HIGH SCHOOL EDITION
Journal of Student Research
Volume 13 Issue 3 (2024)

Role of Soil Microbiome in Abating Intra-Plant Competition

Sanya Dudeja[1] and Harsh Bais[#]

[1]Padua Academy, USA
[#]Advisor

ABSTRACT

With advancing agricultural practices, studying the role of soil microbiome in plant health and fitness has become more important than ever. Plants compete with each other at both intra and interspecies levels, and roots are the major organ that drives this competition. In this experiment, two types of soil were used to study how the absence of soil microbiomes could influence plant growth and competition. Additionally, a third type of soil, which was native soil with the addition of a sole benign microbe inoculation, was integrated into the experiment to explore how the introduction of beneficial microbiomes could impact plants' behaviors. We hypothesized that plants deprived of soil microbiomes may act aggressively in an increasing proximity environment and that introducing a beneficial bacteria could have an overall positive impact on plant growth. The findings showed inconsistent results in determining the impact of microbiomes on plant growth and competition. In our first trial, autoclaving the soil decreased plant-plant competition and enhanced plant growth, however, in the second trial, there was no effect of autoclaved soil on plant competition or growth. The findings also showed that the beneficial bacteria did not affect plant competition or growth, except at a specific density where plant growth was lower with the bacteria added to the native soil than with the native soil control. These results suggest that the effects of microbiomes on plant competition and growth are highly variable and further investigation must be conducted to understand the influence that the microbiome exerts in soil.

Introduction

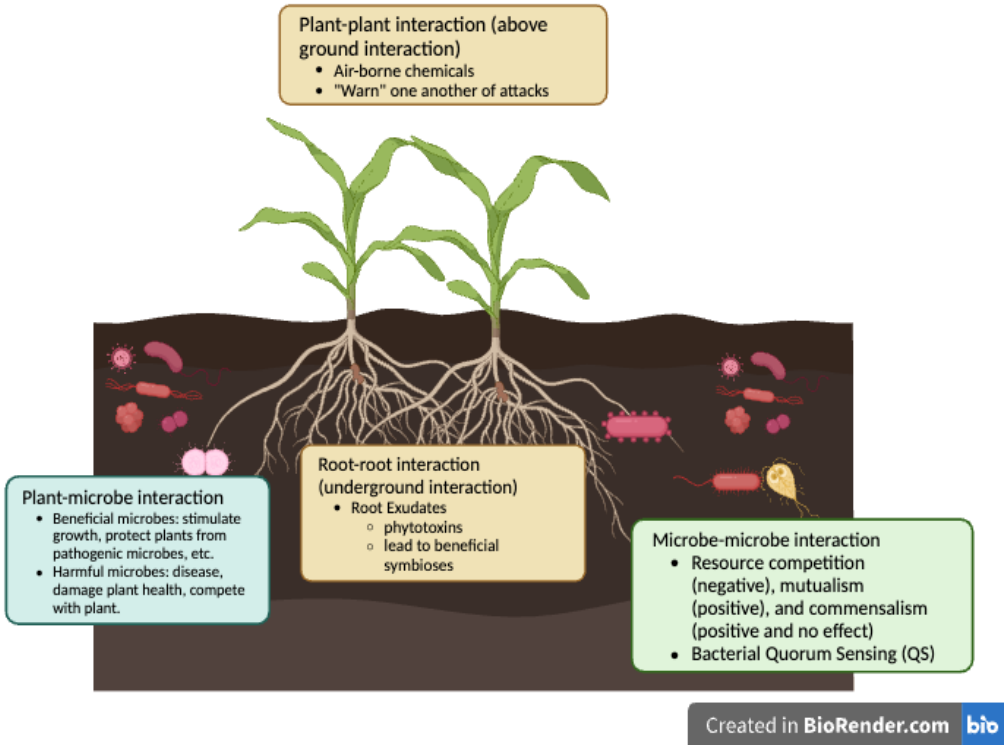
Understanding the Soil Microbiome and its Significance in Plants

The plant microbiome refers collectively to the various microorganisms that live in proximity to a plant and interact with one another in soil. Plants' microbiomes contain three domains: the endosphere, phyllosphere, and rhizosphere (*DOE Explains...the*, n.d.). Out of these three, the rhizosphere is the most significant to note as it is the main point of interaction of the microorganisms that make up the microbiome. The rhizosphere refers to the region that surrounds the roots of the plants and contains microorganisms that can be acellular (lacking any cells), unicellular (containing one cell), or multicellular (having many cells) (*Rhizosphere*, n.d.). Common types of these microorganisms include bacteria, fungi, protists, archaea, viruses, and green algae. These microorganisms in the rhizosphere influence the soil and plant health in various ways. For instance, beneficial soil microbes can break down crop remains, cycle nutrients, and stimulate plant growth. They can also perform nitrogen fixation, solubilization of phosphorus, suppression of pests or pathogens, and improvement of plant stresses. On the other hand, harmful microbes can be detrimental to plant and soil health as they can cause disease or compete for nutrients (*Understanding and Managing*, 2021).

With a variety of different functions, the microbiome can have impacts on the traits of plants. The microbiome can boost or harm the physiological and physical functions of the plant by either enhancing the plant's fitness or hindering it (Dastogeer et al., 2020, p. 1).

Interactions in Soil

The soil is a dynamic ecosystem consisting of complex interactions, all of which can influence the health of both the soil and the crops. Three main types of interactions are significant in soil: Plant-plant interactions, microbe-plant interactions, and microbe-microbe interactions.



Schematic 1. This diagram summarizes the three significant interactions that occur in soil: Plant-plant interactions (aboveground and underground), plant-microbe interactions, and microbe-microbe interactions.

Plant-Plant Interaction

Plants in the same environment have both negative and positive ways of interacting with one another. They interact negatively by competing for resources that exist in limited amounts, such as nutrients, light, and water or they interact with positive impacts on one another through facilitation. For instance, facilitation can occur when one plant species benefits another plant species by fixing nitrogen and adding it to the growing media for the other plant species to use as a nutrient (Faget et al., 2013, p. 253).

Plants exhibit these positive and negative interactions through two different means: aboveground interactions and underground (root-root) interactions. Above-ground interactions take place through air-borne chemicals. These interactions occur particularly in the context of neighboring plants “warning” one another of attacks such as a herbivore eating the plants. On the other hand, underground interactions take place through root exudates. Root exudates, or substances secreted by roots, can change the physical and chemical properties of soil, regulate the microbiome and other interactions in soil, and lead to beneficial symbioses (Wang et al., 2020, p. 1045). Exudates can also be phytotoxins that can inhibit the growth and survival of neighboring plants to minimize competition (Nadarajah & Abdul Rahman, 2021, p. 5).

Plant-plant interaction is very dependent on the proximity of plants as the proximity of plants drives how plants allocate their nutrients and thus drives competition between plants. This is highly prominent in monoculture where planting one species of crop causes issues related to plant proximity and so plants adopt different strategies for nutrient allocation when competing for resources. A specific example of a strategy is

when plants are in high proximity and competition where access to nutrients is reduced and so the plants allocate more nutrients to roots belowground than shoots above ground (Pierik et al., 2012, p. 843). Plant-plant interactions take a variety of forms that all simplify to either beneficial interactions or harmful interactions.

Microbe-Plant Interaction

By the same token, microbe-microbe interactions can harm both species involved, benefit both species, and even benefit one species but not affect the other species (Smid & Lacroix, 2013, p. 149). Soils can contain low amounts of nutrients and high cell densities which can drive competition amongst microbes. Competing for nutrients and space is negative for both species of microbes as one of the species must be more efficient at using resources to outcompete the other. Because of competition, microbes have even evolved distinct ways to position themselves to obtain resources efficiently and enhance their fitness (Brözel, 2022, p. 1). Despite this, microbes have still interacted positively through symbiotic relationships that have benefited both species. Interactions between microbes have been known to enhance the fitness of species involved when the microbes provide one another with beneficial items like nutrients, shelter, etc. This is known as mutualism where both species acquire something advantageous from the interaction and benefit together. Another relationship is when a species benefits but the other is unaffected, also known as commensalism. In this relationship, one species gains something that boosts its fitness, while the other remains unchanged or unaffected. This is not a very common relationship, but it can occur through symbiotic relationships (Smid & Lacroix, 2013, p. 149).

Another mechanism of interaction between microbes that is very important to note is quorum sensing. Though quorum sensing is not specific to a positive or negative interaction, it is a significant means of communication between microbes, or bacteria. Quorum sensing enables bacteria to respond to cell population density by coordinating gene expression (Dlamini et al., 2023, p. 2).

All these types of microbe-microbe interactions play a key role in understanding the extent to which microbes can influence one another and the soil. Moreover, all three of these significant interactions discussed give insights into how dynamic the soil environment is and how soil can be influenced by the different organisms that inhabit it. These interactions can determine the fertility or health of the soil, ultimately determining how plants grow in that soil.

Objectives and Purpose

The purpose of this experiment was to study the impact of the soil microbiome on competition between plants and therefore, its impact on plant growth and germination. This experiment was conducted to explore how removing microorganisms by autoclaving the soil would impact the plants, alongside competition. Furthermore, the effect of adding strains of beneficial bacteria to the native soil on plant competition and growth was also studied. The specific objectives of the experiment were:

1. Analyze the differences present in plant germination and fresh biomass weight in native autoclaved soil versus native soil with differing proximities of plants in each treatment.
2. Examine how introducing a beneficial bacteria strain to the native soil could increase or reduce competition between plants and influence plant germination and fresh biomass weight.

Hypothesis

Alternative Hypotheses (HA)

1. The native soil with intact microbial communities would lower plant-plant competition and lead to higher average corn germination percentage and biomass than the autoclaved native soil in both trials.

2. Adding UD1022 bacteria to the native soil in trial two will reduce plant-plant competition and increase the average corn germination percentage and biomass.

Null Hypotheses (H0)

1. Competition between plants and therefore, the average corn germination percentage and biomass will be unaffected by the removal of microorganisms in both trials.
2. Competition between plants and therefore, the average corn germination percentage and biomass will be unaffected by the addition of the UD1022 bacteria in trial two.

Materials and Methods

Soil Collection and Seeds

Location of Soil Collection

The soil for this experiment was collected from an agricultural field at the University of Delaware in Newark, Delaware. It was collected from the top ~30 cm of the field using shovels. The field where soil was collected was fallow during collection for both trials. However, the field was previously planted with just corn before the collection of soil.

Soil Preparations and Potting

After collection, the soil was thoroughly broken up and mixed to give a uniform texture and loosen the soil. Two trials were conducted with the same procedure for soil collection. In the first trial, the soil was divided evenly into two portions. One of the portions was autoclaved to remove all microorganisms, while the other portion remained unchanged and was the native soil with intact microbial communities. In the second trial, however, the soil was divided evenly into three portions. One of the portions was again autoclaved and the second portion again remained unchanged. However, the third portion was designated the native soil that the beneficial bacteria would be added to. In both trials, after this procedure, each portion of the soil was potted, planted with corn seeds, and placed in the greenhouse.

Experimental Setup and Treatments

In trial 1, there were two treatments: Autoclaved native soil and native soil. Whereas, in trial 2, there were three treatments: Autoclaved native soil, native soil, and native soil with the addition of a specific beneficial bacteria known as UD1022. In both trials, each treatment had 15 pots of soil that was seeded with corn seeds and each treatment also had three varying seed densities. These included 10 seeds per pot, 20 seeds per pot, and 30 seeds per pot. Moreover, each density had 5 reps or pots. Therefore, out of the 15 pots per treatment, there were 5 pots belonging to each of the three seed densities. The control group in both trials was the native soil that was not autoclaved nor had bacteria added to it.

Greenhouse Conditions

Each of the pots was labeled with their corresponding treatment, seed density, and rep, respectively, and then placed in the greenhouse in random order. In the greenhouse, the pots were given the same amount of light and water. The greenhouse gave the plants 12 hours of light per day and had an automatic sprinkler system that watered the plants daily.

Bacteria (UD1022)

The beneficial bacteria added in trial 2 was a *bacillus subtilis* strain or UD1022 that was obtained from Bais Lab at the University of Delaware, Newark, Delaware. It is a plant growth-promoting rhizobacteria that has been used for its growth promotion and disease protection in a wide range of plant species. In this experiment, specifically in trial 2, 10 ml of the bacteria was put in each of all 15 pots belonging to the native soil with UD1022 treatment. 10^8 cells per ml were put in each of these pots.

Parameters of Measurement

Each trial of the experiment was run for three weeks with various data being measured. This data included the number of seedlings germinating and the fresh biomass weight of plants. Data collection and means for both parameters were done in the same way for each of the two trials of the experiment.

Percent Germination

The number of seedlings that germinated for each pot was counted at the end of every week for three weeks.

Equation 1: Then the percent germination for each pot was calculated using the formula:

$$P_n = \frac{Number\ of\ seeds\ that\ germinated\ in\ the\ pot}{Total\ number\ of\ seeds\ planted\ in\ the\ pot} \times 100$$

To simplify the data, the germination percentages were averaged from the five pots in each seed density to obtain a single percentage for that week for each of the three seed densities in each of the soil treatments. This process was replicated every week for three weeks. Finally, after the three weeks, the weekly mean germination percentages were averaged again. The final averages reflected a single number for the mean percent germination for each of the three seed densities in each of the soil treatments, across all three weeks of data collection. The formulas used to calculate all of this were:

Equation 2: The weekly mean percent germination for each seed density in each treatment was calculated using the formula:

$$\mathrm{M} = \frac{1}{n}\sum_{n=1}^{n} P_n$$

n = number of pots in each seed density for each treatment

P_n = percent germination corresponding to each pot calculated in equation 1

Equation 3: The Overall mean percent germination for each seed density in each treatment was calculated using the formula:

$$\frac{1}{w}\sum_{w=1}^{w} M\mathrm{w}$$

w = number of weeks

M =weekly mean percent germination for each seed density in each treatment calculated in equation 2

Fresh Biomass Weight

The biomass weight was taken at the end of the third week. The weight of shoots was measured for each plant in each of the pots in each seed density and soil treatment. Plants were cut off at the surface of the soil and placed on an electronic balance to record the fresh biomass. Then the means of the shoot weight of plants were taken for each pot. Then those means were averaged one more time to obtain weights that would reflect the average shoot weights of all the plants, across all pots, in each of the seed densities of each of the soil treatments. This was done through the formulas of:

Equation 4: The mean fresh biomass weight for each pot treatment was calculated using the formula:

$$A = \frac{1}{s}\sum_{s=1}^{s} B_s$$

s = number of plants in each of the pots
B= biomass weight

Equation 5: The Overall fresh mean biomass weight for each seed density in each treatment was calculated using the formula:

$$\frac{1}{n}\sum_{n=1}^{n} A_n$$

n = number of pots in each seed density for each treatment
A = mean fresh biomass weight for each corresponding pot calculated in equation 4

Data Analysis

The experimental design was completely randomized with 5 replicates of each treatment. As established, the experiment was conducted twice. Statistical analysis was performed using SAS 9.4. The means for each parameter were separated using Tukey's HSD test at a significance level of $p < 0.05$.

Results

Trial 1 Results

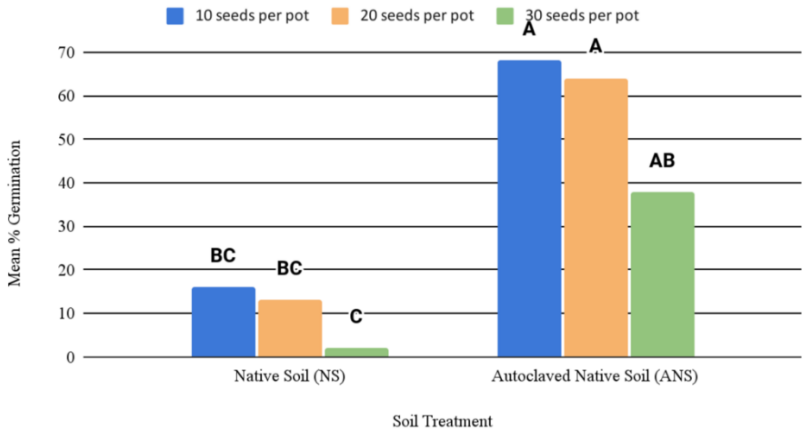


Figure 1. Effect of soil treatment (NS or ANS) and seed density on mean germination percentage in trial 1. Bars followed by the same letter are not significantly different at $\alpha = 0.05$.

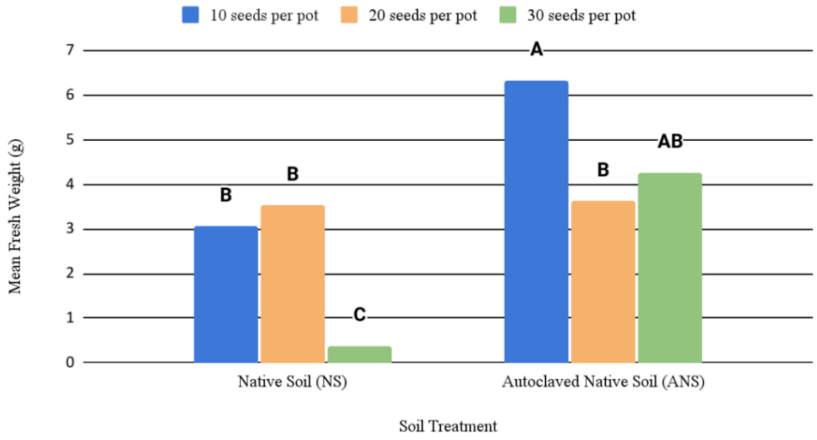


Figure 2. Effect of soil treatment (NS and ANS) and seed density on mean fresh weight of plant shoots (g) in trial 1. Bars followed by the same letter are not significantly different at $\alpha = 0.05$.

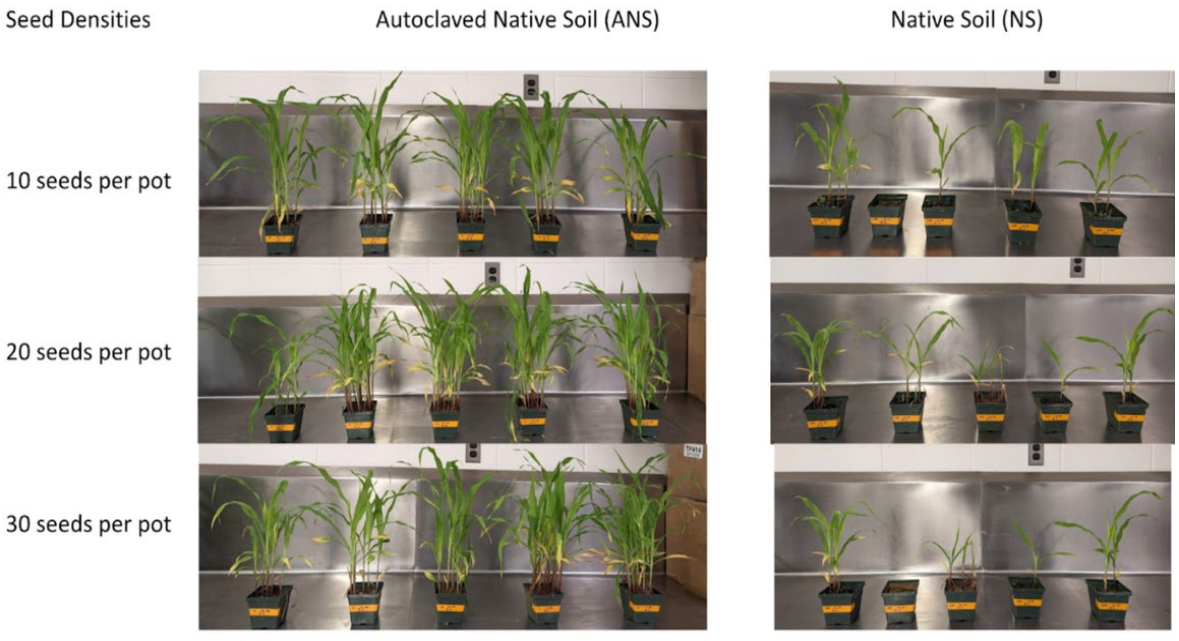


Figure 3. Image of pots of the ANS and NS soil treatments across the three seed densities in trial 1.

Trial 2 Results

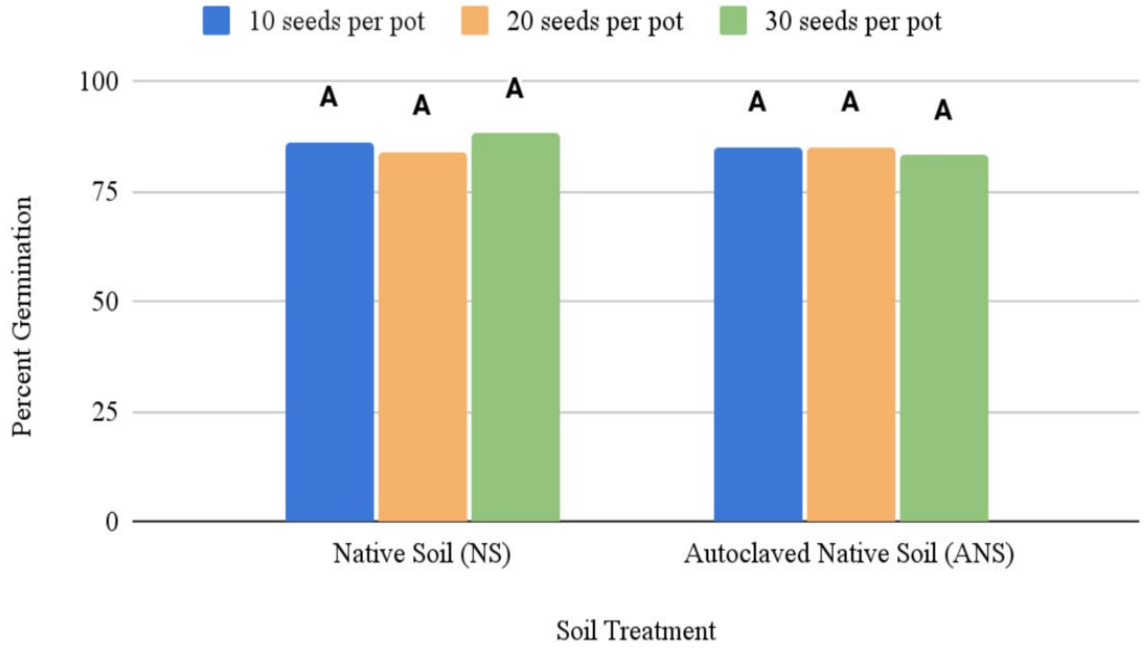


Figure 4. Effect of soil treatment (NS and ANS) and seed density on mean germination percentage in trial 2. Bars followed by the same letter are not significantly different at $\alpha = 0.05$.

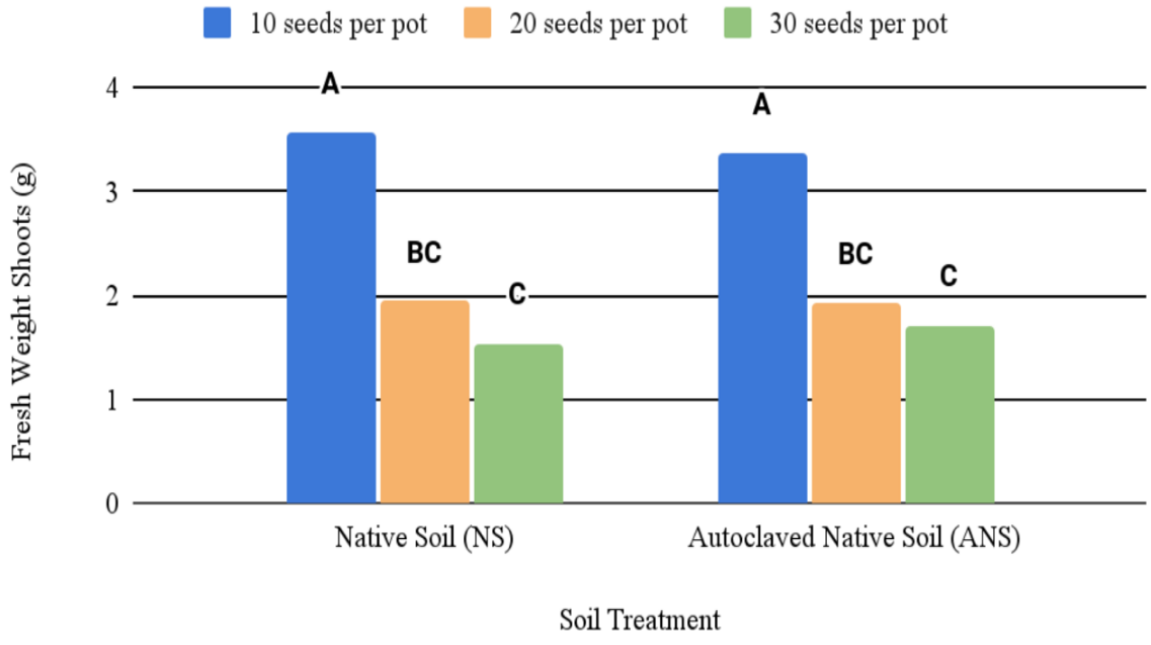


Figure 5. Effect of soil treatment (NS and ANS) and seed density on mean fresh weight of plant shoots (g) in trial 2. Bars followed by the same letter are not significantly different at $\alpha = 0.05$.

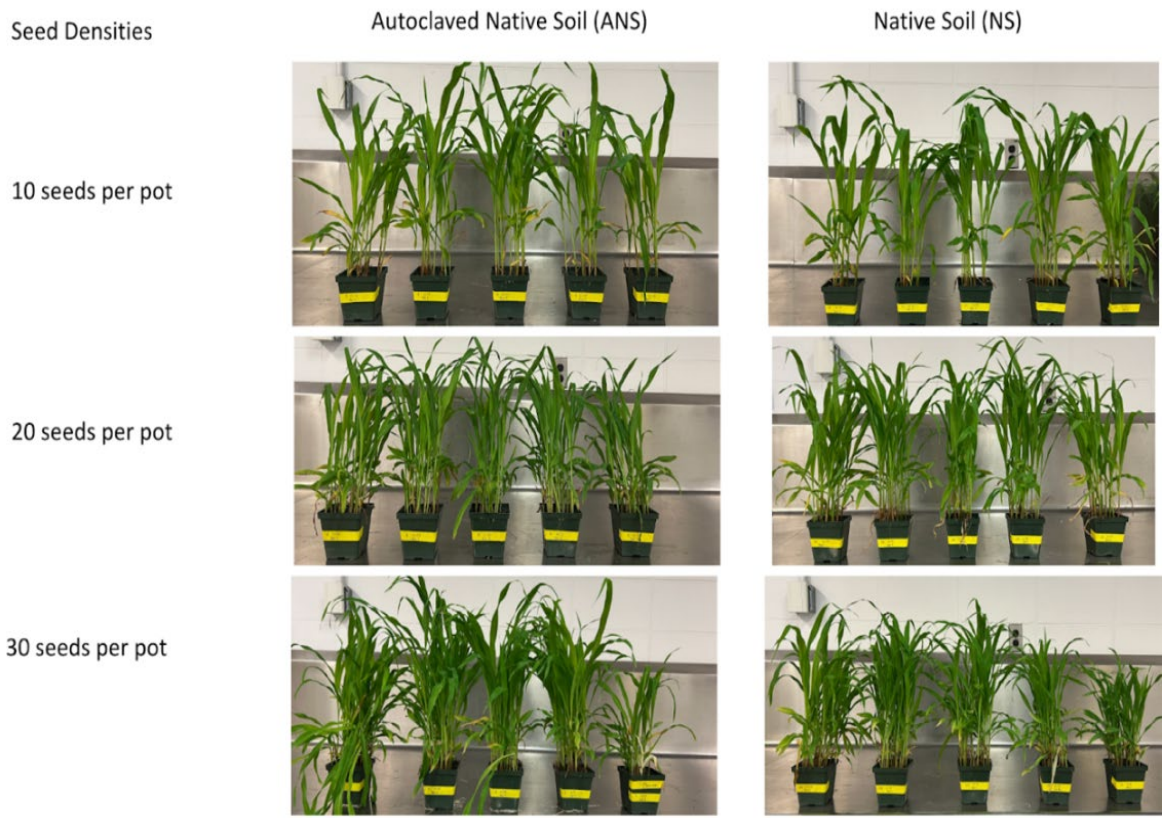


Figure 6. Image of pots of the ANS and NS soil treatments across the three seed densities in trial 2.

Trial 2 Results with UD1022

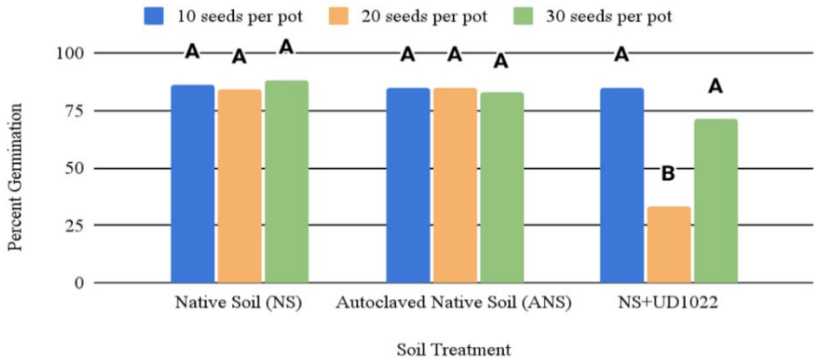


Figure 7. Effect of soil treatment (NS, ANS, and NS+ UD1022) and seed density on mean germination percentage in trial 2. Bars followed by the same letter are not significantly different at $\alpha = 0.05$.

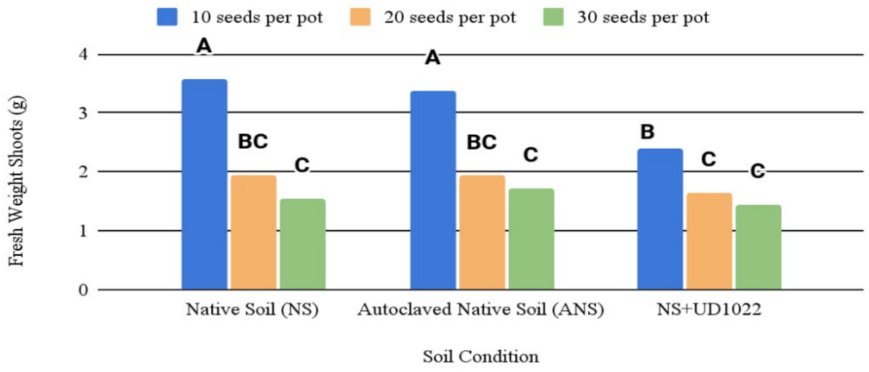


Figure 8. Effect of soil treatment (NS, ANS, and NS+UD1022) and seed density on mean fresh weight of plant shoots (g) in trial 2. Bars followed by the same letter are not significantly different at $\alpha = 0.05$.

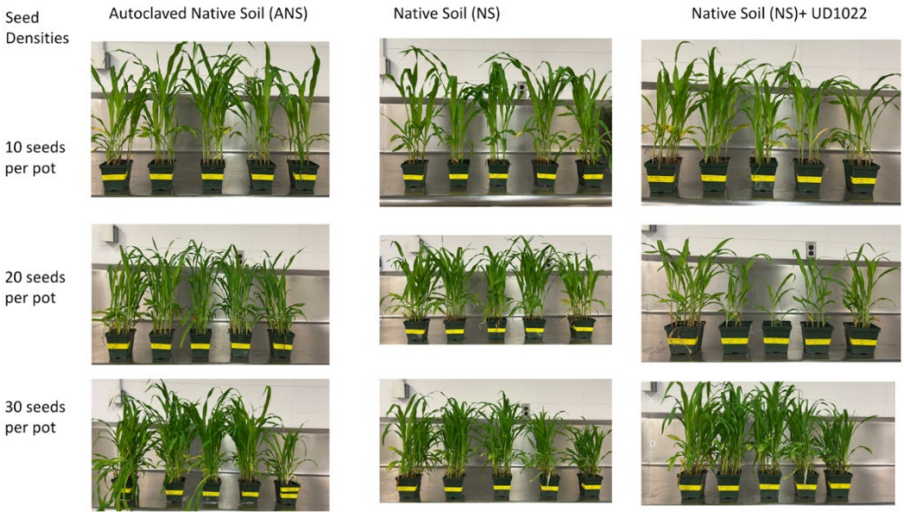


Figure 9. Image of pots of the ANS, NS, and NS + UD1022 soil treatment across the three seed densities in trial 2.

Figure 10. Image of pots of only the NS + UD1022 soil treatment across three seed densities in trial 2.

Discussion

Trial 1

Trial 1 shows that the microbiome plays a significant role in influencing both plant-plant competition and plant growth. The mean percent germination for the autoclaved native soil is much higher than that for native soil at all seed densities, suggesting that the absence of microbes lessens competition between plants and furthers the growth of the plants. Similarly, the mean shoot weight for the autoclaved native soil is generally higher than that for the native soil at all seed densities, except 20 seeds per pot, where they are not statistically different from one another.

Trial 1 also shows that seed density affects plant growth. Higher seed density generally leads to decreased mean percent germination and mean shoot weight for both soil treatments. However, the trial shows that the mean percent germination and mean shoot weight are higher across all different proximities of plants for the autoclaved native soil treatment than the native soil treatment. The native soil treatment's mean percent germination at the highest seed density, 30 seeds/pot, is 95% lower than the autoclaved native soil treatment's mean percent germination at that same density. Therefore, the removal of the microbiome abates competition to a significant degree, and thereby, greatly augments plant growth.

For trial 1, null hypothesis 1 can be rejected as competition amongst plants, average corn germination percentage, and biomass in autoclaved native soil are all affected. However, alternative hypothesis 1 does not hold as contradictory to what was hypothesized, eradicating the microbiome increases plant growth, rather than impedes it.

Trial 2

Autoclaved Native Soil and Native Soil Discussion

Trial 2 shows that microbes hold a limited role in plant growth and competition. Statistically, there is no difference in mean percent germination and mean shoot weight between the autoclaved native soil and the native soil. The mean percent germination and shoot weight do not differ from one another at any seed density between the two soil treatments. Therefore, the null hypothesis 1 fails to be rejected for this trial.

Interestingly, however, the mean percent germinations at the three different seed densities do not statistically differ from one another even within each soil treatment. However, the plant shoot weight does differ based on seed density since as the seed density increases, the shoot weight seems to decline in both soil treatments. All of this suggests that the proximities of the plants initially did not influence how seeds germinated where the initial growth of seedlings occurred invariably across different densities. However, once the seeds did germinate, the way plants allocated nutrients to grow evolved, depending on their current developmental stage. As plants matured into plants from seedlings, seed density played a more prominent role. Competition for nutrients, light, and space increased which offset changes in the allocation of nutrients. Higher seed density led to the mean shoot weight of the individual plants declined, indicating that intense competition was present after plants matured. Since this took place in both soil treatments, it can be concluded that, for this trial, the microbiome not only had no effect on the mean percent germination and mean shoot weight but also it played no role in plant-plant competition and nutrient allocation.

Native Soil and NS + UD1022 Discussion

The NS + UD1022 treatment shows some interesting results, as well. The supplementation of UD1022 to the native soil had virtually no impact on mean percent germination, except at the specific density of 20 seeds per pot where the mean percent germination for the NS+ UD1022 treatment was about 61% lower than the mean percent germination for the native soil treatment. UD1022 supplementation at this specific seed density lowering germination percentage could be due to multiple reasons. UD1022 could be competing with the plants for nutrients, or some plants could simply be sensitive to the changes in the soil environment brought along by UD1022 at this density, which could explain the reduced mean germination percentage.

The mean shoot weight generally remained unaffected by the addition of UD1022, as well. The mean shoot weight for 10 seeds/pot and 20 seeds/pot in the NS + UD1022 treatment is lower than that for the NS treatment. However, there is not a statistically significant difference in the mean shoot weight for the 30 seeds/pot density in both the NS + UD1022 and NS treatments. The closest proximity of plants in both the NS and NS + UD1022 treatment yielded statistically insignificant differences in both mean percent germination and mean shoot weight, indicating the addition of the beneficial bacteria did not affect plant-plant competition, average corn germination percentage, or biomass. The null hypothesis 2, for the most part, fails to be rejected.

Comparison of Trials

Trial 1 shows that microbes hold a significant role in plant growth while trial 2 does not. Trial 1 reveals statistically significant differences in mean percent germination and mean shoot weight when the microbiome is removed from the soil. Trial 2, on the other hand, does not show any statistically significant difference in germination or shoot weight between the autoclaved native soil and native soil treatments. Both trials are inconsistent in terms of determining whether the absence of a microbiome lessens intra-plant competition and thus, influences plant germination and biomass.

Conclusion and Future Work

Summary of Findings

The study found that the effect of microbes on plant competition and plant growth cannot be fully determined as trials 1 and 2 did not reveal consistent results that can be generalized to the plant population. The study did reveal notable findings into how plants were affected similarly by proximity within autoclaved native soil and native soil treatments, suggesting that the presence of the microbiome did not influence at all the effect proximity was having on how plants were germinating and growing. The study also revealed that the supplementation of UD1022 affected plant-plant competition and reduced mean percent germination and mean shoot weight only at a specific density.

Limitations

There are some limitations to these findings that are important to consider. For example, soil collection for the two trials occurred during different seasons. During trial 1, soil was collected in July 2023. While, during trial 2, the soil was collected in March 2024. July is one of the hottest months and it is the peak month for growing crops while March is still quite a cool month and is early for growing crops. This difference in season during the collection of soil could have influenced the composition, moisture, and nutrient level of the soil which may have impacted the results. Another limitation is the growing period. These plants were grown for three weeks,

and that period may not have been sufficient to note any long-term differences between the various soil conditions. Moreover, there could be differences in seed quality such as the age or the health of the seeds across the trials or within the trials that may have caused variability in plant germination and biomass. There could also be an uneven distribution of the UD1022 bacteria when it was applied to the pots which would have inconsistent effects on how the plants grew. All these factors may have been confounding variables that might have influenced the results.

Future Work

In the future, experiments like this one could be conducted over longer periods to explore how plants could be affected by soil treatments across multiple growing seasons. This experiment could also be applied to different species of plants to see how results differ based on the species of plants studied. Different beneficial or even harmful bacterial strains could also be added to further study how supplementation of bacteria to native soil affects the plants. Furthermore, an experiment that would be fascinating to conduct would be the effect of the soil treatments on how plants react to abiotic or biotic stresses. Plants could again be treated with different soil conditions and could then be subjected to a variety of stresses to observe how plants would react to those stresses across different soil conditions. Plants' survival rate and growth could be measured under the conditions of stress. This experiment could be very insightful in revealing the mechanisms plants use to fight against stress and how the microbiome plays a part in those mechanisms.

Acknowledgments

I would like to thank the University of Delaware and Bais Lab for providing the laboratory equipment, pots, seeds, etc. to conduct this experiment. I would also like to thank the Fischer Greenhouse Laboratory for providing space to store the pots and plants used in the experiment. I acknowledge the use of scientific image and illustration software, known as BioRender, to create a diagram that helped explain the introductory information for the experiment.

References

Brözel, V. S. (2022). Microbial interactions in soil. *Microorganisms*, *10*(10), 1939. https://doi.org/10.3390/microorganisms10101939’

Dastogeer, K. M., Tumpa, F. H., Sultana, A., Akter, M. A., & Chakraborty, A. (2020). Plant microbiome–an account of the factors that shape community composition and diversity. *Current Plant Biology*, *23*, 100161. https://doi.org/10.1016/j.cpb.2020.100161

Dlamini, P., Sekhohola-Dlamini, L. M., & Cowan, A. K. (2023). Editorial: Soil-microbial interactions. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1213834

DOE Explains...the Plant Microbiome. (n.d.). Energy.gov. Retrieved May 23, 2024, from https://www.energy.gov/science/doe-explainsthe-plant-microbiome#:~:text=In%20the%20rhizosphere%2C%20the%20plant,such%20as%20drought%20or%20diseases

Dolatabadian, A. (2020). Plant-Microbe interaction. *Biology*, *10*(1), 15. https://doi.org/10.3390/biology10010015

Faget, M., Nagel, K. A., Walter, A., Herrera, J. M., Jahnke, S., Schurr, U., & Temperton, V. M. (2013). Root–root interactions: Extending our perspective to be more inclusive of the range of theories in ecology and agriculture using in-vivo analyses. *Annals of Botany*, *112*(2), 253-266. https://doi.org/10.1093/aob/mcs296

Nadarajah, K., & Abdul Rahman, N. S. N. (2021). Plant-Microbe interaction: Aboveground to belowground, from the good to the bad. *International Journal of Molecular Sciences*, *22*(19), 10388. https://doi.org/10.3390/ijms221910388

Pierik, R., Mommer, L., & Voesenek, L. A. (2012). Molecular mechanisms of plant competition: Neighbour detection and response strategies. *Functional Ecology*, *27*(4), 841-853. https://doi.org/10.1111/1365-2435.12010

Rhizosphere. (n.d.). ScienceDirect.com. Retrieved May 23, 2024, from https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/rhizosphere

Smid, E. J., & Lacroix, C. (2013). Microbe–microbe interactions in mixed culture food fermentations. *Current Opinion in Biotechnology*, *24*(2), 148-154. https://doi.org/10.1016/j.copbio.2012.11.007

Understanding and Managing Soil Microbes. (2021, April 22). Penn State Extension. Retrieved May 23, 2024, from https://extension.psu.edu/understanding-and-managing-soil-microbes

Wang, N., Kong, C., Wang, P., & Meiners, S. J. (2020). Root exudate signals in plant-plant interactions. *Plant, Cell & Environment*, *44*(4), 1044-1058. https://doi.org/10.1111/pce.13892