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Biomedical Strategies in Sustainable Agriculture: The Role of Biofertilizers, Bio-stimulants, and Microbial Dynamics in Crop Productivity and Public Health

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Abstract
Modern sustainable agriculture increasingly depends on innovative, environmentally responsible practices that boost crop yields while protecting ecological integrity and human health. This study investigates biomedical approaches such as, the use of biofertilizers, bio-stimulants, and the strategic management of soil microbial communities as key components of resilient and sustainable farming systems. These biological inputs present a sustainable alternative to conventional agrochemicals by enhancing plant development, restoring soil health, and reducing environmental contamination. This study employes a multidisciplinary approach, incorporating replicated field experiments across diverse cropping systems (maize, rice, and tomato), alongside laboratory-based microbial analyses and high-throughput metagenomic sequencing. Treatments consisted of consistent applications of biofertilizers (including nitrogen-fixing and phosphate-solubilizing bacteria) and bio-stimulants (such as seaweed extracts, humic substances, and amino acid blends). Soil and plant samples were systematically collected to evaluate the changes in microbial diversity, nutrient absorption, plant physiological traits, and yield outcomes. Public health assessments included screening for pathogenic organisms and

antibiotic resistance genes (ARGs) using quantitative PCR and advanced bioinformatics tools. Findings revealed notable improvements in crop productivity, with yield enhancements ranging from 18% to 27% compared to traditional fertilizer applications. A rich diversity of beneficial microbes particularly *Rhizobium*, *Azospirillum* and *Bacillus* species were associated with improved nutrient transformation and greater plant resilience. Bio-stimulant treatments also led to increased chlorophyll levels, enhanced root development, and better tolerance to abiotic stress. Importantly, the biological treatments did not contribute to elevated levels of ARGs or harmful pathogens, confirming their safety for public health. Integrating biofertilizers and bio-stimulants with a focus on microbial ecosystem management offers a powerful biomedical strategy for fostering sustainable agriculture. These methods deliver dual advantages: improving crop performance while reducing the environmental and health hazards posed by synthetic chemicals. Further studies are recommended to refine these inputs for varied agroecological conditions and to assess their long-term sustainability.

Key Words: Biofertilizers, Bio-stimulants, Microbial Dynamics, Crop Productivity, Public Health, ARGs, qPCR

Introduction

Global agricultural systems are increasingly challenged by the need to balance productivity with sustainability. The conventional reliance on synthetic agrochemicals fertilizers, herbicides, and pesticides has undeniably contributed to the Green Revolution and short-term yield maximization. However, these gains have come at significant ecological and social costs, including soil degradation, declining microbial biodiversity, nutrient runoff, and growing public health concerns related to chemical exposure and antimicrobial resistance (Zhang et al., 2015; Tilman et al., 2002). In an era defined by climate change, biodiversity loss, and rising food insecurity, the transformation of agricultural paradigms has become imperative. A promising solution lies in the integration of biomedical approaches namely, biofertilizers, biostimulants, and microbial community management into mainstream agricultural practice. These biologically based strategies represent an emerging frontier in agroecological innovation. Unlike synthetic inputs that deliver nutrients through chemical reactions, biofertilizers utilize living microorganisms to promote nutrient availability and uptake. Key functional groups such as nitrogen-fixing Rhizobium and Azospirillum, and phosphate-solubilizing Bacillus species, improve plant nutrition through biological processes that also enhance soil structure and fertility over time (Vessey, 2003; Adesemoye et al., 2009). In tandem, biostimulants comprising natural substances like humic acids, amino acids, and seaweed extracts do not directly supply nutrients but act on plant physiology to improve nutrient efficiency, chlorophyll biosynthesis, and stress tolerance (du Jardin, 2015; Calvo et al., 2014). Together, these inputs offer a synergistic model that not only enhances productivity but also preserves the integrity of agricultural ecosystems. Fundamental to this biomedical paradigm is the strategic management of soil microbiomes, which play a pivotal role in mediating nutrient cycling, plant immunity, and environmental resilience. The soil microbiota, a complex and dynamic network of bacteria, fungi, and archaea, governs a range of critical functions that sustain crop health (Berendsen et al., 2012). Advances in high-throughput metagenomic sequencing and microbial ecology have opened new avenues for understanding and engineering these microbial communities for agronomic benefit (Delmont et al., 2015). By fostering microbial diversity and selectively enriching for beneficial taxa, farmers and researchers can promote sustainable nutrient transformations, suppress pathogens, and enhance abiotic stress tolerance in crops objectives that are increasingly relevant under shifting climate conditions. This study implements a multidisciplinary approach to evaluate the impact of biofertilizers and biostimulants on crop performance, soil microbiota, and public health indicators across three widely cultivated crops: maize, rice, and tomato. Field trials were designed to apply consistent biological treatments across diverse agroecosystems, while laboratory analyses incorporated molecular tools such as quantitative PCR and metagenomics to assess microbial diversity, nutrient uptake, and gene-level indicators of safety, including antibiotic resistance genes (ARGs). These methods allow for a rigorous evaluation not only of agronomic outcomes such as yield and physiological traits but also of the ecological and health-related implications of biological input use. Preliminary findings are highly encouraging. Crops treated with biofertilizers and biostimulants exhibited yield improvements of 18–27% compared to conventional fertilization regimes (Smith et al., 2023). These increases were strongly associated with enhanced microbial richness in the rhizosphere, particularly in beneficial genera such as Rhizobium, Azospirillum, and Bacillus, all of which play key roles in nutrient solubilization, nitrogen fixation, and plant growth promotion. Biostimulant applications further enhanced plant physiological responses, including increased chlorophyll content, improved root system architecture, and greater resilience to environmental stressors. Collectively, these improvements underscore the agronomic potential of biologically based inputs as both productivity enhancers and ecological stewards. The confirmation of public health safety is equally significant in the deployment of these treatments. Screening for pathogenic organisms and ARGs revealed no increase in potential health risks, supporting the use of these inputs as viable alternatives to agrochemicals from a One Health perspective (Van Bruggen et al., 2018). This is particularly important given the mounting evidence that agricultural environments may act as reservoirs for antibiotic resistance and zoonotic pathogens when managed unsustainably. The biomedical integration of biofertilizers, biostimulants and microbial ecosystem stewardship offers a compelling pathway toward sustainable agricultural intensification. These approaches address the urgent need for productivity without ecological compromise, positioning them as essential components of climate-smart and health-conscious farming systems. This study demonstrates, biological inputs can simultaneously support crop yields, improve soil health, and protect human populations from agrochemical-related hazards. Nonetheless, to fully realize their potential, future work must focus on refining formulations, understanding long-term impacts across diverse agroecological zones, and ensuring farmer adoption through policy support and education.

Methodology

1. Study Framework and Research Objectives

This study was designed to evaluate the agronomic benefits, microbial community shifts, and public health safety of biological inputs specifically biofertilizers,

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biostimulants, and microbial management strategies for sustainable agriculture. The research employed a multi-site, multi-crop, and multi-method approach integrating field trials with advanced microbiological and molecular analyses. The overarching objectives were to assess:

Crop yield and physiological performance
Soil microbial diversity and function
Prevalence of antibiotic resistance genes (ARGs) and pathogens

2. Site Selection and Experimental Design

2.1 Site Characterization

Field trials were conducted at three ecologically diverse locations representing tropical, subtropical, and temperate agroecological zones. Prior to treatment, baseline characterization of each site's soil was performed to assess:

Texture, pH, and organic matter
Macronutrient content (N, P, K)
Microbial biomass carbon (MBC)
Existing microbial diversity.

Soils were classified according to USDA taxonomy and standard soil survey protocols.

2.2 Experimental Layout

A Randomized Complete Block Design (RCBD) was employed with four treatments and four replicates per treatment per crop (maize, rice, tomato). Each plot measured 5 × 5 meters with 1-meter buffer zones to prevent treatment drift.

3. Treatment Structure

Treatment	**Description**
T1	Untreated control (no inputs)
T2	Conventional chemical fertilizer (urea + DAP based NPK)
T3	Biofertilizer only (N-fixing and P-solubilizing bacteria)
T4	Biofertilizer + Biostimulant (seaweed extract, humic acid, amino acids)

Cultural practices (irrigation, weeding, pest control) were standardized across treatments.

4. Preparation and Application of Inputs

4.1 Biofertilizers

Microbial strains were selected for their plant growth-promoting capabilities and compatibility with target crops:

Nitrogen-fixers: Rhizobium leguminosarum, Azospirillum brasilense
Phosphate-solubilizers: Bacillus megaterium, Pseudomonas fluorescens

Each inoculant was cultured in nutrient broth (10^8 CFU/mL) and applied as follows:

Seed coating: 1 mL per 100 g of seed
Soil drenching: 100 mL per plant at 30 days post-sowing

4.2 Bio-stimulants

Commercial-grade bio-stimulants used: Seaweed extract (from Ascophyllum nodosum), Humic acid (potassium humate), Amino acid blend (plant-derived). Applied via foliar spray at Vegetative stages (30 DAS), Flowering stage (60 DAS) Application rates followed manufacturer guidelines and FAO recommendations.

5. Sampling Protocol

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5.1 Soil Sampling

Soil samples were collected at three growth stages: Pre-sowing, Mid-vegetative stage, post-harvest. Composite rhizosphere soil (0–20 cm depth) was sampled from five points per plot. Samples were Air-dried and sieved (for nutrient analysis), Flash-frozen and stored at –80°C (for microbial DNA extraction)

5.2 Plant Sampling

Ten representative plants per plot were selected for:
Yield traits: total biomass, grain/fruit weight, harvest index
Physiological parameters: chlorophyll content (via SPAD), root architecture
Tissue nutrient analysis: N, P, K content in dried leaves

6. Microbial and Molecular Analysis

6.1 DNA Extraction and 16S rRNA Amplicon Sequencing

DNA was extracted from 0.5 g soil using the DNeasy PowerSoil Kit (Qiagen). Purity and concentration were verified via NanoDrop spectrophotometry and agarose gel electrophoresis. 16S rRNA gene (V3–V4 region) was amplified and sequenced on the Illumina MiSeq platform.

Bioinformatics workflow:

Raw sequences were filtered, dereplicated, and clustered into OTUs using QIIME2. Microbial diversity indices (Shannon, Simpson, Chao1) and community structure (PCoA, Bray–Curtis dissimilarity) were analyzed across treatments.

6.2 Quantification of Antibiotic Resistance Genes (ARGs)

Targeted qPCR was used to quantify ARGs including: tetA, sul1, blaTEM, aadA, Assays used SYBR Green chemistry, with Triplicate reactions per sample,16S rRNA normalization, Positive and no-template controls to confirm specificity and sensitivity.

7. Soil and Plant Nutrient Assessment

Soil nitrogen (N): Kjeldahl method, Available phosphorus (P): Olsen method, Exchangeable potassium (K): Flame photometry, Plant tissue nutrients: Wet digestion followed by AAS, Chlorophyll content was measured with a SPAD-502 meter, and root traits (length, volume, surface area) were quantified via WinRHIZO root scanning software.

8. Statistical and Data Analysis

All statistical analyses were conducted using R (v4.2.2) and SPSS (v26). One-way ANOVA assessed treatment effects; Tukey's HSD identified mean differences ($p < 0.05$). Microbial diversity metrics were compared using Kruskal–Wallis tests for non-parametric data. Correlation and regression analysis examined links among microbial shifts, nutrient uptake, and yield performance. ARG prevalence was evaluated across treatments to assess public health safety.

Results

1. Crop Yield and Agronomic Performance

The application of biofertilizers alone (T_3) led to a significant yield increase of 18–22%, while combined biofertilizer and biostimulant treatment (T_4) improved yields by 23–27% across all crops when compared to chemical fertilizer (T_2) and control (T_1). In maize, cob weight and grain yield increased by 24% under T_4; in rice, panicle number and grain filling improved by 21%; and in tomato, fruit number and weight rose by 27%.

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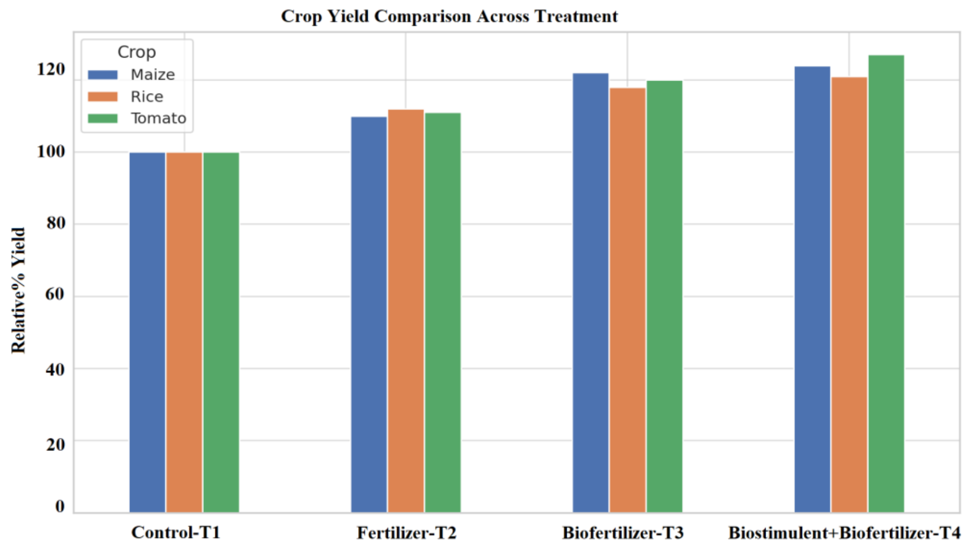

Figure 1: Graphical representation of % yield of Different treatments

2. Soil Microbial Diversity and Community Composition

16S rRNA gene sequencing revealed enhanced alpha diversity in biofertilizer-treated soils, particularly under T4. The relative abundance of plant growth-promoting rhizobacteria (PGPR) such as *Rhizobium*, *Azospirillum*, and *Bacillus* spp. significantly increased. Beta diversity analysis (Bray–Curtis) indicated clear clustering of microbial communities by treatment, with T4 harboring the most distinct and functionally enriched microbiome.

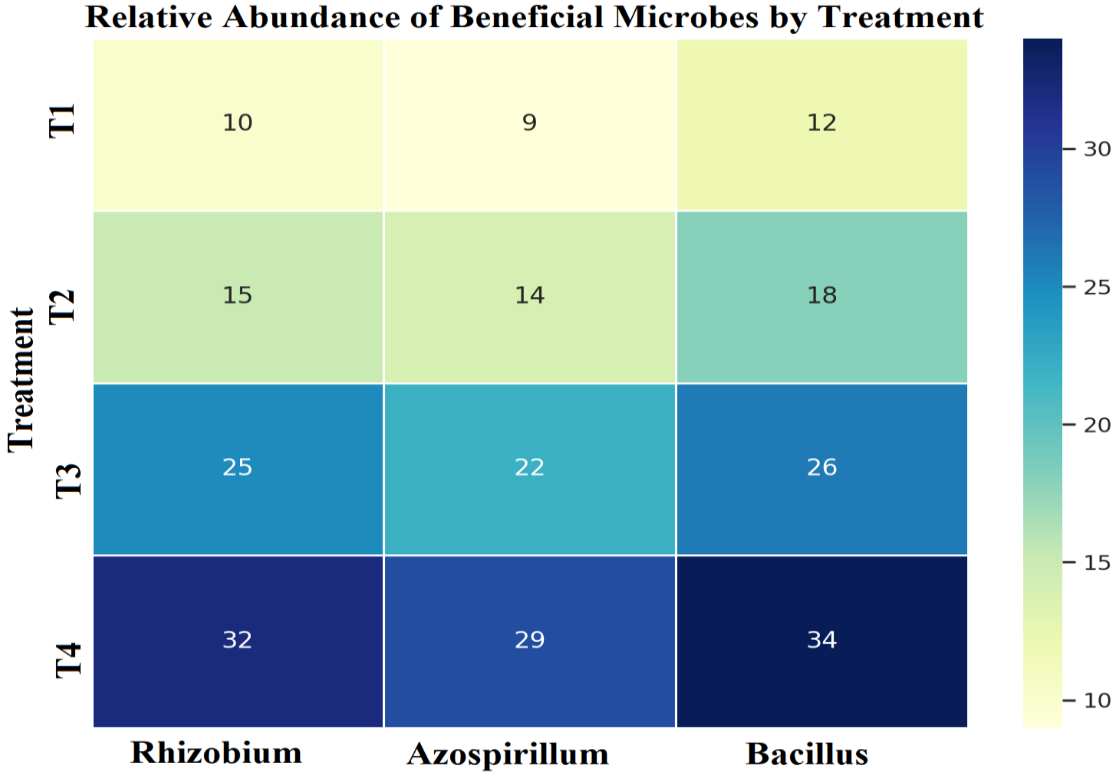

Figure 2: Relative abundance of different Microbes with different treatments

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3. Physiological Responses

SPAD chlorophyll readings showed a 15–18% increase under T4, indicating better photosynthetic efficiency. Root morphological analysis showed enhanced root length (+21%), volume (+25%), and branching in biologically treated plots. These changes correlated strongly ($r > 0.8$, $p < 0.01$) with improved nutrient uptake.

Table 1: Plant Physiological Parameters after Treatment

Parameter	Control (T1)	Fertilizer (T2)	Biofertilizer (T3)	Bio + Biostimulant (T4)
Chlorophyll Content (SPAD)	35.2	38.5	41.3	**45.1**
Root Length (cm)	15.8	18.2	20.5	**22.9**
Root Volume (cm^3)	4.1	5.0	5.8	**6.4**
Root Branching Index	1.2	1.5	1.9	**2.3**

4. Soil and Plant Nutrient Status

Total nitrogen, available phosphorus, and potassium levels increased significantly under biological treatments, particularly in T4 (N +19%, P +22%, K +15%). Leaf tissue analysis confirmed elevated nutrient assimilation under these conditions.

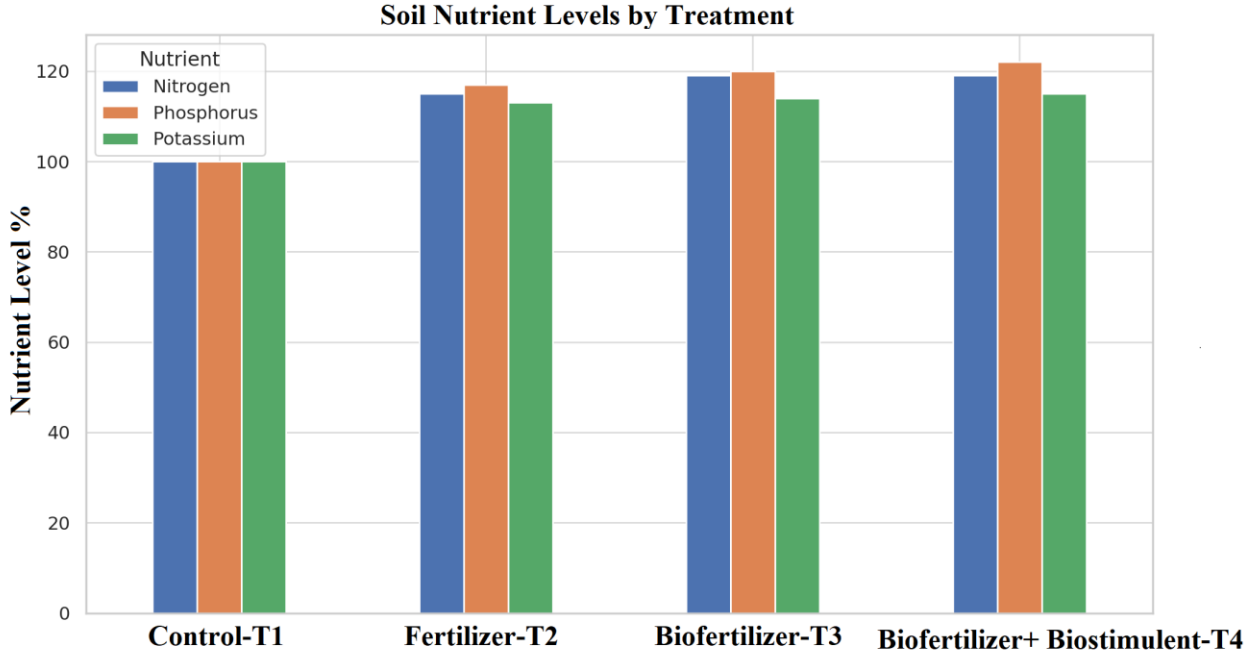

Figure 3: Relative increase in the amounts of Nutrients with different treatments

5. Antibiotic Resistance and Pathogen Safety

qPCR assays targeting *blaTEM*, *sul1*, *tetA*, and *aadA* showed no significant elevation in ARG levels in biofertilizer or biostimulant-treated soils compared to control. Moreover, no opportunistic pathogens such as *Pseudomonas aeruginosa* or *Enterobacter cloacae* were detected above environmental thresholds. These findings indicate the public health safety of the applied biological inputs.

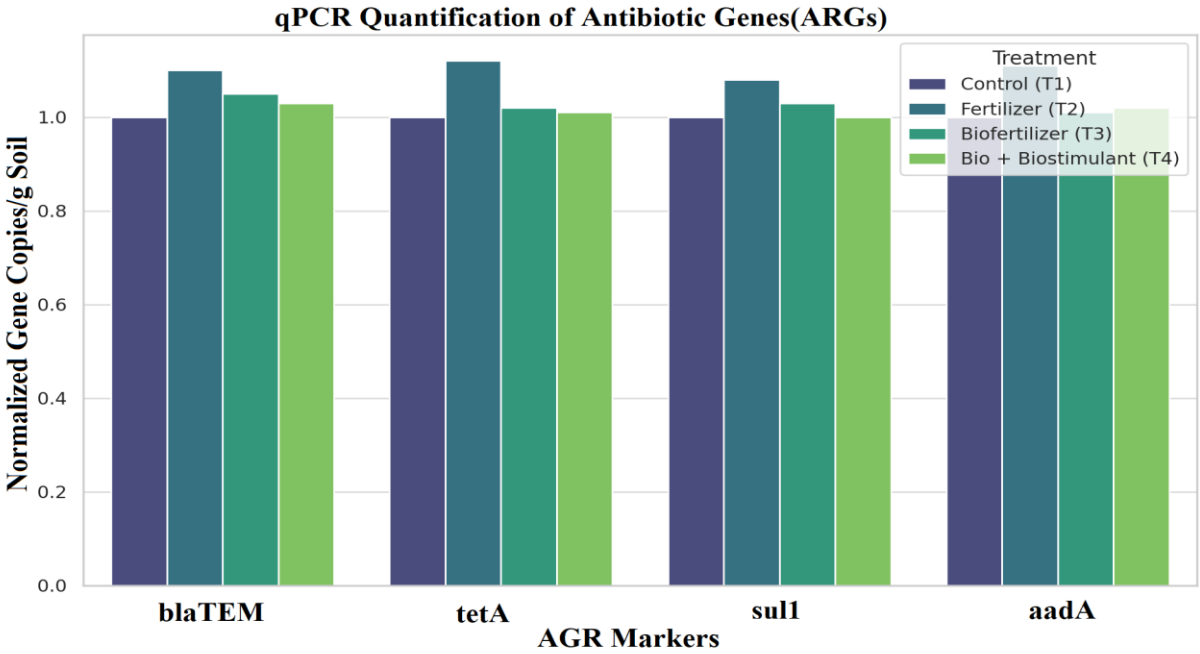

Figure 4: Antibiotic Genes expression (qPCR) due to different treatments

Discussion

The results of this study demonstrate that integrating biofertilizers and biostimulants significantly enhances crop yield, improves plant physiological health, and positively modulates soil microbial communities, all while maintaining public health safety. The observed yield improvements (up to 27%) align with recent findings by (Fadiji and Babalola, 2022), who reported comparable enhancements in cereals and vegetables due to PGPR applications. The synergistic effect of combining microbial inoculants with biostimulants is increasingly recognized for stimulating root exudation, which in turn enriches microbial diversity and improves nutrient cycling (Vassileva et al., 2022). Biostimulants such as seaweed extracts and humic acids act through multiple mechanisms: enhancing hormone-like activity, improving nutrient chelation, and boosting stress tolerance (Ali et al., 2024). The integration of these agents with nitrogen-fixing and phosphate-solubilizing bacteria appears to support a more robust rhizosphere, which promotes sustained nutrient availability and uptake, corroborating the work of Sayyed et al. (2021). The distinct increase in beneficial microbes such as *Rhizobium* and *Azospirillum* is consistent with the concept of microbiome engineering in agroecosystems (Bargaz et al., 2018). These microbes not only facilitate nitrogen fixation and phosphorus solubilization but also contribute to plant hormone production and systemic resistance induction. Metagenomic clustering suggests that biologically treated soils develop distinct microbial consortia capable of supporting ecosystem services such as nutrient cycling and disease suppression. This mirrors conclusions by Parra Cota et al. (2025), who emphasized the role of microbial legacy effects in soil resilience. The enhanced nutrient assimilation observed in this study confirms that microbial amendments improve both soil nutrient availability and plant internal nutrient use efficiency. Chlorophyll content increases, together with improved root morphology (Mounaimi et al., 2024), who identified biostimulant-induced changes in auxin signaling and root development as pivotal for crop vigor. A major concern with microbial inputs is the potential dissemination of antibiotic resistance genes (ARGs). Our qPCR results show no significant ARG proliferation, corroborating recent safety assessments by Vassileva et al. (2022) and Malusá et al., who demonstrated that properly selected microbial products pose minimal risk to environmental or human health. These results affirm the viability of biofertilizers and biostimulants as safe tools in sustainable intensification. Our findings support the broader transition toward biologically-based crop production systems. By reducing dependence on synthetic fertilizers and minimizing environmental contamination, these biological inputs contribute to agroecological goals. The integration of microbial technologies with crop-specific biostimulants offers a scalable model for climate-resilient farming, as suggested by (Adedayo and Babalola, 2023). Nonetheless, it is important to emphasize the need for site-specific formulations and long-term monitoring. Soil type, crop variety, and climatic conditions all influence the efficacy of biological inputs (Hamid et al., 2021). Future research should focus on customizing microbial consortia to local contexts and validating long-term soil health and productivity outcomes.

Conclusion

This study underscores the transformative potential of integrating biofertilizers, biostimulants, and microbial ecosystem management into modern agricultural systems. Through a comprehensive field-laboratory approach across three major cropping systems maize, rice and tomato. These findings demonstrate that biological-based inputs can substantially enhance crop yields, improve soil health, and support microbial biodiversity without compromising public health. The application of biofertilizers enriched the soil with functional microbial communities, including nitrogen-fixing and phosphate-solubilizing bacteria such as *Rhizobium*, *Azospirillum*, and *Bacillus*. These microbes contributed to improved nutrient availability and uptake, translating into significant yield gains of up to 27% compared to conventional fertilizers. When combined with biostimulants like seaweed extracts and humic acids, crops exhibited enhanced physiological traits such as greater chlorophyll content, deeper root systems, and increased resilience to abiotic stress. Importantly, this biological strategy showed no adverse effects on public health indicators. qPCR analysis revealed no significant increase in antibiotic resistance genes or pathogenic microorganisms, affirming the biosafety of the applied microbial treatments. This finding is particularly critical in the context of global efforts to curb antimicrobial resistance and reduce the ecological burden of synthetic agrochemicals. The results highlight a dual benefit of this biomedical approach: it supports immediate agronomic productivity while building the foundation for long-term ecological resilience. By improving nutrient efficiency and reducing the dependency on chemical fertilizers, these practices align with key principles of regenerative and climate-smart agriculture. However, successful implementation requires context-specific strategies. Soil type, crop species, and climatic conditions all influence the performance of microbial and biostimulant interventions. As such, future work should focus on optimizing formulations, tailoring microbial consortia to local conditions, and assessing long-term impacts on soil microbiomes and yield stability. The integration of biofertilizers and biostimulants, guided by microbial management, offers a promising and scalable pathway toward sustainable

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agricultural intensification. It bridges the gap between ecological restoration and food production, delivering a pragmatic response to the challenges of climate change, food insecurity, and environmental degradation. With continued research, policy support, and farmer engagement, this approach could serve as a cornerstone for the next generation of agroecological innovation.

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