

Bush Beans Grow, Flower, and Begin Fruit Production in Washed LHS-1 Supplemented With Less Than 10% Organic Matter

Plant The Moon Challenge Spring 2023

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Challenge:	Plant the Moon
Challenge Division:	Professional
Location:	Our Family Home, St. Louis, Missouri, USA

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ABSTRACT

Returning to the Moon and undertaking long-duration human exploration on the Moon are primary goals of NASA's Artemis Program. To sustain extended human exploration on the Moon, we must learn to grow crops using in situ resources. Some crops have already been grown in lunar regolith simulants, but more research is needed to determine the most efficient way to produce plants in such a harsh environment. Growing crops in lunar regolith simulants, such as LHS-1, requires overcoming multiple chemical and physical challenges, including the absence of essential elements like Nitrogen, Sulfur and Chlorine, toxic concentrations of elements such as Calcium, very high pH, and poor drainage qualities. Many prior studies have sought to overcome these challenges by using large quantities of organic matter (50%), which does not directly address the toxicity caused by high calcium. To expand our knowledge of growing crops on the Moon, we chose to study Bush Bean growth in LHS-1. Through our research, we aimed (1) to grow Bush Bean plants that produced fruit, (2) use less organic matter and amendments than previous research, and (3) demonstrate the efficacy of in situ resources, such as biosolids, on crop production. Two innovative features of our approach are (1) washing LHS-1 with distilled water to reduce toxic concentrations of Ca before using it as a growth substrate, and (2) the design and implementation of a Soil Atmosphere Circulation System to enhance drainage and maintain the soil atmosphere. We show that Bush Beans can germinate, grow, flower, and begin to produce fruit in LHS-1 amended with less than 10% organic matter after 44 days of growth. We believe this growth reflects the value of washing the LHS-1 prior to using it as a growth substrate and of maintaining the soil atmosphere.

INTRODUCTION

Returning to the Moon and establishing a human presence on the Moon is a primary goal of NASA's Artemis program. Essential to any long-duration human presence on the Moon is a reliable food source. It currently costs approximately \$100,000 to send each pound of material to and from the Moon (Jones 2022). Given that cost, it is imperative to limit the amount of material sent between the Moon and Earth. In May 2022, Paul et al. (2022) grew the model plant *Arabidopsis thaliana* in lunar regolith obtained from the Apollo missions to the Moon, demonstrating the potential for growing crops via in situ resource utilization (ISRU).

Multiple crops have already been grown in lunar regolith simulant. Previous studies have grown tomato, cress, radish, rye, rocket, quinoa, and peas (Wamelink et al. 2019, Wamelink et al. 2014). Consistent with the growth experiments in actual lunar regolith (Paul et al. 2022), plants grown in lunar regolith simulant tend to exhibit reduced growth, flowering and fruiting compared to controls. The ability to grow crops on the Moon in sufficient varieties and quantities to support human health remains largely unknown. Much more research is needed for crop production on the Moon via ISRU to become a reality.

Growing crops successfully in lunar regolith simulant presents multiple challenges related to its chemical and physical composition. Chemically, lunar regolith simulants, such as LHS-1, contain insufficient amounts of some elements and excessive quantities of other elements for crops. Plant growth requires at least 16 essential elements obtained from soil (Taiz & Zeiger 2002). Some of these elements, including Nitrogen (N), Sulfur (S), and Chlorine (Cl), are completely absent from LHS-1 (Exolith Labs, 2022). Conversely, elements such as Calcium (Ca) are present in detrimental amounts. LHS-1 contains 9.36% Ca in the form of calcium oxide (Exolith Labs, 2022), which is more than 4X the Ca needs for most plants (Kelling & Schulte 2004, Broadly et al. 2003). Excess Ca leads to toxicity by increasing pH and inhibiting nutrient uptake by plants (White & Broadly, 2003). LHS-1 also contains sodium (Na) in the form of Na₂O, which can contribute to salt stress (Doungous et al. 2022; Shi et al. 2017). Physically, LHS-1 contains particulates that are prone to forming agglutinates. These agglutinates decrease the movement of water and gases in the soil atmosphere, which can inhibit plant growth. Collectively, the chemical and physical properties of LHS-1 make using it as a primary substrate for growing crops a formidable challenge.

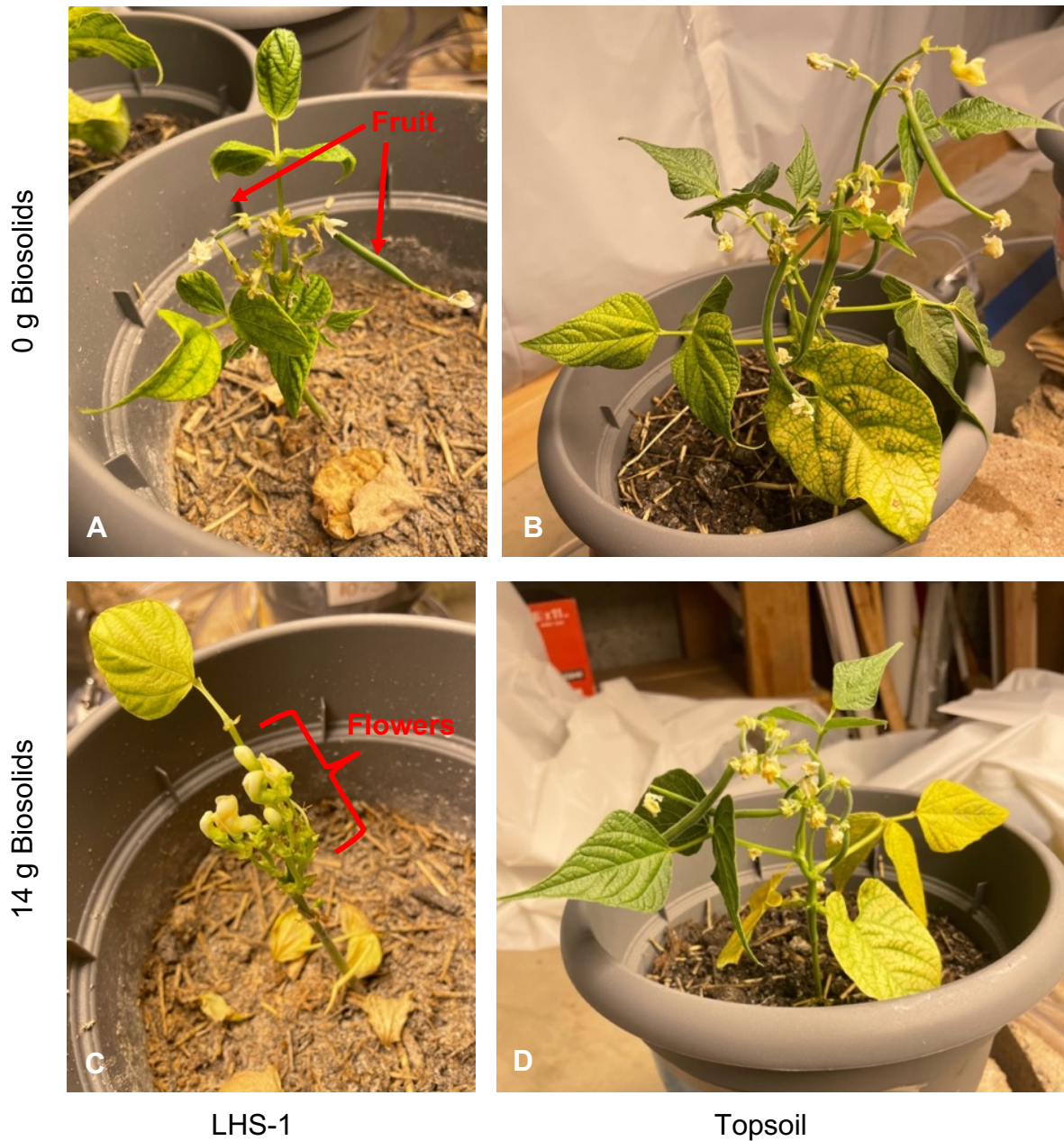
Most recent attempts to grow crops in LHS-1 have relied on large quantities of organic matter (50%), including peat moss, Earth soil, sawdust, worm castings, and compost, as well as fertilizers such as Miracle Grow, ammonium sulfate, urine, and biosolids (PTMC Spring 2021 – Fall 2022). The primary impact of these amendments is to increase nutrient concentration in LHS-1 and improve soil tilth. However, nutrient uptake by plants also depends on the pH of the substrate. LHS-1 has a pH of 8 – 9, which is far too alkaline for most crops to adequately uptake nutrients, even if they are available. Large quantities of organic matter can alter the pH of substrates containing LHS-1, but even a 50:50 mix of LHS-1 to organic matter will not eliminate toxicity caused by Ca. If most crops grow best with approximately 2% Ca, then the Ca content of LHS-1 will need to be reduced by at least 75%. To mitigate discrepancies between the growth requirements of plants and the inherent properties of LHS-1, successful crop production requires the addition of multiple nutrients, development of the soil atmosphere, and reduction of inhibitory chemical concentrations.

To advance our knowledge of crop production on the Moon, we chose to participate in the Plant The Moon Challenge and study Bush Bean growth in LHS-1. We chose Bush Beans

because according to the Old Farmer's Almanac, a trusted source for gardening advice since 1792, they are "easy to grow—even in limited space—and incredibly productive!" (Old Farmer's Almanac, 2023). Also, Bush Beans are nutritionally beneficial for humans, self-pollinating, and we have previous experience growing this crop successfully in our home garden. The guiding principle we used to tackle this challenge was that matter cannot be created nor destroyed. We believe that ISRU on the Moon should be maximized and the amount of materials transported from Earth to the Moon should be minimized. Our objectives for the PTMC Spring 2023 were (1) to grow Bush Bean plants that produced fruit in LHS-1, (2) use less organic matter and amendments than previous research, and (3) demonstrate the efficacy of in situ resources, such as biosolids, on crop production.

In this study, we show that Bush Beans can germinate, grow, flower, and begin to produce fruit in LHS-1 amended with less than 10% organic matter (Figure 1). Two innovative features of our approach are (1) washing LHS-1 with distilled water to reduce Ca before using it as a growth substrate, and (2) the design and implementation of a Soil Atmosphere Circulation System to enhance drainage and maintain the soil atmosphere. We had two hypotheses: (1) Washing LHS-1 with distilled water would reduce its Ca content, and (2) that the more biosolids added to a growth substrate, the greater the plant growth and crop yield. To test our first hypotheses, we assessed the pH of LHS-1 before and after washing. The pH of LHS-1 went from 8 – 9 down to 7 – 8 after washing, which is consistent with a reduction in Ca. To test our second hypothesis, we observed the growth of green beans in LHS-1 supplemented with 0, 3.5, 7, 10.5, and 14 grams (g) of biosolids. Growth varied across pots but did not correlate with the amount of biosolids. The major finding of our study was the flowering and fruit formation of Bush Beans grown in LHS-1 supplemented with less than 10% organic matter. All our Bush Bean plants were still alive at 46 days of growth. We believe this growth reflects the value of washing the LHS-1 prior to using it as a growth substrate and of maintaining the soil atmosphere.

Figure 1. Bush Beans can flower and produce fruit in LHS-1 amended with less than 10% organic matter. Bush bean plants grown in LHS-1 (A, C) or Topsoil (B, D), without biosolids (Top Row) or with 14 g biosolids (Bottom Row) after 46 days. Plants grown in LHS-1 survive to produce flowers (A and B) and fruits (only A). However, they are much small and less productive than plants grown in Topsoil (C and D). Plants grown in both LHS-1 and Topsoil exhibited some chlorosis. However, only plants grown in LHS-1 exhibited senescence.



METHODS

Novel Lunar Regolith Wash Method

LHS-1 contains 9.36% calcium (Ca), which is more the 4X the amount recommended for growing Bush Beans (Kelling & Schulte (2004)). For plants to grow well, this toxic concentration of Ca must be reduced. Given that Ca is soluble in water, we reasoned that we could remove excess Ca from LHS-1 by first washing it with copious amounts of water. To remove Ca from LHS-1, we mixed 5 kg of dry LHS-1 containing 468 g of Ca with 16 L of distilled water. We then used a ladle to remove 12 L of the resulting solution, or an estimated 351 g of Ca. Assuming the calcium dissolved readily in the distilled water, the remaining regolith contained approximately 114 g, or about 2%, Ca. During this process, we also removed other water-soluble elements, including Sodium (Na), Potassium (K), and Magnesium (Mg), as well as clay particles that were held in suspension.

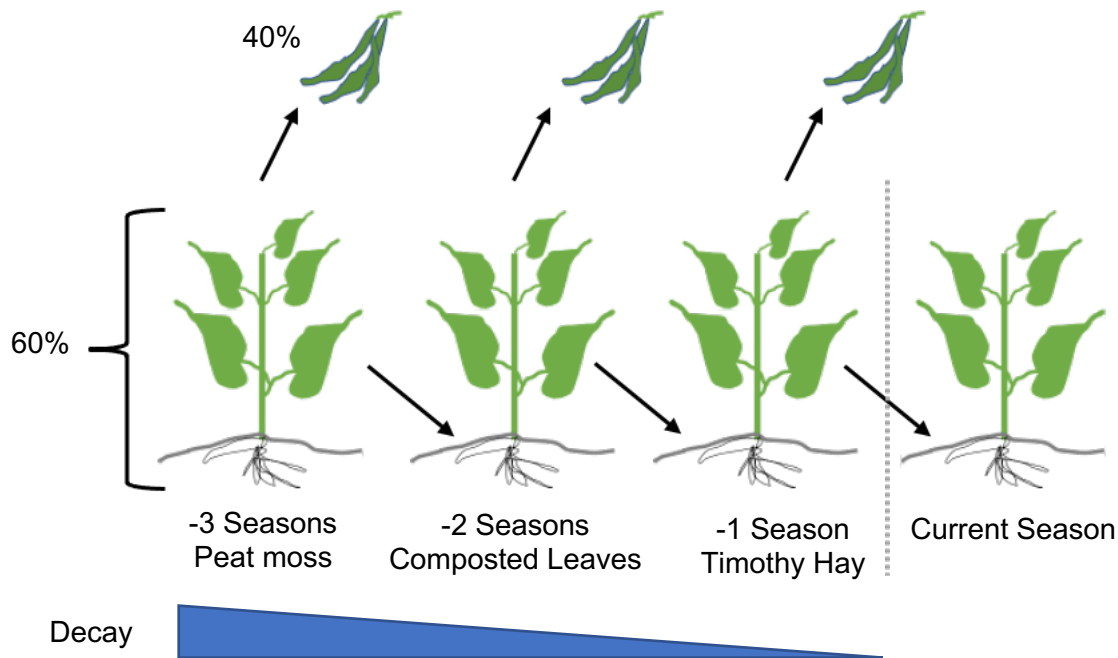
After this wash process, the LHS-1 resembled very wet mud with far too much water for planting. To remove most of the remaining water, we heated the LHS-1 on a camp stove and allowed the water to evaporate into the outdoor air. Due to challenges we encountered attempting this novel approach in a home setting, *e.g.*, all of the containers we used were thrifted from Goodwill and one of them broke during heating, a small amount of LHS-1 was lost. After washing and evaporating most of the remaining water, we ended up with 5,210 g of LHS-1 with 19% water content (approximately 4,220 g dry weight).

Amendments and Fertilizer

LHS-1 lacks multiple elements essential for plant growth, including N, S, and Cl. Additionally, the washing treatment we used to remove excess Ca, likely also removed other water-soluble elements essential for plant growth, including K and Mg. While many prior studies have amended LHS-1 with 50% organic matter to alleviate some of these nutritional deficiencies, reduce pH, and improve soil tilth (PTMC Spring 2021 – Fall 2022), one of our objectives was to reduce the amount of organic matter added to the LHS-1 via ISRU.

To determine the amount of organic matter that could potentially be available in situ on the Moon, we developed the Three Season Compost Model (Figure 2). Based on this model, we used 18 g of organic matter representing three prior growing seasons in each pot. Since Spring 2023 is our first time participating in the PTMC Challenge, we did not have any organic matter from prior growing seasons. To mimic organic matter from three prior growing seasons, we used Timothy Hay for minus 1 season, Composted Leaves for minus 2 seasons, and Peat Moss for minus 3 seasons. These materials represent organic matter in various stages of decay and would ideally be acquired from crops grown entirely on the Moon.

FIGURE 2. Three Season Compost Model. In this model we assume each plant possesses 30 g of organic matter and that 40% of this organic matter comprise fruit that will be consumed by space explorers or used for seeds. The remaining 60% of organic matter comprises roots and shoots that can be composted. In this model each plant yields 18 g of compostable material per season. Given that this is our first season in the PTMC, we chose three different types of organic matter to represent compost from three prior seasons, including Timothy Hay (minus 1 season), Composted Leaves (minus 2 seasons), and Peat Moss (minus 3 seasons).



Although amending LHS-1 with organic matter from 3 prior growing seasons will provide some nutrients and improve soil tilth, it will not provide sufficient nutrition for robust plant growth and food production. First, nutrients lost from human consumption of fruit must also be replaced. To estimate the amount of biosolids that could be reclaimed as fertilizer on the Moon, we developed a Biosolid Reclamation Model (Figure 3). Using this model, we estimated that the maximum amount of biosolids that could be obtained in situ was 14 g per plant per growing season. We wanted to observe whether different amounts of biosolids impacted plant growth, so each pot received 0, 3.5, 7, 10.5, or 14 g of biosolids (sourced from St. Joseph, Missouri Water Protection Agency).

Figure 3. Biosolid Reclamation Model. In this model we estimate the maximum amount of biosolids available per year per plant as a function of the amount of beans consumed and converted in biosolids each year, and the number of plants required for food and seeds. If the nutrition of each person depends on 14 bush bean plants per year, and each person converts the Bush Beans they consume into 195 g of biosolids, then each plants can be fertilized with a maximum of 14 g of biosolids per year.

$$\text{Biosolids (g) produced per person each year} = 195 \text{ g}$$

$$= \frac{100 \text{ g Bush Bean}}{\text{Serving}} \times \frac{26 \text{ Servings}}{\text{Year}} \times 10\% \text{ d. wt. (g)} \times 75\% \text{ (recovery coefficient)}$$

$$\text{Bush Bean plants needed per person each year} = 14 \text{ plants}$$

$$\approx \left(\frac{2,600 \text{ g Bush Beans per person per year}}{226.8 \text{ g Bush Beans per plant}} \right) + 2.5 \text{ Plants for seeds and loss}$$

$$\text{Maximum amount of biosolids (g) available per year per plant} = 18 \text{ g}$$

$$= \frac{\text{Bush Bean plants needed per person each year}}{\text{Biosolids produced per person each year}}$$

$$= \frac{14 \text{ plants}}{195 \text{ g}}$$

Even with the addition of organic matter representing and biosolids, more fertilization was necessary to compensate for nutrients lost to seed production and to account for other known deficiencies in LHS-1. Additional amendments and fertilizers added to each pot included: 21 g Kelp Meal (Neptune's Harvest Kelp Meal, Ocean Crest Seafoods, Gloucester, MA), 0.17 g of 21% Ammonium Sulfate (Hi-Yield), 0.13 g of 18% Super Phosphate (Hi-Yield), and 0.09 g of 60% Potash (Hi-Yield). Kelp meal contains Cl, which is essential for plant growth, as well as cobalt, which facilitates nodule formation and nitrogen-fixation by bacteria in symbiotic relationship with legumes such as Bush Beans. The total amount of organic matter, including Timothy Hay, Composted Leaves, Peat Moss and Kelp Meal, added to each pot was 75 g, or 8.8% for the pots with LHS-1 and 7.5% for the pots with Topsoil (LHS-1 pots received a somewhat greater fraction of organic matter because some LHS-1 was lost during the wash treatment). Ammonium Sulfate, Super Phosphate, and Potash were applied to pots at planting time and after the development of first true leaves according to recommendations by the University of Wisconsin Extension. For an overview of the additions and subtractions we made to the LHS-1 before plants, see Table 1.

Table 1. List of all additions and subtractions made to LHS-1 before planting.

Earth Pots	E-0	E-3.5	E-7	E-10.5	E-14	Units	
Biosolids	0	3.5	7	10.5	14	Grams	Independent Variable
Sphgnum Peat Moss	18	18	18	18	18	Grams	
Composted Leaf Mulch	18	18	18	18	18	Grams	
Timothy Hay	18	18	18	18	18	Grams	
Kelp Meal	21	21	21	21	21	Grams	
21% Ammonium Sulfate	0.17	0.17	0.17	0.17	0.17	Grams	
18% Super Phosphate	0.13	0.13	0.13	0.13	0.13	Grams	
60% Potash	0.09	0.09	0.09	0.09	0.09	Grams	
Ace Hardware Top Soil	1000	1000	1000	1000	1000	Grams	
Moon Pots	M-0	M-3.5	M-7	M-10.5	M-14	Units	
Biosolids	0	3.5	7	10.5	14	Grams	Independent Variable
Sphgnum Peat Moss	18	18	18	18	18	Grams	
Composted Leaf Mulch	18	18	18	18	18	Grams	
Timothy Hay	18	18	18	18	18	Grams	
Kelp Meal	21	21	21	21	21	Grams	
21% Ammonium Sulfate	0.17	0.17	0.17	0.17	0.17	Grams	
18% Super Phosphate	0.13	0.13	0.13	0.13	0.13	Grams	
of 60% Potash	0.09	0.09	0.09	0.09	0.09	Grams	
LHS-1 Lunar Regolith	1000	1000	1000	1000	1000	Grams	
Calcium Oxide	Minus 96	Minus 96	Minus 96	Minus 96	Minus 96	Grams	
Sodium Oxide	Minus 21.75	Minus 21.75	Minus 21.75	Minus 21.75	Minus 21.75	Grams	
Potassium Oxide	Minus 3.75	Minus 3.75	Minus 3.75	Minus 3.75	Minus 3.75	Grams	
Magnesium Oxide	Minus 12	Minus 12	Minus 12	Minus 12	Minus 12	Grams	
Clay	Minus 22.5	Minus 22.5	Minus 22.5	Minus 22.5	Minus 22.5	Grams	

Midway through the growth experiment most plants in both LHS-1 and Topsoil began exhibiting signs of chlorosis. We suspect this stress was caused by our watering method in the first portion of our experiment. To help mitigate this problem, we applied two doses of chelated liquid iron applied according to manufacturer instructions (ferti-lome Chelated Liquid Iron and Other Micronutrients, Bonham, TX).

Seeds and Planting

Blue Lake 274 Bush Beans were grown in either 844 g of LHS-1 or 1,000 g of Topsoil in 8-inch round plastic pots amended with 75 g of organic matter plus fertilizer as described above. We had initially intended to use equal amount of LHS-1 and Topsoil. However, a small amount of LHS-1 was lost during the washing process (see above) and all other components had already been measured and mixed by that time. The total amount of organic matter used in each pot was 8.8% for the pots with LHS-1 and 7.5% for the pots with Topsoil.

Each pot was sown with three seeds approximately 1 inch below the surface of the soil. Seeds were sprinkled with $\frac{1}{4}$ teaspoon of granular Nitrogen-fixing bacteria (Nature's Aid 1×10^8 viable cells per g of *Bradyrhizobium* sp., *Rhizobium leguminosarum* and *R. phaseoli*). Following germination, seeds were thinned to 1 plant per pot.

Novel Soil Atmosphere Circulation System

The physical properties of LHS-1 inhibit the flow of water away from plant roots. Additionally, low gravity on the Moon will exacerbate the poor drainage qualities of lunar regolith. Without sufficient drainage, growth substrates become waterlogged, reducing gas exchange between roots and the soil atmosphere. To grow crops on the Moon, terraforming endeavors must ensure (1) adequate water drainage, (2) soil surface air flow, and (3) subsurface air flow. In the absences of these elements, plants cannot thrive.

For our experiment, we engineered a Soil Atmosphere Circulation System comprised of (1) our Low Gravity Drainage Enhancement System (LGDES), (2) an 18-inch diameter pedestal fan, and (3) our Subsurface Atmosphere Ventilation System (Figure 4). First, to facilitate adequate water drainage in LHS-1 and on the Moon, our LGDES was comprised of a filter, collection reservoir, and vacuum connected via tubing. Following watering, excess water was removed from each pot via suction created by 2.5 gallon 2-HP shopvac (Craftsman). Water from each pot was collected in a corresponding reservoir. Second, to ensure air flow on the soil surface, we positioned an 18-inch diameter pedestal fan (Lasko) on one side of the grow room, 12 inches above the soil surface. Third, to promote subsurface air flow, we installed our Soil Atmosphere Ventilation System (SAVS). The SAVS consists of a 4" square, 12 volt computer fan (Wathai), bespoke pressure collator, and tubing that connects each collection reservoir used in the LGDES to the computer fan. The objective of SAVS was to create equal and subtle air flow from above the soil surface, down through the soil, and away from plant roots. In our approach, air flow from the SAVS was vented into the grow room. The goal of our Soil Atmosphere Circulation System was to overcome the poor drainage qualities inherent to LHS-1 and potentially identify a way to mitigate drainage problems inherent to crop production in low-gravity environments.

FIGURE 4. Novel Soil Atmosphere Circulation System. (A) Low Gravity Drainage Enhancement System (LGDES), (B) 18-inch diameter pedestal fan, (C) Subsurface Atmosphere Ventilation System (SAVS) before installation, and (D) SAVS after installation.



Watering

All pots were watered with 100 – 400 ml of distilled water every 1 to 4 days. The amount of water varied over the course of the experiment. Initially, we sought to keep the growth substrate continually moist to prompt seed germination. As roots developed, we began to allow the topmost surface of the soil to dry between waterings. Following each watering, we extracted excess water from each pot using the shopvac attached to the LGDES for 10 – 20 seconds for pots containing Topsoil and 20 – 30 seconds for pots containing LHS-1 (the two substrates exhibited different rates of water drainage). The amount of water we collected varied from 0 to upwards of 300 ml of water, depending on how moist the soil was prior to watering and how much water was added.

Midway through the experiment we began using a combination of re-claimed water and distilled water. For the first part of the experiment, we combined the extracted water from each pot into a large storage container. Initially, we hesitated re-using this water due to concern about it containing dissolved sodium (Na), which is detrimental for plant growth. Following a note made by Dr. Robert Heinse during the March 22, 2023 Q&A Session about the loss of nutrients into the extracted water and apparent nutrient deficiencies beginning at around 24 days of growth, we opted to begin re-using water extracted during subsequent waterings. We chose not to re-use the extracted water we had collected and combined during the first part of the experiment for two reasons: (1) we did not know how many nutrients were in the solution, and (2) the solution had started to grow algae. Although Na in the collected water may not be ideal for plant growth, we agree with Dr. Heinse's recommendation to re-use extracted water to retain nutrients. Regrettably, we believe we may have lost an unknown, but likely helpful amount of nutrients in the first part of our experiment.

Lighting and Temperature

We used a Spider Farm SF4000 LED Grow Light to expose plants to alternating light/dark periods ranging from approximately 14 hours 30 minutes of light to 15 hours 30 minutes of light. The duration of the light period was increased every 7 – 10 days according to the daylength in Wisconsin during green bean growing season. Wisconsin produces the most green beans of any state in the USA, and thus represents ideal growing conditions for this crop plant. The intensity of the light was increased depending on the growth stage of the plants (germination, leaf growth and flowering), according to manufacturer recommendations. Plants were grown in the basement of our family home with ambient temperatures ranging from 64 to 67°F.

RESULTS

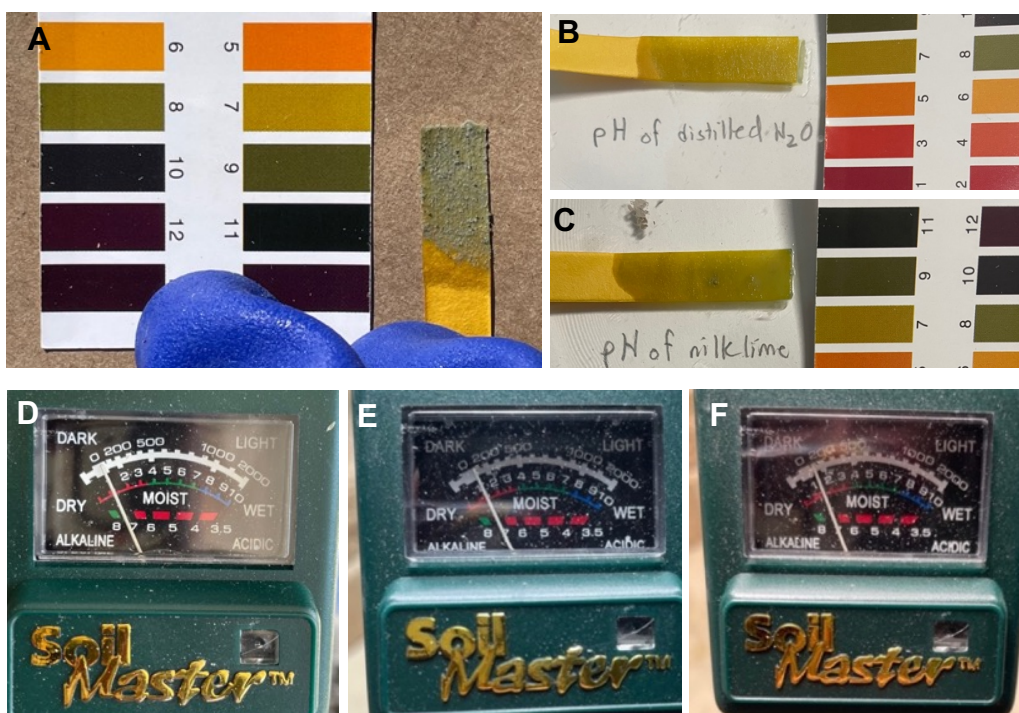
Novel Lunar Regolith Wash Method

Washing LHS-1 with distilled water decreases its pH (Figure 5). Prior to washing, the pH of LHS-1 was 8 – 9. To remove water soluble molecules from LHS-1, we mixed dry LHS-1 with copious amounts of distilled water. We then removed 75% of the resulting water with dissolved molecules (Moon Milk), before evaporating most of the remaining water from the LHS-1. The starting pH of the distilled water was 7, and the pH of the Moon Milk was 8 – 9. This difference in pH is consistent with the dissolution of cations from LHS-1 into the distilled water upon mixing. The pH of LHS-1 after washing was 7 – 8, which indicates an approximately 10-fold

decrease in pH relative to unwashed LHS-1. This decrease is consistent with the removal of cations, such as Ca^{+} , from LHS-1. Notably, the pH of our Topsoil control was also 7 – 8.

In addition to removing soluble ions during our Lunar Regolith Wash, we also removed clay-like particles that remained in suspension. We estimate that we removed 112.5 g of clay-like particles from the 5 kg of LHS-1 we had available for our experiment. These clay-like particles contribute to agglutinates in LHS-1. Removing a fraction of these particles likely also reduced compaction the LHS-1 during plant growth.

Figure 5. Novel Lunar Regolith Wash Method reduced the pH of LHS-1 from 8 – 9 to 7 – 8, which is the same as Topsoil. Shown in the images are (A) pH of LHS-1 before washing, (B) pH of distilled water, (C) pH of Moon Milk, (D) pH of LHS-1 after washing, (E) pH of LHS-1 at planting, and (F) pH of Topsoil at planting.



Novel Soil Atmosphere Circulation System

The LGEDS effectively removes water from pots (Figure 6). After each watering, excess water was extracted from each pot using the LGEDS. Prior to using the system, water tended to pool on top of LHS-1, although it drained readily through the Topsoil. Using the LGEDS facilitated the movement of water through the substrate and out of the bottom of the pot. The amount of water collected varied from 0 – 300 ml, depending on the moisture content of the substrate prior and the amount of water added. The amount of time it took to remove excess water varied between pots with LHS-1 and Topsoil. Water was extracted more quickly from pots containing Topsoil (10 – 20 seconds of suction) compared to pots containing LHS-1 (20 – 30 seconds of suction). Notably, the color of the extracted water difference between the two substrates as well, with the LHS-1 extracts tending to be much darker. The precise reason for this

difference remains uncertain, but it is a bit surprising since the LHS-1 is so much lighter in color than Topsoil.

Figure 6. Low Drainage Enhancement System (LGDES) effectively removes excess water from pots. Show is (Top Row) LHS-1 band (Bottom Row) Topsoil before and after extracting and collecting excess water.



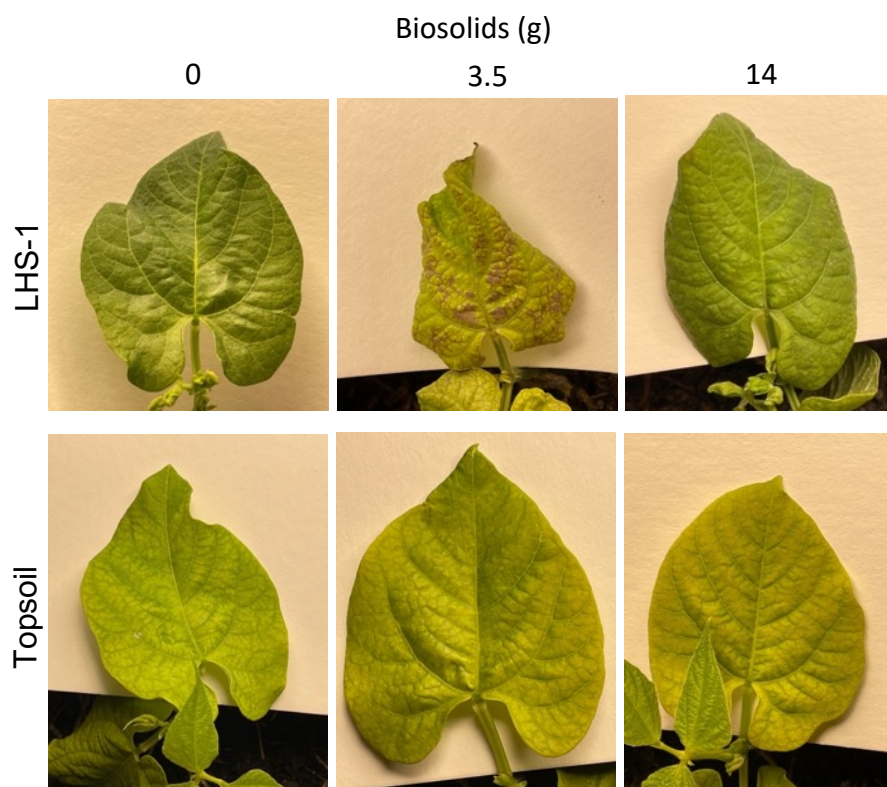
Except during watering and active use of the LGEDS, the room fan and SAVS operated almost continuously. It took us several days post-planting to install the room fan, and several more days to finish the installation of the SAVS. Although we were unable to directly measure the impact of these units on the soil surface and subsurface, several observations are consistent with their efficacy. First, prior to using the room fan we observed mold growth on the surface of the substrate in each pot (data not shown). Once we installed the room fan, the mold diminished, suggesting that the room fan helped to evaporate moisture on the substrate surface. Next, after installing the SAVS, air vented continuously from the computer fan and emitted a mineral-like smell. The substrate also seemed less prone to compaction. Together, these observations suggest that the Soil Atmosphere Circulation System helped to enhance air flow above and within the substrates.

Plant Growth

Seeds germinated in all pots of both LHS-1 and Topsoil. The number of days until germination, which we defined by the emergence of the first true leaves, was 10 to 12 days for LHS-1, and 9 to 16 days for Topsoil. The average number of days to germination was 11 for seeds sown in LHS-1 and 10.3 days for seeds sown in Topsoil (data not shown). Within 12 days of planting, most pots had at least one seed that fully germinated.

For the first 24 days of growth, the most notable difference between plants grown in LHS-1 and Topsoil was leaf color (Figure 7). Plants grown in LHS-1 were medium- to deep-green while plants grown in Topsoil were yellow-green to medium-green. This color difference persisted until about day 22 to 25. By 25 days of growth, variation between the plants became more apparent. For example, by Day 25 the plant grown in LHS-1 supplemented with 3.5 g of biosolids had small, curled leaves that were yellow with dark pigmentation. Meanwhile, plants grown in LHS-1 supplemented with 0 and 14 g of biosolid remained deep-green. We suspect that some of this variance is due to chance, but it could also be related to variation in the rates of the development between the plants. In other words, faster growing plants may exhibit signs of stress earlier because their nutritional demands increase faster. Plants grown in Topsoil also exhibited signs of stress (yellowing leaves), but were more consistent in their appearance overall.

Figure 7. Leaf color varied between plants grown in LHS-1 and Topsoil after 25 days of growth. Plants grown in LHS-1 tended to emerge medium- to dark-green, but they gradually showed signs of stress as they matured (yellowing, curling, dark pigmentation). Plants grown in Topsoil tended to emerge bright green but gradually developed chlorosis as they matured.



While we anticipated that plants grown in LHS-1 were likely show signs of stress, we were surprised that all the plants grown in Topsoil also showed signs of stress. In particular, the leaves of plants grown in Topsoil were large, but yellow with dark venation (chlorosis). This observation suggested a nutrient deficiency. Up until midway through the grow period we did not re-use water extracted via the LGDES following watering. Given that nearly all amendments and fertilizers were applied in the first 14 days of growth, we suspect we inadvertently extracted many of the nutrients we had added along with the water. To address this possibility, we decided to treat all plants for chlorosis with chelated liquid iron and to begin re-using water we extracted via the LGDES during watering.

By Day 45 all plants in both LHS-1 and Topsoil remained alive (Figure 8). Following the treatment with chelated liquid iron, plants in both substrates show signs of improved growth. Development progressed for all plants and all new leaves were medium-green rather than yellow (personal observations). However, as time progressed, the greening was only sustained by the plants grown in Topsoil. Eventually, the leaves on plants grown in LHS-1 began to senesce and gradually fall off the plants.

Figure 8. Growth of Bush Bean plants in LHS-1 and Topsoil supplemented with 0, 3.5, 7, 10.5, or 14 grams (g) of biosolids.

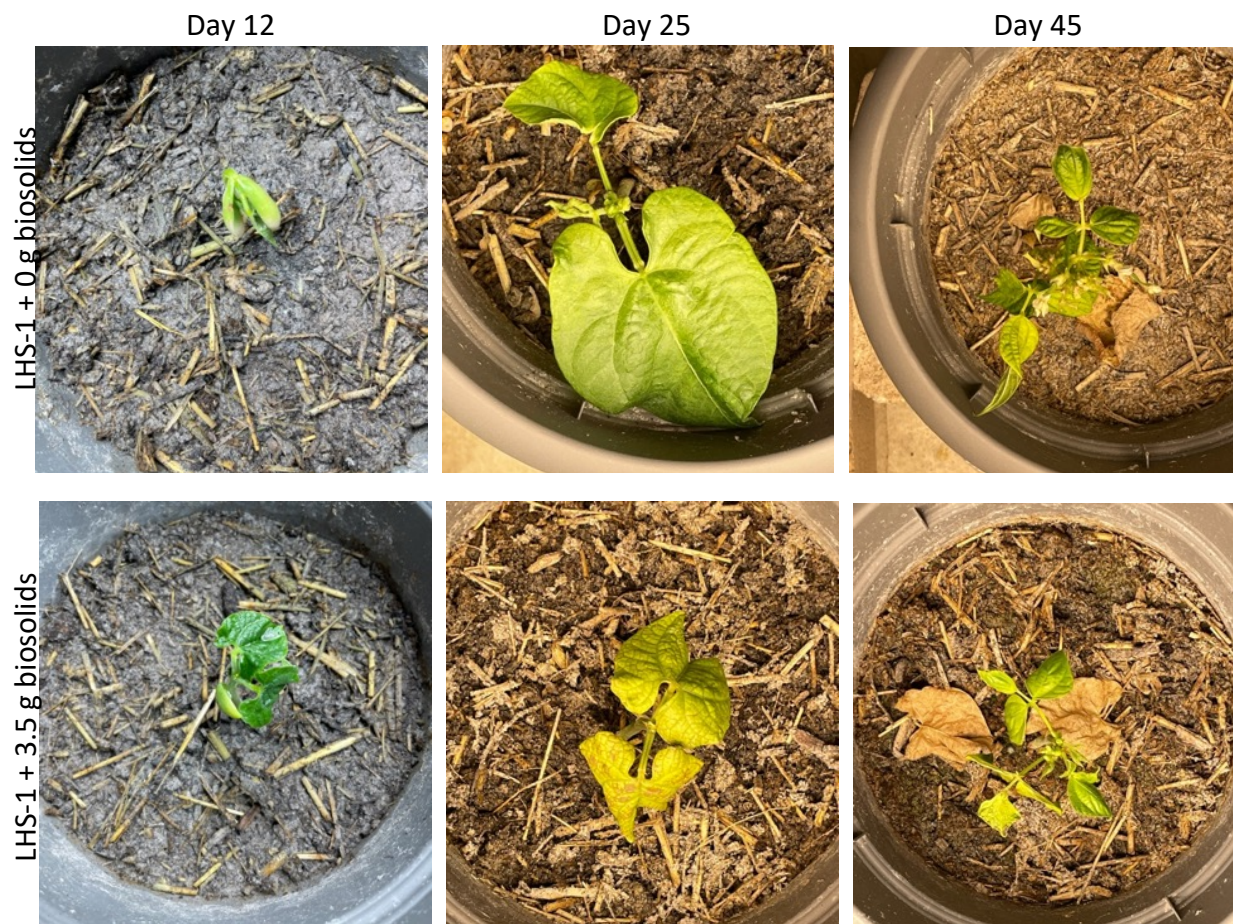


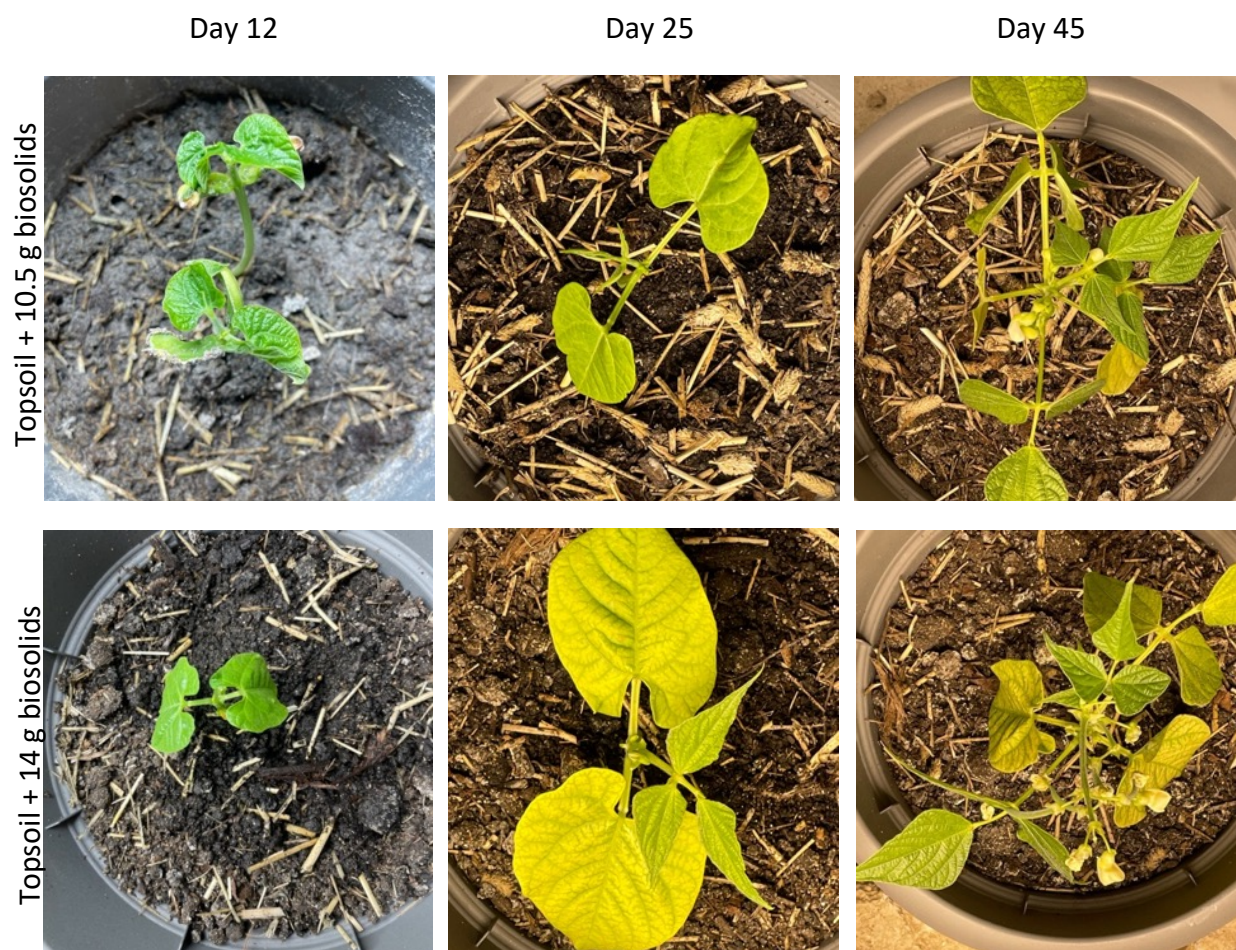
Figure 8. Growth of Bush Bean plants in LHS-1 and Topsoil supplemented with 0, 3.5, 7, 10.5, or 14 grams (g) of biosolids.



Figure 8. Growth of Bush Bean plants in LHS-1 and Topsoil supplemented with 0, 3.5, 7, 10.5, or 14 grams (g) of biosolids.



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Our most notable growth result was the formation of flowers on two plants grown in LHS-1, and fruit on one plant grown in LHS-1 (Figure 1). Given the numerous challenges to growing plants in LHS-1, that this is our first attempt at ever growing plants in this substrate, and that we used less than 10% organic matter in our approach, we are very excited by this result! Compared to plants grown in LHS-1, plants grown Topsoil controls tended to be much large, and exhibited numerous flowers and fruits.

CONCLUSIONS

Participating in the Plant The Moon Challenge Spring 2023 has been awesome! Our family team, including three generations, worked together to learn about the challenges of space exploration and crop production in the wild environments that exist beyond our Earth. As participants in this challenge, we felt like trailblazers trying to find a way to grow plants in a totally new and different way. Lunar regolith possesses numerous challenges for plants adapted

to growing in Earth soil. The innovative contributions of our work include the washing method we used to prepare the lunar regolith simulant for growing plants and the design of a new drainage and ventilation system to maintain the soil atmosphere. As a family we overcame multiple challenges to advance our knowledge, and we hope that the findings of our research help pave the way for future explorers.

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PHOTOS

