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16. ABSTRACT

We report the results of several field and greenhouse projects designed to improve understanding of native plants and their association with arbuscular mycorrhizal fungi in a restoration context. At a site near Los Olivos, California, we tested the erosion control benefits of two live mycorrhizal amendments and a sterile control on a filled embankment. We also evaluated whether the responses vary by the method of application. After three years of field data, we find that the native inoculum cultured from a nearby donor site outperformed a commercially available product in reducing soil erosion. We also tested the utility of applying of a commercial mycorrhizal inoculant as specified by the vendor for use in restoring a cut slope lacking topsoil. We find no evidence for such a benefit, and conclude that other soil amendments and restoration practices should be employed when a cut slope restoration is necessary.

We used an area in San Mateo County, California to investigate some of the biophysical changes that occur when native topsoil is excavated and stockpiled, and to identify whether topsoil can be managed to maintain or improve its viability as a growth medium for native plants while being stockpiled during a construction operation. Our project tracks a general decline in soil quality from excavating and salvaging operations, evident in the loss of soil aggregate stability, increased bulk density, lost soil organic matter, and a sharply reduced nutrient profile. We find that native species can benefit in subtle ways from the application of a cover crop once stockpiled soil has been redisturbed and re-applied for habitat restoration. These benefits would likely accrue over time and a cover cropping regime would need to be implemented with a carefully managed fertilization plan to replace nutrients lost from the initial excavation.

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Prepared for the
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Disclaimer Statement

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Executive Summary

We report the results of several field and greenhouse projects designed to improve the ecological understanding of native plants and their association with arbuscular mycorrhizal fungi (AMF) in a restoration context. AMF are found throughout the world in most terrestrial ecosystems, and form symbiotic associations with their plant hosts. In the context of habitat restoration on nutrient poor soils, the AMF/plant association is generally beneficial to both organisms, and thus can be a valuable tool for restoring the structure and function of severely degraded ecosystems. At a site near Los Olivos, California, we tested the erosion control benefits of two live mycorrhizal amendments and a sterile control on a filled embankment. One of the live treatments used a nearby native donor site as the original source of AMF, while the other live treatment used a commercially available AMF inoculant. We also evaluated whether the responses vary by two common methods of application: hydroseeding and dry broadcast. After three years of field data, we find that the native inoculum cultured from a nearby donor site outperformed a commercially available product in reducing soil erosion. Proximate to this same site, we also tested the utility of applying of a commercial mycorrhizal inoculant as specified by the vendor for use in restoring a cut slope lacking both topsoil and subsoil. We find no evidence for such a benefit, and conclude that other soil amendments and restoration practices should be employed when a cut slope restoration is necessary.

A second component of this research investigated some of the biophysical changes that occur when native topsoil is excavated and stockpiled for later use as a restoration substrate. We asked whether topsoil—as found in its stockpiled condition—can be managed to maintain or improve its viability for shrubs and forbs native to California's coastal mountains. This project took place in San Mateo County, California, in the Devil's Slide area of Highway 1. Our project

tracks a general decline in soil quality from excavating and salvaging operations, evident in the loss of soil aggregate stability, increased bulk density, lost soil organic matter, and a sharply reduced nutrient profile. We find that native species can benefit in subtle ways from the application of a cover crop that includes a mycorrhizal inoculant once stockpiled soil has been re-disturbed and re-applied for habitat restoration. Some native species may also be inhibited by a cover cropping regime, but for restoration purposes, these effects can be readily mitigated by restoring lost soil fertility. Although not particularly strong after one season, the benefits to native species of cover cropping with a mycorrhizal inoculant would likely accrue over time. Additional improvements to native species restoration would be enhanced by a cover cropping regime implemented along with a carefully managed fertilization plan to replace nutrients lost from the initial excavation.

Los Olivos Erosion Experiment

Chapter summary

We tested the erosion control benefits of two live mycorrhizal amendments and a sterile control on a filled embankment in northern Santa Barbara County, California. We also evaluated whether the responses vary by the method of application. Hyphal growth of AMF is known to contribute to the stabilization of soil aggregates, thus we predicted that live AMF amendments should reduce soil loss. We also predicted that inoculation benefits would vary by ecotype of the AMF, and tested whether a native, locally sourced ecotype performed differently than a commercially sourced culture. After three years of field data, we find that the native inoculum cultured from a nearby donor site outperformed a commercially available product in reducing soil erosion.

Introduction

Restoration practitioners and revegetation professionals in California and beyond have widely adopted the routine use of arbuscular mycorrhizal fungi (AMF) as a soil amendment. These symbiotic fungi inhabit the soil and colonize the roots of suitable host plants. The relationship between plants and AMF is often mutualistic, as plants gain access to low-mobility soil nutrients transported by the fungi, while the fungi receive photosynthate from the plants. As a commercially available restoration tool, the product is usually sold in bulk quantities of 50 to 60 lbs. The media consists of granules of baked calcined clay that range in size from 0.5 mm to 2.0 mm and contains severed roots, hyphae and spores produced from the culturing process. In this form, the product is amenable to being applied to the soil in various ways, including hydroseeding and hydromulching equipment, seed drills, and fertilizer spreaders.

The rationale for inoculating a disturbed landscape with AMF is a strong one, as there is substantial scientific and anecdotal evidence that AMF inocula improve the vigor and survivorship of their plant hosts (Reeves et al. 1979, Allen and Allen 1988, St. John 1998, Richter and Stutz 2002, Vogelsang and Bever 2009b), and that mycorrhizal associations have particular benefits for California native species (Vogelsang and Bever 2009a). These fungi—which require the symbiotic association with a plant host to grow—utilize the roots of the vast majority of plant species (Brundrett 2002) and often improve plant performance by increasing access to low-mobility soil nutrients such as phosphorus and zinc (Smith and Read 1997). In a restoration context where degraded landscapes are the norm, reestablishing biota from many taxonomic groups is necessary so that nutrients and materials can once again cycle between organic and inorganic forms (Brussaard 1998). AMF and their associated plant communities therefore underpin the emergence of functional terrestrial ecosystems capable of providing the many ecosystem services that benefit wildlife and the general public.

An important indicator of ecosystem functioning is how well soil is protected from the erosive forces of wind and rain. Sufficient vegetative cover and root development are crucial to protecting soil from the impacts of rain and flowing water (Osborn 1954, Gyssels et al. 2005), and thus the mycorrhizal symbiosis, by improving the vigor or competitive ability of plants, plays an indirect role in soil erosion potential. More directly, AMF are involved in the formation and stabilization of soil aggregates, and aggregate stability has been shown to relate strongly to a soil's erosion potential (Barthes and Roose 2002). In the rhizosphere, soil particles become physically entangled by roots and hyphae and are eventually cemented together from the interaction of various exudates (Jastrow and Miller 1991, Rillig et al. 2002). It is largely unknown, however, whether the routine and ubiquitous use of arbuscular

mycorrhizal inocula have any direct impact on the process of soil erosion independent from plant growth or competitive advantage effects.

In recent years, the widespread use of AMF as a restoration tool has been criticized out of concern for the genetic integrity of natural habitats and also out of concern that introducing novel species into new habitats may produce unintended and undesirable consequences. Conservation biologists highlight good theoretical and empirical reasons for why the source of plant genetics should be considered in ecological restorations. Some of these reasons include the possible loss of genetic diversity or hybridization effects that threaten locally adapted genotypes (Montalvo et al. 1997, Hufford and Mazer 2003, McKay et al. 2005, Fant et al. 2008). As with plant populations, AMF communities have been shown to exhibit ecotypic specificity. For example, serpentine soils in California are known to harbor distinct AMF ecotypes (Schechter and Bruns 2008), and native coastal dune grasses in Florida are known to grow preferentially when the plant ecotype is matched to its local AMF community (Al Agely and Sylvia 2008). Thus, microbial introductions may deserve the same genetic considerations restoration practitioners often apply to plant species.

From an ecosystem functioning and invasion biology perspective, it may be also be important to consider how well a particular fungal inoculation integrates with the resident ecological community (Schwartz et al. 2006, Antunes et al. 2009), or whether the isolates are a good ecotypic match to a particular environment being restored. For example, the germination cues for the host and mutualist may not be synchronized, and some fungal isolates vary substantially in how rapidly they colonize roots, with Glomaceae outpacing other groups (Hart and Reader 2002). This variation in colonization speed could be functionally significant with respect to erosion if a mycorrhizal inoculation contains only fast germinating or fast growing

species that languish and die before any suitable host species is available to support the mutualism. As is often the case in a landscape restoration after construction, perennial plants are not immediately available, and a functioning ecosystem must be rebuilt by starting with fast-growing, erosion control grasses, forbs and other erosion control technologies that might include fiber rolls, mulches, and erosion control blankets. Soil erosion potential would be expected to increase under these circumstances, until sufficient time when the plant community and other AMF could become established. Other inoculations may introduce unseen competitive interactions that impact erosion potential. For example, Bennett and Bever (2009) observed that the AMF isolate *Scutellospora calospora* could outcompete other species for root colonization sites, while at the same time providing inferior growth promotion to its host. Vogelsang et al. (2006) observed another kind of trade-off at the community level, where isolates that excelled at promoting the diversity of a prairie system differed from AMF isolates better at productivity promotion, and moreover, the identity of these key microbial players shifted along an environmental gradient.

Ecotypic specificity of the AMF community would therefore become a prudent consideration in habitat restoration. Most commercial mycorrhizal inoculants are known to contain very few isolates, most typically just one species (*Glomus intraradices*) of unknown origin, and so we hypothesized that ecotypic differences between a widely available commercial product and a cultured inoculum from a donor site proximate to a restoration site would have functional consequences in an erosion test. More broadly, we also wanted to investigate the general effects of live mycorrhizal inoculation on soil erosion, an important ecosystem process that must be mitigated during most large-scale construction projects. AMF are also amenable for use in or in conjunction with the various application technologies

employed by landscape contractors. These technologies include seed drills, straw blowers, and hydroseeding equipment. Thus, we designed our project to test whether plant or soil responses vary with two common application methods.

Methods

Study system

The site designated Los Olivos is named after the nearest town in the Santa Ynez Valley of northern Santa Barbara County, California. The historic ranching town of Los Olivos has an approximate population of 1000 and is 4.3 km southeast of the field site, and both are accessed from Highway 154. Hereafter, Los Olivos refers only to the research site along the Caltrans right of way off Highway 154 (34° 40'52 N, 120° 9'20 W). The site is an abandoned stretch of the highway that was moved approximately 25 m north to its current location. Los Olivos is inland from the Pacific Ocean by 23.5 km, surrounded by the outer south coast and transverse ranges of the Los Padres National Forest system. Annual rainfall amounts are highly variable from year to year, with an average of 397.5 mm distributed mostly over the winter and spring months. The region is characterized by coast live oak forest, blue oak woodland, and valley oak savannah intermixed with coastal sage scrub and annual grasslands.

Table 1. Soil nutrient and fertility profile for filled slope at Los Olivos. Except where noted, all mineral concentration in

Soil test	Mean ppm
Boron	0.4
Ca Saturation (%)	77.9
Calcium	4089
CEC	15.9
Copper	1.0
Iron	53
K Saturation (%)	3.3
Magnesium	394
Manganese	34
Mg Saturation (%)	18.8
NH_4^+	4
NO_3^-	2
Organic Matter (%)	1.2
Phosphorus	9.7
Potassium	241
pH (low)	7.4
pH (high)	8.1
Sulfur	17
Zinc	2

Experimental design

We designed a full factorial two-way experiment using

three soil amendment treatments applied in two different ways. We used randomized blocks replicated five times, and oriented these blocks west to east on a southwest facing slope. The slope was created using a coarse, sandy loam fill material that becomes increasingly rocky towards the eastern most section of the embankment. Overall soil quality was poor, characterized by low levels of available nitrogen (N, as NH_4^+ and NO_3^-), phosphorus (P, Olsen method), copper (Cu), zinc (Zn), boron (B), and soil organic matter, which ranged from 0.7% to 1.6% (Table 1). Soil pH ranged from 7.4 to 8.1. The fill soil exhibited poor structure and low aggregate stability.

We were unconcerned with the poor soil quality exhibited at this site for two reasons. First, we expected any effects from live soil amendments to be more readily observed under poor soil conditions than with good soil conditions, where other properties beneficial to plant growth and soil development might override inoculation effects. Second, better quality native soil was present at the site in areas that had clearly not been mechanically disturbed for several decades or more. This better quality soil was found under mature native shrubs, nearby oak trees, and native perennial grasses and forbs, and we used these areas as our donor site.

Soil amendment treatments consisted of two live cultures containing AMF and an autoclaved control sourced from the live cultures. The live cultures began as a commercially available product (AM 120, Reforestation Technologies International) or as bulk native soil collected from undisturbed areas of Los Olivos. Upon maturity, the live cultures were used to inoculate test plots in the field using either a hydraulically applied technique (via hydroseeding equipment) or broadcast dry and raked into the soil. All test plots then received a common seed mix.

Inoculum preparation, testing, and bioassay

For the amendment designated as "native," field soil from Los Olivos was collected from areas proximate to the native shrub *Artemisia californica* (California sagebrush) or the native grass *Nassella pulchra* (purple needlegrass). Other native forbs were also present in these collection areas (our donor site), including *Sisyrinchium bellum* (blue-eyed grass) and *Bloomeria crocea* (common goldenstar). We used an autoclaved mix of sand and California field soil (1:1 by volume) as our culture growth media, and inoculated 1 L of this sterile media with 30 mL of live field soil. Forty of these cultures were set up in 1.2 L pots. For the amendment designated as "commercial," we substituted 30 mL of the AM 120 product for the live field soil, and set up 20 of these cultures in 1.2 L pots. All cultures were seeded with *Sorghum bicolor*, *Achillea millefolium*, and *Nassella lepida* for host plants and grown in an Indiana University greenhouse for 30 weeks. Host plants were fertilized as needed with a low concentration (4 g L^{-1}) of 15-0-15 nutrient solution, administered 10 mL at a time over the growing period. After 30 weeks of growth, we ceased watering the culture pots and allowed the plants to senesce. Shoots were then cut and the root balls and soil media were chopped to produce the treatment inoculum. We subsampled from these native and commercial cultures to produce approximately 30 L of sterile control inoculum, which we then processed at 121 °C in an autoclave for one hour on three consecutive days.

We used an independent soil laboratory (Spectrum Analytic, Inc., Washington Court House, Ohio) to test the nutrient profiles of both the commercial and native cultures (Table 2). We used this information to verify that our culturing regime was sufficiently nutrient-limited to promote a robust mycorrhizal mutualism, and also to understand to what extent our treatment inoculum functioned as a source of nutrients in our field test plots. The plant macronutrients of N, P, and K all exhibited low availability. The native inoculum expressed slightly higher concentrations of most macro and micronutrients, which we attribute to the compositional differences in the respective starter inocula. We do not consider these nutrient differences biologically important at the scale for which we were interested, however.

Table 2. Nutrient profiles of cultured inoculum applied to Los Olivos test plots. Except where noted, all mineral concentrations are in ppm.

Soil test	Native	Commercial
Boron	0.4	0.3
Ca Saturation (%)	90.1	91.6
Calcium	5478	5194
CEC	16.6	16.4
Copper	1.1	1
Iron	75.2	62.9
K Saturation (%)	0.7	0.4
Magnesium	208	177
Manganese	63	56
Mg Saturation (%)	9.2	7.9
NH_4^+	6	4
NO_3^-	1	1
Organic Matter (%)	0.8	0.9
Phosphorus	13	12
Potassium	53	33
pH	7.7	7.7
Sulfur	40	42
Zinc	4.2	1.8

Commercial and native cultures were also tested for the mycorrhizal inoculum potential using a greenhouse bioassay. The project schedule constrained us in the timing of this particular bioassay, such that we were not able to complete the test for inoculum potential prior to implementing the treatments in the field. Ideally, we would have preferred to adjust inoculum volumes in the test plots to ensure comparable live propagules were being added to

every plot. As described below, plots received equal volumes under the assumption that differences between the numbers of live propagules in the commercial and native amendments were negligible. We mixed 10 mL of each inoculum type with 90 mL of sterile sand in 125 mL "conetainers" and replicated this setup five times. We included the autoclaved mix into this assay as a negative control. Surface sterilized *Sorghum bicolor* seeds were added to each conetainer and watered to initiate germination and growth of the assay. All plants were harvested at 30 days, and the roots were washed, cleared with 10% KOH, and stained with trypan blue. 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).

Site preparation and experiment installation

In May, 2006, a Caltrans maintenance crew mowed the existing vegetation along the shoulder and adjacent slope of Highway 154 where we would be implementing our erosion experiment. Three weeks later, we roto-tilled approximately 0.10 of an acre along the slope to accelerate mycorrhizal and seed bank degradation (Figure 1). Tilling also served to destabilize the existing fill material, and thus mimic construction activity where soils are restructured during grading and compacting operations. We then covered this tilled area with 4 mil thick clear plastic sheeting and secured the sheeting with sand bags and wooden stakes. The plastic sheeting remained in place for 18 weeks over the summer to solarize (Brito et al. 2009) the area and further promote the degradation of the existing mycorrhizal community. Temperature sensors installed above and below the plastic sheeting during the third week in October indicated 65 °C differences were common between day and night time temperatures, and that a maximum

temperature of 69.9 °C was reached during our sample period. We also installed a RG3-M data logging rain gauge and temperature sensor programmed for hourly readings (Onset Computer, Bourne, Massachusetts) at the site for accurate precipitation monitoring throughout the growing season.

Subsequent mycorrhizal and seed bank growth assays on soil collected from the solarized area and the unsolarized area revealed a 35% decline in mycorrhizal density in the solarized area. Seed banks were impacted less by the solarization, where we observed a significant decline in species richness ($F_{1,4} = 7.20$; $P = 0.05$), with smaller declines in the total abundance or biomass of plant species germinating after a six week germination assay. Nevertheless, we interpret the significant reduction of mycorrhizal density as a successful component of our site preparation.

Figure 1. Tilling and solarizing filled slope to accelerate the degradation of existing mycorrhizal fungi.

We installed 30 test plots across the tilled and solarized area of the slope (Figure 2). Each plot measured 1 x 9 m. Six inch wide wooden planks framed each plot so that all water and eroding soil would flow down into a one meter wide flared end section (known here as the "tray") designed for culvert and drainage applications (Hancor, Santa Fe Winwater, Santa Fe Springs, California). Each tray clamped onto a 20 foot corrugated double-wall pipe 12 inches in diameter, which was used to collect and store water that flowed away from each plot. We inoculated test plots in one of two ways: (1) broadcast dry and then hand raked into the soil or (2) hydraulically applied using hydroseed equipment. Plots received equal volumes (3 L) of inoculum, regardless of application method. After completing the inoculations, all plots were hand seeded with pre-measured seed mixes (Table 3). Plots were then hydromulched with a bonded fiber matrix consisting of wood fiber mulch and soil stabilizing compound applied at the area equivalent rate of 2000 lbs of fiber per acre. We labored to keep the mulch layers consistent across all treatments, but after the mulch had settled, we observed some variation in mulch thickness between the dryly broadcast plots and the hydraulically applied plots. Two sand-filled hyphal in-growth traps per plot were buried at a depth of 10 cm for the purpose of obtaining total protein estimates from rhizosphere biota. We assembled the traps from PVC tubes, where both ends were covered with a fine (20 μm) open mesh nylon fabric to exclude root penetration, but allowed for hyphal growth. Traps were filled with fine-grained sand washed free of any protein residues. Electrical resistance soil moisture sensors were later installed adjacent to all plots to evaluate variations in moisture availability across the slope at 20 cm. Plots were watered thoroughly for one week to help settle the soil and promote seed germination.

Figure 2. Los Olivos test plots prior to inoculation.

Table 3. Plant species added to Los Olivos test plots.

Species	Common Name	Mass (g) /plot
Artemisia californica	California sagebrush	20
Eriogonum fasciculatum	California buckwheat	30
Hordeum vulgare	Regreen barley	50
Vulpia microstachys	vulpia	50
Gnaphalium californicum	Everlasting	10
Lotus scoparius	California broom	30

Data collection: soil and water

Initial soil samples were collected for mineral and soil aggregate stability analyses. Samples from the filled slope were analyzed for pH, percent organic matter, available nitrogen (N) in the forms NH_4+ and NO_3-, available phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sulfer (S), boron (B), zinc (Zn), iron (Fe), copper (Cu), and manganese (Mn).

Average bulk density of these soils was determined at 1.32 g mL^{-1}. In many systems, the stability of soil aggregates in water is an indicator of soil erosion (Barthes and Roose 2002). Air dried soil samples passing through a 2 mm sieve and retained on a 1 mm sieve were agitated in water for 20 minutes after being pre-soaked for 10 minutes. The oven dried mass of aggregates remaining after this procedure is used to estimate the percentage of the mass that is stable in water. At the end of the first growing season, additional soil samples were collected from each plot to assess whether treatment effects could be observed on aggregate stability and soil bulk density.

Also at this time, we estimated erosion using sediment cover and total sediment mass from the trays at the base of each test plot. For sediment cover, digital photos from the same camera angle were taken of the accumulated sediment in each tray (Figure 3). The junction of the tray and the pipe along with an interior ridge at the entrance of the pipe functioned advantageously as a check dam to confine nearly all the sediment to the flared section of the tray. For each digital image, we generated a histogram in Adobe Photoshop software by selecting areas of each tray of the light-colored sediment against the black plastic background (Figure 3, inset image). This feature allowed us to count pixels in the selected image for precision estimates of percent sediment cover. We analyzed images taken after each of three growing seasons. For sediment mass, we collected all tray sediment, dried it for 48 hours at 110 °C, and weighed it. Small quantities of fine soil particles drained into each pipe, and after the first season, these fines were settled out of the drainage water and dried and weighed separately. The contribution of the fines from the pipe to the total sediment mass from the tray was negligible, however, and no meaningful differences were detected in separate analyses. Therefore, the small quantities of fines in the pipe were excluded in subsequent years in favor of the more efficient erosion estimates of sediment cover and sediment mass from the tray. For the first growing season,

rodent burrows were evident after three months, so we quantified the number of burrows in each plot and noted whether rodent tailings were accumulating in the tray.

Variable hydrological and soil moisture conditions across the slope were a concern in this project. The randomized block design would allow us to control for any hydrological gradient in the analysis, but to test for any within block or treatment effects we deployed soil moisture sensors in each plot that could be read by a digital soil moisture meter (Delmhorst Instrument Company, Towaco, New Jersey) at different times throughout the year. The electrodes on most of the *in situ* soil moisture sensors had failed by the end of the second season, so we analyzed and report on only the first year end of season data from an early morning read and a late afternoon read (a repeated measure). We quantified the water stored in each pipe by direct volume measurements the first year, and by mass measurements the second and third year, where approximately three times the amount of rain had accumulated than what was experienced in the first year. We converted water mass to volume estimates using the specific gravity properties of water, where 1 kg = 1 L. An automated data logging rain gauge monitored accumulated precipitation for each season.

Data collection: vegetation

At the end of the growing season for each of three years, we harvested vegetation from each plot using a 20 x 50 cm Daubenmire sampling frame placed in four random locations throughout each plot. Plants within the boundary of the sampling frame were clipped, sorted to species, and bagged in preparation for drying at 65 °C for a minimum of 72 hours. Dried plants were then weighed by species. Percent vegetative cover was estimated visually after each season,

but varied only for the first season. Percent cover for the second and third season was at or near 100% for all plots.

Figure 3. Sediment at the base of a test plot as used for sediment cover and sediment mass estimates. For both, all visible areas of sediment in the tray and the pipe were included. For sediment cover, sediment indicating pixels were first replaced with a false color to distinguish sediments from the plastic background, and then divided by the total number of pixels (inset image in upper left).

Data collection: Bradford total proteins

At the end of the first year's growing season, hyphal traps were retrieved and the interior sand removed for protein extraction, using the methods of Wright (1996) and Janos et al. (2008). Briefly, we treated the contents of each trap with 10 mM solution of $Na_4P_2O_7$ (pH=9.0) and

autoclaved for 60 minutes to solubilize glomalin and other proteins bound to sand particles. Supernatants were clarified on a centrifuge (10,000 rpm for 15 min) and 50 μL subsamples transferred into a 96 well plate, along with 50 μL of phosphate buffered saline and 50 μL of Bio-Rad protein dye. After mixing, we scanned well plates with plate reader to obtain estimates of optical density, which we could then compare with a standard curve prepared from a bovine serum albumin solution and calculate the mg g^{-1} of total protein in the sand. Due to technical difficulties with the extraction procedure, some samples were lost.

Data analysis

We analyzed percent mycorrhizal colonization data of the commercial, native, and sterile treatment inocula using a one-way analysis of variance (ANOVA) to compare means. Post hoc comparisons were made using Tukey's HSD test. Estimates of percent sediment cover and total sediment mass at the end of each season were analyzed using a repeated measures multivariate analysis of covariance (MANCOVA) with linear contrasts between the live and sterile AMF treatments and the native and commercial AMF treatments. The rodent burrows observed in the first season were included as a covariate in the model, providing the best and most stable fit consistent with field observations. We generated a series of Pearson's correlation matrices by block and application method to identify plots where percent sediment cover and total sediment mass did not exhibit strong positive relationships. Soil moisture and water harvest estimates were analyzed with a repeated measures MANOVA with the same linear contrasts stated above. Water harvest estimates were natural log transformed (ln(1+Y)) to satisfy variance assumptions. We used a repeated measures MANOVA for analyzing above-ground productivity and species richness responses for all three years. Biomass estimates were natural log transformed (ln(1+Y))

to satisfy variance assumptions. As with soil data, we compared vegetation data using linear contrasts between the live and sterile AMF treatments and the native and commercial AMF treatments. Bradford total protein estimates from the hyphal traps were analyzed with a two-way ANOVA with linear contrasts of the mycorrhizal treatments. We used SAS version 9.1 for all statistical analyses.

Results

We observed significant mycorrhizal colonization differences among the treatment inocula ($F_{2,12} = 132.62$; $P < 0.0001$), with the mycorrhizal density of the cultured commercial amendment 49% greater than the density observed in the cultured native amendment. Both live amendments were significantly different than the sterile control (Figure 4). This observation violated our assumption that live propagules would be roughly equivalent between the commercial and the native treatment inocula.

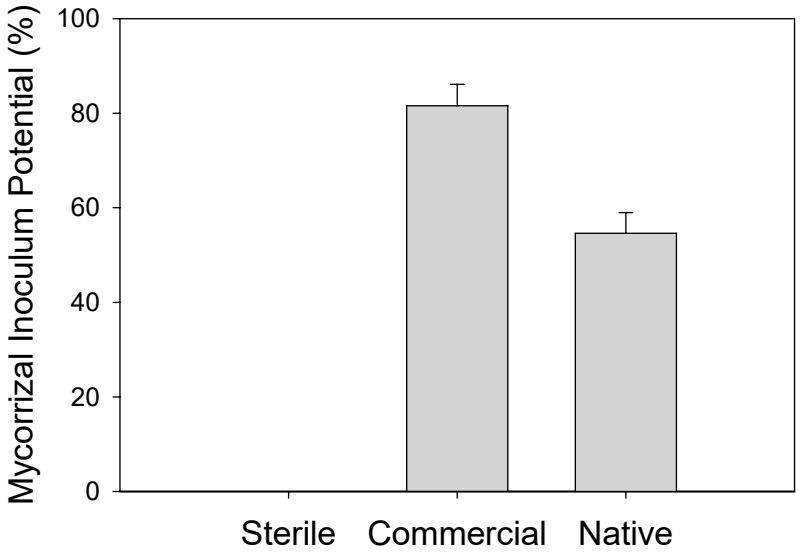


Figure 4. Bioassay of treatment inoculum revealed greater density of mycorrhizal propagules in the culture grown from AM 120.

Rainfall amounts over the course of the three year project stayed well below the long-term average of 397.5 mm for this region of California. During the first growing season, precipitation was especially low, with only 145.4 mm of precipitation

accumulating by June, which was well into the summer drought period that typically prevails until October. The second year was the wettest of the three, with 358 mm of accumulated precipitation, while the third year was still very dry, recording only 255.6 mm total precipitation (Figure 5).

Our Pearson's correlation analysis on percent sediment cover and total sediment mass generally revealed strong positive relationships ($r > 0.85$) between the two variables for each year. Exceptions to this rule were in plots where an abundance of large, coarse fragments (rocks and sand) had eroded into the tray along with the finer textured mineral components, thus confounding the otherwise linear relationship between sediment cover and sediment mass. For this reason, we view the percent sediment cover analysis to be the more reliable response variable of the two, as it better represents the silt and clay components responsible for soil fertility. When eroded into waterways, these same fine textured components also contribute more to the degradation of water quality.

Treatment effects

The multivariate test of overall differences among groups revealed significant treatment effects for both sediment mass and sediment cover, the two erosion estimates. Hydraulically applied amendments were more effective at reducing soil loss than the dryly broadcast application for sediment mass (Wilks' Lambda $F_{3,17} = 4.37$; $P < 0.05$) and sediment cover (Wilks' Lambda $F_{3,17} = 3.95$; $P < 0.05$). Overall soil amendment effects were not significant in the MANCOVA for sediment mass (Wilks' Lambda $F_{6,34} = 1.24$; $P = 0.31$), but were significant for sediment cover (Wilks' Lambda $F_{6,34} = 2.49$; $P < 0.05$). The main effects of

application method and AMF treatment were apparent in the repeated measures ANOVA for sediment mass (Application $F_{1,19} = 8.72$; $P < 0.01$; AMF $F_{2,19} = 3.04$; $P = 0.07$) and sediment cover (Application $F_{1,19} = 6.46$; $P < 0.05$; AMF $F_{2,19} = 6.84$; $P < 0.01$). For sediment mass, the univariate analysis for each year reveals weak application and AMF effects that increase over time, with the hydraulic application effects and the native mycorrhizal effects consistently yielding the lowest quantity of sediment. For sediment cover, the same statistical trends are observed, with the exception that AMF treatment effects are significant in the first year ($F_{2,19} = 4.51$; $P < 0.05$). Linear contrasts on the AMF treatments revealed the consistent erosion reduction benefits of the native inoculum (Table 4). These planned comparisons directly test our hypothesis of functional differences between a widely available commercial product and a cultured inoculant from a nearby donor site. As *a priori* tests, linear (or orthogonal) contrasts allow treatment means to be compared independently by further partitioning treatment sums of squares from our main statistical model. From Table 4, significant effects are evident for both sediment mass and sediment cover.

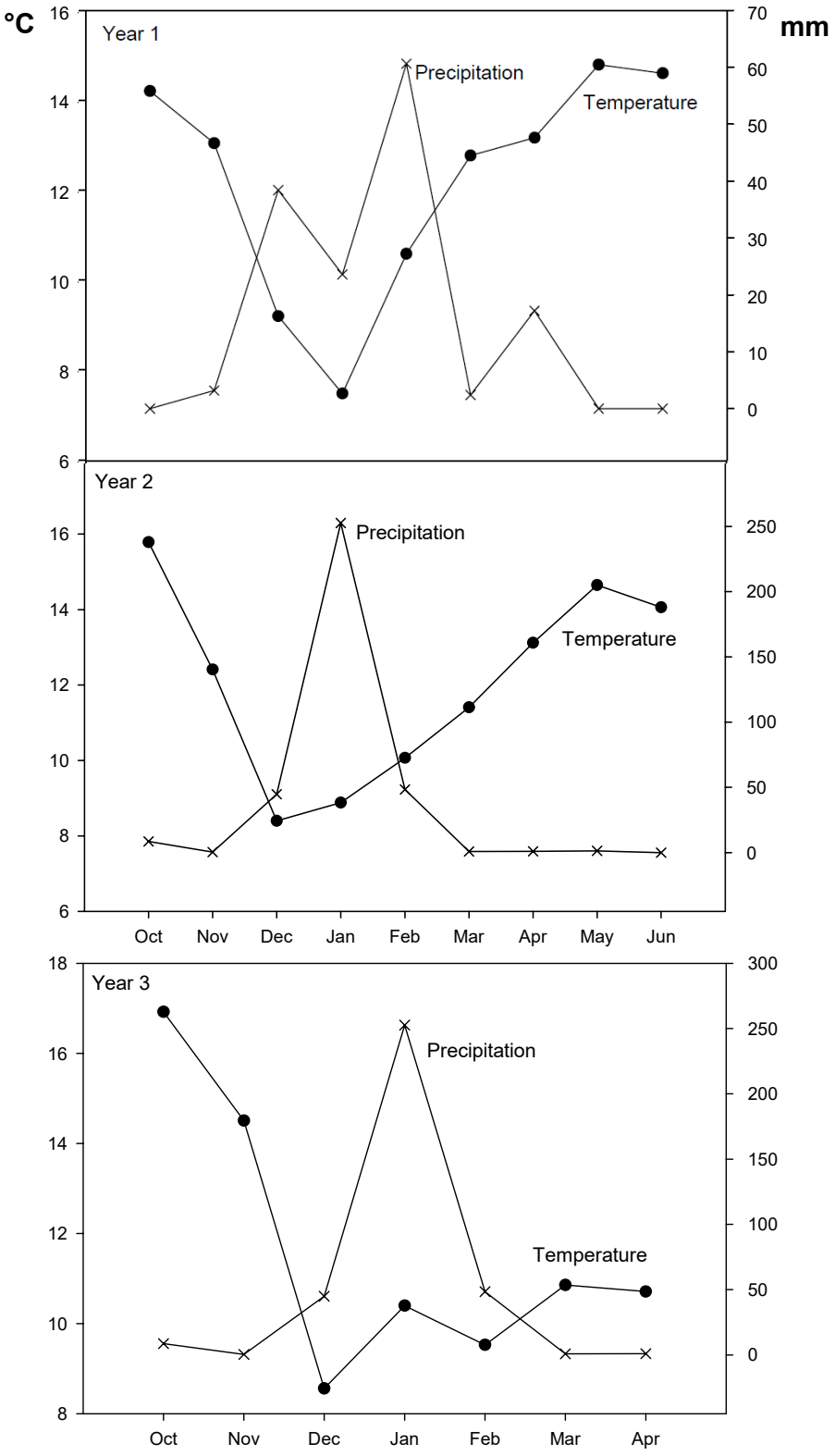


Figure 5. Seasonal hydrographs for Los Olivos. Note that all scales vary from year to year.

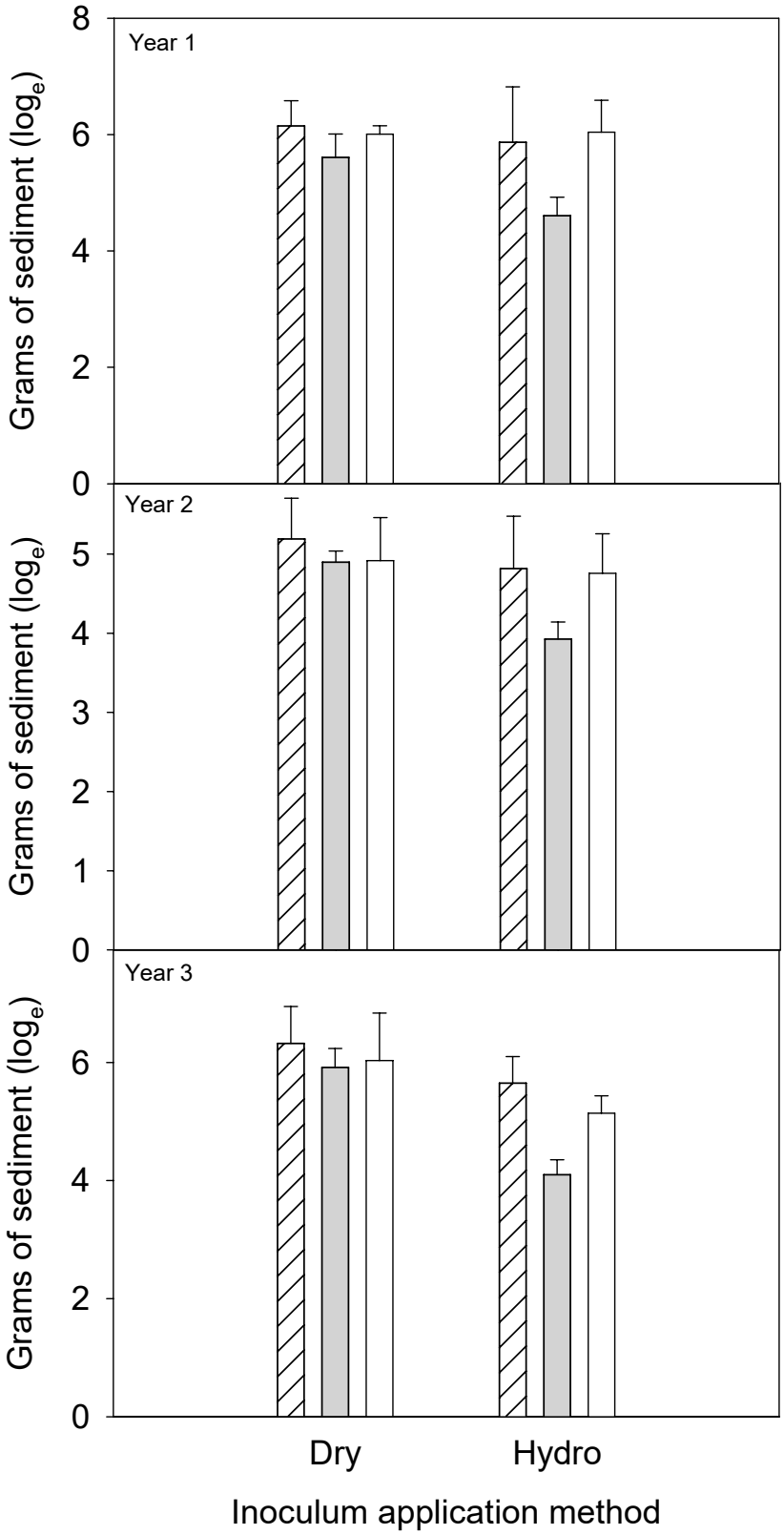


Figure 6. Sediment mass from test plots for each of three growing seasons. Inoculation treatments are commercial (hatched), native (gray) and sterile (open). All error bars are ± 1 se.

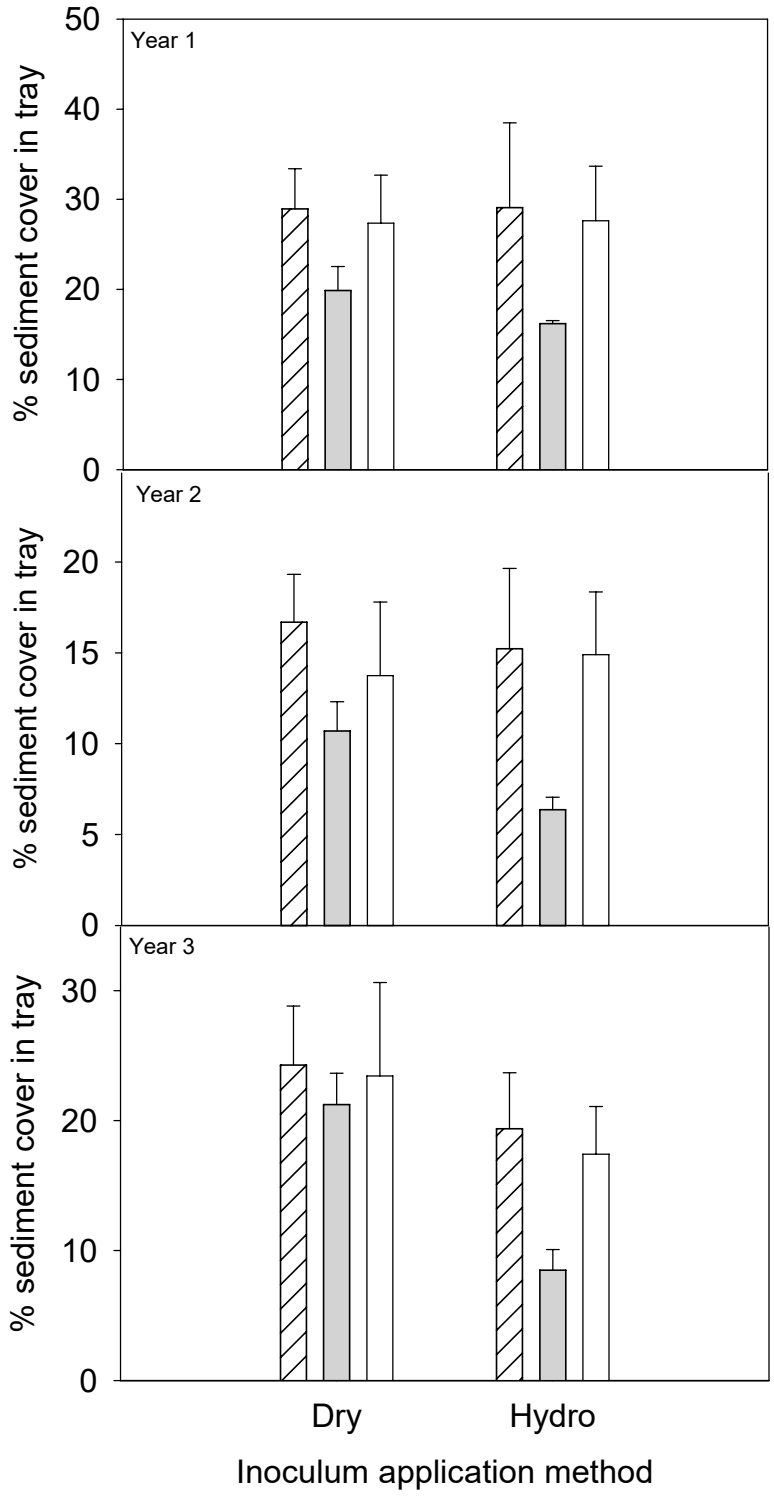


Figure 7. Erosion response as measured by sediment cover at the base of test plots. Inoculation treatments are commercial (hatched), native (gray), and sterile (open).

Table 4. Linear contrasts from univariate ANOVAs for two sediment response variables. Consistent benefits of using native mycorrhizal inoculum are evident for both response variables (where P < 0.10).

Erosion Response	Contrasts	SS	MS	F	P
Sediment Mass	Year 1				
	Live vs. Sterile	0.728	0.728	0.6	0.449
	Native vs. Commerical	4.341	4.341	3.56	0.074
	Year 2				
	Live vs. Sterile	0.061	0.061	0.18	0.676
	Native vs. Commerical	2.073	2.073	6.15	0.023
	Year 3				
	Live vs. Sterile	0.120	0.120	0.1	0.751
	Native vs. Commerical	5.305	5.305	4.59	0.045
Sediment Cover	Year 1				
	Live vs. Sterile	56.065	56.065	0.740	0.401
	Native vs. Commerical	623.858	623.858	8.220	0.010
	Year 2				
	Live vs. Sterile	0.232	0.232	0.010	0.911
	Native vs. Commerical	308.968	308.968	17.240	0.001
	Year 3				
	Live vs. Sterile	0.030	0.030	0.000	0.983
	Native vs. Commerical	274.791	274.791	4.180	0.055

Bulk density after the first year trended lower in plots treated with the hydraulic application and in the native mycorrhizal plots, but none of these trends were statistically significant in either the ANOVA or in the linear contrasts between the AMF treatments. The aggregate stability of these soils in water was enhanced slightly with hydraulic applications and was highest in the native mycorrhizal plots treated with dry inoculum (Figure 8). Linear contrasts indicate significant native mycorrhizal effects on improving the stability of the aggregate size we tested ($F_{1,20} = 4.21$; $P = 0.05$).

Soil moisture after the first year was generally higher in the afternoon than in the morning, and this effect of time was significant in the overall MANOVA (Wilks' Lambda $F_{1,20} = 4988.88$; $P < 0.0001$). For both morning and afternoon readings, we observed a general trend where soil moisture was highest in the hydraulically applied treatments and in the native mycorrhizal treatments, but none of these trends were statistically significant in either the

overall MANOVA or in any of the univariate tests. Water harvests at the end of each season varied strongly over time (Wilks' Lambda $F_{2,19} = 94.07$; $P < 0.0001$), as expected with variable rainfall, but exhibited no interactive or main effects due to treatment regime.

No significant trends in vegetation were observed throughout the three year project (Figure 9). Above ground biomass responded primarily to available moisture, with the first year exhibiting the least amount of growth and the second year producing the most, corresponding to the observed precipitation patterns. In the first year, the native, hydraulically applied plots exhibited enhanced productivity relative to all other treatments, but these were not significant differences. In both total harvested biomass and species richness, we detected no significant interactive or main effects from our treatments, and observed no consistent trends. In all three years, species composition was dominated by non-native species from the existing seed bank. Two non-native annual forbs, *Brassica nigra* (black mustard) and *Erodium cicutarium* (filaree) dominated each plot for the first two years, with the abundance and productivity of both species declining sharply in the third year as the abundance and productivity of non-native annual grasses increased. These grasses include *Avena barbata*, *Bromus diandrus*, *Bromus madritensis*, *Bromus mollis*, and *Hordeum jubatum*. *Vulpia microstachys*, a native annual grass, was also present each year, but most abundant and productive in the first year. *V. microstachys* and the sterile barley *Hordeum vulgare* were the only two seeded species that germinated and grew in the first year. In subsequent years, only *V. microstachys* could be found reliably, although we did identify small seedlings of the native shrub *Artemisia californica* in two plots during the second year. Otherwise, native species were virtually absent, and inoculation treatment had no apparent benefit for enhancing native plant abundance or cover.

The concentration of total proteins extracted from the hyphal traps was generally low, most likely due to very low plant productivity and low rainfall in the first year. We deployed two hyphal traps in each plots, and we observe no patterns in the analysis from either trap.

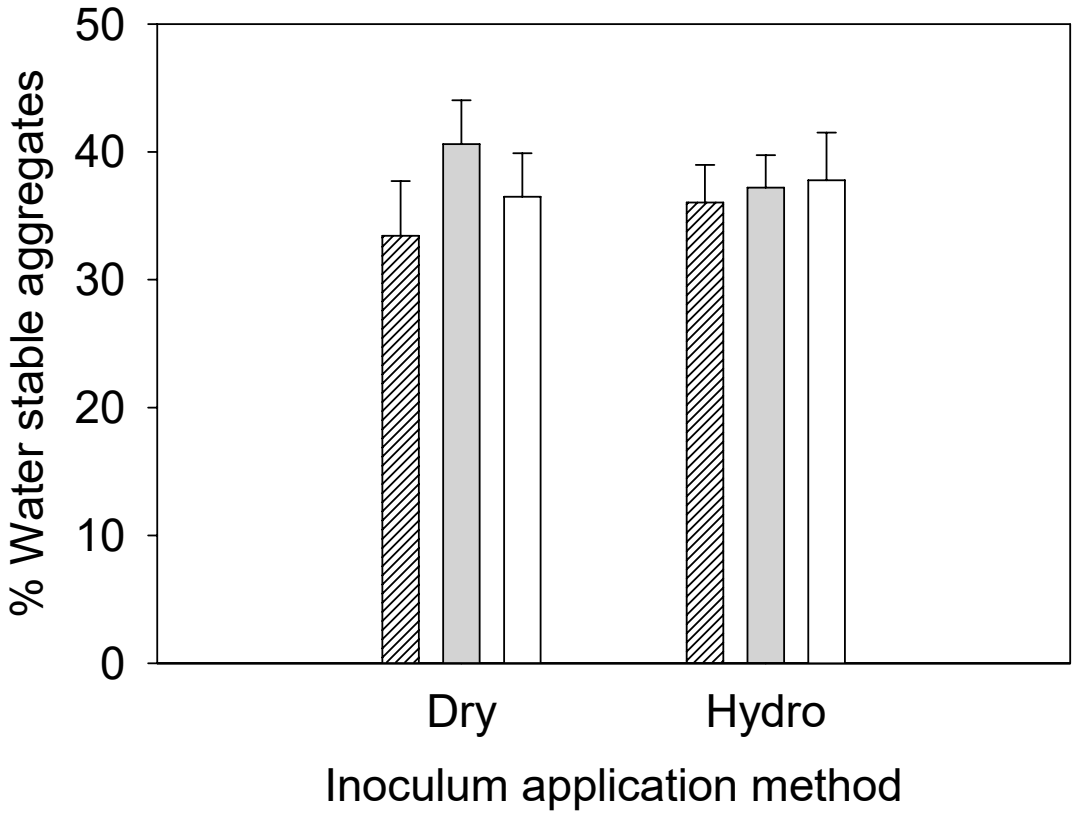


Figure 8. Aggregate stability of soil from test plots after first growing season. Inoculation treatments are commercial (hatched), native (gray), and sterile (open).

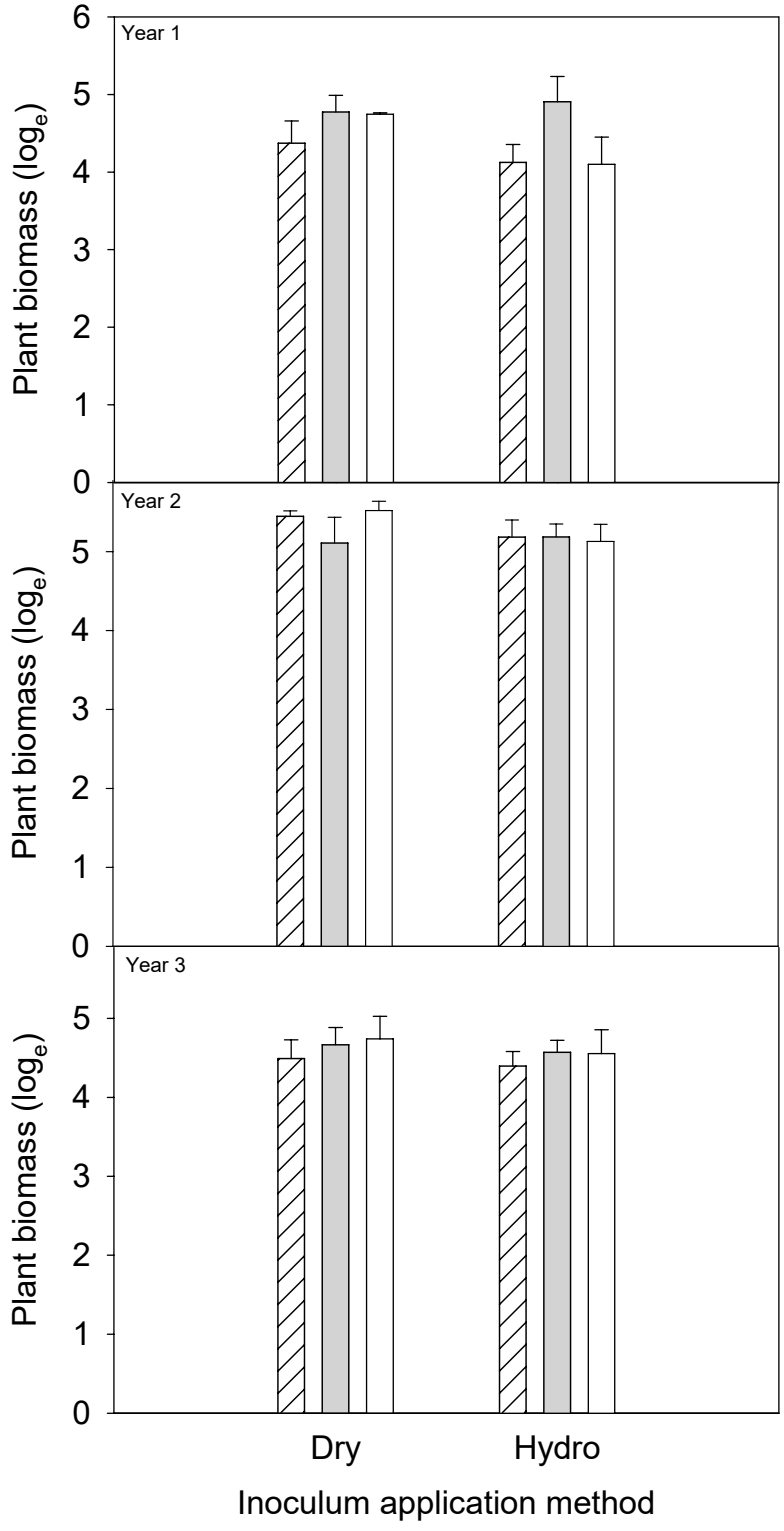


Figure 9. Above-ground productivity for each of three years. Inoculation treatments are commercial (hatched), native (gray), and sterile (open).

Discussion

For all treatments, total soil erosion was highest during the first growing season, an expected result due to the fact that plots were newly graded and mean vegetative cover by the end of the season was approximately 60%. For each of the three years under observation, the live mycorrhizal amendment cultured from a native donor site at Los Olivos exhibited superior performance with respect to reducing erosion. We observed this general pattern regardless of application method (dry or hydraulically applied). Hydraulically applied treatments outperformed dryly applied treatments, with most of the erosion reduction being driven by the native inoculum.

We calculated sediment yields by treatment to understand what these results would mean for actual restoration at this site using these materials. The hydraulic treatment was most dramatic, where compared to the control plots that received a sterile inoculum, we observed an 85% reduction in erosion from the native amendment in the first year. In the second and third years, we observed a 69% and a 68% reduction, respectively. The mean sediment yield in the first year for the control plots equaled 351 g m^{-2}, which translates to over 3100 lbs of soil per acre. The native inoculum would reduce this to an average of just under 500 lbs/acre. For the dry application, the reduced erosion effects are much more modest. Intriguingly, the year to year changes of the native inoculum to the control increase rather than decrease over time. We see a 9% reduction after the first year, with 39% and 64% reductions in the two subsequent years. We note that the effects of rodent burrowing activities have not been removed from these projections, as they were in the statistical models, nor have we accounted for any bias in the data due to rocks that collected in the trays. Nevertheless, we find compelling evidence that ecotypic specificity in the microbial community can reduce soil erosion potential, and

general support for our hypothesis that ecotypic specificity in a mycorrhizal inoculation can have functional consequences.

It is noteworthy that the native inoculum reduced soil erosion more than the commercial inoculum even though the commercial culture exhibited much higher inoculum potential (Figure 4). That is, the density of live propagules of AMF in the commercial culture as applied to the test plots was much higher than what we observed in the native culture, due to the fact that we implemented the treatments in the field under the assumption that both cultures would exhibit similar densities. The results of our inoculum bioassay revealed this to be an incorrect assumption, and suggest that the benefits of the native inoculum could be much greater that what we report here.

The commercial culture did not yield any meaningful reduction in erosion, as indicated by Figures 6 and 7. Indeed, in most instances, the commercial culture yielded more sediment than the sterile controls, but none of these differences were statistically significant. The linear contrasts that used both erosion responses (sediment mass and sediment cover) offer more insight into the ecotypic differences of the inocula, where we see no overall benefits of adding live inoculum, which was an unexpected result. The weak performance of the commercial culture is largely responsible for the absence of an inoculation benefit. Contrasts between the native and the commercial culture reveal the more striking differences between the ecotypes, all of which suggest the native ecotype survived or functioned better under these experimental conditions.

The extremely arid conditions, particularly in the first year, may have been an important determinant in the outcome of this experiment. If, as shown by Hart and Reader (2002), that colonization strategies differed between mycorrhizal species populating each culture, with the

commercial culture dominated by fast-growing Glomeraceae species, then an extremely dry year could have favored slower-growing, slower germinating species locally adapted to the climate regime specific to Los Olivos. It is possible that under normal or above normal rainfall conditions, we would not have observed any such differences in erosion reduction. The enhanced vegetative growth observed during the first year in plots receiving the native culture offers some evidence that these plots had the most viable populations of mutualists, but none of the vegetative responses were statistically strong. We further note that California shrub and grassland ecosystems are characterized by arid and highly variable moisture regimes, thus enhancing the ecological relevance of these results. Supplemental irrigation is generally not feasible in these environments—conditions that would likely favor locally adapted ecotypes.

When we look at the other response variables, these all suggest the native plots harbored the most viable populations of AMF. Although also not statistically strong, soil bulk density tended to be lower and soil moisture tended to be higher in plots receiving native inoculum. Low bulk density indicates less compaction and is more conducive to enhanced root growth and more biotic activity in the rhizosphere. Soils with enhanced mycorrhizal activity also tend to dry out more slowly (Auge et al. 2001). For aggregate stability, the differences were significant for the dry plots receiving native inoculum, as these soils were more stable in water.

We put less confidence in the observed differences between the hydraulically applied treatments and the dryly applied treatments due to difficulties in applying mulch layers of equal thickness on the scale of our test plots. For this reason, we suspect that the erosion reduction effects we observed in the hydraulically applied plots were due to the increased thickness of the mulch layer relative to the dryly applied plots, which also received a top

dressing of mulch. While we have confidence in the statistical trends, we interpret these as a mulch thickness effect, and indeed, it suggests hydroseeding with multiple applications is the preferred method for effective erosion control. Due to the low concentrations of proteins extracted from the hyphal traps buried in each plot, we hesitate to draw any conclusion other than to note a much higher "signal to noise" ratio would be needed for these traps to work in a field setting. The very low rainfall in the first was most likely the determining factor on how much microbial activity these particular traps could capture.

Conclusions and Recommendations

We find strong support for the benefits of mycorrhizal inoculation as a restoration tool that can help reduce soil erosion, but we find these benefits cannot be applied broadly to all inoculants. In our experiment, clear differences were found between cultures from different ecotypes, with a native, site-specific culture outperforming the commercial product. We believe a highly variable and dry initial growing season may have magnified the ecotypic differences, but note that arid regions are characterized by highly variable precipitation. Therefore, we recommend the use of native inoculum cultured from nearby donor sites as a valuable addition to restoring degraded landscapes. Construction sites often employ multiple methods of erosion control, including the use of fiber rolls, proper slope contouring and soil compaction, in addition to revegetation, and native inoculum would complement these other strategies. Further research will be necessary to identify the exact mechanisms responsible for the erosion reduction benefits we observed, and also to identify the geographical limits of ecotypic specificity. We also recommend the use of hydroseeding to hydraulically apply mycorrhizal inoculum as an effective restoration tool. We do not, however, recommend against other application methods, as we

believe the effects of application method we observed were due to inherent differences in the thickness of the mulch layer.

Los Olivos Cut Slope Experiment

Chapter summary

We tested the utility of applying of a commercial mycorrhizal inoculant as specified by the vendor for use in restoring a cut slope lacking topsoil or subsoil. The low fertility conditions suggested AMF would benefit overall plant cover in general and native species in particular. However, we find no evidence for such a benefit, and conclude that other soil amendments and restoration practices should be employed when a cut slope restoration is necessary.

Introduction

Road cuts qualify as one of the most difficult landforms for restoration practitioners to revegetate. Cut slopes are often steep and prone to erosion or raveling, depending on the particular geologic properties of the site. Cut slopes are characterized by the absence of topsoil, which leaves a substrate lacking in available plant nutrients and exhibiting poor water holding capacity. Throughout the arid western U.S., cut slopes can remain barren for decades (Potter et al. 1985). Ensuring seeded species form symbiotic relationships with arbuscular mycorrhizal fungi (AMF) should improve revegetation efforts due to the ability of AMF to access a limited pool of minerals inaccessible to non-mycorrhizal plant roots.

Previous work has shown that California native species associate strongly with AMF (Vogelsang and Bever 2009a), suggesting native species should benefit disproportionately in the presence of mycorrhizal inoculation. It is not known, however, whether or to what extent native species benefit in the presence of a commercially available inoculant, as previous work by Vogelsang and Bever (2009) used native soil communities. By attempting to restore a

severely degraded cut slope using a commercial AMF product, we had an opportunity to test the generality of these earlier findings in an ecologically relevant way.

The objective of this study was to test whether the benefits of mycorrhizal inoculation from a commercial vendor would be evident on a highly exposed slope devoid of topsoil or subsoil. We hypothesized that the use of mycorrhizal inoculum on a low-fertility cut slope would enhance the productivity and diversity of a restored plant community. We further hypothesized that native species would benefit more from mycorrhizal inoculation than non-native species.

Methods

Study system

Los Olivos refers to the research site along the Caltrans right of way off Highway 154 (34° 40'52 N, 120° 9'20 W), as described in the previous chapter of this report. Los Olivos is inland from the Pacific Ocean by 23.5 km, surrounded by the outer south coast and transverse ranges of the Los Padres National Forest system. Annual rainfall amounts are highly variable from year to year, with an average of 397.5 mm distributed mostly over the winter and spring months. The region is characterized by coast live oak forest, blue oak woodland, and valley oak savannah intermixed with coastal sage scrub and annual grasslands. This experiment took place upland from the Los Olivos Erosion Experiment on an abandoned road cut. The sandy loam soil was poor and exhibited a lower CEC than what we observed for the erosion experiment conducted at this site. Available N was low (NH_4^+ = 7 and NO_3^- = 4 ppm). We used the Olsen method (Olsen et al. 1954) to determine available P due to the high

concentration of Ca and Mg (Elrashidi), and P availability was low. Organic matter was also low at 1.2%, indicating very poor fertility potential.

Experimental design

We designed an experiment using a live and sterile inoculant that would be applied with an erosion control/coastal sage seed mix applied as a bonded fiber matrix. We used a completely randomized design replicated five times, and oriented these blocks west to east on a north facing slope.

Inoculum preparation, testing, and bioassay

We tested the mycorrhizal inoculant (AM 120; Reforestation Technologies International) using a bioassay by mixing 10 mL of live AM 120 with 90 mL of sterile sand for each of five replicates grown in a 125 mL conetainer. We repeated this inoculation procedure in another set of five replicates using 10 mL of sterile AM 120 as a control. All conetainers were seeded with surface sterilized *Sorghum bicolor* and watered to induce germination. All plants were harvested at 30 days, and the roots were washed, cleared with 10% KOH, and stained with trypan blue. 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).

Site preparation and experiment installation

In June of 2006, we dug a 20 x 2 m area along the contour near the top of the slope to remove all topsoil and subsoil from the test area. The thin layer of topsoil and subsoil (approx. 15 cm) was removed to test the revegetation benefits of live inoculum in the absence of soil, a condition common to road cut construction projects. We cut down to the weathered rock layer. The area remained exposed throughout the summer until we implemented the experiment on October 23, 2006. Ten 1 x 1 m^2 plots were designated within the cut area, with a 1 m buffer zone separating each plot from its neighboring plot. We bordered the cut area with a fiber roll staked into the slope for erosion control and to serve as a barrier against soil and seeds mobilized by rain from above (Figure 10). We used the vendor recommended rate for mycorrhizal inoculation, which equaled 0.7 kg/100 m^2, or 60 lbs per acre. According to product information sheet, this rate would supply approximately 3,600,000 living propagules per acre. At the scale of our test plot, this translates to just under 900 live propagules per plot. The five plots designated as "sterile" received a mix of 140 g of AM 120 mycorrhizal inoculum and seed applied as a bonded fiber matrix. The sterile AM 120 had been processed at 121 ^{o}C in an autoclave for one hour. After cooling for 24 hours, the autoclave cycle was repeated to ensure all live propagules were killed. The seed mix consisted of the following species and quantities: *Artemisia californica* (California sagebrush) 100 g; *Eriogonum fasciculatum* (California buckwheat) 150 g; *Hordeum vulgare* (regreen barely) 250 g; *Vulpia microstachys* (vulpia) 250 g; *Gnaphalium californicum* (everlasting) 50 g; and *Lotus scoparius* (California broom) 150 g. The seed mix and mycorrhizal inoculum were mixed with water, approximately 1 kg of wood fiber mulch, and a guar-based tackifier in a hydroseeder. After spraying this bonded fiber matrix onto the five test plots, we rinsed out the hydroseeding tank and created another mix of bonded fiber matrix using 140 g of

live AM 120. After both treatments had been applied, the tank was again cleaned and a second mulch application was prepared using approximately 4 kg of wood fiber mulch, and we applied this layer over the entire experimental array.

Figure 10. Setting up the Los Olivos cut slope experiment.

Data collection

At the end of the growing season for each of three years, we estimated total percent vegetative cover visually for each plot. We then harvested vegetation from each plot using a 20 x 50 cm Daubenmire sampling frame placed in three random locations throughout each plot. Plants within the boundary of the sampling frame were clipped, sorted to species, and dried at 65 °C for a minimum of 72 hours. Dried plants were then weighed by species. We used the Shannon-Wiener index to calculate H' using the function $H' = - \sum(p_i)(\text{Log}_e\ p_i)$, where $p_i = m_i / M$, m_i is the mass of all shoots for a given species and M is the total shoot mass for the community.

Data analysis

We performed a one-way repeated measures MANOVA for percent vegetative cover, total above-ground biomass, and species diversity (H'). Surveyed species were designated as native or non-native, according to The Jepson Manual (Hickman 1993) and we constructed separate MANOVAs to test for differences in response between native and non-native species by assessing biomass and diversity. All biomass estimates were natural log transformed (ln(1+Y)) to improve variance behavior. We used SAS version 9.1 for all analyses.

Results

Percent vegetative cover did not change significantly over time (Wilks' Lambda $F_{2,7} = 2.39$; $P = 0.16$). For all three years, percent cover was higher in the live inoculated plots (Figure 11), but this trend was not significant (Wilks' Lambda $F_{3,6} = 1.57$; $P = 0.29$). Above-ground biomass, our estimate of total productivity, did change significantly over time (Wilks' Lambda $F_{3,17} = 4.37$; $P < 0.05$), due to enhanced biomass in the first year (Figure 12), with lower levels of

growth in each subsequent year. There were no significant trends due to mycorrhizal inoculation. Species diversity increased significantly with time (Wilks' Lambda $F_{2,7} = 42.59$; $P < 0.0001$), but these increases were not due to mycorrhizal effects (Wilks' Lambda $F_{3,6} = 2.14$; $P = 0.20$; Figure 13). Native and non-native species did not exhibit significant differences in growth promotion or in diversity promotion due to inoculation. Mycorrhizal inoculation was associated with increased diversity in the second and third years for both native and non-native species, but none of these increases were statistically strong.

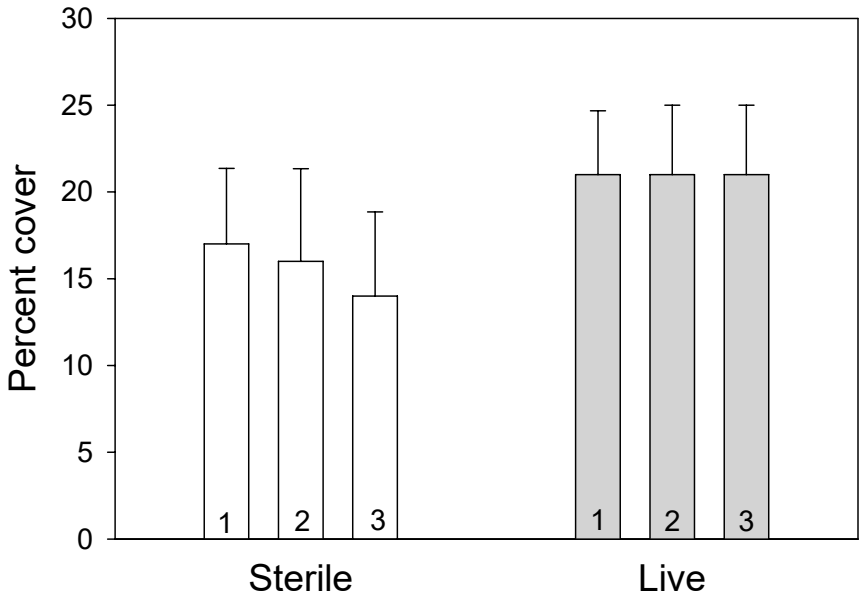


Figure 11. Vegetative cover of cut slope experiment. The number inside the bars indicate the respective growing season.

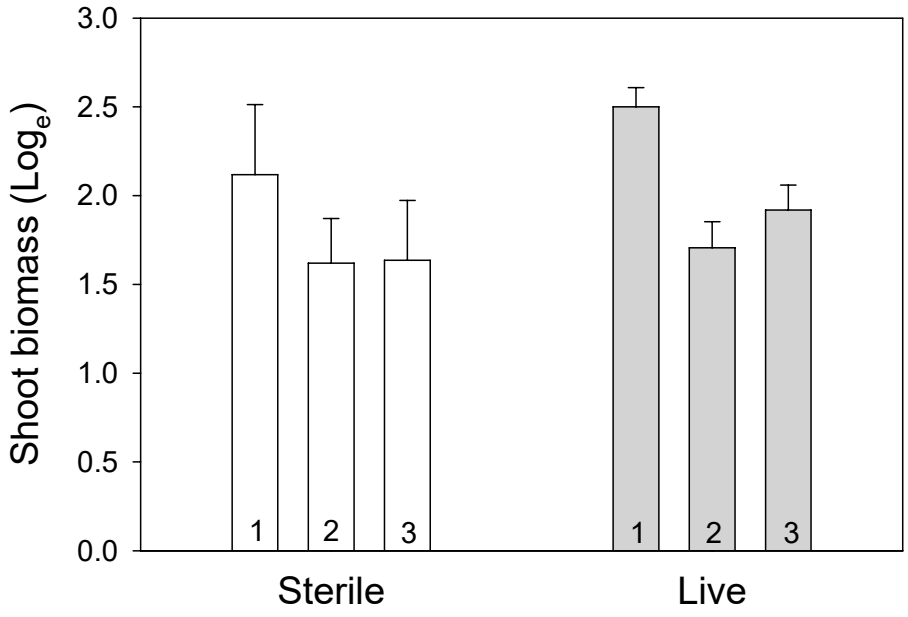


Figure 12. Above-ground productivity for each of three growing seasons in cut slope experiment.

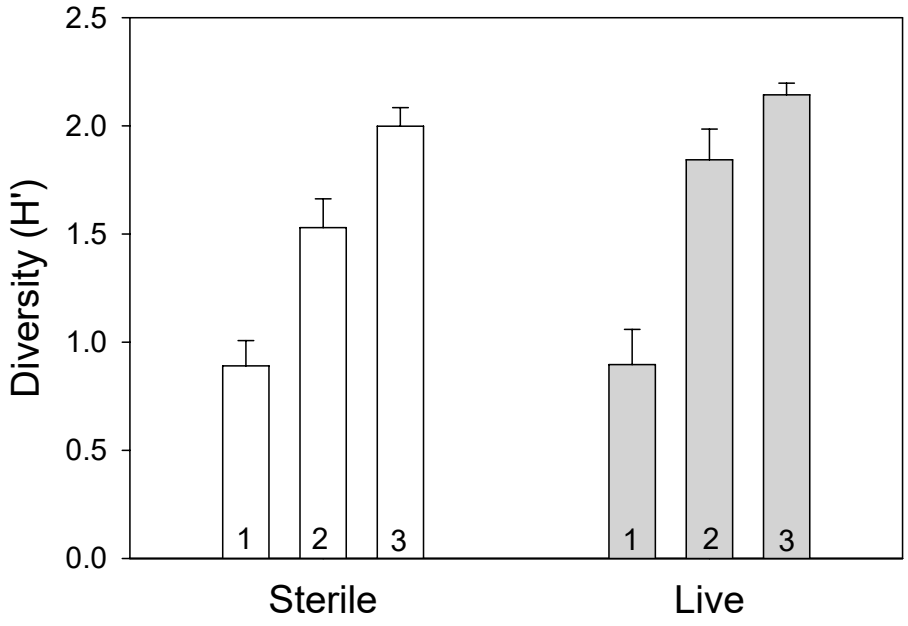


Figure 13. Species diversity for each of three growing seasons in cut slope experiment.

Discussion

This study tested the overall benefits of mycorrhizal inoculation on a cut slope lacking topsoil and subsoil. We see weak evidence for inoculation benefits in vegetative cover, but this pattern is not apparent in the biomass data or in the diversity estimates. Mean percent cover was generally low at less than 20% experiment wide for all three years. The biomass in the first year exceeds what was observed in subsequent years, even though both the second and third years were much wetter than the first. We attribute this to the growth of sterile barley (*H. vulgare*), which we added to the seed mix because of its ability to germinate and grow quickly, thus providing erosion control and potential host plants for mycorrhizae. The barley did not reappear after the first year. Our hypothesis that inoculation would improve growth and diversity on this low-fertility cut slope is not supported.

We see evidence in previous work that native species benefit more from the mycorrhizal mutualism than non-native species (Vogelsang and Bever 2009a). But in the present study, we see no convincing evidence of a similar pattern in this system. We note that in our previous work, the mycorrhizal community originated from a native plant community and was not from a commercially available source. Additionally, as observed in the Los Olivos Erosion Experiment (see chapter 1), the commercial inoculant did not exhibit any environmental benefits, and thus the product used on the cut slope may have experienced a similar (and unresponsive) fate. An important difference, however, is that unlike the erosion experiment described in the previous chapter, the cut slope experiment used unmodified inoculum as available from the vendor. Nevertheless, the microbes used here may have been poorly matched to this particular environment under the conditions of the test.

The generally poor site conditions imposed by cutting into the slope and removing the existing thin layer of topsoil clearly influenced vegetative growth. Areas outside the plot boundaries that were undisturbed generally showed good vegetative cover all three years, even while productivity remained low due to the thin, low-fertility soil. With the removal of topsoil, drought periods in the test plots were intensified, and likely contributed to the premature death of numerous seedlings that did not grow beyond approximately two cm in height, and possibly to the premature death of mycorrhizal propagules. The vendor recommended rate of inoculum application (60 lbs per acre) used in this experiment may have been insufficient given the poor site conditions, and it is possible positive effects would have been observed by increasing the application rate three or fourfold, as doing so would increase the probability of some propagules remaining viable long enough to form an effective mycorrhizae.

The inoculum application rate notwithstanding, augmenting the inoculation with additional amendments that include complex organic matter and mineral nitrogen would have likely improved the overall vegetative cover. The cut slope was inherently low in organic matter, at 1.2%, with other research demonstrating important positive interactions with AMF and available organic matter (Albertsen et al. 2006, Gryndler et al. 2009). Organic materials make up the bulk of the bonded fiber matrix, in the form of the guar tackifier and the wood fiber mulch, but it is possible that these substrates were unsuitable to enhance the expected symbiosis. Indeed, there is evidence that polysaccharides found in the tackifier and cellulose from the wood fiber can inhibit mycorrhizal growth (Ravnskov et al. 1999). Moreover, nitrogen availability was extremely low, and thus the addition of the bonded fiber matrix increased the carbon to nitrogen ratio to the point where germinating seeds already stressed from poor available moisture would be further stressed by a lack of available nitrogen. A nitrate fertilizer added to the inoculation

would have mitigated any such deficiency, and we recommend this approach for future attempts at restoring vegetation to cut slopes. We also recommend the inclusion of a more diverse mix of organic amendments such as composted materials to ensure high-quality substrate is available to saprophytic microbes responsible for the breakdown and release of mineral nutrients. Further research is needed to identify optimal levels of nitrogen and composted organic materials that should be applied to cut slope restorations.

Mycorrhizae also associate with and benefit from "helper bacteria," which are known to increase total root colonization (Frey-Klett et al. 2007) and have been found living symbiotically in fungal spores (Bianciotto et al. 1996). Although speculative, it is possible the culturing regime used to produce the AM 120 product selected against co-mutualistic bacteria such as *Pseudomonas* sp., *Bacillus* sp., *Burkholderia* sp., or *Agrobacterium* sp. that have been isolated from the rhizospheres of various AMF species. Further work should test whether helper bacteria are being properly enriched under commercial culturing techniques to rule out any deficiencies in the finished product.

Conclusions and Recommendations

We do not recommend the use of AM 120 as a stand-alone soil amendment for improving the restoration of a cut slope where the substrate is mostly weathered parent material. Rather, AM 120 or a suitable substitute should be used in conjunction with techniques that will replace lost soil organic matter and lost soil fertility. For example, well composted organic materials along with a modest level of nitrate fertilizer (not more than 20 lbs/acre) would have greatly improved percent cover, and these amendments could easily be applied with a hydroseeder. Moreover, we recommend increasing the application rate of AM 120 to 180 to 240 lbs per acre (three to four times the vendor recommended rate) when the substrate being restored lacks

functional topsoil. Other strategies to consider on difficult to vegetate cut slopes might include the use of container plantings and the use of temporary, supplemental irrigation until such time that roots have taken hold.

Topsoil Management Project

Chapter summary

We used an area in San Mateo County (Devil's Slide) to investigate some of the biophysical changes that occur when native topsoil is excavated and stockpiled, and to identify whether topsoil can be managed to maintain or improve its viability as a growth medium for native plants while being stockpiled during a construction operation. Our project tracks a general decline in soil quality from excavating and salvaging operations, evident in the loss of soil aggregate stability, increased bulk density, lost soil organic matter, and a sharply reduced nutrient profile. Managing stockpiled soil with mycorrhizal and/or seed additions will not necessarily mitigate all of these general declines in soil quality. Native species, however, can benefit in subtle ways from the application of a cover crop once stockpiled soil has been re-disturbed and re-applied for habitat restoration. These benefits would likely accrue over time and a cover cropping regime would need to be implemented with a carefully managed fertilization plan to replace nutrients lost from the initial excavation.

Introduction

Farmers, land managers, and restoration practitioners have long recognized the importance of topsoil in plant establishment and growth. Recommendations on the use of reclaimed topsoil have a long history that goes back to research motivated by surface mining industries (Grim and Hill 1974, Vories 1976). Whether the initial landscape disturbance is necessitated by the search for coal, precious metals, or road construction, salvaging and re-applying topsoil has become a routine practice for restoring land upon completion of the project. By improving our understanding of biotic and abiotic changes that unfold in soil as a

consequence of soil salvaging, we can identify the best strategies that will help accelerate revegetation while minimizing some of the negative environmental impacts of the initial disturbance. From early studies on salvaged soil and into the present day, there is a general recognition that topsoil is perishable and its immediate replacement and/or reapplication is the best strategy for restoration success.

Typically, however, immediate replacement is not possible and topsoil must be stored. The effects of the initial disturbance on microbial communities in topsoil are considerable, and include dramatic shifts in microbial community structure (Visser et al. 1984a) and reduced growth of arbuscular mycorrhizal fungi (AMF) (Visser et al. 1984b). Once in storage, mycorrhizal populations are known to decline over time (Rives et al. 1980, Gould and Liberta 1981, Miller et al. 1985) and the soil itself can experience important changes in biochemical and biophysical properties, including the loss of soil carbon, degraded aggregate stability, and altered soil nutrient profiles (Abdul-Kareem and McRae 1984, Harris et al. 1993).

Of special concern in California's coastal shrub communities is the loss or degradation of AMF communities, due in part to the dependence that plant species native to these habitats are known to exhibit with AMF (Vogelsang and Bever 2009a). In a Colorado sagebrush community, for example, denuded stockpiled topsoil disproportionately slows the growth of *Purshia tridentata*, an AMF dependent shrub, whereas species with weak mycorrhizal associations are less sensitive to the disturbance (Stark and Redente 1987). These findings are important in a restoration context, as practitioners generally want to see desired target species (such as native shrubs) re-establish in a reasonable amount of time, but these efforts can be hindered to the degree that the growing conditions for the target species degrade with the

storage and handling of the topsoil. The goals of this study were twofold. First, we wanted to characterize some of the major biophysical changes that occur as a consequence of a topsoil salvaging and stockpiling operation. Second, we wanted to test the hypothesis that disrupted AMF communities could be mitigated with the use of cover cropping and mycorrhizal inoculation. If so, it would suggest a management practice for stockpiled topsoil that could improve native plant recovery once the soil is reapplied.

Methods

Study system

Devil's Slide describes a portion of Montara Mountain, one of California's central coastal range mountains separating San Mateo County from the San Francisco Bay. The Devil's Slide area (37° 34' N, 122° 30' W) is geologically unstable due to the combined effects of steep terrain cut into decomposed granite. California's Highway 1 transects Devil's Slide and thus has long been vulnerable to dangerous rock slides. A tunneling project through the mountain to re-align this unstable road section specified the salvaging and stockpiling of topsoil and its re-application during various revegetation phases of the construction. The coastal scrub community is dominated by the native shrubs *Artemisia californica* and *Baccharis pilularis*, with other sub-dominant species that include *Mimulus aurantiacus*, *Toxicodendron diversilobum*, and *Ceanothus* sp. Shrub canopies grow dense due to abundant rain and coastal moisture that averages 750 mm of precipitation annually, with summer droughts characterizing this Mediterranean-type ecosystem.

Contractors grubbed and mowed the native shrub community and scraped approximately 30 cm of the topsoil from a 4 acre area designated as the disposal site for tunnel excavation

tailings (Figures 14 and 15). The waste rock and subsoil spread throughout the disposal site would later be restored back to coastal sage/scrub by first redistributing the salvaged topsoil over the disposal fill. The site would then be seeded and mulched as specified in the restoration plan. The mowed vegetation along with the existing soil litter was incorporated into the salvaged topsoil.

Project overview

A few weeks prior to the grubbing and soil salvaging operation of the disposal site, we took baseline soil samples to characterize the nutrient quality of the undisturbed topsoil, along with the mycorrhizal density, the stability of soil aggregates in water, and the bulk density. Doing so allowed us to investigate important changes from the pre-disturbance condition to the post-disturbance condition, and then to follow these changes over time. Five months following the grubbing and soil salvaging operation, we created 20 test plots of stockpiled topsoil *in situ*, and brought additional soil to our laboratory for use in greenhouse experiments. Throughout this report, we refer to "baseline data," "Devil's Slide Field" (DSF) and "Devil's Slide Greenhouse" (DSG) to note the various components of this topsoil management project.

Baseline sampling procedure

On 28 April, 2005, we took 10 core samples of soil from a midslope area of the disposal site along a 90 m transect. As with most of the rest of the site, the sampling area was characterized by the two dominant shrubs, *A. californica* and *B. pilularis*. Cores dimensions were 15.2 cm X 4.75 cm (L x W) for a volume of 269.4 mL. These samples were used for mycorrhizal inoculum bioassays, assessed for their aggregate stability in water, and tested by an independent

lab for nutrient availability. We collected a separate set of 5 samples to determine average bulk density. Shortly thereafter, clearing and grubbing operations commenced and the topsoil was removed and stockpiled. Approximately 5 months elapsed from the time the soil was first stockpiled until we created test plots for the DSF experiment.

Experimental design: Devil's Slide Field

We designed a full factorial two-way experiment using the addition of a live or heat-killed erosion-control seed mix as a cover crop treatment, and the addition of a live or heat-killed mycorrhizal soil amendment (AM 120, Reforestation Technologies International) as a mycorrhizal inoculant. Randomized blocks replicated five times contained all four treatment combinations: (1) live seed + live AMF; (2) live seed + killed AMF; (3) killed seed + live AMF; and (4) killed seed + killed AMF. This design allowed us to test the effects of managing stockpiled topsoil using seed and a mycorrhizal amendment readily available to restoration practitioners. Moreover, we could then observe the relevant effects of soil conditioning by these treatments after an additional soil disturbance, mimicking the handling of stockpiled topsoil *in situ*. Thus, we designed this as a two year experiment, with the first year used to "condition" the soil with seed and mycorrhizal treatments, and the second year used to assess the effects of this conditioning on the growth of target species.

Experimental set-up: Devil's Slide Field

Seed mixes and inocula were prepared as individual packets in order to deliver precise quantities to each test plot. Common erosion control species used in this region were selected for their fast-growing properties and their known ability to serve as competent mycorrhizal host

plants. The following species and quantities were included in each packet: *Achillea millefolium* (10 g), *Hordeum brachypodium* (65 g), *Lupinus littoralis* (100 g), *Nassella lepida* (100 g), and *Vulpia microstachys* (120 g), and AM 120 (120 g). Seeds and AM 120 were either sterile or live. Sterile quantities of seed and AM 120 were prepared as a control for any seed or mycorrhizal amendment effects. We autoclaved separate batches of seed and AM 120 for 90 minutes at 121 ^{o}C, allowed these batches to cool, and then processed each in a microwave oven for three minute intervals until dry.

We arranged 20 test plots linearly along the base of a newly restored slope. Plots were created using a track loader that dumped 6 m^3 of stockpiled topsoil from the disposal area forming soil piles approximately 1 m high that spread across a 4 m^2 area. The plots occupied a total area of approximately 1000 m^2, with approximately 1 m between each plot. A silt fence perimeter around each test plot controlled erosion and prevented runoff from the adjacent slope from impacting our study. Plots were graded to a uniform shape prior to seeding or inoculating with AM 120. Seeds and inoculum were sown into each test plot and hand raked. Plots were then sprayed with a bonded fiber matrix of wood fiber mulch and a tackifier at a rate equivalent to 500 lbs (226.8 kg) per acre. This low rate of fiber application was used to stabilize and "water in" the seed and inoculum, and we intentionally minimized the duration of the hydromulching to keep the stockpiles from washing away from the pressure of the spray. Had we been working with larger, well-consolidated stockpiles, our fiber application rate would have been much greater—as much as four or five times greater. The hydromulching equipment had been cleaned, but we wanted to test for the presence of mycorrhizal propagules in the BFM, the presence of which could potentially confound our experiment. Of the five spray samples obtained, only two exhibited any evidence of AM hyphae, with a mean colonization of 2.4% on a sorghum bioassay.

Figure 14. Clearing and grubbing operation at Devil's Slide to prepare the disposal site.

We also had the BFM tested for nutrient levels to know if plots were being inadvertently fertilized. Our analysis revealed negligible levels of total N, P, and K.

Figure 15. Devil's Slide disposal area before (top) and after clearing and grubbing.

Figure 16. Hydromulching Devil's Slide test plots.

Devil's Slide Field Year 1

In May of 2006, we harvested vegetation from each DSF plot using three randomly placed locations of a 20 cm x 50 cm sampling frame. All above-ground vegetative parts within this frame were harvested to estimate total shoot biomass by species per square meter. We took soil samples from each plot to assess nutrient availability, total organic matter and bulk density. Plants were oven dried at 65 ^{o}C for 72 hours and weighed.

Devil's Slide Field Year 2

In November of 2006, soil cores were taken to assess for nutrient availability, total organic matter, and aggregate stability. We then tilled each of the test plots as a way to re-disturb the topsoil. Plots were seeded with the following native target species (and quantities by mass): *Baccharis pilularis* (15 g); *Artemisia californica* (15 g); *Eriophyllum confertiflorum* (15 g); *Mimulus aurantiacus* (15 g); and *Lupinus littoralis* (60 g). Plots were not re-inoculated with mycorrhizal fungi or given any other amendments. In June of 2007, all plots were again harvested using the same sampling scheme that was used after the first year's conditioning treatment. Soil samples were again collected to assess nutrient availability, aggregate stability, and percent organic matter. Plants were oven dried at 65 ^{o}C for 72 hours and weighed.

Experimental design: Devil's Slide Greenhouse

We designed a completely randomized, full factorial two-way experiment using the addition of a live or heat-killed erosion-control seed mix as a cover crop treatment, and the addition of a live or heat-killed mycorrhizal soil amendment (AM 120) as a mycorrhizal inoculant. Similar to the DSF experiment, four treatment combinations were used: (1) live seed + live AMF; (2) live seed + killed AMF; (3) killed seed + live AMF; and (4) killed seed + killed AMF. Each treatment was replicated 5 times. Also similar to the DSF experiment, we designed the DSG experiment to first condition the soil with our amendments, and then to test the effects of this conditioning on suitable target species. For the test on target species response, we built onto the first year's design with a 4 x 5, full factorial design replicated five times, for a total of 100 pots. We used the four treatment regimes as levels plus an additional untreated control, and used four target species to assess growth potential.

Experimental set-up: Devil's Slide Greenhouse

For the conditioning component of this experiment, we added 1 L of live Devil's Slide stockpiled soil in each of 20 1.5 L pots. We mixed 5 mL of live or sterile AM 120 in each pot, and then uniformly over seeded each with *A. millefolium* and *V. microstachys*. The

Figure 17. Set up of Devil's Slide greenhouse experiment.

seedbanks of all pots were allowed to germinate and grow, regardless of any live seed additions. We harvested at 26 weeks of growth and stored the pots dry for an additional 33 weeks in preparation for the target species response component of the experiment. Just prior to setting up the target species response experiment, we gently crushed the soil in the original 20 pots for use as inoculum. To test for target species response, we grew seedlings of each of the following species in a sterile mix of peat moss: *Artemisia californica*, *Nassella lepida*, *Eriophyllum confertiflorum*, and *Lupinus littoralis*. Seedlings were then transplanted, one per pot, into 600 mL pots. We filled pots with a 380 mL mix of autoclaved Devil's Slide field soil mixed with sand (1:1 by volume) and 112 mL of inoculum from the conditioning pots. Plants were grown in the greenhouse for 17 weeks under a 14 hour day length regime. We harvested shoots and roots separately, assessed both for total mass, and examined the roots to estimate percent mycorrhizal colonization.

Data Analysis: Baseline

We compared changes over time for two soil macronutrients that soil tests revealed were limiting (phosphorus and potassium) and also for organic matter, which changed noticeably from pre-disturbance conditions. Nitrogen was also limiting in this system, but for this study, we focused on P and K because the presence of these minerals in soil is more seasonally stable than N. Additional comparisons were made for magnesium (Mg) and calcium (Ca) because these minerals exhibited similar trends as P and K, even though soil tests indicate Mg and Ca were never limiting. Four time periods were compared: (1) the pre-disturbed conditions sampled in May, 2005; (2) the post year 1 conditions sampled in June, 2006; (3) the pre year 2 conditions sampled in November, 2006; and (4) the post year 2 conditions sampled in June, 2007. An

additional soil test in September, 2005 was used to assess the condition of the main stockpile that was used to create the 20 test plots, but this test was not replicated and therefore dropped from the analysis (but included in the figures). Although these data are ordered as a time series, the sample periods are not equally spaced nor do we have a sufficient number of time periods for a robust trend analysis. Therefore, we analyze them as a one-way ANOVA and compare means with a post-hoc Tukey test.

Data Analysis: Devil's Slide Field Year 1

We analyzed the vegetative response for the first year using a MANOVA with percent plant cover, total biomass, total native biomass, and total non-native biomass as response variables. Seed and AMF additions were treated as main effects, with a seed x AMF interaction term and treatment block as a covariate. All biomass data were natural log transformed (ln(1+Y)) prior to analysis. Soil responses included bulk density, percent change in P, K, Mg, Ca and soil organic matter. We used the mean values for the undisturbed Devil's Slide soil prior to excavation as the baseline value.

Data Analysis: Devil's Slide Field Year 2

We analyzed the aggregate stability of dried aggregates sieved to the 1mm to 2mm range after being oscillated in water for 20 minutes, and compared this response to pre-disturbance, baseline conditions to assess overall degradation of the stockpiled soil. We used a two-way ANOVA to consider seed and mycorrhizal conditioning treatments from the first year. We analyzed the vegetative response after the second year using a MANOVA with percent plant cover, total biomass, total native biomass, total non-native biomass, and plant species diversity

using the Shannon-Wiener index. We used a separate MANOVA to analyze the response of our native target species that were seeded in after the test plots were re-disturbed. For both statistical models, the seed and AMF additions from the DSF year 1 experiments were used as predictor variables, along with relevant block effects and treatment interactions for a two way test.

Data Analysis: Devil's Slide Greenhouse

For each of the four target species, we analyzed the combined mass of roots and shoots harvested from each pot along with estimates of percent mycorrhizal colonization from root subsamples. For each species, we used a two-way ANOVA to identify significant treatment effects due to seed or mycorrhizal additions of the conditioned soil. Means were compared post hoc with a Tukey test. We also did an experiment-wide ANOVA to assess overall effects on plant growth.

Results

Baseline

Available P, K, Mg, and Ca along with soil organic matter all differed significantly over time (Figs. 18-20). Post hoc means comparisons revealed sharp declines in each of these minerals and soil organic matter from the pre-disturbance condition. Soil organic matter declined consistently with each sampling period until the end of the final year, where organic matter rebounded to nearly the level observed from September, 2005, when the main stockpile was sampled (Fig. 18). After the initial decline in available P, levels increased modestly over time (Fig. 19). Similar patterns were observed for extractable Mg and Ca, and somewhat for K, where an increase was observed only after the year 2 harvest (Fig. 19, 20).

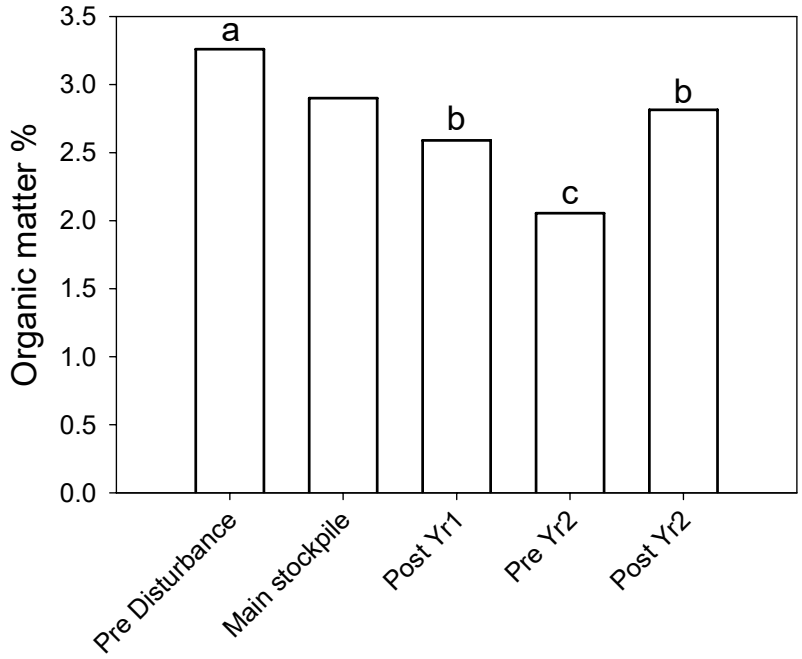


Figure 18. Changes in soil organic matter over time at Devil's Slide. Bars represent means, and those with the same letter are not significantly different (Tukey's HSD test P < 0.05). The main stockpile sample was not included in any analysis.

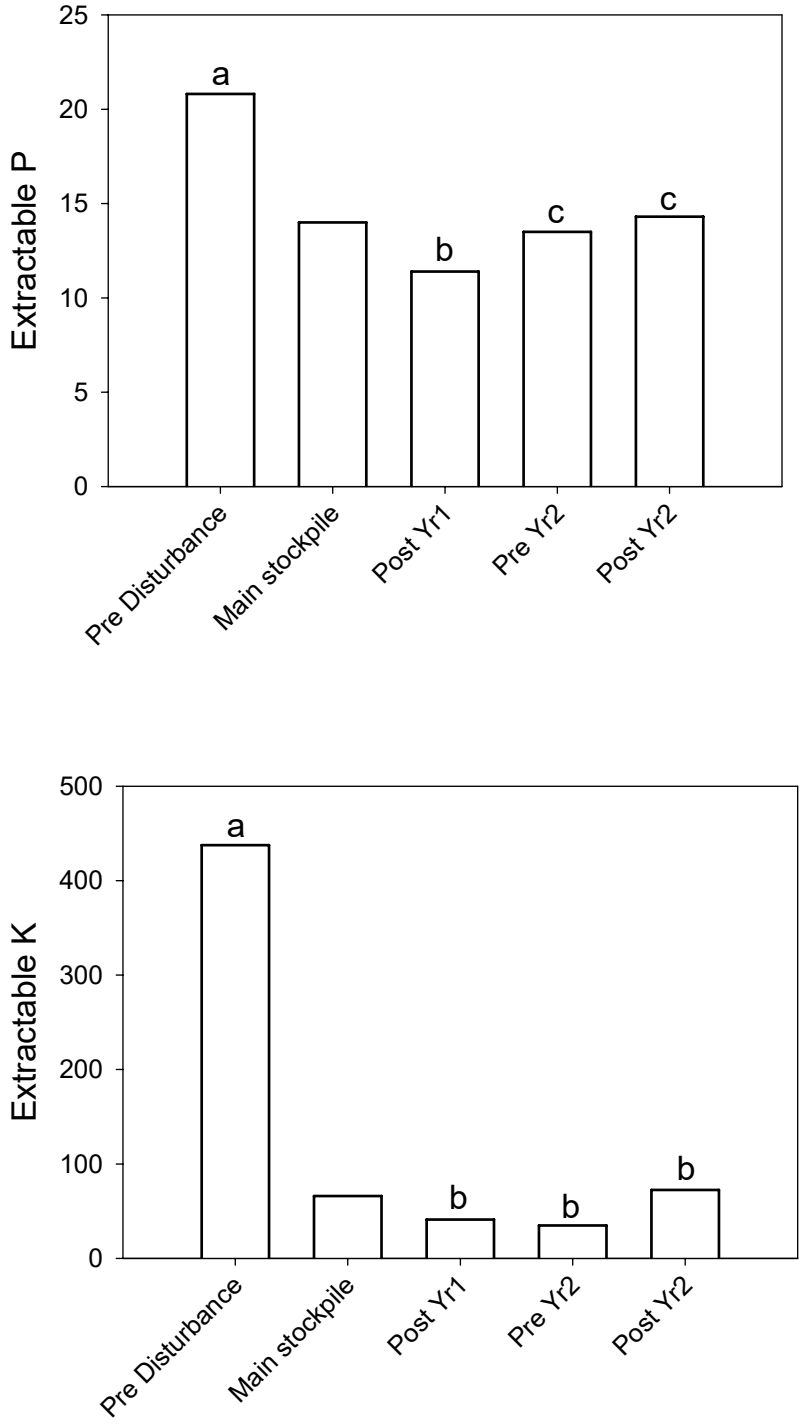
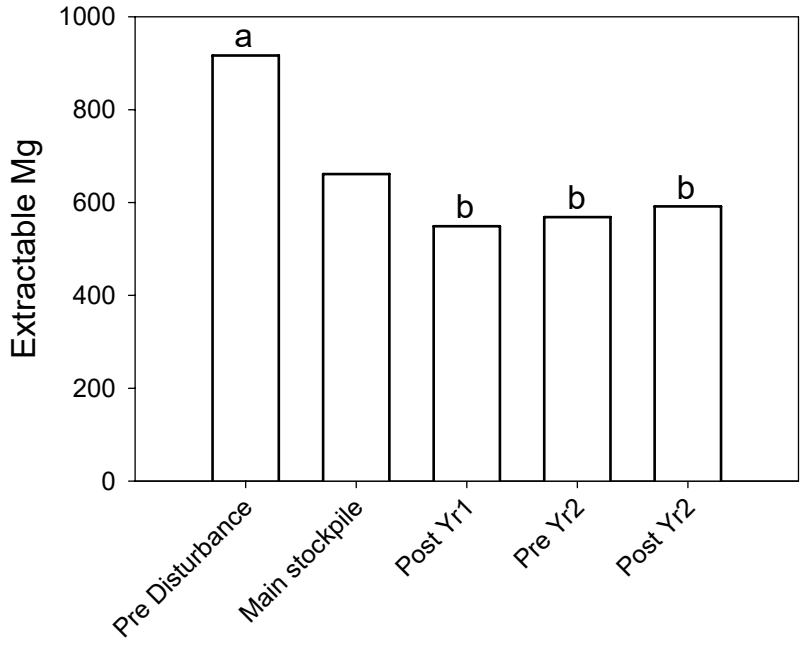


Figure 19. Changes in soil P and K over time at Devil's Slide. Bars represent means, and those with the same letter are not significantly different (Tukey's HSD test P < 0.05).



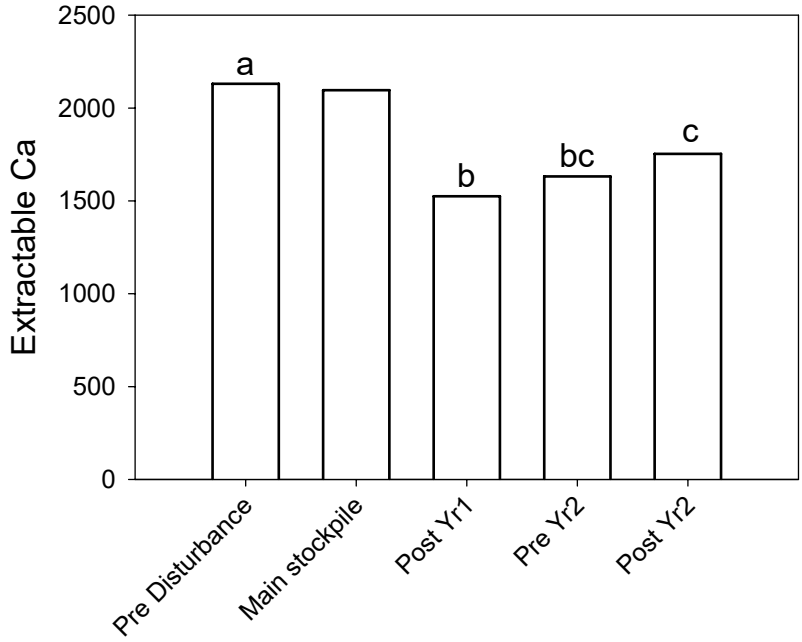


Figure 20. Changes in soil Mg and Ca over time at Devil's Slide. Bars represent means, and those with the same letter are not significantly different (Tukey's HSD test $P < 0.05$).

Devil's Slide Field Year 1

As expected, significant seed effects were observed visually (Fig. 21) and in the overall MANOVA (Wilks' Lambda $F_{4,9} = 208.07$; $P < 0.0001$). The univariate tests reveal the dominance of the non-native annual grasses we used for erosion control, as mean cover, total biomass, and non-native biomass were driving the significance of the MANOVA. Native biomass did not respond significantly to seed additions. Mycorrhizal effects were not significant in the overall MANOVA, but produced clear growth improvement for all four vegetative response variables, with effects especially strong for total native biomass and percent plant cover. The high proportion of variation explained by percent plant cover ($r^2 = 0.98$) motivated a separate univariate analysis, where we observed significant mycorrhizal ($F_{1,12} = 6.10$; $P = 0.03$) and seed effects ($F_{1,12} = 634.82$; $P < 0.0001$). We find no significant changes in the availability of P, K, Mg, and Ca. Additionally, bulk density and soil organic matter were not significant responses. We note, however, that all live treatments reduced soil bulk density, with the live seed treatment reducing bulk density by 9%. Soil organic matter changed the most in the control plots and declined the least in the plots treated with live seeds and AM 120.

Figure 21. Differences in plant cover at Devil's Slide (year 1 experiment). Both plots received AM 120, but only the plot in the lower panel received an erosion-control seed mix as a cover crop.

Devil's Slide Greenhouse

Soil conditioning with AM 120 slightly benefited *Artemisia* and *Nassella*, two of the four target species, but not in any significant way (Fig. 22). Soil conditioning with seed only slightly benefited *Eriophyllum* and *Lupinus*, and significantly depressed the growth of *Nassella* relative to the conditioned control treatment ($F_{1,16} = 37.09$; $P < 0.0001$). We observed no significant interactions in plant biomass response. Mycorrhizal colonization improved in three of the four target species, with significant enhanced colonization observed in *Artemisia* roots ($F_{1,8} = 8.92$; $P = 0.02$). Due to root mortality in *Nassella*, final sample sizes were insufficient for a statistical test on this particular species. Significant overall treatment effects were observed for three of the four target species (*Artemisia*, *Lupinus*, and *Nassella*), when compared to the sterile control. Of the three, only *Artemisia* showed any consistent pattern of benefit with live amendments, with the two live mycorrhizal treatments exhibiting the greatest growth promotion. For the three other species, growth promotion patterns were mixed with no clear trend.

Devil's Slide Field Year 2

We observed a significant AMF x seed interaction ($F_{1,12} = 4.67$; $P = 0.05$) with live soil conditioning treatments resulting in a decline in aggregate stability. From the pre-disturbance condition, aggregate stability decreased an average of 33% in the control plots that received no live seeds or AM 120, and an average of 58% in plots receiving live seeds, 56% with live AM 120, and 55% in plots receiving both treatments. We see no significant overall vegetative response in our MANOVA that included mean plant cover, native and non-native productivity, total productivity, and species diversity. Mean cover and native productivity was slightly

improved in the mycorrhizal plots, whereas non-native productivity was slightly lower. Species

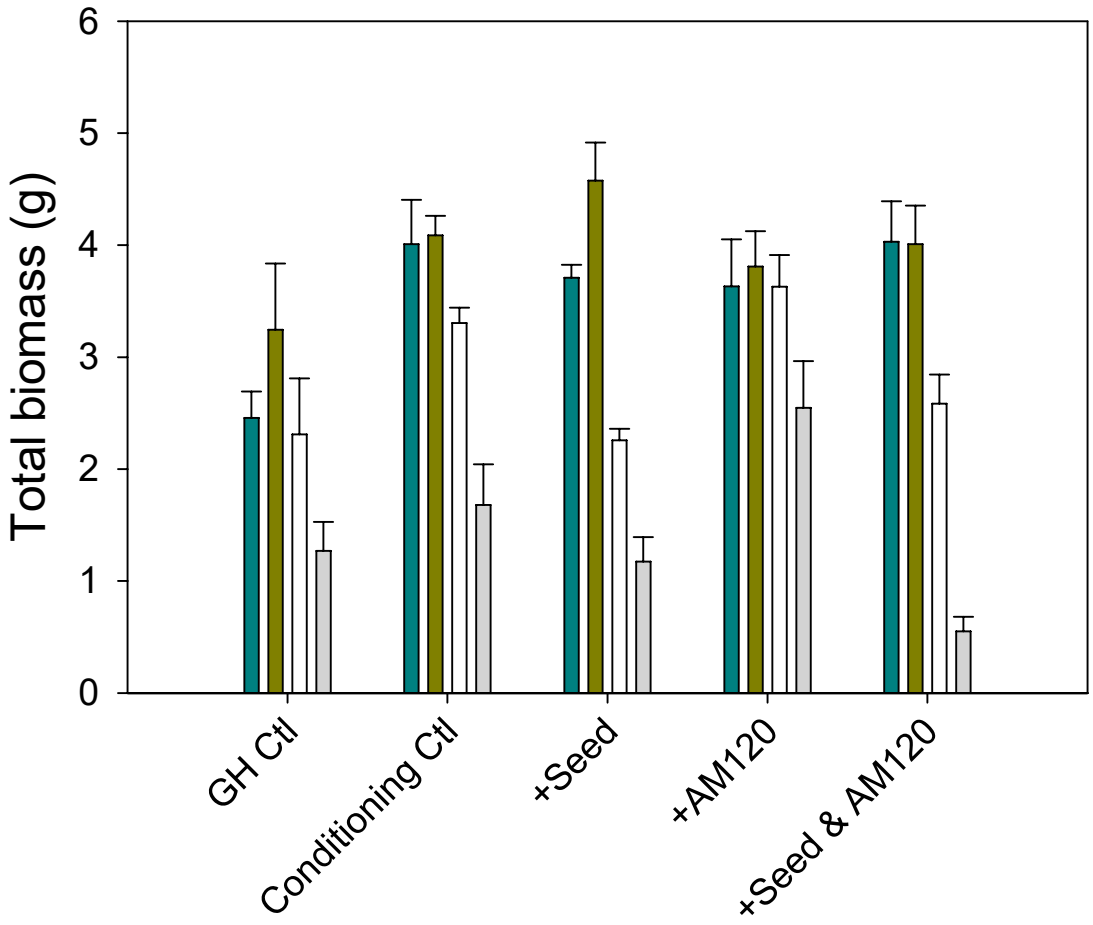


Figure 22. The productivity of four native target species in a greenhouse soil conditioning experiment. The species order for each group is *Artemisa californica* (aqua), *Eriophyllum confertifolium* (brown), *Nassella lepida* (white), and *Lupinus littoralis* (gray). All bars are means ± 1 se.

diversity was also slightly higher (H' = 1.98) under the influence of live AM 120 than without (H' = 1.84). The addition of seed as a conditioning treatment slightly suppressed all general vegetative responses in this experiment, but as with the AM 120 conditioning, no statistical significance was observed.

On the four target species, however, we do see significant conditioning effects of seed in the MANOVA (Wilks' Lambda $F_{4,9} = 5.96$; $P = 0.01$), but no significant mycorrhizal effects. From the univariate tests we can identify that *Eriophyllum* and the legumes were driving the seed effects, as these species were significantly inhibited from the seed conditioning treatments. The mycorrhizal treatments improved the growth of all target species noticeably, but not significantly. *Artemisia* and *Mimulus* are two native species that responded positively to the AM 120 conditioning. On average, *Artemisia* in live AM 120 plots grew 56% larger, and *Mimulus* in these plots grew over 200% larger, but the large error variances associated with these estimates prevented any statistical significance.

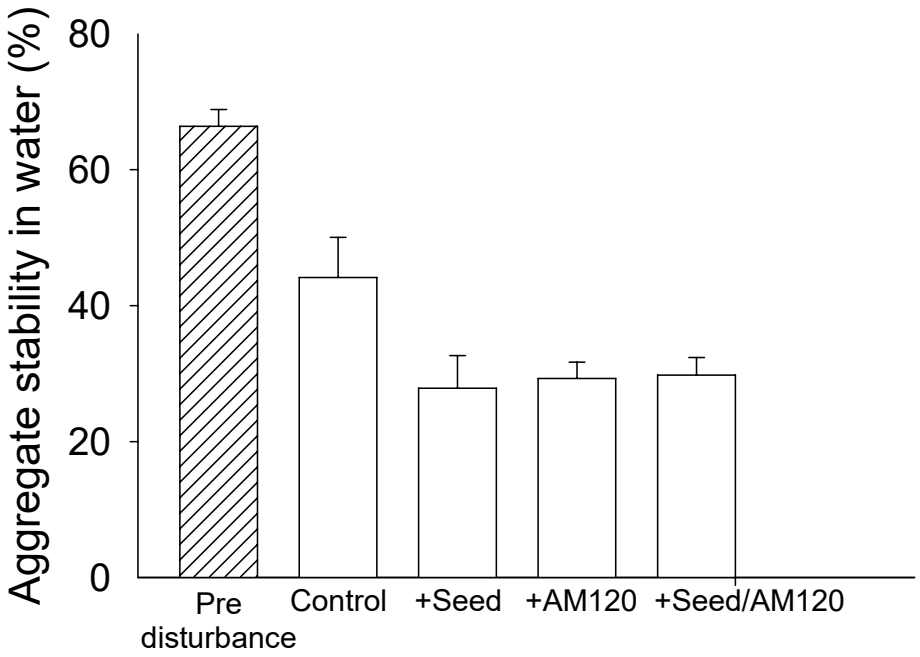


Figure 23. The effects of soil conditioning treatments on soil aggregate stability just prior to re-disturbing the test plots. The pre-disturbance treatment (hatched bar) was not included in the statistical model. All bars are means ± 1 se.

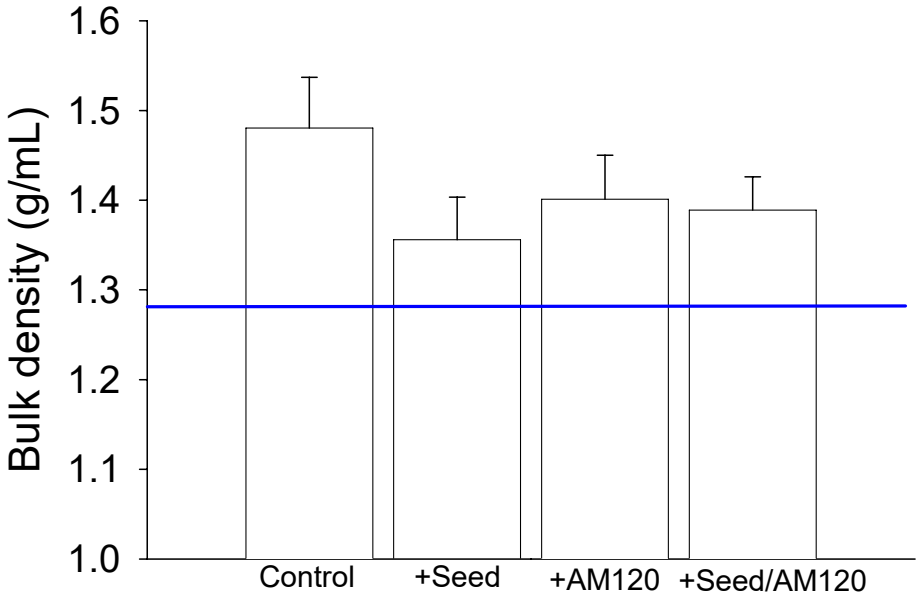


Figure 24. The effects of soil conditioning treatments on bulk density after one year. The blue horizontal line indicates the average bulk density of the undisturbed Devil's Slide soil. All bars are means ± 1 se.

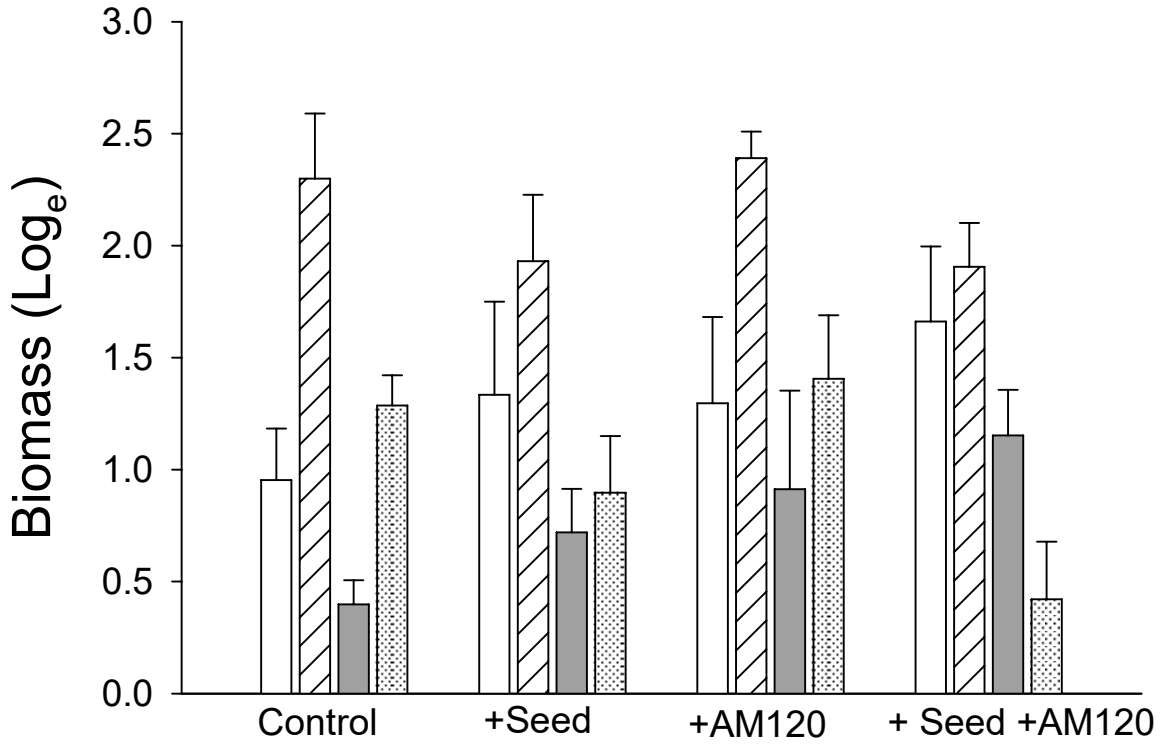


Figure 25. The growth response of four native target species at Devil's Slide after soil conditioning with mycorrhizal and seed cover crops. Open bars are *Artemisia californica*, hatched bars are *Eriophyllum confertiflorum*, gray bars are *Mimulus aurantiacus*, and stippled bars are all legumes grouped together. All bars are means ± 1 se.

Discussion

Available soil minerals and soil organic matter showed a general pattern of decline and then partial recovery over time. For available P, K, and Mg we attribute the initial sharp decline in availability to the effects of the soil salvaging operation. The relative availability of these minerals can vary dramatically with depth (Land and Ohlander 2000), with enhanced availability at the surface that declines with depth as minerals are immobilized in plant biomass or leached. The sandy loam of granitic origin would be highly prone to leaching losses, particularly for K, which was already limiting in this system. We suspect excavating the topsoil inverted and mixed the relatively nutrient-enriched horizons with the lower, nutrient-poor horizons, and the sharp declines observed from the pre-disturbance period to when we sampled soil from the main stockpile likely reflects this phenomenon (Figs. 19-20). Available Ca was the exception, at least for the unreplicated main stockpile sample, but does exhibit sharp declines in mean availability when sampled from the replicated test plots. We further note that the base saturation of K, Mg, and Ca for the excavated and tilled Devil's Slide soil was low overall for the range of cation exchange capacities (CEC) reported for our various soil tests (see Appendix). For example, we calculate optimal levels of K saturation should be two to four times higher than what we observed in the excavated soil. The undisturbed soil exhibited much better K availability and K saturation. These patterns suggest restoration of this site would be enhanced by adding between 70 and 80 lbs/acre of K_2O fertilizer to restore K availability back to its original levels.

The decline of soil organic matter was likely due in part to the excavation and salvaging regime that buried OM-rich layers deeper into the topsoil profile, and also to the decomposition of organic matter generally observed when soils are disturbed in this manner (Rochette and

Angers 1999, Alvaro-Fuentes et al. 2007). From the pre-disturbance condition, we observed a 20% loss of soil organic matter by the time we sampled in June, 2006, following the DSF year 1 experiment. One year had elapsed between these two sampling points. This loss had grown to 37% when these same plots were sampled six months later; leading us to conclude that oxidation of organic matter was the primary factor driving these changes.

Prior to the initiation of the DSF year 2 experiment, plots were again disturbed and much of the above ground biomass was incorporated into the newly tilled plots. Soil organic matter increased sharply by the end of the DSF year 2 experiment, which we attribute to the effects of adding plant residues to the surface, which were then detected as soil organic matter. The loss of soil organic matter can seriously degrade the overall functioning of native ecosystems, as nutrients are more easily lost from the system, water holding capacity declines, and soil compacts more easily. As a management tool to help mitigate some of these undesirable effects, we recommend the use of an erosion-control cover crop for stockpiled topsoil. When this topsoil is re-applied, these fresh plant residues will contribute to the restoration of the active fraction of organic matter that was previously lost due to disturbance. Although the overall contribution to restored soil organic matter will be small, and will vary depending on the surface area to volume ratio of the stockpile, the benefits of cover cropping extends to reducing soil bulk density (Fig. 24) and providing natural erosion protection during soil storage. Other techniques to restore or maintain soil organic matter should also be strongly considered in this system. The granitic, sandy loam soil is characterized by a low proportion of clays that exhibit low charge densities, thus inhibiting nutrient retention (Graham et al. 1997). Therefore, soil organic matter, in its various fractions, becomes even more crucial to binding and exchanging the relatively low abundance of minerals that cycle throughout Devil's Slide.

Our DSF year 1 results produced the desired soil conditioning effects, with significant mycorrhizal and seed responses on plant growth. We interpret the positive mycorrhizal response as evidence that AMF were degraded from the excavation and salvaging operation—an expected result. The question now was whether these responses would have any benefits to native plant restoration once soils were again disturbed. This experiment was not designed to identify an optimal surface area to volume of stockpiled soil, so how well this conditioning regime scales to larger stockpiles is unknown. But with these observed conditioning effects, we now have an empirical basis for testing whether AMF viability can be improved or maintained with cover cropping. The general pattern of nutrient decline (and soil organic matter lost) discussed above suggests that nutrient dynamics have the potential to overshadow mycorrhizal processes in this system. Nevertheless, we predicted that with the inherent low fertility of this site and an abundance of native species, soil microbes were likely to play an important role in restoring the native community.

Our DSG experiment provided mixed results, whereby we see some benefits of AM 120 conditioning, and some benefits of seed conditioning. For our perennial grass target species (*Nassella*), the seed conditioning treatment sharply reduced growth. From our root colonization data, we know that mycorrhizal fungi were actively being cultured in their test pots, and produced a significant colonization response in *Artemisia*, a species known to strongly associate with, and benefit from the mycorrhizal mutualism. The design of this experiment resulted in an abundance of biomass, which may have led to undetected nutrient depletion effects once the soil conditioning component of the experiment was completed.

We used soil aggregate stability as a measure of ecosystem change in our DSF year 2 data set. Our DSF year 1 conditioning treatments produced an overall decline in aggregate

stability, such that the control plots exhibited more stabile soils. In our Los Olivos Erosion Experiment (chapter one), live mycorrhizal treatments enhanced soil stability. We attribute this loss of stability to the general physical properties of the decomposed, granitic soil at Devil's Slide (Graham et al. 1997). Although these soils exhibited good crumb structure as undisturbed samples, their low clay content makes them highly susceptible to erosion, due to poor clay cohesion. We suspect the addition of live soil amendments such as AMF and seed, combined with the effects of lost soil organic matter, promoted the degradation of soil aggregates. The mechanism from the live treatments that contributed to this lost aggregate stability is unknown. One possibility is that enhanced hyphal and root growth in the rhizosphere penetrated and disrupted the already weakly cohesive clays. Over time, as the ecosystem recovered from the disturbance, we would expect to see the restoration of aggregate stability as soil organic matter rebuilds and root exudates contribute to the formation and stabilization of new aggregates.

Overall vegetative responses were evident, but mostly unremarkable after one season. We are intrigued, however, by the general trends. The live mycorrhizal plots showed improved percent cover and increased native productivity. Moreover, we observed increased plant diversity and decreased non-native productivity. These are all encouraging trends due to the addition of AM 120, even though none of these trends were statistically strong after one season. The addition of seed as a conditioning treatment weakly inhibited percent cover, native and non-native productivity, and species diversity, but these effects were not significant and likely negligible over the long term. Our four target species, all of which were slow growing perennials, were improved by the AM 120 conditioning, but no strong statistical benefits were observed. With the exception of *Artemisia*, AM 120 conditioning improved growth relatively

more than seed conditioning alone. *Artemisia* benefited similarly with both treatments, with additional enhancements from the combined effects of seed and AM 120.

Seed effects inhibited *Eriophyllum* and the legumes significantly, and we suspect the inhibition may have been due to nutrient depletion effects, as subsequent analyses indicate reduced base saturation in seeded plots. Moreover, a regression analysis to identify trends in base saturation as a function of DSF year 1 productivity indicates negative relationships for K, Mg, and Ca. For Mg and Ca, these regressions are significant ($P < 0.05$). That is, as plots were more productive, soils became more depleted. Nutrient depletion effects with increasing productivity are not surprising, especially given the low inherent fertility of this site and the fact that fertility declined even further with the salvaging operation. What these findings suggest is that the non-significant mycorrhizal growth trends we observed and the general benefits accrued by the addition of a live inoculant should increase over time. Restoring native plant cover would also benefit from a carefully managed fertilization scheme, whereby lost nutrients would be replaced without overwhelming the system with N, P, or K such that weedy species gain a competitive advantage or that the efficacy of the mycorrhizal mutualism is reduced.

Conclusions and Recommendations

We recommend careful attention to a soil's initial quality prior to undertaking any restoration plan where salvaged topsoil will be re-applied. In the absence of access to a site prior to excavation activities, soil maps to identify nearby donor sites where representative samples could be acquired should be used for this purpose. Restoration practitioners should be aware of the large potential changes in soil structure, bulk density, soil organic matter, and nutrient loss in order to properly mitigate these overall declines in soil quality. We recommend cover cropping

as a valuable tool to offset some of these problems, and mitigation strategies may include fertilizer applications and organic matter additions once the stockpiled soil is re-applied. We further recommend cover cropping to reduce erosion of stockpiled topsoil and we see subtle evidence that cover cropping may have important long-term benefits for maintaining the mycorrhizal community. To increase the probability of realizing these long-term benefits, we recommend including perennial native species in the cover crop seed mix to ensure deep root penetration, and to complement the effects of faster growing annual species.

Appendix

Soil Analyses Reports for Devil's Slide Project, from Spectrum Analytic Inc.

Soil Analysis Report

Spectrum Analytic
1087 Jamison Road NW
Washingon Court House, OH 43160-8748
www.spectrumanalytic.com

Report To	Prepared For	
IU-DEPT OF BIOLOGY 1001 E THIRD ST JH142 BLOOMINGTON, IN 47405-7005	IU-DEPT OF BIOLOGY KEITH VOGELSANG	Sampled 05-04-2005 Tested 05-06-2005

Sample Number	Lab Number	pH		Organic	Analysis Result* and Rating				CEC	Base Saturation			Mehlich-3 PPM and Rating						
		Soil pH	Buffer pH	Matter %	Phosphorus P	Potassium K	Magnesium Mg	Calcium Ca		K %	Mg %	Ca %	Sulfur S	Boron B	Zinc Zn	Iron Fe	Copper Cu	Mang. Mn	Alum. Al
D1	F08418	6.3	6.6	3.6	19 L	188 G	599 V	1687 M	15.9	2.5	27.6	39.7							
D2	F08419	6.1	6.4	3.3	14 L	154 M	644 V	1534 M	18.0	1.8	26.2	31.9							
D3	F08420	6.0	6.4	2.5	14 L	164 M	519 H	1371 M	16.5	2.1	23.1	31.2							
D4	F08421	6.1	6.4	3.1	16 L	255 G	500 H	1386 M	16.6	3.3	22.1	31.3							
D5	F08422	6.1	6.4	3.4	17 L	368 H	444 H	1307 M	16.1	4.9	20.2	30.3							
D6	F08423	6.0	6.5	3.5	12 L	228 G	424 H	1298 M	14.5	3.4	21.5	33.6							
D7	F08424	6.0	6.5	3.1	14 L	330 H	515 H	1363 M	15.6	4.6	24.2	32.8							
D8	F08425	5.9	6.4	3.8	15 L	241 G	417 H	1260 M	15.5	3.3	19.7	30.5							
D9	F08426	6.1	6.7	3.1	13 L	314 H	513 V	1480 M	13.6	5.0	27.7	40.8							
D10	F08427	6.3	6.6	3.2	14 L	363 H	632 V	1519 M	15.9	4.9	29.1	35.8							
RSE1	F08428	6.0	6.6	4.3	20 L	224 G	630 V	1845 M	16.8	2.9	27.5	41.1							

* *Results: P, K, Mg and Ca are extracted by Mehlich-3 (ICP) and are reported in ppm*
Ratings: L=Low M=Medium G=Good H=High V=Very High

Sample Number	Lab Number	Bray P1 lbs/A	C, Total Organic %	NH4-N ppm	NO3-N ppm
D1	F08418	27	2.09	4	12
D2	F08419	19	1.91	5	9
D3	F08420	19	1.45	5	10
D4	F08421	22	1.8	5	8
D5	F08422	22	1.97	6	7
D6	F08423	17	2.03	6	7
D7	F08424	19	1.8	5	8
D8	F08425	21	2.2	5	8
D9	F08426	18	1.8	6	7
D10	F08427	24	1.86	6	7
RSE1	F08428	25	2.49	6	8

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HID:3304-0500-2440-0004

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Soil Analysis Report

Spectrum Analytic Inc.
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www.spectrumanalytic.com

Report To	Prepared For	
IU-DEPT OF BIOLOGY 1001 E THIRD ST JH142 BLOOMINGTON, IN 47405-7005	IU DEPT OF BIOLOGY	Sampled 09-28-2005 Tested 09-29-2005

Sample Number	Lab Number	pH		Organic Matter	Analysis Result* and Rating**				CEC	Base Saturation			Mehlich-3 PPM and Rating**					
		Soil pH	Buffer pH	%	Phosphorus P	Potassium K	Magnesium Mg	Calcium Ca		K %	Mg %	Ca %	Sulfur S	Boron B	Zinc Zn	Iron Fe	Copper Cu	Manganese Mn
DS-SP01	G19347	6.3	6.9	2.9	14 L	66 L	661 V	2096 G	14.0	1.0	34.5	55.9	20 G	1.1 M	4 G	154 V	0.8 G	65 G

* Analysis results for P, K, Mg and Ca are extracted by Mehlich-3 (ICP) and are expressed in ppm
** Ratings: L=Low M=Medium G=Good H=High V=Very High

Sample Number	Lab Number	Bray P1 lbs/A	Texture	Sand %	Silt %	Clay %	
DS-SP01	G19347	22	Sandy Loam	69	21	10	

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Report To	Prepared For	
IU-DEPT OF BIOLOGY 1001 E THIRD ST JH142 BLOOMINGTON, IN 47405-7005	KEITH VOGELSANG	Sampled 06-15-2006 Tested 06-20-2006

Sample Number	Lab Number	pH		Organic	Analysis Result* and Rating**				CEC	Base Saturation			Mehlich-3 PPM and Rating**					
		Soil pH	Buffer pH	Matter %	Phosphorus P	Potassium K	Magnesium Mg	Calcium Ca		K %	Mg %	Ca %	Sulfur S	Boron B	Zinc Zn	Iron Fe	Copper Cu	Manganese Mn
DS1	D05786	5.8	6.4	2.5	13 L	25 L	647 V	1711 M	18.4	0.3	25.8	34.8	18 M	0.7 L	3 M	140 V	0.5 G	44 G
DS2	D05787	6.1	6.5	2.5	10 L	30 L	528 H	1495 M	15.5	0.4	24.9	36.1	15 M	0.6 L	2 M	138 V	0.5 G	46 G
DS3	D05788	6.2	6.6	2.2	12 L	52 L	641 V	1641 M	15.8	0.7	29.8	39.0	15 M	0.7 L	2 M	133 V	0.5 G	60 G
DS4	D05789	6.1	6.6	2.7	10 L	34 L	455 H	1322 M	13.2	0.6	25.3	37.6	12 M	0.5 L	2 M	123 V	0.4 G	46 G
DS5	D05790	6.0	6.5	2.2	11 L	37 L	427 H	1317 M	14.1	0.6	22.1	34.9	13 M	0.5 L	2 M	125 V	0.5 G	43 G
DS6	D05791	6.2	6.6	2.7	11 L	34 L	549 H	1670 M	15.2	0.5	26.6	41.3	16 M	0.7 L	2 M	121 V	0.5 G	44 G
DS7	D05792	6.1	6.5	2.7	11 L	26 L	524 H	1487 M	15.5	0.4	24.8	36.0	14 M	0.6 L	2 M	135 V	0.5 G	45 G
DS8	D05793	4.5	5.8	3.0	12 L	54 L	513 H	1408 L	23.6	0.5	16.0	22.4	18 M	0.7 L	3 M	124 V	0.4 G	49 H
DS9	D05794	5.8	6.5	2.5	12 L	41 L	542 H	1500 M	15.7	0.6	25.3	35.9	15 M	0.7 L	2 M	131 V	0.5 G	52 G
DS10	D05795	6.0	6.5	2.9	13 L	48 L	565 H	1517 M	15.9	0.6	26.0	35.7	15 M	0.6 L	2 M	144 V	0.5 G	46 G
DS11	D05796	6.1	6.5	2.2	10 L	32 L	546 H	1506 M	15.7	0.4	25.5	35.9	17 M	0.6 L	2 L	141 V	0.5 G	39 G

* Results: P, K, Mg and Ca are extracted by Mehlich-3 (ICP) and are reported in ppm
** Ratings: L=Low M=Medium G=Good H=High V=Very High

Sample Number	Lab Number	NH4-N ppm	Bray P1 lbs/A	C, Total Organic %	NO3-N ppm
DS1	D05786	3	24	1.45	6
DS2	D05787	4	24	1.45	4
DS3	D05788	3	26	1.28	5
DS4	D05789	3	22	1.57	4
DS5	D05790	3	24	1.28	6
DS6	D05791	3	24	1.57	5
DS7	D05792	3	22	1.57	5
DS8	D05793	4	22	1.74	5
DS9	D05794	5	22	1.45	5
DS10	D05795	3	24	1.68	4
DS11	D05796	3	20	1.28	3

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HID:3304-0286-6150-0005

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Soil Analysis Report

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Report To	Prepared For	
IU-DEPT OF BIOLOGY 1001 E THIRD ST JH142 BLOOMINGTON, IN 47405-7005	KEITH VOGELSANG	Sampled 06-15-2006 Tested 06-20-2006

Sample Number	Lab Number	pH		Organic	Analysis Result* and Rating**				CEC	Base Saturation			Mehlich-3 PPM and Rating**					
		Soil pH	Buffer pH	Matter %	Phosphorus P	Potassium K	Magnesium Mg	Calcium Ca		K %	Mg %	Ca %	Sulfur S	Boron B	Zinc Zn	Iron Fe	Copper Cu	Manganese Mn
DS12	D05797	6.0	6.5	2.3	13 L	65 L	562 H	1636 M	16.4	0.9	25.1	37.4	15 M	0.8 M	2 M	139 V	0.5 G	47 G
DS13	D05798	6.3	6.6	2.3	9 L	19 L	529 H	1577 M	14.6	0.3	26.5	40.4	12 M	0.6 L	2 M	128 V	0.4 G	43 G
DS14	D05799	6.2	6.6	2.8	10 L	40 L	567 V	1522 M	14.8	0.6	28.2	38.7	13 M	0.6 L	2 M	148 V	0.5 G	43 G
DS15	D05800	6.1	6.6	1.9	9 L	49 L	492 V	1273 M	13.3	0.8	27.2	35.9	15 M	0.5 L	2 L	136 V	0.4 G	42 G
DS16	D05802	5.9	6.4	3.1	16 L	50 L	621 V	1702 M	18.2	0.6	25.0	35.0	19 M	0.6 L	3 M	159 V	0.5 G	64 G
DS17	D05803	6.0	6.5	3.0	13 L	55 L	517 H	1542 M	15.7	0.8	24.2	36.8	17 M	0.6 L	2 M	142 V	0.4 G	55 G
DS18	D05804	6.1	6.5	3.1	11 L	48 L	602 V	1766 M	17.1	0.6	25.8	38.6	17 M	0.7 L	2 M	134 V	0.4 G	52 G
DS19	D05805	6.2	6.5	2.7	11 L	39 L	689 V	1631 M	17.3	0.5	29.3	35.5	14 M	0.7 L	3 M	142 V	0.4 G	49 G
DS20	D05806	6.1	6.5	2.5	11 L	44 L	457 H	1269 M	14.2	0.7	23.6	33.5	15 M	0.5 L	2 M	131 V	0.3 G	50 G

* Results: P, K, Mg and Ca are extracted by Mehlich-3 (ICP) and are reported in ppm
** Ratings: L=Low M=Medium G=Good H=High V=Very High

Sample Number	Lab Number	NH4-N ppm	Bray P1 lbs/A	C, Total Organic %	NO3-N ppm
DS12	D05797	3	24	1.33	6
DS13	D05798	3	20	1.33	3
DS14	D05799	3	24	1.62	3
DS15	D05800	3	18	1.1	3
DS16	D05802	3	36	1.8	3
DS17	D05803	3	24	1.74	4
DS18	D05804	3	24	1.8	5
DS19	D05805	3	18	1.57	5
DS20	D05806	3	22	1.45	3

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Report To	Prepared For	
IU-DEPT OF BIOLOGY 1001 E THIRD ST JH142 BLOOMINGTON, IN 47405-7005	IU DEPT OF BIOLOGY KEITH VOGELSANG	Sampled 09-10-2007 Tested 09-11-2007

Sample Number	Lab Number	pH		Organic	Analysis Result* and Rating				CEC	Base Saturation			Mehlich-3 PPM and Rating						
		Soil pH	Buffer pH	Matter %	Phosphorus P	Potassium K	Magnesium Mg	Calcium Ca		K %	Mg %	Ca %	Sulfur S	Boron B	Zinc Zn	Iron Fe	Copper Cu	Mang. Mn	Alum. Al
DS1	A29178	6.1	6.4	2.8	16 L	75 L	586 H	1724 M	18.1	0.9	23.7	35.7							
DS2	A29179	6.0	6.5	2.0	14 L	79 L	576 H	1639 M	16.5	1.0	25.5	37.2							
DS3	A29180	6.1	6.5	2.3	13 L	50 L	530 H	1524 M	15.7	0.7	24.7	36.4							
DS4	A29181	6.1	6.6	2.4	14 L	74 L	517 H	1538 M	14.5	1.1	26.1	39.7							
DS5	A29182	6.2	6.6	2.4	19 L	82 L	568 H	1780 M	15.8	1.1	26.3	42.2							
DS6	A29183	6.2	6.6	2.7	14 L	71 L	545 H	1827 M	15.8	1.0	25.3	43.4							
DS7	A29184	6.2	6.5	3.3	13 L	82 L	598 V	1792 M	17.3	1.0	25.4	38.9							
DS8	A29185	6.1	6.4	3.5	15 L	86 L	663 V	2019 M	19.8	0.9	24.5	38.2							
DS9	A29186	6.2	6.6	2.7	13 L	69 L	526 H	1598 M	14.8	1.0	26.1	40.5							
DS10	A29187	6.0	6.4	3.5	16 L	64 L	605 H	1790 M	18.5	0.7	24.0	36.3							
DS11	A29188	6.3	6.4	3.5	14 L	77 L	690 V	2069 M	20.2	0.8	25.1	38.4							

* ***Results: P, K, Mg and Ca are extracted by Mehlich-3 (ICP) and are reported in ppm***
Ratings: L=Low M=Medium G=Good H=High V=Very High

Sample Number	Lab Number	Bray P1 lbs/A
DS1	A29178	12
DS2	A29179	12
DS3	A29180	14
DS4	A29181	12
DS5	A29182	16
DS6	A29183	12
DS7	A29184	10
DS8	A29185	12
DS9	A29186	11
DS10	A29187	13
DS11	A29188	10

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Report To	Prepared For	
IU-DEPT OF BIOLOGY 1001 E THIRD ST JH142 BLOOMINGTON, IN 47405-7005	IU DEPT OF BIOLOGY KEITH VOGELSANG	Sampled 09-10-2007 Tested 09-11-2007

Sample Number	Lab Number	pH		Organic	Analysis Result* and Rating				CEC	Base Saturation			Mehlich-3 PPM and Rating						
		Soil pH	Buffer pH	Matter %	Phosphorus P	Potassium K	Magnesium Mg	Calcium Ca		K %	Mg %	Ca %	Sulfur S	Boron B	Zinc Zn	Iron Fe	Copper Cu	Mang. Mn	Alum. Al
DS12	A29189	6.0	6.5	3.5	16 L	80 L	593 V	1791 M	17.2	1.0	25.2	39.0							
DS13	A29190	6.2	6.4	3.1	14 L	74 L	592 H	1960 M	19.1	0.8	22.8	38.6							
DS14	A29191	6.2	6.5	3.2	16 L	69 L	645 V	1809 M	17.7	0.8	26.8	38.4							
DS15	A29192	6.1	6.5	2.7	14 L	86 L	643 V	1751 M	17.5	1.1	27.0	37.6							
DS16	A29193	6.2	6.4	2.7	15 L	72 L	633 V	1852 M	18.9	0.8	24.5	36.7							
DS17	A29194	6.2	6.5	2.7	14 L	69 L	551 H	1625 M	16.3	0.9	24.8	37.4							
DS18	A29195	6.3	6.6	2.5	12 L	74 L	603 V	1926 M	16.6	1.0	26.6	43.5							
DS19	A29196	6.1	6.6	2.3	8 L	41 L	594 V	1447 M	14.7	0.6	29.7	37.0							
DS20	A29197	6.1	6.4	2.5	16 L	72 L	574 H	1607 M	17.6	0.9	23.9	34.3							

* ***Results: P, K, Mg and Ca are extracted by Mehlich-3 (ICP) and are reported in ppm***
Ratings: L=Low M=Medium G=Good H=High V=Very High

Sample Number	Lab Number	Bray P1 lbs/A
DS12	A29189	13
DS13	A29190	9
DS14	A29191	10
DS15	A29192	10
DS16	A29193	11
DS17	A29194	12
DS18	A29195	10
DS19	A29196	6
DS20	A29197	12

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Report To	Prepared For	
IU-DEPT OF BIOLOGY 1001 E THIRD ST JH142 BLOOMINGTON, IN 47405-7005	IU DEPT OF BIOLOGY KEITH VOGELSANG	Sampled 09-10-2007 Tested 09-11-2007

Sample Number	Lab Number	pH		Organic	Analysis Result* and Rating				CEC	Base Saturation			Mehlich-3 PPM and Rating						
		Soil pH	Buffer pH	Matter %	Phosphorus P	Potassium K	Magnesium Mg	Calcium Ca		K %	Mg %	Ca %	Sulfur S	Boron B	Zinc Zn	Iron Fe	Copper Cu	Mang. Mn	Alum. Al
DSTOP1	A29198	6.4	6.6	2.8	21 M	41 L	614 V	2065 M	17.1	0.5	26.3	45.2	16 M	0.8M	4 M	130 V	0.7 G	54 G	
DSTOP2	A29199	6.4	6.5	3.8	19 M	69 L	800 V	2634 M	21.9	0.7	26.8	45.1	16 M	0.9M	4 G	139 V	0.8 G	53 G	

* ***Results: P, K, Mg and Ca are extracted by Mehlich-3 (ICP) and are reported in ppm***
Ratings: L=Low M=Medium G=Good H=High V=Very High

Sample Number	Lab Number	NH4-N ppm	Bray P1 lbs/A	NO3-N ppm	
DSTOP1	A29198	4	18	10	
DSTOP2	A29199	5	17	10	

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Report To	Prepared For	
IU-DEPT OF BIOLOGY 1001 E THIRD ST JH142 BLOOMINGTON, IN 47405-7005	KEITH VOGELSANG	Sampled 02-04-2008 Tested 02-06-2008

Sample Number	Lab Number	pH		Organic	Analysis Result* and Rating				CEC	Base Saturation			Mehlich-3 PPM and Rating						
		Soil pH	Buffer pH	Matter %	Phosphorus P	Potassium K	Magnesium Mg	Calcium Ca		K %	Mg %	Ca %	Sulfur S	Boron B	Zinc Zn	Iron Fe	Copper Cu	Mang. Mn	Alum. Al
GFS7	E32895	4.6	7.4	0.1	12 L	78 M	14 L	97 L	0.6	26.5	16.2	57.3	55 H	0.3L	1 L	59 H	0.5 G	6 H	
GFS8	E32896	4.3	7.1	0.1	695 V	874 V	3 L	33 L	2.0	92.8	1.1	6.1	42 H	0.1L	1 L	68 H	0.3 M	1 H	
GFS9	E32897	6.1	7.5	0.1	25 L	44 L	1 L	3 L	0.1	83.6	6.5	9.9	11 M	0.1L	1 L	9 M	0.4 G	1 G	
GFS10	E32898	6.4	7.5	0.1	3 L	29 L	8 L	27 L	0.2	28.1	26.4	45.5	12 M	0.1L	L	28 G	0.2 G	1 G	
DS1 11 06	E32899	6.1	6.5	2.7	17 L	81 L	679 V	2029 M	18.8	0.9	26.5	40.6							
DS2 11 06	E32900	6.0	6.6	2.0	22 M	89 L	585 V	1792 M	16.0	1.2	26.8	42.0							
DS3 11 06	E32901	6.1	6.6	2.7	17 L	73 L	611 V	1769 M	16.1	1.0	27.9	41.3							
DS4 11 06	E32902	6.1	6.6	2.5	15 L	70 L	586 V	1725 M	15.7	1.0	27.3	41.2							
DS5 11 06	E32903	6.0	6.6	2.9	18 L	81 L	620 V	2080 M	17.3	1.0	26.2	45.0							
DS1 6 07	E32904	6.0	6.5	2.8	19 L	78 L	628 V	1927 M	18.0	0.9	25.6	40.1							
DS2 6 07	E32905	6.0	6.6	2.5	14 L	82 L	593 V	1785 M	16.0	1.1	27.1	41.8							

* ***Results: P, K, Mg and Ca are extracted by Mehlich-3 (ICP) and are reported in ppm***
Ratings: L=Low M=Medium G=Good H=High V=Very High

Sample Number	Lab Number	P, Olsen ppm	NH4-N ppm	Bray P1 lbs/A	NO3-N ppm	
GFS7	E32895	25	294	6	510	
GFS8	E32896	239	114	551	306	
GFS9	E32897	894	6	48	10	
GFS10	E32898	9	6	1	10	
DS1 11 06	E32899			7		
DS2 11 06	E32900			18		
DS3 11 06	E32901			11		
DS4 11 06	E32902			7		
DS5 11 06	E32903			10		
DS1 6 07	E32904			9		
DS2 6 07	E32905			8		

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Soil Analysis Report

Spectrum Analytic Inc.
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Report To	Prepared For	
IU-DEPT OF BIOLOGY 1001 E THIRD ST JH142 BLOOMINGTON, IN 47405-7005	KEITH VOGELSANG	Sampled 02-04-2008 Tested 02-06-2008

Sample Number	Lab Number	pH		Organic	Analysis Result* and Rating				CEC	Base Saturation			Mehlich-3 PPM and Rating						
		Soil pH	Buffer pH	Matter %	Phosphorus P	Potassium K	Magnesium Mg	Calcium Ca		K %	Mg %	Ca %	Sulfur S	Boron B	Zinc Zn	Iron Fe	Copper Cu	Mang. Mn	Alum. Al
DS3 6 07	E32906	6.1	6.6	3.1	17 L	79 L	665 V	1985 M	17.3	1.0	28.2	43.1							
DS4 6 07	E32907	6.0	6.6	2.3	17 L	75 L	592 V	1857 M	16.3	1.0	26.7	42.8							
DS5 6 07	E32908	6.0	6.5	2.9	21 M	81 L	575 H	1905 M	17.5	1.0	24.0	40.7							
RSW2 9 05	E32909	5.9	6.6	4.1	23 M	214 G	627 V	2295 M	18.5	2.5	24.9	46.6							
RSW3 9 05	E32910	6.0	6.6	3.3	18 L	157 M	583 H	2154 M	17.5	1.9	24.4	46.2							
RSW4 9 05	E32911	6.0	6.7	4.4	22 M	185 G	571 H	2031 M	15.8	2.5	26.5	48.2							
RSW5 9 05	E32912	6.1	6.8	3.8	18 L	230 G	586 V	1942 G	14.5	3.4	29.7	50.3							
RSW6 9 05	E32913	6.1	6.6	3.8	20 L	195 G	560 H	1803 M	16.1	2.6	25.5	42.0							
SPO2 11 06 1	E32914	5.7	6.5	3.4	26 M	96 L	757 V	2338 M	20.5	1.0	27.0	42.7							

* **Results: P, K, Mg and Ca are extracted by Mehlich-3 (ICP) and are reported in ppm**
Ratings: L=Low M=Medium G=Good H=High V=Very High

Sample Number	Lab Number	Bray P1 lbs/A
DS3 6 07	E32906	10
DS4 6 07	E32907	9
DS5 6 07	E32908	11
RSW2 9 05	E32909	10
RSW3 9 05	E32910	8
RSW4 9 05	E32911	9
RSW5 9 05	E32912	8
RSW6 9 05	E32913	7
SPO2 11 06 1	E32914	14

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Soil Analysis Report

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Report To	Prepared For	
IU-DEPT OF BIOLOGY 1001 E THIRD ST JH142 BLOOMINGTON, IN 47405-7005	KEITH VOGELSANG	Sampled 02-04-2008 Tested 02-06-2008

Sample Number	Lab Number	pH		Organic	Analysis Result* and Rating				CEC	Base Saturation			Mehlich-3 PPM and Rating						
		Soil pH	Buffer pH	Matter %	Phosphorus P	Potassium K	Magnesium Mg	Calcium Ca		K %	Mg %	Ca %	Sulfur S	Boron B	Zinc Zn	Iron Fe	Copper Cu	Mang. Mn	Alum. Al
SPO2 11 06 2	E32915	5.8	6.6	2.5	21 M	81 L	618 V	1953 M	16.8	1.0	26.9	43.5							
SPO2 11 06 3	E32916	5.9	6.6	3.1	24 M	91 L	731 V	2262 M	18.8	1.0	28.5	45.0							
SPO2 11 06 4	E32917	5.7	6.6	3.1	26 M	94 L	764 V	2339 M	19.4	1.0	28.9	45.3							
SPO2 11 06 5	E32918	5.8	6.6	2.9	28 M	91 L	720 V	2243 M	18.7	1.0	28.3	45.0							

* **Results: P, K, Mg and Ca are extracted by Mehlich-3 (ICP) and are reported in ppm**
Ratings: L=Low M=Medium G=Good H=High V=Very High

Sample Number	Lab Number	Bray P1 lbs/A
SPO2 11 06 2	E32915	13
SPO2 11 06 3	E32916	14
SPO2 11 06 4	E32917	16
SPO2 11 06 5	E32918	14

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References

Abdul-Kareem, A. W. and S. G. McRae. 1984. The effects on topsoil of long-term storage in stockpiles. Plant and Soil **76**:357-363.

Al Agely, A. and D. M. Sylvia. 2008. Compatible host/mycorrhizal fungus combinations for micropropagated sea oats: II. Field evaluation. Mycorrhiza **18**:257-261.

Albertsen, A., S. Ravnskov, H. Green, D. F. Jensen, and J. Larsen. 2006. Interactions between the external mycelium of the mycorrhizal fungus Glomus intraradices and other soil microorganisms as affected by organic matter. Soil Biology & Biochemistry **38**:1008-1014.

Allen, E. B. and M. F. Allen. 1988. Facilitation of succession by the nonmycotrophic colonizer *Salsola kali* (Chenopodiaceae) on a harsh site: effects of mycorrhizal fungi. American Journal of Botany **75**:257-266.

Alvaro-Fuentes, J., C. Cantero-Martinez, M. V. Lopez, and J. L. Arrue. 2007. Soil carbon dioxide fluxes following tillage in semiarid Mediterranean agroecosystems. Soil & Tillage Research **96**:331-341.

Antunes, P. M., A. M. Koch, K. E. Dunfield, M. M. Hart, A. Downing, M. C. Rillig, and J. N. Klironomos. 2009. Influence of commercial inoculation with Glomus intraradices on the structure and functioning of an AM fungal community from an agricultural site. Plant and Soil **317**:257-266.

Auge, R. M., A. J. W. Stodola, J. E. Tims, and A. M. Saxton. 2001. Moisture retention properties of a mycorrhizal soil. Plant and Soil **230**:87-97.

Barthes, B. and E. Roose. 2002. Aggregate stability as an indicator of soil susceptibility to runoff and erosion; validation at several levels. Catena **47**:133-149.

Bennett, A. E. and J. D. Bever. 2009. Trade-offs between arbuscular mycorrhizal fungal competitive ability and host growth promotion in Plantago lanceolata. Oecologia **160**:807-816.

Bianciotto, V., C. Bandi, D. Minerdi, M. Sironi, H. V. Tichy, and P. Bonfante. 1996. An obligately endosymbiotic mycorrhizal fungus itself harbors obligately intracellular bacteria. Applied and Environmental Microbiology **62**:3005-3010.

Brito, I., M. r. d. Carvalho, and M. J. Goss. 2009. Techniques for Arbuscular Mycorrhiza Inoculum Reduction. Pages 307-318 *in* A. Varma and A. C. Kharkwal, editors. Symbiotic Fungi: Principles and Practice. Springer-Verlga, Berlin.

Brundrett, M. C. 2002. Coevolution of roots and mycorrhizas of land plants. New Phytologist **154**:275-304.

Brussaard, L. 1998. Soil fauna, guilds, functional groups and ecosystem processes. Applied Soil Ecology **9**:123-135.

Elrashidi, M. A. Selection of an appropriate phosphorus test for soils. USDA NRCS, Lincoln, NE.

Fant, J. B., R. M. Holmstrom, E. Sirkin, J. R. Etterson, and S. Masi. 2008. Genetic Structure of Threatened Native Populations and Propagules Used for Restoration in a Clonal Species, American Beachgrass (Ammophila breviligulata Fern.). Restoration Ecology **16**:594-603.

Frey-Klett, P., J. Garbaye, and M. Tarkka. 2007. The mycorrhiza helper bacteria revisited. New Phytologist **176**:22-36.

Gould, A. B. and A. E. Liberta. 1981. Effects of topsoil storage during surface mining on the viability of vesicular-arbuscular mycorrhiza. Mycologia **73**:914-922.

Graham, R. C., P. J. Schoeneberger, M. A. Anderson, P. D. Sternberg, and K. R. Tice. 1997. Morphology, porosity, and hydraulic conductivity of weathered granitic bedrock and overlying soils. Soil Science Society of America Journal **61**:516-522.

Grim, E. C. and R. D. Hill. 1974. Environmental protection in surface mining of coal. NTIS Report Pb-238 538.

Gryndler, M., H. Hrselova, T. Cajthaml, M. Havrankova, V. Rezacova, H. Gryndlerova, and J. Larsen. 2009. Influence of soil organic matter decomposition on arbuscular mycorrhizal fungi in terms of asymbiotic hyphal growth and root colonization. Mycorrhiza **19**:255-266.

Gyssels, G., J. Poesen, E. Bochet, and Y. Li. 2005. Impact of plant roots on the resistance of soils to erosion by water: a review. Progress in Physical Geography **29**:189-217.

Harris, J. A., P. Birch, and K. C. Short. 1993. The impact of storage of soils during opencast mining on the microbial community: a strategist theory interpretation. Restoration Ecology **1**:88-100.

Hart, M. M. and R. J. Reader. 2002. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytologist **153**:335-344.

Hickman, J. C., editor. 1993. The Jepson manual: higher plants of California. University of California Press, Berkeley.

Hufford, K. M. and S. J. Mazer. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. Trends in Ecology & Evolution **18**:147-155.

Janos, D. P., S. Garamszegi, and B. Beltran. 2008. Glomalin extraction and measurement. Soil Biology & Biochemistry **40**:728-739.

Jastrow, J. D. and R. M. Miller. 1991. Methods for assessing the effects of biota on soil structure. Agriculture Ecosystems & Environment **34**:279-303.

Land, M. and B. Ohlander. 2000. Chemical weathering rates, erosion rates and mobility of major and trace elements in a boreal granitic till. Aquatic Geochemistry **6**:435-460.

McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild, and J. A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. New Phytologist **115**:495-501.

McKay, J. K., C. E. Christian, S. Harrison, and K. J. Rice. 2005. "How local is local?" - A review of practical and conceptual issues in the genetics of restoration. Restoration Ecology **13**:432-440.

Miller, R. M., B. A. Carnes, and T. B. Moorman. 1985. Factors influencing survival of vesicular-arbuscular mycorrhiza propagules during topsoil storage. Journal of Applied Ecology **22**:259-266.

Montalvo, A. M., S. L. Williams, K. J. Rice, S. L. Buchmann, C. Cory, S. N. Handel, G. P. Nabhan, R. Primack, and R. H. Robichaux. 1997. Restoration biology: A population biology perspective. Restoration Ecology **5**:277-290.

Olsen, S. R., C. V. Cole, F. S. Watanabe, and L. A. Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate U.S. Department of Agriculture Circular 939, Washington D.C.

Osborn, B. 1954. Effectiveness of cover in reducing soil splash by raindrop impact. Journal of Soil and Water Conservation **9**:70-76.

Potter, L. D., R. C. Reynolds, and E. T. Louderbough. 1985. Mancos shale and plant community relationships: field observations. Journal of Arid Environments **9**:137-145.

Ravnskov, S., J. Larsen, P. A. Olsson, and I. Jakobsen. 1999. Effects of various organic compounds growth and phosphorus uptake of an arbuscular mycorrhizal fungus. New Phytologist **141**:517-524.
Reeves, F. B., D. Wagner, T. Moorman, and J. Kiel. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid west. I. A comparison of incidence of mycorrhizae in severely disturbed vs. natural environments. American Journal of Botany **66**:6-13.
Richter, B. S. and J. C. Stutz. 2002. Mycorrhizal inoculation of big sacaton: Implications for grassland restoration of abandoned agricultural fields. Restoration Ecology **10**:607-616.
Rillig, M. C., S. F. Wright, and V. T. Eviner. 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant and Soil **238**:325-333.
Rives, C. S., M. I. Bajwa, A. E. Liberta, and R. M. Miller. 1980. Effects of topsoil storage during surface mining on the viability of VA mycorrhiza. Soil Science **129**:253-257.
Rochette, P. and D. A. Angers. 1999. Soil surface carbon dioxide fluxes induced by spring, summer, and fall moldboard plowing in a sandy loam. Soil Science Society of America Journal **63**:621-628.
Schechter, S. P. and T. D. Bruns. 2008. Serpentine and non-serpentine ecotypes of Collinsia sparsiflora associate with distinct arbuscular mycorrhizal fungal assemblages. Molecular Ecology **17**:3198-3210.
Schwartz, M. W., J. D. Hoeksema, C. A. Gehring, N. C. Johnson, J. N. Klironomos, L. K. Abbott, and A. Pringle. 2006. The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. Ecology Letters **9**:501-515.
Smith, S. E. and D. J. Read. 1997. Mycorrhizal Symbiosis. Second edition. Academic Press, San Diego.
St. John, T. 1998. Mycorrhizal inoculation in habitat restoration. Pages 17-19 Land and Water.
Stark, J. M. and E. F. Redente. 1987. Production potential of stockpiled topsoil. Soil Science **144**:72-76.
Visser, S., J. Fujikawa, C. L. Griffiths, and D. Parkinson. 1984a. Effect of topsoil storage on microbial activity, primary production and decomposition potential. Plant and Soil **82**:41-50.
Visser, S., C. L. Griffiths, and D. Parkinson. 1984b. Topsoil storage effects on primary production and rates of vesicular-arbuscular mycorrhizal development in *Agropyron trachycaulum*. Plant and Soil **82**:51-60.
Vogelsang, K. M. and J. D. Bever. 2009a. Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. Ecology **90**:399-407.
Vogelsang, K. M. and J. D. Bever. 2009b. The Use of Native Plants and Mycorrhizal Fungi for Slope Stabilization and Topsoil Management
Indiana University, Bloomington.
Vogelsang, K. M., H. L. Reynolds, and J. D. Bever. 2006. Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. New Phytologist **172**:554-562.
Vories, K. C., editor. 1976. Reclamation of Western Surface Mined Lands. Ecology Consultants, Inc., Fort Collins.
Wright, S. F. and A. Upadhyaya. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Science **161**:575-586.