes to produce sufficient recoverable material. All procedures were performed under ambient temperature conditions.



**Figure 4.1.** Soil samples loaded onto to wet sieving apparatus are assessed for their stability in water.

### *Data Analysis*

We analyzed the inoculum potential data for our field survey of native and exotic plant communities using a two-way ANOVA with community type and site as main effects. We performed this test over both the error variance and the variation among individual sampling sites to assess the generality of the response. We used a conservative approach to scoring colonization to reduce the inclusion probability of saprophytic and other non-mycorrhizal fungi; only root sections exhibiting characteristic mycorrhizal structures such as arbuscules, vesicles, and coiled hyphae inside root cells were included. We performed a separate regression analysis on the response variable significant from the MANCOVA model (see above) to evaluate the relationship of aggregate stability with percent mycorrhizal colonization. We used SAS for all statistical analyses (SAS 1999).

Water stable aggregate data for the greenhouse soil aggregate experiment were analyzed using a multiple one-way analysis of covariance (MANCOVA) followed with a series of a priori orthogonal contrasts (Littell et al. 1991). This approach was desirable to preserve independence among comparisons. We used backward elimination to identify and exclude non-significant ( $P > 0.10$ ) covariates such as shoot productivity, soil moisture, and percent sand. Dry root mass remained in the final model. Soil aggregate data were arcsine transformed prior to analysis to satisfy MANCOVA assumptions. We also compared mean root, shoot, and total productivity with a separate MANOVA.

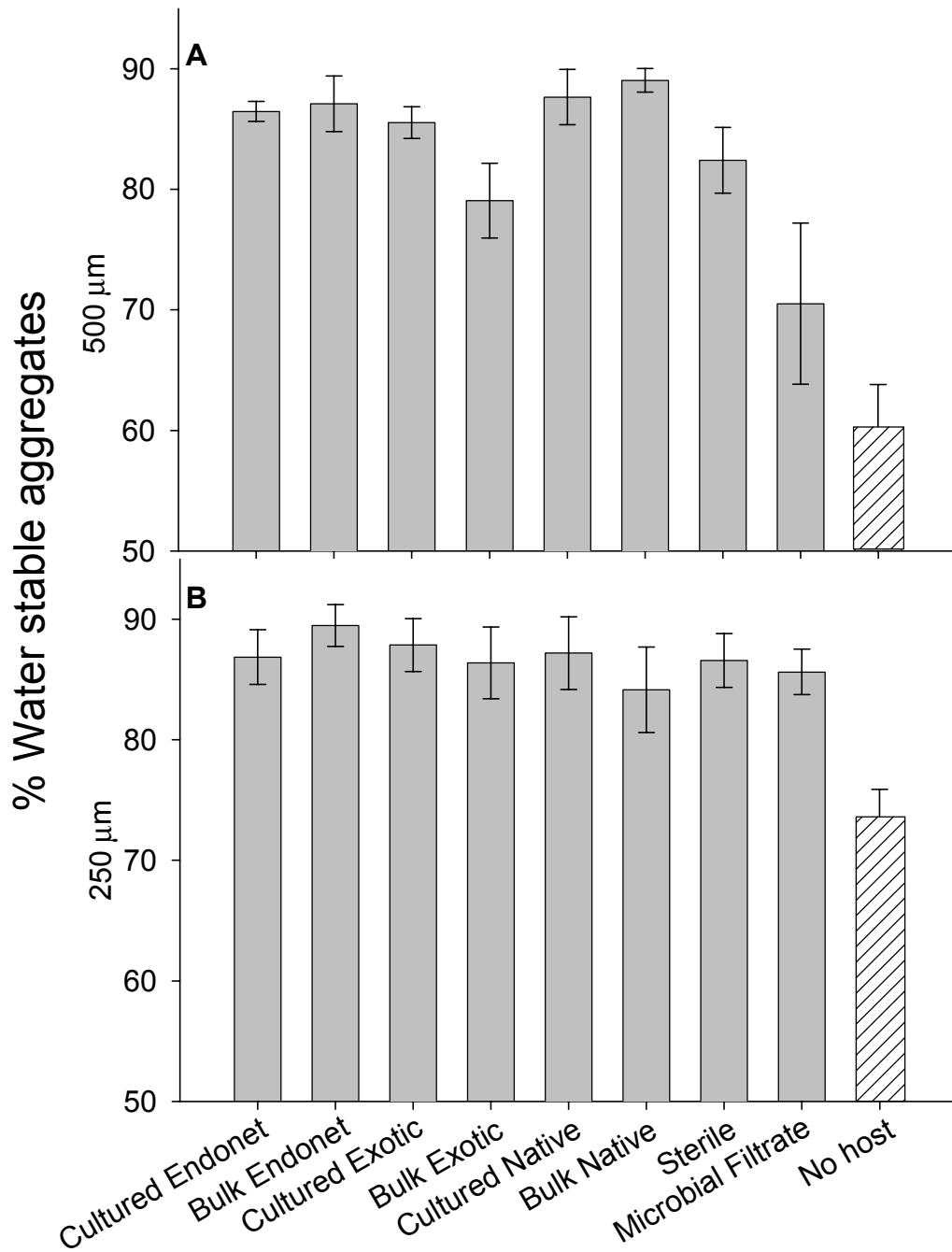
Water stable aggregate data for the mesocosm soil aggregate experiment were analyzed using a two-way MANCOVA. We analyzed for the main effects of species

richness and community type using soil aggregate and percent colonization as response variables. We included the above-ground productivity from the first year as a covariate.

## RESULTS

### *Soil aggregate stability*

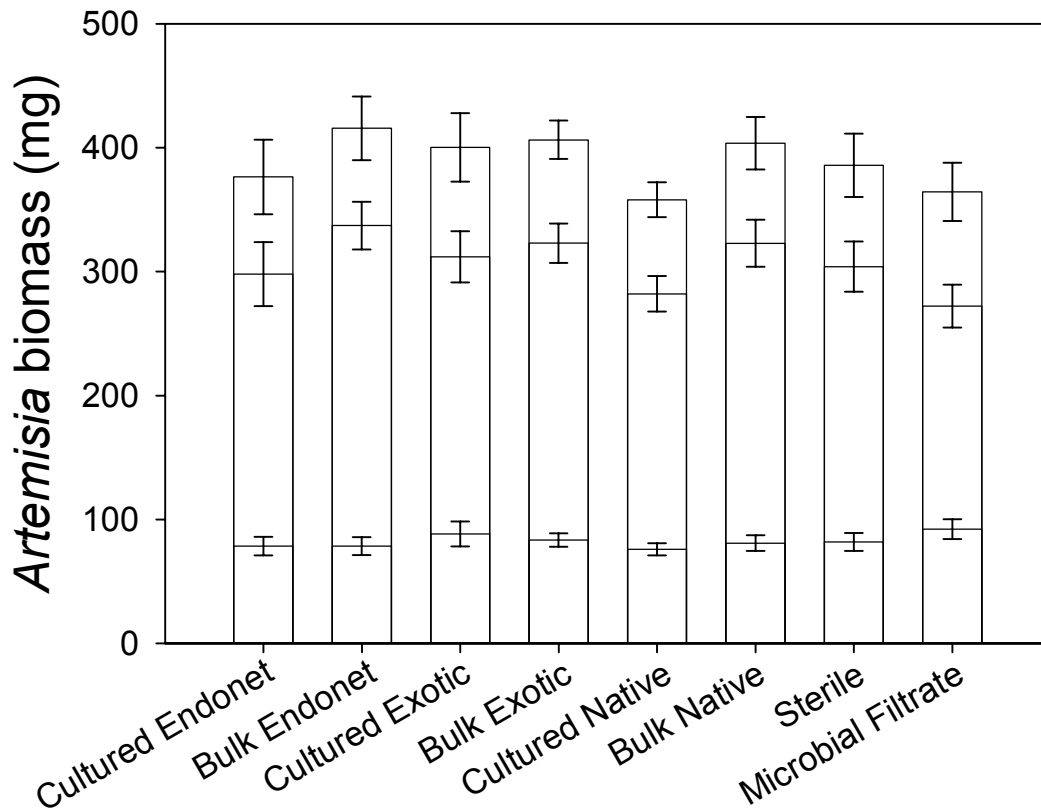
*A. californica* seedlings treated with bulk or cultured native inoculum resulted in the greatest increase in soil aggregate stability for aggregates greater than 500  $\mu\text{m}$ . For these macroaggregates, a priori contrasts were significantly higher for the native inocula than for the exotic inocula ( $F_1 = 5.66$ ;  $P = 0.0201$ ), indicating that one or more of the components of soil community structure (species composition, diversity, or propagule density) can influence function beyond that achieved with microbial presence alone. The same sized aggregates processed on the smaller 250  $\mu\text{m}$  sieves revealed no overall treatment differences. The significant MANCOVA on these two response variables (Wilks'  $\lambda = 0.710$ ,  $F_{14,140} = 1.87$ ,  $P = 0.0347$ ) followed from the effects of the largest macroaggregates. Overall soil aggregate stability increased in treatment groups with AM fungi compared to the sterile and the microbial filtrate control (Fig. 4.2). This significant increase ( $F_1 = 11.18$ ;  $P = 0.0013$ ) occurred in the absence of any significant productivity differences among treatments (see Fig. 4.3). Dry root mass covaried negatively with aggregate stability ( $P = 0.0722$ ). The non-mycorrhizal sterile control exhibited enhanced aggregate stability relative to the non-mycorrhizal microbial filtrate ( $F_1 = 5.00$ ;  $P = 0.0284$ ).



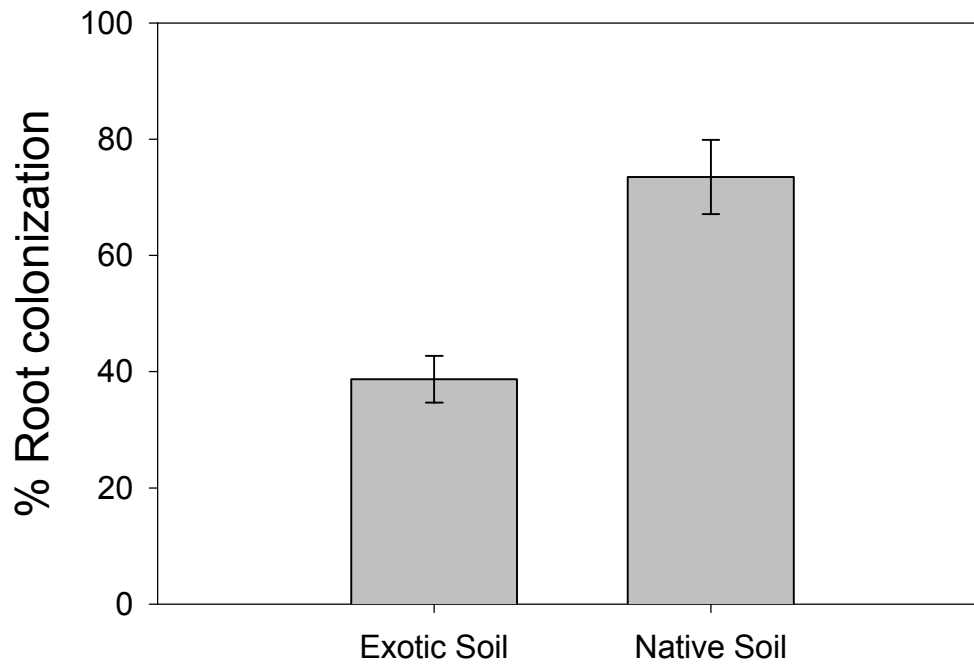
**Figure 4.2.** Mean soil macroaggregates remaining stable after agitating in water on 500 µm (panel A) and 250 µm (panel B) mesh sieves. Aggregates were dry sieved to 1-2 mm diameter and pre-wetted in a vapor chamber prior to wet sieving. On 500 µm sieves, soil organisms derived from native host communities (cultured and bulk) produce a significantly higher percentage of stable aggregates than soil organisms from exotic host communities. No differences appear between any communities on the smaller 250 µm sieves. The hatched bars indicating aggregate stability degradation in the absence of host plants or inoculum were not included in the analysis. Error bars represent  $\pm$  SE .

### *Mycorrhizal colonization*

Roots sampled from the bulk native soil treatment exhibited greater mycorrhizal colonization than roots from the bulk exotic soil treatment ( $t_{18} = 4.23$ ;  $P = 0.0005$ ) (Fig. 4.4). These differences indicated the presence of community-level changes between the native and exotic mycorrhizal communities. Regression analysis on these data revealed a significant positive relationship between the water stability of aggregates  $> 500 \mu\text{m}$  in diameter and percent mycorrhizal colonization ( $N = 20$ ,  $b = 0.30$ ,  $P = 0.0219$ ; Fig. 4.5).

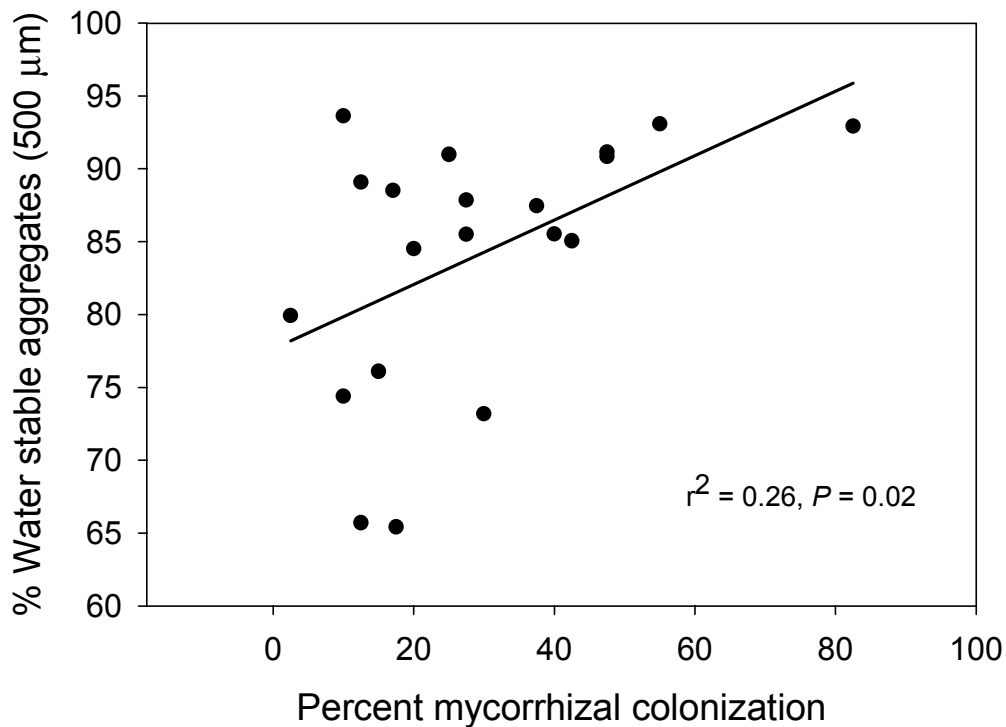


**Figure 4.3.** Productivity of the California sagebrush host species at harvest. Values are means  $\pm$  SE for roots (low bars), shoots (middle bars) and total biomass (top bars). No significant differences in productivity occurred due to soil treatment.



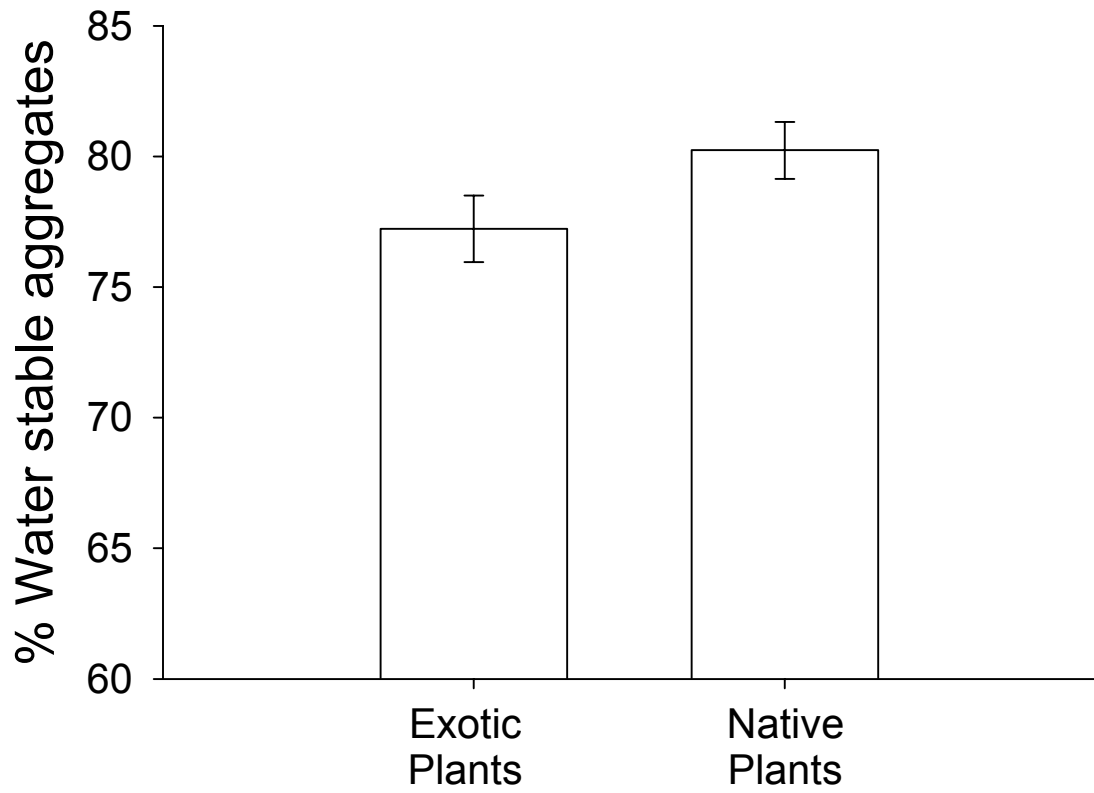
**Figure 4.4.** Arbuscular mycorrhizal root colonization in California sagebrush (*Artemisia californica*) after associating with topsoil used as inoculum. Seedlings were inoculated with soil pooled from multiple native or exotic host communities collected from naturalized sites, and then grown for 25 weeks in a greenhouse. Roots associating with bulk native topsoil exhibited greater mean mycorrhizal colonization than roots associating with bulk exotic topsoil (means  $\pm$  SE).



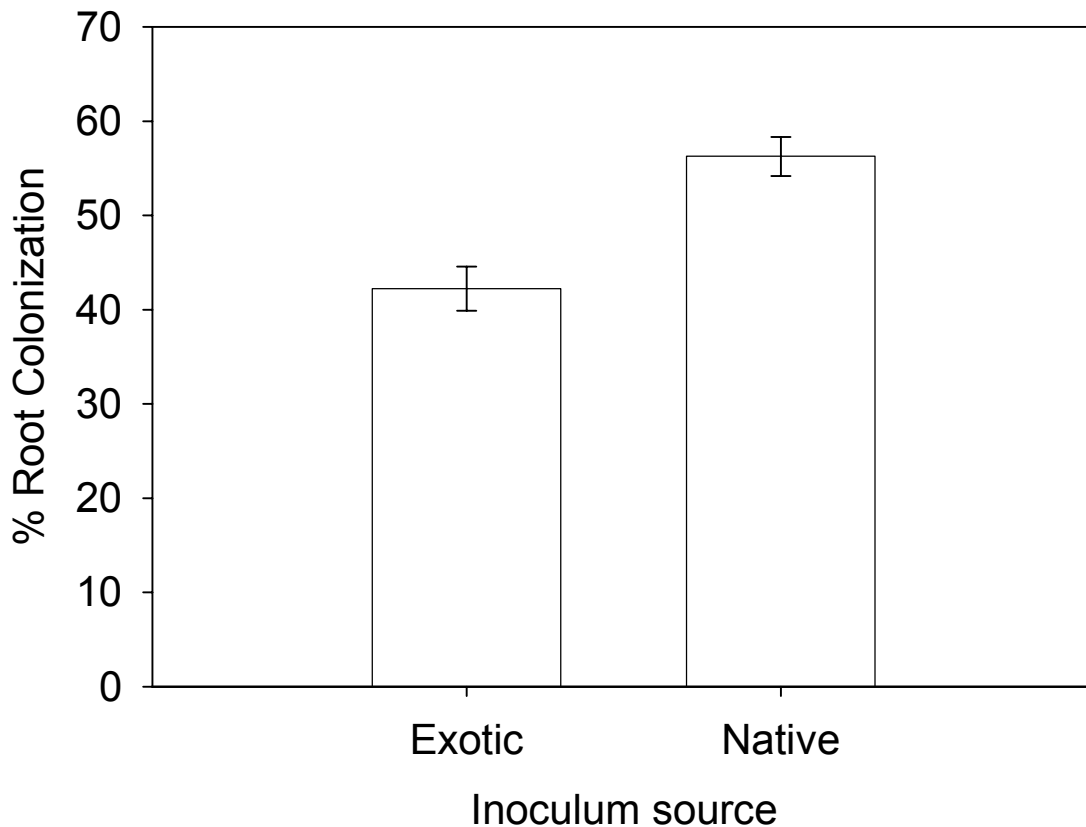


**Figure 4.5.** Percent water stable macroaggregates regressed over percent mycorrhizal colonization for native and exotic topsoil treatments (N = 20). Colonization occurred on California sagebrush grown for 25 weeks in a greenhouse.

The mesocosm soil aggregation experiment produced results similar to the greenhouse soil aggregation experiment with respect to the native plant/microbe interactions. After one growing season, pots containing native plants exhibited improved soil stability ( $F_{1,153} = 8.4, p = 0.0042$ ; Fig. 4.6) and this effect was due to community type, not species richness. Productivity from the first year covaried positively ( $F_{1,153} = 4.3, p = 0.0395$ ), as expected given the physical function of plant roots in soil aggregate formation and stabilization. Percent colonization of sorghum roots induced by mesocosm soil also exhibited significant significant effects of community type ( $F_{1,153} = 10.5, p = 0.0015$ ; Fig. 4.7). Pots with native plants clearly produced more viable mycorrhizal communities.



**Figure 4.6.** The difference in soil aggregate formation and stabilization after 1 growing season. Native plants, due to their stronger association with AM fungi, promote improved soil stability.



**Figure 4.7.** Arbuscular mycorrhizal colonization of sorghum roots after associating with native or exotic mesocosm communities for 1 year (means  $\pm$  SE). Native plants promote AM fungal communities, and as a consequence, these plant/microbe interactions promote improved soil stability (see Fig. 4.6).

## DISCUSSION

Soil microbial community structure can directly influence a physical soil property such as aggregate stability. These results demonstrate the importance of soil organisms in general and AM fungi in particular with respect to altering the stability of macroaggregates greater than 500  $\mu\text{m}$ . In other systems, mycorrhizae have been identified as critical components of the stabilization process (Jastrow et al. 1998, Bethlenfalvay et al. 1999, Rillig et al. 2002). In this chapter, we found that soil communities from intact native grassland sites increased aggregate stability when grown with a common native host. This increase occurred relative to the sterile control, the

microbial filtrate control, and the soil communities from exotic-dominated sites. Moreover, we found that this effect can arise independent of any particular host species and appears to be a general phenomenon of plant species native to California. In the greenhouse soil aggregation experiment, aggregate stabilities increased in all treatments with a host plant, the increases observed from exotic topsoil were 12% less than the increases in native topsoil, with less of a disparity (2%) between the cultured exotic and native inocula (Fig. 4.2). It is important to note, however, that the orthogonal analysis pooled the two exotic treatments together to make a linear comparison with the two pooled native treatments. Thus, the differences reported here are more conservative than a post hoc analysis focused just on the differences between the exotic and native bulk soil treatments. In the mesocosm soil aggregation experiment, aggregate stabilities changed significantly after just one year of growth. The physical soil matrix and the soil microbial communities were common to all pots upon initiation of the experiment, thus the changes observed after one year were due to the associations formed by the plant communities.

Ecosystem function, as measured by the water stability of these soil macroaggregates, appears directly related the structure of the soil microbial community. Specifically, AM fungi are likely to be the guild responsible for this function. Though contributions from other organisms cannot be ruled out, treatments where AM fungi were excluded contrast strongly with those treatments that included AM fungi, with increased stability associated with AM fungal presence ( $p = 0.0013$ ). The differential mycorrhizal root colonization on the California sagebrush is also consistent with the observed aggregate stability patterns, and suggests that AM fungal abundance in the

native soil was likely the major structural change. The regression analysis on the macroaggregates greater than 500  $\mu\text{m}$  provides additional evidence that changes in AM fungal abundance can alter ecosystem function. Whether these mycorrhizal communities were altered directly by the exotic hosts is not clear; the observed structural changes in the soil communities may be associated with exotic species for some other reason. Disturbance history, for example, can alter the structure of mycorrhizal communities (Moorman and Reeves 1979, Jasper et al. 1991, McGonigle and Miller 1993).

#### *Plant productivity effects*

Given the importance of roots in stabilizing macroaggregates, we would expect a positive relationship with respect to root biomass, yet in the greenhouse soil aggregation experiment, the observed function appears independent of host plant productivity in this system. Shoot productivity was independent of aggregate stability and root productivity exhibited a negative relationship. In contrast, Rillig et al. (2002) used five plant species in a California grassland field experiment and found strong positive above and below ground productivity effects on aggregate stability. Similarly, we found positive effects of productivity in our mesocosm soil aggregation experiment. The reason for this disparity is unknown. Perhaps roots and soil aggregates interact differently when constrained by small pots than under field conditions. However, other investigations using containerized roots have also produced positive relationships, as observed in a northern California mesocosm system that used PVC tubes (Eviner and Chapin 2002). The relationship between productivity and soil aggregation seems more likely to be a host-specific phenomenon, or one determined by an interaction between plant host, soil

microbial community, and soil type. Eviner and Chapin (2002) report direct plant effects on soil aggregate stability, along with strong host-specific effects on bacterial biomass, fungal length, and plant biomass. Moreover, Eviner and Chapin (2002) use one exotic grass (*Bromus hordeaceus*) and four native forbs (*Plantago erecta*, *Lasthenia californica*, *Hemizonia congesta*, and *Lotus wrangelianus*) and found the lowest aggregate stabilities associated with the exotic species—results consistent with the present study. Although the present study differs in important methodological ways, our focus on the structure of the soil community itself suggests that altered ecosystem function can arise directly from an altered mycorrhizal community, and indirectly from the effects of host plant community. We are aware of one other study that suggests the structure of the soil community can affect soil aggregate stability (Requena et al. 2001). However, the investigation by Requena et al. (2001) does not clearly distinguish productivity effects from soil community effects.

#### *Non-mycorrhizal effects*

We expected lower levels of aggregate stability in both the sterile and microbial filtrate treatments because mycorrhizal fungi were excluded from these groups. Yet, the contrast between these treatments indicates enhanced stabilization in sterile soil (Fig. 4.2A). Growth of saprophytic fungi likely explains the difference in aggregate stabilization observed in these two treatments, since saprophytic fungi also exhibit soil cementing properties (Tisdall et al. 1997). As a ubiquitous component of most greenhouses, saprophytic fungi were likely to colonize throughout the experiment, and were observed on the soil surface in several replicates. In the microbial filtrate pots,

inoculation with soil bacteria likely competed with these saprophytes and thus may have limited the fungal contribution to aggregate stability.

Aggregates smaller than 500  $\mu\text{m}$  in diameter in this system exhibited stabilities characteristic of microaggregates. We observed no treatment effects for 250  $\mu\text{m}$  aggregates, even though we doubled the agitation cycle in the wet-sieving apparatus. Stability tends to increase in microaggregates, which are those smaller than 250  $\mu\text{m}$  (Tisdall and Oades 1982, Oades and Waters 1991). Microaggregates are influenced more by encrusted plant materials and clays rather than the roots and hyphae that stabilize macroaggregates (Oades and Waters 1991).

#### *Ecological consequences*

Many ecological studies have long focused on competitive aspects of successful exotic plant invaders, but increasingly, there is a need to understand how exotic species alter fundamental ecosystem processes. These altered processes may be readily apparent, as seen in fire-prone grasslands (D'Antonio and Vitousek 1992), or less conspicuous, as seen in some deciduous forest soils where exotic species alter nutrient cycling and soil pH (Ehrenfeld et al. 2001). Though often overlooked, the role of exotic invasions on soil communities and soil ecosystem function is no less important than understanding mechanisms of species introduction. Soil erosion, for example, is associated with many negative large-scale environmental impacts such as nutrient loss, waterway eutrophication, soil organic matter loss, and productivity declines (Pimentel and Kounang 1998). In nearly one third of U.S. croplands, erosion rates still exceed sustainable losses (USDA 2000). If the soil flora associating with exotic-dominated

plant communities are less effective soil stabilizers, then these interactions would have clear implications for landscape-level processes such as soil erosion. There is a considerable need to test for this possibility, and to identify those systems susceptible or resistant to the effects of altered host communities. Although processes that alter the structure or function of soil microbial communities are not identified here, the ability of the plant community to maintain viable soil mutualists is likely to be an important factor.

Individually and as a group, both native treatments exhibited the greatest macroaggregate stability. Native topsoil also resulted in greater mycorrhizal colonization than exotic topsoil on California sagebrush, a common native host species. Native plant grown in a community also produced improved soil stability compared to weedy exotic plants grown in a community. These results suggest that host community type can serve as a proxy for an inconspicuous but potentially important ecosystem function. Host community type may include community composition and structure, species abundance, or in this case, the origin of the dominant species. Native communities that become dominated by exotic species could signify underlying shifts in the structure and function of the soil community. Thus alteration of water infiltration, erodibility, and soil carbon sequestering could be anticipated, since these functions are known to relate to aggregate stability (Bryan 1974, De Ploey and Poesen 1985, Jastrow and Miller 1991). Land management and restoration efforts focused on the functional aspects of exotic species relative to native flora should have a clear interest in this potential.



## **CHAPTER 5: A field test of the importance of mycorrhizal inoculation on revegetation and soil stability**

### **INTRODUCTION**

Soil disturbance, such as that caused by road construction, degrades soil communities. As soil communities play an important role in the maintenance of plant communities (Wardle 2002, Bever 2003) and in soil aggregation (Jastrow and Miller 1991), the degradation of soil communities can impair revegetation efforts and increase susceptibility to erosion.

For both difficulty in revegetation and erosion susceptibility, the degradation of the mycorrhizal community is of particular concern. Mycorrhizal fungi can play an important role in plant establishment in disturbed environments (Medve 1984, Allen and Allen 1990, Gange et al. 1990) and in maintaining plant species diversity (Grime et al. 1987, Van der Heijden et al. 1998, Bever 2002). Mycorrhizal fungi also play an important role in resistance to soil erosion, both through physically enmeshing of soil aggregates in fungal hyphae and through the production of a glue like substance, called glomalin that holds soil particles together (Jastrow et al. 1998, Wright and Updahyaya 1998). As a result, inoculation of disturbed areas with mycorrhizal fungi should have the twin benefits of increased establishment of native plants and the improved stability of soil aggregates. Improved establishment of natives in field inoculation trials has been observed in Midwestern prairies (Smith et al. 1998, Bever et al. 2003), but evidence in California is less direct.

In our laboratory experiments, we found that native plant species have greater dependence on mycorrhizal fungi than do exotic plant species and that native plant

species grow particularly well with soil mutualists derived from areas dominated by native plant species (Chapter 2, 3). On the other hand, exotic plant species grew better in sterile soil and in soil derived from exotic dominated sites (Chapters 2, 3). In Chapter 4, we report greater soil aggregation when native plant species associate with mycorrhizal fungi, particularly when the fungal community derives from native sites. Both of these effects suggest that inoculation in the field should result in a decrease in the erosion and decrease in maintenance of Caltrans roadcuts.

In the present experiment, we test for improved establishment of natives and improved aggregation in the field using a large well-replicated plot experiment. We test for the effect of inoculation in general and of different inocula sources (either local and diverse fungal cultures, or commercially available strains). We also test for two different approaches to inoculation: direct inoculation with field soil and prior culturing of inocula. Our experiment takes place on a recapped landfill.

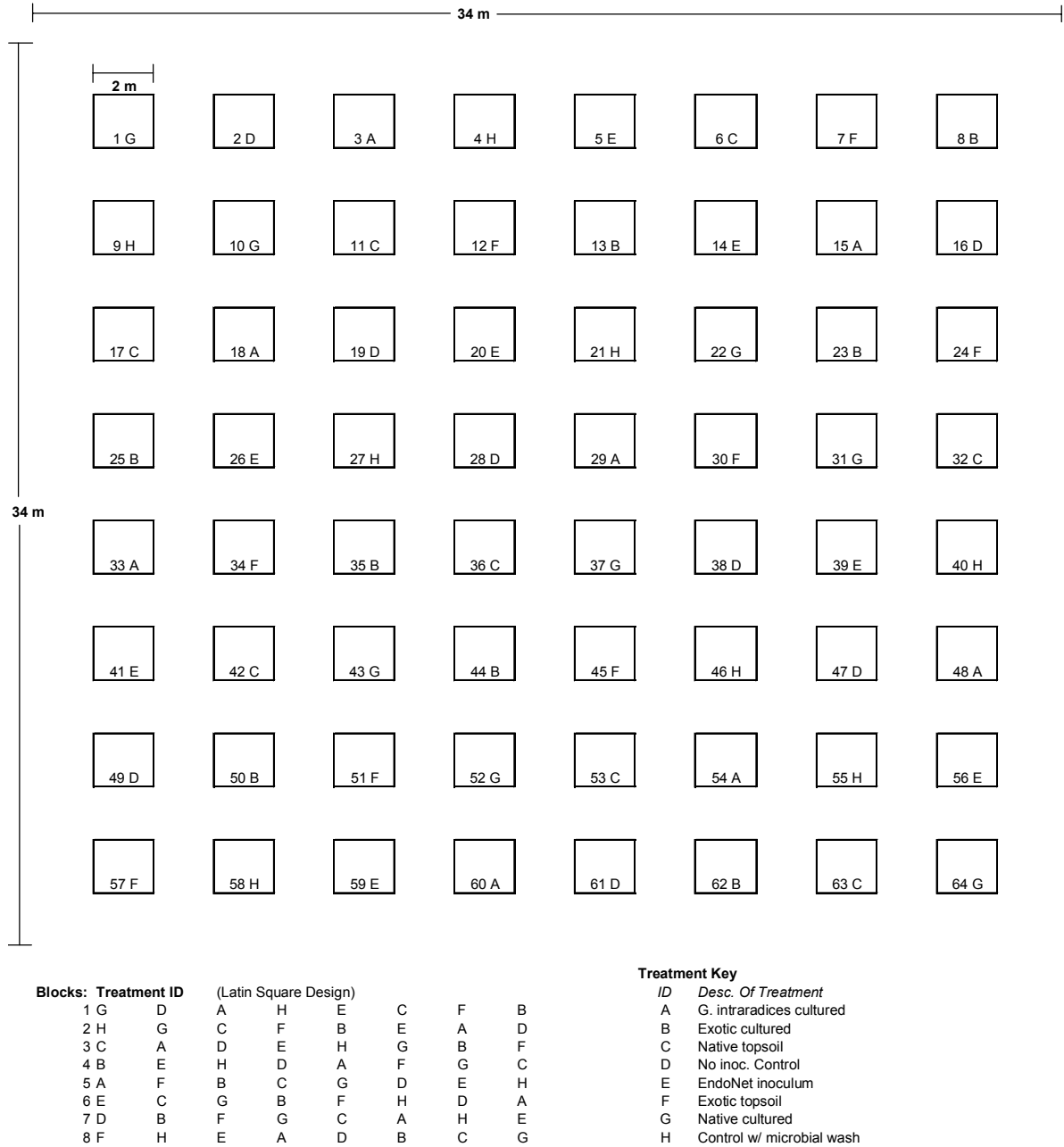
Our major findings are:

- Native plant species tended to have higher cover with mycorrhizal inoculation.
- Soil aggregate stability also increased with mycorrhizal inoculation.
- Soil aggregate stability also increased with the establishment of native plant species.

## **METHODS**

### *Experimental design:*

In this experiment, we test the effectiveness of mycorrhizal fungi from three different sources: vegetated areas in southern California dominated by native plant species, vegetated areas in southern California dominated by naturalized exotic plant species and commercial inocula. Each of these three inocula treatments were produced in two different ways. The native and exotic derived inocula were produced directly from field soils collected immediately prior to field inoculation and from soil. We also had two versions of the commercial inocula: cultured under similar conditions as the other cultured inocula and directly as purchased from the company. We compare the inoculated plots to two controls: one that was uninoculated and a second that was inoculated with non-mycorrhizal components of the other inocula. In total there were eight inocula treatments, which were replicated eight times (64 plots) and arranged in a randomized Latin Square design (Fig. 5.1). Each plot is 2 meters wide by 2 meters deep. We left a 2 meter uninoculated border between plots and along the edge of the blocks to reduce contamination.



**Figure 5.1.** Treatment map for field inoculation experiment at recapped landfill.

*Study site*

The study is located on top of a recently recapped land fill that is currently part of the campus of University of California, Irvine. This site had previously been topped with brackish fill from Newport Beach Back Bay. As a result of the high salinity of this

layer, plant establishment had been very poor. In an attempt to correct for this poor establishment, the University layered an additional fifteen inches of subsoil over the entire landfill. Our experiments are located in a region in which this top layer was obtained from underneath an office building and is therefore relatively uniform and has reduced mycorrhizal inoculum.

*Experimental protocol*

Plots were laid out in October and November 2000 (Fig. 5.2). We inoculated at a rate of 1,000 cubic centimeters of inoculum per square meter and raked our amendments into the top 5cm of soil. We distributed our seed mix evenly over the surface and tapped down the soil. Finally, the area was covered with rice straw at an application rate of 8,000 lbs per acre (as per Caltrans specifications) to protect the inoculum and seed and to add organic matter.



**Figure 5.2.** 64 field plots were inoculated in the fall of 2000 to test the effectiveness of mycorrhizal fungi from various sources. The inoculum was raked into the soil, the plots were given a common seed mix, and then covered with straw mulch.

#### *Source and preparation of treatment inocula*

Our experiment tests revegetation success and soil stabilization using eight treatments comprised of seven microbial communities. Six of the microbial communities used for live inoculum consisted of either unamended bulk sources (such as topsoil) or were specifically cultured from soil communities that originated as a bulk source. The unamended bulk sources were as follows: (1) *Glomus intraradices* as Endonet, a commercially available mycorrhizal inoculant in a soilless medium; (2) Exotic Bulk, a topsoil from an exotic-dominated site; and (3) Native Bulk, a topsoil from a native-dominated site. The cultured sources include: (4) *G. intraradices* Culture, derived from a commercially available source (*VAM 80*, Tree of Life Nursery, San Juan

Capistrano, CA); (5) Exotic Culture, which originated from an exotic-dominated site; and (6) Native Culture, which originated from a native-dominated site. Cultures began as 500 mL of starter inoculum in a sterile mix local soil and sand, and then grown under natural conditions with native grasses, legumes, composites, and other suitable host species.

A seventh (7) microbial community that lacked AM fungi began as an aqueous solution containing all other inocula and was then sieved repeatedly to exclude particles  $> 38 \mu\text{m}$ . The sieved solution was then mixed into a carrier medium of autoclaved soil and sand. For the field component of this project, the eighth (8) treatment was the absence of any inoculum. For the greenhouse component, we substituted autoclaved soil to control for any small-scale nutrient effects.

Our cultured exotic and native inoculum originated from adjacent areas currently dominated by native or exotic plant species at Starr Ranch (Orange County, CA). Freshly collected soils from these sites were used as starter inoculum by mixing it with sterile soil and sand and planting sterile host plants into this mixture. We planted several species of host plants into each pot, including garlic, several species of native grasses, as well as native composites and legumes. These cultures were started in April of 1999 were maintained for two years for eventual use in this restoration experiment. During this time, the AM fungi infected their host plants, proliferated, and sporulated while the plants died back in mid summer. We are comparing the effect of these two inoculum mixes to commercially available inoculum (*Glomus intraradices*) and to a cultured form of *Glomus intraradices* prepared in the same manner as our other AM cultures. The topsoil to be used directly as inoculum was obtained from a native grassland site and

exotic dominated grassland site on UCI property. The soil was collected during the summer months and stored dry and cool until used in our plot establishment.

### *Seed Mix*

Our seed mix included a diverse assemblage of native species characteristic of Orange county perennial grasslands. To provide a stronger test of the treatments, we also included exotic weed seeds that are typically problems in restorations. We hoped that including these exotic weed species would reduce the heterogeneity of weed seed presence at our site. Table 5.1 lists the actual species used. Our seed was hand-collected from Starr Ranch and the UCI Ecological Preserve, where possible, or purchased from commercial seed vendors. Seed application rates varied by species and was dependent on seed availability and seed size. For most species, however, we applied a minimum of 200 pure live seeds per square meter. We calculated the pure live seed from germination assays.



**Table 5.1.** Species list of native and naturalized exotic plants seeded into field inoculation plots.

<b>Family</b>	<b>Genus</b>	<b>Species</b>	<b>Common Name</b>
Asteraceae	<i>Achillea</i>	<i>millefolium</i>	Yarrow
Liliaceae	<i>Allium</i>	<i>praecox</i>	Onion
Liliaceae	<i>Allium</i>	<i>haematochiton</i>	Onion
Liliaceae	<i>Allium</i>	<i>penisulare</i>	Onion
Amaranthaceae	<i>Amaranthus</i>	<i>palmeri</i>	Tumble pigweed
Asteraceae	<i>Artemisia</i>	<i>californica</i>	California sagebrush
Asclepiadaceae	<i>Asclepias</i>	<i>sp.</i>	unkn milkweed
Poaceae	<i>Avena</i>	<i>fatua</i>	Wild oat grass
Liliaceae	<i>Bloomeria</i>	<i>crocea</i>	Common goldenstar
Brassicaceae	<i>Brassica</i>	<i>nigra</i>	Black mustard
Poaceae	<i>Bromus</i>	<i>hordeaceus</i>	Soft chess
Poaceae	<i>Bromus</i>	<i>carinatus</i>	California brome
Asteraceae	<i>Centaurea</i>	<i>melitensis</i>	Tocalote
Liliaceae	<i>Dichelostemma</i>	<i>capitatum</i>	Blue dicks
Primulaceae	<i>Dodecatheon</i>	<i>clevelandii</i>	Shooting star
Asteraceae	<i>Encelia</i>	<i>californica</i>	California encelia
Euphorbiaceae	<i>Eremocarpus</i>	<i>setigerus</i>	turkey mullein
Polygonaceae	<i>Eriogonum</i>	<i>fasciculatum</i>	California buckwheat
Asteraceae	<i>Eriophyllum</i>	<i>confertifolium</i>	Broad-leaved Golden Yarrow
Geraniaceae	<i>Erodium</i>	<i>cicutarium</i>	Red-stemmed filaree
Papaveraceae	<i>Eschscholzia</i>	<i>californica</i>	California poppy
Asteraceae	<i>Gnaphalium</i>	<i>californica</i>	Everlasting
Asteraceae	<i>Grindelia</i>	<i>robusta</i>	Big gumweed
Asteraceae	<i>Grindelia</i>	<i>stricta</i>	Gumweed
Asteraceae	<i>Hazardia</i>	<i>squarrosa</i>	Saw-toothed goldenbush
Asteraceae	<i>Hemizonia</i>	<i>fasciculata</i>	Fascicled tarweed
Asteraceae	<i>Heterotheca</i>	<i>grandiflora</i>	Telegraph weed
Asteraceae	<i>Isocoma</i>	<i>menziesii</i>	Coast goldenbush
Asteraceae	<i>Lactuca</i>	<i>serriola</i>	Prickly lettuce
Asteraceae	<i>Lasthenia</i>	<i>californica</i>	Coastal goldenfields
Asteraceae	<i>Lessingia</i>	<i>filaginifolia</i>	California aster
Poaceae	<i>Lolium</i>	<i>multiflorum</i>	Italian ryegrass
Fabaceae	<i>Lotus</i>	<i>scoparius</i>	Deerweed
Fabaceae	<i>Lotus</i>	<i>purshianus</i>	Spanish lotus
Fabaceae	<i>Lupinus</i>	<i>bicolor</i>	Miniature lupine
Lamiaceae	<i>Marrubium</i>	<i>vulgare</i>	common horehound
Poaceae	<i>Melica</i>	<i>imperfecta</i>	Oniongrass
Fabaceae	<i>Melilotus</i>	<i>alba</i>	White clover
Poaceae	<i>Muhlenbergia</i>	<i>rigens</i>	Deergrass
Poaceae	<i>Nassella</i>	<i>cernua</i>	Nodding needlegrass
Poaceae	<i>Nassella</i>	<i>lepida</i>	Foothill needlegrass
Poaceae	<i>Nassella</i>	<i>pulchra</i>	Purple needlegrass
Asteraceae	<i>Picris</i>	<i>echioides</i>	Bristly ox-tongue
Polygonaceae	<i>Rumex</i>	<i>crispus</i>	Curly dock
Chenopodiaceae	<i>Salsola</i>	<i>tragus</i>	Russian thistle
Lamiaceae	<i>Salvia</i>	<i>apiana</i>	White sage
Caryophyllaceae	<i>Silene</i>	<i>laciniata</i>	Mexican pink
Liliaceae	<i>Sisyrinchium</i>	<i>bellum</i>	Blue-eyed grass
Poaceae	<i>Sporobolus</i>	<i>airioides</i>	Alkali sacaton
Asteraceae	<i>Stephanomeria</i>	<i>virgata</i>	Twiggy wreath-plant
Lamiaceae	<i>Trichostema</i>	<i>lanceolatum</i>	Vinegarweed
Fabaceae	<i>Trifolium</i>	<i>gracilentum</i>	clover

### *Assessing Revegetation Success*

We monitored the shifts in plant cover and composition on all plots by conducting vegetation surveys near the end of the growing season. Surveys for the past two years were timed to coincide with plant phenology, which largely depend on

meteorological conditions during the growing season. During the first year, the surveys on the slope and flatland experiment were conducted over three weeks in late April and early May. During the second year, our survey began during the second week in April. We have used the point intersection method, taking two transects randomly located through each plot and then identifying and recording all plants located beneath 33 fixed points along the transect. From these measures, we can calculate percent cover. We chose not to perform destructive samples during the first year in order to avoid disturbing the plots.

#### *Assaying soil characteristics*

Prior to inoculating and seeding our plots, we removed soil samples which were thoroughly characterized for their chemical and textural properties, which allows us to establish a baseline for subsequent statistical analysis. We also removed soil samples following the first growing season to assess changes in water stable aggregate stability.

#### *Field Soil Aggregation Analysis*

In the summer following the first growing season, five soil cores were removed from each of the 64 plots. Air-dried soil samples were dry sieved to capture aggregates greater than 0.5 mm and less than 1 mm. For each sample, approximately 8 g of these aggregates were loaded onto a 7.6 cm diameter sieve (0.5 mm screen) and vapor-wetted in vaporizing chamber for 45 minutes. After hydration, these aggregates were agitated in a wet-sieving apparatus for 10 minutes (stroke length 1.3 cm, 35 cycles  $\text{min}^{-1}$ ) as described by Kemper and Rosenau (1986). Receiving cans mounted below the test sieves collected soil particles washed through the sieve. Those particles washed through during the first agitation cycles were considered water unstable (WU). A second

agitation cycle was used to separate the water stable (WS) fraction from the sand remaining in the sieve. Both fractions were transferred into a stainless steel evaporating pans and oven dried at 110 °C to a constant mass. Estimates of aggregate stability are calculated as a percentage of the stable to unstable particles, minus the sand:  $Y = 100 \times WS / (WU + WS)$ .

*Statistical Analyses:*

We are analyzing our measures of plant establishment and soil stability using one-way analysis of variance followed by a series of orthogonal *a priori* contrasts. We tested for the overall effect of mycorrhizal inoculation by contrasting the uninoculated control against the average of the three inoculation treatments. We expected that mycorrhizal inoculation would improve native plant establishment and increase the stability of soil aggregates. We used mean separation tests to test for differences among the three treatments with inoculation. Total cover was included as a covariate for the field soil aggregate stability analysis.

**RESULTS**

*Vegetation establishment:*

**Year 1**

After the first year, exotic plants on average covered 63.8% of the plots, while native plants covered 5.8% of the plots. The productivity of exotic plants was not significantly dependent on the inoculation treatment, however the productivity of exotic plants was highest in the uninoculated plots. The productivity of the exotic plants was

strongly positively responsive to nitrate concentration in the soil ( $p < 0.0001$ ) and weakly positively responsive to soil pH ( $p < 0.05$ ).

The establishment and productivity of native plants tended to be increased by inoculation with mycorrhizal fungi ( $p < 0.11$ ). The relative abundance of native plants decreased with increasing concentrations of nitrate in the soil, reflecting the increased competitive ability of exotic plants as a function of soil nitrate. Native cover also declines with Sodium Adsorption Ratio (SAR), calcium concentration and exchangeable K concentration ( $p < 0.02$ ,  $0.02$ , and  $0.04$ , respectively). The SAR effect reflects the high salinity of the site—apparently the salinity reaches sufficiently high levels to decrease the performance of native plants relative to the exotics (such as ice plant). It is possible that the negative relationship with calcium and exchangeable K result from the same effect. Of the native plants that occurred frequently enough for individual analyses, *Lasthenia californica* was more abundant in plots inoculated with living soil or the *G. intraradices* inoculum than in control plots. From the first year data, we would hope to see greater effects of inoculation in future years as the native plants become better established.

## **Year 2**

The second year was very dry and as a result there was very low vegetation cover. The cover by exotic plants dropped to 24% and the cover by native plants dropped to 0.25%. The cover by exotic plants decreased with the increasing cover by native plants in the previous year ( $p < 0.07$ ), suggesting an inhibitory effect of the native species. Since the native plants were not great abundant this year, it is likely that this effect was mediated through changes in the soil environment, perhaps through an

increase in the density of mycorrhizal fungi. Exotic plant productivity also decreased with increasing Sodium Adsorption Ratio (SAR) and increased with magnesium concentration. The decrease in productivity of the exotic plant species with SAR in the second year suggests that the salinity is greater problem with reduced rainfall. The native plant cover was too low for meaningful statistical analysis.

### **Year 3**

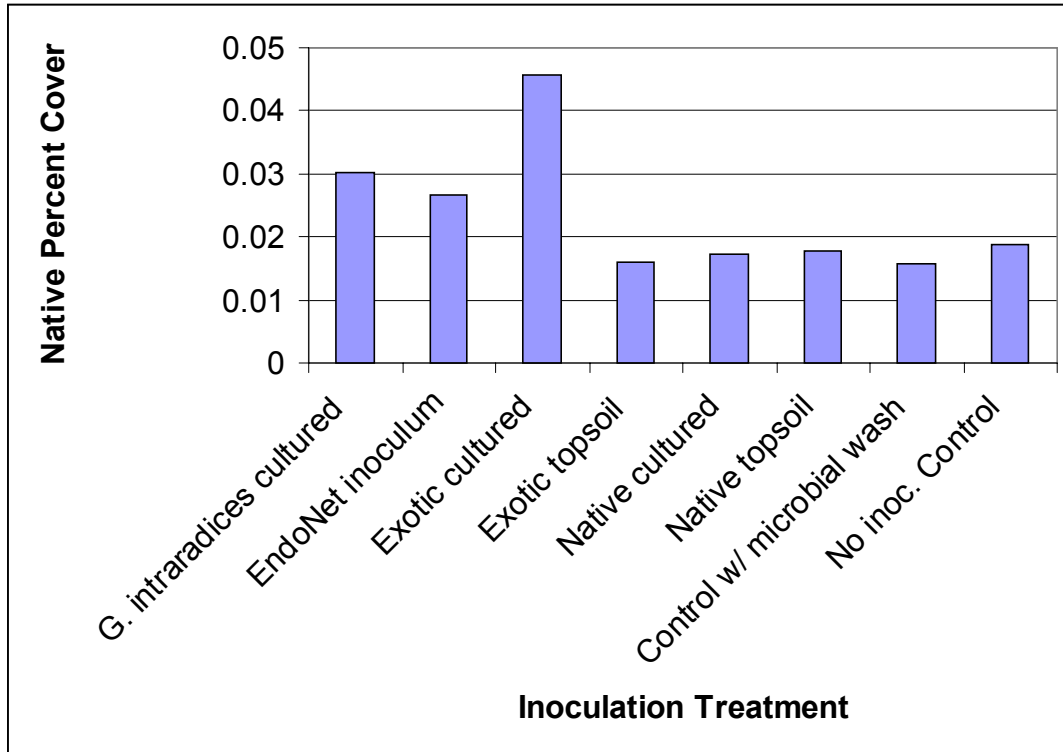
The native plants did not recover from the drought in the second year, with native cover remaining at 1.2% (Fig. 5.3). It is likely that the salinity became an increasing problem for the native species, thereby preventing their re-establishment. Exotic cover remained over 60%, however, the species composition had shifted toward salt tolerant species.



**Figure 5.3.** Field inoculation experiment after 3 years. Plots are dominated with naturalized exotic species.

## **Overall Vegetation Response**

When averaged across all years, the success of native plants was positively affected by mycorrhizal inoculation, but this effect was marginally significant ( $p < 0.13$ ). Native cover was particularly high with the cultures originating from exotic dominated areas (Figure 5.4). This is surprising because the mycorrhizal density was lower than the inocula cultured from exotic sources than the inocula cultured from the native source. However, it might reflect different abilities of the fungi to survive the disturbance of setting up the experiment and the highly saline environment in the experiment. Native cover was also higher in plots inoculated with soil cultures than with fresh field soil ( $p < 0.05$ ). Again, this could result from the fungi in the cultured inocula being more likely to be in the resistant form of spores and therefore better able to survive the stress of disturbance in the experimental establishment.



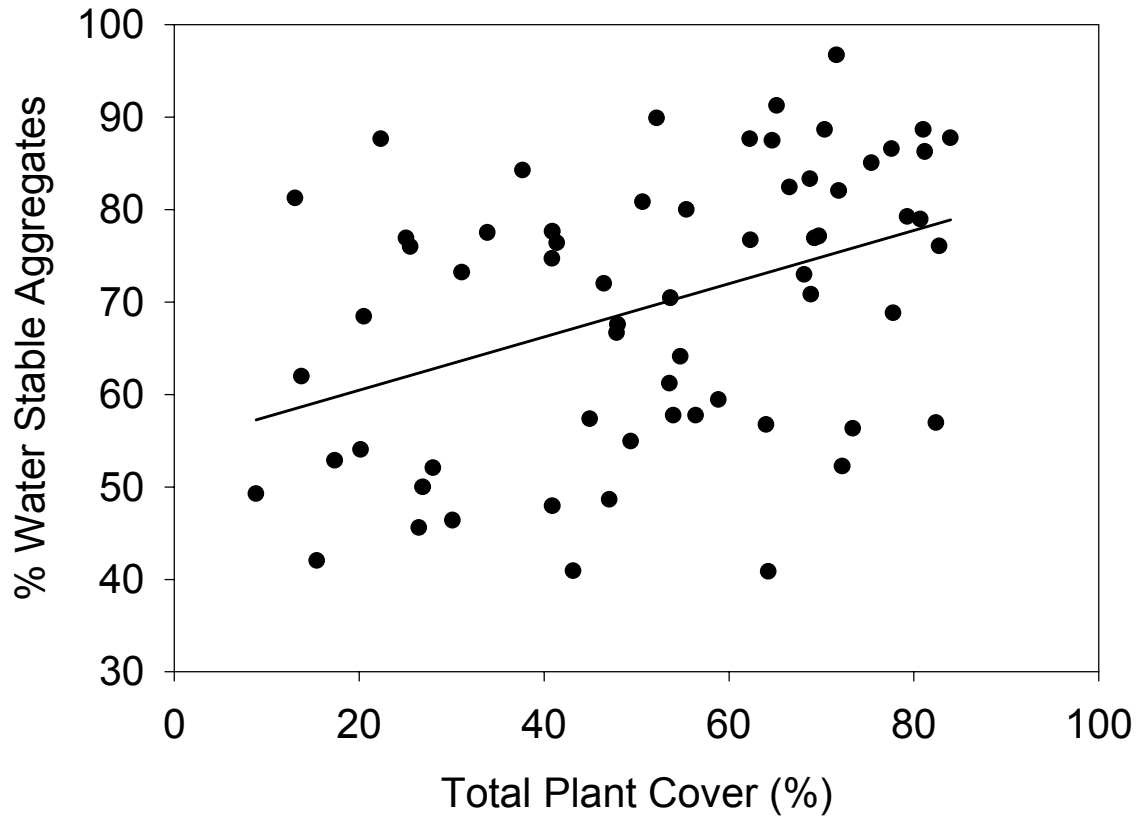
**Figure 5.4.** Average percent cover by native species. While the overall native cover was low, three inocula successfully increased the average native cover, the commercial inocula and the cultured soil community derived from exotic dominated vegetation.

While the overall density of native species was responsive to inoculation, the overall density of exotic species was unaffected by inoculation, but instead was spatially variable because of abiotic factors. The average density of exotic species was strongly positively responsive to the available nitrogen (both ammonium and nitrate,  $p < 0.05$  and  $0.0001$ , respectively), reflecting the high responsiveness of weedy species to nitrogen. Density of exotic species was also negative affected by the electrical conductivity (EC) of the soil ( $p < 0.0001$ ), reflecting the negative effects of salinity.

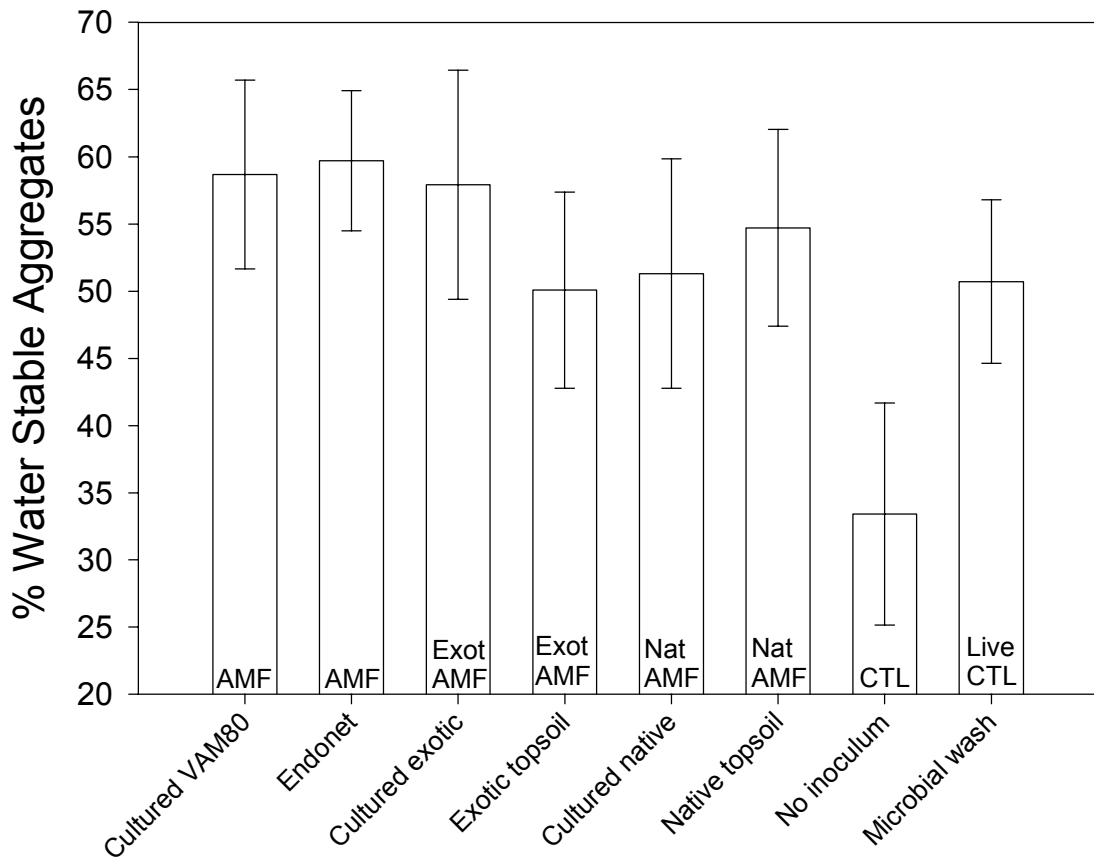
*Field Soil Aggregation:*

Soil aggregate stability covaried positively with total plant cover (Figure 5.5). We also observed a significant increase in soil aggregate stability ( $F_1 = 10.10$ ;  $P = 0.0028$ ) in plots inoculated with mycorrhizal fungi compared to the control plots (Figure 4). No distinctions among the sources of mycorrhizal inoculum were evident, however, *a priori* contrasts revealed a significant effect of the live microbial wash treatments compared to the uninoculated control ( $F_1 = 10.50$ ;  $P = 0.0024$ ). These results strongly implicate that soil organisms in general are critically important to improving soil structure. The treatments that had effective mycorrhizal fungi, however, exhibit only slight improvements beyond the benefits produced by the non-mycorrhizal soil community.





**Figure 5.5.** Field soil aggregate stability following the first growing season. Plant cover, which estimates productivity, accounts for most of the variation in aggregate stability.



**Figure 5.6.** Field soil aggregate stability following the first growing season by inoculum type. Live soil shows general improvement over plots lacking live inoculum.

## DISCUSSION

The performance of the field trial was hampered by combination of the drought in the second year and the high salinity in the land fill cap. Unfortunately, the second cap was not sufficient to mask the negative effect of the layer of brackish deposits. The site remains largely unvegetated and remains in need of reclamation.

Even so, our experiment does give a measure of confirmation of the effectiveness of inoculation with mycorrhizal fungi. While native species never reached high

densities, they did reach highest densities in plots inoculated with mycorrhizal fungi (Figure 5.4). Moreover, both density of natives and inoculation with mycorrhizal fungi increased soil aggregate stability (Figures 5.5 and 5.6). Each of these responses were as expected from our previous laboratory and greenhouse experiments which gives us greater confidence in their interpretation (Chapters 2, 3, and 4) From these results, we have even greater confidence of the practical importance of inoculation with mycorrhizal fungi.

The magnitude of responses observed in these field trials, however, likely underestimate the importance of mycorrhizal fungi, because the inocula were not adapted for the high salinity of the site. Similarly, the observed differential responses of the inocula may not be representative of the efficacy of the mycorrhizal fungi, but instead may reflect the differential resistance of the inocula to disturbance and high salinity. In this field trial, we observed the greatest response of native plants and the greatest aggregation with cultured exotic soil communities and the commercial inocula, while in our greenhouse trials we observed the greatest responses to native inocula (Chapters 2, 3, and 4). The native inocula may have performed better, if we had performed the trial in a non-saline environment.

We also observed better response from cultured exotic inocula than from fresh field soil. This may also reflect the severity of the conditions in that the fungi present in the field soil may not have been present as spores, which are resistant to disturbance, but rather as hyphae, which is vulnerable to disturbance. It remains possible that field soil could be used to better effect if it is collected and stored properly and used in similar environments as were it was obtained (see Chapter 9 for a study of soil stockpiling).

Overall, we are encouraged by the results of the experiment in that they confirm the major results obtained from more carefully controlled greenhouse experiment. We think that the experiment should be repeated in a non-saline environment and field tested on a roadcut more characteristic of landscapes used by Caltrans.

## CHAPTER 6: A field test of different inocula on soil erosion

### INTRODUCTION

Soil erosion by water is perhaps the most ubiquitous of all geologic phenomena, and as such, factors that prevent or promote erosion are generally well known.

Vegetative cover has long been recognized for its ability to intercept raindrops, thus neutralizing a powerful erosive force. Unintercepted raindrops can dislodge soil particles from larger aggregates, destroy soil structure, and mobilize large amounts of soil once water begins to flow over the soil surface. In addition to providing soil cover, plant roots hold soil via physical entanglement, and interact with soil microbes to alter the structural properties of soil and improve water infiltration. Arbuscular mycorrhizal (AM) fungi are known to be a crucial biotic component to the soil aggregating process (Miller and Jastrow 1990). These symbiotic soil-dwelling fungi colonize plant roots and release sticky glycoproteins into the rhizosphere. These glycoproteins, identified as glomalin (Wright and Upadhyaya 1996), cement soil particles into stable aggregates. Road improvement projects, however, often result in a loss or severe decline of naturally occurring soil mutualists such as AM fungi. Thus, establishing native vegetation that functions as a natural system becomes very difficult without first recognizing which biotic components are missing from the site. If AM fungi and other crucial organisms are absent, then an inoculation of suitable microbes should contribute meaningfully to the restoration of ecological function.

In Chapters 4 and 5, we show how the native plant/microbe interactions result in improved soil aggregate formation and stabilization. In terms of erosion control, we

would predict from this result that combinations of native plants and their soil mutualists should result in the most stable field soils and therefore yield the lowest quantity of sediment over the course of a season. We also hypothesize that inoculating a degraded site with AM fungi should be superior to the lack of inoculation if AM fungi are indeed important to the functioning of this system. In this investigation, we wanted to assess the relative benefits of inoculating a site with mycorrhizal fungi, and to compare differences among multiple inoculum sources.

In this chapter, we report results of a field test where we find:

- Inoculation with AM fungi cultured from native plant communities does not necessarily result in improved soil holding capacity.
- Inoculation with live soil, regardless of the source, can improve soil holding capacity.
- Inoculation with live soil, regardless of the source, may contribute to improved water infiltration.

## **METHODS**

### *Study site*

The study site traverses a recently recapped land fill that is currently part of the campus of University of California, Irvine. This site had previously been topped with brackish fill from Newport Beach Back Bay. As a result of the high salinity of this layer, plant establishment had been very poor.

### *Experimental protocol*

In October and November 2000, we designated and installed 20 1 x 2 m plots spanning 60 m horizontally across a slope of approximately 20% grade. Four inoculation treatments were randomly assigned within each of five blocks. We inoculated at a rate of 1,000 mL of inoculum per square meter and raked our amendments into the top 5 cm of soil. We distributed our seed mix evenly over the surface and tapped down the soil. Finally, the area was covered with rice straw at an application rate of 8,000 lbs per acre (as per Caltrans specifications) to protect the inoculum and seed and to add organic matter.

### *Erosion data collection*

Each plot ran lengthwise down the slope and was bordered by plastic edging to form a discrete 2 m<sup>2</sup> watershed. During a rain event, water received in each plot would flow down into a galvanized steel catchment and drain into a five gallon receiving bucket nested inside a 20 gallon plastic container (Fig. 6.1). Approximately 6 mm of precipitation was required to initiate overland surface flow. Following cessation of the rain, we exchanged a clean bucket for the one filled with water and sediment (Fig. 6.2). We measured water from the filled buckets with a dipstick and reclaimed the sediment using a 0.1 M solution of “Alum” (Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> nH<sub>2</sub>O) as a settling agent. We stirred in 50 mL of Alum to each five gallon bucket of turbid water to facilitate settling, and then poured off the supernatant from the sediment. We poured the sediment into a drying pan and placed these pans into a drying oven at 110 °C for 48 hours. We quantified the mass of the dry sediment. We acquired erosion data eight times during the 2000-2001 rainy season, nine times during 2001-2002 rainy season, and six times during the 2002-2003

rainy season. Water data can be informative for detecting variation in precipitation amounts across the landscape and/or variation in watershed performance, and thus can be used to correct sediment yields to the actual runoff received. During the first year, prolonged heavy rains resulted in two overflow events, so that reliable water data could not be obtained on these two occasions. During the second year, our field technician overlooked water data for the first rain event.



**Figure 6.1.** Field erosion demonstration experiment using 20 plots inoculated with mycorrhizal fungi.

*Source and preparation of treatment inocula*

This experiment tests the soil holding functions of three microbial communities and a self-inoculated control. We used the same cultured AM fungal communities described in Chapter 5. These sources include: (1) *G. intraradices* Culture, derived



from a commercially available source (*VAM 80*, Tree of Life Nursery, San Juan Capistrano, CA); (2) Exotic Culture, which originated from an exotic-dominated site; and (3) Native Culture, which originated from a native-dominated site. Cultures began as 500 mL of starter inoculum in a sterile mix local soil and sand, and then grown under natural conditions with native grasses, legumes, composites, and other suitable host species.

Our cultured exotic and native inoculum originated from adjacent areas currently dominated by native or exotic plant species at Starr Ranch (Orange County, CA). Freshly collected soils from these sites were used as starter inoculum by mixing it with sterile soil and sand and planting sterile host plants into this mixture. We planted several species of host plants into each pot, including garlic, several species of native grasses, as well as native composites and legumes. These cultures were started in April of 1999 were maintained for two years for eventual use in this demonstration experiment. During this time, the AM fungi infected their host plants, proliferated, and sporulated while the plants died back in mid summer.



**Figure 6.2.** Collecting water and sediment from field erosion experiment.

### *Seed Mix*

Our seed mix included a diverse assemblage of native species characteristic of Orange county perennial grasslands. To provide a stronger test of the treatments, we also included exotic weed seeds that are typically problems in restorations. We hoped that including these exotic weed species would reduce the heterogeneity of weed seed presence at our site. Table 5.1 in the previous chapter lists the actual species used. Our seed was hand-collected from Starr Ranch and the UCI Ecological Preserve, where possible, or purchased from commercial seed vendors. Seed application rates varied by species and was dependent on seed availability and seed size. For most species, however, we applied a minimum of 200 pure live seeds per square meter. We calculated the pure live seed from germination assays.

### *Erosion data analysis*

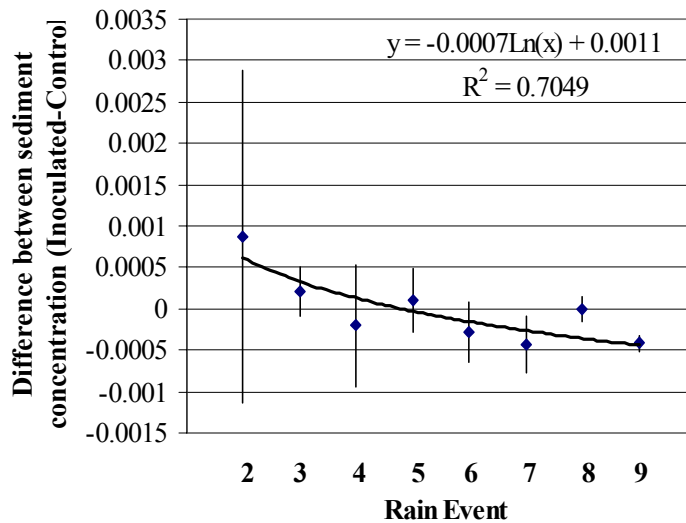
Erosion data consist of total dry soil received per rain event (in grams) and sediment concentration (mg/mL) to correct for variations in water harvest. Our initial analyses use sediment concentration as the response variable. For each treatment our erosion estimates were repeated over time and thus were analyzed using a one-way multivariate analysis of variance (MANOVA) for repeated measures with a priori contrasts. We used Wilks' lambda ( $\lambda$ ) as our test statistic. For each event, we are interested in the overall inoculum effects compared to the uninoculated treatment, and whether there are differences between exotic and native sources of inoculum. We constructed contrast statements to test our expectation that inoculated plots will yield less soil than uninoculated plots, and that the cultured native inoculum yield less soil than the cultured exotic inoculum. Separate analyses are performed for each rainy season.

## **RESULTS**

Inoculation effects in the erosion demonstration experiment were not anticipated to present during the first year, given that the plots had just been installed and lacked sufficient vegetative cover until well into the rainy season. As such, it is not surprising to report no overall treatment effects evident during the first rainy season. The initial rain event suggested inoculated plots held soil better than uninoculated plots, and that cultured native inoculum outperformed cultured exotic inoculum. However, these effects were undetectable with subsequent storms ( $\lambda = 0.1537$ ;  $p = 0.4481$ ). Significant overall block effects appear ( $\lambda = 0.0125$ ;  $p = 0.0082$ ), and are restricted to rain events

early in the season. These block effects diminish over time, and are not significant for the final rain event of the first year.

More consistent treatment effects were observed during the second rainy season. We observed a significant decreasing trend of reduced sediment concentration in the inoculated plots compared to the controls (Fig. 6.3). Five of the eight orthogonal contrasts revealed slightly more soil holding ability in the inoculated versus the uninoculated plots, and this difference was significant in the final rain event of the



**Figure 6.3.** A comparison of inoculated versus uninoculated erosion plots. Sediment concentration declines over the 2001-2002 rainy season in plots inoculated with mycorrhizal fungi. The overall trend is significantly negative.

season ( $F_1 = 14.69$ ;  $p = 0.0033$ ). Additionally, in seven out of eight events, cultured exotic inoculum held soil marginally better than cultured native, and this trend was significant once late in the season ( $F_1 = 6.04$ ;  $p = 0.0338$ ).

Treatment and block effects were not detected in the overall MANOVA.

In the final year, the plots treated with native inoculum exhibited marked improvement in their water holding capacity. We observed a mean difference of less than 6% between the top performing exotic inoculum plots (169.9 L of runoff) and the second best native inoculum plots (179.6 L of runoff). In the first and second years, the

differences between these two treatments were 12.9% and 27.4%, respectively. In the final year, the amount of runoff released in the exotic plots was significantly different from the Endonet treated plots, but not different from any of the other treatments.

## **DISCUSSION**

After three years of field data, we find no significant year to year differences by inoculum type in the quantities of soil eroded from the test plots. These plots were treated cultured inocula originating as a (1) commercial product (Endonet), (2) soil from a site dominated by exotic species, (3) soil from a site dominated by native species, (4) a self-inoculated control. In the second field season, we found that plots treated with live inocula were releasing less sediment over the course of the season, however, this trend was not evident in the final field season. The lack of response in the final year may relate to the poor overall rate of establishment of native plants (see Chapter 5). This in itself may reflect the high salt conditions which continued to be a problem at this landfill site.

In terms of inoculating with AM fungi, however, we do find evidence of improved ecological function where water infiltration improved over time in the inoculated plots. For the first two seasons, mean water harvest for the exotic inoculum treatment was consistently lower than mean water harvests for all other treatments, and the native inoculum treatment yielded the greatest amount of surface runoff. None of these differences were statistically significant, however, so we adopt a cautious approach to our secondary hypothesis. Future research should quantify infiltration rates directly to investigate this phenomenon.

Curiously, these emerging differences in runoff did not translate to differences in sediment yield. The reasons are unclear, but it may be due to inherently poor site conditions such as high salt levels that impeded early vegetation establishment. By the final year, all plots exhibited the same levels of continuous vegetative cover. Erosion plots were dominated by exotic annual grasses, clover, and a herbaceous forb known as lamb's quarters (*Chenopodium alba*). Near the base of each plot, a ubiquitous species of iceplant (*Mesembryanthemum nodiflorum*) tended to dominate at the interface of the plot boundary and the catchment basin. This exotic salt-tolerant species characterized the landscape at this site, and may have contributed to the poor performance of the native species we seeded into the test plots. For the final year, we find evidence that the iceplant and the other exotic vegetation may have masked the differences in sediment released into our traps, while allowing much of the water to pass through. A regression analysis reveals that for the first two years, the single best predictor of sediment yield was the amount of water runoff, as expected for any soil mobilized in a hydrological system. In year 3, however, the amount of runoff was not a good predictor of year 3 sediment loads. This finding suggests system function had indeed changed by the final year, and supports the previous analysis that water infiltration had improved due to mycorrhizal inoculation. Without proper plant establishment, we would not anticipate treatment effects of AM fungi. We believe improved plant establishment and thus stronger treatment effects would be observed with less salty initial site conditions, or with a watering regime designed to mitigate the effects of salty soils (i.e. irrigation to augment rainfall).

## **CHAPTER 7: A test of mycorrhizal inoculum dispersal and viability using a hydroseeder**

### **INTRODUCTION**

Hydroseeding and hydromulching refers to a mechanized system of establishing vegetative cover across a landscape. Hydroseeding equipment generally consists of a water tank coupled to a pump and mixer apparatus that can churn seed, mulch, fertilizer, and inoculum into a slurry to be forced out under pressure through a hose. The low material costs, which range from 1.5 cents to 2.5 cents per square foot, make hydroseeding technology an attractive option for revegetating or restoring degraded land. Many restoration practitioners either use or have considered using live soil amendments such as arbuscular mycorrhizal (AM) fungi, but there have not been any published data to indicate how effective hydroseeders are at dispersing AM fungal propagules, or whether those propagules remain viable to form associations with plant roots. In this study, we tested three commercially available products known to contain AM fungi to determine if hydroseeding/mulching equipment can effectively disperse mycorrhizal inocula.

In this chapter, we find:

- Mycorrhizal fungal products are easily discharged through the hydroseeder/mulcher we experimented with.
- 30 lbs of inoculum per 3000 gallons of water represents the low-end of propagule density.
- The Conservaseed product “Endonet” promoted the greatest root colonization of three products tested.

## **METHODS**

We designed a two-way experiment to test the effects of three mycorrhizal products at two different application rates on the ability to form mycorrhizae with Sudan grass (*Sorghum bicolor*). Prior to setting up this bioassay, we first needed to use a hydroseeder/mulcher in order to pre-treat our products in a manner similar to how these products would actually be used in revegetation project. In April of 2003, we contracted with Nakae & Associates of Orange County, CA for the use of their Bowie Imperial 3000 Hydromulcher. This machine features a 3000 gallon tank and incorporates a 4 X 3 centrifugal pump to agitate the slurry inside the tank. Operating pressures range from 70 – 80 psi, with a maximum pressure of 100 psi. A technician from Nakae & Associates operated the equipment.

To assess actual mycorrhizal viability of commercial products as received from the vendor, we used 30 lbs of inoculum from three different commercial vendors: (1) AM120 from Reforestation Technologies International (RTI) (Salinas, CA); (2) BioGrow from Mycorrhizal Applications, Inc.(Grants Pass, OR); and (3) Endonet from ConservaSeed (Rio Vista, CA). Each mycorrhizal product was mixed with 3000 gallons of water and Hydro Mulch® wood fiber from Conwed Fibers (Hickory, NC). The wood fiber mulch was used as a binding agent to aid in the mixing and dispersal of the inocula.

For each mycorrhizal product to be tested, we began with a rinsed hydromulcher tank filled to capacity with clean water. We added wood fiber mulch, 30 lbs of inoculum, and allowed the slurry to mix thoroughly. The technician then sprayed the slurry across a maintenance yard and the spray was collected in an array of five gallon buckets (Fig. 7.1). This process was repeated for each of the three inoculum products.



Slurry samples of each vendors' product were pooled into a single bucket. We then used the slurry from each vendor trial as live inoculum in a replicated bioassay set up later that same day. Treatments for the bioassay consisted of two volumes of "live" hydrospray along with the requisite controls for the added wood fiber mulch and for the autoclaved soil growth medium.



**Figure 7.1.** Discharging water, mulch, and mycorrhizal inoculum through a hydroseeder/mulcher.

### *Propagule dispersal density*

We wanted to assess whether mycorrhizal propagules were being dispersed during all phases of equipment operation. The possibility existed that inoculum could settle out of solution during the agitation process since these products incorporate a dense, calcined clay medium to facilitate dry dispersal. The possibility also existed that spores, hyphae, and other small propagule fragments would float when added dry to water. We believe that including wood fiber mulch as a binding and dispersal agent

minimized these possibilities. Indeed, our visual inspections throughout the hydromulching process confirmed that each vendor's product was being well-mixed and ejected efficiently. Nevertheless, for the first vendor trial (RTI), we subsampled the ejected hydrospray three times during the course of emptying the water tank. Samples were obtained when the tank was full, then again when the tank was approximately half full, and finally when the tank was nearly empty. We inspected these subsamples in the our laboratory to get an estimate of the number of mycorrhizal propagules per mL

#### *Mycorrhizal percent colonization*

We filled 32 seedling cones (3.8 cm diameter × 21 cm depth; Stuewe & Sons, Inc., Corvallis, OR) with autoclaved soil and sand mixed 1:1 (v:v) previously sieved to exclude aggregates > 4.75 mm. We used a clay loam soil collected from a site adjacent to the UCIEP for this purpose (see Chapters 3 and 4). Each cone then received either 10 or 50 mL of slurry as its inoculum for each of the three vendor products. All cones were seeded with at least three surface sterilized sorghum seeds, randomized, and cared for at the Culver City (CA) home of an Earthworks technician for three weeks. All plants were harvested at 24 days and shipped immediately to Indiana University for root processing and scoring. Roots were washed, cleared with 10% KOH, and stained with trypan blue (Brundrett et al. 1994). The stained roots were randomly subsampled and mounted onto glass slides for inspection under a light microscope. We estimated the mycorrhizal colonization percentage of each sample visually according to methods adapted from McGonigle et al. (1990).

### *Data analysis*

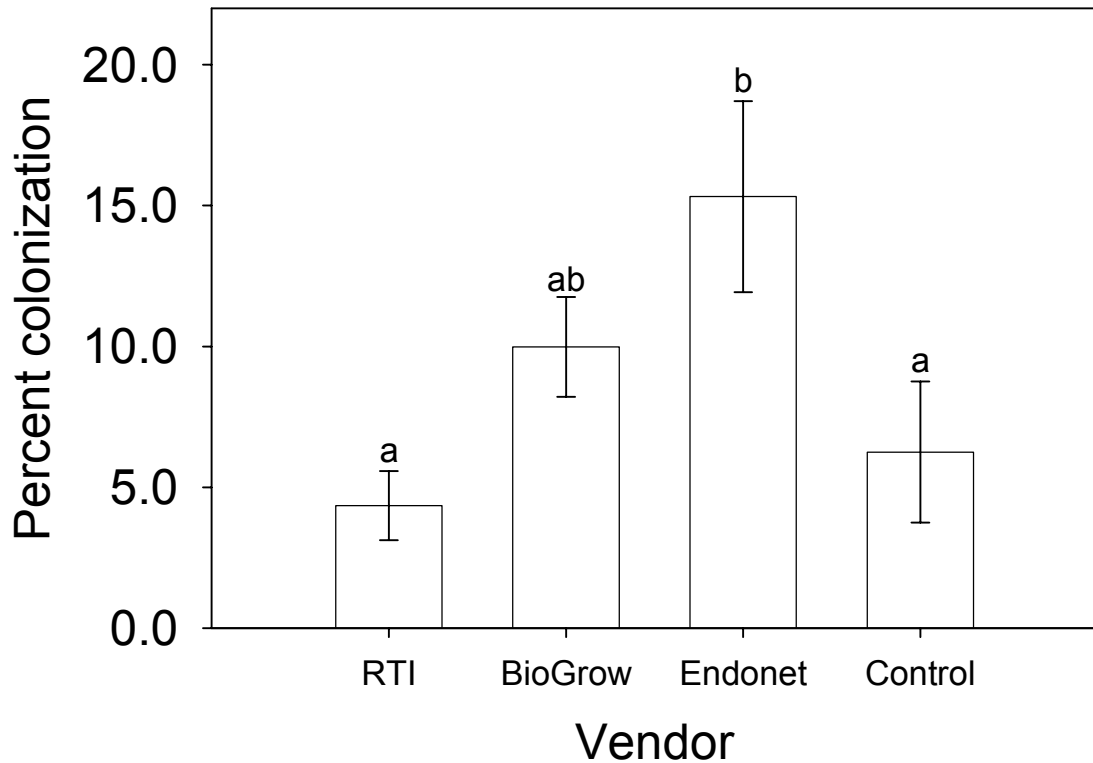
We used a two-way ANOVA to assess the effects of vendor and slurry volume on the percent mycorrhizal colonization of sorghum roots. We used Tukey's post hoc test to compare differences among vendors and slurry volumes. Data were arcsine square root transformed prior to analysis.

### **RESULTS**

Upon visual inspection during the hydromulching process, mycorrhizal propagules were clearly being mixed and ejected through the Bowie 3000 equipment. No free-floating spores were evident when we estimated propagule dispersal density in the laboratory. However, all samples contained root fragments, which are known to harbor infective mycorrhizal propagules such as hyphae and spores. We calculated a mean of 3.0 propagules/mL in each of our three subsamples. The root fragments quantified were not assessed for their specific infectivity, so the effective or viable density may actually be lower.

There was a significant vendor effect on the mycorrhizal colonization of the sorghum roots ( $F_{2,24} = 5.19$ ;  $p = 0.0134$ ). This indicates that commercial products vary considerably in the density or quality of the mycorrhizal propagules cultured. Overall root colonization by vendor product was low (below 20%), and only one vendor (Endonet) exhibited colonization that was significantly greater than the two controls (Fig. 7.2). The results suggest that inoculum quantity (or the density of propagules included within a given product) would need to be increased two to threefold in order to be effective at a landscape level.

We found a marginally significant effect of slurry volume ( $F_{2,24} = 2.94$ ;  $p = 0.0721$ ). This effect comes from comparing the live treatments to the controls; there were no differences among the two volumes of live slurry used for this assay. Unfortunately, this lack of difference among the live slurry treatments does not provide much information with respect to identifying the best target volumes. It does, however, indicate that even small amounts of inoculum are capable of producing mycorrhizae.



**Figure 7.2.** The mycorrhizal colonization observed in sorghum roots for three commercially available products. Bars are means  $\pm$  1 SE. Bars with letters in common do not differ significantly.

## DISCUSSION

Hydroseeding/mulching equipment is clearly an effective way to disperse commercially available mycorrhizal products. We determined this visually while processing the three commercial products selected for this experiment. Entangled granules of calcined clay and root fragments were abundant throughout the wood fiber mulch. Subsequent estimates of 3.0 propagules / mL were low and somewhat surprising given our expectations from the field trials. We also observed low levels of root colonization when we used the live slurry to inoculate sorghum roots. Thus, these two results are consistent with each other and suggest the quantities we used represented the low-end of what should be specified for actual use in restoration project. We recommend a two or threefold increase in either the inoculum quantity or the inoculum propagule density to ensure a sufficient number of live propagules are available to colonize germinating seeds.

The Conservaseed product “Endonet” promoted the greatest root colonization on our test plants (sorghum). We note that the three products were tested sequentially in the same order they appear in Figure 7.2, and so it is conceivable that cross-contamination occurred among the products. If so, this may partially account for the pattern of increasing percent colonization with each vendor’s product. That is, propagules from one vendor’s product may have remained in the tank and thus contributed to the density of propagules in the next vendor’s product. We discount this possibility, however, because the tank was emptied and rinsed between trials, and any propagules remaining would likely be greatly diluted when the tank was refilled. We mention this only as an

alternative possibility, and note that overall colonization levels for all three products remain very low.

We designed this experiment to test whether mycorrhizal inoculum can be effectively discharged and remain viable to colonize plant roots. However, we did not test for the actual benefits of these products as they would be used in a restoration context, so it remains an open question whether including mycorrhizal inoculum in hydroseeded operations provides superior vegetation establishment over not including AM fungi. We suspect there are quantifiable benefits, but the proper field experiments remain to be conducted to ascertain the benefits of inoculation, and whether hydroseeding/mulching procedures are the most desirable way to produce self-sustaining native plant communities.

## CHAPTER 8: Conditioned seed technology: a test of *Achillea millefolium* coated with Sow-EZ™

### INTRODUCTION

The Sow-EZ product produced by ConservaSeed (Rio Vista, CA) was developed as an alternative technology to hydroseeding. The Sow-EZ process coats seeds of a species of interest with a mix of soil amendments known to provide benefits to plants. According to the product literature, the Sow-EZ coating includes calcium carbonate, calcium sulfate, a mycorrhizal inoculant, blue-green algae, a macro algae inoculant, a beneficial soil bacterium (*Bacillus*), a saprophytic fungus (*Trichoderma*) plant hormones, and polymers to keep everything adhered to the seed. The finished product can be dispersed easily from broadcast spreaders, seed drills, or aircraft, and thus may be a cost-effective alternative to hydroseeding or container planting.

We tested the efficacy of the Sow-EZ process on the ability of the coated species to become mycorrhizal when grown in a sterile planting medium. If a viable mycorrhizae forms under sterile growth conditions, then this would provide a meaningful test of the assumed purpose of the product, which we believe is to promote mycorrhizal colonization of a suitable host species. We note, however, that in the product literature we reviewed, no specific claims are made with respect to mycorrhizal colonization. Of the multiple claims made, most fall into the category of seed handling and dispersal. One claim states that the Sow-EZ product “improves each seed’s likelihood of germination and survival.” The mechanism or causative agent for improved germination and survival is implied, but never explicitly stated. We use this implication to hypothesize that mycorrhizal colonization should be evident in conditioned seeds, and

differ from colonization observed in unconditioned seeds when grown in sterile soil. Moreover, we can test whether germination differs in conditioned versus unconditioned seeds without reference to any causative agent. None of the other vendor claims for this product are investigated in this report.

In this chapter, we report:

- The seed conditioning process may potentially work as a means of pre-inoculating seeds with useful soil microorganisms.
- The conditioning process of *A. millefolium* with mycorrhizal fungi does not necessarily result in mycorrhizae formation as seedlings.
- The conditioning process of *A. millefolium* with soil microorganisms in general does not result in improved plant vigor or growth promotion
- Conditioned *A. millefolium* seeds do not appear to germinate differently from unconditioned seeds.

## **METHODS**

We received seeds of common yarrow (*Achillea millefolium*) from the Caltrans Office of State Landscape Architecture. Yarrow is a native perennial herbaceous forb common throughout California grasslands. Our previous work with this species certifies its mycotrophic status. Caltrans acquired conditioned and unconditioned yarrow seeds from the vendor (ConservaSeed). The conditioned seeds were coated with the Sow-EZ product. Both the conditioned and unconditioned seeds originated from the same seed lot. Upon visual inspection, conditioned seeds could be classified into two distinct size categories. Size variation appeared to be due to the thickness of the Sow-EZ product as it adhered to the seed; some individual seeds exhibited very thick coats while other seeds



exhibited thin, light coats of inoculum. In some cases, seed clumping caused the apparent size variation, so that it was possible for two or more seeds to adhere together and thus be uniformly coated during the Sow-EZ conditioning process. With size variation evident, we further hypothesized that colonization intensity would be associated with inoculum quantity, such that thickly coated seeds should exhibit greater mycorrhizal colonization than lightly coated seeds.

We used a 1mm mesh sieve to objectively sort the conditioned seeds into thickly coated or lightly coated categories. It was not possible to distinguish thickly coated clumped seeds from thickly coated single seeds, so all thickly coated seeds separated by the dry-sieving procedure were treated as a single group. Thus, we designated three treatment groups: (1) thickly coated conditioned seeds; (2) lightly coated conditioned seeds; and (3) unconditioned seeds as a control. We replicated each treatment 10 times.

We planted all seeds with a mix of field soil and sand (1:1 by volume) that had been autoclaved twice for a minimum of 90 minutes. The field soil originated from a construction site adjacent to the UCI Ecological Preserve in Irvine, CA. We filled 30 containers with the sterile soil/sand mix and seeded in 5 seeds per cone for each of the three treatment groups. Following seedling emergence, seedlings were thinned to 1 individual per pot. Plants were fertilized approximately every fourth week with a dilute solution of phosphorus-free fertilizer (20-0-20) for a total of 210 mg of N added throughout the growth period. All plants were grown for 134 days under ambient light and temperature conditions in an Indiana University greenhouse (Fig. 8.1). Plants were harvested by clipping each individual at soil level. Root and shoot materials were processed separately. We washed and weighed the fresh root material and then

subsampled each root ball for microscopy evaluation. All shoots and remaining fresh roots were then oven dried to a constant mass at 65 °C. Subsampled roots were cleared in 10% KOH and stained according to the methods of McGonigle et al. (1990) and inspected under a light microscope for evidence of mycorrhizal colonization.

To test for germination, we counted conditioned and unconditioned seeds and placed them on filter paper inside sterile petri dishes. We replicated this procedure for another set of seeds, moistened the filter paper, and placed the dishes under a grow light for 14 days. Germinated seedlings were counted at 4, 6, and 14 days. We report the mean values for the conditioned and unconditioned treatment groups.



**Figure 8.1.** *Achillea millefolium* grown from conditioned and unconditioned seed (Sow-EZ) after 18 weeks of growth.

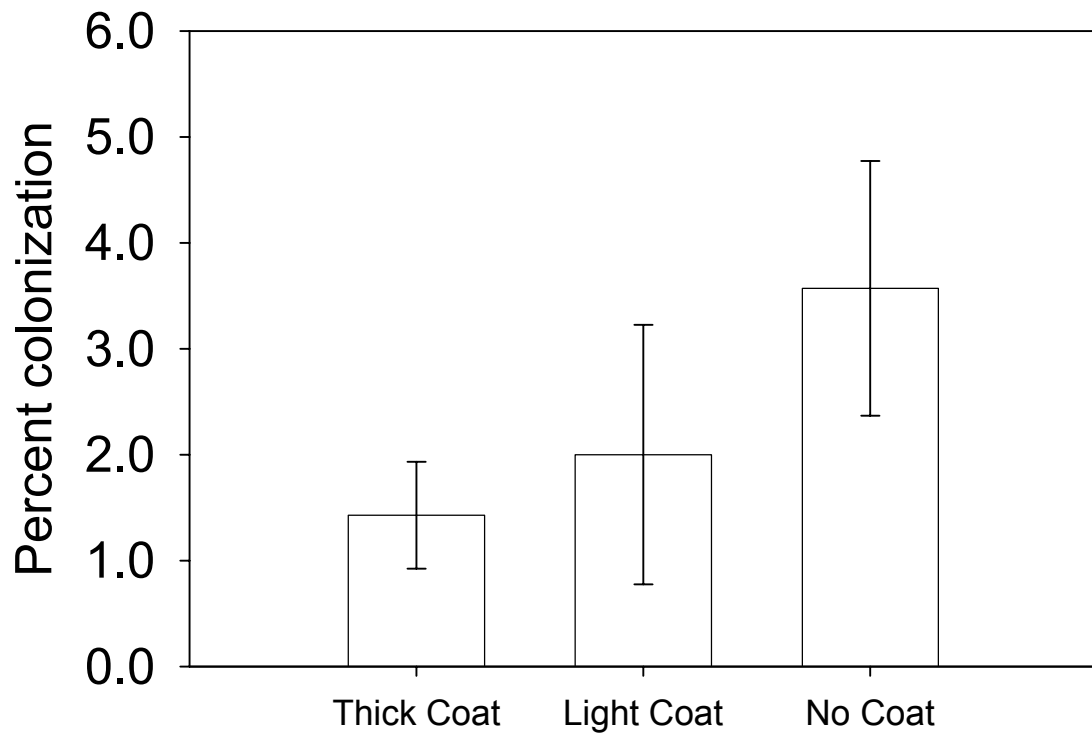
### *Data analysis*

We analyzed biomass data (roots and shoots) and percent colonization data using a one-way ANOVA.

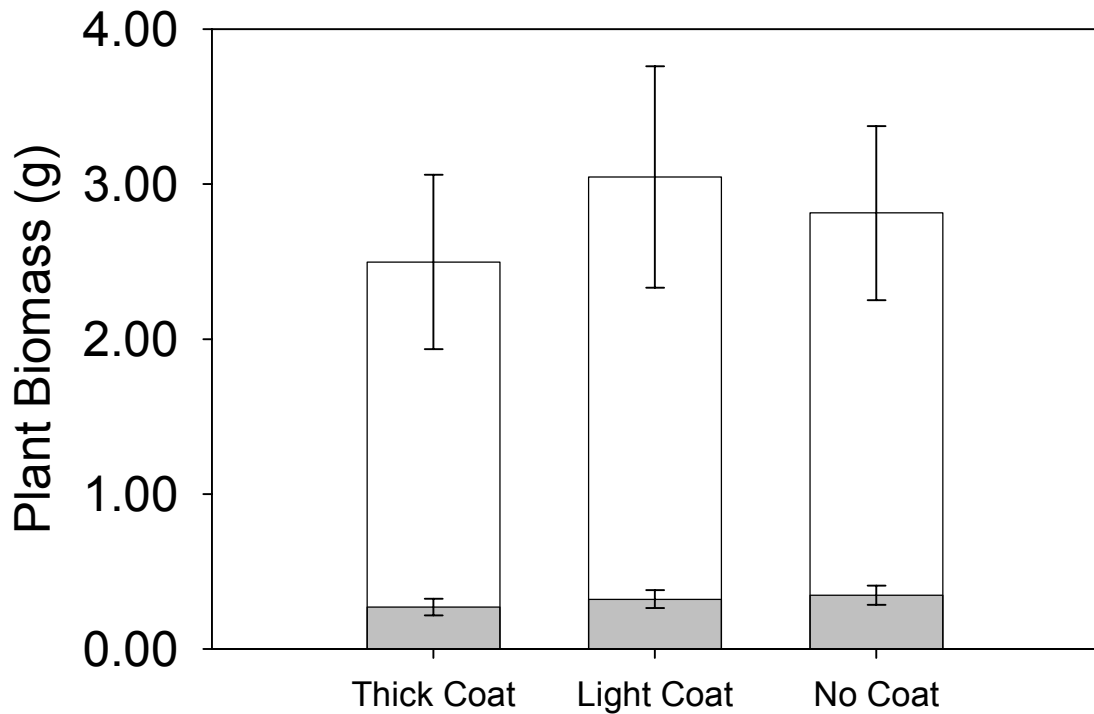
## RESULTS

We observed no differences in percent colonization between thickly coated and lightly coated conditioned seeds, or among the conditioned seeds and the unconditioned controls (Fig 8.2). If viable AM fungal propagules were present in the coating of the conditioned seed, then mycorrhizal colonization should be evident throughout the root samples. Various saprophytic (non-mycorrhizal) fungi were evident throughout both the conditioned seed treatments, but largely absent in the unconditioned seed treatment. It is thus likely that these saprophytic fungi were among those non-mycorrhizal species included by the vendor. For example, *Trichoderma*, a cellulolytic fungus known to be antagonistic to pathogenic fungi, was listed in the product literature. The identity of these saprophytic fungi remains unknown, and it is unclear if they belong to the genus *Trichoderma*, but their presence provides qualitative evidence that the Sow-EZ seed conditioning process can deliver viable fungi to the rhizosphere. We note, however, that the overall fungal colonization levels are very low. We observed no differences in shoot or root biomass among these treatments (Fig. 8.3), an observation that is consistent with the root colonization results.

We observed no meaningful survivorship differences among the groups. Of the 10 replicates planted for each of the three treatments, 8 thickly coated, 5 lightly coated, and 7 uncoated seedlings survived until harvest. Mean germination of the conditioned seeds after 14 days = 55.5%, and germination for the unconditioned seeds = 58%. These differences are negligible and not significant, and we note that  $n = 2$  for the germination assay.



**Figure 8.2.** The mycorrhizal colonization of *Achillea millefolium* after 19 weeks of growth. All treatments exhibited minimal colonization, and the conditioned seed treatments did not differ from the unconditioned seed.



**Figure 8.3.** Biomass differences among conditioned and unconditioned *A. millefolium* seed after 19 weeks of growth. Open bars are mean values for the fresh roots, and shaded bars are mean values for the oven-dried shoots.

## DISCUSSION

These data do not support our hypothesis of mycorrhizal colonization enhancement in seeds conditioned with the Sow-EZ product. After 19 weeks of growth, we would expect abundant overall levels of colonization throughout any treatments where viable mycorrhizal propagules were present. The low levels of mycorrhizal colonization, coupled with the lack of between treatment differences, suggest viable mycorrhizal propagules were absent from the conditioned seeds.

We observed no meaningful differences in either germinability or survivorship, which were two specific product claims we evaluated.

Given these results, we do not recommend the use of Sow-EZ conditioned seed if the product is being used as a substitute for viable mycorrhizal inoculum. Conditioned seed may be desirable for other purposes, however. For example, the product literature suggests conditioned seeds are not attractive to birds and other granivores. We did not evaluate this claim, but if conditioned seeds are indeed ignored by wildlife then the product could have potential uses in areas where seed predation is a concern. The product literature also suggests conditioned seeds are less prone to aerial drift due to the altered ballistic properties. We believe this claim is both plausible and likely, but note that this, too, was outside the scope of our test.

## **CHAPTER 9: Soil stockpiling for use as live soil inoculum: a review**

### **INTRODUCTION**

Severe soil disturbances are known to deplete existing communities of arbuscular mycorrhizal fungi (Moorman and Reeves 1979, Reeves et al. 1979) and disrupt hyphal networks (Bellgard 1993). Given the importance of these soil mutualists to many native species throughout the arid west, incorporating AM fungi into revegetation or restoration projects has become common. For large-scale road construction projects, one of the easiest and most cost-effective techniques for including indigenous soil microbes into a revegetation plan is to use salvaged topsoil. This topsoil can originate from the initial construction site or can be transported to the revegetation site from a suitable donor site that exhibited the desired plant community. Regardless of the source, salvaged topsoil must often be stockpiled prior to being used as replacement soil.

As a form of soil disturbance, excavating and stockpiling soil will dramatically alter soil microbial communities in general and mycorrhizal fungi in particular (Visser et al. 1984a, Visser et al. 1984b). As a planned disturbance, however, stockpiling represents an important opportunity to address the post-disturbance restoration process. Thus, understanding the changes to the soil biota becomes a crucial part of any revegetation effort where the use of indigenous soil microbes is warranted. Soil stockpiling has become common throughout the surface mining industry, where regulations stipulate that impacted sites must be mitigated or restored following the completion of mining activities. As such, there has been considerable research interest

on the effects of stockpiling on mycorrhizal viability, and the various storage conditions that maintain or degrade the inoculum potential of salvaged topsoil.

## **METHODS**

For this study, we reviewed the scientific literature to assess the results of studies where topsoil stockpiling was the primary treatment. We focused primarily on investigations of mycorrhizal viability and/or infectivity (also known as inoculum potential) although most studies quantified a variety of physical and biological soil responses.

## **RESULTS AND DISCUSSION**

Rives et al. (1980) produced the earliest known empirical data on the effects of time on mycorrhizal viability. In general, they found that mycorrhizal populations would decline over time, but that high colonization levels can still be observed even after 4 years of storage. Gould and Liberta (1981) repeated the study first reported by Rives et al. (1980) and found evidence of seasonal effects with respect to collection time. Conditions such as temperature, exposure to sun, soil moisture, and the quantity and quality of host species will influence the biology of mycorrhizal fungi. However, these early investigations considered only the effects of time.

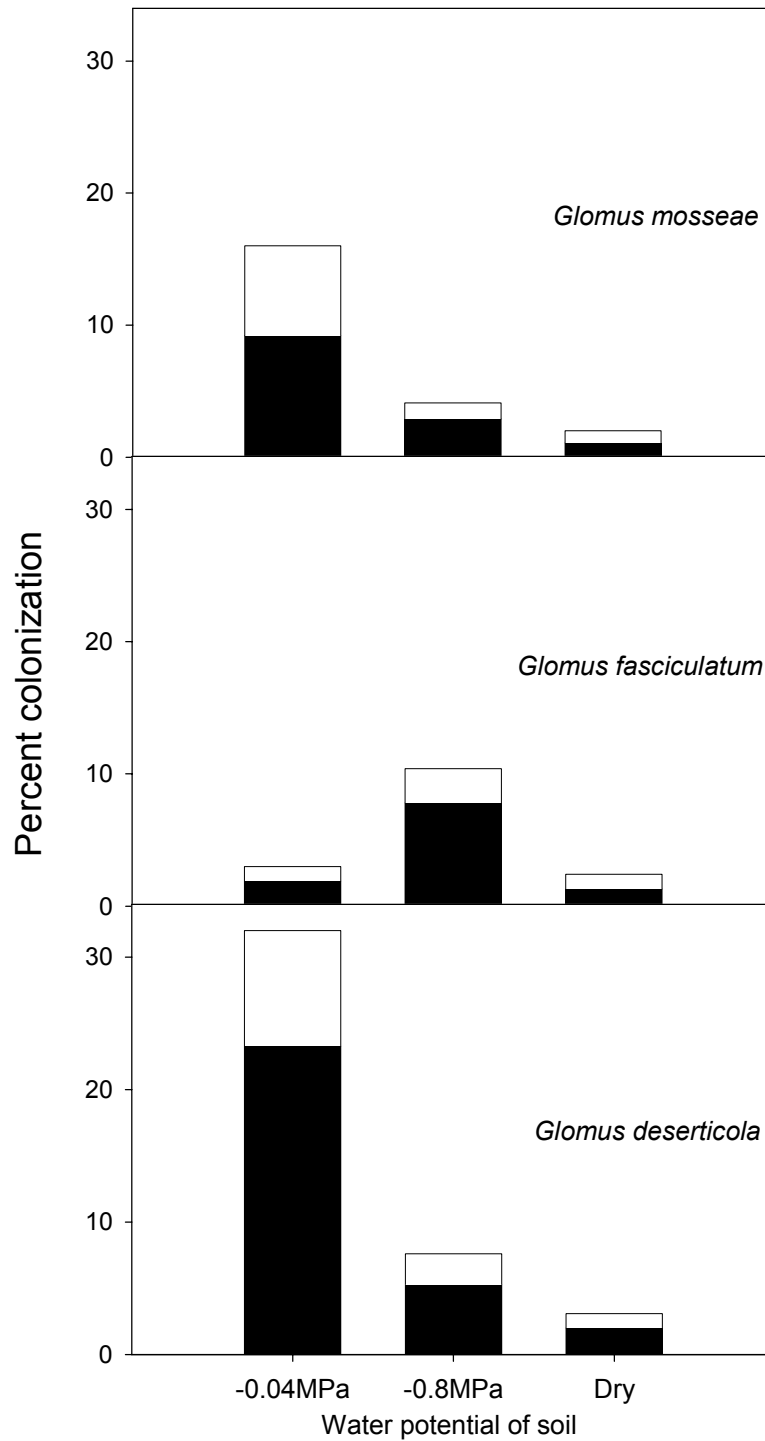
A study published by Miller et al. (1985) investigated soil stockpiled for 6 years, and did consider soil moisture. They found that mycorrhizal viability of soil from a cold desert high-elevation Wyoming scrub community was generally stable for 2 years, and then declined rapidly thereafter. These workers also observed low infection when soils were relatively moist, but then found that with drier soils, length of storage time became



a better predictor of inoculum viability. An increase in soil moisture can also improve mycorrhizal viability. For example, in controlled laboratory conditions, Wagner et al. (2001) found that at ambient temperatures, it was beneficial for mycorrhizal spores to be stored in relatively moist soil (10% moisture). Wagner et al. (2001) also report that temperature effects are more critical than soil moisture effects with respect to spore survival. Under cold storage at 4 °C, the half-life of *Glomus claroideum* spores increases from 2 years to 3.5 years. We note, however, that stockpiled soil for most California projects is likely to experience a wide range of temperatures, and long-term cold storage would not be feasible. In another controlled laboratory experiment, Ruiz-Lozano and Azcon (1996) found that wetter soils improved infectivity over storage in dry soils for three common species of *Glomus* (Fig. 9.1).

The previous two findings run counter to conventional storage recommendations that promote dry storage as the preferred method. We note, however, that controlled laboratory studies for questions related to stockpiling field soil must be interpreted with caution given the dramatic environmental differences between controlled indoor and uncontrolled outdoor studies. Even so, these findings do suggest stockpiled soil should be managed for the proper soil moisture conditions. It is unclear what represents optimal soil moisture conditions, and we suspect this factor would vary by region, ecosystem, and plant community. For example, mycorrhizal fungi native to grasslands adapted to continental or tropical climate regimes might remain viable under moisture conditions very different from fungi native to grasslands in Mediterranean-type ecosystems. Ruiz-Lozano and Azcon worked in the Mediterranean-type ecosystems of Granada, Spain with the local mycorrhizal fungi, and found that infectivity decreased considerably as soils

dried. Ruiz-Lozano and Azcon (1996) inoculated only with spores in their investigation, and so it may be that excluding other mycorrhizal propagules such as hyphae and infected root fragments contributed to their results.



**Figure 9.1.** Mycorrhizal colonization of *Lactuca sativa* roots after 6 months of soil storage under variable moisture conditions. Two dilutions are shown for each fungal species; 1/4 (white bars) and 1/16 (black bars). Data reproduced from Ruiz-Lozano and Azcon (1996).

Claassen and Zasoski (1993) published a comprehensive study on the use of topsoil for road construction revegetation. They assessed soil quality by measuring nutrient levels, nutrient availability, and productivity potential. A comparison between topsoil stockpiled for five months, fresh topsoil, and dried topsoil revealed no effects with respect to nutrient properties. In a comparison between a live topsoil treatment and a subsoil treatment, the biological activity of the topsoil contributed to important changes in plant community composition, improved productivity, and yielded greater production of mycorrhizal roots. After five months of storage, stockpiled soil did not differ from fresh soil in its mycorrhizal potential. Threshold volumes exceeding 20 % of live soil were also necessary to detect a mycorrhizal response, which suggests that inoculum density is a crucial factor in the use of mycorrhizal amendments.

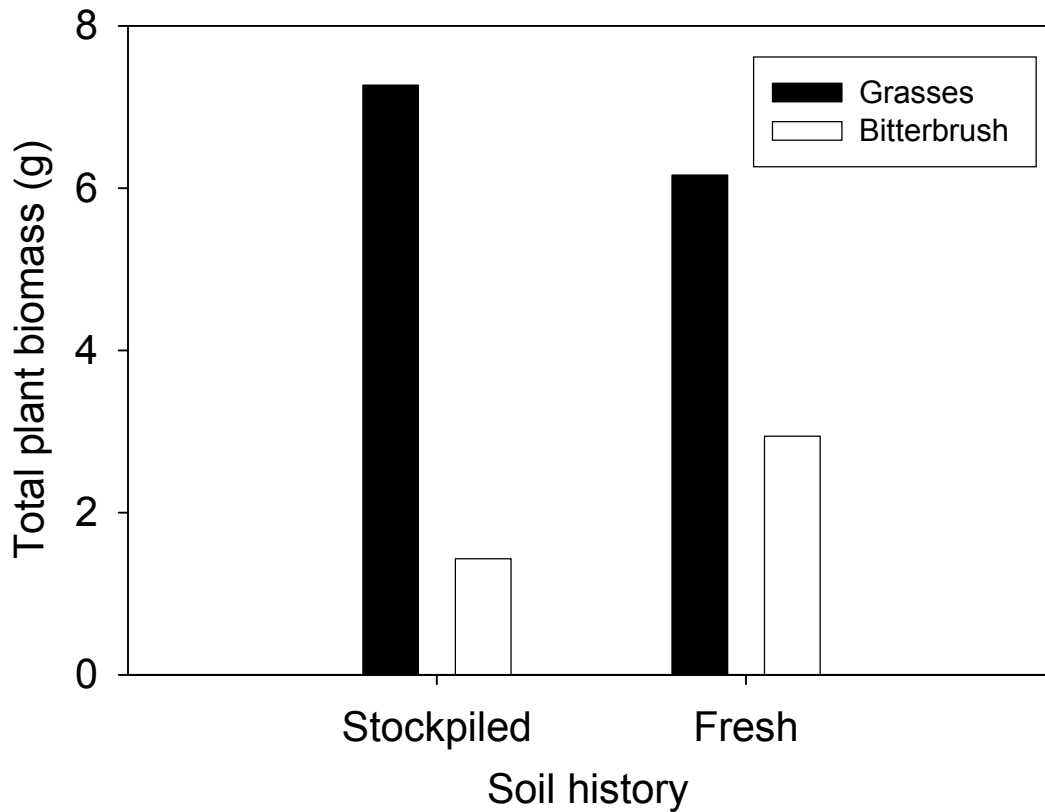
Although not found by Claassen and Zasoski (1993), changes in chemical and physical properties are possible in stockpiled soils. Given the mechanized handling and degree of mixing inherent in the harvesting of topsoil, it would be surprising not to observe such changes. Abdul-Kareem and McRae (1984) working with 18 different soil stockpiles in Britain report dramatic losses in soil aggregate stability due to stockpiling, as well as important changes in soil carbon, nutrient availability, and nitrogen composition. These workers also found reduced mycorrhizae formation and lower microbial biomass in stockpiled soils relative to undisturbed soil.

An important question that few researchers have investigated directly is how well do stockpiled soils promote the growth of desirable plant species? Stark and Redente (1987) report that stockpiling soil will negatively affect species dependent on mycorrhizae, but have no adverse effects on grass species such as western wheatgrass

that exhibit only weak relationships with AM fungi. These researchers used bitterbrush seedlings (*Purshia tridentata*), Indian ricegrass (*Oryzopsis hymenoides*) and western wheatgrass (*Agropyron smithii*) as indicator species grown with fresh soil and soil stockpiled for 5 years. Although all three species can be colonized by AM fungi, the bitterbrush is highly dependent on the mutualism, and performed poorly on stockpiled soil relative to the fresh field soil. The other two species are known to be less dependent on AM fungi, and consequently, grew better in the stockpiled soil that had been severely disturbed (Fig. 9.2). The results reported by Stark and Redente (1987) are consistent with the data we report in Chapters 2 and 3, and would be expected if AM fungi are being degraded during the stockpiling process.

Without exception, all studies we reviewed found that topsoil removal and stockpiling resulted in some kind of detrimental effect on its biotic activity. These detrimental effects varied widely, however, which suggests there is much to be learned with respect to removing and managing salvaged topsoil. The use of topsoil is clearly an important tool for site restoration, and any detrimental effects on stockpiled soil must be considered against alternative strategies. For example, the complete loss of topsoil from a site would create a severe restoration problem because the valuable soil resource plants require takes hundreds if not thousands of years to develop naturally, and thus cannot be easily replaced without tremendous expense and effort. With respect to revegetation success and the use of stockpiled soil, research is needed to understand the loss of mutualistic species such as mycorrhizal fungi in California's ecosystems, and also the relative importance of the various biophysical changes known to occur throughout the

soil harvesting process. Future research should also focus on ways to maintain the biological activity of stockpiled soil.



**Figure 9.2.** A comparison of the production potential of stockpiled and fresh field soil from the Piceance Basin of northwest Colorado. Bitterbrush, which is more highly dependent on AM fungi, grows better in fresh field soil, whereas the grasses, which exhibit less mycorrhizal dependence, grow better in soil with a disturbance history. Figure generated from data in Stark and Redente (1987).

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