

## Beneficial Soil Microbes in Sustainable Agriculture: Applications, Mechanisms, and Future Prospects

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**Abstract**—The beneficial soil microorganisms are crucial in sustainable agriculture in terms of increasing crop productivity, improving soil health, and decreasing reliance on synthetic agrochemicals. This broad survey is a review of the diversity of useful soil microbes such as plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi (AMF), actinomycetes, and cyanobacteria and their processes of action in nutrient cycling, growth promotion, biocontrol, and soil structuring. The use of them as biofertilizers and biopesticides has been promising, and the meta-analyses have reported that biomass gains 47 percent and phosphorus uptake gains 105 percent in plants inoculated with them (1). Nevertheless, issues such as sporadic field performance, competition, and native microbiomes, regulatory, and adoption limitations by farmers are still present. New technologies like metagenomics, artificial microbial communities, CRISPR-based genome editing, and Artificial Intelligence are changing how we can learn and exert control over soil microbial communities to their agricultural advantage. The review is based on the recent development between 2015-2025, addresses the existing limitations, and outlines the future research directions that could be microbiome engineering, integration of precision agriculture, and climate-smart uses. To effectively utilize the positive microbes in the soil, it is necessary to manage the complexity of the ecology, create powerful formulations, coordinate the regulatory systems, and arrange the work of multiple stakeholders to reach sustainable agricultural intensification in the changing climate.

**Keywords:** Plants growth promoting rhizobacteria, arbuscular mycorrhizal fungi, biofertilizers, sustainable agriculture, soil microbiome, metagenomics, synthetic communities, climate-smart agriculture, precision farming.

### 1. Introduction

#### 1.1 Agricultural problems in the world and the Soil microbiome.

The 21<sup>st</sup> century is a challenge to agriculture. By the year 2050, food consumption will be 70 percent higher than it will be needed to satisfy the demands of an increasing global population projected to be 9.7 billion (2). At the same time, decades of extensive agricultural activities based on the use of synthetic fertilizers and pesticides have had disastrous effects on the environment such as soil degradation, water contamination, greenhouse gas emissions, and the disappearance of biodiversity (3). Climate changes have also increased environmental pressures such as drought, flooding, heat waves and salinity which pose a threat to the world food security (3). The overlapping of these problems creates a necessity to radically change to the consideration of the

sustainable agricultural systems capable of supporting or even enhancing productivity, but also restoring ecosystem balance.

Microorganisms in soils are an untapped source of dealing with these challenges. Soil has a tremendous microbial diversity with approximately  $4-5 \times 10^3 - 10^{10}$  microbial cells in the world with  $10^8-10^9$  bacteria,  $10^7-10^8$  viruses and  $10^5-10^6$  fungal cells that are present in one gram of soil (4). Microorganisms make up about 17% of the total biomass of the world and serve important ecosystem roles such as cycling of nutrients, decomposing organic materials, and enhancing the health of plants (4). The main soil area directly affected by root secretions, the rhizosphere, has 10-100 fold greater abundance of microbes than bulk soil, containing  $10^8 - 10^{11}$  cultivable cells per gram of soil which is estimated to contain  $10^4$  microbial species (4). It is a thick microbial community that develops a complex relationship with plant roots which has a significant effect on plant growth, nutrition, and tolerance to stress.

## 1.2 Historical Development and Present State

The history of the discovery of localized beneficial interactions between plants and microbes can be traced as far back as 100 years ago when nitrogen fixing associations between *Rhizobium* and legumes were first observed, which culminated in the initial commercial production of bacterial inoculants by the 1890s. Nevertheless, the development of synthetic nitrogen fertilizers in the aftermath of the Haber-Bosch process eclipsed biological alternatives in most of the 20<sup>th</sup> century. The last two decades have been characterized by a resurgence of interest in useful soil microbes due to increasing awareness of the need to adopt sustainable agriculture practices, and revolutionary changes in molecular biology and technologies in sequencing (5).

Lederberg and McCray coined the term microbiome, which means the community of resident microorganisms that live within a particular host or environment, the structures and metabolites of such a community, and the environment (2001) (2). The next-generation sequencing (NGS) technologies developed since have continued to open up the global understanding of culturable and non-culturable soil microbes with remarkable diversity and functional complexity (3,5). The combination of high-throughput sequencing, cultivation-independent methods, and multi-omics has changed the nature of soil microbiology as a science of description to one of prediction and an engineering science.

## 1.3 Scope and Objectives of this Review

The review is a synthesis of recent developments in the beneficial soil microbe research and application with a focus on peer-reviewed sources published since 2015-2025. We discuss the variety of positive microorganisms, their action, recent application in sustainable agriculture, and new technologies that are bound to transform the area. We critically assess the obstacles that curtail large-scale implementation, which are ecological complexity, formulation concerns, regulatory obstacles and socioeconomic constraints. Last but not least, we pinpoint the research priorities in the future and comment on how microbiome based solutions can be integrated with precision agriculture, organic agriculture systems, and climate-smart farming programs. We aim to be the reference point of researchers, practitioners, and policymakers attempting to capitalize on soil microbiomes to create sustainable food production in the evolving world.

## 2. Biodiversity of Wanted Soil Microbes

The reason is that the growth of plants depends on the presence of beneficial rhizobacteria (PGPR). This is because growth of plants is pegged on the availability of useful rhizobacteria (PGPR).

The growth promoting rhizobacteria of plant growth consist of a heterogeneous group of free-living bacteria which colonize the roots of the plant and promote plant growth in a number of ways. Proteobacteria, Actinobacteria, and Bacteroidetes form the largest part of rhizosphere bacterial communities (73-80 of total bacterial reads) and bulk soil (46-56) (4). Genera of core microbiomes always linked to plant roots in various plant species are Sphingomonas, Burkholderia, Pseudomonas, Bradyrhizobium, Rhizobium, and Mesorhizobium (4).

The fixation of nitrogen by bacteria is an essential functional group. The most important, next to photosynthesis, biological process is biological nitrogen fixation that fixes 473 Tg of atmospheric N<sub>2</sub> per year (4). Symbiotic nitrogen fixers include Rhizobium, Bradyrhizobium, Mesorhizobium, and Sinorhizobium, which form specialized nodules on legume roots. These bacteria reduce atmospheric N<sub>2</sub> to plant-available ammonia within oxygen-protected nodular environments, enabling legumes to thrive without synthetic nitrogen inputs. Free-living and associative nitrogen fixers including Azotobacter, Azospirillum, Gluconacetobacter, Beijerinckia, Klebsiella, and Bacillus species colonize root surfaces or intercellular spaces of non-leguminous crops, contributing modest but agriculturally significant quantities of fixed nitrogen (6).

**Phosphate-solubilizing bacteria** enhance phosphorus availability through production of organic acids including gluconic, 2-ketogluconic, lactic, isobutyric, isovaleric, acetic, glycolic, oxalic, succinic, and malonic acids (6). These acids lower rhizosphere pH and chelate cations bound to phosphate, converting insoluble phosphate minerals into plant-available forms. Key phosphate-solubilizing genera include Arthrobacter, Azotobacter, Bacillus, Beijerinckia, Burkholderia, Pseudomonas, Rhizobium, and Pantoea (4,6).

**Siderophore-producing bacteria** synthesize low-molecular-weight iron chelators with extraordinarily high affinity for Fe<sup>3+</sup>, making iron bioavailable to plants while simultaneously limiting iron availability to phytopathogens. Over 500 siderophores have been identified, including pyoverdine, catechol, hydroxamate, carboxylate, and phenolate types (6). Major producers include Pseudomonas fluorescens, Azotobacter, Bacillus, Enterobacter, and Streptomyces species.

### 2.2 Mycorrhizal Fungi

Mycorrhizal fungi form mutualistic symbioses with approximately 80% of terrestrial plant species, dramatically expanding root system effective surface area and enhancing nutrient acquisition (7).

**Arbuscular mycorrhizal fungi (AMF)** belonging to the phylum Glomeromycota represent the most widespread and agriculturally important mycorrhizal type. AMF classification includes 3 classes, 5 orders, 14 families, 29 genera, and approximately 230 described species (7).

Key AMF genera include **Rhizophagus** (formerly Glomus), representing 16.39% of AMF abundance in field studies. Rhizophagus irregularis (formerly Glomus intraradices), particularly strain DAOM-197198, is the most extensively studied AMF species with a sequenced genome and widespread use in commercial inoculants (7). **Funneliformis mosseae** (formerly Glomus mosseae) is the most widely distributed AMF species globally, easily propagated in culture and commonly employed in agricultural research (7). Other important genera include Acaulospora (14.75% abundance), Diversispora (8.20%), Claroideoglomus, Gigaspora, and Scutellospora (7).

AMF form highly branched arbuscules within root cortical cells that serve as nutrient exchange interfaces. Extraradical mycelia extend beyond phosphorus depletion zones surrounding roots, reaching several centimeters into soil with network densities up to 25 m per gram of soil (7). This extensive hyphal network represents 20-80% of soil microbial biomass and contains 15% of soil organic carbon (7).

**Ectomycorrhizal fungi (EcM)** have an association with about 2 per cent of plant species, mostly woody plants such as Pinaceae, Fagaceae, Betulaceae and Salicaceae (7). In comparison to AMF, EcM never enter cell walls; on the contrary they create external mantles and Hartig nets between root cortical cells. EcM have a particularly vital role in agroforestry and in restoration of forests, and genera such as *Pisolithus*, *Suillus*, *Laccaria* and *Amanita* have been shown to confer increased nutrient uptake and stress tolerance in tree species.

### 2.3 Actinomycetes

Actinomycetes are gram-positive bacteria that grow in the form of branches in hyaliform filaments. These organisms are important in the process of organic matter decomposition especially the degradation of recalcitrant compounds such as cellulose, lignin and chitin. The most dominating actinomycetes in agricultural soil are streptomycetes which exist in large numbers in agricultural soils and generate various secondary metabolites that possess antibiotic properties and inhibit soil-based pathogens (8). The *Streptomyces rochei* and species of *Streptomyces* have been promising with particular applications in enriching the biomass and drought resistance of plants, and field trials have reported 20 percent biomass and chlorophyll content improvements in trees under water stress levels (8).

*Frankia* species create nitrogen fixing root nodules in actinorhizal plants such as *Alnus*, *Casuarina*, *Elaeagnus* and *Myrica*, which can be cultivated in the nitrogen-deficient grounds. Actinorhizal symbioses are especially useful in agro forestry, soil erosion control and land restoration.

### 2.4 Cyanobacteria and Algae

Cyanobacteria and algae also help in improving the soil fertility by fixing nitrogen, producing organic matter and also forming biological soil crust. *Nostoc*, *Anabaena*, *Cylindrospermum* and *Calothrix* species of free-living cyanobacteria fix atmospheric nitrogen which adds 20-30 kg N per hectare yearly in rice paddies (9). Complex associations of cyanobacteria, algae, lichens, mosses, and heterotrophic bacteria are known as biological soil crusts and they help stabilize soils, control erosion, increase soil fertility and biodiversity in dryland ecosystems (10). Such crusts are important especially in eroded soils where they trigger ecological succession and enable the formation of micro-ecosystems that support the growth of vascular plants.

## 3. Mechanisms of Action

**Nutrient cycling and acquisition:** This process plays a vital role in the photosynthetic pathway and involves the breakdown of molecules within the stroma to produce energy and drive photosynthesis. Nutrient Cycling and Acquisition This is a critical part of the photosynthetic pathway that includes the breakdown of molecules in the stroma to generate energy and trigger photosynthesis.

The agricultural contribution of microbes to plant nutrition is the most important nitrogen fixation. Nitrogenase enzyme complex involves reduction of  $N_2$  in the atmosphere producing ammonia ( $NH_3$ ) at massive energy expense (16 ATP molecules per mole  $N_2$  fixed). *Rhizobium-legume* symbiotic relationships have the capacity to repair 100-300 kg N per hectare in a year, which fully

meets the legumes nitrogen demands (6). Associative nitrogen fixers such as *Azospirillum* provide a contribution of 5-30 kg N per hectare to cereal crops which corresponds to 5-20 per cent of the total plant nitrogen under optimum conditions (6).

**Phosphorus solubilization and acquisition** occur through multiple mechanisms. Bacteria and fungi secrete organic acids that lower rhizosphere pH and chelate calcium, iron, and aluminum cations bound to phosphate, converting insoluble forms ( $\text{Ca}_3(\text{PO}_4)_2$ ,  $\text{AlPO}_4$ ,  $\text{FePO}_4$ ) to soluble phosphate ions. AMF provide particularly effective phosphorus acquisition, with hyphae exhibiting phosphorus uptake rates 6-fold higher than root hairs and transfer rates 10-fold faster (7). Meta-analyses demonstrate that AMF inoculation increases plant phosphorus concentration by 27% on average and total phosphorus uptake by 105% (1,7). AMF express specialized phosphate transporter genes (PT4 family) in arbuscules that facilitate high-efficiency phosphorus transfer from fungus to plant (7).

**Iron acquisition** through microbial siderophores overcomes iron limitation in alkaline and calcareous soils where iron solubility is extremely low. Pyoverdine produced by *Pseudomonas fluorescens* exhibits iron-binding constants exceeding  $10^{30} \text{ M}^{-1}$ , effectively scavenging iron from soil minerals and making it bioavailable to plants while simultaneously sequestering it away from phytopathogens lacking compatible siderophore receptors (6).

### 3.2 Plant Growth Promotion

**Phytohormone production** the rhizosphere microbes have a significant impact on the development of plants. The major auxin is indole-3-acetic acid (IAA) synthesized by a wide range of PGPR such as *Pseudomonas*, *Azospirillum*, *Bacillus*, *Rhizobium*, and *Bradyrhizobium* (4,6). IAA enhances cell elongation, lateral root formation and adventitious root growth which matastically enhances the surface area of the root to absorb water and nutrients. Gibberellins (GA 1, GA 3 and GA 4) stimulate the elongation of stems, germination of seeds and flowering. *Pseudomonas fluorescens* and *Rhizobium* species produce cytokinins which induce the division of cells and inhibit senescence (6).

ACC deaminase synthesis is a distinctive pathway of increasing the stress tolerance of plants. This enzyme splits 1-aminocyclopropane-1-carboxylate (ACC) the immediate precursor of ethylene hence reducing the level of ethylene in stressed plants (4,6). High levels of ethylene prevent root growth and hasten senescence. ACC deaminase-producing bacteria such as *Pseudomonas*, *Bacillus*, *Azospirillum* and *Burkholderia*, help plants to continue growing during drought, salinity, flood, and heavy metal stress conditions that increase the accumulation of ethylene (11).

Exopolysaccharide (EPS) production increases soil moisture retention on the surrounding of roots which is essential in drought tolerance. *Proteus penneri*, *Pseudomonas aeruginosa*, and *Alcaligenes faecalis* release EPS that form hydrated gels, which enhance water-holding capacity of soil, and prevent root drying (8). EPS is also involved in the formation of biofilms that help in the colonization of bacterial to root surfaces and in the survival of bacteria in soil.

### 3.3 Biocontrol and induced Resistance.

Production of antibiotics by useful microbes that grow in the soils inhibits the pathogens in the soils by direct antagonism. *Pseudomonas fluorescens* is a producer of 2,4-diacetylphloroglucinol (DAPG), pyrrolnitrin, and phenazine antifungal agents which have a broad spectrum of activity against *Fusarium*, *Pythium*, *Rhizoctonia* and other pathogens (6). The *Bacillus* species produce

lipopeptide antibiotics such as iturin, fengycin and surfactin which interfere with the membranes of the fungal and bacterial pathogens (6). Most of the naturally occurring antibiotics are produced by streptomyces species which offer effective control of pathogens in the rhizosphere (8).

Production of lytic enzymes directly causes degradation of cell walls of pathogens. Chitinases break down chitin polymers in the cell walls of fungi, and 6 7 3-glucanases break down the glucan components (6). These enzymes are synergistic in their effects, they cause a structural attack on the integrity of pathogen limited infection and disease.

Induced systemic resistance (ISR) is an indirect form of biocontrol in which helpful microbes condition plant immune systems to generate improved defense reaction(4, 6). The ISR-inducing bacteria trigger the jasmonic acid and ethylene signaling pathway that causes systemic expression of the defense-associated genes. Plants with ISR have less disease symptoms when later exposed to a variety of pathogens, which offers wide-spectrum protection against diseases but without the fitness costs of constitutive expression of defense.

The establishment of the pathogen is constrained by competition and exclusion in niches via fast colonization of the root surfaces and ingestion of root exudates. The beneficial colonizers occupy the sites of infection before the pathogen can do so and serve to deplete nutrients needed to grow the pathogen and hence exclude the pathogen in the rhizosphere.

### 3.4 Soil Structure Improvement

Aggregate formation and stabilization can greatly affect the soil physical aspects such as water infiltration, soil aeration, soil erosion potential and penetration by roots. AMF can play the role of soil aggregation in various ways (7). Extraradical hyphae entrap and emphasize soil particles, and the binding of hyphae happens at a fine scale as the diameter of hybala ranges between 2-20  $\mu\text{m}$  (7). GRSP secreted by AMF hyphae is a biological glue that causes aggregates of soil particles to be held together in a stable manner (7). GRSP elevates the hydrophobicity of soil and preserves the intact state of organic matter against microbial attacks, and the level of GRSP is highly associated with the stability of aggregates (the correlation coefficients are greater than 0.7) (7). AMF elevate the quantity of macroaggregates of large size, and decelerate the disaggregation of macroaggregates, and maximum effects are at about 5 months post-inoculation (7).

The rhizosphere bacteria increase the soil structural stability by improving the process of EPS production and bacterial aggregation. Biofilms form micro-ecosystems having different PH, nutrient, and water content that promote the surviveability and activity of bacteria. Biofilms in degraded soils trigger the recovery of soil structure by adhering to primary particles to form microaggregates which later combine to form macroaggregates (10).

Microbial transformations mediate the sequestration of carbon in the soil organic matter pools. The necromass of fungi, especially those that include melanin, is difficult to decompose and acts as a long-term storage of carbon (12). Warming and conservation agriculture practices enhance organic carbon in the soil by promoting fungal succession and carbon use efficiency by microbes (12). Field experiments that have been conducted over a period of 10 years indicate that the accrual of carbon in agricultural soils by microbes can increase significantly when both the management practices that promote fungal development and hygalae network protection are present (12).

#### 4. Sustainable Agriculture Sustainable Agriculture has a variety of applications in Sustainable Agriculture.

##### 4.1 Biofertilizers: Usability and Effectiveness.

Biofertilizers -microbial inoculants that include live microorganisms that improve the availability of nutrients, is a sustainable substitute to synthetic fertilizers. Syntheses of 187 publications indicate that AMF inoculation leads to 47, 16, 27, 67 and 105 percent whole-plant biomass, nitrogen concentration, phosphorus concentration, and nitrogen uptake and phosphorus uptake, respectively, over non-inoculated plants (1,7). Single AMF species yield significantly high effects than mixed species inoculation and have positive correlations with colonization rate (1,7).

The field trial findings show that there are variable yet usually significant yields advantages. *Rhizophagus irregularis* (700 spores per plant) inoculation of maize improved the harvest season shoot dry weight by 142 percent when the plants were placed under low phosphorus conditions (4.8 ppm Olsen P) (7). This was found to have improved root system architecture with more branched and denser roots and higher nutrient content of 20 elements that were measured (7). Different dual inoculation of maize grown in the field showed a tolerance to drought as biomass of the plants produced in field inoculated with *R. irregularis* and *Bacillus megaterium* raised by 42.7 percent and chlorophyll content by 13.4 percent over the non-inoculated plants in drought conditions (7).

More variable results are demonstrated in wheat field trials. In an organic field trial in Tuscany, Italy where 12 wheat genotypes were inoculated with *R. irregularis* (3,444 spores /m<sup>2</sup>) there was a statistically significant increase in root colonization, root biomass, shoot zinc concentration at the early growth stages and grain zinc concentration at harvest, though no significant increase in grain yield was observed (7). This indicates that inoculant efficacy is context-dependent and indicates that micronutrient biofortification can be a more dependable benefit than yield improvement in certain systems.

The most successful application of biofertilizers in the world is the rhizobium inoculants of legumes. When Rhizobium inoculants are properly developed, the legumes are able to fix 100-300 kg of N to the soil each year which will fully avoid the use of synthetic nitrogen fertilizers (6). Strain-legume specificity, large inoculum cell counts (10<sup>-9</sup> cells per seed), the use of suitable carrier formulations that are viable, and the prevention of molybdenum deficiency that inhibits nitrogenase activity are considered as success factors.

##### 4.2 The biocontrol in practice is biopesticides

Bio pesticides Microbial biopesticides are an alternative to synthetic pesticides that are environmentally benign and not based on toxic substances. *Trichoderma* species are the most commercially successful types of fungi biocontrol agents, where products are aimed at a range of soil pathogens, such as *Fusarium*, *Rhizoctonia*, *Pythium* and phytoplankton pathogens (13). *Trichoderma* survival strategies are mycoparasitism (direct parasitism of pathogen hyphae), the production of antibiotics, nutrient competition, and ISR (13). On-field trials show that there is a reduction of 22-52% in the severity of plant diseases when *Trichoderma* is applied (7).

The biopesticides based on *Bacillus* utilize lipopeptide antibiotics and ISR in controlling the foliar and soil diseases. *Bacillus subtilis* strain N11 is effective in the control of banana wilt disease, and it reduces the pathogen on a field-level (5). Several *Bacillus* species are also registered as

biopesticides in various countries around the world with wettable powders, water-soluble granules and liquid suspensions found under the formulations.

*Pseudomonas fluorescens* products attack *Pythium*, *Phytophthora*, and *Fusarium* with the production of DAPG and pyrrolnitrin. The field efficacy depends significantly on the soil type, temperature and the composition of indigenous microbial community, which points to the fact that ecologically-aware application strategies are needed (14).

The biopesticide market in the world is currently estimated to be USD 11.41 billion but is estimated to expand to USD 11.1 percent year-on-year (8). Nevertheless, the biopesticide market is now comprising a small percentage of 8 percent of the total crop protection market (65 billion dollars) so there is a lot of room to expand as regulations are further liberalized and products are formulated to be more powerful (14).

### 4.3 Soil Restoration and Rehabilitation

Others with beneficial microbes are regarded as game-changers in rehabilitation of impaired lands that have been subjected to physical soil erosion, chemical property degradation, and contamination (15). The restoration of nutrient cycling, the enhancement of soil structure, and the reestablishment of revegetation use PGPR, nitrogen-fixing bacteria and mycorrhizal fungi in the process of degraded soil rehabilitation (10,15).

Restoration research in Brazilian semiarid areas indicates that rehabilitated areas exhibit greater abundance of generalist microbes than do degraded areas dominated by specialists (16). The soils in the restoration have better percentages of Acidobacteria and Actinobacteria, more intricate bacterial association with mostly positive associations, and greater potential to cycle nutrients (16). These were associated with better vegetation cover and recovery of ecosystem functionality.

In Chinese Loess Plateau rehabilitation after 20 years of ecological restoration, Chinese Loess Plateau had more complex bacterial networks than agricultural soils with 77% more node and link number and enriched keystone taxa (17). Increased resilience of microbial networks was positively associated with soil PH recovery and nutrient recycles (17).

The heavy metal bioremediation takes advantage of the microbial metal sequestration, transformation, and immobilization, which help to diminish the phytotoxicity. Fungi enhance the effectiveness of the heavy metal removal in the contaminated soils and also promote the plant growth through the growth promoting process (15). Synergistic effects Co-inoculation of metal-tolerant PGPR and AMF has synergistic advantages in phytostabilization and phytoextraction technologies.

The establishment of biocrust boosts the restoration of degraded drylands. Biofuels on cyanobacteria stabilize soil surfaces, minimize soil erosion, fix nitrogen in the atmosphere, and provide microhabitat where vascular plant growth can occur (10). Application of biocrust is a low cost restoration approach to arid and semiarid degraded soils.

### 4.4 Modularity with the Contemporary Farming Systems

Precision agriculture integration involves the use of sensor technologies, data analytics and automated application systems while combining them with microbial inoculants. The cloud-based systems can be used to monitor soil biological characteristics, disease pressure, and also indicators of plant stress in real time and to use specific microbial applications where and when they are most

useful (8). The latest technologies of seed coating provide slow-release microbial inoculants, which enhance the success of establishment (8). Spores of microorganisms and especially *Bacillus* species are desirable as seed coats because they are more resistant to storage and the environment. The systems of organic farming are based on the beneficial microbes to use in lieu of the forbidden synthetic inputs. The abundance and activity of the soil microbes are enhanced in organic management, and meta-analyses demonstrate significant positive impacts on the carbon and nitrogen biomasses, phospholipid fatty acids, and enzyme activities (urease, protease, dehydrogenase) in the soil (18). Microbial community complexity and functional diversity are restored by 5-year changes between traditional and organic management (18). Organic systems are very resilient to drought stress since organic maize has yields of 6-7 tons per hectare under drought stress against 2.2 tons per hectare under conventional management (18).

FAO pegs their climate-smart agriculture (CSA) initiatives upon microbial technologies that will be integrated to increase productivity, adaptation, and mitigation (19). Mechanisms of microbial drought tolerance such as osmotic adjustment, EPS production, improved root architecture and activation of antioxidant defense allow crop to survive a more unpredictable precipitation pattern (19). Carbon sequestration through microbiomes is part of the greenhouse gas mitigation agenda (12). The Eastern Mediterranean region, which is a climate change hot-spot, has put much emphasis on developing microbial inoculant to aid the tolerance to drought and salinity (19).

Experiments run by the European Union EXCALIBUR project on tomatoes, apples, and strawberries proved that tomatoes, apples and strawberries grow well with bioinoculant carefully designed fertilizers that lead to a reduction in chemical fertilizers by 30-50 percent without impairing crop performance (8,12). Optimal outcomes were achieved in cases where microorganisms were obtained within the same geographical location as the location of application, and the significance of locally-adapted strains was realized (12).

## 5. Challenges and Limitations

### 5.1 Inconsistent Field Performance and Ecological Complexity

Although encouraging laboratory and greenhouse outcomes are obtained, microbial inoculants often do not produce such levels of benefits even in the field (14,20). Meta-analyses attest to the fact that field efficacy is not reliable limiting scale adoption (20). There are several reasons behind poor performance. Competition in native soil microbiome is a tough nut to crack as native microbes will normally outcompete introduced inoculants in conditions and nutrients (14,21). The bacteria diversity in soil limits the invasion of inoculants by competing with resources and niche occupancy, and communities of greater diversity are more resistant to inoculant colonization (21).

A recent set of meta-analyses assessing 114 studies revealed that native core microorganisms have a higher potential to promote plant growth compared to non-native inoculants, indicating that, locally-sourced ecologically-compatible isolates could be better than cosmopolitan commercial products (21). Inoculants are assimilated food to natives, and microbes growing in non-soil conditions die quickly after being emitted to the field soils (14). They might be required to undergo recurrent inoculation, yet the invasion of soil communities is exceptionally resistant to repeated applications (21).

The variability of the environment has severe effects on the performance of inoculants. Extremes of temperature, pH, moisture stress and salinity influence microbial survival, colonization and activity (20). The state of production (usually a liquid culture that is nutrient-rich and at the correct

temperature and pH) is incompatible with the conditions in the field, leading to physiological stress and death (20). Genotype of crops, the type of soil and the practices used to achieve agriculture are further complicated by context-related responses that make it challenging to predict the inoculant efficacy (20).

### 5.2 Barriers to Formulation, Quality Control and Commercialization

Failure to control quality is rife in the commercial microbial inoculant industry. An international assessment of 28 commercial AMF products of Australia, Europe, and North America, revealed that 84 per cent contained no viable propagules in sterilized soil, and that 1- of 25 products promoted plant biomass in non-sterilized field soil (7). The evaluation of 23 commercial products in a 2025 meta-analysis showed that the propagule counts reported and the viable spores were at most 100% different, and only 12% of commercial inoculants had viable mycorrhizal fungi (7). Contamination with pathogens such as *Oplidium* was found in several products and unknown synthetic fertilizers concealed in the actual benefits of microbes (7).

The problems of formulation are related to viability in storage, transportation and field use. Normal shelf-life of 6 months is 20-25C restricts distribution choice (20). Field efficiency decreases because of desiccation in storage. Although efficient, peat-based carriers are of concern to the environment. There are other carriers that need to be developed and proven. Multi-species inoculants have different stability needs, which makes optimization of the formulations challenging (20). Encapsulation technologies present opportunities though the consistency of quality at commercial scale is still a problem (20).

Scalability Production Scalabilities of obligate biotrophs such as AMF that cannot be cultured in the absence of host plants is an obstacle. It is costly, time consuming and can be contaminated (7,20). Even the inoculants that involve fermentation infrastructure have a problem in producing large quantities at a cost-effective manufacturing process without compromising the quality (20).

### 5.3 Barriers to Regulation, Economy and Adoption

The problem of regulatory fragmentation provides barriers to market access. The world has no internationally agreed regulatory framework with unequal legal definitions of biofertilizer, biostimulant, and other similar terms in different jurisdictions (22). European Union restricts the marketing of microbial inoculants to 4, which are *Rhizobium*, *Azotobacter*, *Azospirillum*, and mycorrhizal fungi (22). The regulation of the United States is divided between EPA (biopesticides), USDA-APHIS (interstate movement), and state agencies, and no particular federal framework of biofertilizers existed until recently (22). Registration entails a long period of field trial data lasting 2 or more years, toxicological, and environmental safety studies, which are lengthy and costly processes (22).

There are high barriers to adopting farmer. According to 48-52% of the farmers, cost issues and uncertainty of the payoff would be the largest obstacles to adoption (23). The confusion in the market caused by more than 400 companies supplying biologicals and little information on how to choose it prevents the decision-making process (23). In 2022, only 23% North American farmers applied biostimulants in most farms, and 55, 33, and 12 percent were satisfied, uncertain, and dissatisfied, respectively (23). Poor knowledge regarding awareness of benefits, poor training programs and superficial knowledge regarding the process of handling and application also limit adoption (20,23).



The distribution infrastructure, which is meant to support chemical components that are stable, does not suit living organisms in need of certain temperature and humidity levels. Remote rural areas have problems with availability and the small-scale farmers can hardly access good products (20). The complexity of the application and the lack of integration with the current gear are also an obstacle (23).

## 6. Prospects and Future Research.

### 6.1 omics Technologies and Systems Biology Approaches

Metagenomics offers an integrated evaluation of the genetic potential of the soil microbes that have not to be cultivated. The approach to shotgun metagenomics and metabolomics allows seeing the nature of structure-function relationships involved in nutrient cycling and soil health (24). Knowledge-based microbiome engineering using network analysis of metagenomic data has shown itself useful, with 2022 researchers using network analysis to predict selective activation or suppression of particular microbial groups with specific metabolites (dopamine, vitamin B12) to suppress pathogen growth (24). Multi-omics integration characterizes the both structures and functions, and demonstrates the mechanisms of the interaction between the microbes and the metabolites in complex soil systems (24).

Metatranscriptomics reveals dynamic expression of functional genes and in this way the real time information about the activities that are going on in the metabolism of the microbial communities is given (25). RNA sequencing, in contrast to DNA-based metagenomics, which measures possible functions, provides information on which genes are expressed in particular circumstances (25). Metatranscriptomics has identified changes in rhizosphere microbiomes at the level of the kingdoms in response to rising CO<sub>2</sub> levels, which the DNA-based methods had failed to identify (25). Its uses include the observation of the differences between temperatures on polymer hydrolysis, syntrophic oxidation, and methanogenesis in rice field soils, which gives the system-level insights into microbial food webs (25).

Metaproteomics is a response that uses the identification of proteins in the active state to clarify the current genetic possibilities to actual function. The two-dimensional liquid chromatography greatly enhances the depth of coverage in the soil metaproteomes, which facilitates the determination of the main functional proteins involved in the nutrient conversion processes and stresses response (24). Activity-based protein profiling provides innovative methods of addressing the plant-microbe interaction on a molecular scale.

### 6.2 Engineering Synthetic Microbial Community and Microbiome.

Genome-scale metabolic model-based synthetic microbial communities (SynComs) that are selected based on functional traits are more reliable in the field compared to single-strain inoculants (26,27). Recent research utilised multi-genome metabolic modelling of 270 metagenome-assembled genomes to design minimal communities (MinCom) containing essential microbial metabolic compounds and plant-relevant compounds (26). This has been a focused strategy that allows rational design and not trial-and-error.

Principles of successful SynComs are: (1) compatible microorganisms with proven colonization properties are chosen; (2) metabolic complementation that enables efficient use of resources without ruinous competition; (3) preservation of community diversity with time despite relative differences in growth rates; and (4) such synergistic interactions as cross-feeding and cross-



protection (26,27). Bacteriocin and quorum sensing system-based automated design produces stable steady states of cross-protection mutualism, whereby density of one subpopulation prevents self-limitation of others (27).

The best examples of these successful applications have been 16-member tunable synthetic communities of switchgrass that have gained maximum robustness and diversity and synthetic communities that have been engineered to enhance soybean tolerance to drought through functional assembly of root-associated microbial consortia (27). Isolating, engineering, and reintroducing individual microbes into the system are bottom-up strategies complemented with top-down strategies employing synthetic ecology and horizontal gene transfer to modify microbiomes in vivo (28).

### 6.3 CRISPR and Genome Editing to Improve Functionality

Applications of CRISPR/Cas9 in systems of plants support disease resistance, efficiency of nutrient use and compatibility with microbiomes (29,30). Susceptibility genes (TaPDIL5, OsDjA2, OsERF in wheat and rice) are knocked out to confer widespread resistance to the virus (29). Alteration of carotenoid cleavage dioxygenase genes in sorghum alters the strigolactone production, which influences the root exudate composition and the formation of microbiomes in the rhizosphere (29). New systems such as LrCas9 of probiotic *Lactobacillus rhamnosus* could provide effective plant genome engineering systems with a prospect of higher regard of genome-edited crops (30).

Use in beneficial microbes Applications of CRISPR interference (CRISPRi) and activation (CRISPRa) as transcriptional regulators do not involve genome editing, and dCas9 fused to transcription activators can activate gene expression 3-fold, with which growth-promoting traits can be enhanced (30). Base editing (cytosine and adenine base editors) permits specific nucleotide transitions, whereas prime editing that entails the merge of nCas9 and reverse transcriptase permits insertion, deletion and base changes without the formation of two strands (30).

The biocontainment measures to ensure biosafety are that the field release of CRISPR-modified microbes should be robust. There are several layers of containment in kill switches, auxotrophy systems and synthetic protein designs (30). Nonetheless, the regulatory systems of genetically modified microorganism are still scattered in the world and might hamper its application (22).

### 6.4 Applications of Artificial Intelligence and Machine Learning

Machine learning models forecast soil health and crop productivity based on the microbiome at the cost of a steadily growing accuracy. Random Forest and Support Vector Machine models can make 12 predictions of soil health metrics based on 16S rRNA amplicon data with Kappa of approximately 0.65 when categorical and R<sup>2</sup> of approximately 0.8 when numeric metrics are used (31). Biological ratings of health are more predictable than chemical or physical ratings and this indicates that microbial communities are closely coupled with the biological functions of the soil (31). Predictive value Taxa have distinct ecological relationships such as Pyrinomonadaceae, Nitrososphaeraceae, and Candidatus Udaeobacter (31).

The accuracy of Drought stress detection with the help of random forest classifier on bacterial microbiome is 92.3% accuracy at genus level which shows generalization ability with various plant species (32). SHAP (Shapley Additive Explanation) values can be used to gain interpretable feature importance, i.e., particular taxa whose abundances can be used to predict stress status (32).

This allows creation of microbiome-based diagnostic instruments to be used in the precision agriculture.

Optimization of agricultural practice using machine learning shows that fertility source, tillage and cover cropping affect the assembly of microbes at various depths in soil (33). The combination of AI and multi-omics data makes it faster to select a strain, predicts the phenotype of plants, and designs a consortium to meet the requirements of a particular application (31,32,33). The next generation forest artificially intelligent soil quality indices that include land management approaches, genomic characteristics, vegetation cover, and soil variables will transform soil health surveillance and management (33).

## 7. Conclusion

Useful soil microorganisms are invaluable partners to a sustainable agricultural practice in the 21<sup>st</sup> century. The remarkable plurality of the PGPR, mycorrhizal fungi, actinomycetes, and other useful microorganisms offers versatile solutions on how to improve crop nutrition, growth, disease suppression and soil physical effects. Meta-analyses attest to significant advantages when the ecological conditions are suitable to the establishment of inoculants and biomass increases of about 50% and nutrient uptake increases by two using the responsive systems. Experiments in biofertilizer use in legumes and new achievements in the use of mycorrhizal inoculants, Trichoderma Biopesticides, and multi-strain consortia show good feasibility.

Nevertheless, the potential of soil microbiomes needs to be achieved despite significant obstacles. Limited adoption is due to inconsistent performance of the field based on the complicated ecological interactions with the native microbiomes, variability of the environment and limitations in formulation. The failure of quality controls in commercial products, fragmentation of regulations through jurisdictions and the lack of awareness in farmers are an obstacle to the development of the markets. All these issues require multidisciplinary solutions that combine the basic microbial ecology, formulation science, regulatory harmonization and proactive involvement of the farmers.

The combination of revolutionary technologies provides unprecedented opportunities. The microbial community structure, function and dynamics are being revealed at a molecular scale through metagenomics, metatranscriptomics and metaproteomics. Synthetic biology facilitates logical design of microbial consortia having predictable, consistent functions. CRISPR genome editing offers accurate methods of refining useful attributes without jeopardizing biosafety. Machine learning and artificial intelligence leverage predictive insights on complex multi-omics datasets to have strain selection and optimization of application faster. These technologies together with precision agriculture applications, climate-smart applications and organic farming systems are set to change microbiome-based solutions to be used in niche applications to mainstream agriculture practice.

The way ahead needs further investment in basic research of the explanation of the rules of microbiome assembly, regulation of interactions between plants and microbes, and the mechanisms of variability of field performance. It is necessary to develop strong formulations whose shelf-life is long, the choice of the strain based on the region with respect to the compatibility with the native microbiome, and long-term monitoring in the field. Standardized international regulatory frameworks with a balanced promotion of innovations and environmental protection, farmer training programs with clear value propositions, and collaboration between the

state and the business sector, which enables the transfer of technology, will speed up the process of adoption. With the help of exploiting positive soil microbes using technology-based and ecologically-inspired strategies, agriculture is able to shift to regenerative systems that recover soil health, make the climate more resilient, and provide food to a growing world population.

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