Plant the Moon Challenge Final Report of Team Bio Luna, New Mexico State University

Team ID: 9177

# Title: Soil Amendment and Microbial Inoculation of Lunar Regolith

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## Introduction

Future long-term manned space missions to the Moon and other extraterrestrial bodies will require a sustainable food supply. The ability to grow food in situ would be ideal for maintaining human life. However, the unconsolidated rocky material found on the Moon (regolith) has not undergone pedogenic processes and does not possess the emergent qualities of soil that allow for plant growth. Regolith is lacking in available nutrients, organic matter, and biological life. Understanding how nutritious foods can be grown on these local substrates will facilitate lengthier exoplanetary explorations such as near future NASA Artemis expeditions. Research initiatives such as the Plant the Moon Challenge (PTMC) offer the next generation space biologists and environmental scientists an opportunity to contribute their ideas in developing innovative solutions to this challenge.

In Spring 2021 the NMSU Zia Luna PTMC student team carried out an experiment using compost and vermiculite amended lunar regolith to grow beans, green onions, and mushrooms. Vermiculite was used to aerate the soil and compost was used as a source of organic matter. While no growth deficiencies were observed for mushroom crops, it was noticed that the plant crops suffered from what appeared to be nutrient deficiency or toxicity. However, analysis of leachate did not indicate particularly low concentrations of essential nutrients nor high concentrations of potential toxic elements, so it was speculated that there may have been an important microbial component missing from the "soil".

On Earth there are many beneficial microorganisms helping plants to achieve their full potential. Microorganisms are important for releasing and transforming nutrients into forms that can be uptaken by plants. For example, symbiotic mycorrhizal fungi can tap into occluded inorganic phosphorus pools and extend the nutrient acquiring capacity of plants while rhizosphere microbes can protect the plant against disease. Some legume plants, like beans, may acquire rhizobia, which colonize the roots and fix nitrogen which is exchanged with the plant host for carbon.

In this round, our 2022 student team modified the experimental design with the addition of microbial inoculation of only one species vs a consortium of species. The inoculants chosen here were biological soil crust (biocrust) and the photosynthetic cyanobacteria species *Symplocastrum torsivum*. Cyanobacteria are key stone species in dryland soils weaving together soil particles, fix carbon and nitrogen and create biocrust, a living soil aggregate. Once formed, biocrusts house diverse microbial communities of other organisms such as other bacterial, fungal, and macroscopic taxa associated with the cyanobacteria and are responsible for nutrient cycling and bioweathering. The goal of this experiment was to provide insights as to what sort of soil amendments should be used and what microbial communities should be encouraged when attempting to start a self-sustaining soil system on a space station.

## Theory

For this project there were two independent factors: the type of microbial inoculum and regolith amendment. The dependent effect of these factors was tracked through the measurement of plant growth (height, leaf number, total plant biomass), plant health (leaf morphology and

damage), and regolith chemistry (pH, electrical conductivity, leachate composition). A negative control (regolith only) and a positive control (potting soil) were used as a comparison to observe potential growth effect.

Substrate amendments: In order to support bean growth, compost was added as a source of organic matter under the justification that on an established space station, any excess organic waste from food or horticultural practices could be added to an extant compost pile and recycled back into gardening. In a real situation, it would be advised to bring some amount of starter compost to establish an initial crop and subsequently, plant material from previous harvests and food waste could be used to establish a compost heap. The addition of vermiculite selected in order to provide aeration to the soil, and, in the context of a space mission, can be used as a packing material, acting as a buffer during shipping and thereafter being used for plant growing.

Microbial Inoculum: For this experiment, 3 inoculation treatments were proposed. Inoculating with cyanobacterium *Symplocastrum torsivum* would demonstrate the capacity of one organism to support plant growth through aggregate formation and the initiation of bioweathering. Inoculating with biocrust soil would demonstrate the capacity of a consortium of organisms to support plant growth through providing a multitude of biogeochemical reactions including carbon, phosphorus, and nitrogen cycling and other micro and trace nutrient cycling in addition to aggregate formation and bioweathering. Not inoculating pots would be a control. Compost also inherently hosts its own microbial consortium, however, microbes in compost are mostly heterotrophic bacteria, fungi and microscopic animals. A treatment of sterilized compost was added to test for any aid compost microbes might render.

Our project aim was to test a variety of experimental conditions to cultivate heirloom Anasazi beans (*Phaseolus vulgais* L.) in lunar regolith simulant. The Anasazi bean variety is a drought tolerant plant crop adapted to low fertility soils and have been selected and bred under arid climate for millennia.

This project had four primary hypotheses:

H1: If nutrient content is limited in regolith, then the addition of compost will produce more above ground biomass (quantified by plant height and above ground biomass weight) and overall healthier crops (quantified by leaf damage area).

H2: If nutrient content is limited and substrate is too dense in regolith, then the addition of compost plus vermiculite will produce more above ground biomass (quantified by plant height and above ground biomass weight) and overall healthier crops (quantified by leaf damage area).

H3: If beneficial soil microorganisms are not present in the regolith, then the inoculation of pots with microorganisms will produce more above ground biomass (quantified by plant height and above ground biomass weight) and overall healthier crops (quantified by leaf damage area).

H4: If increasing microbial biodiversity increases biogeochemical functionality, then inoculating pots with a consortium of microorganisms will produce more above ground biomass (quantified by plant height and above ground biomass weight) and overall healthier crops (quantified by leaf damage area).

**Measurement Methods**

Experimental design: Our full factorial experimental design included two factors: a regolith amendment factor and a microbial inoculum factor (Table 1). Five treatments were assigned as regolith amendments: 100% regolith, 50% regolith + 50% vermiculite, 50% regolith + 50% compost, 50% regolith + 50% autoclaved compost, 50% regolith + 25% compost + 25% vermiculite. For each of those regolith amendment treatments, three types of inoculation were carried out: None, *Symplocastrum torsivum*, and biological soil crust. For each of these conditions, there were five replicate pots filled 500 ml substrate, except in the regolith + sterilized compost treatment where there were only 4 pots and one of those reps only contained 400ml substrate material due to limited regolith material (= a total of 72 experimental pots). The 100% regolith with no inoculation was treated as a negative control. An additional five pots were filled with potting soil as a positive control (although only 3 of the 5 ended up seeded).

**Table 1.** Treatment listed at the top and inoculant listed on the left. Regolith + Sterilized Compost treatment differs in pot number due to limited materials.

| | Regolith Only | Regolith + Compost | Regolith + Sterilized Compost | Regolith + Compost + Vermiculite | Regolith + Vermiculite |
|---|---|---|---|---|---|
| Single Cyanobacterial Species | 5 pots with 500 ml each | 5 pots with 500 ml each | 3 pots with 500 ml, 1 with 400 ml | 5 pots with 500 ml each | 5 pots with 500 ml each |
| Biocrust (mixed species) | 5 pots with 500 ml each | 5 pots with 500 ml each | 3 pots with 500 ml, 1 with 400 m | 5 pots with 500 ml each | 5 pots with 500 ml each |
| None | 5 pots with 500 ml each | 5 pots with 500 ml each | 3 pots with 500 ml, 1 with 400 m | 5 pots with 500 ml each | 5 pots with 500 ml each |

Biocrust samples were collected from the Jornada LTER, NM (32°30’37.2” N; -106°44’32” W). We collected lichen crusts focusing on *Clavascidium* spp. and *Peltula* spp., two common biocrust lichens, for our experiment. Biocrust samples were homogenized by gently crushing the soil crust aggregates through a 2 mm sieve. Biomass of the cyanobacterium species *Symplocastrum torsivum* was grown in liquid Z8 media (Carmichael 1986) in flasks starting as a subculture from the terrestrial cyanobacterial culture collection of the NMSU Dryland Microbes lab.

The experiment began on February 7. We used 9cm x 9cm x 13cm square tapered pots. A coffee filter was placed as a liner within each pot to minimize loss of substrate from the drainage holes. Regolith-only pots were filled with 500ml of material measured in a 250ml graduated cylinder, which was filled and tapped against the counter until the material settled and refilling to 250ml to standardize volume. The same procedure was carried out for all pots filled with 50%

regolith and 50% amendment (by volume) but adding 250ml of regolith only. Pots with the vermiculite and compost amendment were filled with 50% regolith (250ml), 25% compost, and 25% vermiculite (125ml for each). Measured volumes of regolith material were poured into a large plastic bag and shaken to homogenize and loosen the material before pouring into pots. The compost added was sourced from the NMSU Compost Club's compost pile and sterilized compost was autoclaved twice before addition to pots. Pots were placed into 40oz Ziplock bags in order to catch draining leachate for analysis. Three Anasazi beans (*Phaseolus vulgais* L.) were planted at 2cm and carefully buried. Pots were watered with 80ml initially and 10ml added until leachate began draining from the bottom of the pot.

After watering, assigned microbial pots were inoculated (Fig. 1). Approximately 9ml of crushed biocrust was sprinkled on the moist surface of biocrust treatment pots. Biomass of the cyanobacterium species *Symplocastrum torsivum* was teased apart with sterile tweezers, gently applied to the pot, and spread across the surface of the single cyanobacterial treatment pots (no more than 3g added).

Figure 1. Initial inoculation treatments for regolith only immediately after application.

After the initial watering and leachate analysis, leachate was collected on the Tuesday of weeks 3, 6, 8 and 10. Pots were placed into 40oz Ziplock bags to catch the leachate. 60ml of water was added to each pot and allowed to drain for five hours. The bags were then collected, and the five replicates of each treatment were composited into a 250ml flask to have enough volume to conduct analysis. Each composite sample was then partitioned into two 25ml vials. One vial was preserved with 1+1 trace pure $HNO_3$ until the pH is less than 2 to bring the elements into solution. Leachate was analyzed for 28 elements (Al, As, B, Ba, Be, Cd, Co, Cr, Fe, Mn, Mo, Ni, Pb, Se, Tl, V, Zn, Bi, Ca, Li, Mg, P, Sr, K, Si, Na, S, Cu) via Perkin Elmer Optima 4300DV ICP-OES Method EPA 200.7. The second vial is analyzed within two days of collection via the Technicon Autoanalyzer method EPA 353.2 for nitrate composition and method SM 4500 Cl-E for chloride. The leachate was also analyzed for pH and EC using

Fisherbrand accument Benchtop Laboratory Meter and methods EPA 150.1 for pH and SM 2510B for EC.

Pots were initially placed under grow lights in a laboratory growth room (Fig. 2) and watered every other day with 30ml with a 16hr/8hr light dark cycle. One replicate of each treatment plus a positive control were blocked on a tray flat (Fig. 2) Growth room temperature was measured at ~20-25˚C throughout the day. After 5 days (20 ml) of Z8 media was added to assist in the growth of algae. Culling took place after any one plant in each pot was taller than 5cm, plants were cut back to the surface of soil with scissors sterilized with ethanol.

Figure 2. Tray containing all treatments in week 1.

After 2 weeks, pots were moved to the greenhouse (Fig. 3 B-D) where they were watered with 60ml every day, unless pots appeared damp at the surface. In the greenhouse, trays were rotated every week to combat uneven lighting. During the greenhouse move one of those control plants snapped and died shortly after. After 3 weeks 50ml of Hoagland solution was added as a fertilizer to each pot. Only one addition of fertilizer was added to support the plants while the algae was slowly establishing at the surface of the pots.


Figure 3. A) Plants after 2 weeks. B) Plants after 3 weeks. C) Plants after 8 weeks. D) Plants after 10 weeks.

Emergence of the beans was tracked daily. Every Monday, plant height and leaf number were measured and recorded (until week 7 for height and week 6 for leaf number). Plant height was measured from the base of soil to the apical meristem.

Measurement of pH was carried out at the beginning, in the middle and end of the experiment to minimize disruption of microbial biomass (week 1, week 6, and week 10). In order to collect pH, a pH probe was pressed to the surface of water saturated regolith until measurement stabilized.

At the conclusion of the experiment on April 15th, plant height, root weight, extent of algal colonization, bean size, bean count, bean weight, quantification of rhizobia colonization, and above ground biomass were examined. Extent of algal colonization was assessed by imaging the surface of every pot and making a visual assessment of: No cover, partial cover, or total algal cover. For microscopic investigation of microbial cover, each pot in reps 3 and 5 was sampled using sterile technique. A sterile 0.5cm circular cookie cutter was used to collect 3 $<0.5cm^3$ soil cores, samples were stored at 4˚C until imaged with inverted Zeiss Axiovert 100 microscope. Above ground biomass was separated out into beans, stem, and leaves and preserved for plant tissue analysis. Roots were carefully separated from surrounding soil and washed for 10 sec in phosphate free soap (micro 90) diluted with reverse osmosis (RO) water and 10 sec in RO water. Surface and subsurface biomass were left to air dry for future tissue analysis. Roots were additionally weighed after a week of drying.

Analysis of all plant data was carried out in R studio (1.4.1717) (R 4.1.2). An anova was carried out for analysis of pH differences within each week data was collected. Leachate chemistry data was explored with a principal component analysis in order to categorize differences in grouping trends. Specific leachate analysis was carried out in Excel.

**Analysis & Results**

The percentage of emergent plants was greatest in the control, followed by treatment RVC and RV, R, RC, and RS, respectively (Fig. 4B). Microbial inoculation showed no difference in plant emergence (Fig. 4A).

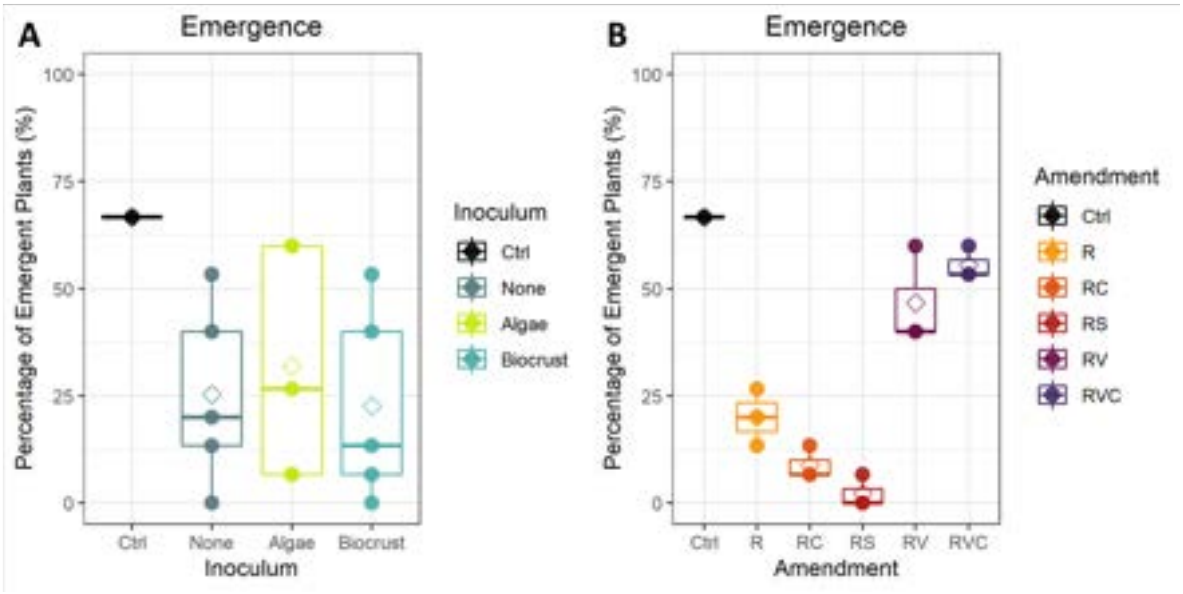


Figure 4. Percentage of plants that emerged from each treatment, where *Ctrl* is potting soil positive control, *R* is regolith only, *RC* is 50% regolith + 50% compost, *RS* is 50% regolith + 50% sterilized compost, *RV* is 50% regolith + 50% vermiculite, and *RVC* is 50% regolith + 25% vermiculite + 25% compost. *None* indicates no microbial inoculation, *Algae* indicates inoculation with cyanobacteria *Symplocastrum torsivum,* and *Biocrust* indicates inoculation with biocrust.

For pH in week 1, the potting soil control (Ctrl) treatment was significantly different from all other treatments (<0.0005), RB was significantly different from RA and RCN (<0.05), and RVCB was significantly different from RA and RVCN (<0.05). For pH in week 6, the potting control (Ctrl) was significantly different from all other treatments (<0.05). For pH in week 10, the potting soil control (Ctrl) was significantly different from all other samples (<0.05), except RSA, RVA, and RVB (Fig 5, Table 2).

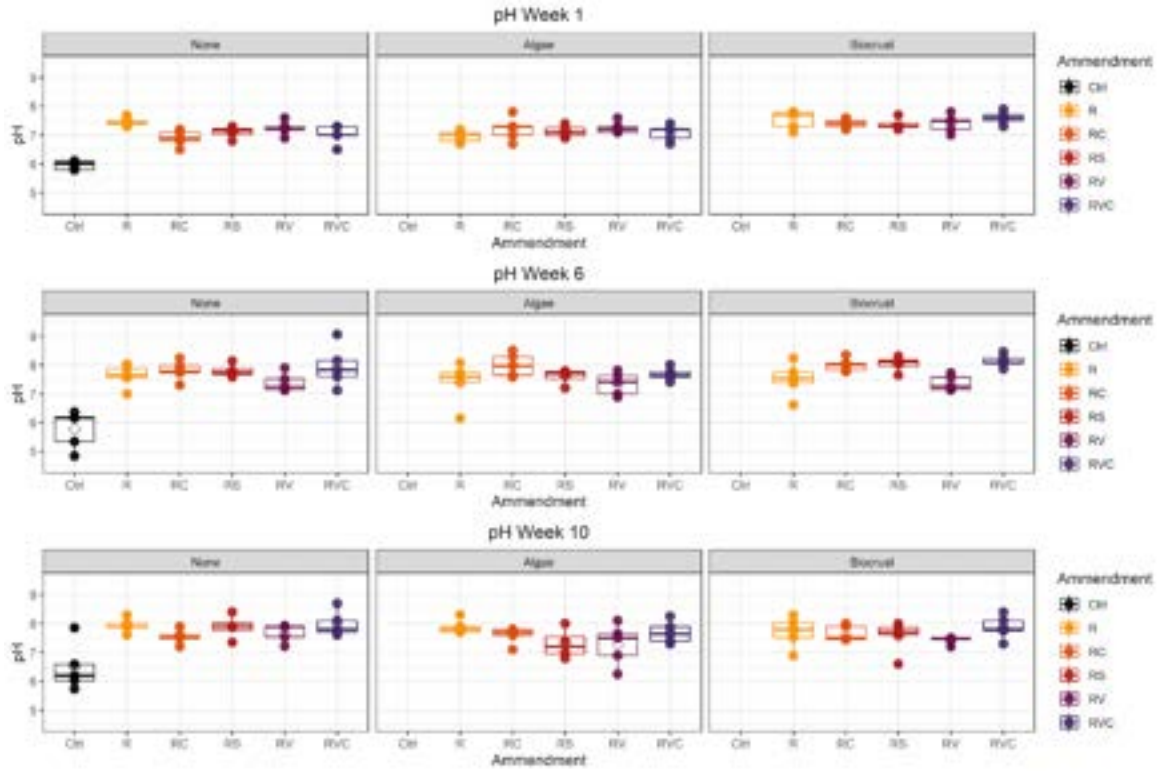


Figure 5. pH for Week 1 (top), Week 6 (middle), Week 10 (bottom), where *Ctrl* is potting soil positive control, *R* is regolith only, *RC* is 50% regolith + 50% compost, *RS* is 50% regolith + 50% sterilized compost, *RV* is 50% regolith + 50% vermiculite, and *RVC* is 50% regolith + 25% vermiculite + 25% compost. *None* indicates no microbial inoculation, *Algae* indicates inoculation with cyanobacteria *Symplocastrum torsivum,* and *Biocrust* indicates inoculation with biocrust.

Table 2. pH for Weeks 1, 6, and 10 where *Ctrl* is potting soil positive control, *R* is regolith only, *RC* is 50% regolith + 50% compost, *RS* is 50% regolith + 50% sterilized compost, *RV* is 50% regolith + 50% vermiculite, and *RVC* is 50% regolith + 25% vermiculite + 25% compost. *N* indicates no microbial inoculation; *A* indicates inoculation with cyanobacteria *Symplocastrum torsivum,* and **B** indicates inoculation with biocrust.

| Treatment | Week 1 | Week 6 | Week 10 |
|---|---|---|---|
| **Ctrl** | 5.96 | 5.78 | 6.48 |
| **RA** | 6.96 | 7.39 | 7.88 |
| **RB** | 7.54 | 7.50 | 7.71 |
| **RN** | 7.46 | 7.62 | 7.93 |
| **RCA** | 7.22 | 8.00 | 7.60 |
| **RCB** | 7.40 | 8.00 | 7.66 |
| **RCN** | 6.90 | 7.81 | 7.54 |
| **RSA** | 7.13 | 7.60 | 7.30 |
| **RSB** | 7.38 | 8.03 | 7.56 |
| **RSN** | 7.10 | 7.80 | 7.89 |
| **RVA** | 7.26 | 7.35 | 7.28 |
| **RVB** | 7.40 | 7.37 | 7.43 |
| **RVN** | 7.24 | 7.38 | 7.69 |
| **RVCA** | 7.08 | 7.68 | 7.70 |
| **RVCB** | 7.60 | 8.14 | 7.87 |
| **RVCN** | 7.02 | 7.95 | 7.98 |

An anova was run for each plant measurement (plant height, root biomass, bean biomass, and total above ground biomass) using treatment as an explanatory variable. There were no significant differences (height P=0.37, root biomass P=0.63, bean biomass P=0.063), except in total above ground biomass where treatment was significant (P=0.04), but there were no individual differences within the data. Generally for biomass measurements, trends remained broadly similar whether total above ground biomass, bean biomass, or stem only biomass was the focus (Fig. 6). The greatest average biomass (for bean and total) after the potting soil control was seen in the RVC treatments (Fig. 6A, D). The biomass found in RVC treatments was similar to the biomass measures found in RC and RS treatments, however, these treatments only had one rep due to low instances of emergence. Treatments RV and R had the second lowest and lowest biomass measurements, respectively. Root biomass of RVC was highly variable but included pots with the greatest amount of root biomass (Fig. 6C). Treatments RVC, RV, RS, and potting soil control overlapped in their root biomass for Algal and Biocrust inoculation but treatment RV with no inoculation had lower root biomass. Treatment R had the lowest root biomass averages, with the treatment with no inoculation being the lowest of the inoculations.

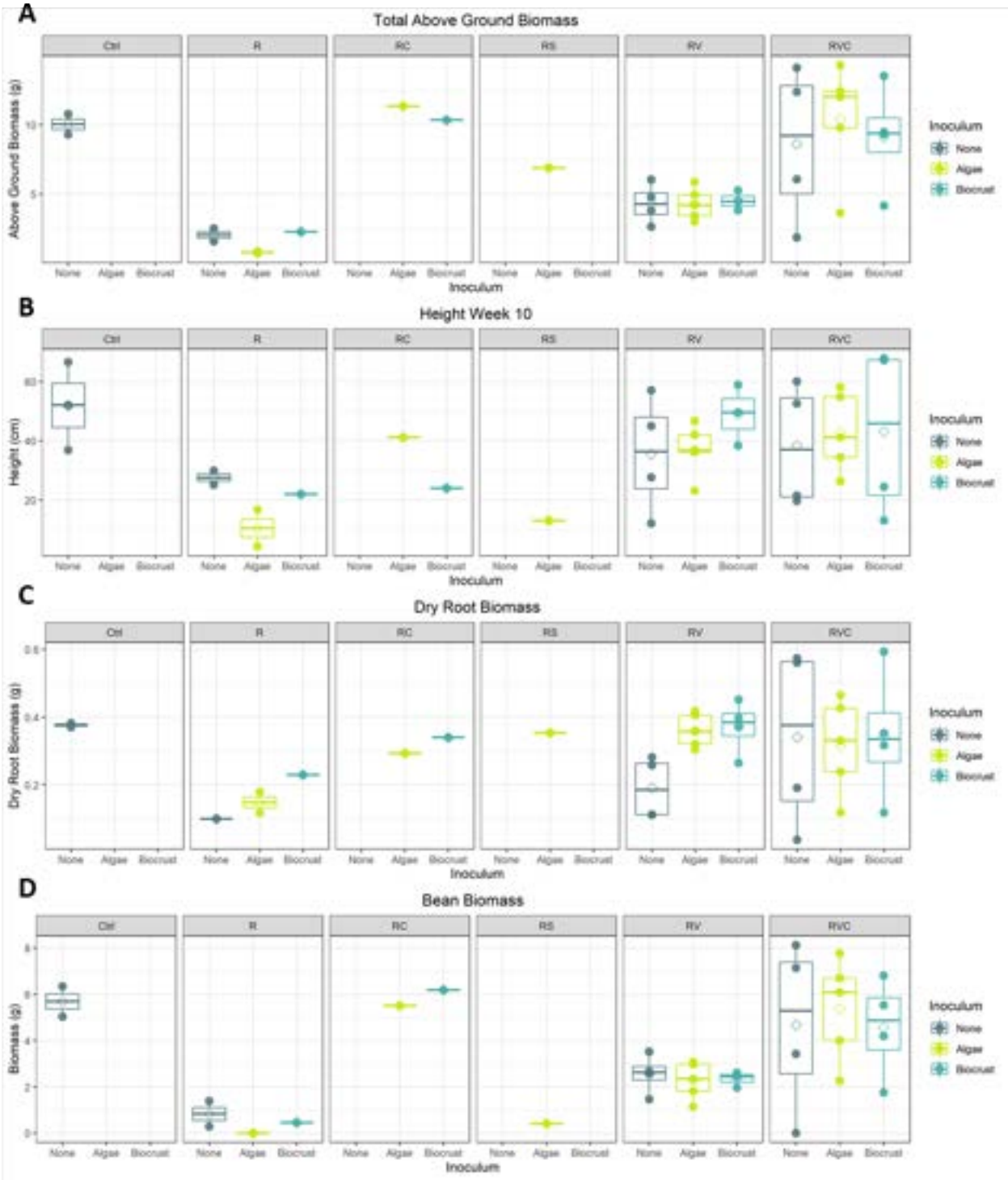


Figure 6. Total above ground biomass (A), plant height at week 10 (B), dry root biomass (C), and bean biomass (D) grouped by Amendment, where *Ctrl* is potting soil positive control, *R* is regolith only, *RC* is 50% regolith + 50% compost, *RS* is 50% regolith + 50% sterilized compost, *RV* is 50% regolith + 50% vermiculite, and *RVC* is 50% regolith + 25% vermiculite + 25%

compost. *None* indicates no microbial inoculation, *Algae* indicates inoculation with cyanobacteria *Symplocastrum torsivum,* and *Biocrust* indicates inoculation with biocrust.

The presence of rhizobia formed nodules (Fig. 7) was noted in 100% of Ctrl pots with live plants (2), rhizobia were noted in none of the R or RS pots, 50% of RC pots with plants (2 pots), 33.4% of RV pots with plants (4 out of 11), and 45.5% of RVC pots with plants (5 out of 11).

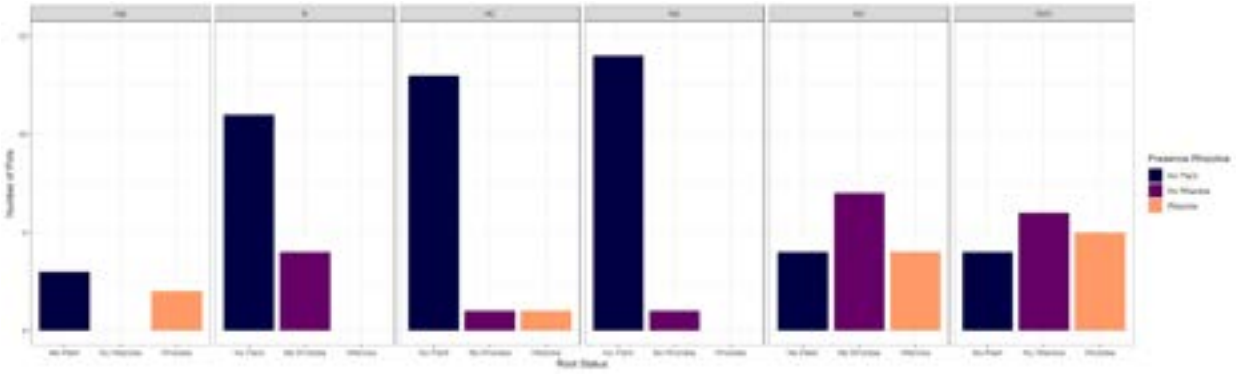

Figure 7. Tally of pots with rhizobia presence, where Ctrl is potting soil positive control,where *Ctrl* is potting soil positive control, *R* is regolith only, *RC* is 50% regolith + 50% compost, *RS* is 50% regolith + 50% sterilized compost, *RV* is 50% regolith + 50% vermiculite, and *RVC* is 50% regolith + 25% vermiculite + 25% compost. *No Plant* indicates no emergent plant within the pot, *No Rhizobia* indicates plant presence but no nodules, *Rhizobia* indicates the presence of nodules.

The first flowers were spotted in week 7 and beans began developing shortly after. Our experiment could have been terminated in week 8 or 9 based on the apparent life cycle of these plants. By the end of 10 weeks most plants appeared to be at the end of their life (Fig 8C). Possibly, the plants could have lived longer if not for nutrient issues, leaf spotting was first noticed in week 5 and after 6 weeks leaves were falling off plants to the extent that leaf counting was halted (Fig. 8B).

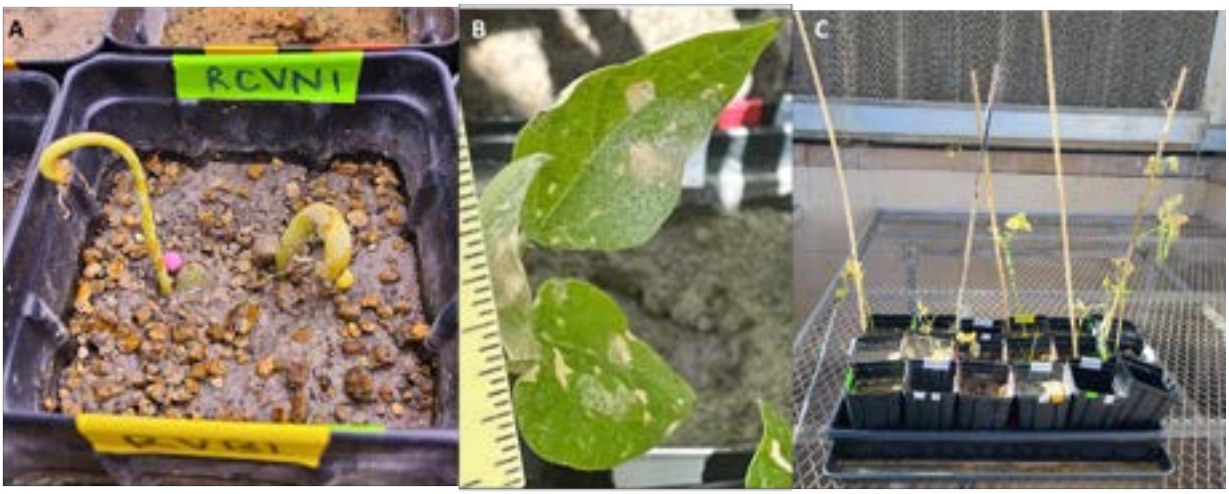


Figure 8. A) Roots emerging before the stem. B) Leaf spotting. C) Week 10 tray.

PCA plot grouping was strongest according to week, pots becoming more and more similar (as demonstrated by narrowing ellipses in (Fig. 9) as weeks went on. Component 1 explained 56.2% of variability and eas strongly correlated with Mg, EC, Bi, Ca, NO3, Sr, K, Na, Ba, PB, S, Cd, Chloride, B, Li, P, As, Mn, and pH (-0.998, -0.997, -0.997, -0.996. -0.99, -0.99, -0.99, -0.99, -0.99, -0.98, -0.97, -0.95, -0.94, -0.94, -0.84, -0.86, -0.75, -0.71, and 0.71, respectively). Component 2 explained 12.6% of variability and was moderately to strongly correlated with Fe, Co, Ni, Cr, Zn, and Si (correlation of 0.91, 0.8, 0.79, 0.73, 0.69, and 0.54, respectively).

The leachate data was reviewed for trends and possible indicators of plant growth limiters. Most macro and micronutrients decreased after successive watering events. Two macronutrients, phosphorus and nitrate, were of particular interest. The phosphorus concentration was below 20 ppm from the beginning of the growing period and the nitrate concentration was below 5 ppm in all treatments except the control by day 36 (Fig. 10). These nutrient levels are insufficient for most crop plants. Additionally, leachate aluminum concentration increased in all treatments, exceeding 2 ppm by day 36 except for RC. Aluminum is toxic to most plants and may have unfavorable effects on plant growth.

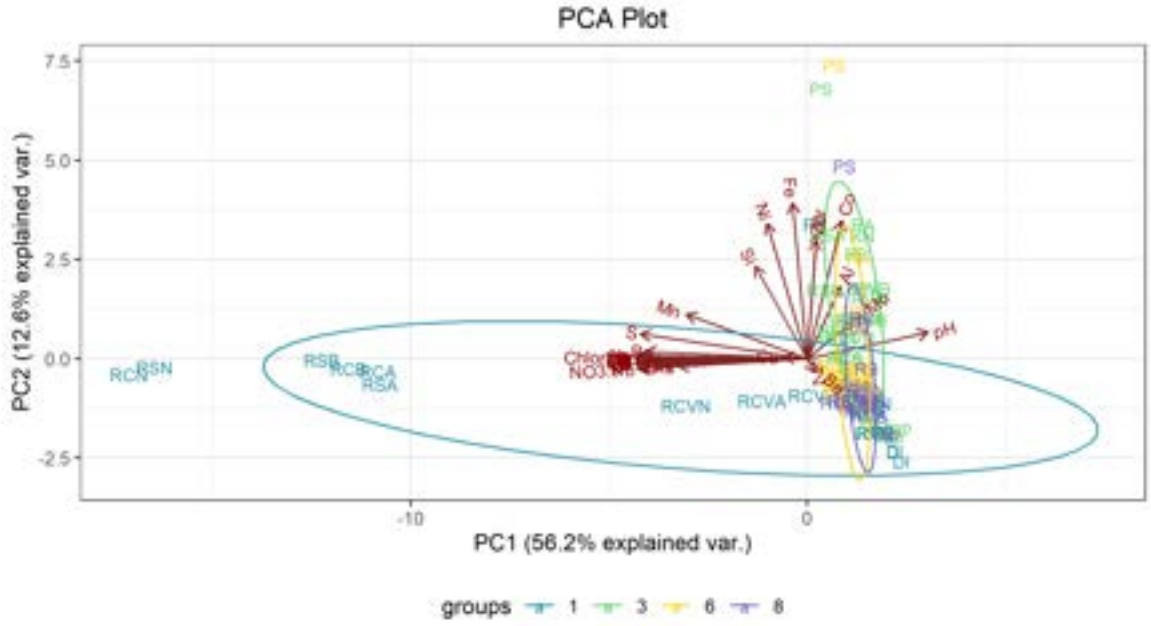


Figure 9. PCA plot for leachate analysis where groups are colored according to the week collected (weeks 1, 3, 6, and 8) and points are named according to treatment.

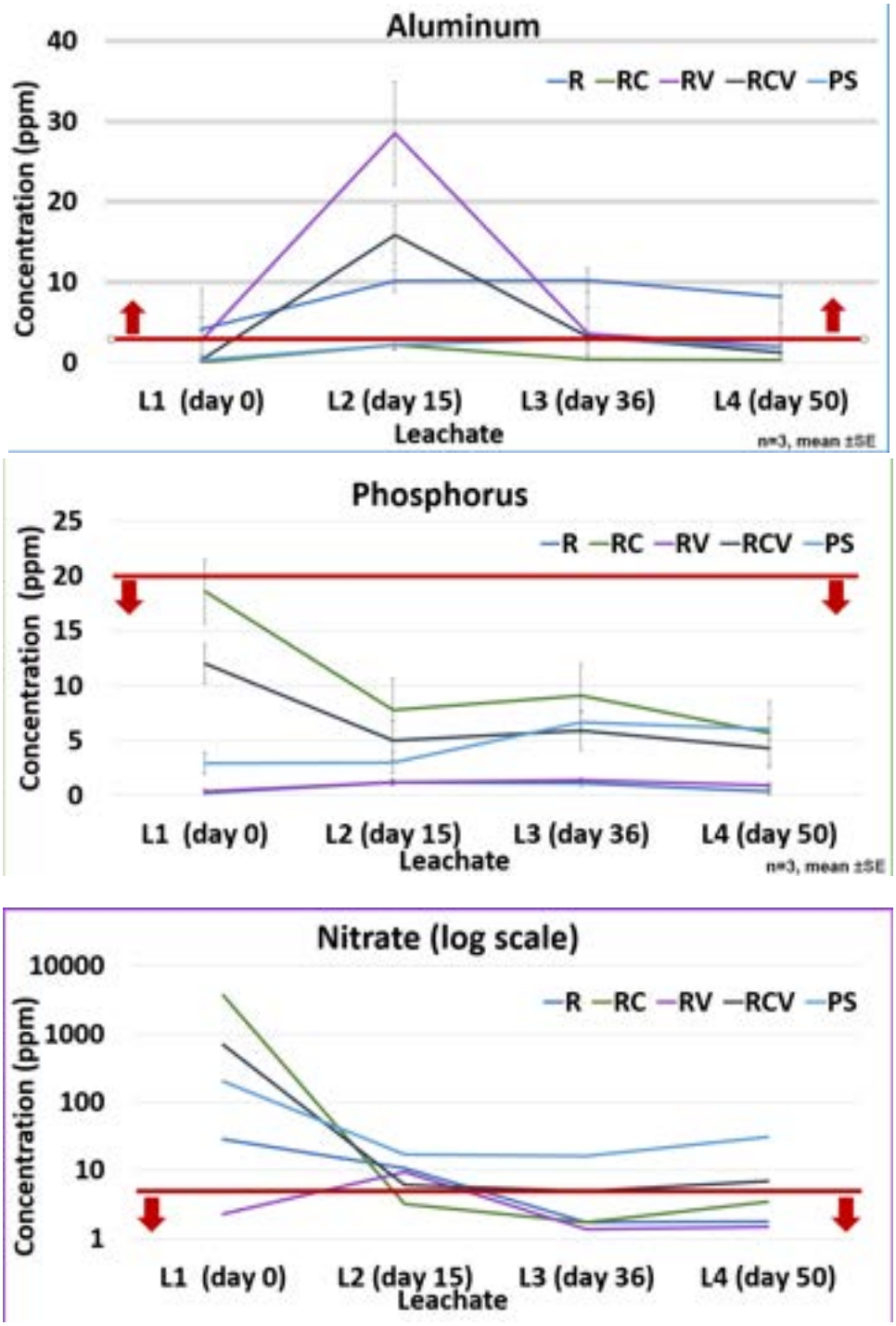


Figure 10. Leachate Aluminum (Top), Phosphorus (Middle), and Nitrate (Bottom) concentrations (ppm) over the course of the experiment, where *R* indicates Regolith only, *RC* indicates Regolith + Compost, *RV* indicates Regolith + Vermiculite, RCV indicates Regolith + Compost + Vermiculite, and *PS* indicates potting soil. Each treatment was a composite sample.

Nearly all tested samples were colonized by diatoms and green algae (Fig. 11), likely acquired from the air inside the greenhouse or from the greenhouse tap water. Evidence of

colonization by filamentous cyanobacteria and fungi were also found across an assortment of treatments without any strong pattern. This lack of pattern indicates widespread cross contamination of pots. All reps were held in one tray, making it easy for contamination while watering or by airflow. If this experiment were to be repeated, more biomass should be used to ensure strong establishment in pots that were inoculated to contrast to the slow contamination of other pots. Establishment of visible algal cover was greatest in biocrust inoculated pots (Fig. 12, 13), but there appeared to be more instances of algal establishment in the non-inoculated pots than those inoculated with *Symplocastrum torsivum.*

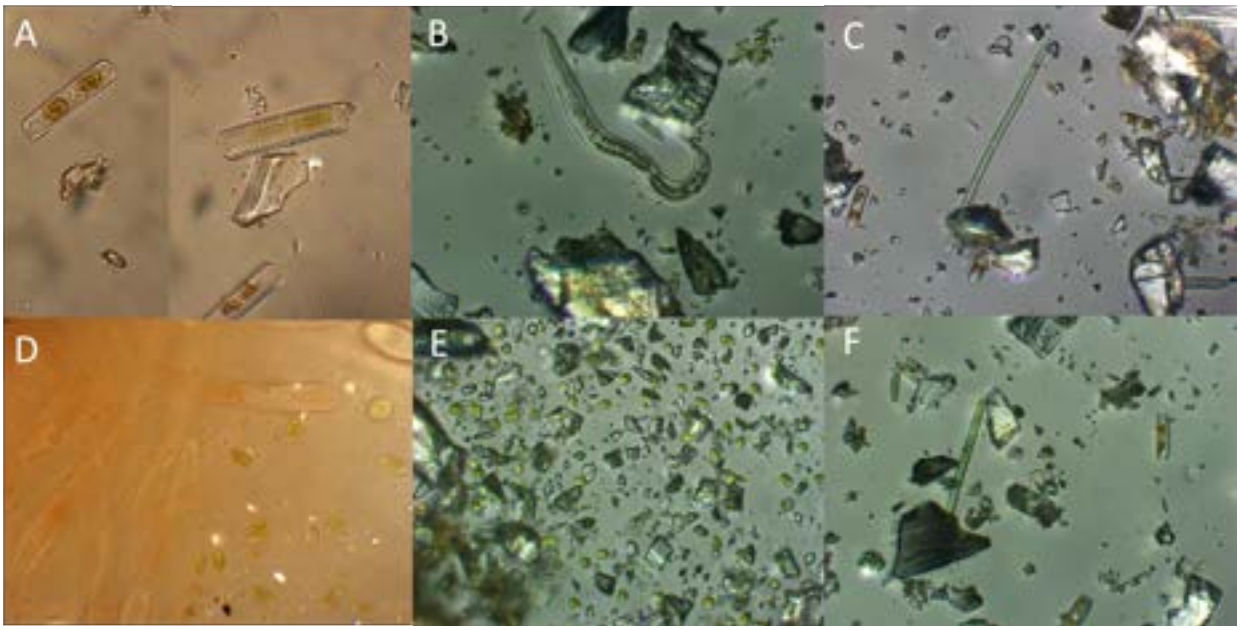


Figure 11. Images taken with a Zeiss Axiovert 100 microscope. A) Diatoms at 100x. B) Nematoad 40x. C) Filamentous cyanobacteria 40x. D) Fungi 100x. E) Green algae 40x. F) Filamentous cyanobacteria 40x.

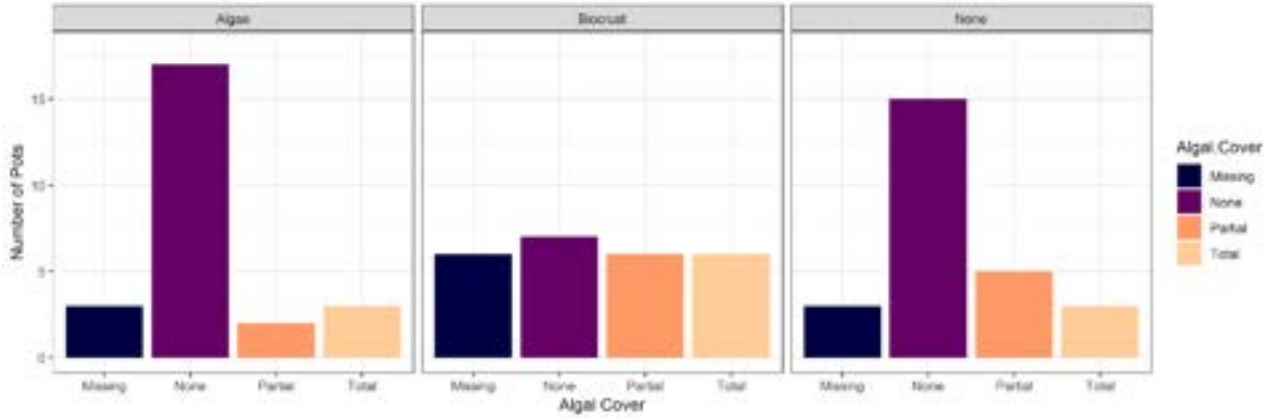


Figure 12. Tally of pots with categories of algal cover, grouped by type of inoculum where *None* indicates no inoculation, *Algae* indicates inoculation with cyanobacteria *Symplocastrum torsivum,* and *Biocrust* indicates inoculation with biocrust and where *Missing* indicates that the

picture of algal cover was missing, *None* indicates no visible cover, *Partial* indicates patchy partial cover, and *Total* indicates substantial cover.

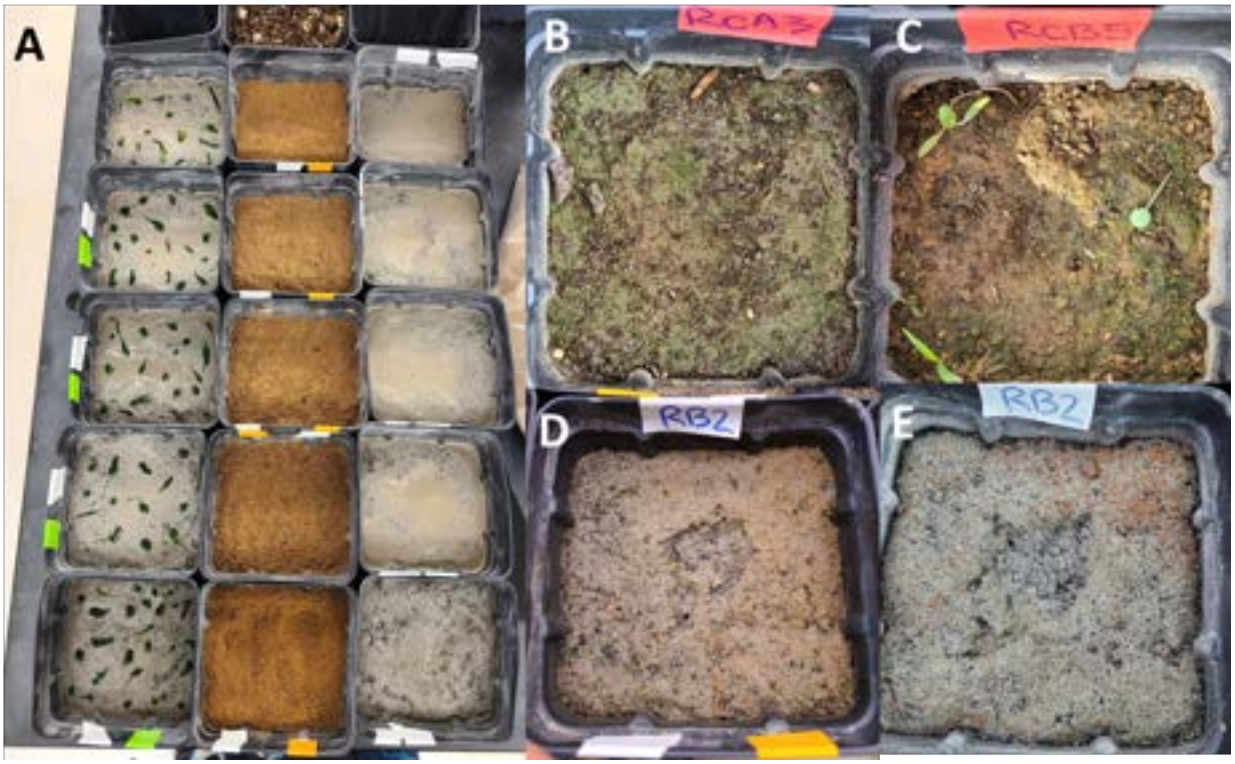


Figure 13. A) Initial inoculation treatments for regolith only immediately after application. B-C) Two pots were algae visibly established at the surface. RB2 after 3 weeks (D) and after 10 weeks (E) to show that the regolith material coated the added soil.

**Discussion & Conclusions**

This year our student team competed in the plant the moon challenge for the 2nd time. Many observations and patterns seen during last year's experiment of bean seedling emergence, early establishment, and overall plant growth in lunar regolith were supported with data obtained this year. *However, we gained new insights into the specific constraints of plant growth in regolith, regolith chemistry, and the types of microbes that can colonize this material.*

*Emergence*

A greater percentage of emergent plants in RV and RVC than other treatments (Fig. 4B) is likely due to the presence of vermiculite providing more pore space aerating the soil. It was noted that in a number of pots (RN3, RVB3, RA3, RCN4, RVCN1, RVCB2, RVCN3), roots emerged before the stem of the plant (Fig. 8A). One possible explanation could be that the regolith material was so dense and difficult to penetrate that the path of least resistance was upward to the surface. The low number of emergent plants in RC and RS and R may likely be due to the density of the material suffocating the plants before they could break the surface. The addition of organic matter in treatments RC and RS may have exacerbated seedling mortality by

introducing microbes that rotted the seedlings in contrast to the R treatment which had more instances of emergence.

*Leaf damage*

Extensive leaf damage was seen across all treatments, including the potting soil controls. One possible explanation for the damage could be over/underwatering of some pots. The regolith material alone holds water for extended periods of time without allowing for drainage, meanwhile, amended samples each had different water holding capacity. All pots were watered with the same amount, possibly leading to the development of anoxic zones within some treatments. When cutting open pots for root analysis some pots (especially the RS treatment) smelled like hydrogen sulfide, which suggests the development of anaerobic activity. In the case of leaf damage in the potting soil controls, a lack of watering was the likely culprit because the potting soil dried so much more quickly. Another possibility for leaf damage in treatments would be aluminum toxicity and/or nitrogen and phosphorus deficiency as indicated by leachate analysis (Fig. 10). Additionally, the 1st week of leachate analysis was the most dissimilar to the following weeks and indicates that with successive watering nutrient composition became more and more similar (Fig. 9) likely because nutrients were being flushed from the regolith.

*Biomass*

No difference in above ground biomass between RVC, RC, and RS was detected (Fig. 6A) due to only one rep exiting in the latter two treatments as a product of low emergence (Fig. 4B). However, the fact that these three treatments had similar levels of biomass in comparison to the lower biomass in RV and R suggests that the addition of compost as a soil amendment increases plant biomass. Additionally, treatment RV had the second lowest biomass measures next to the lowest biomass measures seen in treatment R, suggesting that the density of the material also had some impact on the plant's capacity for growth, possibly interfering with plant nutrient uptake. Root biomass in treatment RV was similar to that of RVC, RC and RS, while treatment R had the lowest root biomass averages (Fig. 6C). This indicates that the presence of vermiculite and compost were beneficial for root growth. There was an indication that inoculation may have benefited root growth as in treatments R and RV the no inoculation treatments had the lowest biomass levels.

*Microbes*

The presence of root nodules was expected in the potting soil and treatments with compost (RVC and RC) because those treatments would contain an assortment of soil microbes, rhizobia among them. However, nodules were also observed in the RV treatment (though not in the RS or R treatments). This may indicate that there are Rhizobia associated with the bean seeds themselves that establish when conditions are optimal (as they may have been RV due to aeration by vermiculite).

Inoculation treatments did not have a significant effect on our response variables measured, except possibly root biomass. These treatments could have had an effect if more inoculum material was added. No more than 3g of algae were added to each pot.This was not

enough algae biomass to cover the entire surface and when water was applied, the algae became coated with the fine particles of the regolith (Fig. 13). We recommend future experiments to add more biomass, perhaps in combination with some inert material like small glass beads to allow the algae to colonize the surface of the pot. Similarly, only 9ml of biocrust was applied to each pot, only enough to cover the surface (less than 2 mm). Again, when watered, the material was coated by the fine particles of the regolith. The biocrust microbial consortia established on the regolith better than the algae (Fig. 12)and was able to establish a surface aggregate in a number of pots. To assess this treatment again, more biocrust material should be applied to the surface of the pot. Additionally, a number of pots with no inoculation were colonized by algae and investigation with the microscope indicated widespread contamination between treatments. In order to prevent contamination, a downward facing fan may be required to prevent the spread to algae, separation of treatments into different locations, or application of substantially more biomass. In future experiments would also benefit from a standardized method of crust establishment assessment. The surface of each pot should be allowed to dry completely before the end of the experiment in order to test surface stability for biocrust formation.

*Hypotheses*

H1: If nutrient content is limited in regolith, then the addition of compost will produce more above ground biomass (quantified by plant height and above ground biomass weight) and overall healthier crops (quantified by leaf damage area).

Above ground biomass and bean biomass was greater in treatments including compost (RVC, RC, and RS (though there was only 1 RS rep)), than those without (R and RV). By the time of the experiment conclusion many plants were so damaged that proper analysis to quantify leaf damage was not possible.

H2: If nutrient content is limited and substrate is too dense in regolith, then the addition of compost plus vermiculite will produce more above ground biomass (quantified by plant height and above ground biomass weight) and overall healthier crops (quantified by leaf damage area).

Above ground biomass and bean biomass was greater in RVC treatments on average than RV and R treatments, however RVC treatments were the most variable in their results. RV biomass was greater than negative control (R). RV and RVC also had greater instances of emergence than other treatments. This indicates that the substrate was dense enough to effect emergence and growth and plants benefited from the combination of compost and vermiculite.

H3: If beneficial soil microorganisms are not present in the regolith, then the inoculation of pots with microorganisms will produce more above ground biomass (quantified by plant height and above ground biomass weight) and overall healthier crops (quantified by leaf damage area).

There was no significant effect of inoculation on any of the measured values, above ground biomass aside from some indication of increased root biomass in R and RV treatments.

H4: If increasing microbial biodiversity increases biogeochemical functionality, then inoculating pots with a consortium of microorganisms will produce more above ground biomass (quantified

by plant height and above ground biomass weight) and overall healthier crops (quantified by leaf damage area).

Not enough microbial inoculum  may have been used to investigate plant growth promoting or inhibiting effects of soil microbes. Contamination led to colonization throughout treatments.

*Conclusions*

The addition of vermiculite led to increased emergence and plant biomass likely due to increase of pore space in the regolith material. The addition of compost produced more biomass in surviving plants. The combination of regolith and compost produced the most favorable conditions for plant growth. The inoculation treatments had no significant effect, likely because not enough microbial biomass was used in each pot to establish distinctive treatments. Future experiments should focus on adding additional amendments or fertilizing in order to eliminate toxins and deliver plants limited nutrients like nitrate and phosphate nutrients. A standardized watering regime would also be desired to balance the amount of water being delivered to treatments with different water holding capacities. Additionally, future experiments involving inoculation should add a greater amount of inoculum to the surface of pots or use amendment that will stop the regolith from coating and "swallowing" all microbial additions. Although this experiment has concluded for the sake of this competition, further analysis of plant tissue and the week 10 leachate analysis will still be carried out by students working on funded projects.

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**References**

**Appendices**

All data associated with this project can be found at:
https://github.com/mhoellrich/PTMC-Spring-2022