THE USE OF MYCORRHIZAL FUNGI IN THE RESTORATION OF HIGHLY DISTURBED SOILS AT HEIL RANCH, BOULDER COUNTY, COLORADO U.S.A.

REPORT TO BOULDER COUNTY PARKS AND OPEN SPACE
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ABSTRACT

The goal of the study was to explore the value of using different kinds of mycorrhizal inoculum (commercially available and native soil-borne fungi) to determine the best methods for restoring a degraded road within the Heil Ranch Open Space, a Boulder County Parks and Open Space property. A primary goal was to test practical methods that Boulder County could potentially use on other restoration projects. The study site was an abandoned dirt road overgrown with weedy, non-native plants, and treated with a spring prescribed burn and mechanical seeding of native grasses. Control, commercial inoculum, and native soil inoculum treatments were randomly located within the roadbed and samples were collected for the analysis of percent vegetation cover, biomass, dominant species, and mycorrhizal infection rates. A large amount of variation was documented within plots and treatments for both percent vegetation cover and biomass production, especially between the two years of sampling (2002 and 2003). Cover and biomass were significantly greater in 2003, presumably due to the increased precipitation and subsequent non-drought conditions. Additionally, the second year of the study revealed a greater prevalence of native species in all treatments, although most of the native plants were not species included in the revegetation seed mix. Germination of seeded material may have been negatively affected by the lack of available moisture in 2002 or the low viability or adaptability of the non-local native seed material. Overall, the native soil treatment had the greatest amounts of biomass, consistently high percent cover measurements, and the greatest prevalence of native species. This treatment had some of the lowest mycorrhizal infection rates, while the control treatments had the highest fungal infection levels. The commercial mycorrhizal inoculant had the lowest infection rates, and consistently low percent cover and biomass results. All plots and treatments tended to increase in the prevalence of native species during the second year of the study, possibly due to non-drought conditions or the potentially positive effects of the prescribed burn on the non-native vegetation. Although the primary weed species (cheatgrass and Japanese brome) remained prevalent throughout the two years of sampling, clear changes could be documented in the composition of native species between the two years. The changes in native species may be a successional change related to the disturbance (fire), although no clear advantages could be documented from the fungal inoculants.

INTRODUCTION

Vesicular arbuscular mycorrhizal fungi (VAM) have been shown to play an important role in the restoration of disturbed and degraded soils and ecosystems (Reeves et. al. l979; Allen 1991; Read et. al. 1992; Wilson et. al. 200l). This research has shown that among the benefits to plants from root infection and colonization by VAM are improved uptake of nutrients, principally phosphate, but also ions such as copper, zinc, chloride, sulfate and ammonium. In addition, VAM provides considerable protection from root pathogens as well as giving plants the ability to withstand higher soil temperatures, higher soil salinity, and wider extremes of soil pH (Palm and Chapela, 1997). The USDA has demonstrated that VAM produce Glomalin, a compound that increases soil aggregation and infiltration, as well as water-holding capacity. Similar fungi have been termed Arbuscular Mycorrhizal Fungi (AMF), instead of Vesicular Arbuscular Mycorrhiza (VAM) and therefore some disagreement exists in determining the appropriate name. The controversy has persisted because not all endomycorrhizal fungi produce vesicles, and arbusculars are not always present (CSIRO 2002). Throughout this paper all endomycorrhizal fungi will be termed VAM. In the spring of 2002, an experimental road restoration project was initiated in the Geer Canyon of Heil Ranch Open Space as part of the Boulder County Parks and Open Space Small Grants Program. Using techniques that could be applied to larger scale habitat restoration projects, Denver Botanic Gardens tested the effects of commercial mycorrhizae and native soil inoculums on the vegetative cover and biomass production of a mountain meadow following a prescribed burning and broadcast seeding of native species within a degraded roadbed.

In natural undisturbed ecosystems, propagules of VAM are concentrated in the uppermost few centimeters of soil. When the upper portion of the soil is damaged by compaction, degradation, modification, or other detrimental effects; the propagules of natural soil fungi are greatly reduced in numbers and viability. Reintroduction of VAM may hold an answer for the restoration of that natural ecosystem (Tallaksen, l996). This project sought to examine the effects of introducing VAM to disturbed and compacted soils of an old road in need of restoration, by comparing the introduction of a commercial inoculum containing VAM with the introduction of locally collected top soil potentially containing native VAM.

The research site selected for the study was in an essentially tree-less meadow in Geer Canyon on Heil Ranch. In the open mountain meadow, the presence of an unused, dirt road

resulted in the compaction and degradation of the site, leaving an unattractive and weed infested "scar". The roadbed had been covered with a coarse fill that became overgrown with several types of non-indigenous plants, thus restricting growth of native species. As far as could be determined, the area chosen for the study plots was more or less uniform in its vegetative cover and species diversity.

The project consisted of four phases: 1) Choosing a project location and marking out experimental and control plots; 2) Introducing two types of inoculum (commericial and native soil) into the randomly selected plots directly after the prescribed burning and mechanical sowing of a standard seed mix; 3) Monitoring the site for changes in vegetation for a period of two growing seasons (2002 and 2003); 4) Harvesting 10 randomly selected subplots within each plot in order to assess vegetation cover, biomass, dominant species composition, and VAM infection success. Mycorrhizal infection rates were only analyzed in 2002, due to the laborious process of quantifying the number of infections per root cross-section. The experimental project was initiated with the purpose of testing potential restoration methods that could be applied by Boulder County Parks and Open Space to existing natural areas and restoration projects. The primary goal is to evaluate the effectiveness of commercially available mycorrhizal inoculants and native soil inoculants on the restoration of the degraded roadbed using percent vegetation cover, biomass, and diversity of species as the quantitative measurements.

MATERIALS AND METHODS

Originally, the degraded roadbed test site was covered by a relatively dense growth of non-indigenous, weedy plants and a few native grasses and forbs. The field experiment began in late March 2002 by establishing six study plots within the old roadbed, each 9 square meters (3 x 3m). A narrow walkway between each plot was established in order to allow monitoring and photography. Following our marking of the six test plots, Boulder County employees conducted a prescribed burn of the entire meadow in early April, 2002. After the fire we noted that some non-native species, particularly *Anisantha tectorum* (Cheatgrass, also known as *Bromus tectorum*) and *Verbascum thapsum* (Great Mullein), were not completely burned and some living, aboveground growth could be identified within the roadbed. Boulder County then seeded the roadbed area, including our marked plots, with a seed mix commonly used in their revegetation programs. The prescribed burn affected most of the meadow and all of the road, but

only the road was seeded. Appendix A lists the plant species included in the seed mix and application rates. On April 19, 2002, immediately after the broadcast seeding, our research team treated two plots with AM120 commercial inoculum and two plots with a native soil inoculum.

The application of the treatments and controls were randomly applied to the six plots along the roadbed. Appendix B illustrates the experimental design for the restoration project and lists the treatments applied to each plot. The two experimental treatments were applied as follows: the commercially available VAM inoculum product (AM120 Reforestation Technologies International, purchased from Pawnee Buttes Seed Company of Greeley, CO) consisting of propagules of three VAM species of *Glomus (G. intraradices, G. aggregatum, G. mosseae)* was broadcast at the recommended application rate (0.7kg per $100m^2$) for the two experimental plots (3 and 6) and gently raked into the soil surface. An additional application of VAM inoculum was applied to the soil surface, therefore the recommended rate was doubled although only half of the inoculants were mixed with the topsoil. Native soil inoculum was obtained locally from topsoil set aside during construction of a vault toilet at the Heil Ranch Open Space's primary parking area. Soil material exposed to sunlight or near the surface of the storage pile was not collected, due to possible contamination by weed seeds or destruction of mycorrhizal fungi from direct solar radiation or temperature extremes. Approximately three gallons of the topsoil were broadcast evenly over each of the two experimental plots (4 and 5) and gently raked. The control plots were not inoculated or manipulated, although they received the same burning and seeding treatments as the experimental plots. Two additional control plots were installed within the roadbed on August 13, 2002. The seventh and eighty plots were added to aid in the assessment of control plots, since the two original control plots had been randomly assigned to the top of the meadow near the current road, which had a gentler slope and appeared to be more disturbed. Plot 7 was sampled for cover, biomass, and mycorrhiza, but plot 8 was only sampled for the mycorrhizal quantification.

Within the seven test plots, potential edge effects were reduced by creating 2m x 2m plots in the center of each 3m x 3m plot for the sampling of cover and biomass. Percent vegetation cover and biomass were sampled within each plot using ten randomly placed 20cm x 20cm frames (Appendix C). If sampling frames overlapped with a previous sample, new random coordinates were generated. Overall, 10% of each 2m x 2m plot was sampled and a total of 10 samples were taken from each of the seven plots. A grid placed over the sampling frames for the

analysis of basal vegetation cover divided the sample area into 25 quadrats, each 4cm X 4cm and consisting of 4% of the total area. Using small areas for the estimation of cover reduces variability between observers and increases reliability of the sampling method (Elzinga et. al., 1998). We counted each 4cm X 4cm quadrat with greater than 50% cover and determined the percent cover for the sample based on the number of 'covered' versus 'uncovered' quadrats. After cover was estimated, all of the biomass (living and dead) was cut to ground level in each of the 20cm x 20cm sample frames and collected for laboratory analysis. Biomass quantifies the amount of annual production by harvesting all aboveground standing vegetation. Only biomass that originates basally within the sample frame was collected for the study. Dead biomass (last year's growth) was removed from the current year's growth, although little biomass from previous years remained after the prescribed burn. The collected biomass was dried at 32 to 35 degrees Celsius until no additional weight loss from the evaporation of water could be detected. Samples were dried for seven days before the final weighing of biomass (dry weight in grams) and identification of the three dominant plant species within each sample. The mean percent cover and biomass (grams) were calculated for each plot (n = 10) and each treatment (n = 20 for commercial and native soil inoculants, n = 30 for control). Tables 3 and 4 display the mean biomass per plots and treatments, respectively.

From April through September 2002, Denver Botanic Gardens researchers visited and photographed the test plots every two to three weeks. It was noted on several visits that the severe drought conditions were limiting growth of the vegetation (Appendix D). Because of the drought conditions, we decided to delay collecting of the samples for biomass and percentage cover studies with the hope that late summer rains would promote additional growth. The initial collecting of biomass began on September 26, 2002; after several rains storms had stimulated growth of vegetation within the meadow. In 2003, the cover data and biomass samples were collected between September 24th and October 6th.

A method of ranking the dominant species within each sample was developed based on scoring the three species which dominated the biomass for each of the 70 samples. The purpose was to qualitatively determine the predominant species for each of the plots and treatments. Due to the drought and the dominance of small plants (example – many grass seedlings were less than 2cm tall), it was impossible to separate individual species for weighing. Therefore, the three dominant species were determined visually during the weighing of samples, based on

proportional volume of sample, plant size, and number of plants. The dominant species for each sample received a score of three points, the second most dominant scored two points, and the third most dominant species received one point. The scores for the three dominant species in each plot were tabulated and the dominant species ranked in descending order of prevalence for each treatment (Table 5). Analysis of all biomass samples and determination of the species dominance data was determined by the same individual throughout the study.

In order to assess the amount of mycorrhizal fungi (VAM) present in each plot and treatment, several species of plants were collected from each plot by Vera Evenson (DBG Mycologist) in the fall of 2002. Microscopic study of mycorrhizal colonization of roots were done by carefully removing roots in each study plot to a depth of at least 10 cm. Roots ranging from 1-5 mm in width were collected from each plant. Two root samples were obtained per plant and were cleared in dilute KOH and HCl baths at 90°C for one hour and then stained in a 90°C bath of Trypan blue (Phillips and Hayes 1970; McGonigle et al. 1990). The stained roots were mounted on slides to be tallied for vesicular arbuscular mycorrhizae (VAM). Two slides per plant were prepared with two cover slips per slide, six roots per cover slip. Root segments were 10-20 mm in length. Each cover slip was viewed in four cross-sections to determine presence or absence of VAM fungi. Stained hyphae alone, hyphae with arbuscules, with vesicles, or with both were counted as root infections. There were a total of 96 cross-sections per plant, (48 per slide), from which the percentage of infection for each sample was deduced. Methods used for these studies are those of T. P. McGonigle et. al. (1990), R. B. Mullen and S.K. Schmidt (l993). This aspect of the project was only briefly mentioned in the original grant proposal and the complicated, time-intensive methods had never before been conducted by DBG staff. The importance of quantifying mycorrhizal infections in the study is obvious and therefore DBG paid an intern from the University of Denver to be trained in the methods by Beth Newwingham (USGS Moab Office) and perform the extremely labor intensive work of quantifying the root infections in Dr. Robert Sanford's laboratory at the University of Denver. Mycorrhizal quantification of root samples was not included in the 2003 proposal to BCPOS due to the amount of time and expense necessary to prepare and count root infections.

RESULTS

The mean percent vegetation cover for 2002 and 2003 was calculated for each plot (n = 10) and summarized in Figure 1. In 2002, the results range from a high of 68.4% vegetation cover for plot 5 (native soil inoculant) to a low of 24.8% for plot 6 (commercial inoculant). The 2003 mean percent vegetation cover range from a high of 96.8% for plots 1 and 4 (control and native soil inoculant, respectively) and a low of 64.0% for plot 6 (commercial inoculant)(Table 1). All 2003 means were higher than the 2002 values for the respective plot, although the 2002 cover data did not fit the assumptions of normality, even after multiple transformation attempts (log, square root, arc sin) and therefore the 2002 plot and treatment data was not analyzed using a parametric statistical test. The 2003 cover data fits the assumptions of normality based upon a Systat normal probability test. A paired T-Test (two tailed) of the mean percent cover between plots found the two years to be statistically significant (P = 0.0002, n = 7). This test compared the mean percent cover (n = 70) for each plot between 2002 and 2003; therefore the sample size of the test is small (n = 7). Statistical analysis was completed using Systat 5.05 (SPSS) software for ANOVA's and Microsoft Excel 2000 for T-Tests with all alpha levels equal to 0.05.

An ANOVA of the 2003 percent cover data revealed statistically significant differences for both cover versus plots (P = 0.001, n = 70) and cover versus treatments (P = 0.028, n = 70). A Tukey's post hoc test of multiple comparisons revealed that all plots were significant from plot 6, which is the commercial inoculant treatment with the lowest mean cover of all plots in 2002 and 2003 (Figure 1). There were no other statistical differences between any specific plots comparisons. A Tukey HSD post hoc test of cover versus treatments showed only the comparison of commercial inoculant and native soil inoculant to be statistically significant. Figure 2 illustrates the mean percent vegetation cover for each treatment and statistical significance. In 2002, the control treatment had the highest mean percent cover (52.5%), although the range between the treatments was narrow (7.9%). The 2003 percent cover data ranged from a high of 94.2% for the native soil inoculant to a low of 79.0% for the commercial inoculant (Table 2).

Biomass represents the amount (grams) of vegetation produced within each 20 x 20 cm sampled at the end of the growing season (late September to early October). Table 3 lists the mean biomass per plot and dominant species based on the ten samples collected within each of

the seven plots in 2002 and 2003. In 2002, the native soil inoculant (plot 5) has the highest mean biomass (12.05 grams), followed by plot 3 (9.29 g, commercial inoculum), then plot 4 (7.8 g, native soil inoculant). The second commercial inoculant plot (6) had the lowest mean biomass production (2.86 g). 2002 biomass data was log transformed to meet the assumptions of normality. A one-way analysis of variance (ANOVA) determined statistical significance between plots for biomass production in 2002 (P = 0.001, n = 70)(Figure 3). Plot 5 (native soil inoculant) had the greatest mean biomass and was the only plot in which the dominant species was a native, desirable plant (*Sporobolus cryptandrus*, Sand Dropseed). 2002 Tukey's test determined statistically significant differences between the following plots:

3 (commercial) : 4 (native) at P = 0.012
3 (commercial) : 6 (commercial) at P = 0.011
4 (native) : 5 (native) at P = 0.034
5 (native) : 6 (commercial) at P = 0.031

In 2003, plots 5 and 4 had the largest amount of biomass (13.61 g and 13.18 g, respectively), both are native soil inoculant treatments (Table 3). Plot 7 (control treatment) had the least amount of biomass (7.96 g). ANOVA determined statistical significances between plots in 2003 (P = 0.016, n = 70), although Tukey's test revealed no specific statistical significance when plots were compared directly to each other (Figure 3). A two-tailed Paired T-Test of the 2002 and 2003 mean biomass per plot data revealed statistically significant differences between the two years (P = 0.004, n = 7), with all plots increasing in biomass in 2003.

Table 4 lists the mean biomass and dominant species for each treatment over the two years of the study. In 2002, the native soil treatment had the highest mean biomass (9.925 g, n = 20) and the control treatment had the lowest mean biomass (5.09 g, n = 30). An ANOVA statistical test comparing the biomass production (log transformed) between treatments in 2002 was not statistically significant (P = 0.976, n = 70)(Figure 4). The overall dominant species for each of the treatments in 2002 was non-native. The 2003 data also has the largest amount of biomass in the native soil treatment (13.4 g), but the commercial inoculant treatment has the lowest (9.6 g)(Table 4). The 2003 ANOVA of biomass and treatment was significant (P = 0.019, n = 70) and Tukey's test showed a statistical significance between the native soil and commercial inoculant treatments (Figure 4). The dominant species in the native soil treatment was blue

gramma (*Bouteloua gracilis*), a desireable native prairie species. The other two treatments were dominated by Cheatgrass (Table 4).

The results of the ranking system determined the dominant plant species for each plot and treatment based on the 10 samples collected from each plot in both years of the study. Table 5 lists the dominant species and summarizes the combined scores for each treatment. In general, Cheatgrass (*Anisantha tectorum*), Bindweed (*Convolvulus arvensis*), Japanese Brome (*Bromus japonicus*) and Crane's Bill (*Erodium cicutarium*) were the dominant species in most of the plots and treatments. There are several notably exceptions. In the 2002 control plots, *Artemisia ludoviciana* (Prairie Sage) was the third most dominant species and *Sporobolus cryptandrus* (Sand Dropseed) was the fifth most prevalent species. The control treatment also saw a surge of Blue Gramma and *Poa compressa* in 2003, although the *A. ludoviciana* and *S. cryptandrus* did decrease in prevalence.

The 2002 native soil treatment was dominated by Cheatgrass and the second ranking was a tie between Crane's Bill (*Erodium cicutarium*) and *Sporobolus cryptandrus*, a native grass. The forth most prevalent species was *Artemisia ludoviciana*. *S. cryptandrus* was not part of the seed mix and therefore could have been introduced to the site within the native soil treatment, although it was also found in the control plots. Both of this species decreased in prominence in 2003, although Blue Gramma became the most dominant species in the native soil inoculant treatments.

The commercial inoculant treatments had the least amount of native species in 2002 and were heavily dominated by the brome grasses in both years, although Blue Gramma, *Poa compressa*, and *Artemisia ludoviciana* increased in prevalence during 2003 (Table 5).

The results of the mycorrhizal quantification summarize the mean percentage of VAM infections for each plot, treatment, and species (Tables 6, 7, and 8 respectively). Figure 5 illustrates the mean percentage of mycorhizae infections for each plot and the standard error of the mean for each histogram. Plot 1 (control) had the highest mean percent infection (88.71%) of all plots and plot 5 (native soil inoculant) had the lowest infection rate (48.77%), although this plot had the least amount of samples and species (4 and 2, respectively)(Table 6). The control treatment has the highest mean infection rate (77.78%), while the native and commercial inoculants are nearly tied at 64.52% and 64.18%, respectively (Figure 6 and Table 7). *Bromus japonicus* has the highest mycorrhizal infection rate of all species (93.66%) and *Sporobolus*

cryptandrus has the lowest (50.53%)(Table 8). Most of the native grasses (*Bouteloua gracilis, Sporobolus cryptandrus,* and *Schedonnardus paniculatus*) have relatively low infection rates, except *Buchloe dactyloides* (Buffalo Grass). All forbs have infections rates greater than 74.12%, while the grasses seem to vary greatly. An additional control plot (8) was included in the mycorrhizal quantification analysis, but was not sampled for the cover or biomass studies during either years of the project.

Weather data for a Boulder, Colorado weather station (# 050848) was collected from the Western Regional Climate Center in Reno, Nevada (WRCC, 2003). Based on measurements from 1948 thru 2003, this weather station has a mean annual precipitation of 19.04 inches. In 2002, a total of 13.88 inches of precipitation were received and a total of 21.23 in 2003. Although no data was available for December 2003, this month normally receives little precipitation (mean = 0.68 inches). Appendix D lists the mean and 2002 precipitation data for the Boulder weather station. In 2002, three extremely dry months occurred during the growing season and project timeline: April, June, and July. Each of these months was dramatically below the mean monthly precipitation based on the 55 years of measurement. April received 2.35 inches below the mean, June was 0.95 inches below, and July was 1.73 inches below the monthly mean amount of precipitation.

DISCUSSION

The restoration of natural ecosystems is an essential component in the management of natural resources, especially when open spaces are increasingly surrounded and influenced by development, degraded habitats, and recreational pressures. In order to sustain natural areas in perpetuity, ecologically sensitive management practices are necessary to support the naturally evolved and dynamic systems. At the Heil Ranch, Boulder County Parks and Open Space has initiated programs of prescribed burns and seeding of native species to assist in the restoration of a meadow that has been degraded by an old road. Denver Botanic Gardens has added an experimental aspect to the restoration of this roadbed restoration in Geer Canyon. Using commercially available mycorrhizal fungi and native soil-borne mycorrhizae, we quantitatively and qualitatively tested the effects on vegetation cover, biomass production, and species composition. By adding an experimental component into a restoration project, it is possible to determine methods or techniques that can be beneficial to the goals of an ecological restoration.

A primary goal for this project was to test practical methods that Boulder County could potentially use in future restoration projects, primarily the addition of commercial mycorrhizal inoculum to revegetation seed mixes.

The summer of 2002 was a drought year and this undoubtedly affected the growth of existing vegetation and the germination of seeded material and possibly mycorrhizae activity (Appendix D). Comparison of 2002 and 2003 cover and biomass data illustrates the dramatic differences in vegetation cover (Figures 1 and 3) and biomass production (Figures 2 and 4). Additionally, Paired T-Tests of cover and biomass means between the two years were statistically significant (see results). Following the burning and seeding of the mountain meadow, we saw little growth of vegetation until late August of 2002. Originally, we had planned to collect the biomass and cover data in August, but this was delayed to allow more time for the vegetation to grow. Unfortunately, we saw large amounts of *Anisantha tectorum* (Cheatgrass) seedlings emerging from the ground following the late summer rains. Cheatgrass is an annual (or winter annual) species that is non-native, invasive, considered to be a fire hazard, and harmful to animals because of the spiked awns, which harm the mouths of grazers (Harrington, 1954; Weber and Wittman, 2001). The prescribed burn may not have been intense enough to kill the soil seed bank of Cheatgrass propagules, possibly due to insufficient vegetation or litter along the road to sustain the fire. Cover data and biomass samples were collected between September 26 and October 4 of both 2002 and 2003, although vegetative growth occurred throughout October at the study site (Evenson, Personal Observation).

The vegetation cover data estimates the amount of basal vegetation covering the soil surface within the study plots. Cover can affect many factors of a microsite, including: solar insulation, soil temperature, erosion, plant competition, germination, and recruitment. Areas of high percent cover are less likely to have soil erosion and may be less susceptible to invasion by non-native, weedy species. Unfortunately, many of the dominant species within the roadbed are established non-natives. The mean percent cover by plot (Figure 1) illustrates the large variation in cover between plots within a year, especially during 2002, and between the two years of the study. The ANOVA results highlight statistically significant variation between plots and treatments in 2003, although the 2002 data could not be analyzed using a parametric statistical test due to non-normal data. All plots were significantly different than plot 6 (commercial inoculant), which had the lowest mean cover values for both years of the study and therefore is

considered an outlier. A few trends can be extrapolated from the data; primarily that 2003 had significantly more cover than 2002, presumably due to greater precipitation (Appendix D), and that the control and native soil treatments had higher cover values than the commercial inoculant (Figure 2). A Tukey's test revealed significant differences in 2003 between the native soil and commercial treatments, which had the highest and lowest mean cover values, respectively. The commercial inoculant treatment had the lowest cover values for both years of the study and may not have been effective in the goal of aiding restoration processes (Table 2), although the native soil treatment did not differ statistically from the control and therefore it can not be determined if it was more effective than no experimental treatment. The outlier data (plot 6) of the commercial treatment may be the cause of the statistical differences between the treatments, although all aspects of the study design were random. The dramatic and statistically significant variation (Paired T-Test) between years for both plots and treatments probably shows the affects of climate (precipitation) on the germination and growth of plant material, but does not provide information to whether the increased vegetation cover in 2003 consists of native or non-native species and therefore whether the treatments are assisting in the goals of native plant restoration.

Biomass production data augment the information gathered from vegetation cover studies, primarily by providing species dominance information and balancing the sampling biases of cover estimates when studying areas dominated by graminoids. Grasses tend to cause an over estimation of basal cover and add little weight to biomass samples, while a single native prairie sage (*Artemisia ludoviciana*) or invasive musk thistle (*Carduus nutans*) may add little basal cover, but lots of biomass. Therefore, differences between cover and biomass results should be expected in the summary results. Similar to the cover data, are the documented increases in biomass from 2002 to 2003 (Figures 3 and 4) and statistical significance between years (Paired T-Test). In both years of the study a plot treated with the native soil inoculant had the greatest amount of biomass, although a commercial treatment (plot 6) had the least biomass in 2003 and a control treatment (plot 7) had the least in 2003. The 2002 Tukey test revealed statistical differences between many plots (3:4, 3:6, 4:5, 5:6), although none were significant in 2003 (Figure 3). The lack of growth in 2002 due to drought conditions may have created greater variation in biomass during 2002, which could have caused the numerous specific between plot differences, even though the ANOVA determined significant differences between plots for both 2002 and 2003. The ANOVA only determines if a statistical difference occurs, while the Tukey

test reveals specific plots or treatments that differ significantly. Unlike the vegetation cover results, biomass data determined that the native soil treatments had the highest amount of biomass in both 2002 and 2003 (Table 4).

The species dominance data show interesting changes in the dominant plant species between years and experimental treatments. In 2002, the control and native soil treatments were dominated by Cheatgrass (*Anisantha tectorum*), while the commercial treatment was dominated by Bindweed (*Convolvulus arvensis*), an undesirable, non-native species. While in 2003, the control and commercial treatments were dominated by Cheatgrass and the native soil treatment was dominated by Blue Gramma (*Bouteloua gracilis*), a desirable native which existed naturally in the meadow and was also a component of the revegetation seed mix. Cheatgrass and Japanese Brome (*Bromus japonicus*) are large components of the biomass samples for all treatments and both years of the study. Although none of the treatments show an overall dominance by native species, we can detect several increases in the prevalence of specific native species in all treatments in the 2003 data. Table 5 ranks the prevalence of all plant species found in the biomass samples based upon our qualitative methods of ranking dominance species (see methods). The weights of specific species in the biomass samples could not be determined quantitatively in 2002 due to the small amount of vegetative biomass available (drought related) and the lack of separating species at the time of biomass collection; therefore this method of qualitative ranking was carried over into the 2003 sample analysis. In addition to the strong representation of the ubiquitous weeds (Cheatgrass, Japanese Brome, Bindweed, and Crane's Bill) in all treatments, the 2002 dominance rankings show several native species to be consistently found in all treatments. *Artemisia ludoviciana, Sporobolus cryptandrus,* and *S. asper* ranked high in all three treatments during 2002 (Table 5). The species composition changed dramatically in 2003; with these natives usually decreasing in prevalence, the four non-native weeds formerly listed remaining high in the rankings, and several new native species increasing in dominance. *Bouteloua gracilis* (Blue Gramma), *Poa compressa, Artemisia frigida,* and *Ambrosia psilostachya* (indigenous, ruderal weed) increased in prevalence in several or all treatments in 2003 (Table 5). These increases may be due to the increased precipitation and subsequent growth or germination of native species, which could thereby out-compete the non-natives, or the effects of the prescribed burn in promoting natural processes that benefit the

native species which evolved with fire, or a combination of these and other unmeasureable factors.

The mycorrhizal quantification portion of the study revealed interesting, but inconclusive results due to the collection of varied plant species in each plot and possible problems in the microscopic analysis of VAM infections. Control plots 1 and 2 had the highest infection rates (Figure 5) and overall the control treatment had the highest infection rates (77.78%) of all treatments (Figure 6). These plots and treatments had consistently high % cover and biomass results as would be expected due to the supposedly mutualistic nature of plants and mycorrhizae. Surprisingly, plot 6 (commercial inoculant) had the third highest infection rate and some of the lowest % cover and biomass measurements of all plots. Additionally, plot 5 (native soil inoculant) had the lowest infection rate of all plots, yet had the highest percent cover in 2002 (Figure 1), the second highest percent cover in 2003, and the most biomass of any plot for both years (Figure 3). Inconsistencies such as these, may be due sampling methods that are not sufficiently standardized in the collection and comparison of different plant species, methodologies that are inconsistent (differences in root diameters), or a general lack of understanding concerning the role of mycorrhizal fungi in dynamic ecosystems. We assumed that plots with greater cover and biomass would have higher levels of mycorrhizal fungi and therefore be more 'successful', but this assumption may be wrong or misinterpreted due to a lack of information concerning mycorrhizae and how they relate to plants. Our results do not reveal any increases in mycorrhizal infections due to the experimental treatments. Additionally, the results do not show any reductions in mycorrhizae due to the prescribed burns, since the control plots had the highest percent infections. Table 8 lists the percent infection of all species studied and the corresponding number of plant samples collected. Each sample supplied the material for 96 cross-sections, from which the percent infections were determined. In general, the native grasses had low infection rates, except for Buffalo Grass (*Buchloe dactyloides*). The forbs usually had higher infection rates than grasses and surprisingly the two brome species (Cheatgrass and Japanese Brome) had very different levels of infection (61.46% versus 93.66% respectively). The non-native Japanese Brome had the highest percent infection of all species sampled, while two native grasses (Blue Gramma and Sand Dropseed) had the lowest. Different samples sizes between species and possible problems related to the sampling of different root sizes (at a very small scale, < mm) may create inconsistencies in this progressive aspect of the

study. Additional application and experimentation of this methodology may provide higher quality results, primarily related the capacity of mycorrhizae to infect different plant species at variable rates and root diameters.

This three-tiered approach (percent cover, biomass, and mycorrhizal infections) to determining the best method of restoring a degraded and weed infested road to a natural grassland ecosystem documented a few of the dynamic changes that constantly occur in plant communities. Following the spring prescribed burn we documented only two of the seeded species in the study plots throughout the two years of the project (Blue Gramma and Buffalo Grass), both of which also occur naturally in the area. None of the other seven species in the seed mix were documented in any plots throughout the study. We suspect that the viability of the seed mix was low or the material was mal-adapted to this microsite, possibly due to out of state origins of all the seeds (Appendix A) or selection in the nursery cultivation and increase of the plant material.

Regardless of the revegetation material, this study focused on the affects of mycorrhizal fungi in the restoration of the roadbed. The commercial mycorrhizae did not show any positive effects on vegetation cover, biomass or mycorrhizal infection; although it must be mentioned that the second commercial treatment plot was significantly different in so many of the statistical tests that it could be considered an outlier due to unknown circumstances. Regardless, the data do not exhibit any positive benefits from the use of commercial mycorrhizae. Overall, the native soil treatment had the best results of the three treatments tested, especially in the percent cover and biomass portions of the study. This experimental treatment may have introduced additional seeds to the plots, smothered weed seeds due to the addition of soil, or created the necessary disturbance to give the native species a chance to establish. This treatment did not have any positive affect on mycorrhizal infection rates. Since this study commenced following the prescribed burn, we cannot analyze the effects of the fire or compare the results with pre-fire conditions. The use of native soil as a restoration technique is fairly uncommon and may not be practical for a large-scale restoration. This treatment was included in the study as an alternative to the primary question: does commercial mycorrhizae positively aid in the restoration of a degraded dirt road. This two-year study provides valuable quantitative and qualitative data concerning the restoration of a degraded area following a prescribed burn and mycorrhizal inoculation. It does not determine absolute guidelines for managing a semi-natural ecosystem,

but provides some guidance that can be developed upon with additional experimentation and habitat management practice by Boulder County Parks and Open Space.

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Table 1 – Mean Percent Vegetation Cover by Plot
Sample size (n) = 10 per plot for each year.

Plot	Treatment	Mean % Cover 2002	Mean % Cover 2003
1	Control	64.4	96.8
2	Control	41.2	89.2
3	Commercial Inoculant	64.4	94.0
4	Native Soil Inoculant	35.2	96.8
5	Native Soil Inoculant	68.4	91.6
6	Commercial Inoculant	24.8	64.0
7	Control	52.0	88.8

Table 2 – Mean Percent Vegetation Cover by Treatment

Treatment	Mean % Cover 2002	Mean % Cover 2003	Samples (N) per Year
Control	52.5	91.6	30
Native Soil Inoculant	51.8	94.2	20
Commercial Inoculant	44.6	79.0	20

Table 3 – Mean Biomass Production by Plot and Dominant Species for 2002 and 2003
Sample size (n) = 10 for each plot. Dominant species determined by ranking system based on visual estimate of the three dominant species in each biomass sample (see methods).

Plot	Treatment	Mean Biomass (g) 2002	Dominant Species 2002	Mean Biomass (g) 2003	Dominant Species 2003
1	Control	5.6	Anisantha tectorum (L.) Nevski	12.94	
2	Control	4.14	Anisantha tectorum (L.) Nevski	10.98	
3	Commercial Inoculant	9.29	Anisantha tectorum (L.) Nevski	10.57	
4	Native Soil Inoculant	7.8	Erodium cicutarium (L.) L'Hér. ex Ait.	13.18	
5	Native Soil Inoculant	12.05	Sporobolus cryptandrus (Torr.) Gray	13.61	
6	Commercial Inoculant	2.86	Convolvulus arvensis L.	8.06	
7	Control	5.55	Convolvulus arvensis L.	7.96	

Table 4 – Mean Biomass Production by Treatment and Dominant Species for 2002 and 2003
Dominant species determined by ranking system of three dominant species from all samples for each treatment (see methods).

Treatment	Mean Biomass (g) 2002	Dominant Species 2002	Mean Biomass (g) 2003	Dominant Species 2003	N
Native Soil Inoculant	9.925	Anisantha tectorum (L.) Nevski	13.4	*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths	20
Commercial Inoculant	6.075	Convolvulus arvensis L.	9.3	*Anisantha tectorum* (L.) Nevski	20
Control	5.096666667	Anisantha tectorum (L.) Nevski	10.6	*Anisantha tectorum* (L.) Nevski	30

Table 5 – Results of ranking system for dominant plant species by Treatment

Species are listed in descending order of dominance based on the combined score for all plots within the respective treatment. Native plants are in **bold.**

2002

Rank	Control Plots	Total
1 -	*Anisantha tectorum* (L.) Nevski	**35**
2 -	*Convolvulus arvensis* L.	**33**
3 -	***Artemisia ludoviciana* Nutt.**	**27**
4 -	*Bromus japonicus* Thunb. ex Murr.	**18**
5 -	***Sporobolus cryptandrus* (Torr.) Gray**	**17**
6 -	*Erodium cicutarium* (L.) L'Hér. ex Ait.	**12**
7 -	***Sporobolus asper* (Michx.) Kunth**	**5**
8 -	***Artemisia frigida* Willd.**	**4**
9 -	*Lactuca serriola* L.	**4**
10 -	*Potentilla* sp.	**3**
11 -	*Asteraceae* (unidentifiable)	**2**
12 -	***Schedonnardus paniculatus* (Nutt.) Trel.**	**2**
13 -	*Taraxacum officinale* G.H. Weber ex Wiggers	**2**
14 -	*Alyssum parviflorum* Fisch. ex Bieb.	**1**
15 -	*Poaceae* (unidentifiable)	**1**

2003

Rank	Control Plots	Total
1-	*Anisantha tectorum* (L.) Nevski	**54**
2-	*Bromus japonicus* Thunb. ex Murr.	**36**
3-	***Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths**	**23**
4-	***Poa compressa* L.**	**22**
5-	***Artemisia ludoviciana* Nutt.**	**16**
6-	*Evolvulus nuttallianus* J.A. Schultes	**16**
7-	***Sporobolus cryptandrus* (Torr.) Gray**	**8**
8-	*Bromus inermis* Leyss.	**2**
9-	***Buchloe dactyloides* (Nutt.) Engelm.**	**2**
10-	***Artemisia frigida* Willd.**	**1**

2002

Rank	Commercial Soil Inoculant	Total
1 -	*Convolvulus arvensis* L.	**28**
2 -	*Anisantha tectorum* (L.) Nevski	**23**
3 -	*Erodium cicutarium* (L.) L'Hér. ex Ait.	**20**
4 -	*Bromus japonicus* Thunb. ex Murr.	**9**
5 -	*Poaceae* (unidentifiable)	**9**
6 -	***Artemisia ludoviciana* Nutt.**	**7**
7 -	***Sporobolus asper* (Michx.) Kunth**	**7**
8 -	*Carduus nutans* L.	**3**
9 -	*Asteraceae* (unidentifiable)	**2**
10 -	*Trifolium* sp.	**2**

2003

Rank	Commercial Soil Inoculant	Total
1-	*Anisantha tectorum* (L.) Nevski	**31**
2-	*Bromus japonicus* Thunb. ex Murr.	**27**
3-	***Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths**	**24**
4-	***Artemisia ludoviciana* Nutt.**	**13**
5-	***Poa compressa* L.**	**11**
6-	*Evolvulus nuttallianus* J.A. Schultes	**10**
7-	***Artemisia frigida* Willd.**	**2**
8-	***Heterotheca villosa* (Pursh) Shinners**	**2**

2002			2003		
Rank	**Native Soil Inoculant**	**Total**	Rank	**Native Soil Inoculant**	**Total**
1 -	*Anisantha tectorum* (L.) Nevski	**14**	1-	***Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths**	35
2 -	*Erodium cicutarium* (L.) L'Hér. ex Ait.	**12**	2-	*Anisantha tectorum* (L.) Nevski	30
3 -	***Sporobolus cryptandrus* (Torr.) Gray**	**12**	3-	*Bromus japonicus* Thunb. ex Murr.	29
4 -	***Artemisia ludoviciana* Nutt.**	**11**	4-	***Ambrosia psilostachya* DC.**	10
5 -	*Bromus japonicus* Thunb. ex Murr.	**11**	5-	***Poa compressa* L.**	6
6 -	*Convolvulus arvensis* L.	**9**	6-	***Artemisia frigida* Willd.**	4
7 -	*Lactuca serriola* L.	**9**	7-	***Sporobolus cryptandrus* (Torr.) Gray**	3
8 -	***Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths**	**6**	8-	Alyssum sp.	2
9 -	***Artemisia frigida* Willd.**	**5**	9-	***Artemisia ludoviciana* Nutt.**	1
10 -	***Sporobolus asper* (Michx.) Kunth**	**5**			
11 -	***Buchloe dactyloides* (Nutt.) Engelm.**	**3**			
12 -	***Lepidotheca suaveolens* Nuttall.**	**3**			
13 -	*Asteraceae* (unidentifiable)	**2**			
14 -	*Verbascum thapsus* L.	**2**			
15 -	*Taraxacum officinale* G.H. Weber ex Wiggers	**1**			

Table 6 – Mean Percent Mycorrhizal Infection per Plot (2002 only)

Plot number	Treatment	Mean % Infection	N (# of Samples)	Number of Species
1	Control	88.71	6	4
2	Control	76.78	6	4
3	Commericial Inoculant	58.24	7	3
4	Native Soil Inoculant	71.52	9	5
5	Native Soil Inoculant	48.77	4	2
6	Commericial Inoculant	72.50	5	3
7	Control	NA	0	0
8	Control	65.87	5	3

Table 7 – Mean Percent Mycorrhizal Infection per Treatment (2002 only)

Treatment	Mean % Infection	# of Samples (N)
Commericial Inoculant	64.18	12
Control	77.78	17
Native Soil Inoculant	64.52	13

Table 8 – Mean Percent Mycorrhizal Infection per Species (2002 only)

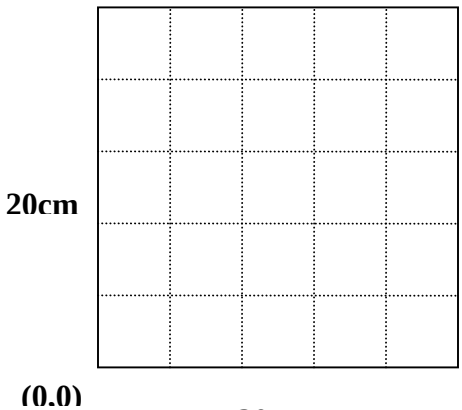
Species	Life Form	Mean % Infection	# of Samples (N)
Artemisia frigida Willd.	forb	74.12	9
Bouteloua gracilis (Willd. ex Kunth) Lag. ex Griffiths	graminoid	50.55	9
Bromus japonicus Thunb. ex Murr.	graminoid	93.66	2
Bromus tectorum L.	graminoid	61.46	2
Buchloe dactyloides (Nutt.) Engelm.	graminoid	90.63	2
Dyssodia papposa (Ventenat) Hitchcock	forb	82.29	2
Erigeron divergens Torr. & Gray	forb	90.90	5
Erodium cicutarium (L.) L'Hér. ex Ait.	forb	81.88	2
Poa pratensis L.	graminoid	77.93	2
Schedonnardus paniculatus (Nuttall) Trelease	graminoid	63.16	2
Sporobolus cryptandrus (Torr.) Gray	graminoid	50.53	5

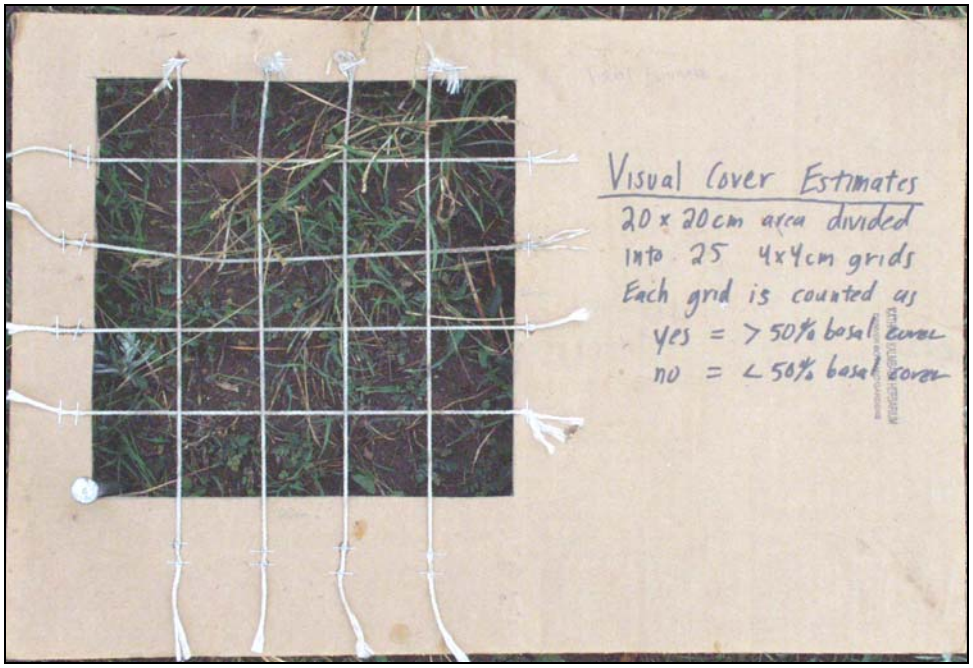
APPENDIX B – Heil Ranch Experimental Design

Seven experimental plots (3 x 3 meters each) were placed within the old roadbed and the following experimental treatments randomly applied: 1 = control, 2 = control, 3 = commercial VAM, 4 = native soil inoculant, 5 = native soil inoculant, 6 = commercial VAM, 7 = control. Within each plot a 2 x 2 meter sampling area was defined to reduce edge effects.

APPENDIX C – Cover and biomass sampling frames

Sampling frames were used to delineate biomass collection areas and define 25 quadrats (each 4cm X 4cm and 4% of sampling area) for estimation of percent cover. Percent cover based on methods of Elzinga et al, 1998.





APPENDIX D – Weather Data for Boulder, Colorado

Source: Western Regional Climate Center website, Desert Research Institute, Reno, Nevada. http://www.wrcc.dri.edu/

Monthly Total Precipitation in Inches Boulder, Colorado (050848)													
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	Cumulative
2002	1.07	0.44	1.5	0.2	3.2	1.18	0.09	1.44	1.52	2.44	.78	.02	13.88
2003	0.16	1.52	5.44	2.99	2.62	0.71	3.52	0.35	0.45	0.16	0.80	No data	21.23

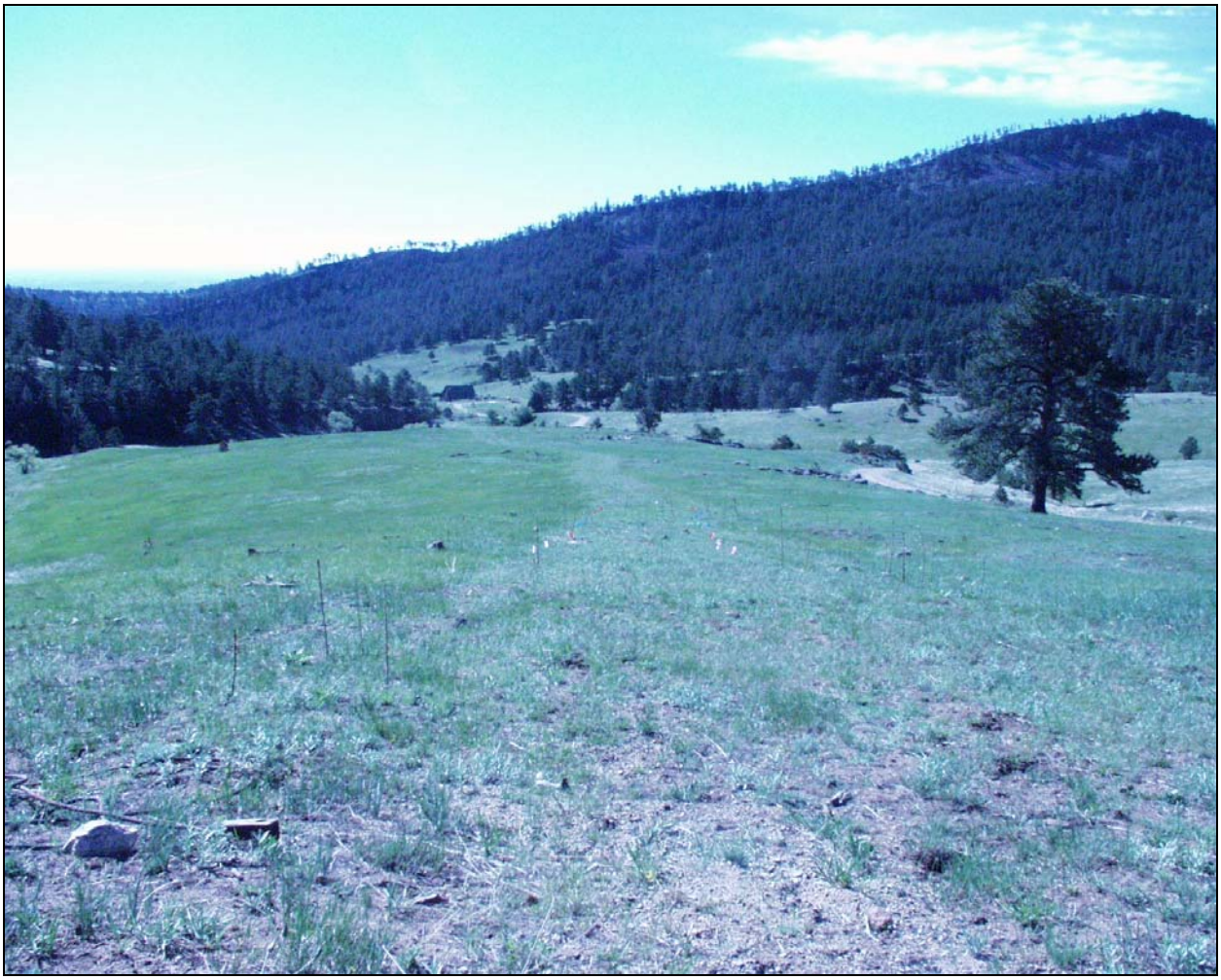
Average Total Monthly Precipitation in Inches Period of Record: 1948 - 2003 Boulder, Colorado (050848)													
JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	Cumulative
0.68	0.77	1.78	2.37	3.09	2.13	1.82	1.59	1.65	1.27	1.22	0.68	19.04

APPENDIX E – Project Photographs

April 10, 2002 Geer Meadow of Heil Ranch Open Space (Boulder County Parks and Open Space) after prescribed burn.

April 10, 2002 Heil Ranch study site. Note discoloration of the old roadbed.

April 19, 2002 applying VAM fungi to study plot (Thomas Grant).



May 30, 2002 Heil Ranch Study Site. Flags marking plots can vaguely be seen along old roadbed.

September 26, 2002 biomass collection (Karen Schoen and Vera Evenson, left to right).