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Rub Some Dirt on It: How the Soil Microbiome Can Restore Plants

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Rub Some Dirt on It: How the Soil Microbiome Can Help to Feed 10 Billion

By:

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CAPSTONE THESIS:

In order to feed the growing population by the year 2050, it is necessary to begin harnessing the properties of the soil microbiome. The soil microbiome is a complex microenvironment that has a massive impact on plants' growth, size, nutritional value, and overall health. By better understanding the soil microbiome, it will then become possible to manipulate it for the improved efficacy and nutritional value of crops. Thus, improving crop yield and crop value as it pertains to the human diet. With these improvements to agriculture feeding 10 billion by 2050 becomes feasible.

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Introduction

By the year 2050, it is expected that the world population will have reached 10 billion people. The massive surge in population will increase in the amount of food necessary for the entire planet to be fed. However, food could be a problem for this drastically increased population, for even today, around 9% of the world's population (690 million people) go to bed with an empty stomach each night (World Health Organization, 2014). There is also the problem of hidden hunger, where many communities and individuals do not receive the correct nutrition for a healthy and vigorous life. How to handle this future problem has been at the forefront of many scientists' and researchers' minds. One possible avenue is manipulating the soil microbiome and its interactions with the crops it is closely associated with. The soil microbiome has complex interactions with the plant and its roots, helping to remove contaminants, provide nutrients, and proliferate growth (Liu et al., 2019). Continued research into this subject matter is necessary to elucidate the complex interactions that occur, so that manipulating these relations may be used to help feed 10 billion people.

The soil microbiome itself is made up of different sections that have other interactions with plants and their associated roots. While researchers are interested in the effects the overall soil microbiome has on plants; they are most intrigued by what is called the plant microbiome. The plant microbiome consists of bacteria, fungi, bacteriophage, protozoa, and viruses. The plant microbiome is made up of three separate parts: the phyllosphere, the endosphere, and the rhizosphere (Zhang et al., 2021). The phyllosphere is the microbiome for above ground plant surfaces. The endosphere involves the microorganisms that reside within the various plant tissues. The rhizosphere is a region of the soil microbiome that consists of

microbes inhabiting the narrow space between the soil and plant roots. The rhizosphere itself also has three different sub sections; these include the endorhizosphere, the rhizoplane, and the ectorhizosphere. The endorhizosphere is the cortex and endodermis of land plants. It is the space where materials can diffuse through freely, known as the apoplastic space. The rhizoplane is directly neighboring the roots and is made up of the root's epidermis and mucilage. The ectorhizosphere is the space where the roots extend into the soil and where the majority of interactions between the soil microbiome and the plant occur (Zhang et al., 2021).

The rhizosphere is a hub of activity between the plant and soil. The primary function of the rhizosphere is to sustain a diverse and helpful microbiome. It is estimated that the microbial organisms outnumber the cells within a plant; with these extra cells working with the plant, an increase in protein-coding and metabolic potentials can occur (Zhang et al., 2021). The bacteria in the soil works with the plant and can vastly increase the plant's production of nutrients and vitamins when the right bacterium are cultivated from the bulk soil. This bacterium is referred to as a plant growth-promoting rhizobacteria (PGPR). These bacteria produce chemicals called phytohormones – auxins, gibberellins, cytokinins, ethylene, and abscisic acid – which are a key influence in plant cell enlargement, division, and extension of roots (Kaushal, 2019). The auxins assist in root development, cytokinins assist in shoot development, and gibberellins assist in seed germination. Each of these travels from the soil into the plant and help promote a greater yield of produce. There are countless genus of PGPR that produce these phytohormones. While it is assumed specific species can have different effects on the plant, there are many still unknowns between direct correlation.

The microenviroment in the rhizosphere is a diverse environment composed of bacteria, fungi, archaea, and viruses. It is estimated that a 1-g sample of rhizosphere soil contains approximately 10^8-10^{12} bacterial cells (Kennedy et al., 2005). The main phyla of bacteria found in the rhizosphere are Actinobactera, Proteobacteria, Cholorflexi, and Firmicutes (Zhang et al., 2021). The bacteria also rely on the fungi to boost diversity in the microbiome and change the composition in the rhizosphere. Viruses like bacteriophages can be both beneficial and harmful depending on what they target. There are some cases were they naturally attack pathogenic bacteria, or some lytic viruses can remove helpful bacteria from the rhizosphere. Each type of microorganism helps create a healthy rhizosphere.

As the rhizosphere builds good microbes and assists in healthy plant growth, its main interaction is with the roots. The root system of a plant is its primary source of communication with the soil below, and because of this, the rhizosphere helps develop the plant's root system. The root system forms in various stages, and in each stage, the microorganisms start their colonization and begin producing the phytohormones to help root development. At the embryotic stage, the primary root is formed, and postembryonic, lateral, and adventitious roots are formed. During this postembryonic stage, communication from soil to the plant is critical because it can increase root growth by auxins. The plant can begin to recognize microbe-derived compounds and change its defense system based on whether it will benefit or harm them (Ortiz-castro et al., 2019). As the plant grows, the composition of the rhizosphere will change with it.

Recruitment from bulk soil to the rhizosphere is a process that helps cultivate good bacteria that will produce these plant-enriching phytohormones. The two key factors in forming

a good rhizosphere are the abiotic soil environment and rhizodeposition (Berg et al., 2009). The abiotic environment consists of water levels, salinity levels, pH levels, and heavy metal levels. All of these factors influence what bacteria can reside in the soil, and each geographical biome selects for particular bacteria based on those conditions. These factors influence what the bulk soil consists of and will then impact what the rhizosphere is composed of by selecting from the bulk supply. The other factor, rhizodeposition, is when the plant selects for certain bacteria by releasing a compound from its roots. One plant can select certain bacteria that are more beneficial for itself compared to another using the same bulk soil. The plant selects the bacteria based on the functions it provides. A study using *Jacobaea vulgaris*, a wildflower also known as "stinking willy", as the key plant, found that transporter genes, Embden-Meyerhof-Parnas (EMP) pathways, and hydrogen metabolism were the functions that this plant selected for. Simultaneously, the bulk soil contained bacteria with functions related to cellular stress responses and carbohydrate- active enzymes (Yan et al., 2017). Each plant requires a unique blend of microbes to flourish.

Ideally, the rhizosphere would only be composed of beneficial microorganisms, but that is not always the case. Pathogenic bacteria and fungi can cause a decrease in nutrient supply for beneficial organisms, diminish root growth, and in turn reduce yield or even kill plants. These pathogenic organisms use many strategies; for example, some strategies focus on killing the plant to feed on the remains while others co-colonize with it to feed off living tissue (Doehlemann et al., 2017). The pathogenic organisms do this by interfering with the plant's defense system or by producing toxins. The plant's defense system can also use the microenvironment within the rhizosphere as an extra component. Elicitors are a molecule

produced by the plant that are derived from beneficial or pathogenic microbes (Ortiz-Castro et al., 2019). These self-defense signals are sent out in the form of salicylic acid, methyl jasmonate, and nitric oxide which triggers secondary metabolites like alkaloids, flavonoids, and phenolics (Zaynab et al., 2018). Along with supporting the plant defense system, the bacteria can also safeguard each other; they can communicate and form biofilms for protection (Zhang et al., 2021). The biofilms coordinate plant and microbe communication to fend off pathogens and this function can also be used as a natural alternative to pesticides.

The rhizosphere is also influenced by abiotic factors like water levels, salinity levels, pH levels, heavy metal levels. These can directly influence plant growth by adding stress and changing the composition of the soil. In turn the plants utilize the microbiome to help counteract these stressors and flourish. Water levels are important in drought conditions; the microbiome biomass as a whole decreases and selects for certain types of bacteria. The main phyla found in drought conditions are Proteobacteria, Bacteroides, and Firmicutes. These phyla generate drought resistance by increasing metabolism and nutrient production, reducing ethylene production, and increasing root water intake (Zhang et al., 2021). Salinity levels important to consider in agriculture because an imbalance can reduce nutrient uptake based on osmotic gradients. High salinity levels show an overall decrease in diversity and richness of bacteria in the soil and select for Actinobacteria. Alcaligenes was found to thrive in saline soils and increased growth two-fold by producing phytohormones (Ahmed et al., 2014).

Another factor that plays into soil condition is the pH. Along with what selections of bacteria are present in the microbiome it also affects the heavy metal concentration in the soil. Bacteria have a small viability range of pH, thus based on what the pH is in the soil, the

presence of certain bacteria may be limited (Zhang et al., 2021). This will limit the diversity and richness of the bulk soil, and a decrease will be seen in the rhizosphere as well. Plant growth can directly impact the pH: plant growth is inhibited by alkaline soils due to reduced ability to uptake minerals, and plants can be harmed by acidic soil due to excessive uptake of toxic heavy metals (George et al., 2012). Heavy metals are the final abiotic factor of microbial selection in soil. The primary heavy metals consist of lead, zinc, cadmium, chromium, and copper. Different metals have different effects on the microbiome and can cause shifts in the presence of entire species. A bacterium, *Pantoea agglomerans*, is a species that has shown high tolerance to heavy metal contamination, while other phyla have decreased in the presence. Each abiotic factor plays a large role in microbiome selection and diversity, impacting the growth of plants.

The relationship between plants and microbes is a symbiotic relationship. Not only are microbes assisting plants through various processes, but also plants assist microbes by aiding them in necessary functions for their continued proliferation. Plants support microbes by releasing various exudates that have different biotic effects on these microbial communities (Zhang et al., 2021). Some of the various factors that affect the exudates released by the plant are temperature, light, and age. Low molecular weight products like sugars and amino acids help provide nutrients for the different microbes. The release of certain sugars and amino acids can help select for specific phyla of microbes. Heavy molecular weight exudates, including proteins and mucilage, help signal microbes to perform specific processes to assist the plant in nutrient uptake or contaminant removal. Volatile and toxic contaminants such as MIPV, HIPV phenanthrene, n-octadecane, and cadmium also help to signal specific processes pertaining to the health of the plant and the soil microbiome it is associated with. (Jiao et al., 2019) These

different exudates help form the symbiotic relationships between the plants and microbes by allowing them to communicate and exchange with one another.

The Plant Growth-Promoting Rhizobacteria (PGPR) are some of the most studied bacteria. When one is pinpointed for a certain development of growth, it could potentially be used in other soils to increase plant yield. The Pseudomonas and the Bacillus genus contain a several enriching bacteria that can live in various environments. The main component of the PGPR is that they produce phytohormones such as auxins and cytokines, and also, they indirectly produce antibiotics, such as hydrogen cyanide (Oritz-Castro et al., 2019). An auxin is derived from tryptophan and is produced from the metabolism of bacterial and fungal species, including indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA). IAA increased the number of lateral roots and root hairs during the postembryonic stage. There is evidence that it decreased root elongation and increased shoot to root ratio. The effects of IAA are beneficial because plants can increase nutrient and water uptake by creating more surface area at the roots. Cytokinins work with auxins, regulating root organogenesis. Cytokinin is derived from purine, is metabolized by many organisms, and was found in *Bacillus megaterium* (Oritz-Castro et al., 2019). It influences the cell division and differentiation. In a shoot cytokinin promotes cell proliferation at the root cap and in the apical and axillary meristems. Increasing cytokinin levels has been seen to increase grain yield (Kieber et al., 2018). Cytokinin and auxins are equally important to normal plant health and growth because they can both promote more significant growth. PGPR are also able to counteract abiotic stresses. When a plant is in a stressful environment with a water deficit and improper salinity levels, it can disrupt photosynthesis and increase photorespiration, creating reactive oxygen species (ROS), which will disrupt the plant's

growth and viability (Bosco de Oliveira et al., 2013). This can be counteracted by the bacteria in cytokinin and auxin production, in changing the root structure and growth, it can allow for increased water and nutrient uptake.

Fungi also play an important role in forming the rhizosphere and, while slightly less studied, rival their bacterial counterparts in helping growth. A key element of fungal colonization in the rhizosphere is that beneficial fungi compete with pathogenic fungi and can parasitize spores, sclerotia, or hyphae of pathogenic fungi (Ortiz Castro et al., 2019). They are heavily involved in the biocontrol of the rhizosphere and work to degrade any fungi that are harmful to the plant. A majority of the diseases seen in plants are caused by a fungal infection. As mentioned above, fungi can also produce auxins that help development and growth of shoots and roots. *Trichoderma* has been found to be very beneficial in the production of IAA for root development. Many other fungi are also beneficial and work with the bacteria in the rhizosphere to create an environment for plants to thrive.

The impacts that the soil microbiome and its interactions with plants have on the human population have massive implications. By manipulating the soil microbiome, both the quality and quantity of our crops can be greatly improved. An increase in the overall health of our plants can also be accomplished. This increase in plant health could be done by selecting for microbes to inhabit the soil microbiomes that prevent the infection of crops by disease-causing microbes and viruses commonly found in the soil microbial communities. Healthy crops are more likely to have higher levels of nutrients within them and provide greater harvest time yields. Another benefit of controlling the soil microbiome is that there will be less need to use pesticides and fertilizers. The use of these products could be supplemented by instead using

different techniques for growing and harvesting crops. For instance, possibly through the use of products such as bioinoculants which are homogenous mixtures of various PGPM, microbiomes could be artificially populated with specific microbes chosen for their beneficial properties (Owen et al., 2015). There is also a technique known as cover cropping which involves using a noncash crop to help fertilize the soil before the planting of cash crops occurs (Wang et al., 2020). Both of these different farming techniques modify the soil's composition to provide a more favorable environment for the crops.

A healthy microbiome is not only beneficial for plants but also advantageous for humans. There are a few ways that a healthy soil microbiome can be helpful. First off, humans have a microbiome of their own. Of great importance is the microbiome within the gut, it is what helps digest food, and each microbiome has a composition that is unique. It can change based on diet, and what people consume contains some of the soil microbiome, so it is incorporated within their gut. These microorganisms assist in extracting nutrients from the food people ingest and help build their immune systems (Hirt, 2020). In addition to processing food, they also provide essential amino acids and vitamins. Humans only synthesize eleven out of twenty amino acids and rely on food for the other nine, they also use food to incorporate the thirteen essential vitamins into their bodies (Hirt, 2020). Even food intake cannot supply everything, B12 is one vitamin that only microbes can synthesize. As our food production has changed over the years, with an increase in in fertilizers, pesticides, and herbicides it has changed the soil microbiome and therefore changed our gut microbiome. The loss of diversity could be what is leading to gastrointestinal diseases, inflammatory diseases, diabetes, and

chronic kidney diseases (Hirt, 2020). Cultivating a good soil microbiome will, in turn, produce a healthy gut microbiome.

The gut microbiome is not the only component that the soil microbiome impacts. As agricultural processes have changed so have the nutrient levels in produce. With an increase in yield and size, the produce is suffering from a dilution effect. The plants uptake the same amount of nutrients as they did before, but now it is distributed to double the size leading to a decrease in nutrition per fruit or vegetable. This is one of the problems leading to hidden hunger, a phenomenon where even when people are consuming enough calories they are still malnourished. Hidden hunger is now affecting an estimated 2 billion people (World Health Organization, 2014). Using good cultivation techniques, plants can naturally increase their nutrient uptake by increasing lateral roots or cultivating bacteria that metabolize those nutrients into the rhizosphere. Hidden hunger is a key factor in maintain a healthy populace; however, the main goal should be to get rid hunger altogether. With the population growth projected to reach 10 billion by 2050, there are more mouth to feed. Increasing crop yield and nutrition is more important now than ever and doing it in a sustainable and healthy way will ensure that humans can continue this around the globe. Cultivating a healthy microbiome is one way to do this; it can increase yield and nutrition in produce and change soil composition. If these changes can be made there will be more land to farm, thus, improving the health of the human population, and restoring damaged ecological habitats.

Presentation of Research

Historical Developments

Soil Microbiology is a relatively new area compared to other disciplines. It is only in the past two decades, though, that the knowledge base of the soil microbiome has expanded (Chu et al., 2020). In early studies, the focus was on pathogenic soil microorganisms, working to mitigate crop loss. In the early 1900s, a professor, H.L. Bolley, was the first plant pathologist at the University of North Dakota. His main focus was on a soil-borne disease, fusarium wilt, and a common root rot of wheat (Zimdahl, 2010). He led the way for more researchers to follow. In 1908 the University of Louisiana conducted a research study that spanned forty years on sugarcane disease (Edgerton, 1950). They sampled a variety of sugar cane fields and tracked the total sugar cane yield of Louisiana. The main focus was on three major diseases: red rot, root rot, and mosaic. Based on soil composition, these could destroy upwards of eighty percent of seedlings. Yield varied drastically from 20 tons to 60 tons, with the average yield around 21 tons; they strived to achieve an average of 30 tons a year. To determine how to increase the yield, they tracked appearances of rot and spikes over the years and compared them with root development and activities. They also examined organisms in the soil, soil fertility to parasitic organisms, production of new cane seedlings, testing of new cane seedlings, and weed control. Connections were beginning to form on how truly complex the interactions between soil organisms and plant growth are. It was not until the 1970s that the technology needed to make a breakthrough in microbiology emerged.

In 1977 Carl Woese and George Fox guided microbiology to what it is today. They were the first to use the 16S sRNA gene in bacteria phylogenetic analysis, which is still used today in genetic sequencing. Using these evolutionary markers, they discovered the three domains of life: Bacteria, Archaea, and Eukarya (Zhulin, 2016). The traditional classification of bacteria was based on phenotypic characteristics. This is not as accurate as genotypic methods, and the 16S sRNA gene is able to identify bacteria to the genus level and occasionally the species level (Wang et al., 2015). The 16s Ribosomal RNA gene codes for the RNA component of 30S subunit of the bacterial ribosome, which is present in all bacteria. It also contains many evolutionary conserved nucleotides, which allows for better classification (Bryne et al., 2018). This technique is highly useful for genus classification and has resolved more than 90% of the isolates; it is sometimes less valuable for species classification because of low discriminatory power, with only 65–83% of strains being identified and the rest remaining unresolved (Wang et al., 2015). Alternative techniques can be utilized to help determine the unresolved identifications. DNA-DNA hybridization is an alternative that has been able to distinguish strains that show 99% similarity in the 16S sRNA sequences. Comparatively, researchers use the internal transcribed spacer (ITS) region as the key identifier. Compared to other techniques, the ITS will most clearly defined barcode gap between inter- and intraspecific variations (Schoch et al., 2012). Classification is key in soil microbiology, because in order to find a correlation between what bacteria are beneficial for plant growth, there needs to be an identification of what bacteria contain said beneficial properties. Recently the first global atlas of bacterial biodiversity was published by the European Union (Chu et al., 2020). It is a good baseline to expand on, and advance species identification, function, and location.

A study done in 2019 by Jiao and associates examined how plant microbiomes and their associated soil microbiomes reacted to contaminants located in the various soil samples. (2019) The plants under examination were exposed to phenanthrene, n-octadecane, and cadmium. Any microbiome stability was attributed to the mechanisms of resistance, resilience, and functional redundancy. There were 21 samples taken over four different time periods from three different experimental plants. There was the lowest level difference in the measured abundance between days 0 and 90. There was a clear pattern of succession shown, with the level of diversity at first severely decreasing. As time progressed, the diversity began to increase as microbes with contaminant-resistant genes proliferated. This proliferation of microbes containing traits for dealing with contaminants shows the importance of resilience. After the 90 days had passed, soil microbiomes containing and without plants had significant changes in their taxonomic and functional levels. The authors concluded that their research showed the importance of microbes in assisting plants in the degradation and removal of toxins. Their research also showed how microbiomes could change rapidly to become better equipped for handling various problems that may arise. Lastly, they concluded that further research should focus on how plants exert selective pressure for microbes with these important contamination-related traits when exposed to said toxins.

Current Research

In recent years knowledge of the inner workings of the soil microbiome and, rhizosphere section of the soil microbiome, have increased exponentially. Application has far succeeded in protecting plants from pathogens. The global community is expansive now, and with the increase of exchange, many invasive species has taken the opportunity to expand. In early

2020, an experiment was published on the process of an invasive weed, *Conyza canadensis*, and the impact it has on the soil microbiome through the various stages. The weed is known to cause changes in soil properties and thus affecting the functions of soil microbiome. In the experiment they used, *Lactuca sativa* a common lettuce plant, as the non-invasive species and grew the plants from seeds in varying concentrations. A total of four seeds were grown in each pot: 0:4; 1:3; 2:2; 3:1, and 4:0. Control soil samples were also placed in pots without plants. Soil samples were taken after four months to determine chemical composition, testing soil organic matter, total/available nitrogen, total/available phosphorus, total/available potassium. The DNA was extracted through PowerSoil DNA Isolation Kit sequences were assembled using FLASH.

The addition of *Conyza canadensis* modified the nutrient and enzymatic composition of the soil. The significant chemical composition change was an increase in total nitrogen, urease, and invertase. The abiotic factors showed a clear separation between the pots. The diversity and richness of the bacteria among the gradient soils were not significant but did alter the bacteria cultivated in the rhizosphere. The dominant phyla were *Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes*. The non-invasive soil was enriched with *Actinobacteria* and *Choloflexi*, and the invasive soil led to a decrease in *Planctomycetes*. The fungal communities did have a significant decrease in diversity and richness, and the composition was also different between gradients. *Chytridiomycota* and *Ascomycota* dominated the soil, and in the invasive soil, a decrease of both occurred. In conclusion, around 50% density of the invasive weed is the threshold for change in soil microbiome. The results showed that the microbiome was changed to be better for the invasive species *Conyza canadensis*, and detrimental for *Lactuca sativa*

which had its nutrients depleted. This study can be applied in agricultural practices to determine how much herbicide needs to be used to still have healthy soil without a significant change in soil microbiome.

Another experiment that shows potential to help change agricultural practices focuses on the use of bentonite to induce a change in the soil microbiome composition. Desertification is a process that happens with overused land and adverse climate conditions-resulting in sandy soil that contains little nutrients and is difficult to farm. Bentonite is a soil conditioner that changes the abiotic composition in the soil. Bentonite is usually composed of 2:1 clay mineral montmorillonite and is beneficial in water retention. Thus, mixing with the sandy soil they predicted would change the function of the soil. In the experiment, they spread bentonite in a cornfield in randomized blocks, took random soil samples throughout the course of a year from plots with bentonite and without bentonite. Lastly yield was measured at the end of the season. The DNA extraction was done using the E.Z.N.A. DNA Kit, and sequences were joined using FLASH. The fungal community had a diversity of eight phyla, with dominate *Ascomycota, Basidiomycota, and Zygomycota.* The bacterial community consisted of 38 phyla with dominance of *Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes, Chloroflexi, and Bacteroidetes*. Maize yield was significantly higher with bentonites than without. Fungal communities were found to be enriched in the soil with bentonite, but the bacterial communities were not significantly shifted. They discovered a significant increase in corn yield in the bentonite areas, along with an increase in fungi diversity, and bacterial communities showed little difference between the two soil types. This is not to say that there could not be more change in the future. These fields showed dramatic change with just one year of

bentonite application; there is potential to further change with additional research and increased seasons. With an increase in fungal communities, it could trigger a positive feedback loop that will increase the bacterial communities. Repairing overused fields can help increase production of food for the upcoming years.

Current research has found that by using different types of agricultural techniques, beneficial manipulation of the soil microbiome can be accomplished. Two such techniques have been studied in detail, showing positive signs of future use as an alternative to many unsustainable and inefficient practices. The first of these new techniques is intercropping, which involves a process of growing various plants within close proximity of one another so that they may help cycle diverse nutrients into the soil, thus helping to establish a healthy and diverse microbiome that can then assist in the growth of the crops (Zhang et al., 2018). The second technique researched was that of cover cropping. Cover cropping involves using a specific noncash crop to help replenish and invigorate the soil microbiome before planting a cash crop (Wang et al., 2020). This enrichment is done by planting the cover crop, allowing it to grow to maturity, and then mowing the crops down before planting the cash crop. There is time allotted for the decomposition of the cover crop into the soil before the cash crop is planted. Both of these techniques showed positive results for further study of their possible use as an alternative to unnecessary agricultural practices.

In the experiment conducted by Wang and coworkers examining cover cropping techniques, there were two different cover crop mixes used (2020). There was a single mix and multi mix; the single mix used a non-legume buckwheat species, whereas the multi-mix used seven summer organic cover crops. The experiment involved a plot with nine separate beds

that were tripled so that the effects of a control, single-mix and multi-mix cover crop could be analyzed. The experiment itself followed the cover cropping technique steps, which involve planting the cover crops, allowing them to grow, mowing them down, allowing them to decompose, and then the panting of the cash crops. The cash crop used for this experiment was broccoli. Microbial data collection was done at three time points, immediately after cover crop planting, immediately before the mowing, and before the harvest of the broccoli crops. Analysis of soil composition showed that between times 1 and 2, organic carbon content decreased significantly, and then between points 2 and 3, it increased significantly. Overall microbial abundance and diversity decreased between points one and two in all three situations. In multi-mix and the control, microbial diversity and abundance increased between points 2 and 3, showing the benefit of cover cropping. Their conclusion stated that their hypothesis of multi-mix being preferable to single mix was supported by their findings (Wang et al., 2020).

Another area of interest is the use of bio-resources to manipulate the composition of the soil microbiome. Bio-resources are any material that is applied to soil to help soil quality, nutrient supply, and plant growth (Orozco-Mosqueda et al., 2018). There are three types of bio-resources: bio-inoculants, bio-amendments, and bio-fertilizers. Bio-inoculants can be made up of bacteria, fungi, and mycorrhizal fungi (Owen et al., 2015). While there have been some promising results, the common thread in clinical studies and trials are results that are inconsistent and varied. The conclusion is while even though results have been erratic, future research is necessitated to show whether the potential of these bio-inoculants can be reached.

An experiment conducted by Deng and coworkers examining the use of VESTA as a bio-inoculant showed many promising results (2019). This study focused on the exposure of

strawberry plant associated rhizospheres and their associated soil microbiomes to the bio-inoculant VESTA. VESTA is a fermented liquid product made up of different microbes, fermentation products, and organic acids. The authors created an experiment that allowed for the collection of control and exposed strawberries grow on two neighboring plots in California. Four replicates were sampled from the control and experimental plots at four different time points: two, three, four, and seven months after planting. Roots from the strawberries grown in experimental fields showed enhanced growth and total water content, characterized by greater fresh weights when compared to the control. The authors inferred that this showed an amendment effect on greater root growth and enhanced soil physiochemistry. The authors also hypothesized that experimental strawberries would show greater biodiversity and abundance than the control. Unexpectedly they found that the opposite was true in both situations, and in fact, the control showed higher marks. They believe that this was due to several biotic and abiotic factors. The authors believed the results while in conclusive, were promising and believed the field should be researched more.

Biofilms are estimated to hold more than 99% of all bacteria in natural environments (Wu et al., 2019). A study done by Wu and associates examined how soil biofilm formation can affect microbiome assembly, diversity, and metabolic activity. Their study aimed to investigate biofilm development, abiotic and biotic determinants, and how biofilm formation changed the microbes' metabolic activity residing within biofilms. Samples were collected for the extraction of biofilms from the surface soil. The samples containing biofilms were then placed into artificial soil; for a control, free-living cells were used. The biomass growth, nutrient consumption, and oxygen concentration were all measured. To measure the biomass diversity,

16s rRNA amplicon sequencing was used. Samples were then exposed to one of three varying levels of glucose (high, medium, low). It was found that when exposed to the high and medium levels of glucose, the levels of biofilm diversity and evenness were increased. The levels of biofilm total biomass concentration dipped below that of the control when exposed to low glucose levels. *Bacillus* and *Paenibacillus* were the primary bacteria found in the biofilms. Rapid oxygen depletion was observed in the biofilm samples; the authors suggested this rapid depletion of oxygen could be due to *Bacillus* biofilm formation. The respiration and nutrient turnover rates were significantly higher (23-times) in the soil containing biofilms when compared to that of the soil containing free-living cells. This study showed the important implications of how Biofilm development can be beneficial to soil microbiome health, composition, and diversity.

Gaps in Current Knowledge

To keep progressing in future research, there are a few knowledge gaps that need to be filled. It is well known that biodiversity decreases further away from the equator. In the microorganism community, this needs to be proven as well. A study done by Delgado-Baquerizo found reduced soil microbiome diversity from the equator down to Antarctica (Chu et al., 2020). Determining if this remains true for the northern hemisphere will be key to mapping soil biodiversity in the future. Another area for research is the change of soil biodiversity with increasing elevation. Mapping what bacteria, fungi, and other microorganisms can live in differing climates can be valuable when planting various crops.

To be able to understand what kind of microbes are most beneficial for plant growth and health, it is important to increase the ability to identify the various species of

microorganisms and what part they play in soil microbiomes. While much is known about the

microbes that are associated with human and animal health conditions, very little is known

about microbes that affect plant health, whether detrimentally or otherwise. The investigation

of these diverse microbial communities is done mainly through DNA sequencing techniques

such as targeted amplicon gene sequencing or metagenomic shotgun sequencing (Zhang et al.

2021). Both of these techniques have various limitations that are associated with soil type and

soil environment.

Bio inoculants have presented some of the most conflicting data out of any area of

research related to the soil microbiome. Variations in experimental design and methods

compound the inconsistency of performance and lack of independent certification (Owen et al.,

2015). Much is still unknown about the complex relationships between microbes and plants and

microbes with other microbes. The soil microbiome itself is a complex microenvironment with

compounding environmental variables that must be accounted for when designing an

experiment that is attempting to elucidate the results of implementing a bio inoculant into

these said microenvironments. An example of this can be found in a study done by Latkovic

involving the foliar application of biofertilizers on plants containing the nitrogen-fixing bacteria

Klebsiella in the soil (2020). While the highest statistically significant levels of microbial biomass

concentration and enzyme activity were found in the plants inoculated with the *Klebsiella* and

fertilized with mineral fertilizer, the proteinase activity was actually recorded to increase with

the use of traditional nitrogenous fertilizer. The authors found that this was correlated with the

highest levels of precipitation distribution. This experimental design error articulates how while

Bioinoculants have shown promising effects it is still important to continue research into the

complex interactions within the microbiome and the soil microenvironment.

Conclusions and Recommendations

Conclusions

Healthy plants require a diverse microbiome. A wide variety of bacteria, fungi, viruses, and archaea form a complex web of interactions that supply plants with nutrients from the soil up. Increasing the diversity and richness has been found to promote growth better than a decrease in diversity. The richness of bacteria and fungi drives the ecosystem multifunctionality of nutrient cycling, organic matter decomposition, and plant productivity (Chu et al., 2020). This is due to the functions of each microorganism; different bacteria metabolize distinct phytohormones and secondary metabolites. So, the more diverse the selection of bacteria are within the rhizosphere, the greater the variety of phytohormones can be produced. In addition to an increase in phytohormone production, bacterial communities' diversity could be beneficial in sharing genes. Horizontal gene transfer through conjugation, transformation, and transduction can help beneficial bacteria protect against pathogens, evolve to increase phytohormone synthesis, or survive in harsher soil conditions. Fungal communities in the microbiome also benefit from a greater diversity. Interactions between fungi and bacteria in the microbiome have been shown to increase bacterial abundance and promote bacterial attachment in root colonization, generating healthier plants (Zhang et al., 2021). Greater diversity can induce new interactions between microorganisms, potentially leading to an ever-better soil composition and produce yield.

Plants specifically select for microbes that assist in promoting the health and growth of the plant (Zhang et al., 2021). Plants do this by selecting for traits present within phyla

containing microbes that are better for filling the plant's niche (Edwards et al., 2015). Plants typically require months to exert pressure on the composition of microbial communities. However, this process is expedited when contaminants are present because the contaminants help filter out microbes that do not have functional traits to assist in contamination removal and clearance (Jio et al., 2019). The patterns of proliferation show clear signs of the succession of the genes conferring resilience. These genes can then be passed on to other microbes present, allowing for the microbiome's increased diversity and composition.

The introduction of bioresources and, more specifically, bio inoculants has drastically altered soil microbiomes' composition (Deng et al., 2019). While the evidence pointed to the reduction of biodiversity and composition when using bio inoculants, this does not necessarily mean that these bio resources cannot be used for the benefit of the plant. By specifically altering the microbiome to contain important PGPM, the efficacy and overall health of agricultural plant growth could be increased while reducing the number of possibly toxic or dangerous microbes present in the microbiome.

Recommendations for Future Research

Human survival depends on our ability to feed the growing populace, and continuous research into microbiome health is solution to this challenge. To better develop the microbiome in real-life agricultural fields to produce better yield, pinpointing exact function of bacteria and fungi is a necessity. Identifying functions and mapping locations of what bacteria are beneficial in an environment will greatly improve and potentially increase agricultural land. Along with an increase in agricultural land, research of the microbiome functions can also assist in ecological restoration. Our ecosystem has relied on the Holocene Epoch's consistency and

heightened human activity has begun to change the landscape across the globe dramatically. By replenishing the microbiome to its healthy original state, or something representative, various environments can be restored. Similar to this concept of restoration is terraforming. Terraforming is a hypothetical process of modifying another planetary body to become more earth-like. This is another area of research that could prove useful in the quest to colonize Mars. By selecting specific microbes that would first transform the atmosphere, then the soil, this is an extensive and cutting-edge concept.

An important avenue of future research could be examining what traits within microbes could assist in higher concentrations of vitamins and nutrients within crops. Understanding what microbes assist in nutrient uptake and what traits within said microbes perform this task is an important first step in manipulating the soil microbiome. After this possible additional avenue of research could examine how to implement these traits into easily manipulated microbes. Additionally, how to populate microbiomes with these microbes to improve plant nutritional value and health.

Literature Cited

Primary Literature

Ahmed, I., Kudo, T., Abbas, S., Ehsan, M., Iino, T., Fujiwara, T., & Ohkuma, M. (2014).

Cellulomonas pakistanensis sp. nov., a moderately halotolerant Actinobacteria. *International*

Journal of Systematic and Evolutionary Microbiology, *64*(Pt_7), 2305-

2311. https://doi.org/https://doi.org/10.1099/ijs.0.059063-0

Deng, S., Wipf, H. M., Pierroz, G., Raab, T. K., Khanna, R., & Coleman-Derr, D. (2019). A Plant Growth-

Promoting Microbial Soil Amendment dynamically alters the Strawberry Root Bacterial

Microbiome. *Scientific reports*, *9*(1), 17677. https://doi.org/10.1038/s41598-019-53623-2

Edgerton, C. (1950). Forty-two years of sugarcane disease research at the Louisiana Agricultural

Experiment Station: a report of pathological and botanical investigations as they have affected

the Louisiana sugar industry during the period 1908-1950. *LSU Agricultural Experiment*

Station Reports. 691. http://digitalcommons.lsu.edu/agexp/691

Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N. K., Bhatnagar, S., Eisen, J. A., &

Sundaresan, V. (2015). Structure, variation, and assembly of the root-associated microbiomes

of rice. *Proceedings of the National Academy of Sciences of the United States of America*,

112(8), E911–E920. https://doi.org/10.1073/pnas.1414592112

Jacquiod, S., Puga-Freitas, R., Spor, A., Mounier, A., Monard, C., Mougel, C., Philippot, L., & Blouin, M.

(2020). A core microbiota of the plant-earthworm interaction conserved across soils. *Soil*

Biology and Biochemistry, *144*, 107754.

https://doi.org/https://doi.org/10.1016/j.soilbio.2020.107754

Jiao, S., Chen, W., & Wei, G. (2019). Resilience and assemblage of Soil Microbiome in response to Chemical contamination combined with Plant growth. *Applied and environmental microbiology*, *85*(6), e02523-18. https://doi.org/10.1128/AEM.02523-18

Kieber, J. J., & Schaller, G. E. (2018). Cytokinin signaling in plant development. *Development*, *145*(4), dev149344. https://doi.org/10.1242/dev.149344

Latkovic, D., Maksimovic, J., Dinic, Z., Pivic, R., Stanojkovic, A., & Stanojkovic-Sebic, A. (2020). Case Study upon Foliar application of Biofertilizers affecting Microbial Biomass and Enzyme activity in Soil and Yield related Properties of Maize and Wheat Grains. *Biology*, *9*(12), 452. https://doi.org/10.3390/biology9120452

Liu, F., Hewezi, T., Lebeis, S. L., Pantalone, V., Grewal, P. S., & Staton, M. E. (2019). Soil indigenous microbiome and plant genotypes cooperatively modify soybean rhizosphere microbiome assembly. *BMC microbiology*, *19*(1), 201. https://doi.org/10.1186/s12866-019-1572-x

Qi, G., Ma, G., Chen, S., Lin, C., & Zhao, X. (2019). Microbial Network and Soil Properties Are Changed in Bacterial Wilt-Susceptible Soil. *Appl Environ Microbiol*, *85*(13). https://doi.org/10.1128/aem.00162-19

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., & Chen, W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc Natl Acad Sci U S A*, *109*(16), 6241-6246. https://doi.org/10.1073/pnas.1117018109

Wang, S., Bao, X., Feng, K., Deng, Y., Zhou, W., Shao, P., Zheng, T., Yao, F., Yang, S., Liu, S., Shi, R., Bai, Z., Xie, H., Yu, J., Zhang, Y., Zhang, Y., Sha, L., Song, Q., Liu, Y., Zhou, J., Zhang, Y., Li, H., Wang, Q., Han, X., Zhu, Y., & Liang, C. (2021). Warming-driven migration of core microbiota indicates

soil property changes at continental scale. *Science Bulletin*.

https://doi.org/https://doi.org/10.1016/j.scib.2021.01.021

Wang, C. H., Wu, L., Wang, Z., Alabady, M. S., Parson, D., Molumo, Z., & Fankhauser, S. C. (2020).

Characterizing changes in soil microbiome abundance and diversity due to different cover crop

techniques. *PloS one*, *15*(5), e0232453. https://doi.org/10.1371/journal.pone.0232453

Wu, Y., Cai, P., Jing, X., Niu, X., Ji, D., Ashry, N. M., Gao, C., & Huang, Q. (2019). Soil biofilm formation

enhances microbial community diversity and metabolic activity. *Environment International*, *132*,

105116. https://doi.org/10.1016/j.envint.2019.105116

Yan, Y., Kuramae, E. E., de Hollander, M., Klinkhamer, P. G. L., & van Veen, J. A. (2017). Functional traits

dominate the diversity-related selection of bacterial communities in the rhizosphere. *The ISME*

journal, *11*(1), 56-66. https://doi.org/10.1038/ismej.2016.108

Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M. H., & Bahadar, K. (2018). Role of

secondary metabolites in plant defense against pathogens. *Microbial Pathogenesis*, *124*, 198-

202. https://doi.org/https://doi.org/10.1016/j.micpath.2018.08.034

Zhang, H., Chen, W., Zhao, B., Phillips, L., Zhou, Y., Lapen, D., & Liu, J. (2019). Sandy soils amended with

bentonite induced changes in soil microbiota and fungistasis in maize fields. *Applied Soil*

Ecology, *146*, 103378. https://doi.org/10.1016/j.apsoil.2019.103378

Zhang, H.-Y., Goncalves, P., Copeland, E., Qi, S.-S., Dai, Z.-C., Li, G.-L., Wang, C.-Y., Du, D.-L., & Thomas,

T. (2020). Invasion by the weed *Conyza canadensis* alters soil nutrient supply and shifts

microbiota structure. *Soil Biology and Biochemistry*, *143*, 107739.

https://doi.org/https://doi.org/10.1016/j.soilbio.2020.107739

Zhang, M. M., Wang, N., Hu, Y. B., & Sun, G. Y. (2018). Changes in soil physicochemical properties and

soil bacterial community in mulberry (Morus alba L.)/alfalfa (Medicago sativa L.) intercropping

system. *Microbiologyopen*, *7*(2), e00555. https://doi.org/10.1002/mbo3.555

Secondary Literature

Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function

of microbial communities in the rhizosphere. *FEMS Microbiol Ecol*, *68*(1), 1-

13. https://doi.org/10.1111/j.1574-6941.2009.00654.x

Bosco de Oliveira, A., Alencar, N., & Gomes-Filho, E. (2013). Comparison between the water and salt

stress effects on plant growth and development, responses of organisms to water stress. *Sener

Akıncı, IntechOpen*. DOI: 10.5772/54223.

Byrne, S. J., Butler, C. A., Reynolds, E. C., & Dashper, S. G. (2018). Chapter 7 - Taxonomy of Oral

Bacteria. In V. Gurtler & J. T. Trevors (Eds.), *Methods in Microbiology* (Vol. 45, pp. 171-201).

Academic Press. https://doi.org/https://doi.org/10.1016/bs.mim.2018.07.001

Chu, H., Gao, G.-F., Ma, Y., Fan, K., & Delgado-Baquerizo, M. (2020). Soil Microbial Biogeography in a

changing World: Recent advances and future Perspectives. *mSystems*, *5*(2), e00803-00819.

https://doi.org/10.1128/mSystems.00803-19

Doehlemann, G., Ökmen, B., Zhu, W., & Sharon, A. (2017). Plant Pathogenic

Fungi. *Microbiol Spectr*, *5*(1). https://doi.org/10.1128/microbiolspec.FUNK-0023-2016

George, E., Horst, W. J., & Neumann, E. (2012). Adaptation of Plants to Adverse Chemical Soil

Conditions. In P. Marschner (Ed.), *Marschner's Mineral Nutrition of Higher Plants (Third

Edition)* (pp. 409-472). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-

384905-2.00017-0

Hirt, H. (2020). Healthy soils for healthy plants for healthy humans. *EMBO reports*, *21*(8), e51069.

https://doi.org/https://doi.org/10.15252/embr.202051069

Kaushal, M. (2019). Chapter 12 - Climatic Resilient Agriculture for Root, Tuber, and Banana Crops using

Plant Growth-Promoting Microbes. In K. K. Choudhary, A. Kumar, & A. K. Singh (Eds.), *Climate*

Change and Agricultural Ecosystems (pp. 307-329). Woodhead

Publishing. https://doi.org/https://doi.org/10.1016/B978-0-12-816483-9.00012-8

Kennedy, A. C., & de Luna, L. Z. (2005). Rhizosphere. In D. Hillel (Ed.), *Encyclopedia of Soils in the*

Environment (pp. 399-406). Elsevier. https://doi.org/https://doi.org/10.1016/B0-12-348530-

4/00163-6

Orozco-Mosqueda, M., Rocha-Granados, M., Glick, B. R., & Santoyo, G. (2018). Microbiome engineering

to improve biocontrol and plant growth-promoting mechanisms. *Microbiological research*, *208*,

25–31. https://doi.org/10.1016/j.micres.2018.01.005

Ortiz-Castro, R., Contreras-Cornejo, H., Macías-Rodríguez, L., & López-Bucio, J. (2009). The role of

microbial signals in plant growth and development. *Plant signaling & behavior*, *4*, 701-

712. https://doi.org/10.4161/psb.4.8.9047

Owen, D., Williams, A. P., Griffith, G. W., & Withers, P. J. A. (2015). Use of commercial bio-inoculants to

increase agricultural production through improved phosphorus acquisition. *Applied Soil*

Ecology, *86*, 41–54. https://doi.org/10.1016/j.apsoil.2014.09.012

Wang, X., Jordan, I. K., & Mayer, L. W. (2015). A Phylogenetic Perspective on Molecular Epidemiology.

In Y.-W. Tang, M. Sussman, D. Liu, I. Poxton, & J. Schwartzman (Eds.), *Molecular Medical*

Microbiology (Second Edition) (pp. 517-

536). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-397169-2.00029-9

Zhang, J., Cook, J., Nearing, J. T., Zhang, J., Raudonis, R., Glick, B. R., … Cheng, Z. (2021). Harnessing the

plant microbiome to promote the growth of agricultural crops. *Microbiological Research*, *245*.

https://doi.org/10.1016/j.micres.2020.126690

Zhulin, I. B. (2016). Classic Spotlight: 16S rRNA Redefines Microbiology. *Journal of

Bacteriology*, *198*(20), 2764. https://doi.org/10.1128/JB.00616-16

Zimdahl, R. L. (2010). Chapter 4 - The founders. In R. L. Zimdahl (Ed.), *A History of Weed Science in the

United States* (pp. 37-60). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-381495-

1.00004-9