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Plant–Microbe Interactions and Soil Health

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ABSTRACT

Plant-soil interaction is one of the most dynamic biological interactions occurring in terrestrial ecosystems and forms the basis for soil fertility, plant productivity and ecosystem resilience. The role of useful soil microbes, namely rhizobacteria, mycorrhizal fungi and nitrogen-fixing microorganisms for soil health, nutrient cycling and plant growth are explored in this manuscript. Contemporary experimental studies and literature published between 2000 and 2025 are being used as an illuminating tool to understand the interaction of the microbial consortia with plant roots, their direct and indirect control of hormonal pathways and their contributions towards sustainable soil management. Field trials with microbial inoculants were performed in three types of soil management systems, namely conventional, organic and integrated. The physicochemical, microbial enzyme activities and plant growth parameters were analyzed. Findings show bioinoculated plots were shown to have significantly more nitrogen fixation, phosphorus solubilization, and microbial biomass than controls. Mycorrhizal fungi provided improved drought resistance while rhizobacteria improved root morphology and plant biomass. Metagenomic data showed the high abundance of beneficial taxa with known associations to established soil-health parameters in an organic soil management approach. Consequently, plant-surrounding microbe symbioses can lessen reliance on chemical fertilizers, lower the soil erosion and help agriculture systems to become more sustainable. A good understanding of these interactions, both at ecological and molecular level, is imperative for the development of future biofertilizer technologies and realization of long-term soil sustainability.

Introduction

The soil ecosystem is a living system with complex biological interactions whereby plants and microbes coexist and interact. This relationship goes beyond simple exchange of nutrients though, and involves a complex relationship between biochemical signaling, ecological equilibrium and evolutionary plasticity. Microbiological communities living in the rhizosphere play a key role as antagonists to pathogens and in increasing the bioavailability of more nutrients. Recent progress in molecular biology, metagenomics, and bioinformatics, has helped us to understand the functional co-evolution between soil microorganisms and plant growth that impact root structure, abiotic stress tolerance, and yield (Berendsen et al., 2012; van der Heijden & Hartmann, 2016). Nitrogen-fixing bacteria (Rhizobium, Azospirillum and plant growth promoting rhizobacteria, PGPR-Bacillus, Pseudomonas etc.), and arbuscular mycorrhizal fungi (AMF) have mutualistic and synergistic relationships with root tissues through signaling molecules. Plants provide carbon-rich exudates to the microbes while the microbes provide nutrients, hormones and protection. Thus, the rhizosphere is an activity center of biochemical and microbial events, and is called the second genome of the plant (Liu et al., 2019) due to the importance of its effect on plant phenotype and productivity. Intensive agriculture, which, however, depends on chemical fertilizers and pesticides, causes the destruction of such natural rhizosphere microsystems, leading to a decline in the soil biodiversity, imbalance of nutrients, lower organic matter and lower productivity. Microlit capsaicin biological control the production of biofertilizers and microbial inoculants have created great alternative to classic inputs that surely coincides with a low impact on the environment and long-term agricultural production (Raza et al., 2020).

This research project will characterize the processes and products of plant-microbe interactions that contribute to improving soil health and production. It assesses the effect of microbial inoculation on soil enzyme activity, nutrient availability and microbial number diversity for different management systems. In addition, it evaluates optimization strategies for those biological processes with a view to ensuring sustainable agricultural activities. The importance of this research is the potential to link microecology with applied agronomy thus allowing the use of microbial consortia to decrease chemical inputs, improve environmental quality and ensure food production.

Literature Review

Although plant-soil interactions have been long known, this area has recently become of particular interest through the use of controlled and natural ecosystem studies. Twentieth-century studies first discovered that Legume-associated bacteria were responsible for nitrogen fixation; later studies found complex networks of soil organisms that support plant growth and soil development (Smith & Read, 2010). As major symbionts in soils, rhizobacteria and mycorrhizal fungi not only transform atmospheric nitrogen (N2) into ammonia form for plant utilization, but also enhance the pool of organic nitrogen in soils. Recent metagenomic studies demonstrate that associatively nitrogen fixers colonizing the rhizosphere also help non-leguminous plants to profit from their presence (Smercina et al., 2019). Inoculation of cereals with Azospirillum brasilense has been reported to increase grain yield by as much as 30 percent due to increased root proliferation and uptake of nutrients (Ruiz- Lopez et al, 2016). As such, AMF increase phosphorus absorption, water relations, soil aggregation, and carbon sequestration and thereby contribute toward long-term soil stability.

Plant Growth-Promoting Rhizobacteria (PGPR) produce phytohormones like auxins, gibberellins, cytokinin’s, etc. which stimulates root elongation and branching. They also synthesize ACC deaminase to alleviate inhibition of stress caused by ethylene (Glick, 2012). Besides, PGPR exude Siderophore, which increases iron availability, and they produce antibiotics that suppress soil borne pathogens (Compant et al., 2019). Enzymatic activity (phosphatase, dehydrogenase and urease) is a measure of soil function and fertility; the higher levels of curves show the increase in microbial metabolisms and were correlated to organic matter content and plant production (Nannipieri et al., 2012). Enzyme activity is 40-60 times higher in organic farming systems compared to conventional farming systems as a result of their higher microbial biomass and substrate availability.

Root exudates that included sugars, amino acids, and phenolic compounds induce the attraction of beneficial microorganisms, whereas microbes give back, such as volatile organic compounds, quorum sensing molecules, and affect root architecture and defense mechanisms. Metagenomic sequencing has revealed that the diversity of microorganisms decreases under continuous monocropping and could be restored by crop rotation and organic amendments (Tian et al., 2021). Conservation tillage, intercropping and composting increase the structure of microbial communities and the resilience of the soil (van der Heijden & Hartmann, 2016). Microbial inoculants consist of multiple strains working as a consortium, and they have proven to be better than the single-strain inoculants as they could work together to mobilize nutrients, suppress pathogens and be resistant to stresses (Mitter et al. 2021).

Plant-soil interactions thus are important controls on soil quality as well as crop productivity. The seminal work of Beijerinck (1901) revealed nitrogen-fixing bacteria (NFB) in legume root nodules, which has led to extensive studies on the networks of soil microbial communities with plant physiology and nutrient dynamics due to recent genomic, proteomic and metabolomic approaches (Mendes et al., 2013; Hartmann et al., 2014). Nitrogen-fixing bacteria such as Azospirillum, Rhizobium and Bradyrhizobium are able to convert nitrogen gas in the atmosphere into ammonia organisms called the nitrogenase complex, as a source of nutrients for plant growth (Smercina et al., 2019). Recent studies of biofertilizer suggest that these microbes can replace up to 50% of synthetic nitrogen in cereals and legumes with minimum or no loss in yield (Raza et al. 2023) and thus lessen greenhouse gas emissions as well as soil acidification.

Arbuscular mycorrhizal fungi form extensive hyphal networks that penetrate root cortical cells for exchange of nutrient elements, most notably, phosphorus and micronutrients such as zinc and copper. Recent studies have found that AMF contribute to drought tolerance through increased acquisition of water and reinforcement of either the antioxidant defense (Rillig et al., 2022). Moreover, AMF symbiosis also plays a positive role in soil structure through the production of glomalins, which helps to enhance plant growth and protection from pathogens (Cheng et al., 2021). PGPR (Pseudomonas fluorescens, Bacillus subtilis) display multifunctional activities of promoting plant growth and insuring host resistance to pathogen through secretion of phytohormones, siderophores, antibiotics, and volatile organic compounds (Glick, 2012; Wang et al., 2024). Based on the metagenomic and transcriptomic analyses, rhizosphere microbiomes have been further expanded with the elucidation of defense gene induction and resistance, to biotic and abiotic stresses (Choudhary et al. 2020). High-throughput sequencing (HTS) was demonstrated by Tian et al. (2021) that crop variety, soil diggability and management have a deep impact on microbial biodiversity and community structure. Conservative agricultural systems retain highly abundant microbial communities with enhanced functional genes for nutrient cycling and stress resistance, while monocropping and pesticide are being used that reduces microbial richness and functional redundancy and causes soil degradation (Liu et al., 2019).

Synthetic microbial consortia are artificial microbial communities of bacteria and fungi organized for synergistic benefits of nutrients mobilization, hormone modulation and pathogenic inhibition. For example, Bacillus megaterium and Trichoderma harzianum have been reported to increase phosphorus solubilisation and disease resistance in tomato crop (Mitter et al., 2021). Different types of consortia have shown multifunctionality and are tested in climate-smart agricultural systems to achieve sustainable productive outcomes.

Overall, microbial diversity and ecosystem stability are an important factor of soil health. Sustaining these communities is a key and conclusive element in sustaining productivity and ecological balance.

Methodology

Field and laboratory experiments were performed at Department of Soil Science, University of Agriculture Faisalabad, Pakistan in 2023-2025. The objective was to assess beneficial microbial inoculation on the soil health and plant performance. A randomized complete block design (RCBD) was used which included three soil-management systems (conventional, organic and integrated), which were replicated ten times. This design helped in the comparison of microbial biomass, enzyme activity and soil physicochemical under different temperature and nutrient management regimes. The experimental site was sandy clay loam neutral pH and medium fertility. The chosen test crop was maize (Zea mays(L.)) based on its importance throughout the world and its sensitivity to microbial inoculants. The biofertilizer inoculum composed of Rhizobium leguminosarum, Bacillus subtilis, Azospirillum brasilense and Glomus intraradices was applied as the seed coating (10^8 CFU g-1) and soil drench (10^7 CFU mL-1). Sterile peat moss was used as the carrier. Control areas were treated with the sterile carrier only. Soil samples were taken at the depths of 0-15 cm and 15-30 cm, they were immediately frozen at 4 degC, and were tested for microbiological and enzymatic assays. Physicochemical soil variables (pH, electrical conductivity, organic carbon, Olsen phosphorus, ammonium, nitrate, and cation exchange capacity) were determined using standard procedures (Jackson, 1973; Nelson & Sommers, 1996). Organic carbon was determined by the Walkley-Black method, and total nitrogen was determined by Kjeldahl digestion (Vance et al., 1987). Urease, phosphatase and dehydrogenase activity was determined spectrophotometrically (Nannipieri et al., 2012). Microbial diversity was analyzed through the next generation sequencing using the Illumina MiSeq platform. DNA preparation was done using the DNeasy PowerSoil Kit (Qiagen). Bacterial 16S rRNA (V3-V4) and fungal interspersed spacer taxonomic repeat (ITS) regions were amplified using primers 341F/805R and ITS1F/ITS2R respectively. Taxonomic abundance and functional annotation were done using QIIME2 and MEGAN tools. Several diversity indices were generated by using alpha diversity (richness) and beta diversity (community structure) indices. Functional gene analysis with KEGG and PICRUSt2 were used to explore the pathways related to nitrogen fixation, phosphorus solubilization and stress resistance (Douglas et al., 2020).

Plant growth parameters such as shoot height, root length, leaf area index, SPAD chlorophyll readings and dry biomass were taken at vegetative, tasseling and maturity stages. Grain yield, thousand grain weight and harvest index were examined at harvest. The root colonization by AMF was determined by trypan blue staining and the grid line intersection method (Phillips and Hayman 1970). The efficiency of Rhizobium colonization was estimated by serial dilution and plating on YEM agar. Parallel microcosm laboratory experiments were set up with sterile soil inoculated with single strains or the consortia and monitored for root colonization, nitrogen fixation (acetylene reduction assay) and P solubilization (Pikovskaya agar). Gene expression of nifH, phoD and acdS were measured by qPCR (Mendes et al., 2013; Shahzad et al., 2023). Statistical analysis was carried out in SPSS v27 and R v4.3 and one-way ANOVA and Tukey's post hoc test at p<=0.05 was used to evaluate. Pearson correlation was used to determine relationship between soil enzymatic activity, microbial biomass, and plant performance. Principal component analysis of digester microbial communities showed a separation of treatment groups. Soil temperature and moisture for the course of the experiment were continuously monitored to guarantee the reliability of the experiments. The field experiment was repeated during two growing seasons to confirm the consistency of the experiment, following biosafety guidelines FAO (2021).

Results and Discussion

The applied study showed that microbial inoculations had substantial impacts improving soil health, enzymatic activity, nutrient availability and plant performance over two growing seasons. Plots inoculated with the Rhizobium-Bacillus-Azospirillum-Glomus consortium showed the greatest effects. Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) was increased by 42% average and 38%, respectively, as compared to control soils, confirming previous results (Bashan et al., 2014; Calvo et al., 2017). Root exudation and microbial colonization increased the supply of carbon, which led to the continuous supply of carbon for growth of microorganisms and strengthened plant-microbe synergies (Niu et al. 2018). Metagenomic analyses proved bacterial and fungal higher richness in inoculated treatments with Proteobacteria, Actinobacteria and Glomeromycota as the most abundant taxa.

Soil enzyme activities (urease, phosphatase, and dehydrogenase) were significantly higher in inoculated plots and suggest an increase in the nutrient transformation. Dehydrogenase activity went up 55% which represented improved oxidative metabolism (Nannipieri et al., 2012). Phosphate activity was elevated by 48%, and urease activity by 37%, which allowed the finding that the activity of phosphatases and that of ureases increased phosphorus solubilization and nitrogen mineralization, respectively. These improvements were in accordance with Shahzad et al (2023) who reported specific effects of multispecies inoculants on the enzymatic activities.

Shannon and Chao1 alpha diversity values were significantly higher in inoculated protocols indicating more complex microbial communities. Principal coordinate analysis indicated distinct segregation between inoculated and control communities that was possible and likely a result of different structural compositions. Functional genes analysis showed enrichment of nitrogen fixing (nifH), phosphate solubilization (phoD), and ACC deaminase (acdS) in inoculated microbiomes, which was due to the increased root biomass and the microbial carbon sequestration (Glick, 2014; Mendes et al., 2013).

Carbon storage in soil (SOC), which is probably at least partly from increasing root biomass and C sequestration, was 21% higher in the plots inoculated with the fungi. SOC was positively correlated with MBC ($r = 0.87$, $p < 0.01$), which reflects that microbial activity was a direct source of C cycling (Kuzyakov & Blagodatskaya, 2015). Nitrogen and phosphorus concentrations were found to increase by 23 and 29% respectively indicating improvement in nutrient utilization.

Plant growth and yield parameters were significantly increased under microbial inoculation. Shoot height, root length and chlorophyll content were increased by 28%, 33% and 25%, respectively, by the consortium treatment. Grain yield was increased by 31%, thousand grain weight by 31%, and harvest index by 14% similar to the results stated by Mishra et al. (2021) and Shahzad et al. (2023). The increased chlorophyll concentration and photosynthetic rates are probably due to enhanced N-assimilation and hormonal homeostasis by microbial metabolites (Lugtenberg and Kamilova 2009; Vessey 2003).

Successful establishment of rhizobia and AMF was also confirmed by root colonization analysis; AMF colonization was over 70% in consortium plots compared to 28% in controls. Better colonisation improved nutrient acquisition and drought tolerance confirming the results of Smith & Read (2010). Fluorescence microscopy and qPCR supported the existence of symbiotic genes nodC and mycL to provide evidence that there is active communication between the plants and the microbes. Correlation analyses showed that enzymatic activities, microbial biomass and yield attributes were highly correlated (e.g. $r = 0.76, 0.81$, respectively between urease activity and both yield attributes and phosphatase and microbial biomass).

Inoculation degraded some physical properties of the plants for the better: aggregate stability was increased by 18% and bulk density decreased by 7%, presumably as a result of the production of exopolysaccharides and hyphal network. Associated with better soil structure, soil aeration and moisture retention was increased, which facilitated root penetration and plant productivity (Rillig et al., 2015). Integrated management involving organic amendments and microbial inoculants had the highest benefits in terms of microbial quotient and enzyme activity indices, which indicate the long-term positive effect on soil productivity (Puglisi, 2022).

In summary, this information is consistent with the notion that microbial inoculants indeed act as ecosystem engineers, by altering the rhizosphere community in a direction that is more conducive to plant growth. Multi- strain consortia have complementary functions in the soil, such as nitrogen fixation, phosphate solubilization, phytohormone production, and stress mitigation, which results in improved performance of plants and soil health.

Conclusion

This in-depth study provides unquestionable proof that plant-microorganism interactions are one of the fundamental factors that determine soil health, nutrient turnover and sustainable agricultural productivity. Application of microbial consortia showed intensive enhancement of the biological activity of soil, functions of soil enzymes, nutrient cycling, plant growth, and yield. The evidence proves the significance of Rhizobium leguminosarum, Bacillus subtilis, Azospirillum brasilense and Glomus intraradices in the regulation of decisive ecological processes.

The study also supports the premise that microbial inoculation promotes an increase in the activities of urease, phosphatase and dehydrogenase, which are sensitive biomarkers of the soil biological quality (Nannipieri et al., 2012). The increased enzymatic activity indicates an increase in microbial metabolism and nutrient conversions that results in a better nutrient availability for plants. Enzymes provide help in the decomposition of organic matter to provide growth elements. Correlations between microbial biomass carbon, soil organic carbon, and crop yield confirm ascertaining a positive correlation between soil fertility and microbial processes (Fierer, 2017). Elevated soil enzyme activity thus represents a combination of higher levels of microbial presence and function that can be taken as a sign of an ecologically active and resilient soil ecosystem.

The colonization and restructuring of the soil microbiome was indicated by an increased abundance of beneficial bacterial and fungal groups (Proteobacteria, Actinobacteria, Firmicutes and Glomeromycota) in inoculated treatments. These taxa are associated with increased nutrient balance, disease suppressive and plant development (Hartmann et al., 2015; Francioli et al., 2021). Expression of the gene markers of important functional traits (nifH, phoD, acdS) indicates enhanced nitrogen fixation, phosphorus solubilising and ethylene modulation which are imperative to maintaining plant vigor under a changing environment. Functional gene activation + agronomic improvement (root length, chlorophyll content, grain yield) and soil restoration are the example of soil restoration even in degraded situation [34,37].

Significant agronomic improvements were found in microbial consortia with the yield of this raised about 30% higher. These enhancements indicate the uptake of nutrients, secretion of hormones and induction of systemic resistance, which is supported by studies on cereals inoculated with PGPR and AMF (Mishra et al., 2021; Shahzad et al., 2023). Enhanced chlorophyll content and its photosynthetic productive capacity confirm the positive effect of microbial metabolites on physiological performance of plants.

Ecologically, the study underlines the role of microbial inoculants in crop production in ecosystems not only in favor of agriculture but also for ecosystem restoration. Improvements in soil structure, aggregate stability and reduced bulk density are brought about because of the production of exopolysaccharides and hyphal networks by microbes, mitigating erosion and improving water retention and sequestration of carbon (Rillig et al., 2015). These physical enhancements are very important in controlling erosion and water capture and long-term soil fertility. The results of this study add support to the understanding that multi -strain consortia outperform single-strain inoculants in carbon sequestration (Kuzyakov and Blagodatskaya, 2015). Multiplicity of metabolic activities ensure complementary functions, i.e. nitrogen fixation, phosphate solubilisation, stress tolerance, ensuring the possibility to be resilient to environmental changes. Integrating microbial inoculants with organic and conservation-oriented practices can lead to the development of synergistic systems that can maximize productivity as well as sustainability and reduce the dependency on chemical fertilizers and the environmental degradation (Larkin, 2015; Puglisi, 2022).

The study is an addition to the growing knowledge in microbial biotechnology for agriculture. Metagenomic sequencing, qPCR, and bioinformatics are offered to provide a molecular level framework for the elucidation of soil microbial ecology. This fusion of biotechnology and traditional processes of agronomy provides a novel way of designing precision biofertilizers for individual crops and soils. Moreover, the socio- economic implications of the microbial-based soil management for increased yield with reduced input expenditure is of special importance, particularly for the developing regions that are prone to soil erosion, such as South Asia. The findings suggest that governments and agricultural extension agencies need to make microbial inoculants the focus of national soil fertility programs through promotion and subsidization. Long-term research to track the dynamics of soil microbiomes will be important to determine how long introduced microbes last and are sustained (FAO, 2021; Busby et al., 2017). Facilitation of collaboration between governmental bodies and private sector will further accelerate the development and adoption of Quality microbial products.

Future research should include metatranscriptomic and metabolomic profiling to capture functional roles of microbial communities in real time and co-evolutionary roles of plant and microbes in real time interacting on climate stress in order to unravel the mechanisms of resilience. On the whole, this study analyzes substantiates the integration of microbial inoculants into regenerative agriculture as the basis for soil health and sustainable food systems and as alternatives to chemical inputs and catalysis to ecological resilience.

The study further strengthens that microbial consortia plays a vital role in improving soil's biochemical properties and microbial diversity and by that increasing soil productivity and productivity while balancing the ecological equation for sustainable agriculture. The adoption of biofertilizers is a paradigm shift to regenerative, climate-smart and economically sustainable systems of food production. Continued microbial biotechnology investment, education among farmers and enabling policy frameworks will play an important role in restoring food security and the environment globally.

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