



Strategic Role and Benefit of Microbiomes in Agriculture

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Abstract: *The objective of this paper is to investigate the strategic role or benefit of microbiomes in Agriculture. This Expository study was motivated by the fact that plants depends on their microbiome and the microbiome of the surrounding soil, for access to organic nitrogen, phosphorus and micronutrients which are necessary for their growth. Therefore the root and soil microbiomes are logical place to start when considering agricultural improvements. However, after a comprehensive literature review on the benefit of microbiomes in agriculture, the following findings suggests that despite the presence of pathogens and conditions favourable to infection, some regions produces plants that are less susceptible to disease than other areas. The soil in these areas in turn supports plant health via the microbiome. Moreso, some soil bacteria and fungi form relationships with plant roots that provide important nutrients like nitrogen or phosphorous. Fungi can colonize upper parts of plants and provide many benefits including drought, heat tolerance, resistance to insects and resistance to plant diseases.*

Keyword: *Microbiomes, Agriculture, Organic Nitrogen, Plant disease, Fungi, Phosphorus.*

INTRODUCTION

Tight links exist between complex microbial populations and plant roots. Plant and gut microbiomes are recognized for their roles in food intake, defense against pathogens and abiotic stress, and provision of metabolic capacity (Sissitsch & Mitter, 2014). Additional evidence points to the plant microbiome as a continuation of the host phenotype (Alekklett and Hart, 2013). Plant-microbe interactions are very specialized, and the host's genotype, physiology (such as root exudates and metabolites), and environmental variables all influence the plant microbiota (Rasche et al., 2006; Lundberg et al., 2012). The literature demonstrates that the advantages of microbiomes in agriculture are a hot research area that is gaining global interest and receiving financing from a large number of funding organizations. To a great degree, however, the intricate relationship between the bacterium and the plant remains a mystery. Thus, it is necessary to evaluate the literature in order to stay up to date on the subject's present state of knowledge and to maximize this new scientific field's potential benefits for mankind as a whole. Therefore, the purpose of this research is to promote the use of bacteria in agriculture by increasing our understanding of microbiomes.

The collection of microorganisms coexisting in a certain environment is known as the microbiome. According to Hunter (2016), microbiomes are specific to humans, animals, and plants, but they

are also present in soils, seas, and even buildings. According to Igbinosa et al. (2018), the term "microbiome" can also refer to the group of bacteria or other microorganisms that live in a given environment and form a "mini-ecosystem." Communities of symbiotic, commensal, and pathogenic bacteria, as well as fungi and viruses, inhabit our bodies and make up our human microbiome (Chukwuka et al., 2023). These communities are found in every part of our body, including our skin, genitalia, lips, eyes, and, of course, our intestines. They are present in distinctive, complementing mixtures. The term "microbiota" refers to a variety of bacterial clusters found in different parts of the body, such as the gut microbiota, often referred to as "gut flora," skin, oral, and vaginal microbiota. While some of these bacteria, known as commensal bacteria, are only there to get by, others, known as symbiotic bacteria, provide a mutually beneficial interaction. Pathogens are opportunistic bacteria that cause illness and are always present, but in much lesser quantities. However, not all pathogens cause disease; in fact, some of them may even be helpful (Chukwuka et al., 2023).

Joshua Lederberg, the 2012 Nobel winner, originally used the term "microbiome" in his work "Infectious History" in Science Lundberg et al. In an attempt to provide a more complete genetic picture of homosapiens as a living creature, he proposed referring to the collective genome of our native bacteria (Microflora) as our "microbiome." Prescott (2017), however, refuted the assertion that Joshua Lederberg, a microbiologist and Nobel laureate, invented the term "microbiome" in 2001. Hundreds of recent research studies, including those published in pediatric journals by Hoffman et al. (2015), portray the declaration of coinage as fact. This assertion is corroborated by more study articles. Despite these assertions, Lederberg did not define or originate the terms "microbiome" or "microbiota," as demonstrated by Prescott's (2017) very convincing evidence. He asserted that his widely referenced 2001 article never used the term "microbiome." In fact, microbiota is a fundamental word in microbiology that has been around for at least half a century (Prescott 2017).

2.1 Functions of Microbiome in Agriculture

According to Roossinick (2008), soil microbes, such as fungus and bacteria, are vital to the breakdown of organic matter and the recycling of leftover plant material, which is why microorganisms are so crucial to agriculture. Certain fungus and bacteria in the soil interact with plant roots to supply essential nutrients like phosphorus and nitrogen (Roossinick 2008). Despite making up less than 1% of a soil's total mass, soil organisms are essential to the survival of all plants and, by extension, all animals. Following is Roossinick's (2008) description of a few of their essential roles:

2.1.0. Organic matter is broken down by soil microbes: Microorganisms are crucial to the breakdown of organic matter because they enable plants to obtain and utilize nutrients. Microbes come in a variety of forms and are adapted to inhabit a wide range of organic matter types.

2.1.1. Soil microorganisms assist in nutrient recycling: Soil bacteria are essential in restoring nutrients to their mineral forms so that plants can reclaim them. We call this process mineralization.

2.1.2. Humus is created by soil bacteria: Humus is a dark brown, jelly-like material that can stay in the soil for millennia after the microbes have consumed all of the available food. Humus promotes the development of soil structure and aids in the soil's ability to hold onto moisture.

Humus contributes significantly to a soil's ability to exchange cations by binding to positively charged ions (cations) of plant nutrients thanks to the negatively charged spots all over its molecules. Additionally, humus may decrease plant diseases.

2.1.3. Soil bacteria produce polysaccharides, gums, and glycoproteins that bind soil minerals together and provide the foundation for soil structure. Soil aggregates are further bound together by plant roots and fungus hyphae. Plant development requires a certain level of soil structure.

2.1.4. Soil microorganisms fix nitrogen: The capacity of certain microbes, primarily bacteria, to change atmospheric nitrogen (N₂ gas) into ammonia (NH₃) is crucial to agriculture. While some bacteria are free-living in the soil, others are associated with plant roots; Rhizobium bacteria, for example, are found in the roots of legumes. The term nitrogenfixation refers to the conversion process. Approximately 60% of the nitrogen fixed on Earth comes from biological means. Industrial fertilizers, on the other hand, only make up 25%. The relevance of biological nitrogen fixation in food production is expected to grow as energy costs rise and artificial nitrogen fertilizers become more expensive.

2.1.5. Soil organisms encourage plant development: Auxins, gibberellins, and antibiotics are just a few of the compounds that some soil bacteria create to encourage plant growth.

2.1.6. Soil microorganisms' aid in the control of illnesses and pests: The most well-known use of soil microbes in pest management is the commercial spore production of the soil bacteria *Bacillusthuringiensis* (Bt) to manage agricultural pests that are caterpillars. Certain Bt strains are also utilized to manage flies and beetles. Numerous *Trichoderma* strains have been created as biocontrol agents to combat fungal infections mostly affecting the roots of plants. Other fungal genera are employed in the management of insect infestations.

2.1.7 Plant Microbiomes to Enhance Crop Yields and Ensure Food Security

A few instances of advantageous plant-microbe interactions have been thoroughly studied and examined in relation to nitrogen's significance in agricultural systems. Among these is biological fixation by rhizobia, which forms a symbiotic relationship with legumes and serves as the foundation for crop rotations in which legumes help to preserve soil fertility. Moreover, arbuscular mycorrhizal fungi internally colonize around 80% of soil plant species. Arbuscules and vesicles are produced in this symbiosis by the hyphae, which are crucial for the plant's uptake of nutrients. Furthermore, the well-studied (Clay, 1988) symbiotic defensive mutualism between Pööideae grasses and endophytic fungus of the *Epichloë* is crucial for pasture productivity. Aside from these widely recognized mutualistic relationships between plants and microbes, beneficial microorganisms have received little attention in agricultural production plans. However, it is anticipated that plant microbiome functions will be a crucial part of crop production in the future due to the established functional importance of the plant microbiome, the effects that can be seen upon the inoculation of specific microorganisms, and the fact that both plants and microorganisms carry genetic determinants needed for their interaction. Agricultural activities including fertilizer and pesticide treatment, as well as a variety of host-driven parameters like plant genotype, influence the makeup of the plant microbiome (Mader et al., 2002). It is probable that such structural alterations will also impact microbiome functioning, even if we still don't fully understand how. Organic farming methods often strive to make the greatest use of natural resources and retain biodiversity, but conventional agriculture has not yet begun to examine potential effects on the functioning of plant related microbiota owing to existing practices (Mader

et al., 2002). For example, crop rotations including legumes are implemented, and typically, a greater variety of plants is utilized or preserved, leading to a more effective investigation and preservation of microbial activities. Fertilizer or pesticide treatments may be better chosen in accordance with or demonstrating the least negative effects on desired plant microbiome functions, in line with the broader trend to increase the sustainability of agricultural practices, such as various soil preparation techniques (Clay, 1988). A selection factor might include the impact on the plant microbiome in addition to effectiveness.

Moreover, dose effects may need to be taken into account. Pesticides and fertilizers used in excess may affect microbiome activity more negatively than dosages that are still effective. Individual microorganisms are now being used by industry, primarily as biofertilizers or microbial plant protection products. The industry's interest in microbial products is growing quickly as a result of the increased need for substitutes for the present fertilizers and pesticides, which is being driven by national strategic plans that aim to limit the use of chemicals in agriculture (Bulgarelli 2018). The effectiveness and consistency of desired effects of microorganisms under different field conditions represent a major bottleneck for product development, despite the high potential that such microbial inoculants have frequently demonstrated in lab and greenhouse experiments (Bulgarelli 2018). As a result, there is a pressing need to enhance application methods, selection procedures, and, most importantly, our understanding of how microbes and plants interact in the field.

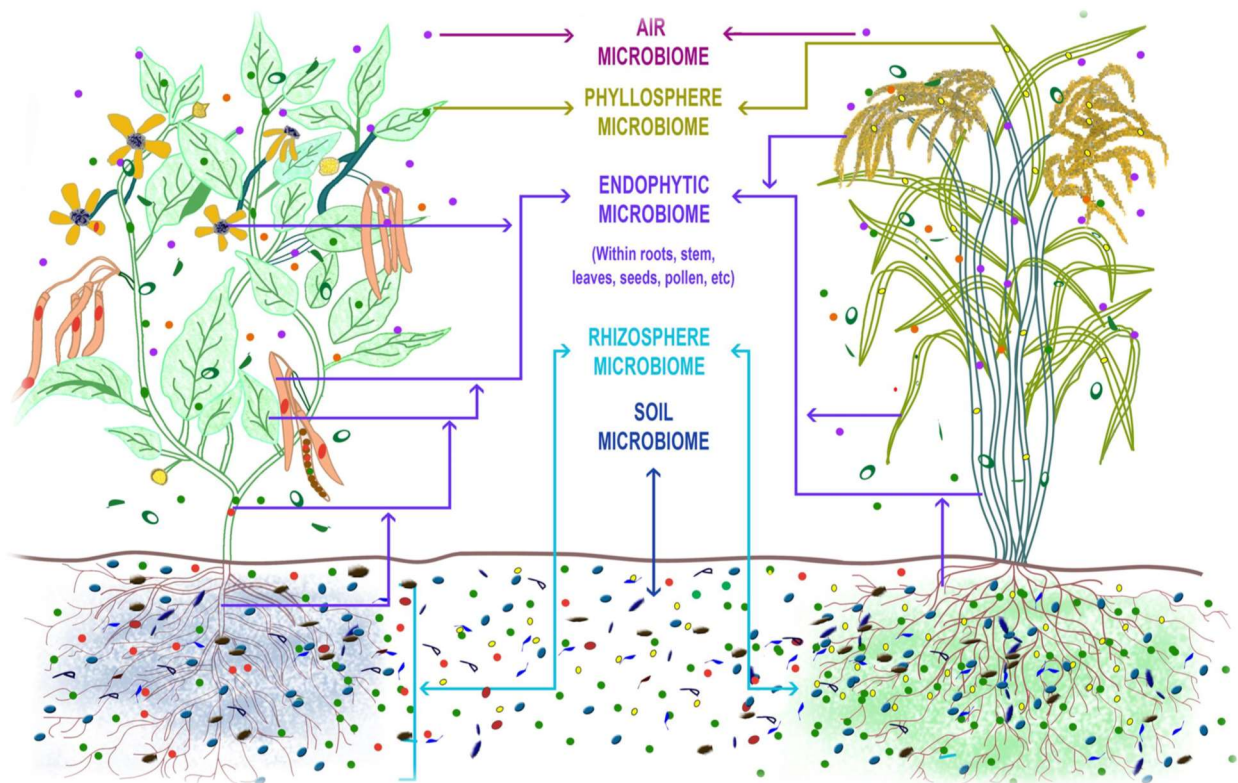


Figure 1: The plant Microbiome.
(Source: Gopal and Gupta 2016)

With model plants cultivated under gnotobiotic settings, a wealth of knowledge on the processes underlying plant–microbe interactions has been collected. Scientists are now beginning to appreciate how critical it is to employ appropriate plant cultivars and have a deeper understanding of microbial activity in the field in order to increase field efficiency. Such knowledge will indicate the (field-relevant) circumstances in which a microbial strain manifests desirable activities, or if co-colonizing microorganisms facilitate or obstruct particular activities of an inoculant strain. This will significantly increase the efficacy of microbial products, together with enhanced formulations and application methods. In the future, it could be able to create microbiomes that support plant growth and health by better using the complimentary and synergistic processes of different strains. Concepts about the transplanting of microbiomes may also be created; for example, plant microbiomes that thrive in unfavorable environments may be used as inoculants or as a model for creating "synthetic" microbiomes (Delaux et al., 2014).

Genetic factors that interact with microorganisms allow plants to react to the microbiota; host-microbiome evolutionary links have been demonstrated (Bouffaud et al., 2014; Delaux et al., 2014). This suggests that plants might be enhanced to interact with beneficial bacteria more effectively by breeding, selection, or genetic enhancement. While plants have mostly been enhanced and chosen for increased resistance and yield in recent decades, it is anticipated that effective interaction with helpful microbes will be a further breeding goal in the future. Applications might include crops that reduce relationships with particular diseases and enhance mutualists, plants that activate specific microbiome components, or breeding legumes for enhanced interaction with well-known rhizobial symbionts (Bouffaud et al., 2014; Delaux et al., 2014).

A deeper comprehension of the molecular pathways through which plants interact with mutualists will facilitate the creation of appropriate breeding targets and screening strategies. The discovery of plant lines guaranteeing enhanced resource efficiency, tolerance of abiotic stress, and protection against pests and diseases may eventually result from genetic alteration or plant breeding. Sustaining the positive microbiome activities of plants is especially crucial for yield stability and for permitting plant development under poor (and even unforeseen) environments like pathogen infection or drought. Plant microbiomes have the potential to significantly increase agricultural productivity on a global scale, but they will be especially crucial for plant growth in situations when resources for fertilization, irrigation, and disease treatment are few. This is true in many regions of the world where the use of minimal input agriculture is widespread and access to enhanced germplasm or agricultural amendments is scarce. Utilizing plant microbiome functions more effectively would help agricultural productivity in particular under these circumstances and promote the bio-economy of less developed nations by building strain collections from local settings and delivering microbial inoculants (Bulgarelli 2018).

A advantage of plant microbiome in sustainable agriculture is the three-way symbiosis that occurs between plants, mycorrhizal fungi, and rhizobia bacteria. These symbioses are common in legumes but uncommon in most cereal crops, despite the persistence of the underlying signaling pathways. Cereals should be able to get nitrogen and phosphorus without fertilizer if they could be genetically modified to create the necessary elements for these symbioses to form, especially the root nodules for mycorrhizal fungus and rhizobia bacteria (Hunter, 2016).

3.0 Four key ways where agriculture may benefit from using the microbiome

According to Martin (2018), microbes cohabit and interact with a wide range of host creatures to create the basis of every ecosystem on Earth. The soil microbiome and plant microbiome, as well as their interactions, are of importance in the field of agriculture. Microbes are present in every plant tissue. They are called endophytes when they are found in the stems, leaves, and seeds, and epiphytes when they are located on the surfaces of these same tissues (source reference). The rhizosphere of a plant, which consists of the bacteria, archaea, and fungus (including mycorrhizal fungi) that live in and around the root system of the plant, is the most researched agricultural microbiome (Martin 2018). In return, the plant roots provide nutrients (such as sugars and amino acids) that support the microorganisms. They also offer fixed nitrogen, access to micronutrients, and defense against diseases in the soil. The seeds with their own microbiomes support the plant's next generation, whereas the rhizosphere is mostly drawn from the soil to sustain the developing plant.

To understand the effects of agricultural microbiomes and how to modify them over time, researchers in the discipline of agricultural biosciences, or agbio, are fusing traditional and cutting-edge methods. They employ shotgun metagenomics, transcriptomics, and traditional culture methods to create community snapshots through the sequencing of universal barcode regions. They intend to use the microbiome to make agricultural products even more durable, delicious, abundant, and nutritious.

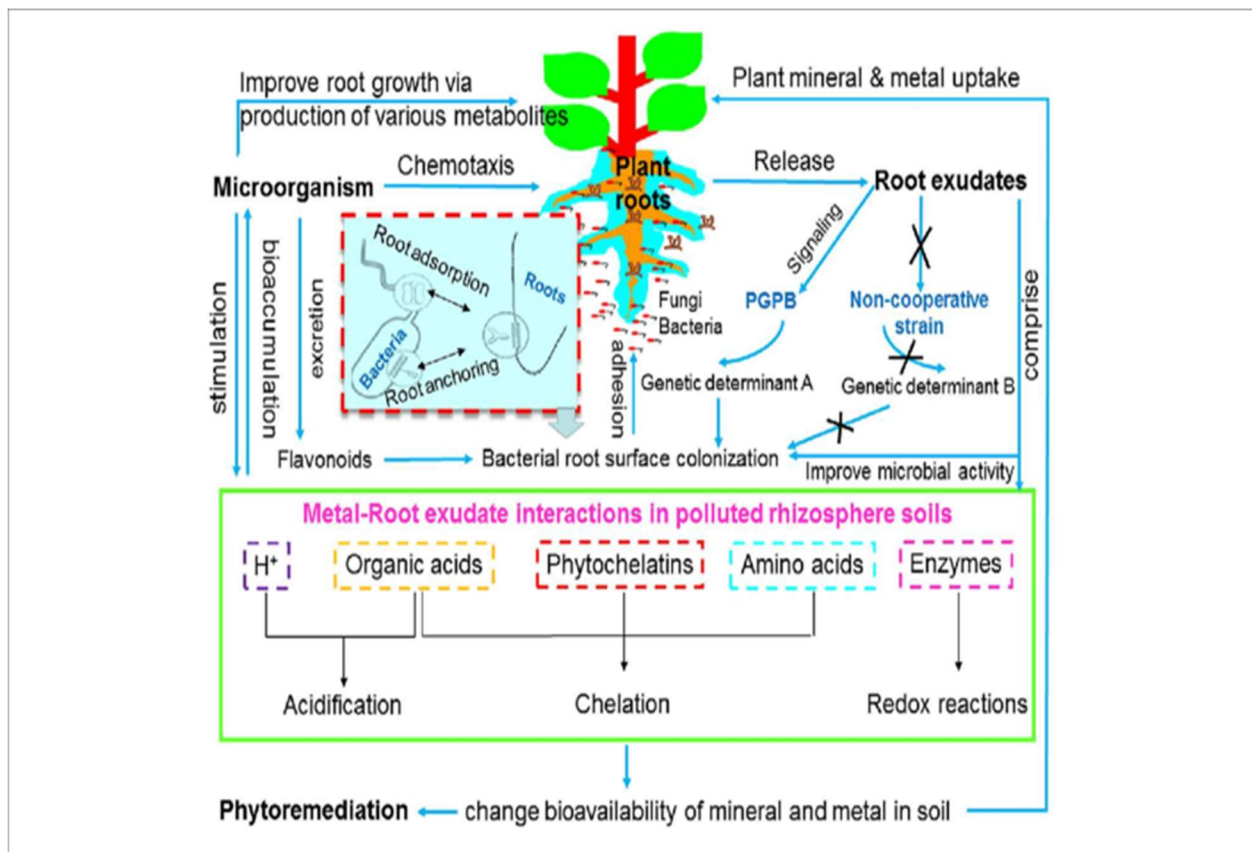


Figure 2: Overview of plant-microbe interaction in nutrient recycling

Source: Ma Y. et al. (2016).

4.0 Below is a brief summary of four areas of potential for microbiome in agriculture:

4.1 Yield Improvement: For access to organic nitrogen, phosphorus, and other micronutrients required for development, plants rely on the microbiome of their roots and the surrounding soil. Thus, while thinking about agricultural improvements, it seems sense to start with the root and soil microbiomes (Ricky & Sullivan 2017). A key factor in raising agricultural yields is healthy soil. Since ancient times, legumes have been used in crop rotation due to their capacity to enhance soil health. More recently, research has revealed that legumes accomplish this by influencing nitrogen and nitrogen-fixing microorganisms (rhizobia) in root nodules. Even though these rhizobia have been around for decades, when legumes are first planted, they are still employed as inoculants.

Additionally, there are biological inoculants of soil and root bacteria that may be used to increase oil output, alleviate salt and drought stress, and increase the bioavailability of phosphate, nickel, and potassium. A product in the early stages of development aims to increase the vigor of seedlings, while another is under development to provide nitrogen fixation to non-legumes. Research on inoculum for endophytes of the stems and leaves is being pushed by the advantages of bacteria beyond the roots ((Ricky & Sullivan 2017).

Inoculation is only one example of how many new technologies are moving the emphasis from individual organisms to a community perspective. The hormone lipochitooligosaccharide (LCO) is the signaling molecule that nodule-forming plants and their rhizobia utilize to communicate. Its application speeds up the natural microbiota's and injected bacteria's root colonization. Modulating whole microbial populations as opposed to just a few species is at the forefront of the microbial inoculate business.

4.2 Nutritional enhancement

While improving crop productivity is a major goal, nutritional content should also be taken into consideration. Research has shown that during the past century, the nutritional content of many of our food crops has declined (Ricky & Sullivan, 2017). Numerous reasons, such as the industrial selection of fast-growing, low-nutrient crop kinds, the depletion of soil nutrients, and disturbances of microorganisms in the surrounding soil and plants, are probably to blame for this loss. Understanding the part that endophyte and rhizobia communities play in reviving these crops' nutritional profiles has been the focus of recent research. In the future, microorganisms could contribute to current crops' increased nutritious content.

4.3 Taste Modification

meals cultivated on sandy soils lack the richness and flavor of meals grown in soils with high organic matter content. The microbiome's constituent organisms may even modify taste by producing compounds that affect their host plants' metabolisms. End goods like wine, coffee, chocolate, and herbs and spices that rely on specific flavor profiles would find this especially interesting. For example, the chemical makeup of completed wines contributes to "terroir," and the bacteria and fungus in wine fermentations, which are molded in part by circumstances in the

vineyard, correspond with this information (Ricky & Sullivan 2017). The flavor and scent of herbs growing in enriched surroundings will be more intense. Researchers are always trying to better understand how different bacteria and fungus affect these particular ecosystems.

4.4 Management of pathogens and pests

There are several risk factors that might interfere with a plant's life cycle throughout growth. Because of its endophytes, the plant possesses its own protective mycobiome. These microbes create secondary metabolites that are poisonous to insects and other herbivorous pests, such as terpenes and alkaloids. A major factor in the quick development of biopesticide technology is customer desire for safer alternatives to chemical pesticides. These products make use of defense mechanisms against agricultural diseases. Numerous microorganisms can be employed to manage other creatures, including but not limited to insects, fungus, nematodes, bacteria, and viruses, that might be negatively impacting the ecology and plant growth. Similar to the next crop-enhancing technologies, novel biopesticides will take into account not just a single beneficial organism but also an intricate web of collaborating microorganisms (Raschee et al., 2006).

Viruses are typically thought of as disease-causing agents. This is so because the organisms that have been researched are the ones that cause sickness. Though most of the plants don't appear to be unwell at all, around half of them contain viruses. It appears that the viruses are coexisting well with the plants. Recently, neglecting to water certain virus-infected plants caused stress. Surprisingly, all of the virus-infected plants were far more resilient to stress and drought than the control group in this experiment.

5.0 Enhancing Agriculture through Plant Microbiome Manipulation

It is becoming more and clearer that, similar to animals, plants are not independent entities but rather are home to a wide variety of microbes.



Figure 5: MODELING THE MICROBIOME

SOURCE: SIMON FRASER/SCIENCE 2018

Using next-generation sequencing, Schulze-Lefert (2015) of the Max Planck Institute for Plant Breeding Research in Cologne, Germany investigated the bacterial communities inhabiting the roots of the model plant *Arabidopsis thaliana*. For long years, researchers have known that a wide range of microorganisms interact with roots, but their makeup remained mostly unknown. He was astounded by the astounding taxonomic variety of bacteria that a single, microscopic root could support when the sequencing results started to come in. Nevertheless, despite the seeming turmoil, there was order (Raschee et al., 2006). Members of the phyla Actinobacteria, Bacteroidetes, and Proteobacteria were almost always abundant, which allowed the root specimens to be distinguished from their surroundings.

These results were corroborated by subsequent research conducted in different labs, which suggested Firmicutes as another important component of the plant microbiome. Apart from these bacterial taxa, several fungal and eukaryotic microorganisms have also been identified using plant genomic surveys. Furthermore, every one of these groups of organisms is establishing itself not only in the soil around the roots of plants but also in other tissues like leaves ((Raschee et al., 2006). New concerns were quickly raised by this research: why were some microorganisms more

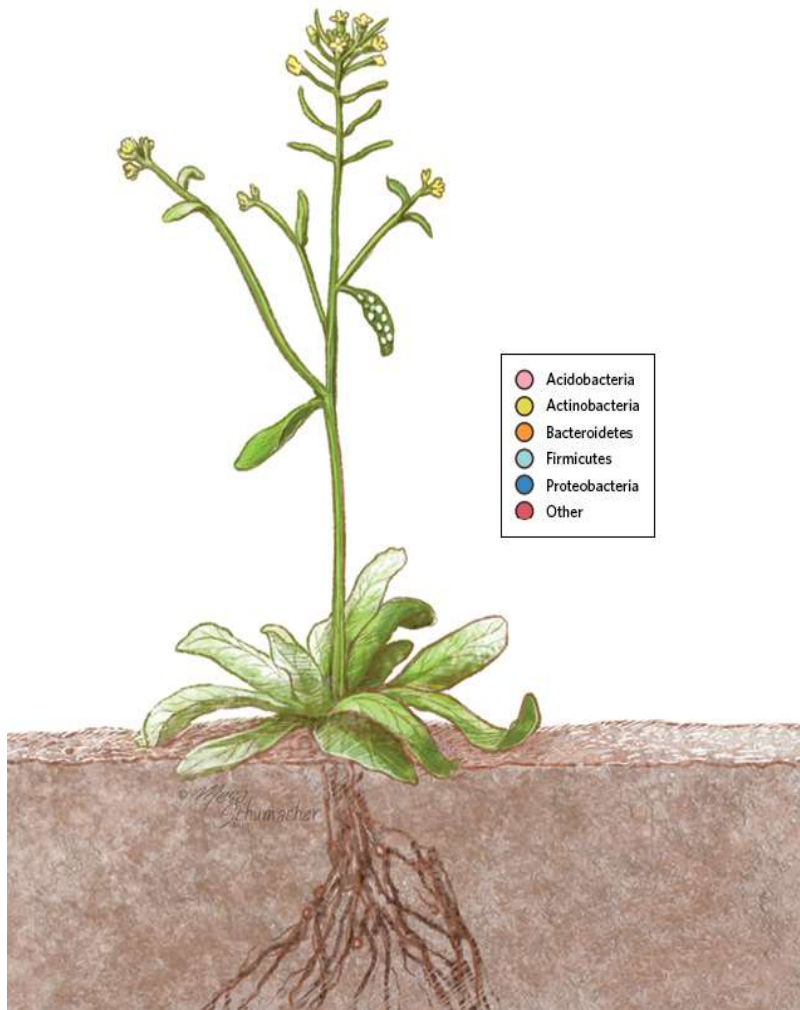
prevalent in leaves and roots? How did these colonies of microbes get together? Above all, how did they impact the health of the plants?

Recently, scientists have started looking into the functional effects of these bacterial, fungal, and eukaryotic symbionts in addition to conducting genomic surveys of the microorganisms found in different plant tissues. Agriculture might undergo a transformation if a deeper comprehension of the molecular dialogue between plants and their microbiome is gained. By 2050, there will be 9.8 billion people on the planet, a 30% increase from the current number. Global food supply will be under tremendous strain as a result, and this pressure won't be entirely mitigated by farmers using the agrochemicals they presently employ to boost output and shield crops from pests and diseases. Radical adjustments to the crop-production process are required to support a sustainable food supply for humans; these adjustments may take the form of microbial manipulation.

The rhizosphere, or the area where plant roots meet the soil, as well as the root itself are places where microbes colonize and multiply. These microbes can improve a plant's uptake of minerals, actively synthesize and regulate phytohormones—chemical compounds that affect a plant's growth and development—and shield plants from pests and diseases that come from the soil. For these reasons, in an effort to boost agricultural productivity in a sustainable manner, scientists are trying to modify the microorganisms that inhabit this subsurface environment.

Examining the microbiome of plants

Soil is home to the roots of terrestrial plants and is considered one of the planet's most varied and abundant microbial reservoirs. A single gram of soil is thought to contain hundreds of distinct types of bacteria, not to mention other microbes like fungus, protists, and archaea. It should come as no surprise that the development of relationships with the soil biota marked a significant turning point in the adaptation of plants to the terrestrial environment. According to fossil data, these kinds of interactions with microbiome-associated fungi may have started as early as 400 million years ago.

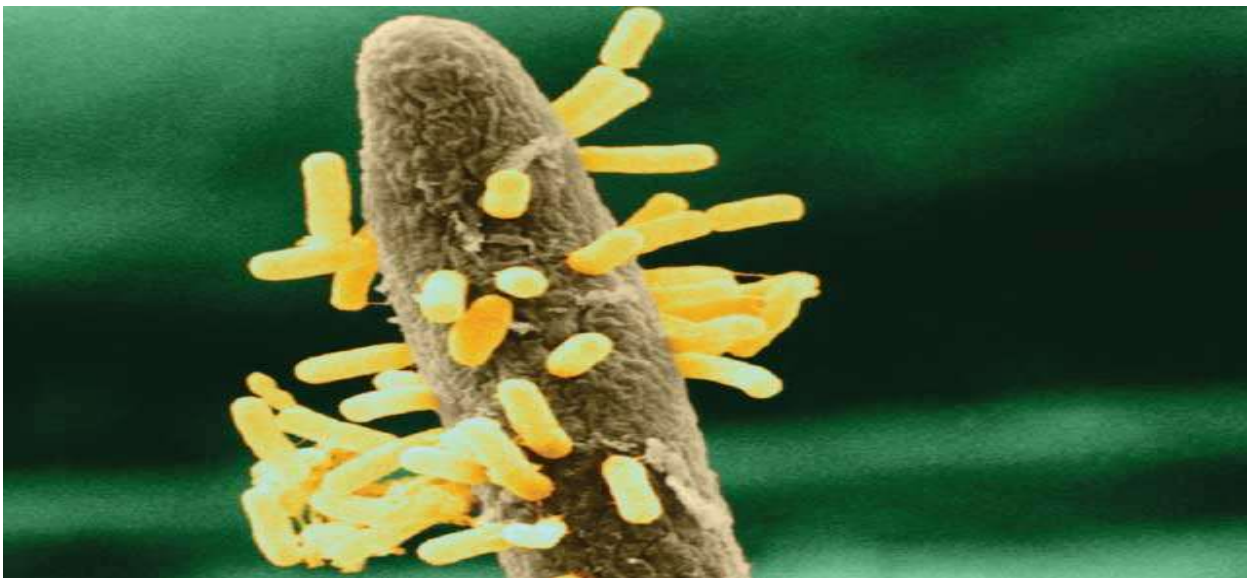


PLANTS MICROBIAL COMMUNITIES:

SOURCE: Bulgarelli (2018)

Comparative research shows that the root microbiome is mostly determined by soil properties including nitrogen and mineral availability (Rascheet al., 2006). The root microbiome controls plants' nutrition dependent on soil, just as digestive tract microorganisms interact with the food that vertebrates eat. Individual plant microbiome members seem to be compartmentalized, which is similar to host/microbe interactions in the animal realm. At least three different microbiomes have been shown to be flourishing at the root-soil interface in studies involving Arabidopsis and rice: the rhizosphere, the rhizoplane (the surface of the root), and the endosphere (the inside of the root). The bacterial populations in various plant species are dominated by Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria in all three compartments. The microbial mix of plants' above-ground parts, including their leaves, is also predictable.

The species compositions of these communities vary greatly throughout hosts, despite the fact that the types of microorganisms that comprise the plant microbiome are generally preserved (Rasche et al., 2006). Rhizodeposition, or the plant's release of organic chemicals into the rhizosphere, appears to be a major component in determining how the microbiome is populated and maintained. Depending on the kind of plant and stage of development, the quantity and makeup of these organic deposits can range from 10 to 16 percent of total plant nitrogen and up to 11 percent of net photosynthetically fixed carbon. This process affects the rhizosphere's physical and chemical makeup, which in turn supplies organic substrates and signaling molecules for microbial development.



ROOT BUGS

SOURCE: Kemen (2016)

Another factor that likely shapes the composition of the plant microbiome is interaction between microbes. In 2016, Eric Kemen of the Max Planck Institute for Plant Breeding Research and colleagues surveyed the microbes thriving in and on wild *Arabidopsis* leaves at five natural sites in Germany sampled in different seasons. They then plotted correlations between the abundances of more than 90,000 pairs of microbial genera identified in their survey, revealing six “microbial hubs” nodes with significantly more connections than other nodes within the network. These hubs were represented by the oomycete genus *Albugo*, the fungal genera *Udeniomyces* and *Dioszegia*; the bacterial genus *Caulobacter*, and two distinct members of the bacterial order Burkholderiales. Given the high degree of connectivity within the communities, it is likely that

these microbial hubs play a disproportionate role in the microbiome, akin to that of keystone species in an ecosystem.

To validate this idea that certain species can drive the composition of the plant microbiome, Kemen's team selected *Albugo* sp. and *Dioszegia* sp. as paradigmatic examples of microbial hubs. *Albugo* oomycetes are eukaryotic pathogens of *Arabidopsis* with an obligate biotrophic lifestyle, meaning that they cannot be cultured outside their host. Consistent with the central role of *Albugo* in the plant's microbial community, *Arabidopsis* that had been artificially infected with *Albugo laibachii* and maintained in potting soil under controlled conditions displayed a bacterial microbiome composition that was less variable across plants than that of uninfected individuals. Conversely, differences between the bacterial microbiomes of three distinct *Arabidopsis* strains were amplified in the presence of *A. laibachii* infection. The fungal microbiome, however, was not significantly affected by the presence of *A. laibachii* and another *Albugo* species.

Kemen's team conducted a parallel set of experiments with *Dioszegia* sp., which—unlike *Albugo* sp.—are culturable under laboratory conditions, and six bacterial isolates from *Arabidopsis* leaves. The results confirmed that the presence of the fungal species can strongly inhibit the growth of *Caulobacter*—plants whose leaves were inoculated with *Dioszegia* sp. showed a 100-fold reduction in the number of colony-forming units of *Caulobacter* sp.—mirroring the significant negative correlation observed between these two groups of microbes in the network analysis. In 2017, Harvard University's Roberto Kolter and colleagues demonstrated that such microbial interactions are not limited to *Arabidopsis*. The researchers developed a simplified version of the maize root microbiome, consisting of seven bacterial strains previously identified in sequencing surveys (Raschee *et al.*, 2006). By using a leave-one-out approach to colonizing naive maize plants, they demonstrated that removal of *Enterobacter cloacae* disrupts the composition of the microbial community, which became dominated by *Curtobacterium pusillum*, while the other five species had nearly disappeared. Interestingly, this effect was limited to plant colonization: when the seven strains of bacteria were monitored in a substrate that did not contain maize seedlings, the community's composition was significantly different from the one retrieved from roots, and the regulatory role exerted by *E. cloacae* was not detected.

These studies suggest that individual members of the microbiome can have a disproportionate role in assembling and stabilizing the community. Deciphering the interactions within and between the various taxa populating leaves and roots will be required to understand the regulation of the plant microbiome (Kolter 2017).

From composition to function

For years, researchers have observed that, despite the presence of pathogens and conditions favorable for infection, some regions produce plants that are less susceptible to disease than other areas. The soils in these areas, it turns out, support plant health via the microbiome (Kolter 2017).

Researchers are making strides in understanding the mechanisms underlying this support. In 2011, for example, a team led by Rodrigo Mendes, then at Wageningen University and Research Centre in the Netherlands, demonstrated that disease suppression was linked to the recruitment

of a specific population of Pseudomonadaceae, a family of the phylum Proteobacteria. Using a PCR fingerprinting approach, the researchers discerned that this population could be grouped into ten haplotypes, which the team designated A to J. Of these, haplotypes A, B, and C represented some 90 percent of the isolated bacteria. When inoculated in soil, a representative strain of haplotype C suppressed the incidence of disease caused by the fungus *Rhizoctonia solani* on sugar beet roots, while, surprisingly, strains from haplotypes A or B did not.

Similarly, in their study published (2017), Kolter and colleagues found that maize plants inoculated with seven selected bacterial strains showed significantly delayed development of *Fusarium verticillioides*, the causal agent of maize blight. This phenomenon was mediated by the specific strains chosen, and not by bacterial colonization per se, as seed treatment with a laboratory strain of *Escherichia coli* did not protect maize seedlings from pathogen development. Likewise, the seven strains together were required for the protective effect: inoculation with individual strains resulted in significantly less protection against *F. verticillioides* (Kolter 2017).

This method of combining sequencing data with microbial isolation is becoming a powerful tool to formulate testable hypotheses and gain novel insights into the function of the plant microbiome. Like Kolter, researchers are assembling microbial isolates into synthetic communities (SynComs) of known composition and testing their effects on host plants. This approach was once considered a daunting task, as only a very limited fraction, often less than 1 percent, of soil biota was considered culturable under laboratory conditions. But in 2015, Schulze-Lefert's laboratory teamed up with Julia Vorholt's group at ETH Zurich in Switzerland to investigate the proportion of *Arabidopsis*-associated bacteria that can be cultured, and found the 1 percent statistic to be a vast underestimate.

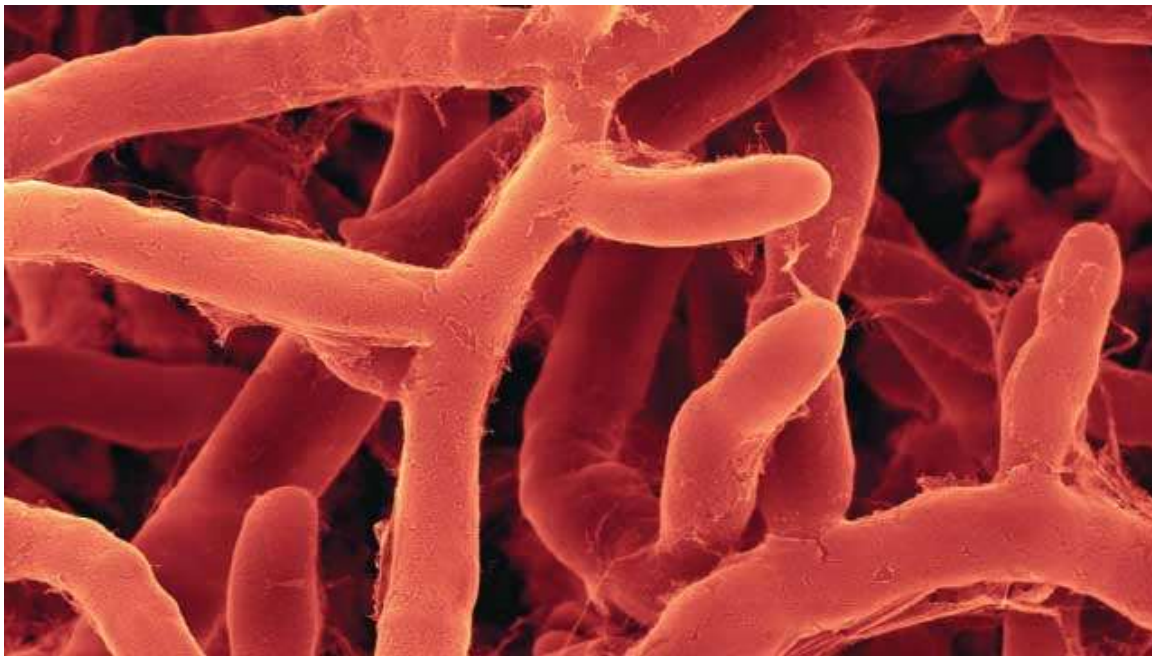


Figure 4: FUNGAL FINGERS DENNIS KUNKEL MICROSCOPY/SCIENCE
SOURCE: Kolter 2017.

Comparing the taxonomic relationships among some 8,000 colony-forming microbes from leaves and roots of plants using cultivation-independent sequencing surveys of leaf and root microbiomes, the researchers demonstrated that more than 50 percent of the dominant members of the *Arabidopsis* microbiome can be cultured in vitro. Taking advantage of this finding, the team assembled SynComs representative of the microbiota of the *Arabidopsis* roots and leaves and tested the communities' capacities to colonize these tissues on plants grown in a sterile substrate—the botanical equivalent of germ-free mice. These experiments revealed that, upon plant inoculation, root and leaf isolates form microbial communities resembling the natural microbiomes of those tissues, demonstrating that the SynCom approach accurately recapitulates the effects of a complete microbiota. Since then, numerous researchers have begun to develop SynComs to further explore the function of the plant microbiome. For example, Jeff Dangl of the University of North Carolina at Chapel Hill and colleagues used the SynCom approach to explore the role of the root microbiome in phosphate uptake (Kolter 2017).

In nature, less than 5 percent of the phosphorus content of soils is available to plants. To circumvent this limitation, farmers rely on the application of chemical fertilizers, but this approach is not sustainable in the long term. Thus, understanding how plants and their associated microbes can thrive under sufficient and limiting phosphorus supplies is a priority. There is a huge body of literature documenting the contribution of arbuscular mycorrhizal fungi to phosphorus uptake in plants, but the role of the bacterial microbiota remains mysterious. Scientists are looking to manipulate soil microbes to sustainably increase crop production—and novel insights into the plant microbiome are now facilitating the development of such agricultural tactics.

In experiments with *Arabidopsis*, which does not engage in symbiotic relationships with mycorrhizal fungi, Dangl and his colleagues compared the microbiomes of wild-type plants with those of mutant lines that had impaired phosphate starvation responses (PSRs)—a set of morphological, physiological, biochemical, and transcriptional activities evolved by plants to cope with phosphorus deficiency. Using a SynCom represented by 35 taxonomically diverse bacterial isolates from *Arabidopsis* and related plants, the researchers demonstrated that wild-type plants and mutants, grown on agar plates, assemble distinct root communities when exposed to both low and high phosphorus concentrations. Remarkably, SynCom inoculation reduced accumulation of phosphorus when plants were grown under limited conditions but not when plants were grown in the presence of abundant phosphate, suggesting that bacteria and plants compete for the element.

By monitoring a core set of 193 marker genes, the team observed that SynCom inoculation greatly enhanced PSR-related transcription in wild-type plants. When the researchers transferred inoculated wild-type plants grown with limited phosphorus to plates with sufficient supplies, they observed a striking result: 20- to 40-fold increases in phosphorus concentration in the plant stem, as compared with mock-inoculated controls. Such a dramatic increase in phosphorus uptake was not detected in inoculated plants initially grown with sufficient phosphorus. Therefore, initial plant-bacteria competition for phosphorus might be part of an adaptive mechanism to maximize PSR in plants (Maderet. *al.* 2002).

Further investigation into the binding sites of transcription factors on *Arabidopsis* DNA revealed that PHR1, a master regulator of PSR, and its paralog PHL1 contribute to transcriptional regulation of plant immunity (Chukwuka & Amahi 2021). In particular, *phr1;phl1* mutant plants display enhanced activation of plant immunity genes in response to phosphate starvation and to

SynCom inoculation, compared with wild-type plants. Together, these data suggest that the nutritional status of the host is a driver of microbiome composition; through master regulators of mineral starvation, plants can modulate immune responses, which could, in turn, shape microbiome composition (Maderet.al. 2002).

In soil, microbial engineering could be as simple as adding food sources or vitamins that beneficial microbes need, adding antibiotics that target detrimental microbes, or infusing fertilizers with probiotic cocktails of plant-friendly microbes (Maderet.al. 2002).

The interconnectedness of soil microbes also makes them nearly impossible to study in isolation in the laboratory. There's a catch, however. Without knowing what each of the microbes in soil do it's impossible to decide which microbes to add or subtract to maximize crop yields. The microbial social network is not only beneficial to plants, but essential to the entire community of organisms living in soil. Microbes often cannot function without the support of their social network since they're constantly interacting with their neighbors by trading food, removing toxic waste, and sending signals about changes in the soil around them.

Adding or removing the wrong types of microbes could upset the delicate balance of the community, with disastrous results for plant growth. As a result, scientists have to understand not just the microbes that directly affect plant growth and soil health, but all of the surrounding microbes that play an indirect role in supporting the entire microbial community.

Unfortunately for scientists, the interconnectedness of soil microbes also makes them nearly impossible to study in isolation in the laboratory, which is how we have typically figured out how different microbes work. Isolated from the other soil microbes they normally interact with, microbes brought into the lab simply die.

Hitting the side of the barn

Soil researchers are turning straight to the source for answers: DNA. As the blueprint for life, the DNA in every microbe's genome defines what it can do: what it eats, how it interacts with other microbes, and whether it provides any direct benefits for plants. If scientists can map the genomes of every microbe in the complex soil community, they can figure out which microbes are involved in plant growth and start to untangle the complex web of soil communication. The key to mapping these genomes is a relatively new technique known as genome binning. Rather than attempting to untangle individual cells in the laboratory, scientists are mashing up all of the microbes in a spoonful of soil and sequencing the entire DNA that spills out at the same time. The process is known as "shotgun" sequencing for its molecular-scale resemblance to shooting birdshot at the broad side of a barn.

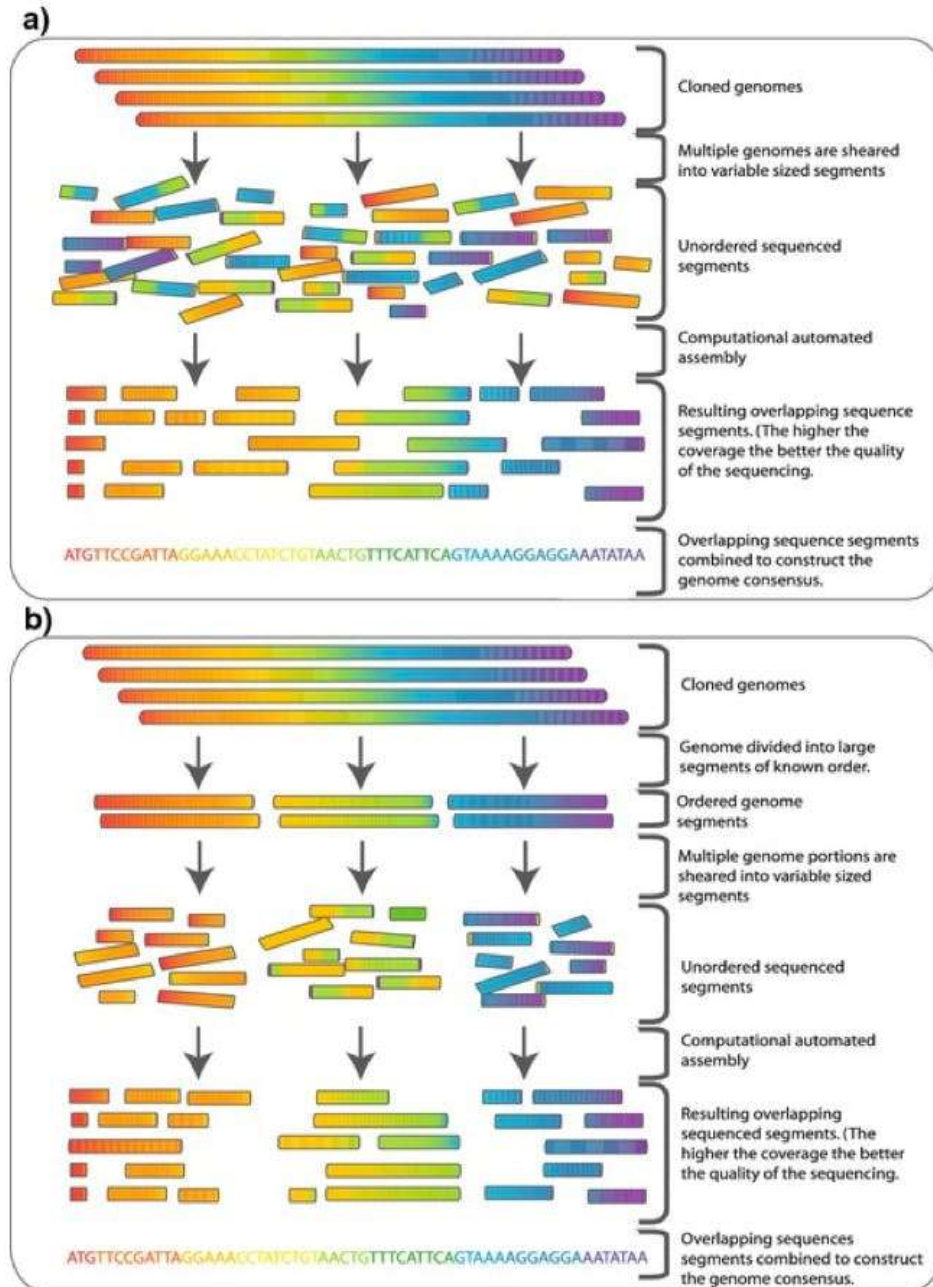


Figure 5: shotgun sequencing process.

Millions of brief, fragmented DNA sequences are produced by shotgun sequencing, each of which represents a tiny portion of the genome of one of the 100,000 distinct microorganisms present in that soil. Determining which of those bits belongs to which microorganism is the difficulty. In basic microbial communities including only a few hundred species, genome binning has proven effective. However, due to the enormous amount of microorganisms that reside in soil, soil scientists have not been able to succeed until lately.

Accepting the "grand challenge"

A computer program that must determine which DNA pieces belong together and which don't is at the core of genome binning. In order to do that, the computer looks for matching DNA sequences in each individual DNA fragment. This shows that two samples produced by shotgun sequencing are, in fact, successive segments of the entire genome of the same creature. However, the number of potential DNA sequences and deceptive partially overlapping sequences rises along with the diversity of species contributing to the pool of sequenced DNA fragments. The technique fails to bin any genomes at all when an excessive number of distinct species are provided because it is unable to reliably determine whether overlapping pieces actually originate from the same genome (Maderet al., 2002).

For soil scientists, binning has come to be regarded as the "grand challenge" since soil samples include so many different types of organisms. And it looks like a team of scientists at Pacific Northwest National Laboratory under the direction of Dr. Janet Jansson could be about to take on the problem.

These researchers used a relatively unusual strategy to solve the overlap issue. Rather than attempting to enhance the computer program that aligns DNA sequences, they contemplated the potential outcomes of enhancing the DNA pieces themselves. The most advanced DNA sequencing technique could only generate fragments up to 250 units long until recently. Since there isn't much information in these brief sequences, there is a good risk that two sequences will appear to match but aren't really compatible.

Yet the most recent DNA sequencing technology, which was just revealed, is 40 times better than the next best technique for sequencing DNA fragments up to 10,000 units long (Maderet al., 2002).

5. MATERIALS AND METHODS

The researcher used a qualitative research method to carry out this study. Exploratory research is the main component of the qualitative method, which is modified to understand the motivations, viewpoints, theories, and views in order to address the study issue. This study relied on secondary source of data through the extensive review of literatures on Microbiomes in Agriculture.

6. RESULTS

After a comprehensive literature review on the benefit or the role of microbiomes in agriculture, the following findings were discovered and presented as conclusions.

1. For years, researchers have observed that, despite the presence of pathogens and conditions favorable to infection, some regions produce plants that are less susceptible to disease than other areas. The soils in these areas, it turns out, support plant health via the microbiome.

2. Comparative studies indicate that soil characteristics such as nutrient and mineral availability are major determinants of the root microbiome. Just as digestive tract microbes interact with the food consumed by vertebrates, the root microbiome mediates the soil-based diet of plants.
3. Sequencing data indicates a staggering taxonomic diversity of bacteria that a single, tiny root can host. Sequencing data also suggests that microbiomes act as a fertilizer to agricultural plants; thus boosting plant yields.
4. Some soil bacteria and fungi form relationships with plant roots that provide important nutrients like nitrogen or phosphorus. Fungi can colonize upper parts of plants and provide many benefits, including drought tolerance, heat tolerance, resistance to insects and resistance to plant diseases (Roossinck 2008).
5. Some examples of beneficial plant–microbe interactions include biological fixation by rhizobia, which establish a symbiosis with legumes and represent the basis of crop rotations including legumes contributing to the maintenance of soil fertility (Clay, 1988).
6. Although plant microbiomes have high potential to improve overall crop production worldwide, they will be particularly important for plant production under constrained conditions, where limited resources are available to irrigate, fertilize or treat plant diseases. This is the case in many parts of the world, where low input agriculture is common practice and improved germ plasm or agricultural amendments are hardly available. Making better use of plant microbiome functions will particularly support agricultural production under these conditions and foster the bio-economy of less developed countries providing microbial inoculants and establishing strain collections from local environments.

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