

A REVIEW ON SOIL AND PHYTOMICROBIOME FOR PLANT DISEASE MANAGEMENT

ABSTRACT:

Pests and pathogens significantly reduce crop yields and cost the global economy USD 540 billion annually. The phytomicrobiome is becoming a cornerstone of a comprehensive rethink of agricultural management practices, with microbiome-assisted plant breeding focusing on three principles: minimal soil disturbance, continuous soil cover with crops, cover crops, or a mulch of crop residues, and crop rotation. Plant diseases, phytomicrobiomes, and agricultural practices can all affect plant health. Conservational agricultural practices like minimum tillage and no-tillage have been implemented to reduce anthropogenic activity and preserve microbial diversity. Mulching is a common practice in agriculture to stop moisture loss, maintain soil temperature, control weed growth, and stop soil erosion. Monoculture farming is the practice of cultivating a single crop continuously over several growing seasons on the same field. Intercropping systems encourage the growth of beneficial fungi, such as mycorrhiza, endophytes, saprophytes, decomposers, and bioprotective fungi, and can benefit forest ecosystems by creating disease-suppressive soils. By combining conservation tillage and crop rotation, farmers can reduce disease pressure by disrupting the life cycles of soil-borne pathogens linked to particular crops or genotypes. Composted manure and plant residues can control plant pathogens like *Pythium*, but the effect is thought to be due to microbial competition or plant host resistance. Green manure can control plant diseases brought on by pathogens in *Rhizoctonia*, *Verticillium*, *Sclerotinia*, *Phytophthora*, *Pythium*, *Aphanomyces*, and *Macrophomina*, but manure-derived fertilizers may contain antibiotic resistance genes and mobilomes, which could pose risks to both human and animal health. Climate change impacts crop yields by reducing crop physiology and productivity, increasing pathogen diversity, and affecting pathogen genetic traits and speciation. The state of the environment and the availability of suitable hosts significantly impact the ability of these pathogens to survive and spread. However, PGPRs are unpredictable and soil warming can interfere with their effectiveness. Interdisciplinary collaborations between plant biologists, microbiologists, climatologists, and agronomists are required to create effective strategies to reduce the effects of climate change on plant health, crop production, and ecosystem stability.

Keywords: Phytomicrobiome, Soil, pests, fertilizers, pathogens, manures and plant residues

1. INTRODUCTION:

“Food security will become a top concern as the world population is expected to increase to 9.7 billion people by 2050 and 10.4 billion by 2100” [1]. “Food insecurity is a result of pests and plant-threatening pathogens that reduce crop yields by 20 to 40 percent globally and cost the global economy USD 540 billion annually” [2,3]. “The phytomicrobiome and its interactions with the environment are becoming a cornerstone of a comprehensive rethink of agricultural management practices as people become more aware of the harm that chemical pesticides cause to human, animal, and environmental health. Research on the microbiome has expanded quickly to include many fields, including medicine, food science, marine science, forestry, aquaculture, and agriculture. The term “microbiome” refers to a broad range of metabolites and structural components found in microorganisms, including proteins and their subunits. The microorganisms connected to any internal or external part of a plant, from the surface to the ground, as well as the outcomes of their activity, is referred to as the phytomicrobiome” [4]. “In situ manipulations of resident microorganisms through agronomic practices without the need for industrial-scale production are two broad phytomicrobiome-based approaches that may provide alternative strategies for plant disease suppression and management. Direct introduction of microorganisms as inoculants to ecosystems is another. It is based on three principles: (i) minimal soil disturbance or absence of tillage; (ii) continuous soil cover with crops, cover crops, or a mulch of crop residues; and (iii) crop rotation” [5]. “Conservation agriculture is a prime example of this strategy. While breeding is not a primary focus of conservation agriculture, which places more emphasis on

farming methods, it is important to note that microbiome-assisted plant breeding, which involves choosing plants based on their improved capacity to attract beneficial microorganisms, can be viewed as falling under the second category from a wider perspective” [6]. “The discovery of the precise genetic loci in charge of these traits has made this significantly more likely” [6]. “The review will focus on the implications for improving ecosystem resilience as it examines the difficulties and barriers involved with identifying and using the phytomicrobiome for plant disease management. It will also look at possible effects of climate change on phytomicrobiome functioning, including plant pathogens. The research field’s applicability spans forestry and landscape management in urban and non-urban settings, among other areas” [7].

2. Plant diseases, phytomicrobiomes and agricultural practices

“The phytomicrobiome, which signals the root and modifies its rhizosphere microbiome, is crucial for supporting soil health, plant health, and disease mitigation” [8]. Due to their antagonistic ability and use of management techniques like tillage and crop rotation, suppressive soils serve as the first line of defense against particular nematodes and pathogens [9]. With enrichment in *Pseudomonas* species, suppressiveness can be either general or specific, connected to different diseases. *Flavobacterium*, *Chryseobacterium*, *Burkholderia* and *Streptomyces* are some additional functional guilds accountable for the suppression of soil-specific microbial populations [9, 10]. Following World War II, agricultural practices like the use of pesticides and chemical fertilizers have seriously harmed the microbial life that inhabits arable soils. In order to improve soil fertility and plant pathogen suppression, sustainable agricultural practices modulate the phytomicrobiome for the entire agroecosystem.

2.1 Tillage

Tillage is a common management technique for soil distribution and weed containment. Conventional tillage (CT) can alter the chemical composition of soil and harm soil aggregates, which reduces the diversity and abundance of soil microbiota [11-15]. “Extended tillage led to nutrient-poor soils and wind-mediated soil erosion, as seen in the Dust Bowl events in the US and Canada. In order to reduce anthropogenic activity and preserve microbial diversity, conservational agricultural practices like minimum tillage (MT) and no-tillage (NT) have been implemented” [16,17]. “Positive outcomes from MT and NT include improved arbuscular mycorrhizal colonization, elevated soil carbon levels, and microbial activity. Increased total nitrogen and available nitrogen, as well as an expansion of bacterial communities involved in nitrogen cycling, have all been linked to long-term NT” [18,19]. “A destructive pathogen that causes *Fusarium* crown rot or *Fusarium* head blight, *Fusarium graminearum*, does not thrive as well in soils under MT because they have more diverse bacterial communities. Our ability to draw firm conclusions about disease management is, however, constrained by the incomplete evaluation of the direct impact on plant disease suppressiveness. In systems like *Pythium ultimum*—*Lepidium sativum* (cress) and *Fusarium graminearum*-*Triticum aestivum* (wheat), some studies have shown that long-term NT or continuous application of MT can enhance soil suppressiveness” [20,21]. “Other studies in the Netherlands’ arable rotation systems have found little effect of tillage practices on soil suppressiveness against *Rhizoctonia solani* and *Streptomyces scabies*. When used in conjunction with other conservational agronomic techniques, NT has frequently shown to effectively suppress diseases like take-all brought on by *Gaeumannomyces graminis* var. *Fusarium pseudograminearum* and *Fusarium* crown rot are brought on by *F. culmorum*” [22].

2.2 Mulching

In order to stop moisture loss, maintain soil temperature, control weed growth, and stop soil erosion, mulching is a common practice in agriculture. However, it has both beneficial and detrimental effects on the health of the soil and plants. Particularly in arid regions, inorganic mulching techniques like plastic film mulching (PFM) are preferred because they have immediate economic advantages like increased crop yield, improved crop quality, reduced water usage, and lower inputs. PFM also encourages soil solarization, increases microbial activity, and reduces soil-borne plant diseases. It has been demonstrated that long-term mulching improves plant root growth, increases Arbuscular Mycorrhizal Fungi (AMF) colonization, and increases bacterial diversity [23,24]. “However, it also has drawbacks, including increased carbon/nitrogen metabolism, accelerated biodegradation of soil organic matter (SOM), and the emergence of a new microbial community niche known as the “plastisphere,” which may house potential pathogenic organisms” [25,26-29]. “To address these issues, biodegradable plastic films such as those made of starch-based polymers have

been introduced. Mulches made of organic materials, such as leaves, straw, or wood chips, are an alternative to inorganic plastic mulches. By adding vital elements to the soil, such as carbon, nitrogen, and phosphorus, organic mulching enhances soil physical properties and nutrient uptake by plant roots” [30,31-34]. “Organic mulches can maintain a favorable soil environment for plant development and growth, which is less conducive to pathogen proliferation and disease establishment” [35,36]. “In the early stages of decomposition, plant residues in organic mulches readily decompose, enriching the soil with a nutrient pool and encouraging the activity of bacterial communities involved in decomposition, denitrification, and nitrification processes” [37,38]. They can control the temperature and moisture of the soil, lessen the arrival of aphids and fungus spores, and perhaps even lessen the prevalence of some diseases. However, depending on the specific type of mulch used, the efficacy of organic mulches may differ. It is essential to use disease-free mulch and to stay away from mulch made from sick plants if you want to stop the introduction or spread of plant pathogens. While mulching can have a number of advantages for enhancing plant and soil health, it's important to understand that other cropping techniques, like crop rotation and soil amendments, can also help prevent disease and promote nutrient-rich soil [35].

2.3 Polyculture vs Monoculture

Monocultural farming is the practice of cultivating a single crop continuously over several growing seasons on the same field. Field farming systems, horticulture, and agroforestry all struggle with this system. Farmers often substitute monocultures of high-value cash crops for intercropping and crop rotation as the world's population rises in an effort to increase yields, profits, and manageable costs [39,40]. But because long-term monocropping disturbs the soil's microecological environment, it has a negative impact on soil health and has led to an increase in plant diseases. A legacy of low Soil Organic Carbon (SOC) and nitrogen, as well as a proliferation of saprotrophic and pathogenic microbes, is left behind by the negative plant-soil feedback over time, ultimately resulting in decreased yields and nutrient-poor soils [41–41]. After a significant disease outbreak, persistent monoculture of susceptible hosts can occasionally paradoxically cause specific suppression against the causative agent. For instance, ongoing barley and wheat monoculture has resulted in a phenomenon known as "take-all decline" (TAD), in which the pathogen *Gaeumannomyces graminis* var. *tritici*. Due to the enrichment of various fluorescent *Pseudomonas* species, can be specifically suppressed. producing 2,4-diacetylphloroglucinol (DAPG), an antimicrobial. While the crops themselves can recruit and sustain such a symbiotic relationship, the antagonistic microorganism strains that are responsible for this suppressiveness must have a strong affinity for crop roots, facilitating their colonization. The development of diverse fungal taxa with various ecological functions, such as mycorrhiza, endophytes, saprophytes, decomposers, and bioprotective fungi, is facilitated by intercropping systems. For instance, intercropping maize with *Atractylodes lancea* acidified the rhizosphere soil and encouraged the accumulation of advantageous PGPR like *Streptomyces*, *Bradyrhizobium*, *Candidatus Solibacter*, *Gemmatirosa*, and *Pseudolabrys* [45]. Intercropping systems also benefit forest ecosystems by creating disease-suppressive soils when trees are planted in multiple species as opposed to monoculture [46,47]. Similar to intercropping, crop rotation can enhance soil health by fostering a variety of microbiomes and modifying the microbiome of the rhizosphere. Farmers can reduce disease pressure by disrupting the life cycles of soil-borne pathogens linked to particular crops or genotypes by combining conservation tillage and crop rotation. By using this method, the populations of pathogens that may have accumulated in the soil are reduced, lessening their negative effects on subsequent crops. The crops that are included in a rotation can affect how well it works to improve crop productivity and soil health. While grasses help build soil organic matter and improve soil structure, grain legumes can fix atmospheric nitrogen to increase soil fertility. Crop rotations must include non-host plants in order to minimize yield losses brought on by soil-borne diseases. Crop productivity is increased and soil health is optimized when at least three different crops are included in a crop rotation plan [48]. For improving overall soil health, crop rotation with carefully chosen crops that encourage the development of advantageous endophytic and rhizosphere microbial communities is essential. This strategy encourages the development of soils that are more nutrient-rich and have a phytomicrobiome that controls pathogens. To meet crop nutrient needs while maintaining microbial diversity through soil nutrient enrichment, fertilization becomes an additional strategy if monoculture is chosen over polyculture to produce higher yields and profits. To further improve plant defense against phytopathogens, various soil amendment techniques are also used.

2.4 Soil amendments

2.4.1 Fertilization

Traditional tillage methods and intensive monocropping techniques have depleted the soil, leaving it nutrient-poor with low levels of organic carbon and microbial diversity. In order to restore the nitrogen and phosphorus levels in agricultural fields and increase crop yields, fertilization is required. However, using chemical fertilizers continuously has drawbacks, including harming the health of the soil, endangering the lives of animals and people, and increasing air pollution [49,50]. Concerns about eutrophication are made worse by the fact that soil can leach over 50% of nitrogen and 90% of phosphorus into groundwater. The phyllosphere microbiome and root endophytes are not significantly affected by long-term fertilization, whereas the soil microbiome is more sensitive and protists are most affected [33,34]. Long-term fertilization resulted in a 30% reduction in phagotrophic protist diversity, which raises concerns about the potential loss of functionally significant microbial taxa as a result of abiotic changes brought on by fertilization. Copiotrophic bacteria outcompeted oligotrophs in fields that had both mineral and organic fertilizer applied, while organic fertilization increased the overall bacterial community's richness and diversity [51]. To maintain a balance that promotes plant health while lowering the risk of disease development, inorganic/mineral fertilizers should be closely monitored.

To comprehend the precise nutritional circumstances that affect pathogen growth, more study is required. Depending on their trophic modes, plant pathogens react to nitrogen fertilization differently. While facultative parasites exhibit a decrease in infection severity with high nitrogen supply, obligate parasites show an increase in infection severity with increased nitrogen supply. Due to their capacity to improve soil quality by boosting beneficial microbes and nutrient composition, biofertilizers like vermicompost are regarded as sustainable agricultural practices. Biocontrol agents like *Bacillus* spp. are added to biofertilizers made from mature compost. *Trichoderma* species, too, have been found to inhibit the *Fusarium* wilt illness. By directly suppressing pathogens or by altering the local microbial communities, the use of biofertilizers can improve soil health [52, 53, 54]. Organic fertilizers, such as compost, manure, or slaughterhouse waste, provide vital nutrients to plants and enhance soil aggregation, water retention, soil organic carbon, and overall soil health. Additionally, they support microbial diversity, richness, and activity in the soil as well as enzyme activity [13,55-57]. Plant pathogens like *Pythium* can be controlled by composted manure or plant residues, but their effects are thought to be due to microbial competition or plant host resistance. Vineyard pruning waste is one type of composting material that can have an impact on the microbial environment and activities, resulting in suppressive composts with higher relative abundances of Ascomycota and fungi from the genera *Fusarium* and *Zopfiella*. When peat is added to compost, the pH of the soil rises, suppressing pathogens like *Fusarium* that prefer acidic soils. Plant diseases brought on by pathogens in *Rhizoctonia*, *Verticillium*, *Sclerotinia*, *Phytophthora*, *Pythium*, *Aphanomyces*, and *Macrophomina* can be controlled by green manure, particularly from Brassica crops [7]. However, it is crucial to be aware that manure-derived fertilizers may contain antibiotic resistance genes (ARGs) and mobilomes, which could pose risks to both human and animal health, whereas plant residue-based fertilizers frequently contain plant pathogens. Manure is frequently preprocessed before being applied to land in order to reduce the spread of ARGs [58,59]. To address these worries and create plans for the safe and responsible application of organic fertilizers in agriculture, efforts should be made.

2.4.2 Chemical pesticides vs biofungicides

Farmers frequently use pesticides to manage and control pests and plant pathogens that can harm crops. Overuse, however, can result in resistance, decreased fertility, and negative effects on the environment, human health, and soil health. High-risk organisms like *B. cinerea* can arise as a result of overuse [60]. Pesticides can also negatively affect soil diversity, which leads to a loss of biodiversity and a decline in the health and productivity of ecosystems. Additionally, pesticides can harm beneficial microorganisms that are necessary for maintaining soil health and nutrient levels. A combination of sustainable farming methods and prudent pesticide use is crucial to reduce these potential disruptions. The resilience of the agroecosystem is increased by maintaining a diverse rhizosphere microbiome [61–63].

2.4.3 Biochar

A useful tool for soil improvement and carbon sequestration is biochar, a carbon-rich solid created through pyrolysis [64]. It is the perfect substrate for microbial growth due to its high porosity and substantial surface area. By modifying the bacterial and fungal communities in the soil, promoting beneficial bacteria, and suppressing plant pathogens, biochar reduces the negative plant-soil feedback.

By adding PGPR and fungi to the root microbiome, it also induces systemic resistance in plants, lowering their susceptibility to soil- and airborne pathogens [65,66,67-70]. However, given that the mechanisms affecting soil biota health are still not fully understood, worries about long-term safety and implications persist. Predicting biochar's long-term effects is difficult because the chemistry of the material can change depending on the feedstock and production conditions. By promoting long-term carbon sequestration and influencing greenhouse gas fluxes in soil, biochar can also help mitigate climate change by lowering greenhouse gas emissions. As a result, biochar is an effective tool for sustainable agriculture that addresses both environmental and food security concerns [71-73].

2.4.4 Chitin & Derivatives

Arthropod and fungal cell walls are primarily made of chitin, a polymer of N-acetylglucosamine. Its derivatives, such as chitosan and oligosaccharide derivatives are used in a variety of industrial, agricultural, and household applications [74]. Chitosan can trigger defense mechanisms in host plants and has broad-spectrum antimicrobial properties against viruses, bacteria, fungi, oomycetes, and nematodes. Chitosan can prevent the growth and development of post-harvest pathogenic fungi like *Colletotrichum*, *Botrytis cinerea*, and *Rhizopus stolonifer* as well as soil-borne pathogens like *Verticillium dahliae* [74]. Additionally, chitosan increases bacterial chitinase expression and regulates the abundance of Actinobacteria and Oxalobacteraceae members, which enhances the soil's ability to suppress plant pathogens. Potato wart disease, a fungus brought on by *Synchytrium endobioticum*, was shown to be suppressed by the addition of crab shell (23% chitin) to infected soil [75, 76]. In contrast to crop rotations, which showed a decrease in resting spore populations when intercropping potato with rye and sunflower, chitin-mediated management of potato wart disease was less successful.

2.4.5 Bentonite

In arid and semiarid areas, bentonite clay is used to improve the soil. It enhances plant growth and quality by increasing plant available water (PAW) by holding a significant amount of water within its crystals [77]. By creating the ideal moisture environment, bentonite also encourages microbial activity in the soil and the cycling of nutrients. By absorbing heavy metals like cadmium and lead from contaminated agricultural soils, it functions as an important tool for soil detoxification [78,79]. Through improved soil moisture retention and the formation of macroaggregates, bentonite also affects the fungal communities, which results in a decrease in the activity of phytopathogens like *Alternaria*, *Bipolaris*, *Fusarium*, *Leptosphaeria*, and *Microdochium*. This heightened competition from advantageous microorganisms may aid in the control of disease in the soil. To increase the chances of biocontrol agents like *Bacillus subtilis* surviving and functioning against particular plant pathogens like *Rhizoctonia solani*, bentonite has been used in recent biocontrol techniques [80].

2.5 Bio control agents

Biocontrol agents, which are primarily based on microbial inoculants, are a method for reducing pests and diseases through the use of living organisms or their derivatives. Through a variety of mechanisms, including competition with pathogens, antagonistic activity, inducing systemic resistance in plants, and/or direct lysis of the pathogenic organisms' cell walls, these agents aid in the alleviation of plant disease. Numerous microorganisms, including PGPR, endophytes, rhizosphere bacteria, and mycorrhizal fungi, are employed as biocontrol agents. While some biocontrol agents, like PGPR, may be generally advantageous for all plants, others may be host- or pathogen-specific [81, 82, 83]. To aid in the development of a healthy agroecosystem, researchers are investing in the engineering of phytomicrobiome. Selecting potential biocontrol agents may include additional criteria for resilience to climate change. Numerous agronomic techniques, including crop rotation, cover crops, natural selection, and organic mulching, have proven successful in enhancing soil health and disease resistance. The effectiveness of these techniques can, however, differ based on elements like soil types, climatic conditions, and crop genotypes. It is essential to investigate fresh and cutting-edge methods for managing plant diseases if we are to successfully address the issues brought on by climate change. Utilizing phytomicrobiome-based approaches, such as microbial inoculants and practices that take advantage of the complex interactions between plants and their microbial communities to promote disease suppression and improve crop resilience, is one promising area of research. To utilize the full potential of phytomicrobiome-based approaches, however, there are obstacles and pitfalls that must be overcome [84, 85].

3. Phytomicrobiome-Based Approaches- Challenges

Agronomic practices can have an impact on how well an agroecosystem functions, and the phytomicrobiome is essential for plant health. Agronomic adjustments can lessen the impact of plant diseases. Although successful examples demonstrate effectiveness in controlling plant diseases, obstacles such as conceptual, computational, and non-target effects still exist.

3.1 Conceptual Difficulties

A comprehensive comprehension of the interactions between plants and their microbiomes is necessary for the effective application of phytomicrobiome-based approaches. This includes having a thorough understanding of the life cycles, ecology, and etiology of the pathogens that cause plant diseases [86–90]. According to Bass et al., the pathobiome concept refers to the group of organisms associated with the host that have lower health status as a result of interactions between those organisms and the host. The Meloidogyne-based disease complex (MDCs), which causes severe yield loss in important food crops worldwide, is an example of such collaborative work. This complex results from the interaction of phytopathogenic fungi and root-knot nematodes (RKN). The pathobiome concept also covers tree diseases brought on by intricate host, microbiota, and insect interactions [91, 92]. For effective plant disease management strategies, it is essential to comprehend how the phytomicrobiome as a whole contributes to the initiation, promotion, or mitigation of disease development [93-95]. The following issues would need to be resolved in order to develop phytomicrobiome-based strategies for plant disease control: (i) Who are the members of the pathobiome and phytomicrobiome at taxonomic and functional levels? (ii) How do they interact with one another, the plant host, and their surroundings? What is the long-term effect on the development and spread of the disease? Prior to assessing the overall effect on disease incidence and severity, recent studies have concentrated on comparing the microbiota associated with symptomatic versus healthy tissues, suppressive versus conducive soils, and investigating the microbiota shift under various agronomic treatments and/or upon pathogen infection. There is growing interest in investigating the interactions between pathogenic agents and the local microbiota using co-occurrence network analysis based on metabarcoding data [96-98]. Positive correlations between a pathogenic taxon and other taxa may contribute to the development and spread of the disease, according to the widely accepted underlying theory, and can therefore be used to identify pathobiome members. Contrarily, negative correlations between taxa that contain a pathogen may imply antagonistic interactions and aid in the identification of potential biocontrol agents. Alternative biological explanations, such as a prey-predator model or taxon aggregation because of dispersal constraints or related niche requirements, may, however, account for positive or negative correlations [96-99].

3.2 Computational Difficulties

By offering more in-depth insights into microbial interactions, metabarcoding has revolutionized microbial interactions. To prevent misunderstandings and draw conclusions with the appropriate level of caution, it is crucial to be aware of the restrictions and limitations associated with this approach. Only genus- or species-level identification is currently possible using metabarcoding techniques like the 16S rRNA gene or Internal Transcribed Spacer (ITS) [100]. Since isolates from the same genus may be phytopathogens, non-pathogenic organisms, or even biocontrol agents, accurate taxonomic identification is essential for identifying pathogenic organisms. For some pathogens, additional barcodes have been created down to the species level, including those for the translated elongation factor (TEF-1), -tubulin, RNA polymerase II second largest subunit (RPB2), and cytochrome c oxidase subunits (COI), but a metabarcoding strategy aimed at these particular barcodes has not yet been created [101-104]. As shown in the study by Belair et al., taxonomic-level resolution can be evaluated by creating a phylogenetic tree based on Bayesian inferences. A multi-affiliation output is offered by the FROGS pipeline to take into account errors in taxonomic assignment. Finding taxa with notable differences in abundance between the treated and control conditions is another common goal. However, metabarcoding restricts the way that data can be expressed, making it compositional [105,106].

Since they are lost during sequencing, absolute abundances cannot be inferred from relative abundance. One could estimate the size of the overall population and then infer the population size of each taxon based on its proportion in order to access absolute abundances. It is possible to use traditional microbiological methods that count colony-forming units in Petri dishes, but only populations that are

viable and cultivable are taken into account. Traditional qPCR may not be as effective as digital droplet PCR, which does not require a calibration curve. Because taxa have different numbers of 16S rRNA and ITS gene copies, it still represents a significant bias [107].

Before extracting the DNA from samples, Tkacz et al. suggested a technique that corrects the initial microbial density by adding a synthetic spike. Due to the method's infancy and dependence on microbial eukaryotes, it is not yet widely used. Long-read and single-molecule sequencing methods, like Pacbio or Nanopore SMRT sequencing, may lessen PCR bias and improve taxonomic assignment accuracy. Understanding how microorganisms interact to start or stop pathogenesis and promote or mitigate disease progression is limited by co-occurrence networks. Recent advancements in metagenomics technology, including metagenomics, metatranscriptomics, metabolomic analysis, or metaproteomics, in conjunction with culture-dependent analysis (culturomics), may provide new insight into the underlying mechanisms of microbial interactions in the context of plant diseases. To better understand the underlying functional mechanisms of lesion formation, Broberg et al. compared the metagenome, metatranscriptome, and metaproteome of inner bark tissues in AOD symptomatic versus asymptomatic trees [108]. Using metabarcoding and shotgun metagenomics techniques, Gao et al. also carried out a thorough investigation comparing the taxonomic and functional profiles of microbial communities linked to Fusarium wilt-affected and healthy chili pepper plants.

Finally, although a simplified system lacking the full range of the phytomicrobiota, the use of synthetic communities may present important opportunities to establish causal relationships and advance our knowledge of the individual or collective role of microorganisms and their influence on plant phenotypes [109].

3. Evaluation of Side Effects

Plant disease-mitigating inoculants face difficulties adapting to production conditions and establishing a successful colony. Considerations like formulation, dosage adjustments, and strategic positioning of treatments become crucial factors to optimize colonization and disease protection efficacy [110]. While in situ manipulations of resident microorganisms through agronomic practices are unaffected by these issues, approaches involving the introduction of microbial inoculants are most affected [120].

Another issue is how introducing microorganisms will affect the ecosystem and how they will leave a legacy [111-114]. Numerous studies have demonstrated that the application of microbial inoculants significantly altered soil microbial communities when compared to control treatments, though the demonstration of their beneficial effects was not mentioned [112]. The degree to which these shifts, brought about by the introduction of inoculants, were linked to the agroecosystem's dysfunctional functioning is critical to understanding the risks associated with this type of practice.

Composting or adding manure are known to cause changes in the microbial communities and add a lot of microorganisms to the ecosystem. Except for cyanobacteria blooms, which are partly caused by excess nutrients from fertilizer runoff, particularly from farmers, these practices have never been linked to significant direct microbial invasions. After the introduction of microorganisms, no evidence of microbial invasions has been documented, though it may be challenging to find given how cryptic microbes are [115]. A scientific body of knowledge regarding microorganisms' safety as well as their capacity to produce potentially harmful secondary metabolites is necessary for the development of microbial inoculants. In order to successfully replace aflatoxin producers in maize fields or nut tree orchards, non-aflatoxin producing strains are being used in the US or some African nations. Aflatoxin restoration in non-toxigenic strains through mating has also been raised as a concern regarding the long-term impact of continuous application of biocontrol strains on the native population structure [116, 117, 118]. "Information on the mode of action, the potential for related species and strains to produce relevant metabolites or toxins, adverse effects seen in (eco)toxicity tests, and the use of population genomics to examine the impact of biocontrol strains on native populations should all be gathered in order to assess the risk". [121] The most recent consolidated version of European Regulation 1107/2009 calls for a number of protections before product approval, including microorganisms deposited at an internationally renowned culture collection, validated analytical techniques, and proven efficacy in guarding plants against specific pests or pathogens [119].

6. Conclusions

This review discusses a variety of phytomicrobiome research topics, including difficulties with standardization, translation, and side effects. The phytomicrobiome is essential for the health of ecosystems and soils, and it holds promise for reducing plant diseases and boosting resistance to climate change. It is difficult to identify and use the phytomicrobiome for disease management, and climate change may change how it interacts with plant pathogens, making disease management strategies more difficult. For forestry, agriculture, and urban landscaping, harnessing the phytomicrobiome has significant potential advantages. To comprehend the mechanisms underlying its effects and develop workable management strategies, more research is required.

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