NASA Santa Susanna Field Laboratory Replacement Soil Testing

FINAL TECHNICAL REPORT

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EXECUTIVE SUMMARY

The National Aeronautics and Space Administration (NASA) is currently preparing for environmental clean-up activities on NASA administered federal land at the Santa Susana Field Laboratory (SSFL) in Ventura County, California. These clean-up activities will include the removal of chemical contaminants that remain in the soil from past operations at SSFL. The Soil Ecology and Restoration Group (SERG) at San Diego State University (SDSU) has undertaken two different studies to assist NASA in determining the suitability of available backfill material for use as replacement soil in the restoration of the clean-up areas after contaminated soils have been removed from the site. Soil microbial community deoxyribonucleic acid (DNA) analyses were conducted on the backfill material and reference samples from native habitats occurring at SSFL to document the taxonomic diversity of each. Additionally, greenhouse seed germination and plant development trials were conducted to determine the suitability of the backfill material for the germination and initial growth and development of native southern California plant species.

DNA analysis confirmed that the microbial community of the backfill material differed taxonomically from that of the reference soils; soil microbial biomass was an order of magnitude lower in the backfill material, and soil DNA levels mirrored soil organic matter content. Results from the greenhouse trials suggest that although native plant seeds were able to germinate in the backfill material, plant growth and development were severely limited when compared to plants grown in a standard greenhouse potting mix. These results suggest that the backfill material currently available for use at SSFL may be inadequate for the long-term growth and development of native plant species and soil microbes. The very low organic matter content of the material is the most obvious deficiency. Organic amendments would likely improve growth of plants and beneficial soil microorganisms in the proposed backfill material. Additionally, if intact areas of native soil could remain on the site to serve as a source of inoculum, and if suitable organic matter were added so that native plants and microbes could develop, then the backfill material might eventually attain a community more typical of native soils, and would lose its urban/anthropogenic characteristics.

INTRODUCTION

The Santa Susana Field Laboratory is located on 2,850 acres in the Simi Hills, nearly thirty miles northwest of downtown Los Angeles, California in southeastern Ventura County. SSFL began operating in 1948 and became a center for the development and testing of rocket engines for defense and space exploration purposes. It was also a test site for advanced energy research programs. SSFL is divided into four "Administrative Areas" with additional undeveloped areas of land to the north and south. Areas I, III, and IV and the undeveloped areas are owned and operated by the Boeing Company. NASA is responsible for Area II, consisting of 409.5 acres, along with 41.7 acres in Area I. The U.S. Department of Energy (DOE) has long held a lease on land in Area IV (NASA, 2014).

All rocket engine testing activities at SSFL were discontinued in NASA's areas by 2006 and environmental clean-up activities are now underway to remove chemicals in the environment that remain from past operations. NASA, Boeing, and DOE are each responsible for clean-up in areas in which they operated. California's Department of Toxic Substances Control (DTSC) is overseeing the clean-up. NASA's overarching goal in its clean-up efforts at SSFL is to protect public health and safety and the environment, as well as the cultural and natural resources onsite. In August 2007, NASA, Boeing, DOE, and DTSC signed a Consent Order for Corrective Action that addressed the clean-up of soils and groundwater at SSFL. Subsequently, in December 2010, NASA and DTSC executed an Administrative Order on Consent for Remedial Action (AOC) with specific requirements to complete the characterization and clean-up of soils in NASA-administered areas. As part of the clean-up action at SSFL, the AOC will require that NASA remove large volumes of contaminated soil from SSFL and replace it with suitable soil to restore the site (NASA 2014) (DTSC, 2010).

Following the removal of contaminated soil, one of the key goals at SSFL will be the restoration of a fully functioning ecosystem suitable for the establishment and long-term persistence of native plant and animal species. To restore the site, NASA must import backfill soils that can meet the strict look-up table (LUT) values in the AOC. Among NASA's concern is that AOC-compliant replacement soils, while chemically acceptable, may not be able to sustain restoration activities, without serious amendments. These soil amendments could potentially make the backfill materials unusable by exceeding LUT values. NASA contacted SDSU's SERG to provide expertise in native southern California plant and soil ecology, soil microbial communities, and the vital role this complex plant/soil system plays in supporting native flora and fauna.

SERG and soil microbial ecologist Dr. David Lipson of SDSU have undertaken two different studies to assist NASA in determining the suitability of the replacement soils for the restoration of the clean-up areas at SSFL. The first study focused on the analysis of the proposed fill material and relevant reference soils collected from two native southern California plant communities located at SSFL (oak woodland and chaparral) to assess the microbial community of the replacement soil in terms of its taxonomic diversity.

Secondly, greenhouse trials were conducted using established native plant propagation techniques to determine the suitability of the replacement soils for the germination and initial growth and development of various native southern California plant species common to the plant communities of SSFL. Seed germination and growth rates for native plant species in the replacement soils were compared to a group of the same plant species grown in a standard greenhouse potting mix.

METHODS

Microbial Community Analysis

To test the capacity of the replacement soil to sustain a native microbial community, 20 gram (g) samples of the fill material in sterile pint jars were brought to 45% of field capacity with sterile water and inoculated with 1 milliliter (mL) of 10:1 suspension of sterile water to reference soil (either oak woodland or chaparral, shaken for 20 minutes (min) at 120 revolutions per minute (rpm), vortexed for 1 min at high speed, and allowed to settle for 1 min. Controls received 1 mL of sterile water. After two weeks of incubation at room temperature, soil respiration was measured to test for a stimulation of microbial activity in response to the inoculation. Soil respiration was measured by monitoring carbon dioxide (CO_2) increase in the jars for 5-minute intervals using a PP Systems EGM gas analyzer. After four weeks, the incubations were harvested, and DNA was extracted from the three inoculation treatments (using Qiagen™ soil DNA kits), along with oak woodland and chaparral references soils. DNA concentrations (measured with a Qubit System™) served as a proxy for microbial biomass in the samples. DNA was sequenced using the Illumina MiSeq© platform. Shotgun metagenomes were uploaded to MG-RAST and data were analyzed using the Refseq database to assign reads to taxonomic groups to the level of genus.

Greenhouse Trials

The objective of the soil testing effort was to examine the suitability of the backfill material NASA will be using as replacement soils where contaminated soils are removed at SSFL. To test the replacement soil, SERG germinated and grew selected native coastal sage scrub (CSS) and chaparral plant species in both the replacement soil and a standard greenhouse potting mixture, referred to as greenhouse soil in this report. The plants were grown in the respective soils under climate-controlled conditions in the SERG greenhouse at SDSU for eleven weeks to collect seed germination and yield data. The following describes the procedures undertaken to complete this project.

Soil Collection from SSFL Site

SERG obtained 30 gallons of backfill material, referred to as replacement soil in this report, from the NASA representatives at SSFL on 10 October 2019. This fill soil was chosen by NASA for its availability in bulk, its sandy clay loam texture (similar to that of the native soils at the site), and its ability to meet the strict look-up table values of the AOC (CH2M Hill, 2017) (NASA, 2017).

Seed Collection and Selection

Chaparral, coastal sage scrub (CSS), and coast live oak woodland and riparian forest are the dominant vegetation community types at SSFL (NASA, 2014) and the seed lots selected for use in the greenhouse trials were from native plant species that are common in these vegetation

community types. All seeds used for the greenhouse trials were collected by SERG in eastern San Diego County, California from habitats similar to those found at SSFL. Following the collection and a drying period, the non-seed structures were removed from the seed lots and the remaining material was sifted or blown to remove non-seed matter, leaving only cleaned seed. The seeds were then packaged in plastic containers and kept in cool, dry storage for later use. Several common CSS/chaparral/riparian plant species readily available in this seed inventory were considered for use in this project. Table 1 below lists the final six species that were chosen for the soil experiment.

Table 1. Native Plant Species Selected for the Greenhouse Trials.

Species Abbreviation	**Species**	**Common Name**	**Vegetation community**
ACMGLA	*Acmispon glaber*	Deerweed	CSS
CLAPUR	*Clarkia purpurea*	Purple Clarkia	Chaparral
ERIFAS	*Eriogonum fasciculatum*	California Buckwheat	CSS
SALAPI	*Salvia apiana*	White Sage	Chaparral/CSS
SISBEL	*Sisyrinchium bellum*	Blue Eyed Grass	CSS
STIPUL	*Stipa pulchra*	Purple Needlegrass	CSS/Oak Woodland

Seed Germination Testing

To assess the percentage of Pure Live Seed (PLS) of the six plant species used, a germination test using a 1000 parts-per-million (ppm) Gibberellic acid solution was performed on the seed lots from each of the species. Gibberellic acid (GA3) is a plant hormone that promotes rapid cell growth and induces a faster rate of seed germination in many plant species. This procedure would quantify, as a percentage, how many seeds could be expected to germinate when sowed in soil. A random sample of 100 seeds was taken from each of the six chosen species' seed lots and evenly placed on germination paper saturated with 1000 ppm GA3 solution (Figure 1). After placing 100 seeds on the wet germination paper, a second sheet of GA3 wetted germination paper was placed over the first sheet to cover the seeds. The wet germination papers were then rolled up and stored in an upright, open Ziploc™ plastic bag at room temperature to allow for respiration while encouraging vertical growth. Moisture content of the germination paper was checked every two to three days, and additional 1000 ppm GA3 solution was reapplied to the paper as necessary to maintain a consistent moisture level. After seven days from the start of the germination tests, the germination papers were unrolled and seeds that had germinated were counted and then removed from the paper sheets. The same counting procedure was repeated on the 14th day. Although most seeds are expected to germinate within a two-week period, the test duration was extended to allow for longer germination times. After nine weeks, the germination tests were deemed completed and the percentage of Pure Live Seed (PLS) for each species was calculated by multiplying Mass Purity by Germination Percentage. As the seed

lots were all previously cleaned, they had a 100% mass purity value. Germination percentage was calculated as the final number of germinated seeds divided by number of seeds tested (100) multiplied by 100. The resulting PLS values would serve as a guideline on how many germinants could be expected for each plant species in the soil experiments to follow.

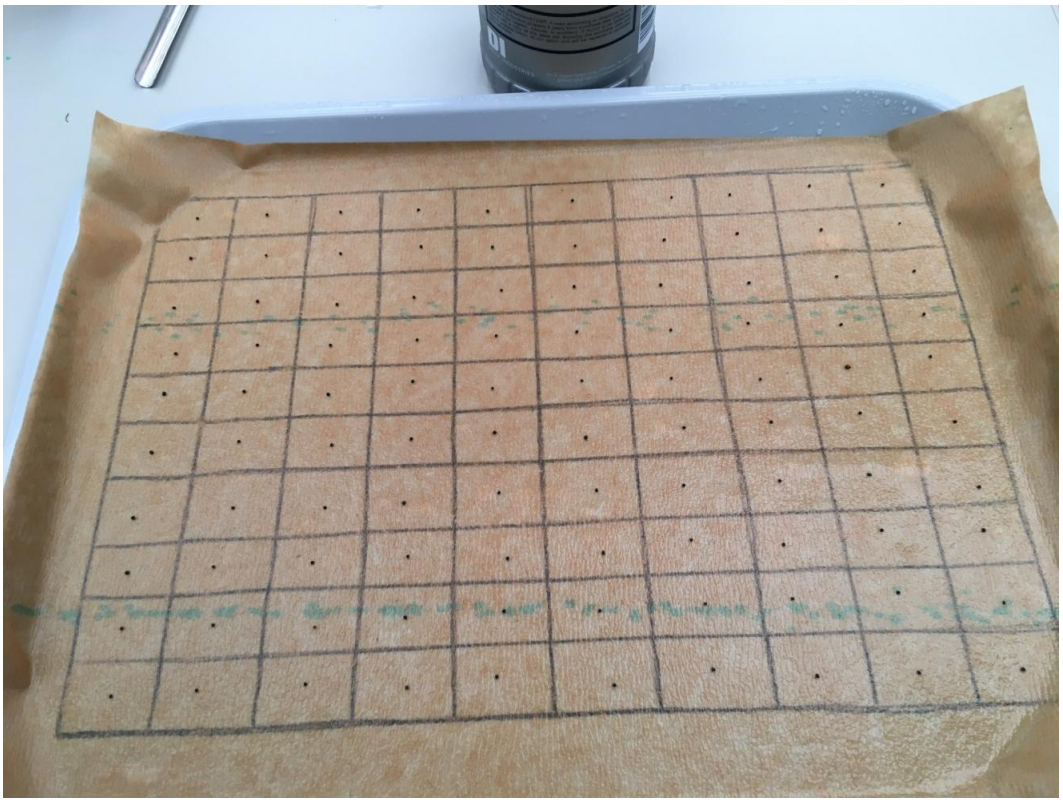

Figure 1. Seeds placed on GA3 moistened germination paper

Germination and Growth Experiment Replacement Soil vs. Greenhouse Soil

The soil experiment entailed an 11-week procedure, conducted in the SERG greenhouse at SDSU, where the six plant species were grown in both backfill material (replacement soil) and a standard greenhouse potting mix (greenhouse soil). The greenhouse soil mixture was comprised of two parts vermiculite, two parts commercially available potting soil, and one part medium grit sand. This is the standard soil mix SERG uses for common southern California CSS and Chaparral plant propagation in its greenhouse and nursery operations. Seeds of each of the six species were sown into one rack of 200 Ray Leach Cone-tainer™ cells**,** with 100 cells used for each soil type. Each cell holds a volume of approximately 66 cubic centimeters (cm^3), and each plant rack holds about 13.2 liters of soil. Prior to sowing the seeds, the plant racks and cells were cleaned with a soapy water and bleach solution to sanitize. Prior to use, both the replacement soil and greenhouse soil were passed once through a course mesh sieve with a pore size of 1 centimeter (cm) to remove any large pieces of material such as gravel or bark. The cells were then filled with replacement soil, 100 cells on the left side of the rack, and

greenhouse soil, 100 cells on the right. After the cells were well compacted and pre-watered, as seen in Figure 2, one seed was placed in each plug with about 1 cm of space remaining at the top of the cell.

Figure 2. STIPUL seeds in cells filled with replacement soil (left) and greenhouse soil (right).

After the seeds were placed in the cells, the seeds were covered with approximately 0.5 cm of the appropriate soil (replacement or greenhouse), watered again, and placed on a greenhouse misting table for the duration of the experiment. Each plant rack was fitted with a protective metal cage that served as a barrier from potential rodent, bird, or other seed predation activity as shown in Figure 3. The automated misting system was set to operate for one minute every 12 hours to ensure the soil moisture in the cells was adequate for seed germination. During the experiment, the availability of moisture as well as the relative humidity and temperature inside the climate-controlled greenhouse were held within an optimal range for plant germination and growth. This meant that any differences seen in the germination and development of the native plants could likely be attributed to the different soils in which they were grown.

Figure 3. Six plant racks on greenhouse misting table.

Twice each week, the wire cages were removed, and the racks monitored for seedlings and photographed to document germination and growth. As new germinants sprouted, those cells were moved to the opposite ends of the rack to keep track of which seeds had germinated in each rack of cells. (Figure 4)

Figure 4. Growth progress photo of SALAPI on 29 November 2018. Sorted germinants at the furthest sides of the plant rack (extreme left and right).

At the end of the 11-week trial, plant measurements were conducted on each of the plants that remained alive. Plants were considered alive if they displayed some green tissue at the end of the trial. Measurements for each plant included maximum height, number of leaves, and maximum leaf size. When measuring maximum height, eudicots were evaluated by stem height (Figure 5) whereas monocots are measured by their longest leaf blade. Maximum leaf size was measured by the length and width of the largest leaf found on the individual.

Figure 5. Measuring stem height on SALAPI.

RESULTS

Microbial Community Analysis

The inoculation treatment resulted in a transient increase in respiration after two weeks, roughly three times higher in the inoculated treatments (Figure 6). However, the inoculations did not result in an increase in microbial biomass, which remained an order of magnitude lower than the reference soils as indicated by soil DNA content (Figure 7). As expected, soil DNA mirrored soil organic matter content (3.0%, 2.9% and 0.4% for oak, chaparral and fill material, respectively).

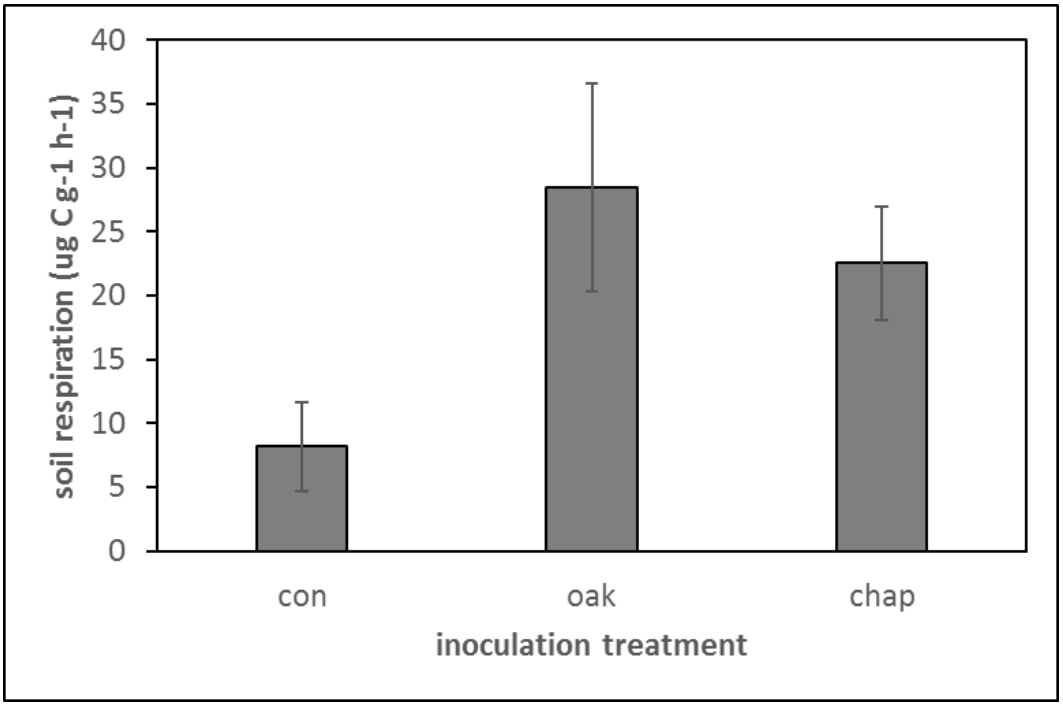

Figure 6. Soil respiration in fill material two weeks after inoculation with suspension of oak woodland or chaparral soil (or sterile water as control).

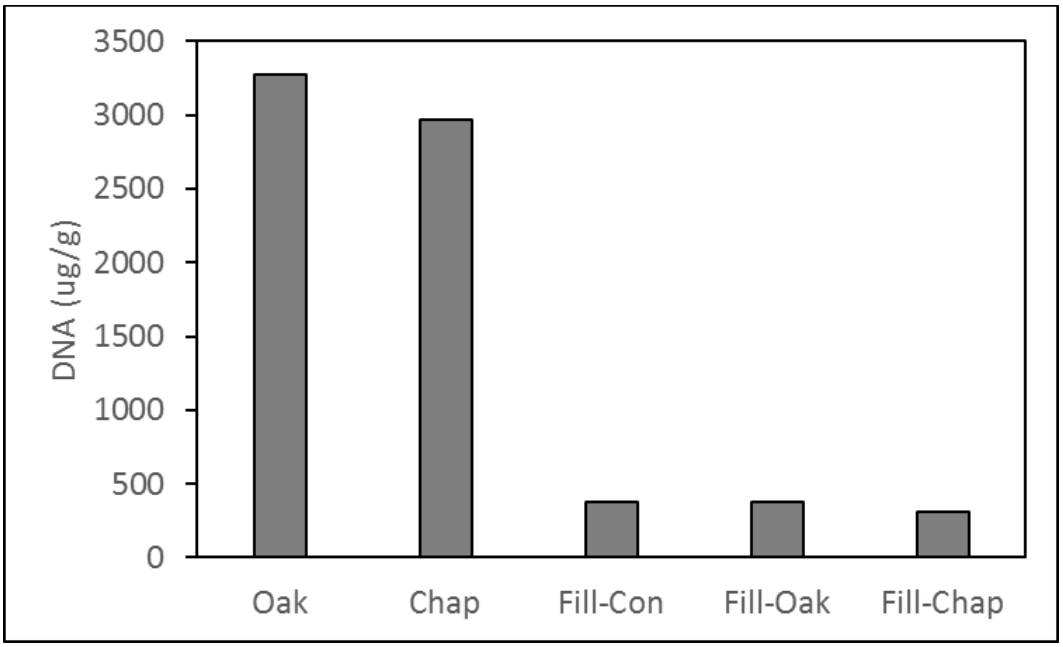

Figure 7. DNA content of reference soils, and fill material after inoculation with suspension of oak woodland or chaparral soil (or sterile water as control), followed by 4 weeks incubation.

Despite the low DNA yields, shotgun sequencing was successful, producing five libraries ranging from 80,697 to 243,636 sequences passing quality control. Average sequence length was 350 base pairs. These metagenomes are publicly available at mg-rast.org as mgm4842585 - mgm4842589.

The microbial community of the fill material differed greatly from those of the reference soils, regardless of inoculation treatment (Figure 8). In a principle component analysis (PCA) based on the 100 most abundant microbial families, the fill material samples clustered close to each other and far from the two reference soils. The inoculation with reference soil suspensions had a negligible effect on the microbial community. In this PCA analysis, the differences between the reference soils and the fill material were driven mainly by the first principle component (PC1), which accounted for 60.6% of the variation in the data. PC1 was positively correlated with several Actinobacteria families which were more relatively abundant in the oak and chaparral reference soils (Table 2). PC1 was negatively correlated with Proteobacteria families that were relatively abundant in the fill material samples. These included groups more generally associated with human or animal hosts than with soils, such as the Enterobacteraceae (enteric bacteria such as *Enterobacter* and *E. coli*), Neisseriaceae (mucous membrane associated *Neisseria* and *Eikenella*) and Vibrionaceae (common pathogen, *Vibrio*). PC2 mainly served to separate the two reference soils, with the oak community represented by several Bacteroidetes families.

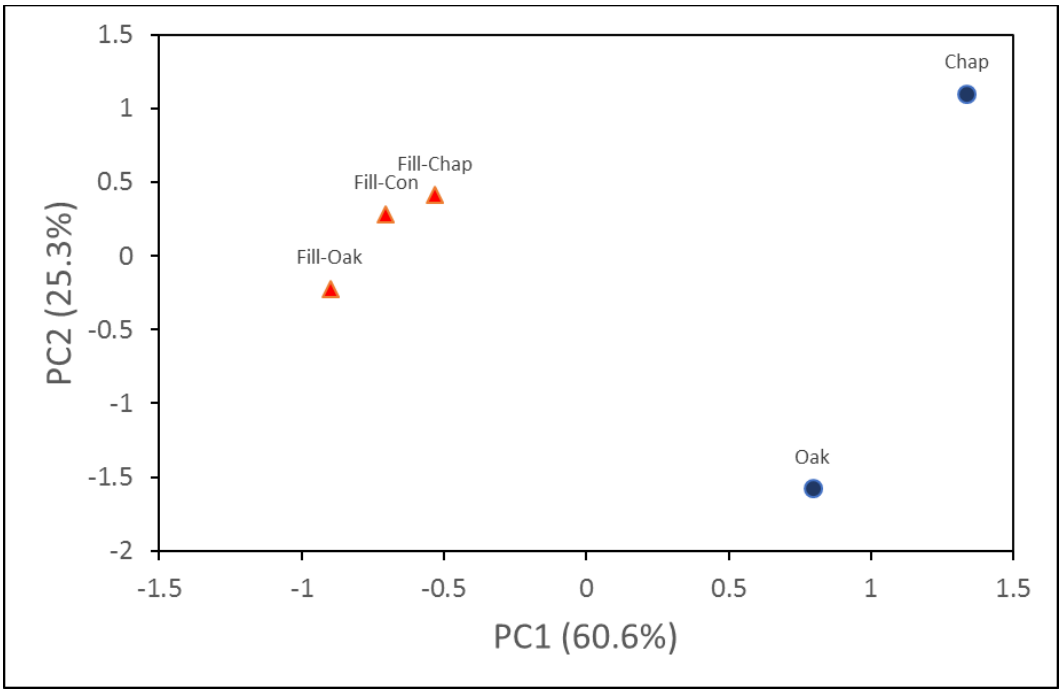

Figure 8. Principle component analysis of metagenomes from the five metagenomes based on the relative abundance of the 100 most abundant microbial families.

Table 2. Microbial Families that Contributed Most to the First Two Principle Components (PC1 and PC2) in the PCA. Top ten positive and negative correlations (Pearson r) shown for both.

PC1 positively correlated	**phylum**	**r**
Geodermatophilaceae	Actinobacteria	0.998
Micromonosporaceae	Actinobacteria	0.997
Pseudonocardiaceae	Actinobacteria	0.992
Ktedonobacteraceae	Chloroflexi	0.986
Catenulisporaceae	Actinobacteria	0.985
Beijerinckiaceae	Proteobacteria	0.981
Methylobacteriaceae	Proteobacteria	0.973
Conexibacteraceae	Actinobacteria	0.97
Nostocaceae	Cyanobacteria	0.97
Clostridiaceae	Firmicutes	0.97
PC1 negatively correlated		
Nitrosomonadaceae	Proteobacteria	-0.995
Neisseriaceae	Proteobacteria	-0.994
Hyphomonadaceae	Proteobacteria	-0.992
Enterobacteriaceae	Proteobacteria	-0.989
Xanthomonadaceae	Proteobacteria	-0.982
Pseudomonadaceae	Proteobacteria	-0.975
Erythrobacteraceae	Proteobacteria	-0.975
Shewanellaceae	Proteobacteria	-0.974
Vibrionaceae	Proteobacteria	-0.969
Sphingomonadaceae	Proteobacteria	-0.968
PC2 positively correlated		
Clostridiales Family XVIII	Firmicutes	0.983
Halobacteriaceae	Euryarchaeota	0.952
Alicyclobacillaceae	Firmicutes	0.947
Desulfobacteraceae	Proteobacteria	0.937
Clostridiales Family XVII	Firmicutes	0.936
Glycomycetaceae	Actinobacteria	0.928
Nocardiopsaceae	Actinobacteria	0.861
Corynebacteriaceae	Actinobacteria	0.848
Nitrospiraceae	Nitrospirae	0.832
Thermomicrobiaceae	Chloroflexi	0.822
PC2 negatively correlated		
Flavobacteriaceae	Bacteroidetes	-0.947
Sphingobacteriaceae	Bacteroidetes	-0.934
Cytophagaceae	Bacteroidetes	-0.928
Bacteroidaceae	Bacteroidetes	-0.904
Gemmatimonadaceae	Gemmatimonadetes	-0.856
Oxalobacteraceae	Proteobacteria	-0.822
Opitutaceae	Verrucomicrobia	-0.787
Caulobacteraceae	Proteobacteria	-0.746
Verrucomicrobia subdivision 3	Verrucomicrobia	-0.628
Haliangiaceae	Proteobacteria	-0.59

On a broader phylogenetic scale, the most obvious difference between the reference soils and the post-incubation fill material is an increased relative abundance of the Proteobacteria phylum in the fill material samples (Figure 9). This increase is at the expense of various common soil phyla such as Acidobacteria, Firmicutes, and Verrucomicrobia. Comparing the combined distributions of reference soils vs. fill material samples, these differences are highly significant by a chi-squared test ($P<0.0001$).

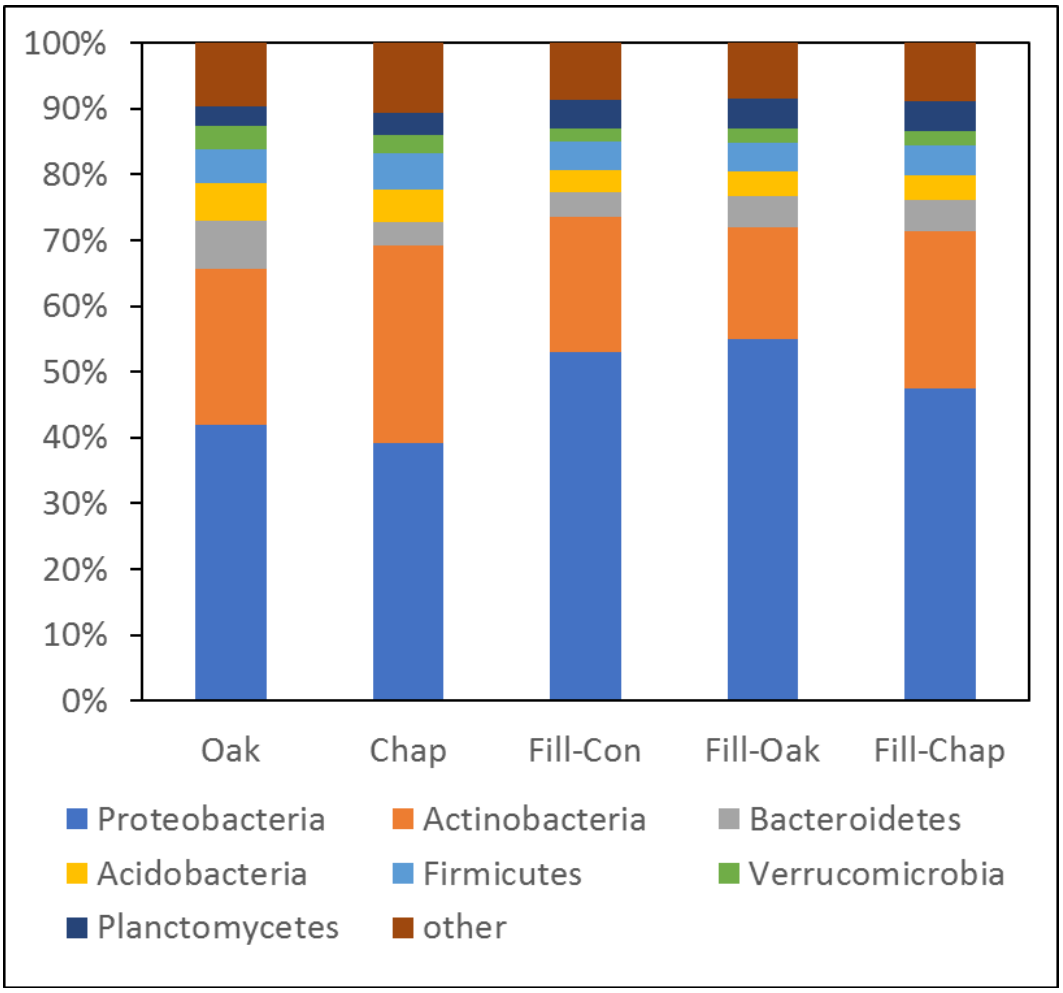

Figure 9. Relative abundance of major phyla in the five metagenomes.

Greenhouse Trials

Figure 10 and Table 3 below show the Pure Live Seed percentages obtained from the Gibberellic acid germination test in comparison to the final percentage of live plants germinated in the Replacement Soil (RS) and Greenhouse Soil (GS) experiment.

Table 3. Pure Live Seed Percentage and Final Live Plant Counts

Six-Letter Code	Species	Pure Live Seed (%)	RS Final Live Plants (%)	GS Final Live Plants (%)
ACMGLA	*Acmispon glaber*	18	3	15
CLAPUR	*Clarkia purpurea*	99	1	64
ERIFAS	*Eriogonum fasciculatum*	47	13	7
SALAPI	*Salvia apiana*	17	4	4
SISBEL	*Sisyrinchium bellum*	14	19	11
STIPUL	*Stipa pulchra*	81	70	87

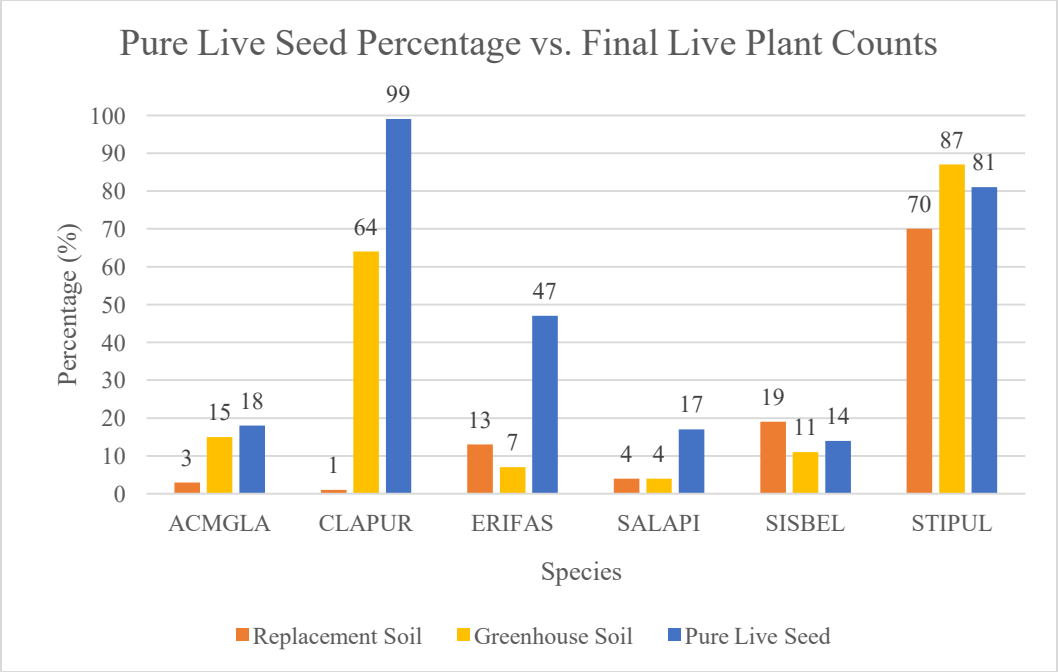

Figure 10. Pure Live Seed Percentage and Final Live Plant Counts

Figures 11 through 16 track the weekly germinant counts from 15 November 2018 to 31 January 2019. Following the progress of *S. apiana*, a larger number of seedlings germinated in the replacement soil initially. Eventually its productivity declined to match that of the greenhouse soil, which retained a consistently low number of germinants throughout the experiment (Figure 11).

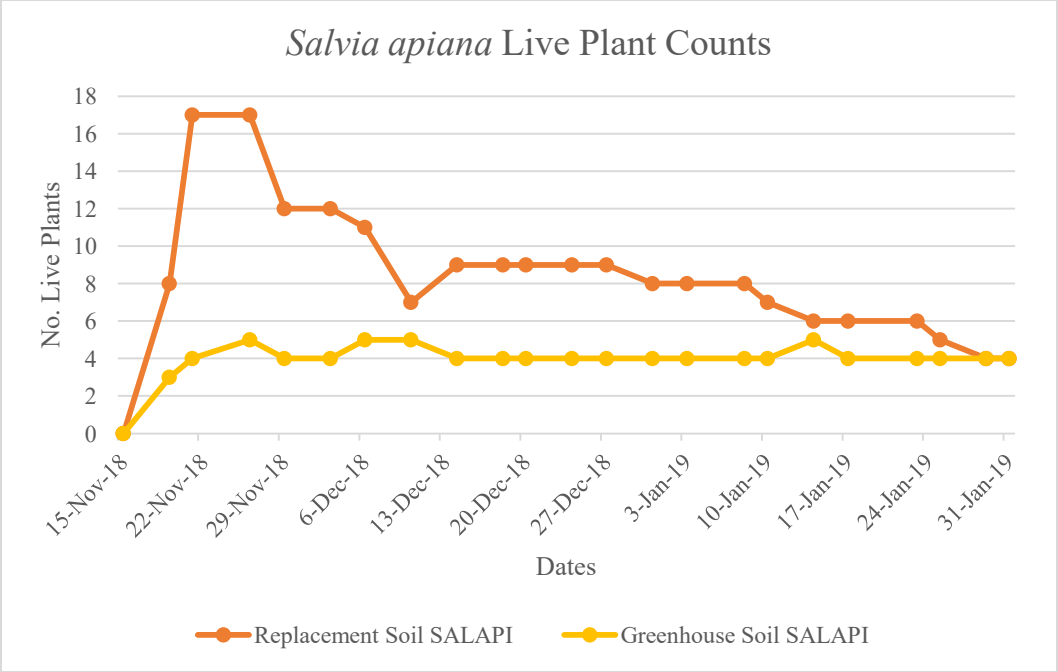

Figure 11. *Salvia apiana* Live Plant Counts for the 11-week period.

S. bellum growing in replacement soil consistently outnumbered those in greenhouse soil (Figure 12).

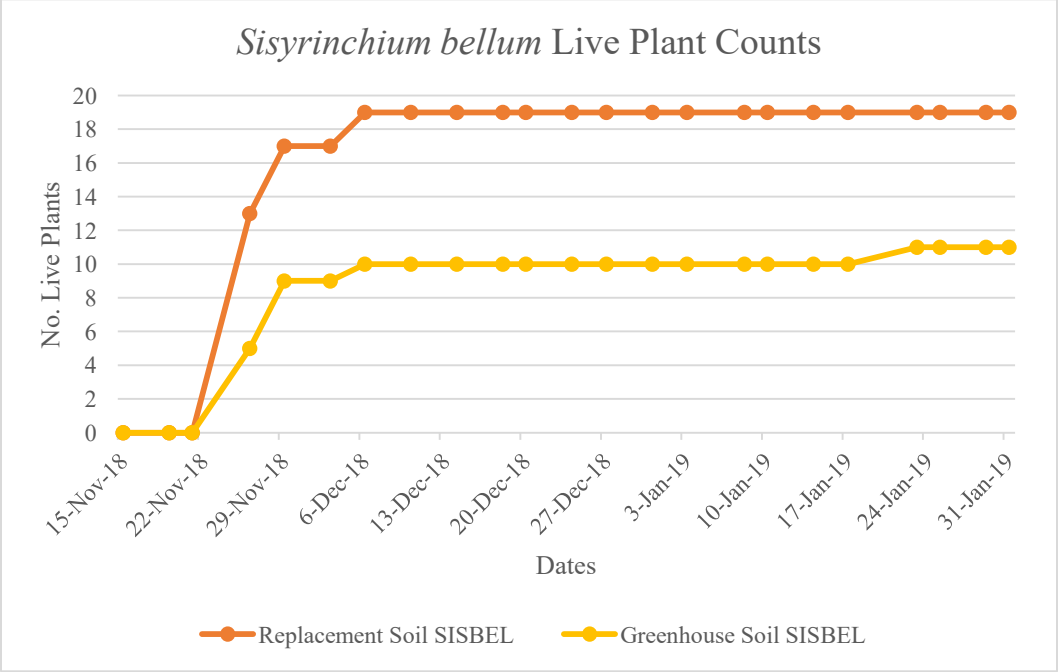

Figure 12. *Sisyrinchium bellum* Live Plant Counts for the 11-week period.

C. purpurea germinated in large numbers in both soil types. Germinants of this species growing in replacement soil started to decline (die off) in numbers in the second half of the experiment,

while those individuals growing in greenhouse soil retained a relatively high count throughout the 11 weeks of the trial (Figure 13).

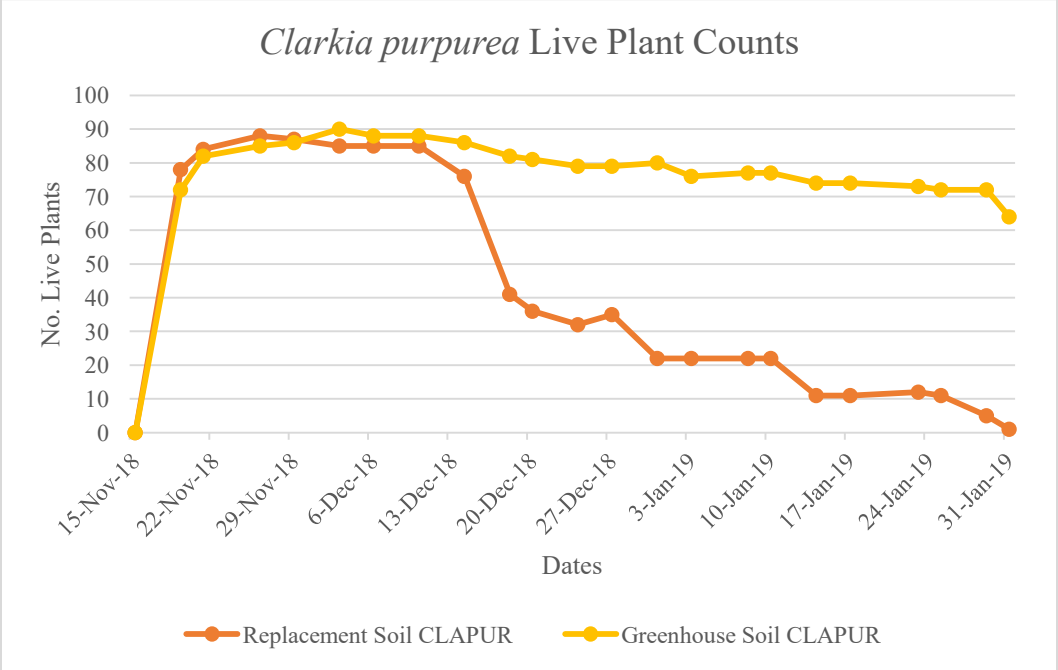

Figure 13. *Clarkia purpurea* Live Plant Counts for the 11-week period.

S. pulchra in both soil types maintained a very consistent number of seedlings throughout, with more germinants in the greenhouse soil than replacement soil (Figure 14).

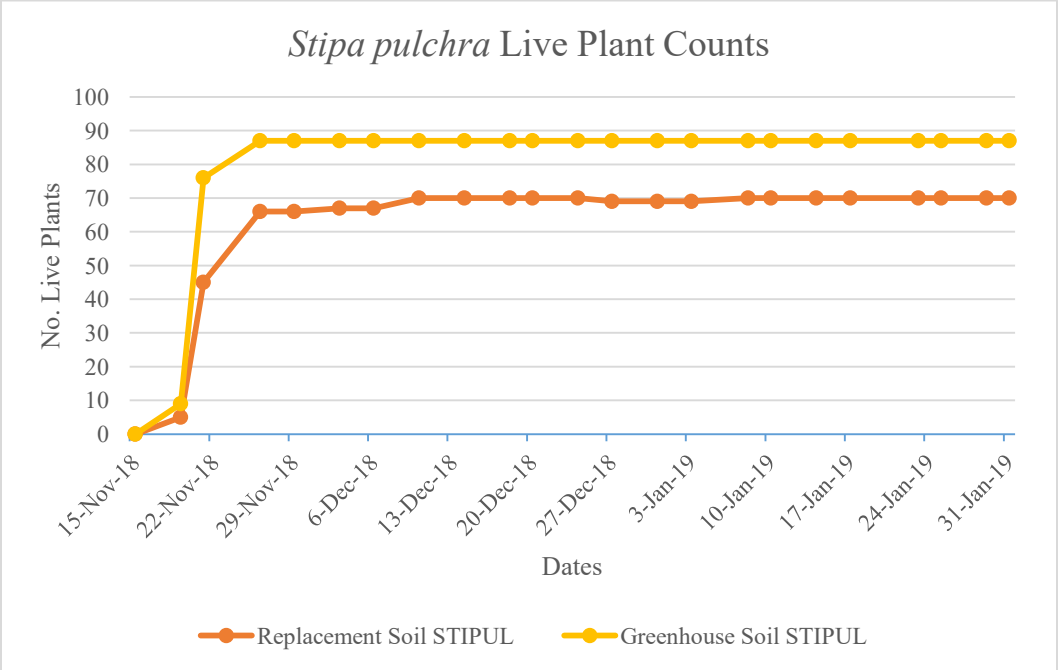

Figure 14. *Stipa pulchra* Live Plant Counts for the 11-week period.

A. glaber in both soil types maintained a very consistent number of seedlings, with greenhouse soil yielding many more germinants than replacement soil (Figure 15).

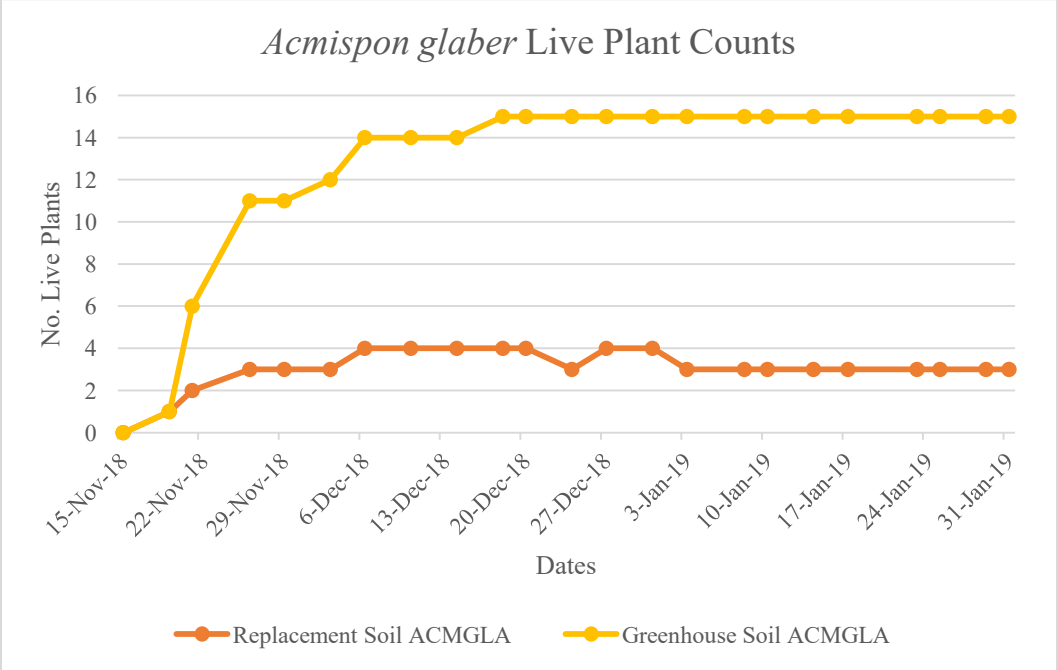

Figure 15. *Acmispon glaber* Live Plant Counts for the 11-week period.

E. fasciculatum seed in both soil types displayed good germination initially and then declined to a low number of surviving individuals by the end of the experiment (Figure 16).

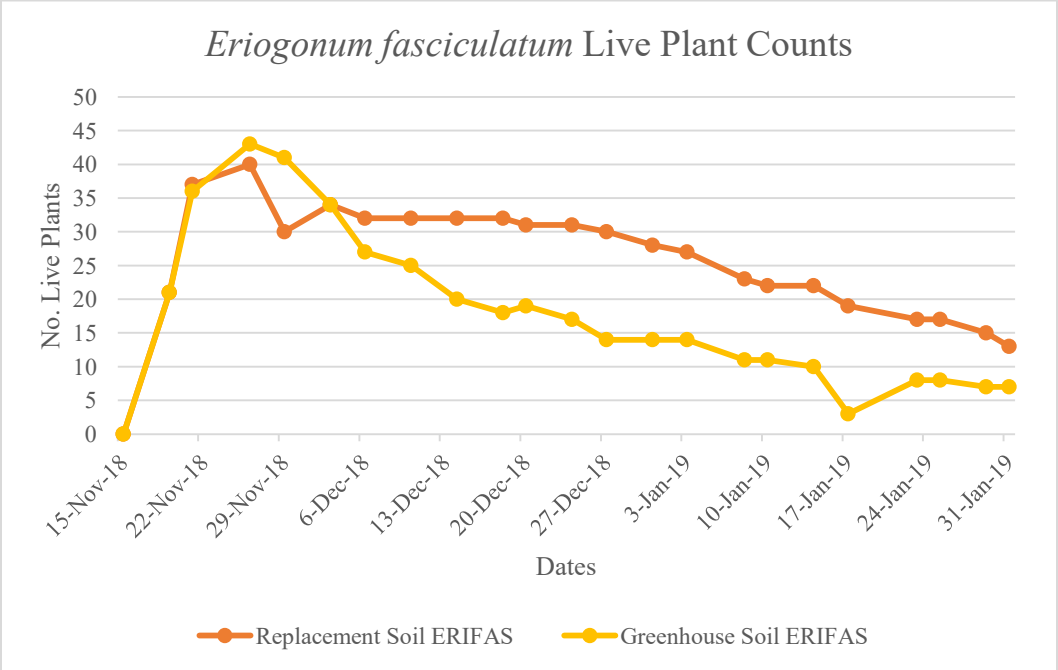

Figure 16. *Eriogonum fasciculatum* Live Plant Counts for the 11-week period.

Figures 17 through 20 compare the average final measurements made on the live plants at Week 11 of the trial. The averages were of maximum plant height, maximum leaf size, and quantity of leaves per individual. When examining plant height, eudicots such as *A. glaber, C. purpurea, E. fasciculatum,* and *S. apiana* were measured from the mouth of the cell to the highest point of the main plant stem. Monocots, including *S. bellum* and *S. pulchra*, were measured from the mouth of the plug to the tip of the tallest leaf blade. As seen in Figure 17, all six species developed into taller plants when grown in greenhouse soil when compared to those grown in replacement soil. The greatest difference in height was seen with *E. fasciculatum,* where the average plant height in replacement soil was 0.02cm tall while the average plant height in greenhouse soil was 17.57cm.

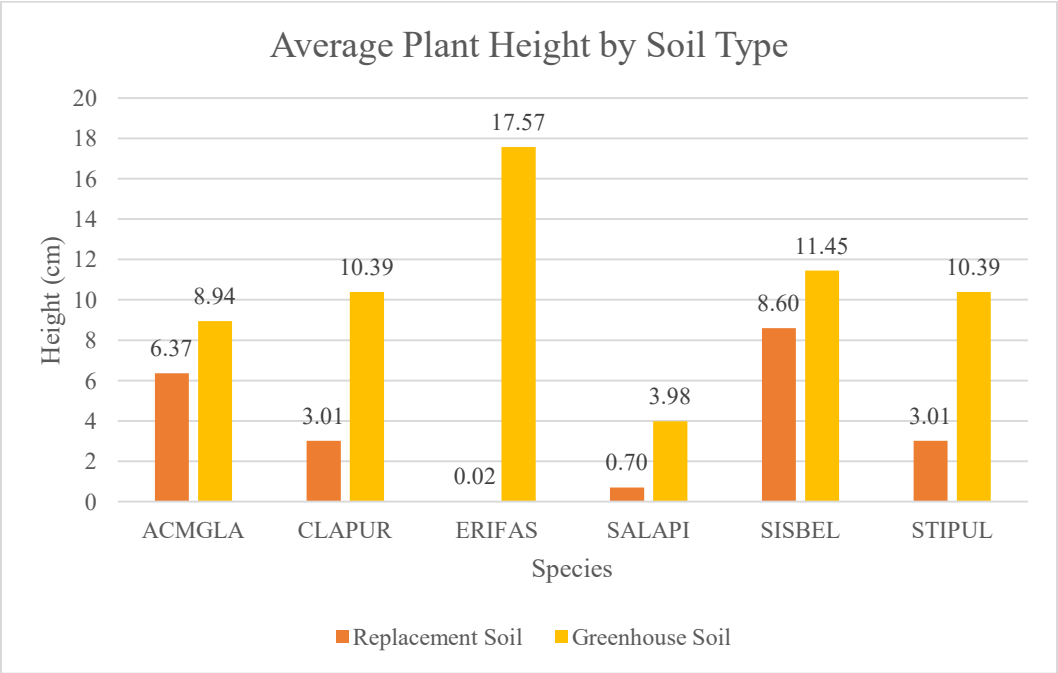

Figure 17. Average Plant Height by Soil Type

Figure 18 below compares the average number of leaves present or emerging on each plant species. Using the number of leaves as an indicator for the plant's development, the greenhouse soil produced plants that were more highly developed with more leaves than the plants that were grown in the replacement soil.

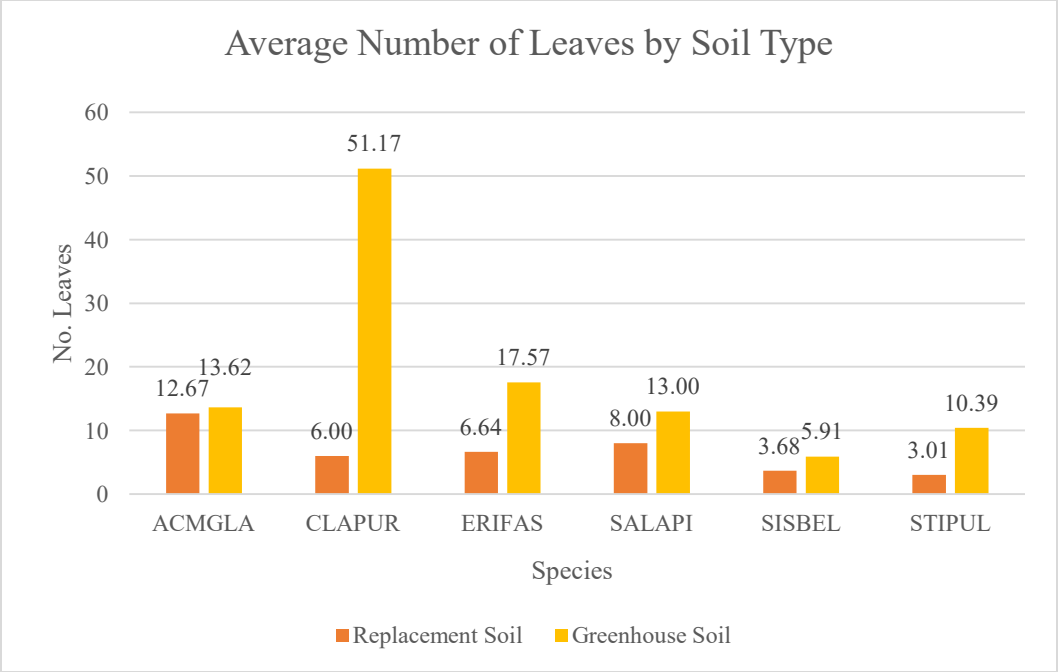

Figure 18. Average Number of Leaves by Soil Type

Figure 19 and 20 below display the average width and length, respectively, of the largest leaf found on each individual plant. Plants growing in greenhouse soil consistently produced larger, more developed leaves in both width and length than those growing in replacement soil.

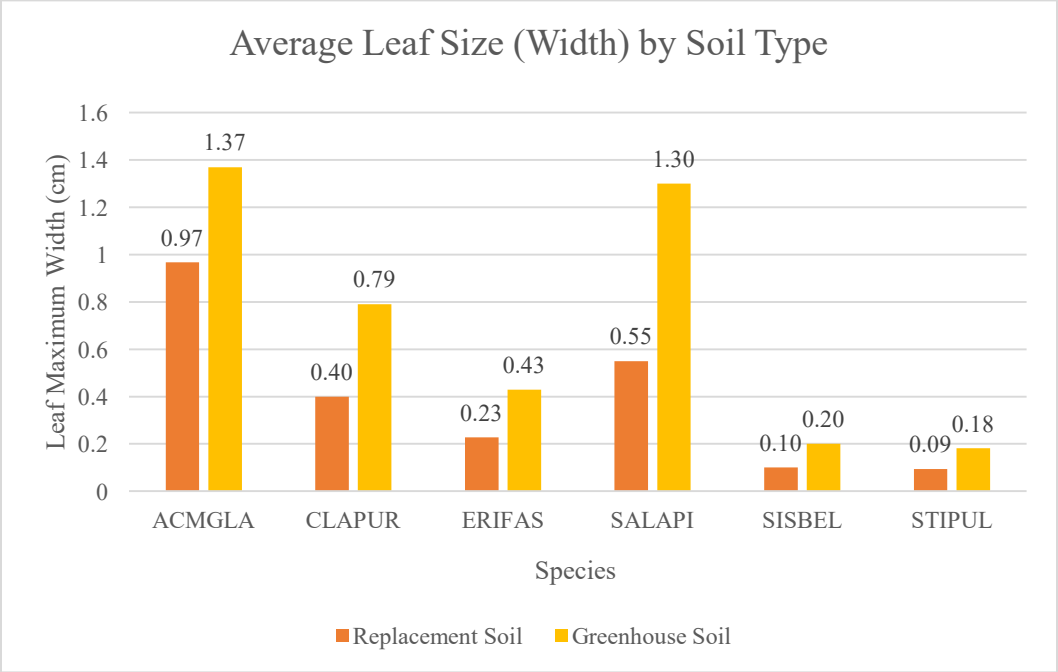

Figure 19. Average Leaf Size (Width) by Soil Type

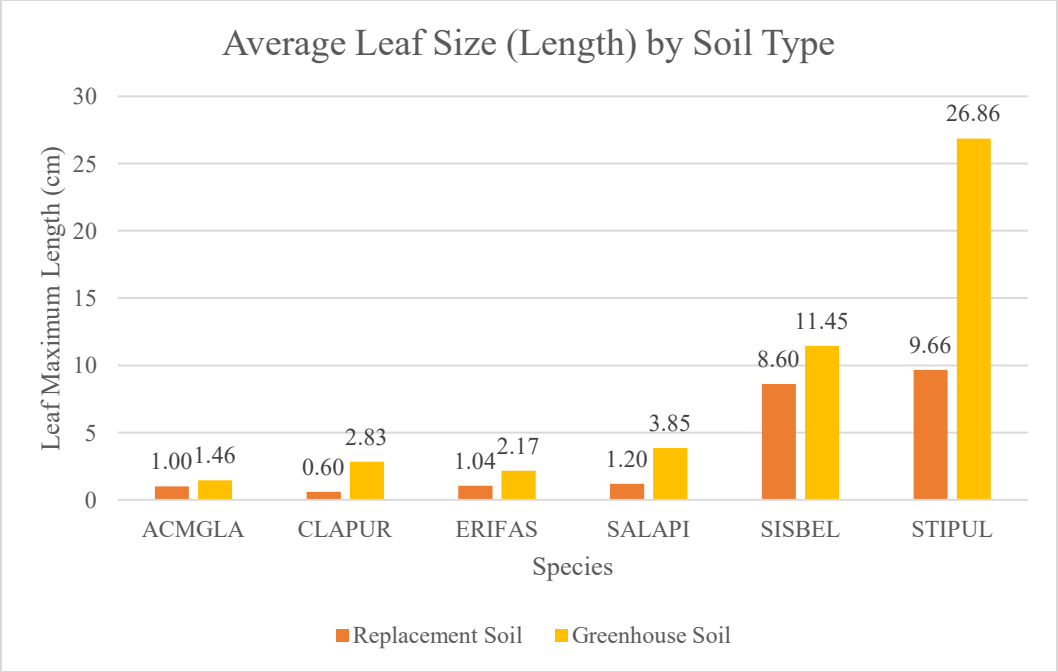

Figure 20. Average Leaf Size (Length) by Soil Type

CONCLUSIONS

Microbial Community Analysis

The fill material supplied for these experiments had extremely low organic matter content and harbored a microbial community that was very different from those of the reference soils from vegetated areas of SSFL. The inoculation of the fill material with suspensions of reference soil produced a transient pulse of respiration but no substantial growth or change of the microbial community. The respiration pulse was likely due to inputs of organic carbon in the soil suspensions. The fill material, whether inoculated with reference soil or not, had a microbial community that indicated contamination from the urban environment from which it was collected, such as body-associated and/or pathogenic groups. With the amendment of organic matter, this material might eventually support normal soil functions. However, the pathogenic potential of the material could be a cause for concern.

Greenhouse Trials

The greenhouse experiment was designed as a bench-scale study where many environmental variables such as temperature, humidity, moisture availability, and seed depth were held constant in order to isolate the effects of the two different soils on native seed germination and plant growth. It should be noted that conditions in the natural environment are very rarely optimal; native plants face a host of stresses in the natural environment including drought stress, temperature and humidity swings, and competition for nutrients and sunlight from other

plant species, both native and non-native. For these reasons, we would expect seed germination and development in the natural environment to be even lower than the results obtained in this greenhouse trial.

The germination percentages achieved for the six native plant species during the greenhouse trial were generally in line with what was expected given the Pure Live Seed (PLS) value calculated for each of the six respective seed lots when grown on germination paper. The exceptions were the two species *Acmispon glaber* and *Clarkia purpurea*, both of which displayed significantly lower germination percentages in replacement soil than in greenhouse soil.

In general, the greenhouse trials showed that native seeds could germinate in the replacement soils as well as, or in some cases, better than in the standard greenhouse potting mix. In particular, the two species *Salvia apiana* and *Sisyrinchium bellum* germinated in greater numbers in the replacement soil than in the greenhouse soil.

The differences between the replacement soil and greenhouse soil were much more clearly illustrated in the development and growth of the six native plant species as shown by the average measurements of the live plants at the end of the 11-week trial. In all six species and without exception, the average plant height, average number of leaves per plant, and average size (length and width) of the largest leaf was greater for the plants grown in greenhouse soil when compared to the plants grown the replacement soil (Figures 17-20).

The results of the greenhouse trials indicate that the currently available replacements soils for use at SSFL may be inadequate for the long-term growth and development of native plant species. One specific example that illustrates that the replacement soil was inadequate for supporting native plant growth and development can be found in the example of *Clarkia purpurea*, a seed lot which had a 99% Pure Live Seed value. As might be expected from seed with such a high PLS value, initial germination was very high for this species in both greenhouse soil and the replacement soil (Figure 13). However, as the trial progressed, many of the germinants of this species growing in the replacement soil began to die off after about 4 weeks, and by the end of the 11-week trial, very few seedlings remained alive in the replacement soil while the greenhouse soil supported 64 live seedlings through week 11 (Figure 21). Additionally, the native bunch grass, *Stipa pulchra* achieved similar germination percentages in both replacement soil and greenhouse soil (70 and 87 germinants, respectively) but the average size and overall development of the grasses grown in the greenhouse soil were significantly greater than those grown in the replacement soil (Figure 22).

Figure 21. *Clarkia purpurea* after 11 weeks (replacement soil left, greenhouse soil right)

Figure 22. *Stipa pulchra* after 11 weeks (replacement soil on left, greenhouse soil on right)

Together, these experiments show that the replacement soil is not an effective medium for growth of native plants or soil microbes. The very low organic matter content of the backfill material is the most obvious deficiency. Organic matter is necessary to sustain beneficial microbial communities, retain moisture and nutrients, and to build good soil structure for root growth and aeration. To bring the soil organic matter content of the replacement material to a level comparable to the reference soils, about 25 g of suitable organic matter per kg of soil would be required. Organic amendments would likely improve growth of plants and beneficial soil microorganisms in the proposed backfill material. However, the large differences between the soil microbial communities of the reference soils and the fill material, together with the presence of human and/or disease-associated bacterial taxa in the fill material, indicate that the starting microbial community is not ideal. If uncontaminated areas of the various native habitats at SSFL could be left in place while contaminated soils are removed and replaced, these “resource islands” could serve as both a native seed source and a source of inoculum. Furthermore, if suitable organic matter were added to the backfill material, the replacement soil would have a greater potential to eventually attain vegetation communities more typical of the native habitats currently occupying the SSFL and soil microbial communities similar to those found in undisturbed native soils not dominated by human and/or disease associated bacterial taxa.

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