

The Role of Cyanobacteria in Enhancing Radish Growth in Simulated Extraterrestrial Soils

Celestial Seedlings

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2.0 Abstract

As humans begin their colonization of the solar system, they will need the resources to do so effectively. Future space missions will have to pay particular attention to food production on foreign celestial bodies. Attempting to grow plants for food production in any kind of regolith is difficult. This is due to several factors including a harsh environment, lack of nutrients/symbiotic organisms, and the composition of the lunar regolith itself, which is fine, abrasive, and sandy.

The Red Rocks Community College Celestial Seedlings team attempted to tackle these problems with an emphasis on the use of cyanobacteria for nitrogen fixation and oxygen production. Experiments compared radish growth in 100% soil, 50-50 soil regolith mix, and 100% regolith. These were then further separated depending on the water received. Sterile autoclaved water was used as a control for one group and pond water for the other. To confirm the presence of cyanobacteria in the substrates, the team employed the use of polymerase chain reaction (PCR) to amplify the DNA and gel electrophoresis to confirm the DNA was cyanobacteria. Results showed the 50-50 mix was the most successful in supporting radish growth with the most abundant biomass being observed in the samples that contained pond water and cyanobacteria. The team's findings suggest that symbiotic relationships such as with cyanobacteria are critical in agriculture production in regolith. Supplying future colonies with food from Earth is impractical and expensive. Celestial seedlings aim to provide valuable data about how to utilize in-situ resources for plant growth such as lunar regolith.

3.0 Introduction

Soil ecosystems are fundamental to the survival of humans and countless other organisms. Among their key contributors are cyanobacteria, primary producers that support food webs and generate various bioactive compounds through processes like nitrogen fixation.

Cyanobacteria improve nitrogen-deficient soils by fixing atmospheric nitrogen and producing compounds that enhance nutrient availability. Nitrogen is vital to plant growth as it contributes to the formation of amino acids, proteins, and nucleic acids, essential to producing chlorophyll, molecules critical for photosynthesis and overall plant development.

Furthermore, cyanobacteria soil crusts stabilize soil to prevent erosion and enhance water retention. This method, named cyanobacteria inoculation, has been explored as a method to restore degraded soils, improve soil health, and counter desertification.

This project explores the potential of using cyanobacteria for lunar agriculture. Due to cyanobacteria's resistance to extreme environmental conditions, they are the ideal candidate for extraterrestrial colonization.

By designing and experimenting with moon regolith and cyanobacteria, this project aims to advance the development of lunar agriculture beyond Earth.

To confirm the presence of Cyanobacteria in the test subjects, we attempted to extract DNA from samples and then ran the extracted DNA through Polymerase Chain Reaction (PCR), which amplifies certain genes in a genetic sequence via specific primers and allows for testing via gel electrophoresis. Primers section out specific sections of a genome, some of which can be used to identify certain organisms. Gel electrophoresis creates a circuit that pulls the negatively charged DNA towards the positive end of an agarose gel, which provides experimenters with a visual representation of the length of a DNA strand, which can be correlated with certain gene sequences. After DNA was run through gels, the quantity of DNA was measured in nanograms per microliter, which confirms or rejects the viability of the DNA for PCR, as a minimum of three nanograms are needed to complete PCR successfully.

3.1 Problem Statement:

Among various seedling options, radish seeds were chosen for its rapid growth rate and nutritional benefits- Vitamin C, potassium, and calcium. These minerals and nutrients contribute to proper muscle and nerve function, bone strengthening, and collagen production- essential considerations for astronauts returning from space missions. The objective question for this experiment is:

Can cyanobacteria improve radish growth in lunar regolith and other soil mixtures?

3.2 Objectives:

The Red Rocks Community College (RRCC) Plant the Moon Project investigates cyanobacteria's presence and role in radish growth with lunar regolith, and sterile soil with different watering conditions.

3.3 Literature Review

Previous Research on Plant Growth in Lunar Conditions

The use of lunar regolith as an agricultural substrate has been a promising option for supporting plant growth in theoretical moon colonies due to its availability. Studies indicate that our largest obstacle to overcome is the usage of molecular oxygen which cannot be extracted directly from lunar silicates and metal oxides (Dalton, B., Roberto, F., Lunar regolith biomineralization: Workshop report).

Studies on Soil Composition and Plant Viability

Previous studies have established a symbiotic connection between cyanobacteria from the order Nostocales and various plant species. Cyanobacteria found within soil have nitrogen-fixing abilities that create biologically available nitrogen and organic carbon, which leads to improved soil fertility and crop production for the organisms they are in symbiosis with (Rai et al., 2019). For this project, we plan on inoculating soil with cyanobacteria through irrigating our experimental plants with pond water.

4.0 Materials and Methods

4.1 Experimental Design:

- Soil Conditions:
 - 2 pots: 180 g lunar regolith
 - 2 pots: 180 g sterilized potting soil
 - 2 pots: 90 g lunar regolith + 90 g sterilized potting soil (50/50 mix)
- Watering Treatments:
 - Weekly pond water collected from a nearby source
 - Half sterilized via autoclave and refrigerated
 - Each soil condition tested with both sterilized and unsterilized water
- Watering Schedule:
 - 25 mL applied on Mondays and Wednesdays
 - 25–50 mL on Fridays based on moisture level assessment
- Soil Sampling:
 - Every Friday: ~0.6 g of soil collected per pot
 - Samples stored at –20°C

- Beginning, middle, and end samples selected for molecular analysis
- See Appendix A for soil processing and DNA extraction workflow

4.2 Planting Procedure:

- Pot Setup:
 - 6 square pots with drainage holes and coffee filters
 - Soil handling conducted under a fume hood
- Handling Procedures:
 - Gloves and surgical masks worn during lunar regolith handling
 - Gloves sterilized with 70% isopropyl alcohol
- Soil Mixing:
 - Soil layered and mixed within each pot
- Initial Watering:
 - Each pot watered with 50 mL of its assigned water type
 - Mixed gently for uniform moisture
 - A 0.6 g soil sample collected post-watering for baseline analysis
 - Detailed collection method available in Appendix A
- Seed Planting:
 - Five radish seeds planted in each pot using a 5-die pattern (1/4-inch depth)
 - One seed per hole, covered with the substrate mixture
- Growth Measurement:
 - On November 22, surviving plants evaluated for:
 - Average radish mass
 - Average leaf area
 - Data grouped by substrate type and water treatment

4.3 DNA Analysis:

- Sample selection:
 - DNA was extracted from beginning, middle, and end soil samples from each pot.
 - Samples were stored at -20°C prior to extraction and analysis.
- DNA Extraction:
 - Extraction was performed using the Zymo Research Quick-DNA Fecal/Soil Microbe Miniprep Kit.
 - The full extraction procedure is available in Appendix A.
- PCR Amplification
 - Two primer sets were used for targeted amplification:
 - 16S rRNA universal primers
 - Cyanobacteria-specific primers
 - PCR master mix preparation and thermal cycling conditions are provided in Appendix B.

- Gel Electrophoresis
 - PCR products were visualized using GelGreen-stained agarose gel electrophoresis.
 - Gel setup, voltage settings, and imaging protocols are described in Appendix C
- Controls
 - Positive control: Known cyanobacteria DNA
 - Negative control: Autoclaved soil with sterilized water (no cyanobacteria)
- Data Collected
 - Cyanobacterial detection determined by presence or absence of bands on the gel blaster
 - Plant growth metrics (height, biomass, and leaf count) recorded and compared across treatment conditions.

5.0 Results

5.1 Plant Growth Outcomes:

- Comparison of growth metrics across soil and water conditions.
 - Sterile water caused plant growth to exceed unsterile pond water in both soil and 50/50 for the first two weeks. Although regolith produced more growth in unsterile water than in sterile.
 - For our unsterilized samples, Unsterilized water was found to aid in the plant's growth in the Regolith, 50/50 regolith/soil and the 100% soil in the following weeks until thanksgiving break. This is a result of the bacterial and microbial presence in our unsterilized pond water.
 - For our sterilized water samples, it still exhibited growth in the 50/50 and 100% soil, but after 1 week, the sterile water-regolith sample became stunted, stopped growing and eventually died.
 - Both regolith plants have died over Thanksgiving Break.

5.2 PCR Results:

Presence or absence of cyanobacteria in each condition:

- Presence of Cyanobacteria in Soil Sterile 10/20 sample found.
- Presence of Cyanobacteria in Soil Unsterile 10/20 sample found.
- Presence of Cyanobacteria in 50/50 Sterile 10/4, 10/11, 11/1, and 11/22 samples found.
- Presence of Cyanobacteria in 50/50 Unsterile 9/30, 10/4 t.2, 11/1 and 11/22 samples
- No presence in Regolith.

Presence or absence of 16S genome in each condition:

- No presence of 16S in Soil Sterile sample.
- Presence of 16S in Soil Unsterile in 10/20 sample found.
- Presence of 16S in 50/50 Sterile in 11/1 and 11/22 samples found.
- Presence of 16S in 50/50 Unsterile in 10/20, 11/1, and 11/22 samples found.
- No presence in Regolith.
- On 11/18 - Our first post-PCR electrophoresis from our (10/4) soil sample we collected.
 - Created agarose wells
 - Ran 16s and Cyano samples through electrophoresis. Positive controls for both lit up. 50/50 sterile lit up for cyanobacteria.
 - Watered with 40 mL with 11/5 water
 - PCR Heat cycle numbers: (**Fig 1**)



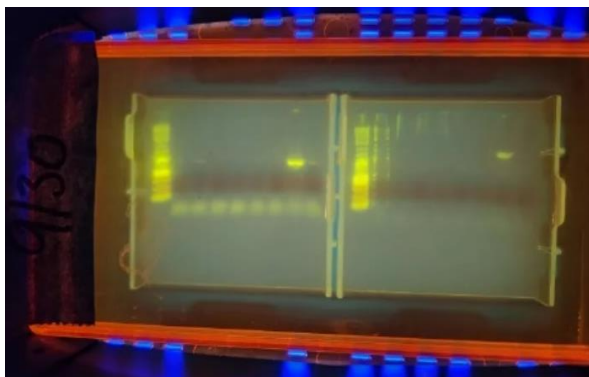


Fig 2 We ran another round of PCR (on the 10/4 soil sample) to double check our results.



Fig 4 Cyano Electrophoresis on 11/18 (for 10/4 sample)

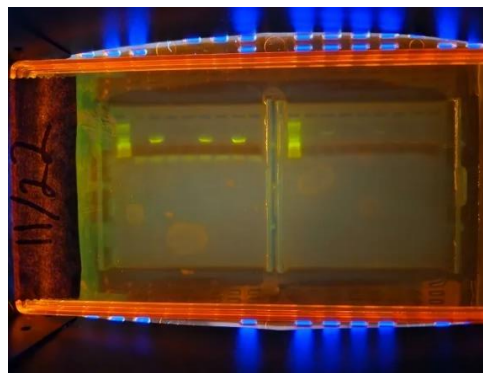


Fig 3 12/19 Electrophoresis result

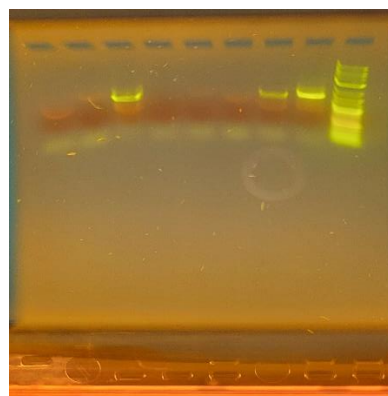


Fig 5 11/1 samples. Both 50/50 samples came through and the controls are working properly.

5.3 Presence-Absence Matrix:

PCR results targeting Cyanobacteria							
	Water Source	9/30	10/4	10/11*	10/20	11/1	11/22
Regolith	Sterile Pond	-	-		-	-	-
	Non-Steril Pond	-	-		-	-	-
Soil & Regolith	Sterile Pond	-	+		+	+	+
	Non-Steril Pond	+	-		-	+	+
Soil	Sterile Pond	-	-		+	-	-
	Non-Steril Pond	-	-		+	-	-
PCR results targeting 16S							
	Water Source	9/30	10/4	10/11*	10/20	11/1	11/22
Regolith	Sterile Pond	-	-		-	-	-
	Non-Steril Pond	-	-		-	-	-
Soil & Regolith	Sterile Pond	-	-		-	+	+
	Non-Steril Pond	-	-		-	+	+
Soil	Sterile Pond	-	-		-	-	-
	Non-Steril Pond	-	-		+	-	-

*10/11 data was not tested before submission date

Using our Sterilized and Unsterilized Pond water, our DNA extraction from the soil and lunar regolith, PCR testing and Gel Electrophoresis trials, we have concluded that the presence of cyanobacteria in a regolith/soil mixture is more prominent than soil or regolith alone. More testing is needed to finalize our results.

Photos from September 30 have been omitted because there was no growth since that is the day the seeds were planted.



Fig 6 & 7. Plant photos of October 4, 2024. Plants have begun to show signs of sprouting, with some green poking through the soil. The black tray holds the plants watered by pond water while the orange tray holds the plants watered by sterilized water.



Fig 10 & 11. Plant photos from November 22, 2024. Both sets of plants in 100% regolith had died by this point. Plants in 100% soil are thriving, especially those watered with pond water. The plants in 50/50 mixture are in worse shape. There is more growth in the 50/50 mix watered with sterile water but are more yellowed than the plants in 50/50 mix watered with pond water.



Fig 12 & 13. Plant photos from December 2, 2024, post growth phase. Upon returning from Autumn break, most of the plants had died except for the plant in 100% soil watered with sterile water. The rest had withered away.

5.4 Alive-Dead Matrix

Plant Growth						
	water source	9/30	10/4	10/16	11/22	12/2
regolith	sterile pond	Alive	Alive	Dead	Dead	Dead
	non-sterile pond	Alive	Alive	Alive	Dead	Dead
soil & regolith	sterile pond	Alive	Alive	Alive	Alive	Dead
	non-sterile pond	Alive	Alive	Alive	Alive	Dead
soil	sterile pond	Alive	Alive	Alive	Alive	Alive
	non-sterile pond	Alive	Alive	Alive	Alive	Dead

Fig 14. Table showing the date of images taken and the status of the plants within the pot. If even one plant remains alive in the pot, then the status of the pot is alive.

5.5 DNA Quantification

DNA Concentration (ng/ μ L)							
	water source	9/30	10/4*	10/11	10/20	11/1	11/22
regolith	sterile pond	0.0		0.0	0.0	0.0	0.1
	non-sterile pond	0.0		0.0	0.0	0.0	0.0
soil & regolith	sterile pond	0.1		9.3	3.4	17.0	28.0
	non-sterile pond	0.0		3.1	0.3	7.7	4.6
soil	sterile pond	0.5		21.0	0.8	14.0	23.0
	non-sterile pond	0.3		22.0	0.6	9.1	9.5

Fig 15. Table showing quantification results of DNA extracted from sample. DNA concentrations above 3.0 ng/ μ L are shown in green, and concentrations below 3.0 ng/ μ L are shown in blue.

*10/11 data was not tested before submission date

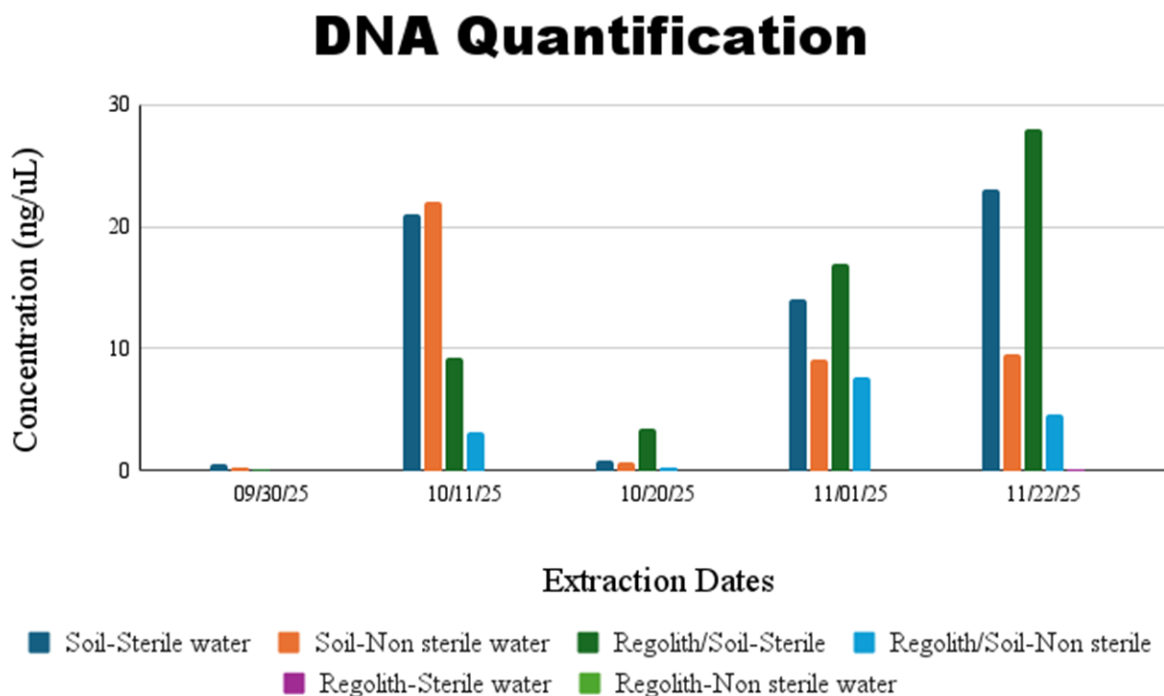


Fig 16. Bar graph representation of DNA Quantification data

6.0 Discussion

6.1 Analysis of Findings

Our findings revealed a correlation between cyanobacteria presence and plant growth. In the case of cyanobacteria detection, the positive control results were consistently positive, and the negative controls were consistently negative, indicating that the controls were reliable and procedural methods for cyanobacteria detection were sound. This suggests that much of the error in our experiment may be attributed to the DNA extraction process in soil-based environments, where cyanobacteria might need to be overly abundant to be detected. Interestingly, the soil and regolith samples were both consistently negative for cyanobacteria, which raises questions about potential procedural or collection errors. It is possible that soil and regolith contain compounds that interfere with bacterial detection, or that moisture at the time of collection played a role. Alternatively, the combination of soil and regolith may have created a ‘false-positive’ effect in earlier stages of analysis. The 50/50 soil-regolith mixture showed the clearest signs of earlier bacterial exposure or cultivation, making it the most effective for detection. However, this raises the question of why the 50/50 combination was more successful compared to other samples.

For the 16S rRNA analysis, the results were predominantly negative, with only a few positive results in the positive controls and late-stage 50/50 samples. Given the limited positive results, there was little new information to extrapolate beyond the conclusions already drawn from the cyanobacteria analysis. This discrepancy between the cyanobacteria and 16S detection methods highlights a need to investigate the procedural differences between the two approaches.

6.2 Insights into Soil and Water Interactions

Our analysis also revealed insights into the interactions between soil and water. While pond water did not result in the dramatic growth differences we had initially predicted, it still proved to be a significant factor. Radishes grown with pond water appeared fuller, brighter in color, and survived longer compared to those grown with sterilized water. For instance, under the harshest conditions (pure regolith with sterilized water), radishes managed to survive to the halfway point before succumbing, providing a useful baseline for future experiments.

Interestingly, the radishes grown in 100% soil with pond water died during the final week of the experiment. This premature death may have been caused by DNA sampling at the plant roots, which disrupted the plant’s environment. However, this finding also suggests that our grow station conditions may not have been ideal for plant longevity. Future improvements to the grow station could help eliminate the possibility of insufficient growth conditions impacting experimental results.

The DNA quantification highlighted key issues with the DNA extraction process, leading to inconsistent results, and potentially explaining the lack of positive PCR results for the regolith samples. Minute concentrations of DNA were observed in two different regolith samples,

indicating some presence of DNA, however the samples likely did not contain enough DNA for the PCR analysis to provide positive results.

6.3 Implications for Space Agriculture

Our findings have important implications for extraterrestrial farming scenarios. For plant growth, anything other than pure regolith proved to be beneficial, even sterilized soil with no added nutrients. Pond water outperformed sterilized water, suggesting that microbe-rich water can significantly enhance plant prosperity. As expected, plants grown in 100% soil showed the largest and fastest growth, while the 50/50 mixture demonstrated moderate growth but died quickly in the latter half of the experiment. Plants grown in 100% regolith exhibited minimal growth. These findings experimentally confirm that regular soil remains the optimal base for plant growth.

6.4 Limitations

Several limitations were identified in this experiment. First, different plants may yield different results, both in terms of growth and bacterial interactions, as each plant requires a unique microbiome. Additionally, the Zymo Research Quick-DNA Fecal/Soil Microbe MiniPrep Kit, while versatile, may not have facilitated the precise detection of microbes required for this study. This DNA extraction kit may have also struggled with extracting DNA from the regolith substrate, as it is not very porous, leading to less DNA extracted, if any. Deviations from the kit's instructions could also have contributed to the absence of microbes in some samples. Furthermore, errors in labeling samples may have caused confusion in data analysis. For instance, inconsistent naming conventions (e.g., 50/50' versus '50/50 soil') may have led to misinterpretation of results. While these discrepancies are unlikely to fully explain the varying outcomes, they underscore the challenges inherent in DNA detection and highlight areas for procedural improvement in future experiments.

7.0 Conclusion

After months of growth, it can be inferred that water enhanced with live microbes can significantly enhance the germination, structure and overall prosperity of radishes grown in different concentrations of regolith. Furthermore, the presence of cyanobacteria in the 50/50 pond water group suggests that the microbes may have been hypothesized and acted to enrich the soil with nitrogen over the growing period. These findings are significant due to their downstream applications in cultivating produce in lunar regolith, suggesting that introducing live cultures of cyanobacteria to seeds in regolith may increase their chances to germinate, grow and thrive despite the unfamiliar substrate.

Going forward, more molecular tests are needed to track cyanobacteria over the growing period to account for the possible false positive found on the first October 4th sample. Future

research focusing on different concentrations of regolith and soil is also needed in order to understand why DNA was only successfully extracted from the 50/50 samples. The optimization of DNA extraction methods in non-porous silica-based synthetic regolith could provide more insights into the growth of bacteria in silica-based substrates. Additionally, molecular testing of the substrate near the roots could suggest whether or not the cyanobacteria found are specifically in a mutualistic relationship with the radish plants, or if enhanced growth is a side effect of their general presence in the substrate. Further experimentation with other species of plants is also recommended, specifically species that have mutualistic relationships with nitrogen fixing bacteria such as legumes to generalize the hypothesized effect of cyanobacteria on plants grown in regolith.

8.0 References

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9.0 Appendix

Appendix A: DNA Extraction Protocol

Protocol for DNA extraction, primers used for detecting cyanobacteria, and thermal cycling conditions.

A beginning, middle and end soil sample will have DNA extracted from it, using the Zymo Research Quick- DNA Fecal/Soil Microbe Miniprep Kit. Methods for extraction can be found below.

1. Add 250 mg of soil sample to a ZR BashingBead™ Lysis Tube. Add 750 µl BashingBead™ Buffer to the tube¹.
2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process for 1 hour.
3. Centrifuge the ZR BashingBead™ Lysis Tube in a microcentrifuge at 10,000 RCF for 1 minute.
4. Transfer up to 400 µl supernatant to a Zymo-Spin™ III-F Filter in a Collection Tube and centrifuge at 8,000 RCF for 1 minute.
5. Add 1,200 µl of Genomic Lysis Buffer to the filtrate in the Collection Tube from Step 4. Mix well.
6. Transfer 800 µl of the mixture from Step 5 to a Zymo-Spin™ IICR Column⁴ in a Collection Tube and centrifuge at 10,000 RCF for 1 minute.
7. Discard the flow through from the Collection Tube and repeat Step 6.
8. Add 200 µl DNA Pre-Wash Buffer to the Zymo-Spin™ IICR Column in a new Collection Tube and centrifuge at 10,000 RCF for 1 minute.
9. Add 500 µl g-DNA Wash Buffer to the Zymo-Spin™ IICR Column and centrifuge at 10,000 RCF for 1 minute.
10. Transfer the Zymo-Spin™ IICR Column to a clean 1.5 ml microcentrifuge tube and add 100 µl (50 µl minimum) DNA Elution Buffer directly to the column matrix. Centrifuge at 10,000 RCF for 30 seconds to elute the DNA.
11. Place a Zymo-Spin™ III-HRC Filter in a clean Collection Tube and add 600 µl Prep Solution. Centrifuge at 8,000 RCF for 3 minutes.
12. Transfer the eluted DNA to a prepared Zymo-Spin™ III-HRC Filter in a clean 1.5 ml microcentrifuge tube and centrifuge at exactly 16,000 RCF for 3 minutes.
13. Store filtered DNA in the lab freezer until PCR is run.

14. The following DNA extraction and gel electrophoresis protocol will be repeated twice. Once with 16S primers and once with cyano primers
15. The extracted DNA will be prepared for PCR using the protocol below.
 - a. We will make the Taq 2X Master Mix from New England Biolabs. We will make a 9X volume solution to ensure we have enough for 6 DNA samples (one from each planter) along with a positive and negative control.
 - b. We will use the protocol below to make a 9X volume master mix.
 - i. Add 112.5 μ l Taq 2X Master Mix.
 - ii. Add 225 μ l Nuclease-free water.
16. Add 4.5 μ l Forward Primer and 4.5 μ l Reverse Primer.
17. Add 90 μ l Nuclease-Free Water
18. Label 8 PCR tubes: one for each soil sample (6), a positive control, and a negative control.
19. Add 24 μ l of the master mix and 1 μ l of extracted DNA to 6 of the labeled test vials. Add 24 μ l of the master mix and a 1 μ l sample of cyanobacteria to the positive control. Add 24 μ l of the master mix and no DNA sample to the negative control.
20. Place all 8 PRC tubes into the thermocycler and set it according to the specifications below.
 - a. Initial Denaturation: 95°C for 10 minutes.
 - b. Denaturation: 95°C for 30 seconds.
 - c. Annealing: 55°C for 30 seconds.
 - d. Extension: 68°C for 90 seconds.
 - e. Steps 20.b-20.d are repeated 30 times
 - f. Final Extension: 68°C for 5 minutes
21. Gel Electrophoresis
 - a. Gel Prep with MiniPcr GelGreen Agarose Tabs
 - i. Add 80 ml of distilled water
 - ii. Soak 2 tablets until fully dissolved
 - iii. Boil solution until clear
 - iv. Let cool briefly and pour into mold

- b. Once the agarose gel has hardened in the mold, set it up in the machine for electrophoresis, and add Tris-Acetate-EDTA buffer until the gel is completely submerged
- c. Add 15 μ l of 6X Purple Gel Loading Dye from New England BioLabs loading dye to the amplified DNA
- d. Place a DNA ladder into one well and 20 μ l of the DNA and loading dye from each sample into 8 other wells
- e. Run gel electrophoresis for 40 minutes at 135 volts

Controls:

- Negative control (autoclaved soil and sterilized water with no cyano).

Data Collection:

- Metrics for plant growth (e.g., height, biomass, leaf count).
- Record of cyanobacteria detection.

Appendix B: PCR Setup

- PCR Master Mix (9X Volume):
 - 112.5 μ l Taq 2X Master Mix (New England Biolabs)
 - 225 μ l Nuclease-Free Water
 - 4.5 μ l Forward Primer
 - 4.5 μ l Reverse Primer
- PCR Tube Setup:
 - Label 8 tubes: 6 soil DNA samples, 1 positive control, 1 negative control
 - Add 24 μ l of master mix + 1 μ l DNA template to each
 - Positive control: cyanobacteria DNA
 - Negative control: no DNA
- Thermal Cycler Conditions:
 - Initial Denaturation: 95°C for 10 min
 - 30 Cycles of:
 - Denaturation: 95°C for 30 sec

- Annealing: 55°C for 30 sec
- Extension: 68°C for 90 sec
- Final Extension: 68°C for 5 min
- Primers Used

Primer Set	Primer Name	Direction
16S rRNA (Universal)	8F	Forward
	1492R	Reverse
Cyanobacteria-Specific	CYA 100F	Forward
	719R	Reverse

Appendix C: Gel Electrophoresis Protocol

- Gel Preparation:
 - Add 80 mL distilled water to a flask
 - Soak 2 MiniPCR GelGreen Agarose Tabs until fully dissolved
 - Heat until the solution becomes clear
 - Cool briefly and pour into casting mold
 - Allow gel to solidify completely
- Gel Setup
 - Place gel in electrophoresis chamber
 - Add TAE buffer until gel is fully submerged
 - Load 1 well with DNA ladder
 - Mix PCR products with 6X Purple Gel Loading Dye (15 µL)
 - Load 20 µL of each sample into wells
- Electrophoresis Conditions
 - Voltage: 135 V

- Run Time: 40 minutes
 - Buffer: Tris-Acetate-EDTA (TAE)
- Visualization
 - Visualize gel under UV transilluminator
 - Photograph gel for record-keeping
 - Assess presence and size of DNA bands relative to DNA ladder