The Impact of Fertilizer Treatment on the Root Microbiome of Hemp and Tomato Plants

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Abstract

In response to the global challenge of managing human waste sustainably, this study was conducted to explore the potential of the leachate byproduct of Clivus Multrum composting toilets as a fertilizer for agricultural use. Traditional wastewater treatment methods are energy-intensive and contribute to water pollution, prompting a need for more sustainable alternatives. Clivus Multrum obtained a permit from New York State to evaluate the potential of human waste compost leachate as a nitrogen-rich fertilizer for farmland, circumventing current US regulations that restrict the use of human waste on farmland without special permission. Our goal was to compare the microbial diversity in the root systems of tomato (*Solanum lycopersicum*) and hemp (*Cannabis sativa* L.) plants treated with three different fertilizers: Clivus Multrum leachate, synthetic fertilizer, and vermicompost. We also aimed to evaluate the abundance of plant growth-promoting microorganisms in each treatment. DNA was extracted from three different root regions—rhizosphere, rhizoplane, and endosphere from 90 plants (45 of each plant type). The 16S and ITS V4 regions were amplified using polymerase chain reaction (PCR) and then sequenced on an Illumina MiSeq platform. Raw sequences were cleaned using DADA2 and then analyzed using Phyloseq and DESeq2 R packages. Results from this analysis indicated minimal significant differences in community composition among the treatments, but notable differences in abundance, particularly in the rhizosphere region. This finding suggests that Clivus Multrum leachate may serve as a viable substitute for organic fertilizer pending state government approval, highlighting its potential in sustainable agricultural practices.

Introduction

Background/Novelty

Human waste management and access to adequate sanitation is a global issue with roughly 4.5 billion people living without appropriate means for wastewater treatment (World Health Organization 2015). Even in the developed world, wastewater management remains a major problem—predominately as a source of pollution to waterways—with 1.2 trillion gallons of untreated sewage flowing into US rivers annually (Wear et al. 2021). Overburdened treatment facilities and lack of commitment by local governments to maintain infrastructure are largely responsible for the discharge of sewage into watersheds (Ahuja 2014). The influx of nutrients caused by the mismanagement of wastewater threatens local ecosystems and diminishes water quality, ultimately leading to poorer human health outcomes (Akpor and Muchie 2011). To make matters worse, the current infrastructure in the US not only pollutes the environment but also wastes clean water and electricity with an estimated 3% of total US power used for wastewater treatment and 30-60% of water use dedicated to toilet flushing in residential/commercial spaces (Anand and Apul 2014). One solution to this ongoing crisis could be the implementation of composting toilets.

Clivus Multrum is a Boston-based composting toilet company that designs systems compatible with commercial, residential, and rural spaces. Once installed, Clivus Multrum toilets use no water/electricity and capture the valuable nutrients stored in human excreta. Contained within a fiberglass frame, the deposited waste is combined with wood chips which serve as a bulking agent and help facilitate the breakdown of material over many years. A solar-powered fan aerates the pile, and a false-bottom drains excess liquid into a separate holding container where it can compost independently from the solid waste. The composting process is often described as odor-free, low maintenance, and yields a pathogen-free and nutrient-rich fertilizer suitable for agriculture. Using the compost/leachate generated by Clivus Multrum toilets could replace the use of synthetic fertilizers and help close the nutrient cycle loop. However, US legislation restricts the use of human excreta in agriculture without specific state permissions, hindering scientific evaluation and creating a knowledge gap on safety considerations.

In 2022, Clivus Multrum obtained a permit from New York State to apply compost leachate to agricultural land with the goal of evaluating it as a nitrogen-rich fertilizer. This study is one of the first of its kind to assess the impact of human waste compost on plant growth and soil microbiology, with the overall objective aiming to improve our understanding of the potential environmental and health risks associated with its application.

Project Aim

The goal of this project was to evaluate Clivus Multrum leachate as a source of plant available nutrients and assess any potential risks associated with its application to agricultural soils. Specifically, this study aimed to evaluate: (1) the impact of Clivus Multrum leachate on plant growth; and (2) its effect on soil microbial communities. We hypothesized that the leachate would perform comparably to the control groups and not significantly alter the soil microbial community.

Objectives

To assess aim (2) we sought to answer three primary questions:

1. Does fertilizer treatment significantly alter the root microbiome of hemp/tomato plants?
2. How does the microbiome change between sampling locations (endosphere, rhizoplane, rhizosphere)?
3. Are plant growth promoting rhizobacteria more prevalent in certain treatment groups?

Materials and Methods

Experimental Design

The ability of Clivus leachate to promote plant growth as a fertilizer and possibly a natural bio-stimulant was assessed in a greenhouse experiment. Week old tomato/hemp seedlings were transplanted into 3-gallon pots containing pasteurized field soil. One of three treatments were applied throughout a 21-day period and each treatment was replicated five times (n=5). Jack's soluble liquid fertilizer (synthetic control) and Black Dirt Farm worm castings (organic control) applied at 100 lbs. of N per acre were used as control groups to compare against Clivus leachate applied at the same rate. Plant height and number of leaves were measured on a weekly basis. At the conclusion of the 21-day experiment, stem diameter was taken, and plants were sampled destructively to swab roots for DNA amplicon sequencing and assess root:shoot ratios. Rhizosphere, rhizoplane, and endosphere regions of the root were sampled and sent for 16S and ITS sequencing.

Initial Bioinformatics Pipeline

Initial sequencing data was cleaned, filtered, and assigned taxonomy by the Fierer Laboratory at the University of Colorado at Boulder, and followed the DADA2 pipeline as laid out in Callahan et al 2016. DADA2 is a bioinformatics tool designed for analysis of microbial diversity and was used to analyze the collected 16S and ITS data. All further analysis was done in the Ecological Genomics class at the University of Vermont.

Phyloseq and DESeq2

We acquired three essential tables: an ASV table detailing the sequence count in each sample, a taxonomic assignment table for individual ASV sequences, and a metadata table containing sample-specific information. These tables were integrated into a phyloseq object using the R (v4.3.2)/phyloseq bioconductor package v1.46.0 (McMurdie and Holmes 2013). ASVs that could not be taxonomically assigned to a bacterial or fungal phylum were excluded from the analysis. Furthermore, ASVs exhibiting a variance greater than 10^6 were filtered out, akin to the removal of ASVs with a read count less than 180, ensuring the dataset's freedom from sequencing artifacts and low-abundance contaminants. PERMANOVA (adonis) tests, was conducted using the R (v4.3.2)/vegan package (Oksanen, 2010), utilized Bray-Curtis distances to evaluate potential associations between treatments, plant type, sample type, and microbiota composition. Normalised (x/ (sum (x)) ASV values from the phyloseq table were used for the PERMANOVA (adonis) test. Statistical significance was defined as P-values < 0.05. Subsequently, the top 11 relevant phyla in the phyloseq object were selected for further analysis, including Acidobacteriota, Actinobacteriota, Bacteroidota, Chloroflexi, Gemmatimonadota, Planctomycetota, Verrucomicrobiota, Proteobacteria, Nitrospirota, and Firmicutes. Alpha diversity was assessed using the Shannon index. Principal Coordinate Analysis (PCoA) was employed to identify and visualize the Principal Coordinate values for each sample. The resulting PCoA plots depicted Axis 1 and Axis 2, visually representing the average phyla abundance across different treatments, sample types, and plant types. We then used the R (v4.3.2)/DESeq2 (Love et al., 2014) package to pinpoint bacterial species exhibiting the most notable changes in abundance at the species level within each sample. Differential abundance was assessed using the Wald test in the DESeq2 package, incorporating three pairs of group comparisons across the various treatments under investigation. We delved into a specific comparison, exploring the differential abundance between the Rhizosphere sample type, which is distinctly clustered in the PCoA plot, and another sample type, Rhizoplane. This focused comparison provided valuable insights into the unique microbial dynamics associated with these distinct sample types, shedding light on their differential abundance patterns, and contributing to a deeper understanding of the microbiota composition within the experimental framework.

Results

Plant Growth

All treatments performed similarly in both plant types. Plant height, number of leaves, stem diameter, and root:shoot ratio showed no significant differences in response to the three treatments.

Community composition and 16S diversity by treatment type and root zones

Shannon diversity of 16S sequence data varied considerably by treatment and region of the root **(Figure 1**). Higher alpha diversity scores were generally associated with the vermicompost treatment group while Clivus and greenhouse fertilizer had more similar scores. Endosphere samples tended to have lower diversity scores while the rhizosphere had greater bacterial diversity. The ADONIS showed significant differences between different treatments, sampling type, and plant type taxa composition ($P < 0.001$ for all three variables) (**Table 1, A**). Analysis of differential taxa abundance (16S) across all treatments revealed significant differences in certain genera from various phyla. **Table 1, B** displays the top 5 significantly abundant taxa across all treatments. A differential taxa abundance analysis was performed to investigate the primary taxa in the rhizophore (**see Table 2, C**).

Community composition and ITS diversity by treatment type and root zones

Shannon diversity of ITS sequence data was similar across treatments, root zones, and plant type (**Figure 6**). Higher diversity scores were generally associated with the vermicompost treatment group, but these differences were marginal. The ADONIS showed evidence of differences between treatment and again for sampling location ($P = 0.042$ and $P = 0.006$, respectively). The ADONIS showed no evidence of difference between plant types ($P = 0.752$).

16S and ITS principal component analysis

Comprehensive PCA plots encompassing treatment type, root zone, and plant type were generated to assess clustering of 16S (**Figure 2**) and ITS data (**Supplemental Figure 2**). For the 16S data, vermicompost samples clustered on axis two further away from the other treatment groups indicating bacterial communities of plants fertilized with vermicompost were more unique than the other treatments (**Figure 2, A**). However, Clivus and greenhouse clusters overlapped considerably indicating similarity between those treatments (**Figure 2, A**). Root zone PCA plots formed three main clusters with endosphere and rhizosphere zones most different from each other along axis one (**Figure 2, B**). Rhizoplane samples overlapped considerably with both endosphere and rhizosphere samples indicating shared bacterial communities (**Figure 2, B**). PCA plots generated by treatment type had considerable overlap in Clivus and greenhouse fertilizer, but vermicompost clustered separately along axis two (**Figure 2, C**). Additional plots were created from ITS data to assess clustering, but no significant clusters were observed between plant type, fertilizer treatment, or root zone (**Supplemental Figure 2**). Of note, a point curve developed on the ITS PCA plot (**Supplemental Figure 2**), further analysis is needed to understand this trend.

Abundance bar plots review/interpretation:
The analysis of differential taxa abundance showed that the top four phyla, Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteriota (**Table 1, B**), collectively accounted for 38%, 28%, 13.5%, and 7.8% of the total taxa abundance, respectively (**Figure 3**).

Discussion

Influence of fertilizer on plant growth
The primary hypothesis of this investigation was that the Clivus Multrum leachate would perform similarly to the synthetic and organic fertilizer controls when applied to hemp and tomato plants. Performance of the fertilizers was first assessed through phenotypic characteristics of the tomato and hemp plants. These measurements included plant height, number of leaves, root-to-shoot mass ratio, and stem diameter. Results from this experiment showed no significant difference in plant outcomes across treatments/plant type indicating the Clivus leachate is a suitable alternative to traditional fertilizers. The root and soil microbiome of each sample were then used as a secondary layer of comparison between treatments.

Does fertilizer treatment significantly alter the root microbiome of hemp/tomato plants?
Shannon's Diversity was used to assess the overall diversity in microbiomes between treatments. Shannon's diversity was chosen as opposed to other indices because it considers richness and abundance, while not overemphasizing rare taxa that were not filtered out in this analysis (DeJon 1975). Vermicompost generally displayed the highest bacterial diversity in the endosphere and rhizoplane of hemp plants and the highest diversity in rhizoplane and rhizosphere of tomato plants. Fungal diversity was highest in the vermicompost treatment, however, there was little to no difference between root sampling location or plant type. This finding agrees with current understanding of vermicompost which has a unique microbiome when compared to other fertilizer sources (Readyhough et al. 2021). The distinct microbial characteristics of vermicompost are largely due to the gut microflora of the worms used in the vermicomposting process. As organic matter passes through the earthworms, it is enriched with a diverse assortment of bacteria and fungi that help facilitate the breakdown of complex material into plant-available forms. This interaction of worms and microbes results in nutrient-rich and microbial diverse castings (worm poop aka vermicompost) known to be beneficial for plant growth (Domínguez et al. 2019). When amended to soil, vermicompost enriches the soil with those microbes which could explain the higher diversity scores observed in this study. In contrast, synthetic fertilizers and the Clivus leachate consist mainly of mineral nutrients which have limited microbiology to pass onto the soil. This would explain the lower diversity scores in the

Clivus and greenhouse treatment groups. Overall, it appears the microorganisms within the vermicompost influence the root microbial diversity of tomato and hemp plants to a greater degree than the Clivus leachate/greenhouse fertilizer treatments in this study.

How does the microbiome change between sampling locations (endosphere, rhizoplane, rhizosphere)?

A secondary question of this investigation was to assess how the microbiome changed between sampling locations along the root:soil interface. The three regions that were sampled in this study were the endosphere, rhizoplane, and rhizosphere. The endosphere can be defined as the region in the root where microbes can colonize the cortex, epidermis, and root hairs. In close proximity, the rhizoplane is simply the external surface of the root and the closely adhered soil particles. Furthest from the endosphere, the rhizosphere is characterized as the region of the soil in the vicinity of the plant roots in which the chemistry and microbiology can be influenced by root exudates (Haldar and Sengugpta 2015). Each zone has its own physical and chemical characteristics which greatly affect how the plant and soil life interact and what bacteria/fungi inhabit each region.

Over the course of the experiment, the three zones were uniquely influenced by the different treatments. Notably, each region formed a separate cluster in the PCA plots with endosphere and rhizosphere samples clustering further away from each other along axis one (**Figure 2**). Clustering reflected spatial differences in root zones with the rhizoplane samples sharing microbial characteristics with both the rhizosphere and endosphere. This is expected as the rhizoplane would include microorganisms from both the plant and the soil microbiome. On the other hand, microbes interacting in the endosphere would be tightly controlled by the host plant while the rhizosphere microbiome would be more heavily influenced by the soil's microbial community (van der Herijden and Schlaeppi 2015). Overall, these findings agree with current understanding of root zone regions and expected diversity scores within those regions.

Are plant growth promoting bacteria more prevalent in certain treatment groups?

Actinobacteria play an important role in root microbial communities, doing so by contributing to nutrient cycling and other aspects of plant health. In addition, they are known to promote plant growth and protect against plant pathogens (Bulgarelli et al. 2013) mainly through competitive biocontrol and the production of antibiotics. Actinobacteria accounted for 7.8% of all ASV counts across all samples of 16S data. In addition, they were prevalent across all treatments and generally had a greater abundance in the endosphere, suggesting members of this phyla colonized the endosphere more consistently than other root microbiome locations.

Bacteroidota is another important phylum to nutrient cycling within the root and soil microbiome of crops (Kruczyńska et al. 2023). This phylum is capable of phosphorus solubilization and nitrogen fixation, making it an important group for absorbing the nutrients made available by the amendment of fertilizers (Wolińska et al 2017). Bacteroidota also promotes plant growth through the production of phytohormone analogs. This phylum was responsible for 13.5% of all ASV counts across all samples. It is in greatest abundance in the endosphere and grows less abundant as the sampling zone moves away from the root and into the soil microbiome. This could indicate the plant being more responsible for the abundance of this phylum compared to the fertilizer treatment. Across treatments, there was not a significant difference in the abundance of bacteroides.

Firmicutes play a critical role in nutrient cycling, plant nutrient acquisition, and plant phytohormone production within soil microbiomes (Zhang and Xu 2008). This phylum is known for its use as a biological control organism of other fungi or pathogens. The abundance of firmicutes was noticeably increased across the species of plant, considerably more ASVs of the phylum were found in hemp compared to tomato plants. However, when analyzed between treatments within each sample type, a consistent root zone and plant species, counts of firmicutes were largely consistent. The lowest firmicute count occurred in the endosphere of the tomato plants that were treated with conventional greenhouse fertilizer. The application of the Clivus treatment did not significantly change the abundance of firmicutes across the root zones of either tomato or hemp plants.

Planctomycetota was observed at very low abundances when compared to other dominant phylum within the soil and root microbiome. Its presence was observed majorly in the vermicompost treatment samples. This specific phylum could have been introduced to the sample from the microbial community present in the vermicompost itself, explaining why no planctomycetes were observed in the Clivus Multrum or conventional greenhouse fertilizer treatment samples. Planctomycetota is known for its carbon and nitrogen cycling capabilities as well as several species within the phylum being able to anaerobically oxidate ammonium (Fang et al. 2022). However, this phylum is not considered a plant growth promoting bacteria and was not included in the family level analysis for this project.

Proteobacteria help to contribute to nitrogen fixation, nutrient availability, and the overall growth of the plant (Bulgarelli et al. 2013). They were the most abundant bacterial phyla found in the 16S data, comprising 37.8% of all ASV's. Proteobacteria frequency was fairly consistent, with the exception of the Clivus Multrum tomato plant

treatment, which saw a higher than expected number. Future analysis should look into the exact composition of this treatment and compare it with the others.

Conclusions

In this study, we found that the Clivus Multrum leachate performed similarly to synthetic and organic fertilizers when applied to hemp and tomato plants, indicating its potential as an alternative to traditional fertilizers. Vermicompost displayed the highest bacterial and fungal diversity in the endosphere and rhizoplane of hemp plants, as well as in the rhizoplane and rhizosphere of tomato plants, suggesting its unique microbiome influences root microbial diversity. Actinobacteria and Bacteroidota were important phyla in root microbial communities, contributing to nutrient cycling and plant growth. The abundance of these phyla varied with plant species and treatments, but the Clivus Multrum treatment did not significantly change their abundance. Overall, the Clivus Multrum leachate could be a viable fertilizer alternative if granted approval by state governments.

Contributions

Project inspiration, background and initial development completed by Noah Olson. ITS analysis, discussion contribution, and initial bioinformatics pipeline methodology interpreted and described by Alex Kissonergis. Phyloseq coding, rarefaction, and plot development completed by Maryam Nouri-Aiin. Plot interpretation and microbiome composition results interpreted by Lucy Toppen. Diversity plots, discussion contributions, and Github repository and project notebook maintained by Carolyn Hanrahan. Analysis choices, rarefaction analysis, interpretation of data, and plots created in collaboration with all group members. Final presentation and paper designed and written collaboratively by all group members.

Scripts

R code, relevant scripts, and a Markdown notebook for this project can be found in the Ecogen_Metagenomics_Project repository on Github at the following link: https://github.com/carolyn-hanrahan/Ecogen_Metagenomics_Project

References

Ahuja, S. (Ed.). (2014). *Water reclamation and sustainability*. Elsevier.

Akpor, O. B., & Muchie, B. (2011). Environmental and public health implications of wastewater quality. *African Journal of Biotechnology*, *10*(13), 2379-2387.

Anand, C. K., & Apul, D. S. (2014). Composting toilets as a sustainable alternative to urban sanitation–A review. *Waste management*, *34*(2), 329-343.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Van Themaat, E. V. L., & Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annual review of plant biology*, *64*, 807-838.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature methods*, *13*(7), 581-583.

Danon, M., Franke-Whittle, I. H., Insam, H., Chen, Y., & Hadar, Y. (2008). Molecular analysis of bacterial community succession during prolonged compost curing. *FEMS Microbiology Ecology*, *65*(1), 133–144. https://doi.org/10.1111/j.1574-6941.2008.00506.x

DeJong, T. M. (1975). A comparison of three diversity indices based on their components of richness and evenness. *Oikos*, 222-227.

Domínguez, J., Aira, M., Kolbe, A. R., Gómez-Brandón, M., & Pérez-Losada, M. (2019). Changes in the composition and function of bacterial communities during vermicomposting may explain beneficial properties of vermicompost. *Scientific reports*, *9*(1), 9657.

Fang, J., Weng, Y., Li, B., Liu, H., Liu, L., Tian, Z., & Du, S. (2022). Graphene oxide decreases the abundance of nitrogen cycling microbes and slows nitrogen transformation in soils. *Chemosphere*, *309*, 136642.

Haldar, S. and Sengupta, S., 2015. Plant-microbe cross-talk in the rhizosphere: insight and biotechnological potential. *The open microbiology journal*, *9*, p.1.

Kruczyńska, Anna, et al. "Bacteroidota structure in the face of varying agricultural practices as an important indicator of soil quality–a culture independent approach." *Agriculture, Ecosystems & Environment* 342 (2023): 108252.

McMurdie and Holmes (2013) phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE. 8(4):e61217

Love, M.I., Huber, W. and Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology*, *15*(12), pp.1-21.

Oksanen, J., 2010. Vegan: community ecology package. *http://vegan. r-forge. r-project. org/*.

Readyhough, T., Neher, D. A., & Andrews, T. (2021). Organic amendments alter soil hydrology and belowground microbiome of tomato (Solanum lycopersicum). *Microorganisms*, *9*(8), 1561.

Rizzatti, G., Lopetuso, L. R., Gibiino, G., Binda, C., & Gasbarrini, A. (2017). Proteobacteria: A Common Factor in Human Diseases. *BioMed Research International*, *2017*, 9351507. https://doi.org/10.1155/2017/9351507

Spain, A. M., Krumholz, L. R., & Elshahed, M. S. (2009). Abundance, composition, diversity and novelty of soil Proteobacteria. *The ISME Journal*, *3*(8), Article 8. https://doi.org/10.1038/ismej.2009.43

Team, R. (2015). RStudio: integrated development for R. *(No Title)*.

van der Heijden, M. G., & Schlaeppi, K. (2015). Root surface as a frontier for plant microbiome research. *Proceedings of the National Academy of Sciences*, *112*(8), 2299-2300.

Wear, S. L., Acuña, V., McDonald, R., & Font, C. (2021). Sewage pollution, declining ecosystem health, and cross-sector collaboration. *Biological Conservation*, *255*, 109010.

Wolińska, A., Kuźniar, A., Zielenkiewicz, U., Izak, D., Szafranek-Nakonieczna, A., Banach, A., & Błaszczyk, M. (2017). Bacteroidetes as a sensitive biological indicator of agricultural soil usage revealed by a culture-independent approach. *Applied Soil Ecology*, *119*, 128-137.

World Health Organization, 2015. Progress on Sanitation and Drinking-water - 2015 Update and MDG Assessment. World Health Organization and UNICEF. WHO Press, Geneva, Switzerland. Available at: https://www.unicef.org/publications/index_82419.html.

Zhang, L., & Xu, Z. (2008). Assessing bacterial diversity in soil: a brief review. *Journal of soils and sediments*, *8*, 379-388.

Tables and Figures

Figure 1

displays Shannon's diversity/alpha diversity metrics for each treatment group (clivus, greenhouse, and vermicompost) by root zone (endosphere, rhizoplane, rhizosphere) and plant type (hemp vs tomato). Higher alpha diversity measures correspond to greater microbiome diversity.

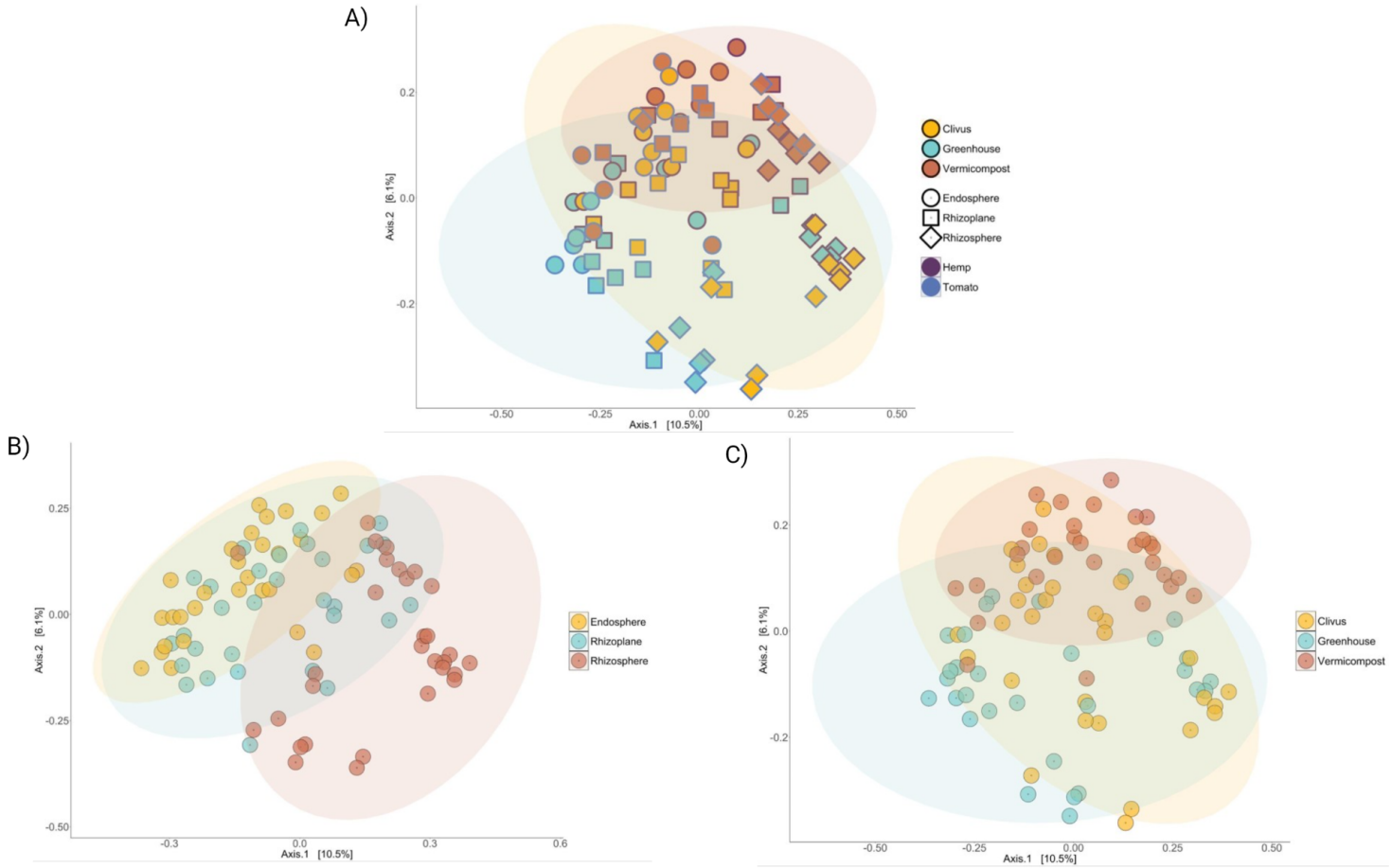

Figure 2

A) Comprehensive PCA plot demonstrating the ordination of taxa based on principal coordinate analysis (PCoA), with data points colored by treatment and shaped by sample type. This plot illustrates a broad overview of the data, showing general clustering by treatment type and root zone. B) shows a PCA plot with two PC axes visualizing clustering by root zone only. C) show a PCA plot with two PC axes visualizing clustering by treatment type only.

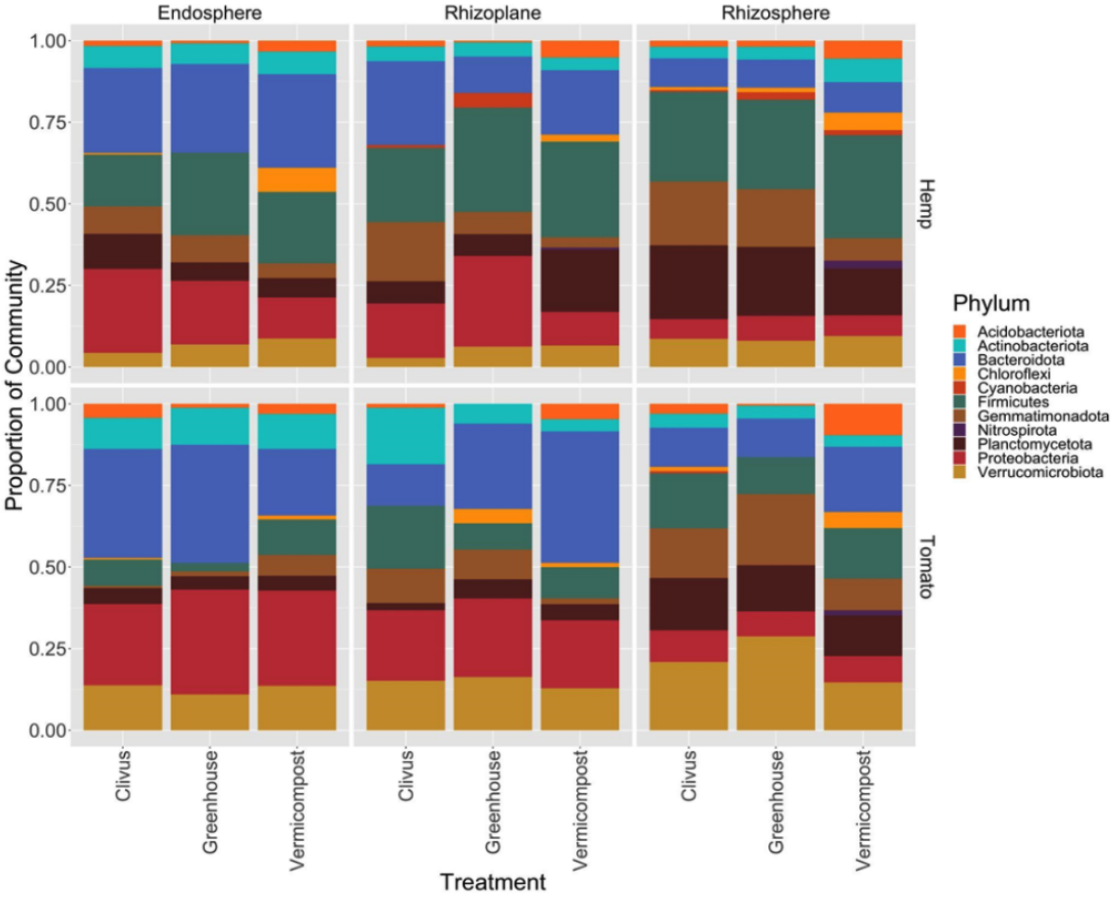

Figure 3

Contains bar plots displaying 11 microbial phyla and the relative composition of them across treatment, plant types, and root zones. Phyla were chosen based on function mainly pertaining to nutrient cycling and plant growth from previous literature. Composition was based on average abundance of each phyla across each treatment and root zone.

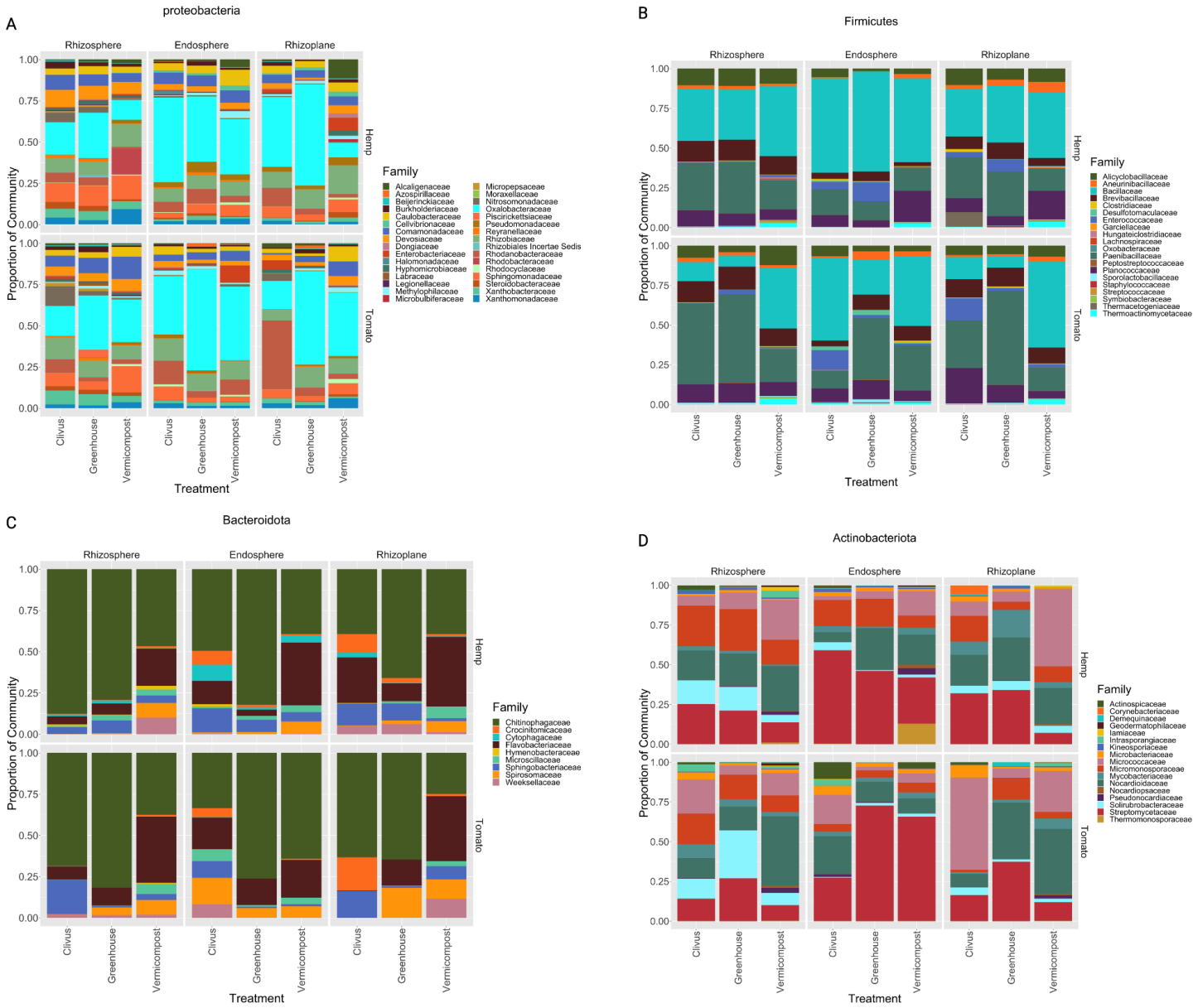

Figure 4

Composition of dominant phyla at the family level, featuring A) Proteobacteria, B) Firmicutes, with a significant presence of the Bacillaceae family, C) Bacteroidetes, where the majority is represented by the Chitinophagaceae family, and D) Actinobacteria, highlighting a noteworthy rise in the Streptomycetaceae family in the endospore.

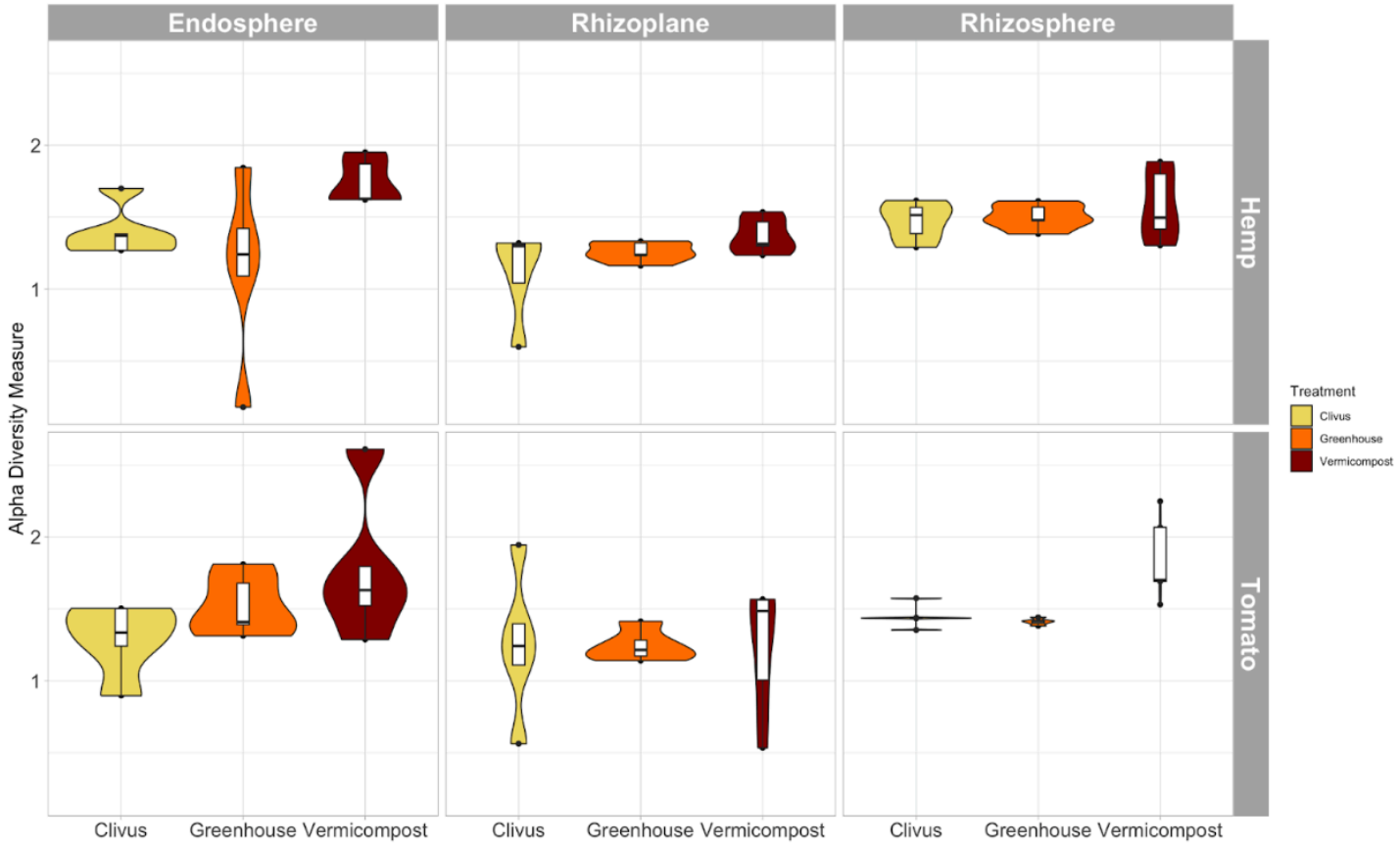

Figure 5

This plot shows Shannon's Diversity metrics for the ITS data. The plot shows diversity measures for each treatment group and by root location and plant type. Higher diversity measures are associated with greater microbiome diversity.

Table 1.
A) Results from PERMANOVA analysis differential taxa abundance across all treatments
B) Analysis of differential taxa abundance across all treatments, displaying the top 5 significantly abundant taxa, C) Differential taxa abundance analysis showing the primary taxa in the rhizophore. baseMean: The average of normalized read counts of each ASV across all samples, Log2 of fold change in abundance of ASVs across different Treatment, and pvalue: p-value from the statistical test (here: Wald test) for differential abundance.

A) *PERMANOVA (adonis)*

Source of variation	*Df*	*SS*	*R2*	*F*	*Pr(>F)*
Treatment	2	2.29	0.08	4.31	0.001
Plant type	1	1.62	0.06	6.09	0.001
Sample type	2	3.10	0.11	5.83	0.001
Residual	84	22.33	0.75		
Total	89	29.344	1.00		

B) *Differential taxa read number analysis Across all treatments**

ASV_ID	baseMean	Log2FoldChange	pvalue	Phylum	Family	Genus
ASV_820	6.31	2.8	0.00	Firmicutes	*Hyphomicrobiaceae*	*Pedomicrobium*
ASV_360	12.85	-1.21	0.00	Proteobacteria	*Sporolactobacillaceae*	*Tuberibacillus*
ASV_918	3.26	-1.41	0.00	Actinobacteriota	*Microbacteriaceae*	*Chryseoglobus*
ASV_881	5.72	-1.98	0.00	Verrucomicrobiota	*Chthoniobacteraceae*	*Chthoniobacter*
ASV_366	10.14	-3.19	0.00	Planctomycetota	*Gemmataceae*	*Gemmata*

** Only top 5 ASVs are presented*

C) *Differential taxa read number analysis Clivus, Hemp, Rhizosphere vs Endosphere**

ASV_ID	baseMean	Log2FoldChange	pvalue	Phylum	Family	Genus
ASV_118	38.81	5.06	0.00	Firmicutes	*Paenibacillaceae*	*Paenibacillus*
ASV_282	18.99	-7.10	0.00	Bacteroidota	*Cytophagaceae*	*Cytophaga*

ASV_246	18.54	-0.74	0.00	Bacteroidota	*Weeksellaceae*	*Chryseobacterium*
ASV_641	7.60	5.03	0.00	Planctomycetota	*Gemmataceae*	*Gemmata*
ASV_1208	5.06	-0.76	0.00	Bacteroidota	*Sphingobacteriaceae*	*Pedobacter*

**Only top 5 ASVs are presented*

Supplemental Figures and Tables

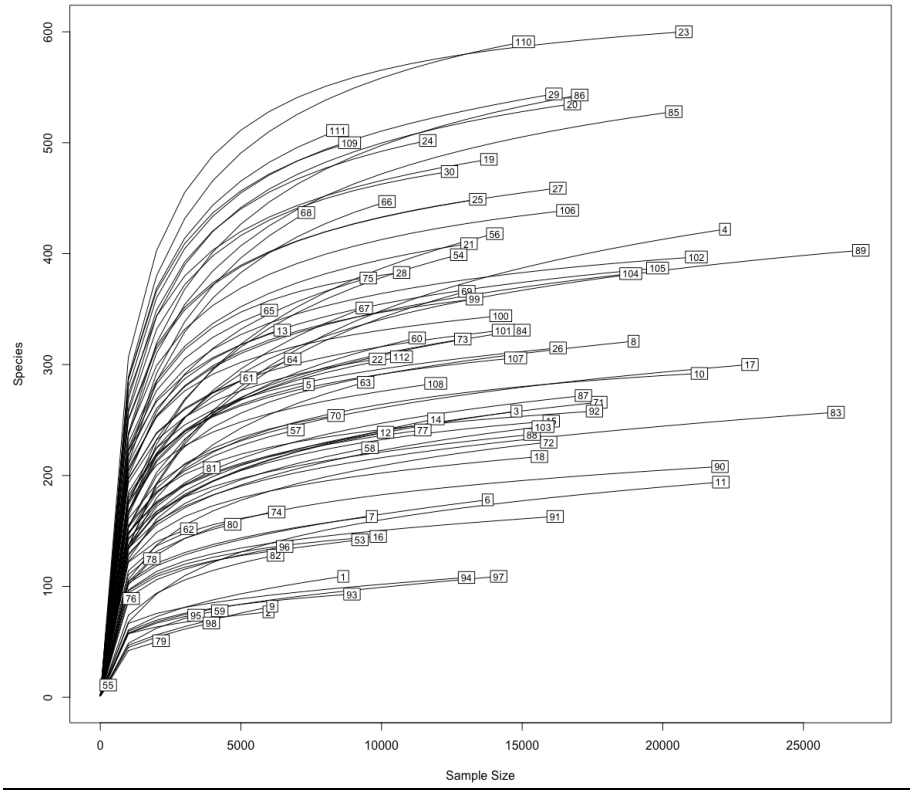

Figure S1

Rarefaction curve illustrating the bacterial community read counts across 90 samples, with a cutoff set at 10^-5 (0.0005) to preserve key taxa for analysis.

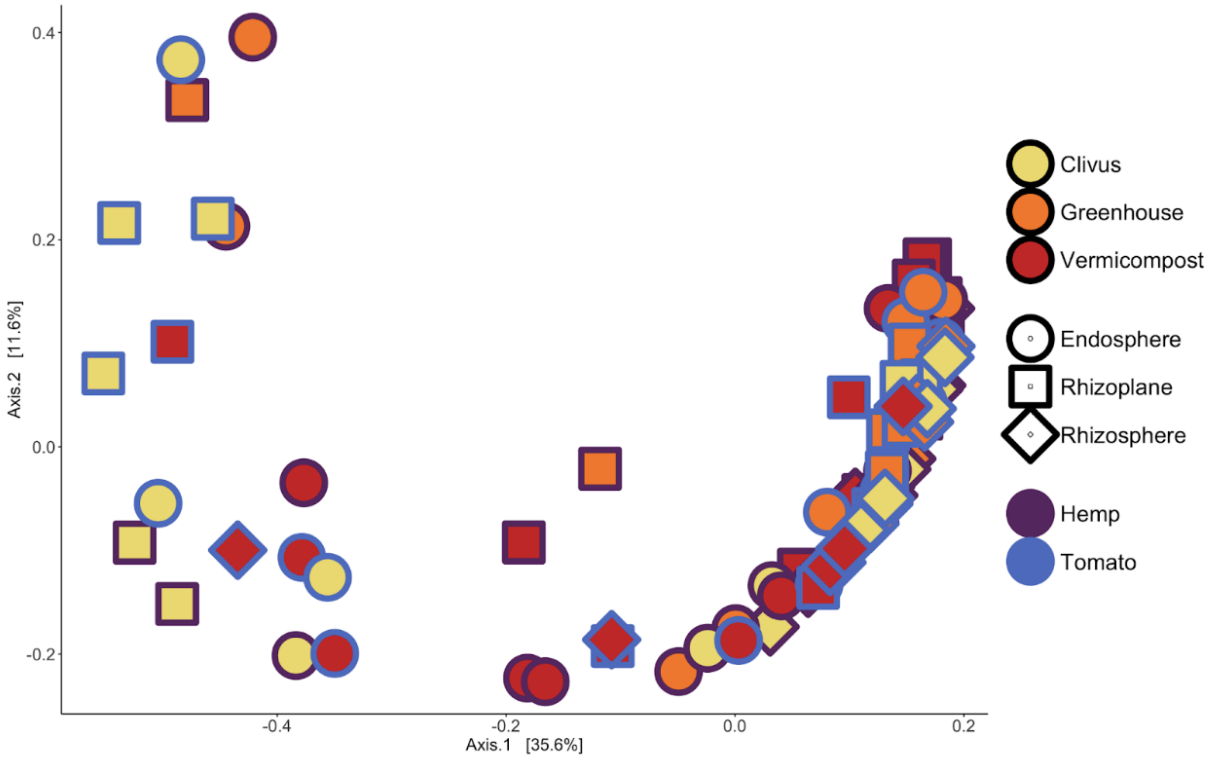

Figure S2

A rudimentary PCA of the ITS data. Treatment (Clivus, Greenhouse, Vermicompost), root sampling location (Endosphere, Rhizoplane, Rhizosphere), and plant type (Hemp and Tomato) are all plotted with colors, shapes, and borders according to the key.