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Artificial Soil Formation And Stabilization Of Material Cycles In Closed Ecological Systems For Mars Habitats

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ARTIFICIAL SOIL FORMATION AND STABILIZATION OF MATERIAL CYCLES
IN CLOSED ECOLOGICAL SYSTEMS FOR MARS HABITATS

by

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Bachelor of Science, North Dakota State University, 2012
Master of Science, University of North Dakota, 2014

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Submitted to the Graduate Faculty
of the
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in partial fulfillment of the requirements

for the degree of

Master of Science

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2014

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This thesis submitted by Joshua Borchardt in partial fulfillment of the requirements for the Degree of Master of Science from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.



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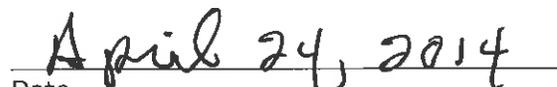


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This thesis is being submitted by the appointed advisory committee as having met all of the requirements of the School of Graduate Studies at the University of North Dakota and is hereby approved.



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Department Space Studies

Degree Master of Science

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ABSTRACT

Scientists are increasingly pressured to investigate novel ways in which to feed astronauts for the first mission to Mars in the 2030s. It is the aim of this thesis to conduct a preliminary investigation for soil formation of NASA JSC Mars-1A Regolith Simulant in an environmentally closed ecosystem to simulate plant growth within these initial habitats, and the prospect of soil formation from a Mars parent material for agricultural purposes. The rhizosphere and plant stress will be the main regions of research focus. It is hypothesized rhizosphere activity will determine the rate of stable soil formation adequate to support the agricultural needs of Mars's first human inhabitants. A *Brassica rapa* (Wisconsin FastPlant™) was grown on several different substrates, and evaluated for plant stress, elemental analysis, soil fertility, and mineralogical analysis to identify the biogeochemical factors related to areas inside and outside of the rhizosphere, which affect soil formation. In addition, multiple plant generations were grown to investigate bioavailability of nutrients within the system, and lay down preliminary approaches for mathematical model development in order to predict & evaluate future conditions and applications under reduced resource availability situations. Overall, the story of early soil formation from a Mars regolith simulant is further defined to aid in the success of our first human adventurers to the red planet.

CHAPTER I

INTRODUCTION

Mars Analogues on Earth

“The growing of crops, arguably, is the most important aspect of food production on Mars and will form the foundation of any self-sustaining colony.”

-Hender 2007

Initial crews to Mars will rely entirely on food transported to Mars, some may be supplemented with fresh grown produce, while the more permanent Martian settlements will have to rely on crop growth and sustainable greenhouses (Hender, 2007). The vast majority of successful Martian closed loop systems will depend on the photosynthetic ability of plants and microorganisms (Cockel, 1999). The composition of Martian soil used for crop growth will be a significant factor affecting plant growth as well (Hender, 2007), with much of the elemental abundance and mineralogy of the Martian lithosphere still not understood (Taylor, 2011). What is known, is that Martian soil is nutritionally low (as observed from Martian meteorites) in N and K, but higher in concentration of other micro and macro nutrients (Hender, 2007). In addition, material cycles on Mars will have significantly different flux rates and buffers, with different ratios of soil, biomass, atmosphere, and oceanic reservoirs (if any), that govern biogeochemical changes of carbon, nitrogen, oxygen, and others (Nelson et al., 2013). Hydroxides in Martian soil should reduce with the presence of water and not pose a significant threat to

crop production, and salts should be able to leach from substrate with an adequate water supply (Hender, 2007). The complete understanding of mineralogical mechanisms in Martian soil which would be present with the introduction of water are not well known in the rhizosphere and requires further study (Hender, 2007).

Higher plants in previous studies have provided up to 400 g dry weight/day of edible biomass from wheat, and roughly 40m² of plant growth area to supply the required 1000g O₂/day metabolized from one astronaut (Kehto et al., 2006). The plants we would use for the first settlement would fall into three broad categories: C3, C4, and crassulacean acid metabolism (CAM) (Hender, 2007). C3 plants, which include 17 of the 21 major food crops and encompass 85% of vascular plant species, react more highly with the presence of CO₂ (Hender, 2007). C4 plants encompass ~5% of Earth's flora and include major crops such as corn and sugar cane, and require less oxygen, nitrogen, and moisture than C3 plants, making them good candidates for initial Martian greenhouses (Hender, 2007). CAM plants fix CO₂ via the C4 process, then stored for a day and use the C3 process at night (Hender, 2007). However, adequate water can cause CAM plants to revert almost entirely back to the C3 process (Hender, 2007). The first Martian greenhouses could most efficiently grow crops that utilize an atmospheric pressure between 500 and 100mbar with around 10% CO₂, high N, and moderate O₂ levels (Hender, 2007). These early, stressful Martian conditions would utilize corn, millet, and sugar cane since they have a more conservative use of water (Hender, 2007).

Exposure to high levels of CO₂ (up to 10,000 ppm) during the initial phases of plant growth have been shown to increase plant size, more leaves and branches, more flowers and fruit, and a more widespread root system, but reduced the amount of O₂

production (Hender, 2007). This process is possible through the partial closing of stomata causing a reduction in moisture loss through transpiration (Hender, 2007). A CES on Mars would require a composition of at least 2% O₂ and less than 0.2% CO₂ (Lehto et al., 2006).

Surface Flow, subsurface flow, and aquatic plant wetlands are three types of wetlands used for wastewater treatment, where subsurface wetlands prove optimal for the surface of Mars (Nelson et al., 2003). These three wetland types utilize systems where water runs over the surface of a shallow substrate basin using emergent and floating wetland plants for mitigation, having water flow beneath a substrate (gravel) where emergent wetland plants are used, and aquatic systems where only floating plants are used (Nelson et al., 2003). Of these three, subsurface flow wetlands can treat about ten thousand gallons of wastewater per hectare, and also provide ample surface area for microbes and substrate filtration (Nelson et al., 2003). A system such as this would be beneficial on Mars as a complimentary source of wastewater treatment and food production.

However, traditional crops grown entirely in hydroponics have been observed to have 50% or more reduced root volume compared to crops grown on soil (Volk, 1996). This observation could support a soil based system instead for plants where the root is the main food source, or if soil organic matter (OM) is required to improve soil fertility for other purposes. In situ resource utilization of soils for crop growth are, in turn, more beneficial for planetary settlements, and provide a considerable safety margin and higher sustainability than hydroponics, which would be predominately used in off-planetary settlements and spacecraft (Nelson et al., 2008). Researchers at NASA JSC also found

that soil systems have a 50% higher productivity in sweet potato compared to hydroponics (Nelson et al., 2008).

Closed Ecosystems/Lessons from Biosphere(s)

A Closed Ecological Life Support System (CELSS) contains three main parts: a producer (crops), consumer (humans), and a decomposer (waste processing), with additional, and sometimes complex processes necessary to decompose some human wastes such as urine and other dissolved minerals (Volk, 1996). Decomposition processes can supplement mechanical components with the proper addition and maintenance microorganisms—including fungi, protozoa, and bacteria that can convert unused plant biomass into more usable forms, which can result in significant planting area savings (Volk, 1996). CESs on Mars will have significantly different compositions than on Earth, however. BIOS 2 had a 100:1 ratio between carbon in living biomass compared to atmospheric, and soil atmospheric carbon compared to atmospheric carbon of roughly 5000:1, whereas Earth's is 1:1 and 2:1 respectively (Nelson et al., 2013).

Various carbon management methods also exist, whether that be storing dried plant matter, mulching and returning matter to the soil (tilling), or the timing of other soil cultivation methods (Nelson et al., 2008). Under a stable climate, and in later stages of ecosystem development, soil Carbon models can assume a constant litter production and soil respiration where a steady state between inputs and outputs are reached (Guelland et al., 2012). However, an entirely closed system with 100% efficiency is not expected on any planetary settlement. An additional concern in small ecologically closed life support systems (ECLSS) is the build-up of trace gasses and atmospheric leakages, where the minimum percentage loss per day should be about 2% (roughly equivalent to the space

shuttle), with 1% being achieved in smaller laboratory biospheres, and about 10% leak rate in Bios 2 (Nelson et al., 2008). Complete closure of a CELSS is a goal, but not an operational necessity for laboratory research (Volk, 1996). In such a system, reduced temperature conditions would also require plants to increase their concentrations of photosynthetic enzymes and Nitrogen to balance other limiting factors like Carbon, water, and light (Hender, 2007). Higher temperatures can also cause inactivation of enzymes and photosynthetic pigments from increased photorespiration, and will require Martian greenhouses to have reliable temperature regulation (Hender, 2007).

Recycling consumables will be required for a long term manned mars mission, and should have well established research and procedures prior to going (Taraba et al., 2006). Urine can be used for ammonium additions to the soil by vapour-phase catalytic ammonium removal (VAPCAR), O₂ generation via static feed water electrolysis, and CO₂ reduction by the Sabatier process (Taraba et al., 2006).

Reduced pressures have shown no significant effect to protein concentration in soy bean, for example (Hender, 2007). The lower magnetic field on Mars should not have a significant effect on plant growth, especially under CO₂ rich conditions (Hender, 2007).

Because Mars has roughly 43% of the Sun light that reaches Earth (Taylor, 2011), and similar day/night cycles, that a controlled lighting of a Martian greenhouse for crops may not be necessary (Hender, 2007). The most significant aspect of a CELSS is how small of an area can be utilized for sufficient crop growth, which is largely dependent on the photosynthetic photon flux tolerance (PPF, light tolerance) of the crop (Volk, 1996). Dwarf wheat has proved to be a good example of a candidate crop where high PPF tolerance and high planting density has not sacrificed plant growth (Volk, 1996). The

biomass production efficiency has been shown to range from 45% for wheat, to 80% and above for lettuce and potatoes (Volk 1996).

Reduced light conditions coupled with increased CO₂ concentrations have also been shown to increase crop yield and photosynthetic response (Hender, 2007). A CO₂ increase of 300ppm above Earth atmospheric levels and optimum water conditions have increased plant growth by up to 31%, and up to 63% under less than optimal water conditions (Hender, 2007; Idso, 1994).

Increasing CO₂ levels in an enclosed greenhouse can have significant plant growth benefits, especially under conditions of nutrient poor soils and reduced pressures (Hender 2007; Idso 1994). Sufficient nutrients and a 300ppm increase in CO₂ resulted in plant growth increase by 51% compared to an increased plant growth of 45% under nutrient poor conditions, and visa versa when CO₂ levels were 600 ppm above normal (Hender, 2007; Idso, 1994). Most notably, when CO₂ levels were increased by 1200 ppm above atmospheric conditions, a 60% increase in plant growth was observed under sufficient nutrient conditions, and a plant growth increase of 207% under poor nutrient conditions (Hender, 2007; Idso, 1994).

CO₂ concentrations for some important agricultural crops that could be grown on Mars are: rice between 1500 – 2000 ppm, algae between 10,000-15,000 ppm. A doubling of normal Earth atmospheric CO₂ can also increase yield in tomatoes, lettuce, cucumbers, and other vegetable by as much as 20 – 50% (Hender, 2007). C₃ crops (rice, wheat, barley, oats) have also shown a 25-65% increased yield with increased CO₂. C₄ crops increased yields from 10 – 55%, and root/tuber crops up to 18-75% increased yield with increased CO₂. Legumes showed an increase of 28-46% with increased CO₂, which

resulted with increased biological nitrogen fixation (Hender, 2007). Increasing ambient temperatures from 20 degrees C to 27-28 degrees C have also benefited crop plant photosynthesis and yield (Hender, 2007).

Radiation levels must also be considered for a source of plant stress. Within these Martian greenhouses, the effect on plant life chloroplast inhibition and percent plant damage ranges from ~22% - 78% (Cockell, 1999). The characterization of the surface radiation must be taken into consideration when designing and implementing a Martian greenhouse.

Radiation in a CES on the Martian surface can be reduced by using building materials that entirely, or partially block UV radiation. Though, as stated before, several types of organisms rely on UV radiation, and the total effect of a system lit by LEDs was not used in this study.

Biospheres 2 & 3

Biosphere 2 (BIOS 2) had a 1.2 hectare (3.14 acre) footprint, 180,000m³ atmosphere, a sustainable high yield agricultural soil-based system, and several considerable engineering challenges (Allen, 2003). With a leak rate of ~10% a year, special consideration was taken towards mitigating harmful buildup of toxic trace gases such as N₂O, CO₂, CO, NO_x, Hydrogen Sulfide, Ethylene, Methane, and Ozone (Allen, 2003).

BIOS 3 used a plant conveyer-like system which maintained roughly the same amount of vegetation biomass in the system to regulate CO₂ levels between 350 and 1700 ppm, which affect plant photosynthesis rates and mitigate toxic CO₂ build-up for humans (Nelson et al., 2008).

BIOS 2 was also able to provide all vitamins (except Vitamin D), amines, enzymes, proteins, fats, and carbohydrates to its inhabitants (Allen, 2003). BIOS 2 did have some agricultural problems with high salinity in one of the plots, and denitrification in the rice paddies (Allen, 2003). However, the two years of soil management inside the BIOS 2 system had maintained an acceptable level of soil fertility, not limited by crop production (Allen 2003; Silverstone et al., 1999).

Though previous studies have shown an optimal crop surface area of around 60m² per person, BIOS 2 allocated 279 m² per person (Hender, 2007).

BIOS 2 also showed a progressive rise in nitrous oxide throughout its roughly three year experiment, and could have used catalytic converters to mitigate the problem, similar to BIOS 3 (Nelson et al., 2008). However, soil bed reactors were affectively demonstrated at regulating other trace gasses such as propane, ethylene, ethane, and methane (Nelson et al., 2008).

BIOS 2 had the first surface-flow wetland wastewater treatment system in a closed ecological system (CES), which achieved a total recycle of wastewater and produced fodder for domestic animals (Nelson et al., 2003). A wetland wastewater treatment organization, Wastewater GardensTM, calculates that a 3-4m² surface area of specially designed surface-flow wetland treatment system is sufficient at removing up to 90% of biochemical oxygen demand (organic compounds that bind up oxygen), up to 80% of P and N, and reduce 99.8% of fecal coliform bacteria without the use of external chemicals (Nelson et al., 2003). In warm weather conditions and high biodiversity, subsurface wetland ecosystems show an increased rate in uptake and higher overall efficiency (Nelson et al., 2003). Measurements from Wastewater GardensTM showed an

increased uptake and treatment efficiency in warm weather vs colder weather climates, proving suitable for greenhouse environments on Mars (though the impact on these systems from the oxidative reactivity of Martian soil is less certain) (Nelson et al., 2003). These systems can also benefit crop growth in Mars greenhouses by providing nutrient rich media to help maintain soil fertility, and supplement some foods which can be grown on the wetland treatment system, such as bananas and rice (Nelson et al., 2003). Wetland plants, with their high transpiration rates, also supplied BIOS 2 with potable water from the wetland's condensation (Nelson et al., 2003). Breathable air was also supplied by cycling it through the soil, where BIOS 3 used a combination of plants and thermocatalytic filters (Volk, 1996).

Soil Formation

Soil structure is defined as the arrangement of pores and particles within a soil, and structural stability influenced by water content and other stress inducing agents added or subtracted to the substrate (Oades, 1993). As observed from retreating glaciers, the chronosequence of soil formation and weathering from increased colonization of plants and microbes have shown to be dependent on Carbon accumulation and turnover, with rates of Carbon accumulation greatest during the initial phase of soil formation (Guelland et al., 2012). Therefore weakly-developed soils could be used as Carbon sinks from atmospheric CO₂ (Guelland et al., 2012). Soil biota also have a significant impact on soil structure, from soil microorganisms to rodents and trees, with soil formation preceding soil stabilization (Oades, 1993). Soil organic matter mineralization by heterotrophs and rhizosphere respiration govern multiple components of total soil respiration in a system (Guelland et al., 2012). One of the major contributors to soil respiration from mature

grasslands and forests is the quick allocation (within a few days) of atmospheric carbon to the rhizosphere (Guelland et al., 2012). Likely, the most important mechanism for soil organic matter stabilization comes from sorption onto mineral surfaces from dissolved organic carbon (DOC) leached from soil (Guelland et al., 2012). Dissolved organic compounds also play an important role in the weathering process by decomposing organic matter, a key characteristic of soil quality, into compounds which accelerate mineral weathering in the rhizosphere of plants (Naderizadeh, 2010). This aspect of soil stabilization has scarcely been studied in initial soils (Guelland et al., 2012). Sorption to secondary oxides of Fe and Al is the major retention process for DOC in mineral soil, and will change over time and with soil maturity (Guelland et al., 2012).

One of the features of Martian soil is the lack of organic matter, though that is not necessarily a limiting factor (Hender, 2007). However, with the addition of organic matter, K, and N, crop growth on Martian soils appears to be feasible based on Apollo era organic matter devoid plant growth experiments (Hender, 2007). The small grain size of martial regolith lends itself to the possibility of becoming compacted and clay like with the addition of water (Hender, 2007). However, the addition of sands, gravel, or organic waste can increase the porosity of the Martian soil to help promote optimal plant growth (Hender, 2007). A balance of biological and physical factors require quantification so a management procedure can be produced for optimum soil structure for crop production (Oades, 1993).

Organic Matter Accumulation

Potassium (K) is an essential element for physiological processes involved with plant nutrition, with the four types of K: soluble, non-exchangeable, exchangeable, and

structural forms being affected by clay mineralogy, and thus, nutrient availability for the plant (Naderizadeh, 2010). Plants are also able to promote the release of K from illite and during the formation process of vermiculite layers (Naderizadeh, 2010).

Mineralogical changes can occur from K uptake in the rhizosphere, which decreases K concentration in the soil (Naderizadeh, 2010). Soil organic matter effects the biological, chemical, and physical properties of a substrate and its ability to resist degradation (Naderizadeh, 2010). Dryland soils require a regular addition of organic matter, as the organic matter content decreases more rapidly, and to a lower percentage than more humid soil types (Naderizadeh, 2010).

Heavy metals usually have a low mobility in soils which are not easily absorbed by most plants unless identified as a “hyperaccumulator,” which are often used in phytoremediation, of which the rhizosphere processes involved are still unclear (Tingqiang, 2011). One of the most important factors in phytotoxicity from heavy metals in soils is the amount of dissolved organic matter (Tingqiang, 2011). Some plants will release organic acids to take up Fe, especially under Fe deficient substrates, and will in turn use dissolved organic matter in the rhizosphere to help mobilize inorganic P (Tingqiang, 2011).

Compost/Artificial Soil Formation

Edmonton, Alberta, Canada, has the largest compost facility in North America, producing ~60,000 tons of compost, solid waste, and biosolids (cocompost), which can be used on agricultural lands, with horticulture, and home gardens (Zhang et al., 2006). Microbes are also able to break down human waste, crop waste, and metabolize trace toxic gasses (Silverstone, 2005).

Nitrogen is the limiting factor for crop growth in most soils, and leaching of N from cocomposits have been observed by several researchers, with N becoming available as early as four months after cocompost application, and decreases until twelve months after application at 25 degrees C (Zhang et al., 2006). Creating a stable Martian soil will minimize crew expenditures and maximize waste recycling back into the system (Silerstone, 2005).

Microbial Interaction

Organic acids from some soil organisms are able to solubilize unavailable forms of K-bearing minerals to bring K into solution (Naderizadeh, 2010). Several studies have also shown increased weathering rates of K in substrates inoculated with nonsymbiotic microflora (Naderizadeh, 2010).

Revegetation of native plants in arid, previously cultivated soils, such as in the American South West, use dry seeding, mulching, chiseling, imprinting, and fertilizer where mycorrhizal fungi became reduced (Banerjee, 2006).

Heterotrophic microbes using ancient Carbon for metabolism have recently been shown to colonize glacial forefields (recently ice-freed soils) prior to autotroph colonization (Guelland et al., 2012). These initial ancient Carbon sources can be from parent material, Aeolian inputs, or organic Carbon resulting from cryoconite holes and cyanobacteria (Guelland et al., 2012). Only in later stages of soil development does accumulated soil organic matter become significant for heterotrophs (Guelland et al., 2012). The resulting dissolved organic carbon (DOC) is a leached product of soil plant components generated by microbial activity (Guelland et al., 2012).

Importing Nitrogen fixing organisms, such as cyanobacteria could become significant sources for dinitrogen fixation in addition to the Sabatier process (Hender, 2007).

Research Hypothesis

Based on the above findings, a test of the stability for the artificial ecological succession to increase soil formation processes will be tested in a partially closed ecosystem. In addition, a rapid growing plant will be used to address the hypothesis that it can indicate and help develop Martian regolith soil fertility of man-made systems. The development of this system will be used to compare soil formation processes of other systems, specifically near the rhizosphere.

CHAPTER II
METHODOLOGY
Experimental Design

One treatment each of JSC Mars-1A Regolith Simulant, Arcillite (Schultz Aquatic Plant Soil™), and Potting Soil mixture (Schultz Professional Potting Soil Plus™), were placed in 6 in. diameter PVC pots in a partially closed ecosystem (CES) under a partially sealed bell jar, with a HAAKE 10^C cooling coil to regulate temperature near 20^C, regulate relative humidity near 35%, and used for multiple plant generations (Figure 1).



Figure 1. Closed ecosystem (CES) chambers and experiment area.

A rhizosphere column modified from Kissoon et al. 2010 (Figure 2) of 3 in. diameter using 37 micron screen were placed in each treatment to separate plant roots radially from the surrounding media (Figure 3). Three plant generations were grown in total: one outside their respective ECS, and two within an ECS. Plants were grown under two 40 in., 40 watt, white-florescent light bulbs for the entire duration, except the first generation which used halogen light bulbs unsuccessfully.

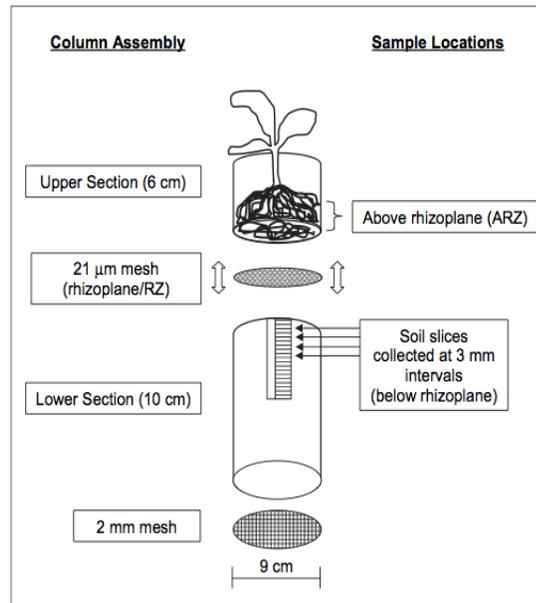


Figure 2. Vertical rhizosphere column assembly. (Kissoon et al., 2010)

The inside of each rhizosphere column (3 in total) had 12 *Brassica rapa* (Wisconsin FastPlant™) seeds which germinated in situ and utilize a 24hr light cycle to minimize eventual planting area by their efficient use of photons (Volk, 1996).

Once the seeds germinated, a total of 10 were saved in each type to grow until flowering, which was when each plant generation was terminated. Tap water was added if needed to maintain adequate hydration of the root zone once every 2-3 days for outside the ECS, and watered inside the rhizosphere column only. Tap water was also added

once half-way through each plant generation inside the ECS, and inside the rhizosphere column only.



Figure 3. Lateral rhizosphere column assembly used in experiment. (Kissoon, n.d.)

Plants were harvested at the flowering stage. Cotyledons, first, second, and third true leaves, flowers, and number of flowers were recorded per treatment type, dried overnight at 170^F, and had their dry weights recorded. Plants were then crushed and re-mixed into the top 1in. of each treatment type. The experiment lasted for a total of three months. All analysis was conducted on Microsoft Excel with subsequent sampling locations represented in Figure 4.

Reflectance Spectroscopy

A reflectance spectrometer (FieldSpec Pro, Analytical Spectral Devices, Inc.) was used to record plant stress on the cotyledon of each plant in each treatment at the flowering stage, for each generation inside of the CES. The spectrometer was placed on each cotyledon for 10 seconds below a 100watt incandescent light bulb, at a spectra setting of 10, a dark setting of 25, and a white reference of 10 (manufacturer

recommendations) prior to saving the spectrograph. No spectrograph was taken if no cotyledons were present. All spectra were averaged for each generation used for analysis.

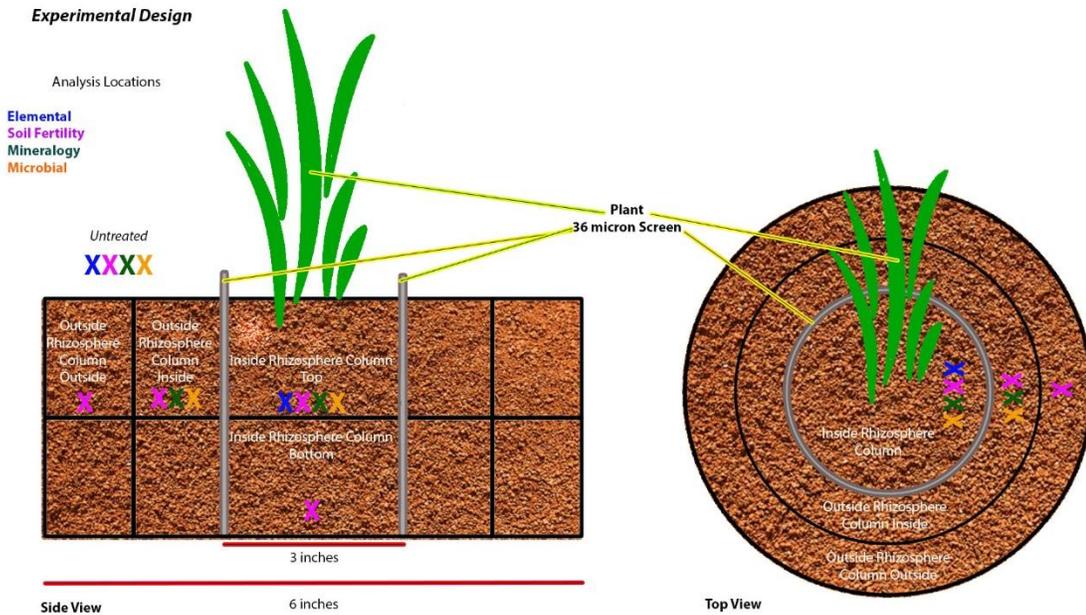


Figure 4. Experimental design and sampling areas.

Soil Fertility

Complete soil fertility analysis was undertaken by AgVise Laboratories (Northwood, ND), a commercial agricultural analysis company. Analysis was conducted from five samples per each of the three substrate types. An untreated sample was provided, as well as two samples inside of the rhizosphere column: a top half and bottom half sample, and two samples from outside the rhizosphere column; the inside half closest to rhizosphere column, and the outside half farthest away. Outside rhizosphere column samples were only taken from the top 1in. of the substrate. Several potting soil sample masses were below the analysis threshold, therefore the two inside rhizosphere samples were grouped together, as well as the two outside rhizosphere samples. Agvise Laboratories analyzed for: pH, organic matter percent (OM%), Salts, Nitrates, P, K, Ca,

Mg, Na, S, Cl, cation exchange capacity (CEC), Calcium carbonates equivalent (CCE), and bulk density. Each measurement was recorded once due to low mass required for triplicate. Therefore, no averages or standard deviations are present with these results.

Soil Elemental Analysis

Elemental analysis was undertaken by the Wet Ecosystem Research Group (WERG) at North Dakota State University (NDSU, Fargo, ND) via a Spectro Genesis SOP Inductively Coupled Plasma – Optimal Emissions Spectrophotometer (ICP-OES). One rhizosphere sample and one untreated sample from each substrate after the final plant generation were analyzed in triplicate for the following elements: Ag, Al, As, B, Ba, Be, Ca, Cd, Ce, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Se, Si, Sn, Sr, Ti, Tl, V, Zn. All samples were randomized for both microwave digestion and ICP analysis. Method detection limit (MDL) calculated by $3 \times \text{stdev} (\text{calibration water blank}) \times \text{extraction volume} \times \text{dilution factor} / \text{dry weight}$. Certified reference material (CRM) (Soil Standard 140-025-001) was also digested for analysis of recovery of elements for nitric acid digestion method. The weight of samples was approximately 500 mg. Several samples were diluted 50 times for Al and Ti.

The final plant generation for each treatment was found to have a cumulative mass bellow method detection limits for the ICP-OES, therefor no elemental analysis was able to be performed on these plant samples.

Soil Mineralogy

Samples that were untreated, inside the rhizosphere column, and outside the rhizosphere column from each of the three substrates were ground in an agate mortar until the resulting powder passed through a 120 μm sieve. Sample powders were

analyzed using an Olympus BTX benchtop X-ray diffraction (XRD) system. The BTX is a transmission XRD instrument measuring Co K α from 5 to 55° 2 θ . During analysis about 50 mg of sample powder was loaded into BTX sample cell equipped with mylar windows. Data was collected for 250 exposures (~80 minute run time). During operation, X-rays pass through the sample powder as it is vibrated within the cell, minimizing preferred orientation of mineral grains. 2-dimensional diffraction patterns were collected by a charged couple device through the entire range of 2 θ during analysis. 2-dimensional XRD patterns were converted into one-dimensional patterns for mineralogical interpretation with MDI Jade analytical software by smoothing patterns and searching the ICDD database for mineral constituents.

Soil Microbiology

Substrate samples for microbial analysis were sent to the Biotechnology and Planetary Protection Group at the Jet Propulsion Laboratory for analysis. Each substrate had samples collected from inside the rhizosphere column, outside of the rhizosphere column, and untreated substrate.

Tag Encoded FLX Amplicon Pyrosequencing (TEFAP) Analysis

Bacterial specific primers 28F (5'-GAG TTT GAT CNT GGC TCA G-3') and 519R (5'-GTN TTA CNG CGG CKG CTG-3') were used to amplify ~500 bp fragments spanning the V1 – V3 hypervariable regions of the bacterial 16S rRNA gene. This primer pair was tailored for TEFAP by adding a fusion linker and a proprietary 8 base-pair barcode sequence at the 5' end of the forward primer, and a biotin and fusion linker sequence at the 5' end of the reverse primer (19). A HotStarTaq Plus master mix kit (QIAGEN) was used to catalyze the PCR under the following thermal cycling conditions: initial

denaturing at 95 °C for 5 min, followed by 35 cycles of denaturing at 95 °C for 30 s, annealing at 54 °C for 40 s, and extension at 72 °C for 1 min, finalized by a 10-minute elongation at 72 °C. Resulting PCR products were purified via Diffinity Rapid Tip (Diffinity Genomics, Inc, West Henrietta, NY) chemistry, and were then pooled accordingly. Small fragments were removed with Agencourt Ampure Beads (Beckman Coulter, Brea, CA).

In preparation for FLX-Titanium sequencing (Roche, Nutley, NJ), resulting PCR amplicon fragment size and concentration were accurately measured with DNA chips using an Experion automated electrophoresis station (Bio-Rad, Hercules, CA) and a TBS-380 Fluorometer (Turner Biosystems, Sunnyvale, CA). All TEFAP procedures were performed at the Research and Testing Laboratory (Lubbock, TX) in accordance with well-established protocols (19).

CHAPTER III

DATA/RESULTS

ICP-OES Elemental Analysis

Rhizosphere samples from Regolith, Arcillite, and Soil after three plant generations of growth were compared to their untreated counterparts for all elements. The elements that showed a significant difference in concentration (mg/kg, $p < .05$) were then assessed by the percent difference in concentration, with up to 100% total change (Figures 5, 6, & 7).

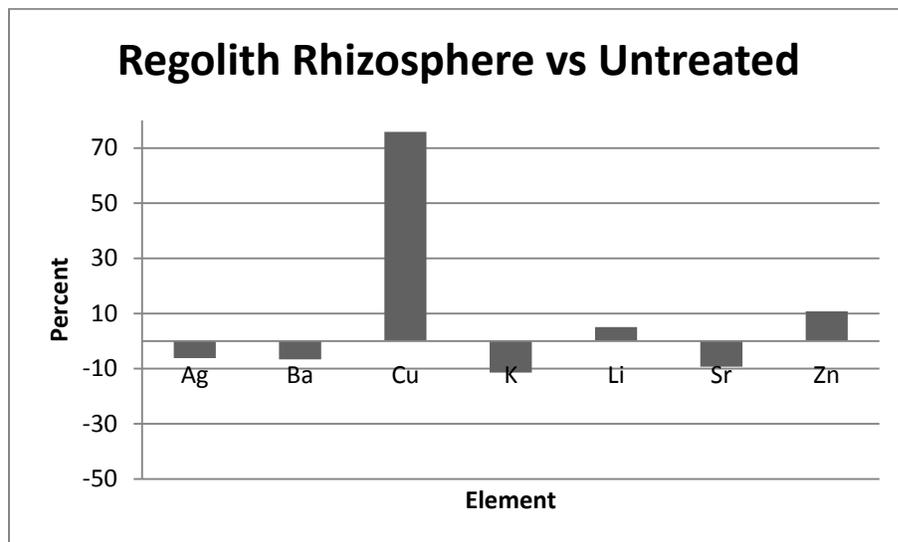


Figure 5. Comparing the percent of change of elemental concentration between JSC Mars Regolith Simulant within the rhizosphere column after the 3rd plant generation, and an untreated JSC Mars Regolith sample.

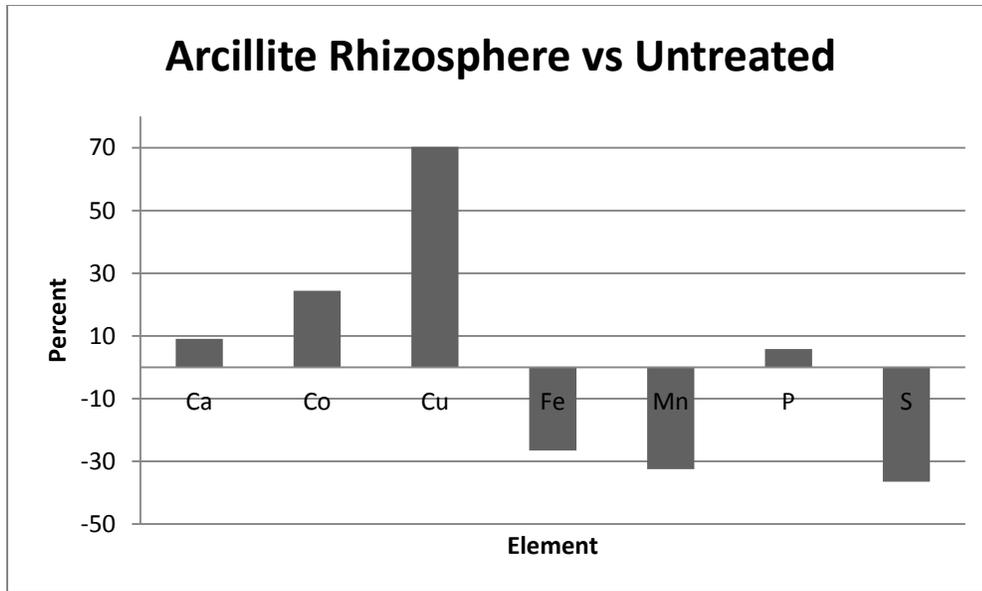


Figure 6. Comparing the percent of change of elemental concentration between Arcillite within the rhizosphere column after the 3rd plant generation, and an untreated Arcillite sample.

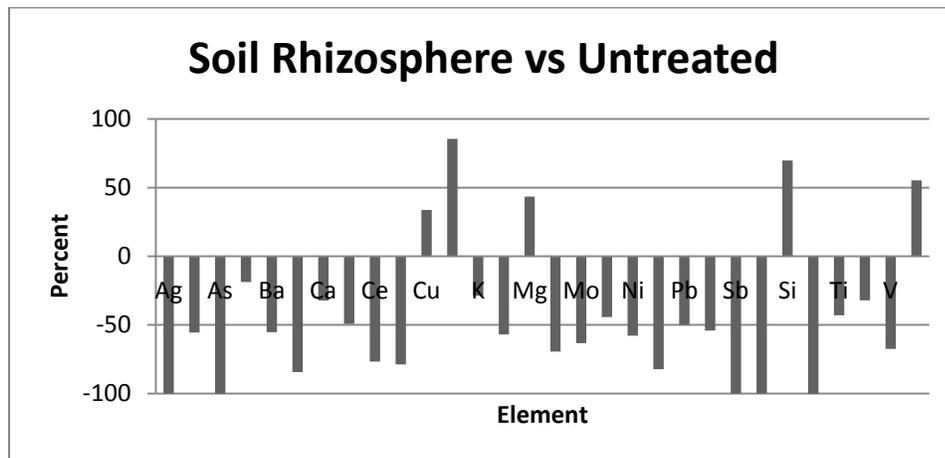


Figure 7. Comparing the percent of change of elemental concentration between the 3rd plant generation Potting Soil within the rhizosphere column, and an untreated Potting Soil sample.

A result showing a negative percentage indicates the primary sample is that much lower in concentration compared to the secondary sample, whereas a positive percentage

results in the primary sample having that much more of an elemental concentration than the untreated sample

A net increase in concentration of Cu, Ti, and Zn within the JSC Mars regolith simulant rhizosphere, with a net decrease in concentration of Ag, Ba, K, and Sr compared to the untreated sample was observed (Figure 5). The results from Figure 6 indicate a net increase in Ca, Co, Cu, and P, and a net decrease in Fe, Mn, and S within the Arcillite rhizosphere compared to the untreated sample. Figure 7 indicates a net increase in concentration of Cu, Fe, Mg, Si, and Zn within the Soil rhizosphere, with a net decrease in concentration of Ag, Al, As, B, Ba, Be, Ca, Cd, Ce, Co, K, Li, Mn, Mo, Na, Ni, P, Pb, S, Sb, Se, Sn, Ti, Tl, V compared to an untreated sample. Another comparison was completed between the significantly different element concentrations within the rhizosphere treated samples of JSC Mars regolith simulant and Arcillite, and JSC Mars regolith simulant and potting soil. Each figure compares the differences in elemental concentrations of the rhizosphere columns after the elements were compared for significant differences ($p < .05$). All other elements were not found to have a significant difference in concentration ($p > .05$). A significant increase in concentration of Ag, Al, Be, Ca, Cd, Ce, Co, Cu, Fe, Mg, Mn, Na, Ni, P, Pb, Sb, Sn, Sr, Ti, Tl, V, Zn in the regolith rhizosphere compared to the arcillite rhizosphere was observed (Figure 8), with decreased concentrations of As, B, Cr, K, Li, and S in the regolith rhizosphere compared to the arcillite rhizosphere.

Figure 9 indicates a significant increase in concentration of Ag, Al, Ba, Be, Cd, Ce, Co, Cr, Fe, Li, Mg, Mn, Na, Ni, P, Pb, Sb, Se, Sn, Sr, Ti, Tl, V, and Zn within the Regolith rhizosphere compared to the soil rhizosphere, and a decreased concentration of B, Ca, K, S, and Si within the regolith rhizosphere compared to the soil rhizosphere samples.

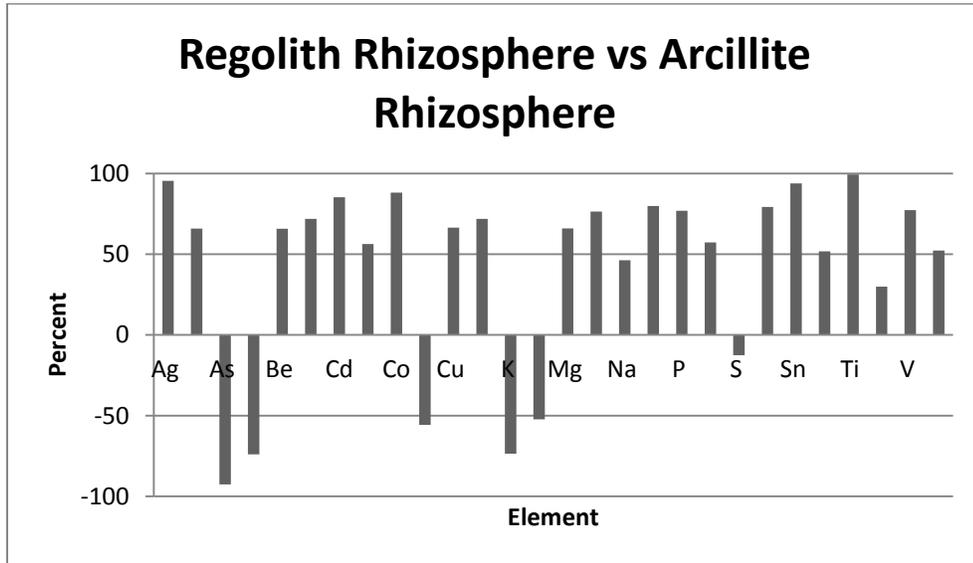


Figure 8. Comparison between the rhizosphere samples of JSC Mars Regolith Simulant and Arcillite of the 3rd plant generation.

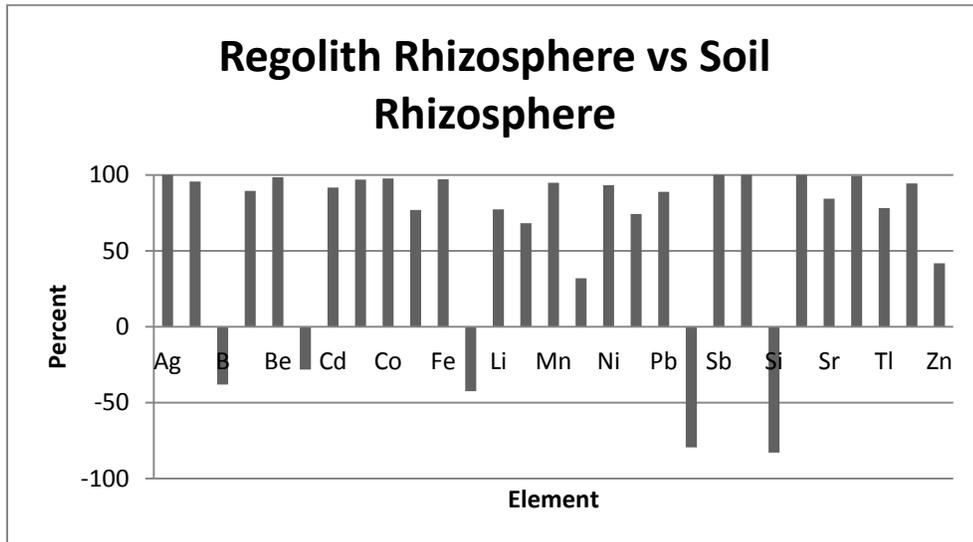


Figure 9. Comparison between the rhizosphere samples of JSC Mars Regolith Simulant and Soil of the 3rd plant generation.

Several significant elemental differences between each treatment is evident in Figures 5 – 9, with each of these treatments having changed in significantly different ways, with the common increase of Cu within the rhizosphere of all treatments (attributed to the copper cooling coil). Regolith samples and soil samples both decreased in Ag, Ba, and K, while both increasing in Cu and Zn. However, Regolith samples increased in Ti concentrations while soil samples decreased in Ti concentrations (Figures 5 & 7). Arcillite and soil samples both decreased in Mn and S concentrations. Arcillite also increased in Ca, Co, and P compared to soil having a decrease in those elements, while Soil samples showed an increase in Fe, arcillite decreased in Fe (Figures 6 & 7). Regolith rhizosphere samples had increased concentrations of Ag, Al, Be, Cd, Ce, Co, Fe, Mg, Mn, Na, Ni, P, Pb, Sn, Sr, Ti, Tl, V and Zn, and a decreased concentration of B, K, and S compared to the Arcillite and Soil Rhizospheres (Figures 8 & 9). Regolith had an increased concentration of Ca compared to Arcillite, but a decreased concentration compared to Soil. Regolith also had a decreased concentration of Cr and Li compared to Arcillite, and an increased concentration of those elements in Soil. In addition, there were no significant differences between As and Cu concentrations between the Regolith and Soil rhizosphere samples, or Ba concentrations between the Regolith and Arcillite samples.

AgVise Soil Fertility

Soil fertility analysis was conducted in four different regions of the experimental setup rhizosphere column after three plant generations of growth, from three different substrates and their untreated counterparts. These comparisons were analyzed on the percentage difference from the largest concentration (identical to elemental analysis).

Therefore, a positive number indicates that measurement was that percentage larger than its comparison, and a negative number indicates that measurement was that percentage smaller than its comparison, out of a 100% scale.

Values from Table 1 indicate the soil fertility of each of the three substrates. All soils were slightly acidic to neutral, and ranged in CEC from loam to loamy sand. Untreated soil, and soil from outside of the rhizosphere, tended to have the highest concentrations of all measured variables, while regolith and arcillite substrates tended to have lower overall concentrations. Regolith pH values spanned all three ranges, while arcillite remained acidic for the entire duration.

Table 1. Indications of soil fertility (AgVise Laboratories) after the third plant generation.

SOIL CHARACTERISTIC	VERY LOW	LOW	MEDIUM	HIGH	VERY HIGH
Nitrate-Nitrogen lbs/acre	0-37 Ri, Ru, Ai, Ao, Au, Si	38-74 Ro	75 – 111	> 111 So, Su	
Phosphorus (P) mg/kg	1-5 Ro, Ru	6-10 Ri, Ai, Au, Si	11-15 Ao	16-20	> 20 So, Su
Potassium (K) Mg/kg	1-40	41-80	81-120 Si	121-160	> 160 Ri, Ro, Ru, Ai, Ao, Au, So, Su
Chloride (Cl) lbs/acre	1-15 Ri, Ru	16-30 Ai	31-45	46-60	> 60 Ro, Ao, So
Sulfur (S) lbs/acre	<1-25 Ru	26-59	60-120	> 120 Ri, Ro	

Table 1 (cont.)

SOIL CHARACTERISTIC	VERY LOW	LOW	MEDIUM	HIGH	VERY HIGH
Magnesium (Mg) mg/kg		0-50	51-100	> 100	Ri, Ro, Ru, Ai, Ao, Au, Si, So, Su
Calcium (Ca) mg/kg	1-250	251-500	501-2,000	2,001-4,500	> 4,500
Sodium (Na) mg/kg	1-40	41-80	81-120	121-160	> 160
Organic Matter (OM) %	.1-1.5	1.6-3.0	3.1-4.5	4.6-6.0	> 6.0
Soluble Salts mmhos/cm	.01 - .25	.26 - .50	.51-.75	.76-2.0	>2.0
Soil pH (pH)		> 7.0 = Basic, <7.0 = Acidic, =7 = Neutral			
Cation Exchange Capacity (CEC)	Sand = 1-8, Loamy Sand = 9-12, Sandy/Silty Loam = 13-20, Loam = 21-28, Clay Loam = 29-40, Clay/peat > 40				
Calcium Carbonate Equivalent (CCE) %	Ri = Loam; Ro = Loam; Ru = Loam; Ai = Sand; Ao = Sand; Au = Sand; Si = Loamy Sand; So = Loam; Su = Loam				
		0-2.5	2.5-5.0	5.0-10	> 10.0
		Ri, Ro, Ru			

R = Regolith, A = Arcillite, S = Soil, i = inside rhizosphere column, o = outside rhizosphere column, u = untreated.

A comparison was made between the three media substrates between the combined region inside and outside of the rhizosphere (Figure 10). A net increase in pH and Organic Matter percent (OM%) within the rhizosphere compared to outside of the rhizosphere was observed in all treatments. In addition, a net decrease in Salts, Nitrates, K, Ca, Mg, Na, and CEC were observed in all treatments. A net increase in P within the Regolith samples were observed, while a net decrease in P within the Arcillite and Soil samples was observed between the inside and outside of the rhizosphere column. Cl showed a decrease in Arcillite, and CEC and bulk density showed a decrease in Regolith samples. Otherwise, Cl, CCE%, and Bulk Density had concentrations that were below the method detection limit of the instrument.

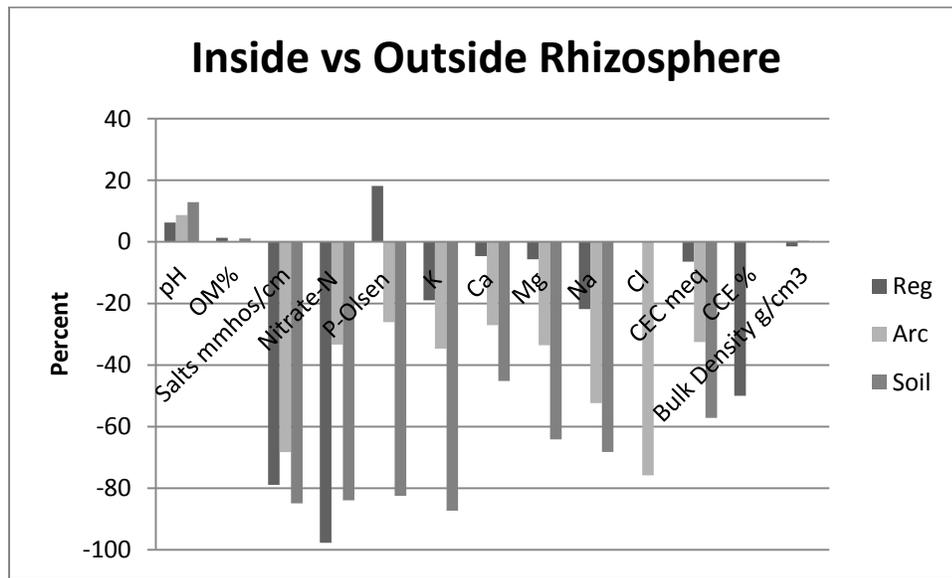


Figure 10. Comparison between JSC Mars Regolith Simulant, Arcillite, and Potting Soil soil fertility results after the third plant generation.

The inside of the rhizosphere was broken into two sections: the top half and the lower half (Figure 4). Soil samples proved to be too small for individual analysis, and were combined into one sample each, used for Figures 10 & 13. For Figure 11, a net

increase in pH, Ca, and Na was observed between the inside top half, and the inside bottom half of the rhizosphere column in both the JSC Mars regolith simulant and Arcillite substrates. JSC Mars Regolith Simulant showed an increase in P, K, Mg, and CEC compared to a decrease in those same measurements for the Arcillite samples. Arcillite showed an increase in Salts compared to a decrease in salts with the regolith samples. An observable decrease in organic matter (OM) and Bulk Density was observed between the top and bottom of the rhizosphere with the regolith treated samples, with no significant difference between those concentrations for Arcillite. Arcillite samples also showed an increased concentration of Cl and CCE% between the top and bottom halves of the rhizosphere, with regolith showing no significant differences between those measurements.

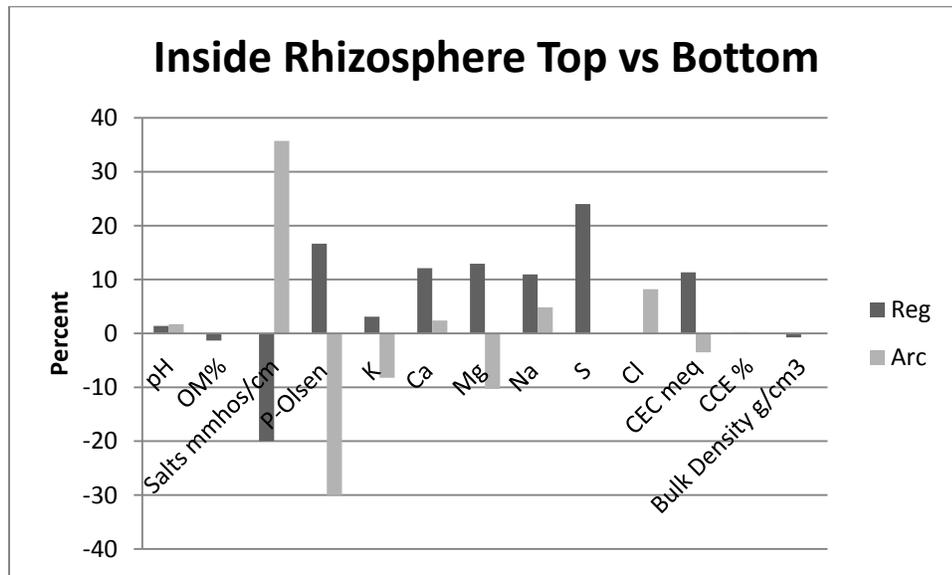


Figure 11. Comparison between the two regions of the rhizosphere from JSC Mars Regolith Simulant and Arcillite soil fertility results after the third plant generation.

The inner most region outside of the rhizosphere, and the outer most region of JSC Mars Regolith and Arcillite treatments were compared in Figure 12. Soil treated samples were pooled to average this area due to each location mass being below the mean detection limit of the instrument used. For Figure 12, an increase in pH, P, and bulk density was observed between the inner and outer zones outside the rhizosphere with the regolith and arcillite samples. In addition, a decrease in Salts, Nitrates, K, Mg, Na, and CEC was also observed between the inner and outer zones outside of the rhizosphere column in both regolith and arcillite treated samples. Arcillite also showed a decrease in Ca concentrations, while regolith showed an increase in Ca concentrations between the inner and outer region outside of the rhizosphere column. Regolith showed a decrease in organic matter (OM) between inside and outside regions outside of the rhizosphere column, however no significant difference occurred between these two regions in CCE%.

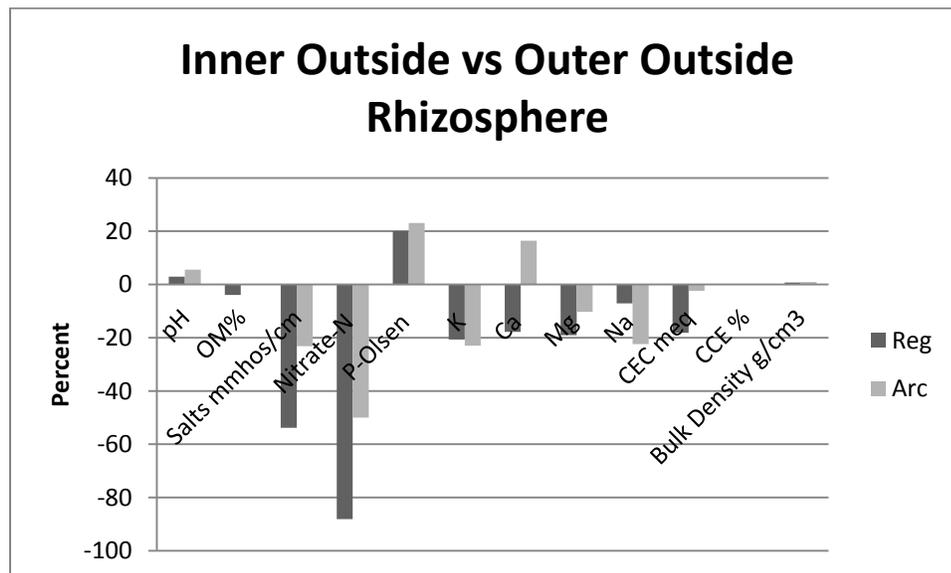


Figure 12. Comparison between the two regions outside of the rhizosphere between the JSC Mars Regolith Simulant and Arcillite samples after the third plant generation.

The two regions inside of the rhizosphere were combined and averaged to determine the differences in soil fertility measurements after three generations of plant growth, and untreated samples (no plant growth, Figure 13). An increase in pH and OM, and a decrease in K was observed in the rhizosphere of all treatments. In addition, an increase in Salts, P, Ca, and Na was observed within regolith and arcillite rhizospheres, while the soil rhizosphere treatments decreased in those measurements. A decrease in Nitrates and CEC was observed in regolith and soil rhizospheres as well, but not arcillite. A decrease in Mg was observed in arcillite and soil rhizospheres, but not in regolith samples. In addition, a decrease in Bulk Density was observed only in the rhizosphere soil treatment.

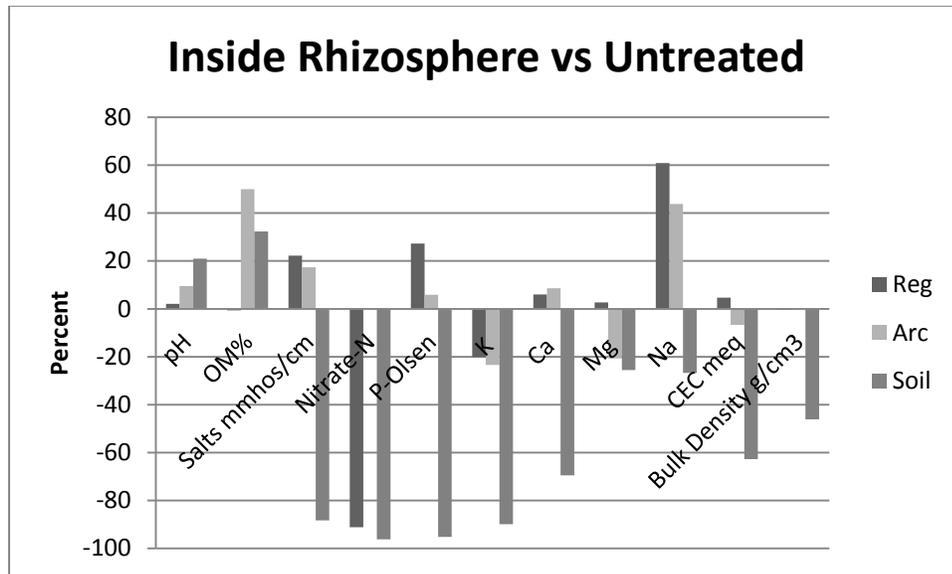


Figure 13. Comparison between the rhizosphere and untreated JSC Mars Regolith Simulant, Arcillite, and Soil samples after the third plant generation.

Mineralogy

Sample mineralogy was conducted via X-ray Diffraction at NASA-Ames Research Center. The findings indicate that no significant change occurred in samples

over the three month duration of the experiment. Untreated samples, which had no plants or water added to them, showed the same mineralogy as samples which were collected after multiple plant generations (Figure 14).

Soil samples (Figure 14. top) contained quartz and clay minerals and X-ray amorphous material which was likely organics. The main crystalline component in regolith samples (Figure 14. middle) is plagioclase feldspar and x-ray amorphous material contributed to organics, nano phase Fe-oxides, and/or poorly ordered clay minerals. Arcillite samples (Figure 14. bottom) contained mostly quartz, opal-CT, and a 2:1 group clay minerals. One observation about Figure 14 C. is that more quartz was observed inside of the rhizosphere column than outside of the rhizosphere column.

Reflectance Spectroscopy

One cotyledon per plant, ten plants per substrate, was recorded and averaged to reveal the reflectance spectrograph of the entire plant generation. A record of the second and third plant generations was recorded, as the FieldSpec Pro™ was not ready in time for the first plant generation. The spectral range collected reflectance signatures between 350nm and 2151 nm. Spectral signatures from 350nm to ~700nm represents leaf pigments in the visible spectrum (VI), ~700nm to ~1300 nm is the Near Infrared (NIR) range that represents plant stress related to cell structure, and ~1300nm to 2151nm is the Shortwave Infrared (SWIR) region that illustrates plant stress related to water content (Govender et al., 2009). In addition, the following wavelengths illustrate photosynthetic activity (Dunagan et al., 2007): Chl *a* = 430nm, Chl *b* and carotenoids = 448nm, carotenoids = 471 nm, Chl *b* = 642nm, Chl *a* = 662nm and 680nm, Green peak = 550nm,

and NIR Reflectance = 800nm. For this analysis, only the spectra between 350 nm and 900nm was used.

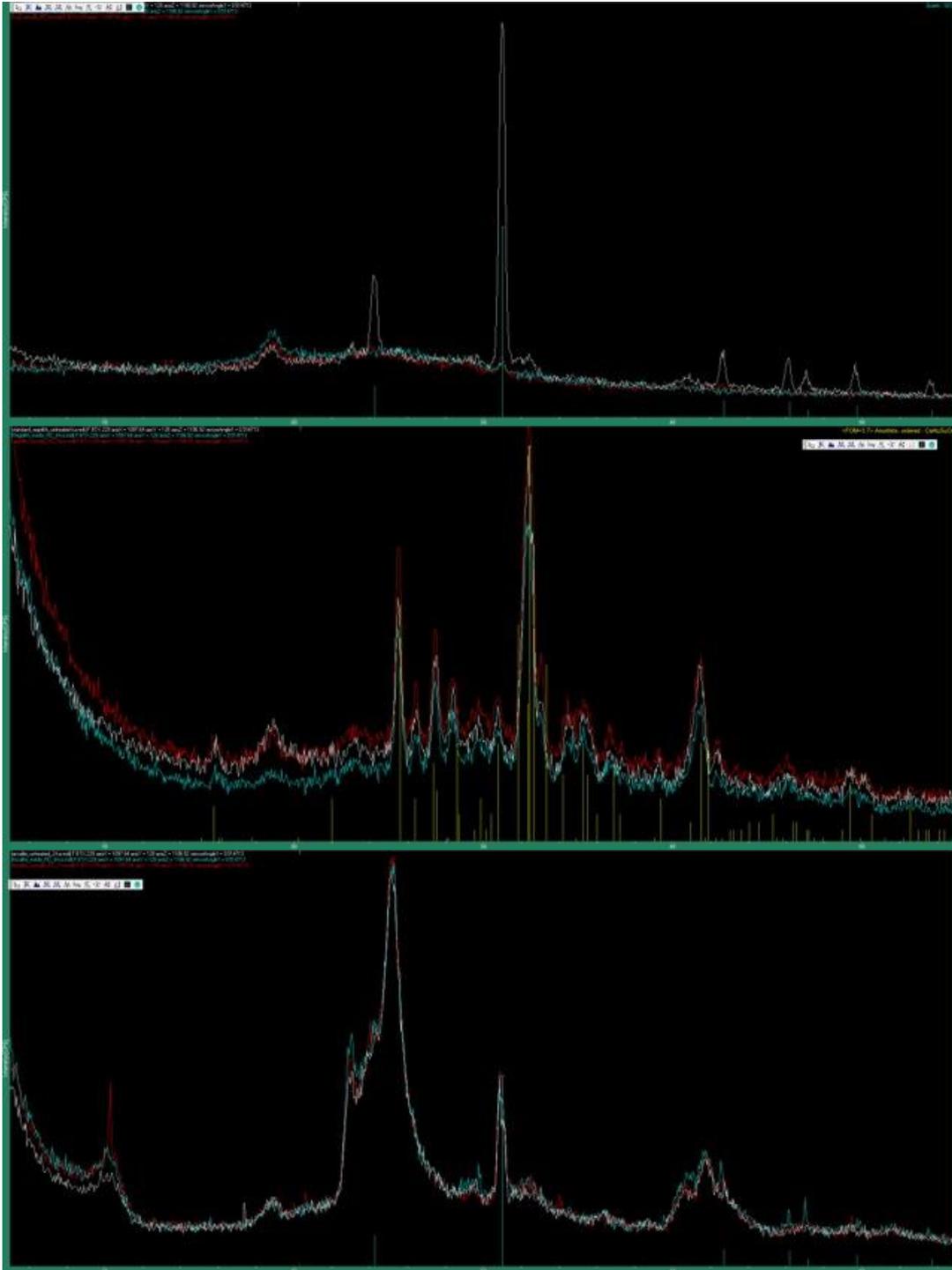


Figure 14. Mineralogy results for Soil (top), Regolith (middle), Arcillite (bottom).

A Normalized Difference Vegetation Index (NDVI) and Ratio Vegetation Index (RVI) were calculated (Equation 1) to determine correlations between biomass of vegetation and leaf area index, nutrient deficiency, and chlorophyll *a* concentrations (Dunagan et al., 2007), An increase in plant stress would be indicated by a decrease in NDVI and RVI values.

$$\text{Equation 1. NDVI} = (R_{800} - R_{679}) / (R_{800} + R_{679})$$

$$\text{RVI} = R_{750} / R_{700}$$

The second and third plant generations grown on the JSC Mars Regolith Simulant showed a decrease in NDVI values, but a very slight increase in RVI values (Figure 15). The second generation also had a reflectance spectra which was higher than the third plant generation, and is indicative that the third plant generation experienced more overall plant stress than the second plant generation.

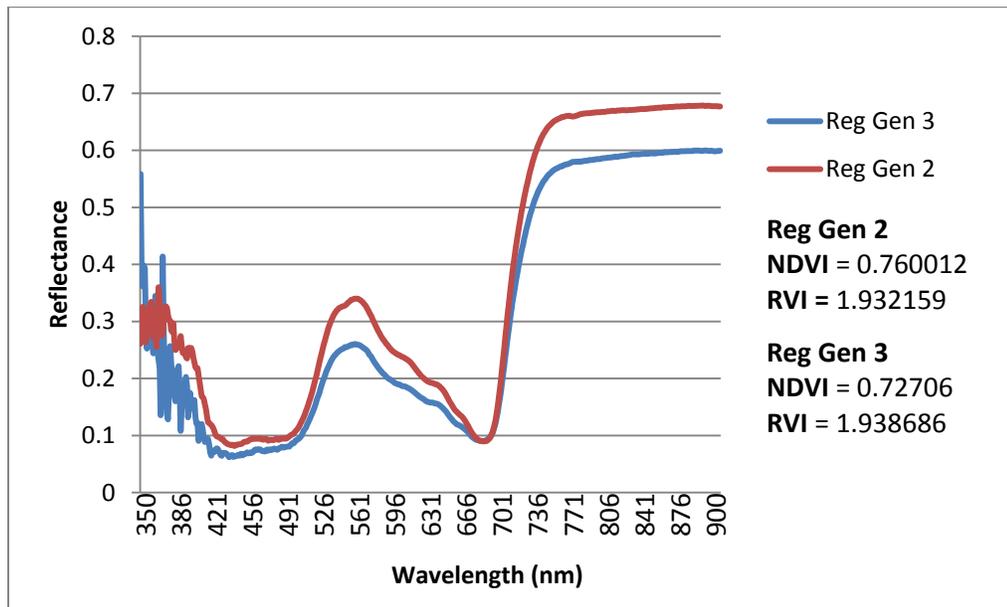


Figure 15. Comparing the average reflectance spectra between 350 and 900 nm of the second and third plant generations on JSC Mars Regolith Simulant, with NDVI and RVI values.

Plants grown on arcillite showed an increase in NDVI and RVI values between the second and third plant generation (Figure 16). However, the spectra between 400nm – 700nm showed a significant decrease in reflectance between the second and third plant generations. Overall, based on NDVI and RVI values, the third plant generation showed less stress than the second plant generation.

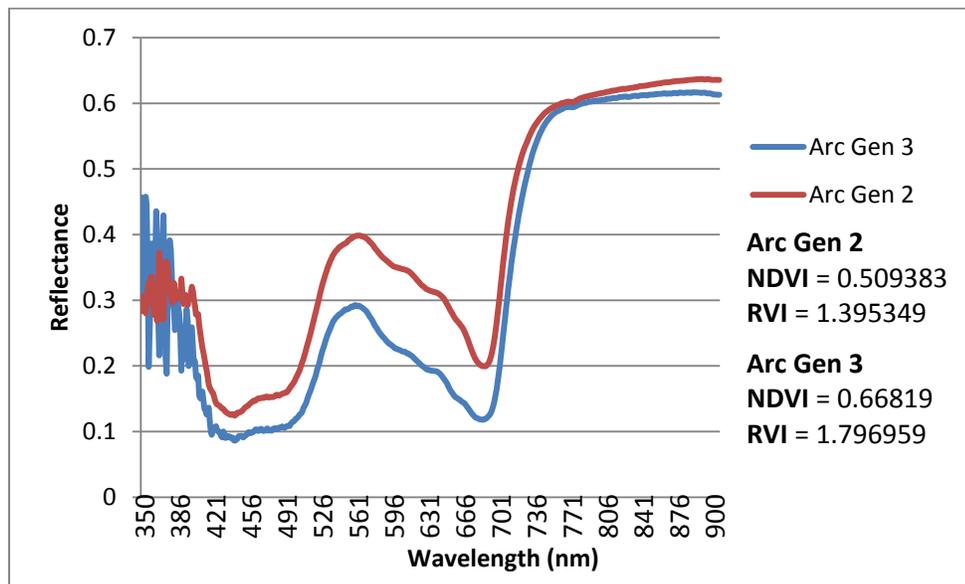


Figure 16. Comparing the average reflectance spectra between 350 and 900 nm of the second and third plant generations on Arcillite, with NDVI and RVI values.

Plants grown on soil showed an overall decrease in stress between the second and third generations (Figure 17). This observation is based on an increase in both NDVI, RVI, and reflectance spectra in the third plant generation compared to the second.

The entire second plant generation was compared in Figure 18. The NDVI values, from lowest to highest, was Arcillite, Soil, and Regolith. The RVI values, from lowest to highest, was Soil, Arcillite, and Regolith. In addition, the soil showed the lowest average reflectance spectra, followed by Regolith (except in the NIR range), and then Arcillite.

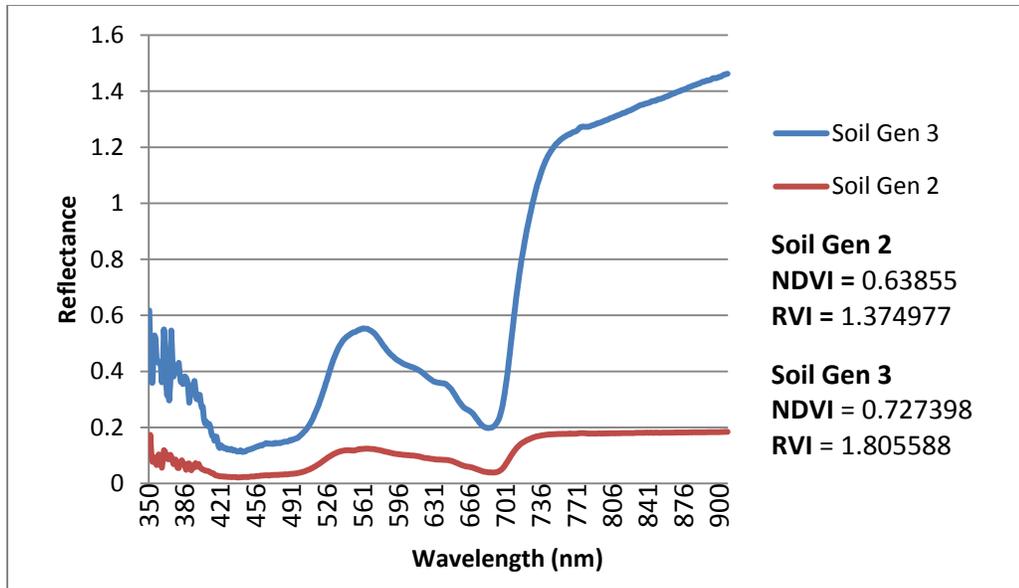


Figure 17. Comparing the average reflectance spectra between 350 and 900 nm of the second and third plant generations on potting soil, with NDVI and RVI values.

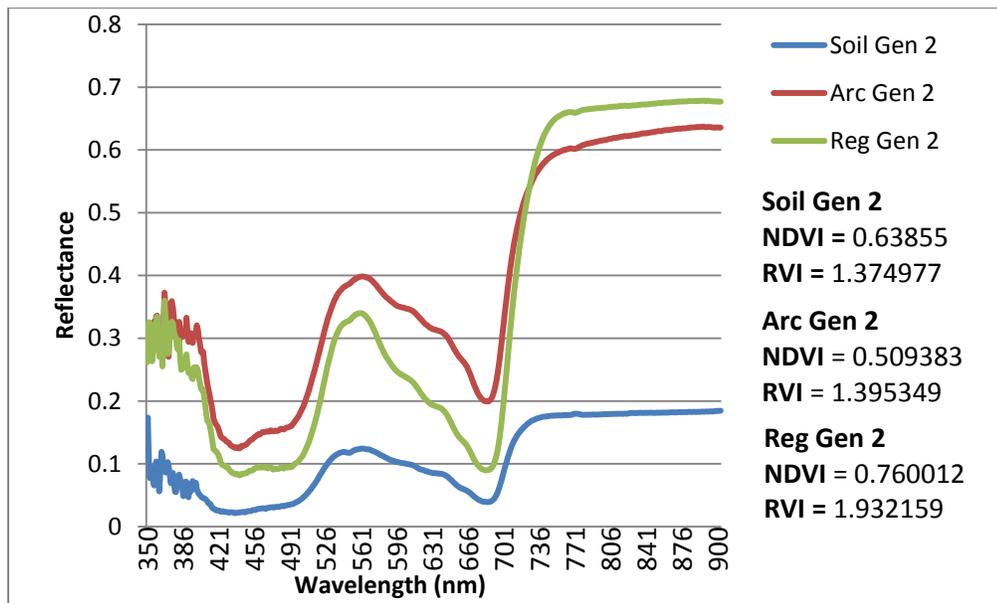


Figure 18. Comparing the average reflectance spectra between 350 and 900 nm of the second plant generations on JSC Mars Regolith Simulant, Arcillite, and potting soil with NDVI and RVI values.

In the third plant generation (Figure 19), soil reflectance had increased above both arcillite and Mars regolith simulant samples. The NDVI values were highest in Soil, followed by Regolith and Arcillite, with RVI values highest in Regolith, followed by Soil and Arcillite. Plants grown on Arcillite remain the most stressed, though the overall reflectance spectra illustrate plants grown on arcillite have more similarities to plants grown on regolith, than plants grown on soil.

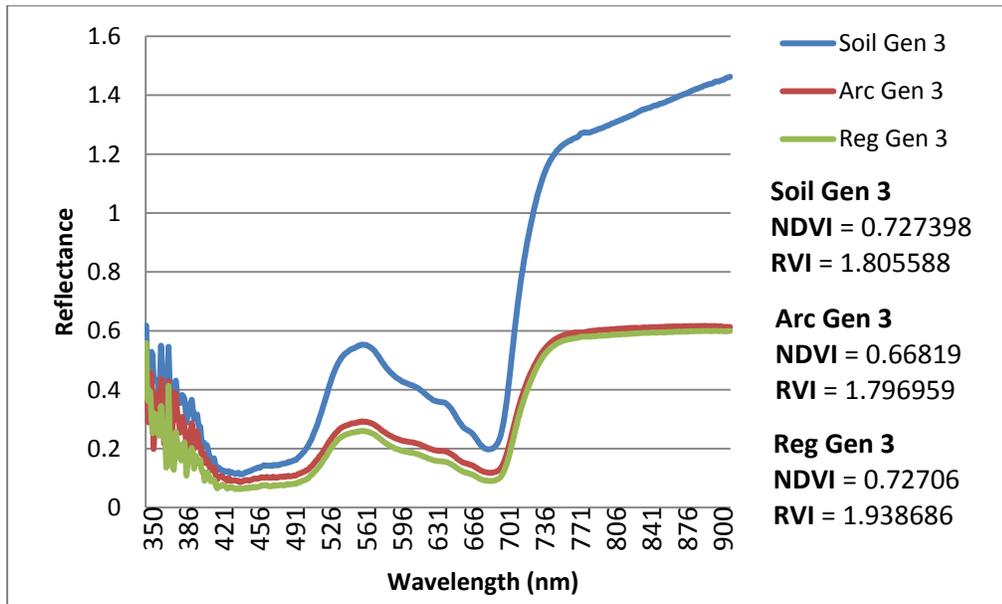


Figure 19. Comparing the average reflectance spectra between 350 and 900 nm of the third plant generations on JSC Mars Regolith Simulant, Arcillite, and potting soil with NDVI and RVI values.

Plant Morphology and Biomass

At the end of each generation of plant growth, plant morphology and biomass was recorded for the amount of shoots that had cotyledons, 1st, 2nd, and 3rd true leaves (TL) present. In addition, the total amount of flowering buds and open flowers were recorded as a means to help predict seed production, and to represent flowering plants where these portions are edible.

The regolith second and third plant generations had an equal amount of shoots that developed cotyledons, 1st, and 2nd true leaves (Figure 20). No shoot developed its 3rd set of true leaves in the regolith samples. The amount of flower buds increased in the third generation compared to the second, but the amount of open flowers decreased in the third generation compared to the second.

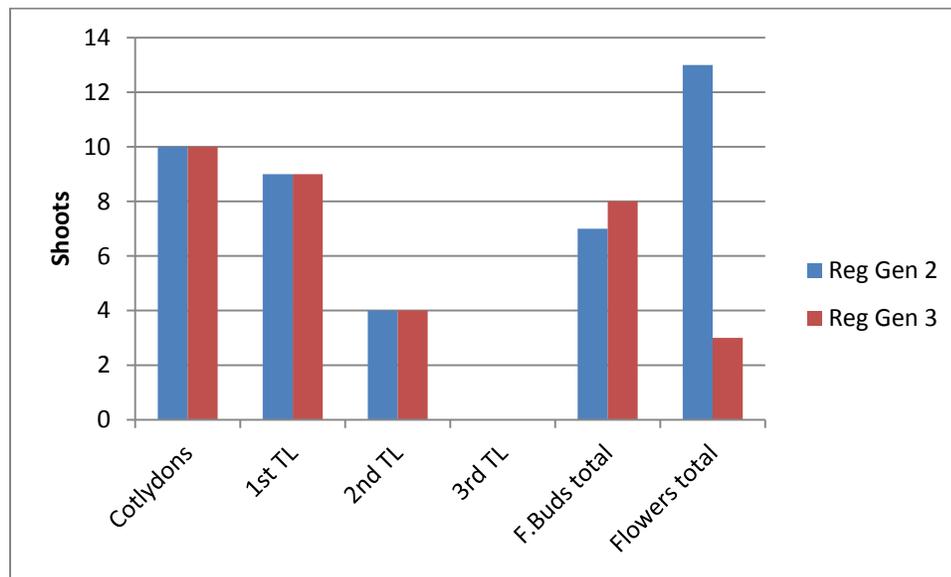


Figure 20. Plant morphology and biomass results comparing the 2nd and 3rd plant generations in JSC Mars regolith simulant.

The second and third plant generations in the arcillite samples showed an increased number of cotyledons and total amount of flowers in the second generation compared to the third (Figure 21). The third generation saw an increase in 1st and 2nd true leaves, and total flower buds, with no 3rd true leaves developing in either generation.

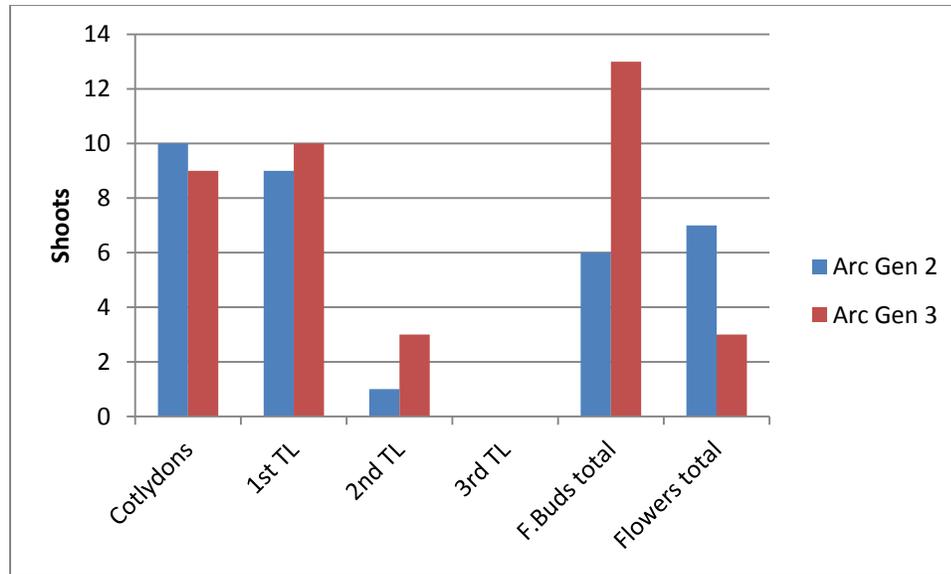


Figure 21. Plant morphology and biomass results comparing the 2nd and 3rd plant generations in Arcillite.

Soil samples showed an equal development of cotyledons and 1st true leaves in the second and third plant generations, a decreased amount of shoots that developed 2nd true leaves and total amount of flower buds and flowers in the third generation compared to the second. However, the total amount of shoots that developed 3rd true leaves increased in the third plant generation compared to the second (Figure 22). In addition, biomass decreased overall in regolith, arcillite, and soil, between the second and third plant generations (Figure 23).

Each substrate treatment had an equal amount of cotyledons present at the time of termination for the second generation (Figure 24). Arcillite and regolith treatments had an equal amount of shoots that developed 1st true leaves, while soil showed an increased amount of shoots that developed 1st true leaves. Arcillite showed a suppressed amount of 2nd true leaves, total flower buds, and total amount of flowers in the second generation compared to regolith and soil samples, with soil developing more 2nd true leaves, total

flower buds, and total amount of flowers than regolith. Soil samples were the only ones to have developed 3rd true leaves.

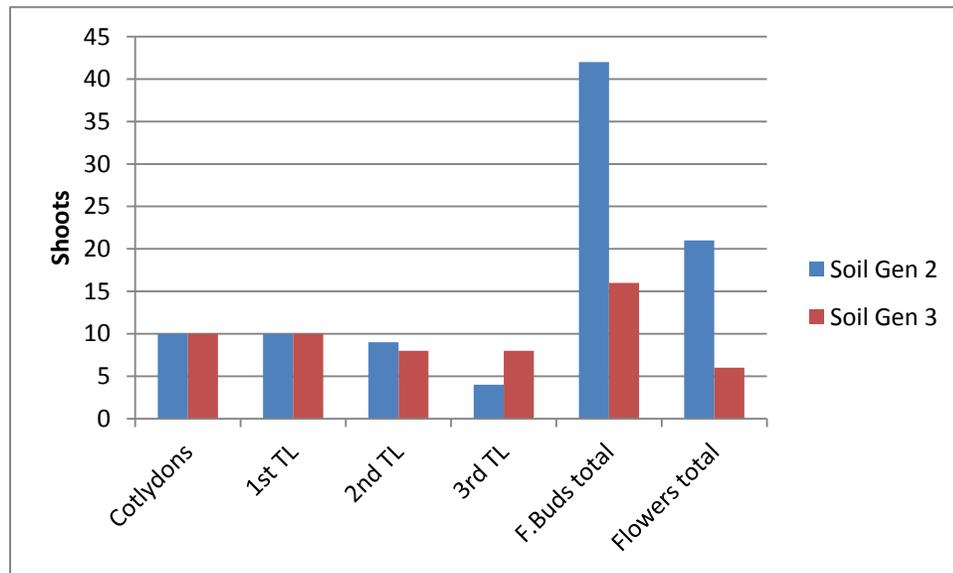


Figure 22. Plant morphology and biomass results comparing the 2nd and 3rd plant generations in Potting Soil.

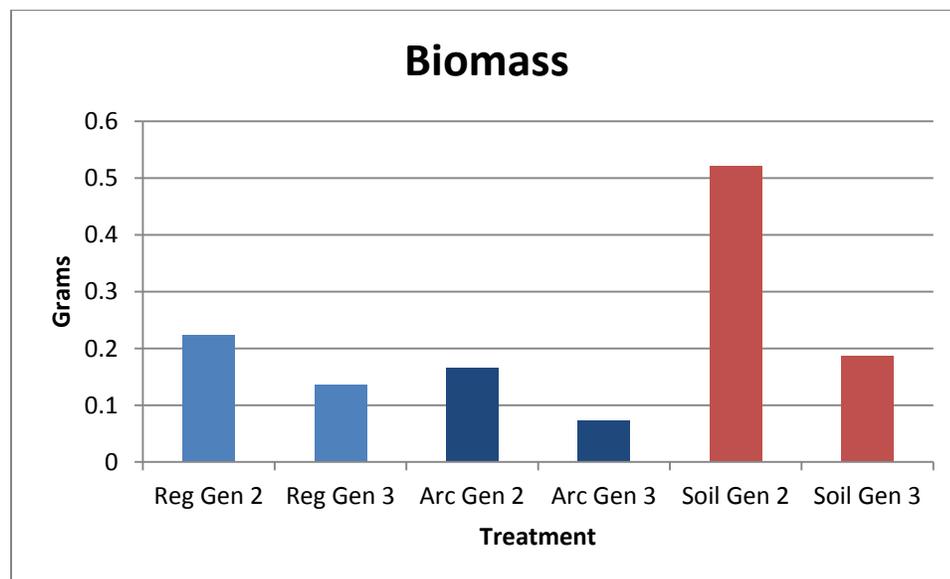


Figure 23. Biomass comparisons between the 2nd and 3rd plant generations of all treatments.

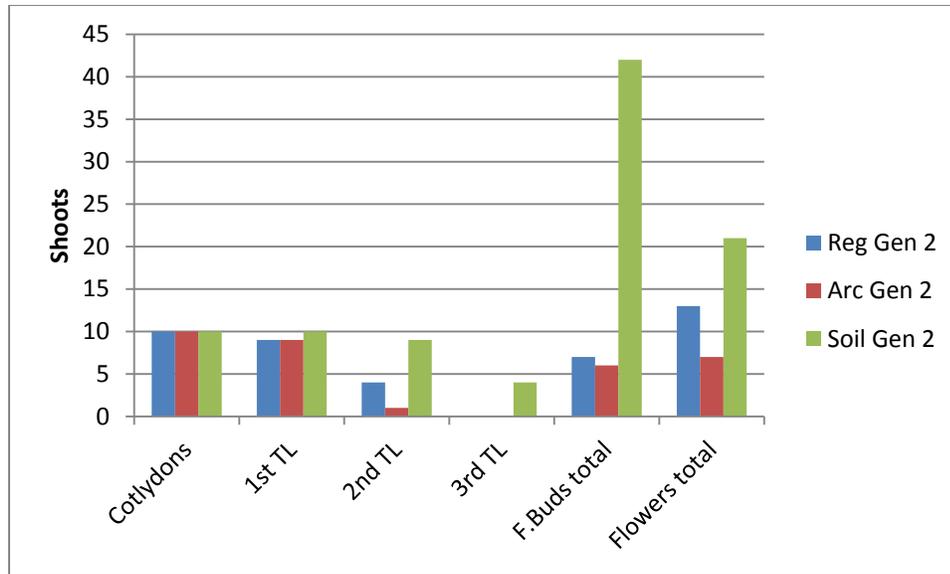


Figure 24. Comparing plant morphology between all treatments of the 2nd plant generation

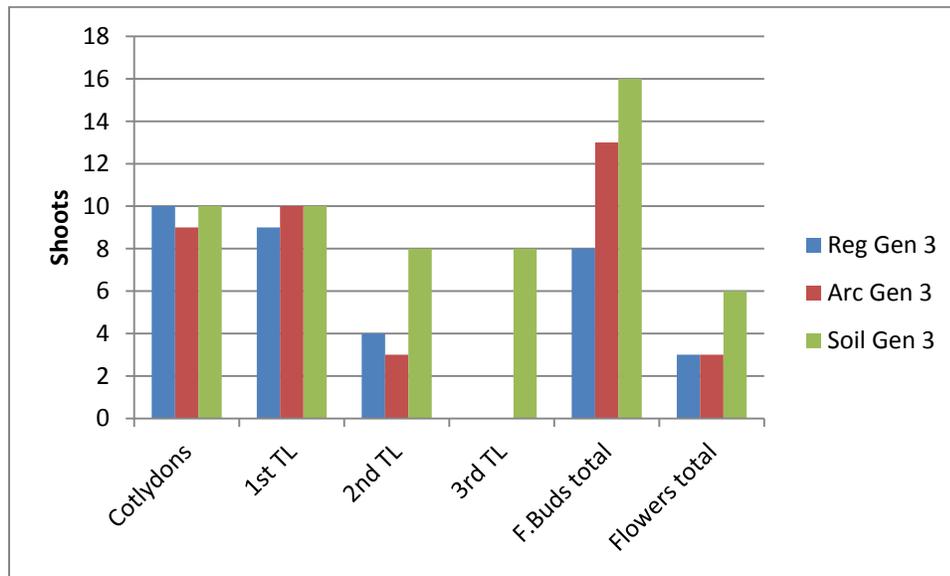


Figure 25. Comparing plant morphology between all treatments of the 3rd plant generation

The third plant generation had an equal number of shoots present with cotyledons in the regolith and soil treatments, with a decreased amount of cotyledons present in the arcillite samples (Figure 25). An equal number of 1st true leaves were present in the Arcillite and soil samples, with a decreased development of 1st true leaves in regolith samples. An equal number of flowers developed in the arcillite and regolith samples, but

an increased number of flowers occurred in the soil samples. Arcillite developed the least amount of shoots with 2nd true leaves, followed by regolith and soil, with soil being the only treatment that developed 3rd true leaves. Regolith treated samples developed the lowest number of flower buds, followed by arcillite and soil treated samples.

Microbiology

A standardized operating procedure (SOP) described by Schloss et al (2011) was implemented for the analysis of the sequence data of this study. Sequences were removed from consideration if they (a) did not contain the primer sequence, (b) contained an incorrect barcode, (c) were < 200 nt in length, (d) homopolymers longer than 8 bp or (e) had a quality score < 25. Unique sequences were aligned using the greengenes reference alignment (McDonald et al., 2012) and trimmed in order to keep 95% of overlapping sequence length. Sequences were classified in accordance with the new Greengenes training set and taxonomy (Werner et al., 2012, McDonald et al., 2012) and clustered into operational taxonomic units at the 0.03 level (i.e., at 97% similarity) (Schloss et al., 2009) after removing chimeras. Rarefaction plots were constructed to show the average number of OTUs detected against the sequencing depth (Figure 26).

1,330 sequences were selected from each sample before calculating community-wide dissimilarity calculations. Sample ArcUn and RegUn (Arcillite and Regolith untreated) had comparatively very less sequences (403 and 405 respectively). These samples were not considered for further analysis, removing the bias due to sequencing depth that would otherwise affect ordination analysis.

Principal Coordinate Analysis (PCoA) is a two dimensional ordination plotting method used to visualize relationship between the samples, and was performed to study the environmental clustering and relatedness of community profiles derived from bTEFAP analyses. The clustering observed in the PCoA plot was congruent with the dendrogram clustering, which implied a close association among different soil samples.

Based on the rarefaction analysis, OUT richness was highly variable among samples. Sample UND-5 had relatively high number of OTUs (603). Sample ArcUn had relatively low number of OTUs (40). The rarefaction curves for many samples were parallel to the X-axis, which indicates sufficient sequencing efforts to cover microbial diversity. Untreated JSC Mars regolith and Arc soil samples had relatively less number of sequences (405 and 403 respectively) indicating less microbial load. OUT richness was highly variable (Figure 26). Sample Soiln showed relatively high (603) number of OTUs while sample ArcUn has less OTUs (40).

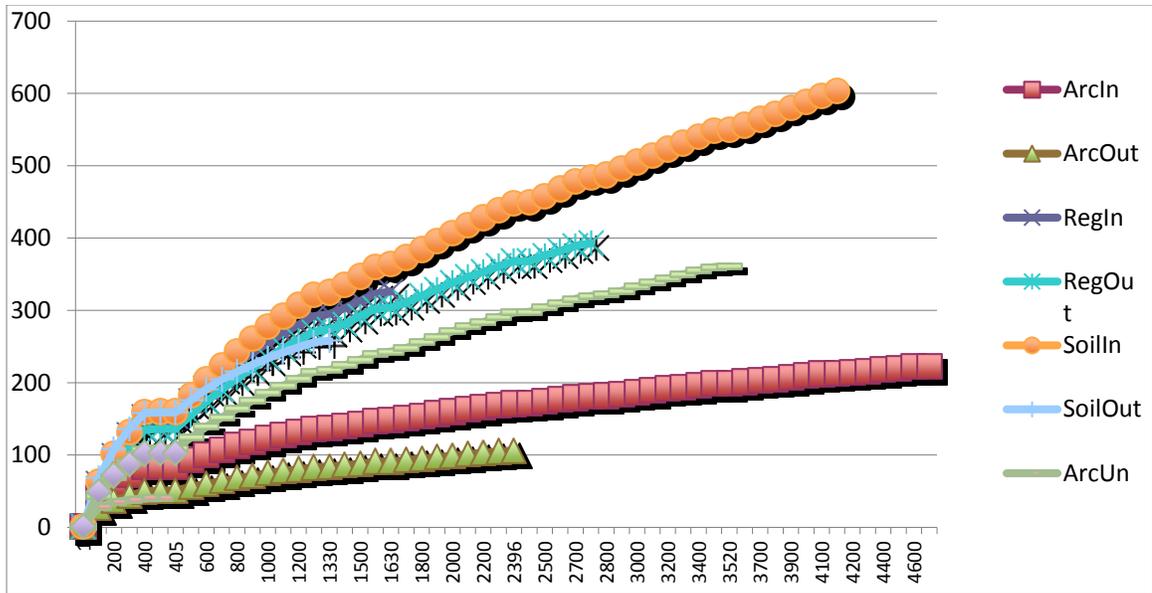


Figure 26. Rarefaction curve depicting average OTUs detected (Y-axis) against sequencing depth (X-axis). Each point represents a subsampled sequences (100), and the average number of OTUs found.

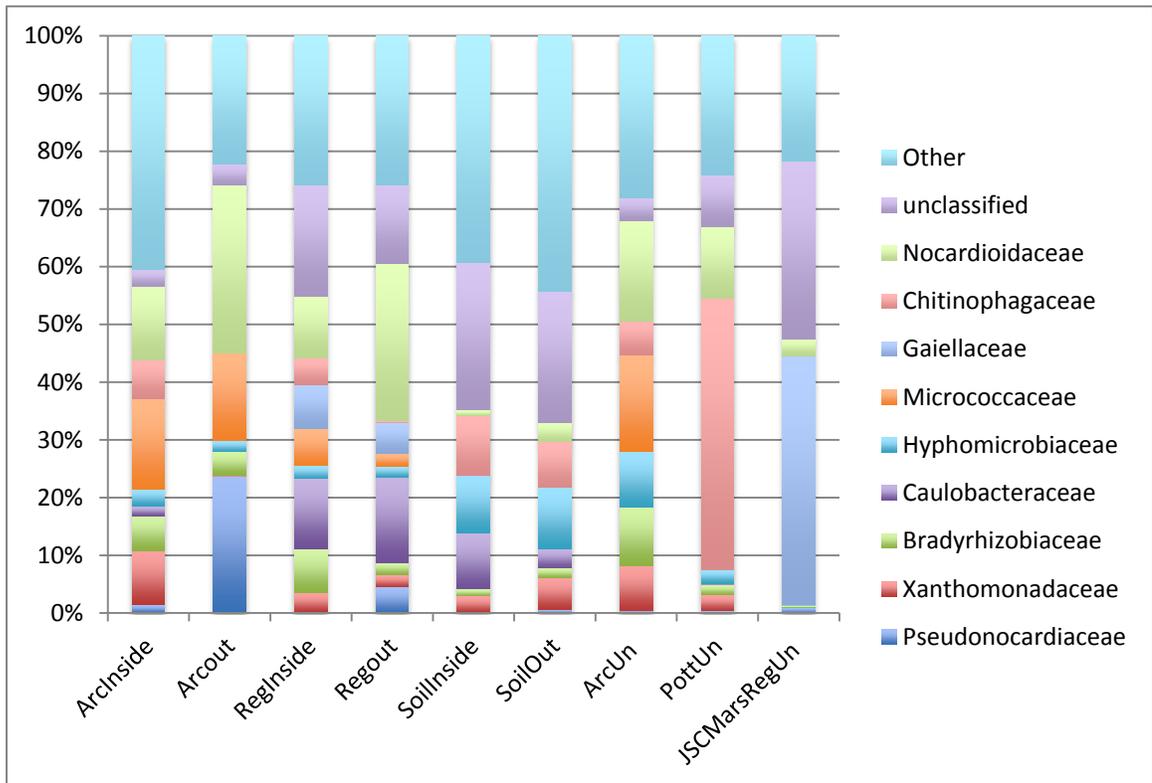


Figure 27. Microbial family level abundance.

A comparison of the abundance of sequences from each sample at the family level was compared (Figure 27). The top 10 families corresponds to on average 70% of each samples sequences. The dominance of *Nocardioideae* (13% average) and *Chitinophagaceae* (9% average) was observed. Sample PottUn showed dominance of sequences classified as *Chitinophagaceae* (47.03%) while samples JSCMarsRegUn exhibited dominance of Gaiellaceae (43.06%). Contrary to our expectation, sample ArcUn showed diverse microbial profile though it had low number of sequences (403).

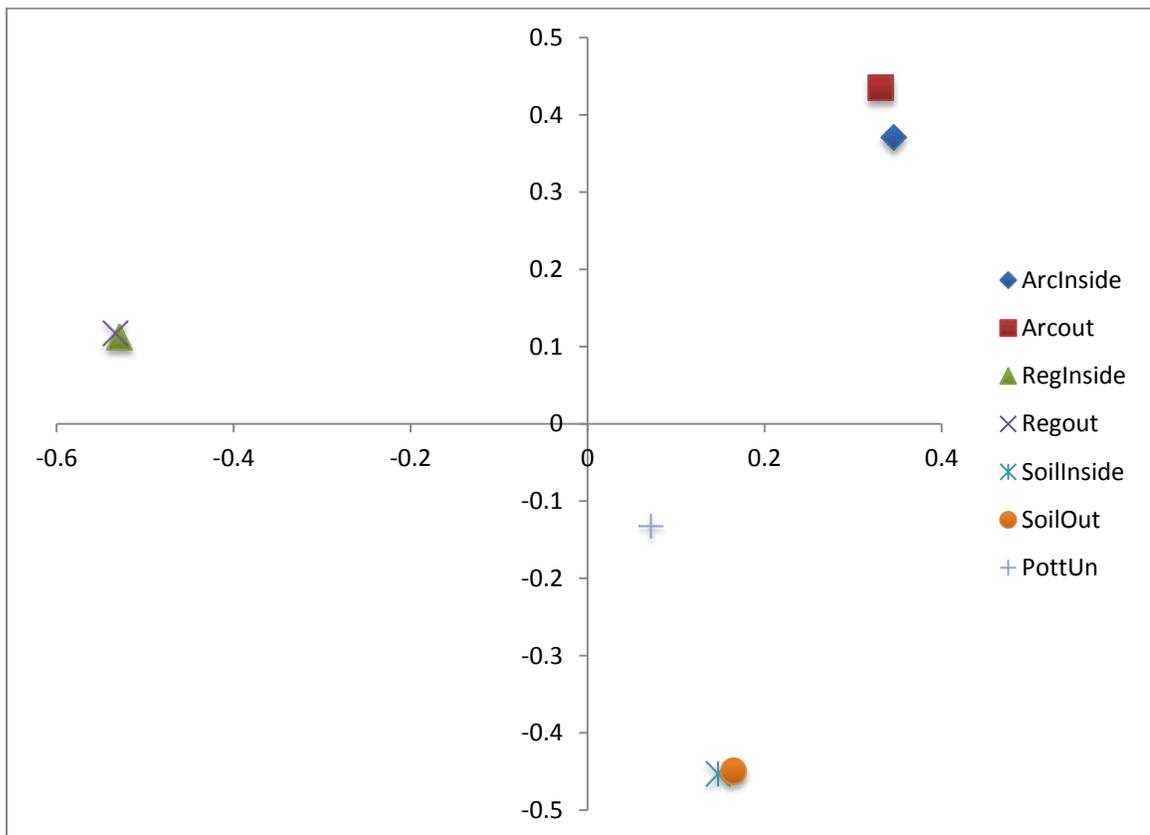


Figure 28. Principal Coordinate Analysis (PCoA) based on Weighted UniFrac distance between samples. PCoA analysis was performed to visualize the complex relationship between samples. Axis1:32.56% variation and Axis2: 29.87% variation.

Based on the ordination analysis grouping of sample pairs based on the soil type was observed along the PCoA 1 axis (Figure 28). Samples collected from inside and outside of the R column from each soil type grouped together indicating similar microbial profiles. Hence, the microbial community composition of sample pairs from each soil type is different. Sample from untreated potting mixture had distinct microbial structure from rest of other samples, which was evident in Figure 21 also.

CHAPTER IV

DISCUSSION

Based on previous experiments and a review of literature, several results were expected to be observed throughout this experiment. These results range from plant morphology, soil fertility, to microbial communities. The expected results and the findings were as follows:

1. Element uptake in the plant will be significantly different from generation 1 and generations 3-5.

The biomass accumulated from the *Brassica rapa* (Wisconsin FastPlants™) chosen for this study were below the mean detection limit (MDL) of the ICP-OES for elemental analysis. In addition, the plants were only able to grow for three generations, as the plants showed slower development in the first and third plant generations due to less than optimal lighting conditions. The first and part of the second plant generations were grown outside of a CES in order to refine optimal growth conditions, the third plant generation was grown entirely within the laboratory closed ecosystems (CES). The added stress observed in the third plant generation compared to the second could be induced by enclosure within the CES. However, the CES provided more optimal watering conditions, and the stress involved was observed in all of the third plant generation. Thus, the added stress is interpreted primarily as being due to the lower

nutrient availability as observed in the soil fertility analysis, and varying lighting conditions that were occasionally present.

2. Media mineralogy will be significantly different within the rhizosphere column compared to outside the rhizosphere, and untreated samples.

The mineralogy did not significantly change in this experiment, though the mineralogy of each substrate was substantially different. However, nutrient availability and soil fertility did significantly change, with a consensus that soils are losing nutrients over time, but increasing in OM%, pH, and an increase of available nutrients close to the rhizosphere compared to farther away. These results are an indication of soil formation, though the long term stability of the system for future plant generations is undetermined.

3. Reflectance spectroscopy will be significantly different between soil and arcillite/regolith treated samples

The data supports this claim: plants grown on a potting soil mixture show significantly different plant stress compared to plants grown on arcillite or mars regolith simulants. Arcillite and regolith samples have similar spectra, though RVI/NDVI values vary, with plant stress between arcillite and mars regolith being similar across plant generations.

The significant change in reflectance spectra of the soil samples between the second and third plant generation is unique and not observed in the arcillite or regolith samples, even though the reflectance spectra was recorded the same way over time. The possibility exists that an increase in incandescent light may be required to further saturate the sampling area to prevent inaccuracies. However, the third plant generation grown on

soil showed more stress in its reflectance spectra, as well as in biomass production and morphology. Therefore, the reflectance spectra does support these additional findings.

4. Plant morphology will be significantly different between soil and arcillite/regolith samples

Plants grown on arcillite and Regolith have similar morphology and biomass accumulation compared to plants grown on soil, which have significantly more biomass, and 2-3 times the amount of flower buds and opened flowers. Biomass across all treatments decreased in the third generation compared to the second, which was not anticipated. Therefore, the response both morphologically and chemically of the system is an indication that soil is still forming, and an interaction within the soil chemistry is still occurring. Future studies may wish to run water (or another nutrient solution) through the substrate prior to planting which could identify the chemical changes in the media attributed to plants and microbes, or pure weathering. These CESs may also benefit by an additional air filtration process which would remove the toxic build-up of trace gasses.

5. Discussion: Different microbial communities may be present based on sample treatments

Diverse microbial community profiles were observed in nearly every sample. Based on the ordination analysis (PCoA) and environmental clustering analysis, difference in microbial community profiles based on sample substrate was also observed, but with relatively similar microbial profiles from the same sample treatment (inside and outside of the rhizosphere column). Untreated soil samples exhibited distinct microbial composition with relatively less richness compared to respective treatment samples. The

likely case for less microbial community richness in the untreated samples is due to these samples not ever having been in the presence of water, warmer temperatures, or the introduction of plants. Untreated samples also had less human contact, and were then less likely to have additional microbes introduced.

CHAPTER V

CONCLUSIONS

Brassica rapa (Wisconsin FastPlants™) biomass proved too low for elemental analysis, but a good indicator of plant stress and development between treatments (a good indicator of soil health/fertility). Wisconsin FastPlants™ also prove limiting in utility to help determine nutrition available to astronauts—a larger plant must be used in this case, though the same family would be beneficial to compare to this study. Plants with larger root biomass should also be investigated, as the potential for biogeochemical changes in the rhizosphere increase with an increased amount of root density within the soil. A larger breed of *Brassica rapa* would also be beneficial in indicating the relationship to plant morphology and the potential yield of crops within its same family.

The elemental/soil nutrient changes were, however, induced over a short duration experiment (3 months). The potential exists with a more targeted strategy to vastly improve nutrient availability and soil formation, in an even shorter amount of time and with a potentially higher plant yield.

In addition, the plants and methods used are inexpensive and quick to evaluate soil formation and fertility for Mars analog habitats and should be expanded upon. Three out of four parameters of soil formation were met during this study (ex. Additions): transformation, translocation, and losses.

Through transformation process, chemical and physical modification, as well as aspects of weathering were observed by the formation of silicate clays, hydrous Fe and Al oxides. The process of translocation was also observed through the movement of inorganic and organic materials both vertically and horizontally. Lastly, losses were observed through the increase of salts and organic acids (possibly from microbial action) closer to the rhizosphere.

Overall, the average biomass and morphology yield of plants grown on JSC Mars-1A regolith simulant is an indication that unique methods specific to Mars will have to be implemented for the success of any settlement there. *In situ* resources will have to be optimized by flooding the surface regolith with water to dissolve hydroxides and other potential compounds toxic to plants. An increase in CO₂ with a variation of temperature compared to Earth conditions will also have to be used, which will decrease the required planting area by increasing plant yield.

Growing plants/crops in an atmosphere which is different from the one astronauts breathe will host its own unique challenges, though by doing so the overall planting area will be reduced, which should be an exponential savings on mission costs. In addition, decreasing the planting area required to sustain each person (roughly 60m²), will decrease the amount of time required to maintain and process the crops grown within the space. Formulating a system which can quickly make Mars regolith receptive to plant growth, and projecting a reliable plant yield for up to ten or more plant generations would be necessary to save both costs and unnecessary use of human time.

CHAPTER VI

SUGGESTED RESEARCH DIRECTIONS

Throughout this study, four specific aspects arose which require future research to predict the growth conditions, plant yield potential, and soil formation on the Martian surface.

Research on Compost/Artificial Soil Formation

Human and other material wastes will have to be collected and recycled to ensure the most closed loop possible in the early planetary settlements on Mars. Studies should be conducted which include compost from expected wastes (urine, feces, grey water, etc.) and incorporated into plant growth studies to investigate the effect recycled wastes and composting have on crop yield. These materials, with chemical compositions in various forms, may also create unique properties within the soil that may lead to more beneficial, or more harmful effects. Deciphering which materials to be recycled or composted to benefit plant growth in a planetary settlement should be better defined.

Larger Plant, More Root and Shoot Biomass, Seed Counts (Achieve 15 OM% From Parent Material), Specialized Microbial Community

By using larger, fast growing plants, a study could be completed which encompasses the entire plant growth and seed production stage. Because the study conducted for this thesis terminated plant growth before the seed production stage, research must be conducted to determine the amount of seeds produced during each plant generation, and determine if the seeds produced show any indication of being better

adapted for growth on Martian regolith. A larger root and shoot biomass has the potential to create more significant changes in soil chemistry—which may also be family or species dependent, and have significant benefits and consequences. Finally, the introduction of specified microbes, such as nitrogen fixing bacteria, earthworms, nematodes, etc., could also be used to improve soil formation, soil fertility, and crop yield.

Longer Duration Experiment in Mars Like Conditions (1-5 Years)

Extending the project for up to five years would be necessary to support an early Martian settlement with confidence. In addition, greenhouse experiments in nearly closed ecosystems at higher altitudes would also be beneficial. Areas above 18,000ft (over half of Earth's atmosphere) have increased radiation with decreased pressures, more similar to expected Mars-like conditions. By conducting these experiments in higher radiation environments, one could identify specific radiation responses in the plant rhizosphere which would relate to soil formation, crop yield, and the microbial community response. An orbital research project, at the International Space Station or elsewhere, is another potential source of high-radiation and a controlled, closed environment. Ultimately, conducting a suite of experiments across radiation levels and pressures will be required, both to determine the most cost effective Mars analog, and to further study the effects these plants have on soil fertility and human health.

Regolith Nutrient Processing (Using Minimum Regolith to Start, Then Increases OM% and Add More), Gypsum

Rapid methods to increase organic matter percent (OM%) in Mars regolith should be further investigate. These systems can benefit from saving the human waste created on the 6 month journey to Mars, and applied to the regolith upon set-up of the early

settlements. These already processed OM from human waste may prove to be a significant contributor to the health and well-being of the initial phases in these settlements. Other methods to increase OM% by recycling unused plant parts, alternative plants grown with the sole purpose of turning into soil OM, and OM derived from other exterior sources should also be investigated. Using one, or several sources in conjunction could pose potential benefits and hazards via the microbial break down and subsequent gasses expelled into the space-system's atmosphere. Benefits and consequences of trace gas build-up with different sources of OM, and during rapid OM accumulation, are understudied in respects to a Mars planetary settlement, and will be required knowledge once humans arrive on the red planet.

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