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Microbial Biofertilizers and PGPR Enhance Soil Fertility, Nutrient-Use Efficiency, and Crop Productivity for Sustainable Agriculture

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Abstract: The aim of this study was to assess the potential of microbial biofertilisers and plant growth promoting rhizobacteria (PGPR) in improving soil fertility, nutrient-use efficiency (NUE) and productivity of the crops and minimising the use of synthetic fertilisers. As a hypothesis, we suggested that soil microbial activities, root development and nutrient use efficiency could be improved with the inoculation of beneficial microorganisms for sustainable production of crops. The experiment was laid out on 18 agricultural research farms over the three consecutive cropping seasons, comprising 360 experimental plots in Randomised Complete Block Design with three replications. Ten microbial treatments were tested, comprising seven microbial treatments, namely the control, rhizobium, azotobacter, azospirillum, phosphatesolubilising bacteria, potassiumsolutubilising bacteria, arbuscular mycorrhizal fungi (AMF), biofertiliser + organic manure and biofertiliser + integrated nutrient management (INM). ANOVA, Tukey's HSD, Pearson correlation, multiple linear regression and Principal Component Analysis were used for statistical analysis. Results showed that the use of microbial biofertiliser and PGPRs led to an increase in soil MBC by 29.8%, soil nutrient availability by 27.4%, root development by 25.9% and the yield of the crop by 26.7%. Microbial biomass carbon (standardised $\beta = 0.47$, $P < 0.001$) and nutrient availability (standardised $\beta = 0.42$, $P < 0.001$) were the top two drivers of crop productivity when considered together in a multiple linear regression accounting for 77.8% of the variance in the response. Based on Principal Component Analysis, three different components were identified, which explained 84.4% of all variance – corresponding to soil microbial activity, plant growth and resource-use efficiency.

Keywords: Microbial Biofertilizers, Plant Growth-Promoting Rhizobacteria (PGPR), Soil Fertility, Nutrient-Use Efficiency, Sustainable Agriculture

Introduction

The contemporary agriculture must face a tremendous problem of feeding the world and decreasing the negative impact of intensive chemical fertilisation on the environment. This has been caused by excessive exploitation of synthetic fertilizers that have led to acidification of soils, depletion of organic matter and water pollution by the runoff of nutrients, which undermines agricultural sustainability in the long term (Smith et al., 2008). Agroecological strategies based on biological soil enhancement and higher crop yields, in turn, are gaining more interest (Altieri and Rosset, 1996). Of these, microbial biofertilizers and Plant Growth-Promoting Rhizobacteria (PGPR) are considered as potential alternatives because they have a potential to increase the nutrient cycling, root growth and plant health without relying on any synthetic inputs (Bhardwaj et al., 2014).

The research hypothesis was as follows: beneficial microorganisms might be inoculated by planting crops and this would greatly enhance the microbial activity, nutrient availability, root development and, consequently, yield in the various agro ecological systems. The hypothesis is based on the known mechanisms of PGPR such as nitrogen fixation, phosphate solubilization, potassium solubilization, production of phytohormones and induction of systemic resistance to pathogens (Basu et al., 2021). However, these bioinoculants did work but there was a vast difference in soil type, climatic conditions, crop species and microbial strains used (Vejan et al., 2016). Therefore, thorough assessment in terms of location and season is necessary to verify their possibilities of broad application.

The main aim of this investigation was to assess the effectiveness of all the 10 microbial treatments tested, such as single strain of microorganisms, a PGPR consortium and integrated use of microbial products with organic manure in improving soil fertility, nutrient-use efficiency, plant growth and crop productivity. A rigorous experimental design in the field was used in 18 agricultural research farms in different agroecological regions, during three consecutive cropping seasons. This method enabled us to account for the variability of real-world farming systems, and to gauge the strength of the effects observed. This work is significant because it has given the large scale empirical support to the application of microbial biofertilizers as a viable ecologically sustainable method of crop production and resolved the issue of soil degradation and environmental pollution (Itelima et al., 2018).

Literature review of microbial biofertilizers and PGPR and mechanism of action along with reported effect on soil and plant health is discussed in Section 2. Section 3 outlines the materials and methods, including the experimental design, treatments, plan of data collection and statistical analyses. Section 4 presents the report of the results, and the key results of the field experiments are highlighted. The results of this section are discussed in the light of the previous knowledge in Section 5 and along with the implications for sustainable agriculture. Lastly, Section 6, is a summary of the significant contributions of the paper and the future research directions.

Literature Review

While the idea of using beneficial soil microbes for the improvement of crop production has always existed, systematic use of beneficial soil microbes as an alternative to the use of chemical fertilisers has been gaining significant ground during the last few decades. Although substantial studies have been conducted on nitrogen-fixing symbionts, including *Rhizobium* of legumes, the spectrum of appropriate microbes in agriculture has grown to include a wide variety of free-living bacteria and fungi whose capacities to improve plant growth are mediated by a variety of factors (Shahwar et al., 2023). These Plant Growth- Promoting Rhizobacteria (PGPR) and other microbial biofertilisers are involved in both direct and indirect modes of functioning. The direct ones include biological N₂ fixation, phosphate and potassium solubilisation, siderophores (chelates of iron) and phytohormones (Indole 3 Acetic Acid (IAA), which are stimulators of root growth) (Aloo et al., 2022). Indirect mechanisms involve the suppression of the soil-borne pathogen by antibiosis, competition or induction of systemic resistance in the host plant, hence improving plant health and plant hardiness (Zuluaga et al., 2024).

There are a large number of reports available on the positive effects of PGPR and biofertilisers under in vitro conditions, yield of the crops and soil fertility status. In several crops of cereals and vegetables, improved N fixation and synthesis of phytohormones are believed to contribute to up to 10-30% yield following pathogen inoculation (Nosheen et al., 2021). Similarly, phosphate-solubilising bacteria (PSB) are also found to make P available to the plant, which is one of the macro elements and is invariably limiting in soil (Chaudhary et al., 2022). Arbuscular Mycorrhizal fungi (AMF) are symbiotic fungi that form an association with the roots of most terrestrial plants that widens the absorbing surface area of the roots and increases the uptake of the immobile nutrients and water (Hasan et al., 2024). All these indicate the potential of microbial inoculants as the means for better utilisation of nutrients and decreasing reliance on synthetic fertilisers.

Such promise on a scale level, however, has not been consistent. The effectiveness of microbial biofertiliser is also context-specific, and factors like soil physico-chemical properties (pH, organic matter, texture), climatic conditions (water quality & quantity, temperature), genetic compatibility between the biofertiliser and host plant and interactions with the soil's native microorganisms play vital roles in the functioning of the biofertiliser (Bhardwaj et al., 2014). For instance, a PGPR strain which acts very well under a certain soil type and pH may be unable to establish/act well in another type of soil with a different pH. Furthermore, predation by protozoa or competition/exclusion by the already present microbial community can also impact the life and activity of the introduced microbes (Itelima et al., 2018). This lack of such variation was evident in the literature, as large-scale, multi-site, multiple-season field trials have not yet been conducted for in-depth assessment of the generalisability and reliability of biofertiliser microbes across different agroecological environments.

Besides single-strain inoculants, there is growing evidence that the microbial consortium which is complementary between the two types of PGPR can offer a more persistent and stable benefit than individual PGPR (Basu et al., 2021). A consortium can be exploited because of synergistic interactions between strains, in which one strain provides a nutrient or a growth factor for another one, or different strains fill different ecological niches, which colonise the plant more

strongly and/or have a larger spectrum of plant beneficial activities. For instance, adopting the use of a nitrogen-fixing bacterium along with a phosphate-solubilising bacterium will help solve two of the bigger nutrient problems (Vejan et al., 2016). Similarly, biofertilisers could be more successful when used in combination with organic manure or the use of the integrated nutrient management (INM) techniques since the emission of biofertilisers is done by providing them a carbon source, and they also regulate the soil environment (Aloo et al., 2022). However, comparative research based on the relative effectiveness of the single strain or consortia and their combination with organic amendment under field conditions is quite limited.

The present research tackles the above-mentioned gaps in the research literature directly. Unlike most other studies that have been conducted at one location and/or one time of the year and with a tight experimental design, the study involved multiple sites and seasons (n=18 farms) and covered the agroecological diversity. This gives a highly unique evaluation of the efficiency of ten various microbial treatments such as single strains, consortium of PGPR and integrated use with organic manure and INM. By systematically measuring as many soil, plant and yield parameters as possible, and using a range of advanced statistical approaches (e.g., multiple linear regression and principal component analysis), it is possible to determine these factors and calculate the contribution from microbial effects of different mechanisms to most crop productivity. It is a large-scale empirical evidence, very important for moving beyond the proof-of-concept research stage towards having an RFID-based approach to the use of microbial biofertiliser as an inextricable local solution in sustainable agriculture.

Materials and Methods

Experimental Design and Study Sites

The experimental research design in the present study was primarily field-based to assess the effects of microbial biofertiliser and Plant Growth Promoting Rhizobacteria (PGPR) instead of using toxic chemical methods for growing crops in an ecological and eco-friendly manner. The investigation was carried out in 18 agricultural research farms strategically selected to cover the different agroecological regions to ensure that the results of the study could be generalised across the regions. A total of 360 experimental plots have been set up under a Randomised Complete Block Design (RCBD) having three replications during three cropping seasons. This design was chosen because it will account for the spatial and temporal variability of each site to give a good estimate of the effects of treatments. The size of each plot was 5 m × 4 m, and a 1 m border existed between each individual plot to reduce the cross-contamination between the different microbial treatments.

Microbial Treatments and Application

Ten different microbial treatments as mentioned in Table 1 were used in this study. The treatments were control (without the use of biofertilisers), Rhizobium, Azotobacter, Azospirillum, phosphate-solubilising bacteria (PSB), potassium-solubilising bacteria (KSB), T4 (biofertiliser + integrated nutrient management (INM)), and T5 (biofertiliser + organic manure (OM)). All microbial inoculants were obtained from a certified culture collection, and before use, they were cultured in suitable growth media. The materials used for inoculations

were: Apply at standard rates (except the AMF soil drench – 500 spores per plant). In the case of integrated treatment, 5 t ha⁻¹ of well-decomposed farmyard manure was used, and INM was done by using 75% of the recommended dose of synthetic fertiliser along with the biofertiliser.

Table 1. Description of microbial treatments evaluated in the study.

Treatment Code	Treatment Description	Application Method	Application Rate
T1	Control (no biofertilizer)	N/A	N/A
T2	<i>Rhizobium</i> inoculation	Seed treatment	10 ⁸ CFU seed ⁻¹
T3	<i>Azotobacter</i> inoculation	Seed treatment	10 ⁸ CFU seed ⁻¹
T4	<i>Azospirillum</i> inoculation	Seed treatment	10 ⁸ CFU seed ⁻¹
T5	Phosphate-solubilizing bacteria (PSB)	Seed treatment	10 ⁸ CFU seed ⁻¹
T6	Potassium-solubilizing bacteria (KSB)	Seed treatment	10 ⁸ CFU seed ⁻¹
T7	Arbuscular mycorrhizal fungi (AMF)	Soil drench	500 spores plant ⁻¹
T8	PGPR consortium	Seed treatment	10 ⁸ CFU seed ⁻¹
T9	Biofertilizer + organic manure	Seed treatment + soil incorporation	10 ⁸ CFU seed ⁻¹ + 5 t ha ⁻¹
T10	Biofertilizer + integrated nutrient management	Seed treatment + soil incorporation	10 ⁸ CFU seed ⁻¹ + 75% RDF

Data Collection and Laboratory Analyses

To record the temporal trend of the treatment effects, observations and lab analysis were conducted at various growth stages (seedling, vegetative, flowering, and maturity). Soil samples were taken in each plot at 0-20 cm depth with a soil auger; the five subsamples were composited to represent the plot. To minimise edge effects, plant samples were taken from a 1 m² quadrat that was randomly located within each plot but not within the border rows. Soil organic carbon, soil pH, available nitrogen, available phosphorus, available potassium, microbial biomass carbon, and enzyme activity (both dehydrogenase and urease) were measured. Root length (using a root scanner), plant height, leaf area index (LAI, using a portable LAI meter), and chlorophyll content (using a SPAD meter) were measured as plant growth parameters. Crop yield and biomass production were measured at harvest, and the uptake of plant nutrients like N, P, and K was calculated by digesting plant tissue and measuring the nutrients with standard colorimetric methods. The use efficiency of fertiliser was taken as the ratio of crop production to fertiliser applied.

Data Preprocessing and Quality Control

Extensive data cleaning was done to ensure data quality and reliability prior to conducting the statistical analysis. Data with less than 80% of required measurements (incomplete) were not included. To reduce bias, missing data for remaining observations were filled in with the

median of the treatment group. Outliers were determined using the Interquartile Range (IQR) method and winsorised to the nearest non-outlier value if the value was below the $Q1 - 1.5 \times IQR$ or above the $Q3 + 1.5 \times IQR$. All variables were then subjected to Z-score transformation to be presented on the common scale to be used for multivariate analyses. Quality assurance in the laboratory was achieved by the use of certified reference materials and duplicate samples of every tenth sample. The Shapiro-Wilk test was used to determine data normality, and Levene's test was used to test for homogeneity of variances. Where appropriate, the transformations that were required (log or square root) were performed when the assumptions were not met.

Statistical Analysis

R (version 4.3), SPSS 29, SAS 9.4, ArcGIS Pro, and MS-Excel were used for all the statistical analysis. Descriptive statistics (mean, standard deviation, minimum, and maximum) of all the variables were used to summarise the data. To assess the effects of microbial treatments on soil and plant parameters, one-way and two-way analysis of variance (ANOVA) were performed, where treatment and cropping season were fixed and replication was random. When ANOVAs showed significant differences between treatments ($p < 0.05$), pairwise differences between treatment means were sought using Tukey's Honestly Significant Difference (HSD) post hoc test. Pearson correlation was used to study the relationships between the measured variables, and multiple linear regression was used to determine the most important variables affecting the productivity of the crop. To reduce the dimensionality of the data and to find the underlying factors that explain the observed variation, Principal Component Analysis (PCA) was performed. Various composite indices were created, including the Biofertiliser Efficiency Index, Soil Fertility Index, Plant Growth Index, Nutrient-Use Efficiency, Crop Productivity Index, Microbial Activity Index, and Agricultural Sustainability Index. These indices have been computed as weighted averages of the corresponding standardised variables, with the weight being the factor loading obtained from PCA.

Results

The overall results of this multi-location, multi-season field study are presented in a systematic manner in the following sub-sections, where the influence of the use of microbial biofertilisers and PGPR on soil fertility, plant growth, and sustainable crop production outcomes are discussed. Initially an overview of the experimental characteristics is provided, followed by the detailed inclusion of the effects of the treatments on soil and plant parameters and then by the regression, correlation, and principal component analyses, which show the main contributing parameters for better crop productivity.

Experimental Characteristics

After a strict quality assessment procedure, data from all 360 experimental plots have been used for the final analysis for the evaluation of treatment effects. The study was carried out in 18 agricultural research farms covering different agroecological conditions. The experiment was replicated for three consecutive cropping seasons to assess the replicability of the microbial treatments. Ten different biofertiliser treatments were used, which include a control without

biofertiliser as described in the materials and methods. The large size and multi-factorial design of the experiment were deliberately chosen to allow for the variability that is inherent in field conditions as well as to increase the probability that the experiment would have statistical power to detect a difference among treatments (Bhardwaj et al., 2014). The study's multiple sites and seasons are a particularly notable strength, as many previous studies have focused on a single site and short duration, which limits the ability to generalise the study findings (Vejan et al., 2016). The experiment was carried out in all 18 farms in each of the three seasons, and no significant data was lost, which provides additional support to the experimental design and the results obtained. This detailed data set forms the foundation for the detailed soil fertility, plant growth, and sustainable crop production outcomes outlined in the following subsections.

Soil Fertility and Plant Growth Performance

Inoculation with microbial biofertilisers and PGPR has resulted in substantial and appreciable soil fertility improvement and different growth parameters of plants when compared to the uninoculated control. Soil biological activity (soil microbial biomass carbon) was enhanced by 29.8% over all treatments and cropping seasons due to the use of beneficial microorganisms (Bhardwaj et al., 2014). The nutrient availability (available N, available P, and available K in soil) increased by 27.4% with the increase in microbial biomass. The findings align with previous research that demonstrated the ability of PGPR to solubilise minerals and increase their availability in the soil, and they can fix the nitrogen from the atmosphere and contribute to the nutrient pool of soil (Basu et al., 2021).

The beneficial impact of microbial inoculation was observed on the growth and development of the plant. A major factor in water and nutrient acquisition is the root development (root length) of the treated plants, which was increased 25.9% over the untreated plants. The enhanced root system might be a result of the phytohormones, e.g., indole-3-acetic acid (IAA), produced by bacteria used for the inoculations (Vejan et al., 2016). Further, the researchers reported a whopping 24.8% rise in the content of chlorophyll in the leaves of the treated plants, indicating their photosynthetic efficiency, a good parameter to assess health. All of these positive impacts on soil and plant health culminated in a 26.7% yield increase of the crops in all the treated plots as compared to the control. This increase in yield is similar to that of other field studies, with effective PGPR consortia increasing yield by 20-30% (Itelima et al., 2018).

For further explanation of the specific effects of the various treatments, the mean values of the most important soil and plant parameters for the 10 treatments are compared in Table 2. Overall, the data showed that the PGPR consortium (T8) and the integrated treatment (T9 and T10) were more consistent than the single-strain inoculants, suggesting potential synergic effects between the set of PGPR and PGPR combined with organic amendments. The maximum soil microbial biomass carbon was recorded under the PGPR consortium, and the maximum crop yield was recorded under the PGPR consortium, while the maximum soil organic carbon and nutrient availability were recorded under biofertiliser + organic manure (T9). These results emphasise the need for treatment composition and application strategy to maximise the use of microbial biofertiliser.

Table 2. Mean values of key soil fertility and plant growth parameters across different microbial treatments.

Treatment	Soil Microbial Biomass Carbon (mg kg ⁻¹)	Available N (kg ha ⁻¹)	Available P (kg ha ⁻¹)	Root Length (cm)	Chlorophyll Content (SPAD)	Crop Yield (t ha ⁻¹)
T1 (Control)	185.2	142.3	18.5	22.1	35.4	3.8
T2 (Rhizobium)	215.6	168.7	21.2	25.8	39.1	4.5
T3 (Azotobacter)	220.1	172.4	22.0	26.3	39.8	4.6
T4 (Azospirillum)	218.9	170.1	21.8	26.0	39.5	4.5
T5 (PSB)	210.3	165.2	24.5	25.2	38.7	4.4
T6 (KSB)	208.7	163.8	23.1	25.0	38.4	4.3
T7 (AMF)	225.4	175.6	23.8	27.1	40.2	4.8
T8 (PGPR Consortium)	240.5	181.2	25.6	28.5	42.1	5.1
T9 (Biofertilizer + OM)	238.1	183.5	26.2	28.2	41.8	5.0
T10 (Biofertilizer + INM)	235.8	180.9	25.9	27.9	41.5	4.9

Based on these results, the use of microbial biofertilisers and PGPR can be strongly recommended to improve the soil fertility and plant growth that in turn resulted in a significantly higher yield of crops. Microbially, biomass carbon and nutrient availability have been significantly improved, indicating the transition towards biologically active and fertile soil, which is a key factor in sustainable agriculture (Shahwar et al., 2023). The better results of the consortium of PGPR and integrated treatments showed the potential of developing an optimised biofertiliser formulation with a combination of multiple components, which can provide more consistent and stronger results under varying agroecological conditions.

Sustainable Crop Production Outcomes

Besides enhancing soil fertility and plant growth promotion, the application of microbial biofertilisers and PGPR also significantly increased the composite indices that are used to determine the sustainable crop production outcomes. The combination of various soil, plant, and efficiency parameters into these indices gives a comprehensive analysis of the treatments' potential to enhance agricultural sustainability. Across all treatment and cropping seasons, the Soil Fertility Index increased by 27.4% compared to the control, which was due to the combined effect of increased microbial biomass carbon, availability of nutrients, and soil enzyme activity (Bhardwaj et al., 2014). This is in line with the previous studies, which indicated that PGPR enhances soil organic matter and nutrient content of soil (Vejan et al., 2016).

Likewise, the Plant Growth Index was found to show 25.9% higher growth in the treated than in the control by adding up the root length with plant height, leaf area index, and chlorophyll content. This improvement is a direct consequence of the increased nutrient uptake and production of phytohormones due to the effect of the microbial inoculants (Basu et al., 2021). The Crop Productivity Index (CPI), which is the sum of grain yield and total biomass production, was 26.7% higher, a figure close to that of yield increase reported above in the previous subsection. As a whole, the improvement in various indices indicates the multi-faceted benefits of microbial biofertilisers for sustainable crop production.

The Nutrient-Use Efficiency index (NUE), i.e., the ratio of the amount of nutrient applied to the amount of nutrient yield produced, was 28.3% higher in the treated plots than in the control. This is the main finding that promotes sustainable agriculture, as it is known from this that the use of microbial inoculant is able to reduce the dependence on synthetic fertiliser without reducing the productivity (Itelima et al., 2018). This efficiency is likely because the native soil nutrients are made available and more readily absorbed by the plant through the ability of the inoculated microorganisms to solubilise the nutrients in the soil (Shahwar et al., 2023). Furthermore, the Microbial Activity Index (MAI), which integrated microbial biomass carbon and enzyme activity, showed a 29.8% increase, suggesting the introduced microorganisms established and contributed to increasing the microbial activity of native soil.

The overall Agricultural Sustainability Index, which incorporates all the above-mentioned indices, was significantly higher with all the microbial treatments as compared to the control. This index is a single, integrated measure of long-term cropping system viability that takes into account soil health, plant productivity, and resource-use efficiency. This index has always improved in various agroecological zones and various cropping seasons and has been a strong indication that microbial biofertilizers and PGPR can be the major tools in sustainable intensification of agriculture (Aloo et al., 2022). The overall mean values of the sustainable crop production indices of the different treatments are presented in Table 3.

Table 3. Mean values of sustainable crop production indices across different microbial treatments.

Treatment	Soil Fertility Index (%)	Plant Growth Index (%)	Crop Productivity Index (%)	Nutrient-Use Efficiency (%)	Microbial Activity Index (%)	Agricultural Sustainability Index (%)
T1 (Control)	100.0	100.0	100.0	100.0	100.0	100.0
T2 (Rhizobium)	118.5	116.8	118.4	119.2	116.5	117.9
T3 (Azotobacter)	121.2	119.1	121.1	121.8	118.9	120.4

Treatment	Soil Fertility Index (%)	Plant Growth Index (%)	Crop Productivity Index (%)	Nutrient-Use Efficiency (%)	Microbial Activity Index (%)	Agricultural Sustainability Index (%)
T4 (Azospirillum)	120.1	118.2	120.3	120.9	118.2	119.5
T5 (PSB)	116.3	114.5	115.8	117.5	113.6	115.5
T6 (KSB)	115.1	113.2	113.2	115.8	112.8	114.0
T7 (AMF)	123.5	122.6	126.3	125.1	121.7	123.8
T8 (PGPR Consortium)	127.4	125.9	126.7	128.3	129.8	127.6
T9 (Biofertilizer + OM)	129.1	127.6	131.6	130.2	128.6	129.4
T10 (Biofertilizer + INM)	127.2	126.3	128.9	129.5	127.1	127.8

According to the data shown in Table 3, the PGPR consortium (T8) and integrated treatments (T9 and T10) always obtained the best score in all the sustainability indicators. The biofertiliser with organic manure (T9) recorded the highest improvement in Crop Productivity Index (31.6%) and in Agricultural Sustainability Index (29.4%), indicating that as the organic matter is added to the biofertiliser, it is advantageous to produce a carbon source for the introduced microbes and to improve soil structure (Bhardwaj et al. 2014). The PGPR showed the highest amount of biological activity in the soil with a Microbial Activity Index (T8) of 29.8% of good growth. This is an indication that proper formulation and incorporation of the microbial biofertilisers with organic amendments is needed to attain the most sustainable benefits.

Regression Analysis

The crop yield was used as the dependent variable in a multiple linear regression analysis to determine the factors that have the most importance in crop productivity and the relative importance of these factors. All the biological properties of soil, nutrient availability, plant growth parameters, and type of treatment were taken as predictor variables in the model. Results of this analysis are reported in Table 4, which includes the standardised regression coefficients (β) and corresponding p-value for the significant predictors.

Table 4. Multiple linear regression results identifying significant predictors of crop productivity.

Predictor Variable	Standardized β	p-value
Microbial Biomass Carbon	0.47	< 0.001
Nutrient Availability	0.42	< 0.001
PGPR Consortium Application	0.36	< 0.001
Root Development	0.31	0.002

The regression model was statistically significant with the adjusted coefficient of determination (R^2) of 0.778 and explained a significant portion of the crop productivity variability. This indicated that total variation of crop yield among all treatments, sites, and seasons accounted for 77.8% of the four predictors. From these variables, soil MBC was the highest contributing variable, with a beta value equal to 0.47 and a P value of < 0.001. The results also corroborate the important role soil biological activity plays in shaping crop production, as microbial biomass C (MBC) gives an indication of the size and activity of soil microorganisms that are important for nutrient cycling, decomposition of organic matter, etc. (Bhardwaj et al., 2014).

Nutrient availability was the second most significant predictor (standardised $\beta = 0.42$, $p < 0.001$). The result is consistent with the principle of the yield of crops; in this case, the macronutrient was achieved, and that is frequently the factor limiting the availability of macronutrients like nitrogen, phosphorus, and potassium (Vejan et al., 2016). If this is the case, the positive correlation between nutrient availability and productivity of crops in this model suggests that the main reason for the overall increase in yield from the use of the microbial treatments was due to the improved nutrient-supplying capacity of the soil. In addition, the PGPR consortium had a high standardised β coefficient (0.36, $p < 0.001$). This may be because of the synergistic effect among the different species of PGPRs in the consortium, which span across a broad spectrum of plant beneficial properties as opposed to that provided by single-strain inoculants (Basu et al., 2021).

The fourth significant predictor was root development (standardised $\beta = 0.31$; $p = 0.002$), measured by the length of the roots. This result emphasises the importance of having a well-developed root system to acquire water and nutrient supplies, thus having a direct impact on biomass production in the aerial part and consequently on the grain yield (Vejan et al., 2016). Calculating root development as one of the most important predictor variables in the model provides an insight into the mechanism based on which microbial biofertilisers improve crop productivity: producing phytohormones that can stimulate root development, the inoculated microorganisms facilitate a plant to access more resources in the volume of soil explored.

The regression model showed a high level of explanatory power ($R^2 = 0.778$), indicating that predictors that were selected account for the majority of the factors contributing to crop productivity in this study. The 22.2% not accounted for in this model may be attributed to other factors (site-specific climatic factors, soil texture, presence of particular pathogens, etc.) that could not be measured.

But the analysis of the results shows that the most significant factors influencing yield are microbial biomass carbon, nutrient availability, PGPR consortium application, and root development, which provide clear indication and guide to make optimal recommendations for biofertiliser application strategies. The results obtained would be useful in developing work that introduces microbial inoculants aiming to maximise crop productivity; however, this should be based on concentrates that increased the biological activity and amount of nutrients available to plants in addition to treatments that stimulated root growth. Finally, the robustness of the PGPR consortium in the regression model indicates that such multi-strain formulations could be considered a good alternative to stable improvement in yield in different agroecologies.

Correlation Analysis

A Pearson correlation analysis was performed to further elucidate the relationships between the key soil, plant and efficiency parameters. From this analysis, the strength and the direction of association between two variables can be quantified, and causal mechanisms relating to the observed crop productivity improvement can be understood. The correlation matrix revealed some significant positive correlations, indicating linkages between the various biological processes which are enhanced by the practices of microbial biofertilisers and PGPR.

The Biofertiliser Efficiency Index had a very high positive correlation with the crop productivity ($r=0.85$, $P<0.001$). This index combines the effects of microbial inoculation on soil fertility, nutrient availability and plant growth and is a complete assessment of the efficacy of the treatment. The same result holds true for the crop yield; resulting treatments with better soil-plant health as measured by the overall high correlation coefficient were also correlated with larger crop yield. Results supported the Biofertilizer Efficiency Index as a good tool to determine the effect of microbial inoculation on the agronomic performance and endorsed the result that the effect of microbial inoculation was manyfold as well as synergistic (Bhardwaj et al., 2014).

The Microbial Activity Index (measurement of soil microbes activity) was also significantly positively correlated to several of the sustainability indicators. That is, there was a significant relationship between soil microbial activity and nutrient-use efficiency with a correlation coefficient of $r=0.81$ at a significant level of $p<0.001$. This result directly validates a correlation between increased and varied activity in the soil microbial community and increased ability of the plant to access and utilise available nutrients and less need of external addition of artificial fertiliser (Vejan et al., 2016). The correlation between soil microbial activity and plant growth was also important ($r=0.79$, $p<0.001$); this indicates that there was indeed a direct link between biological stimulation of soil rhizosphere and improved plant vigour and development. The results are in line with the established beneficial effect of PGPR on the production of phytohormones and nutrients solubilisation, both of which stimulate root and shoot growth (Basu et al., 2021).

Then, further analysis revealed that there were also significant correlations, and it is known that there is a cascade effect of microbial inoculation. The nutrient availability, for instance, was highly correlated with the development of roots ($r=0.76$, $p<0.001$) and with the content of

chlorophyll ($r=0.74$, $p<0.001$). This indicates that the new nutrient production, due to the activities of microorganisms, is beneficial for both root development below ground and for photosynthesis above ground and consequently for the plant productivity as a whole. The correlation of the Plant Growth Index with the Crop Productivity Index was also very high ($r=0.83$, $p<0.001$), thereby supporting the association between augmentation of plant morphological and physiological traits and augmentation of yield. Table 5 summarises the interrelationships that are shown in the correlation coefficients between the most important composite indices.

Table 5. Pearson correlation coefficients among key sustainable crop production indices.

Index	Biofertilizer Efficiency Index	Soil Activity Index	Microbial Activity Index	Nutrient-Use Efficiency	Plant Growth Index	Crop Productivity Index
Biofertilizer Efficiency Index	1.00	0.82**		0.79**	0.81**	0.85**
Soil Microbial Activity Index	0.82**	1.00		0.81**	0.79**	0.78**
Nutrient-Use Efficiency	0.79**	0.81**		1.00	0.76**	0.80**
Plant Growth Index	0.81**	0.79**		0.76**	1.00	0.83**
Crop Productivity Index	0.85**	0.78**		0.80**	0.83**	1.00

Note: ** indicates significance at $p < 0.001$.

The results of the high correlation of the overall indices suggest the universal nature of the provision of benefits due to the use of microbial biofertilisers and PGPR. These treatments do not seem to impact a single parameter but rather seem to trigger a chain reaction of beneficial effects that act on all four parameters, biological activity, nutrient cycling, plant growth and crop yield. As per this particular set of experiments, the correlation between the Biofertiliser Efficiency Index and crop productivity was very high ($r=0.85$), which means that this index may be a handy screening and selection tool in future experiments and fertiliser application. Furthermore, a strong correlation between nutrient-use efficiency ($r = 0.81$) and soil microbial activity suggests that fostering healthy soil microbiomes could also be a good strategy for making crop production more sustainable by reducing synthetic fertiliser use (Itelima et al., 2018). The findings of these correlation results corroborate the result obtained in regression analysis and further establish the importance of soil biological activity and nutrient availability as the two key parameters to improve the crop productivity.

Principal Component Analysis

A Principal Component Analysis (PCA) was also sought to gain further insight into the structure of the data and to identify the principal axes of data variation in the soil, plant and efficiency properties. Dimensionality reduction is a kind of multivariate technique to convert a matrix of many correlated variables to a few unrelated variables. The above-mentioned important variables, i.e., SMC, NAN and RAN, root growth, chlorophyll level, crop output, and all comprehensive measures, were standardised and then analysed.

We could identify three major components by the PCA, with the eigenvalue of each being greater than 1, that explained 84.4% of the total variance in the data. These three together have a high cumulative variance, which means that these three represent a parsimonious description of the intricate relationships among soil fertility, plant growth and crop productivity. The first principal component (PC1) explained 40.8% of the variance, and it was highly correlated with the soil microbial activity and nutrient availability variables. High positive loadings on PC1 were soil microbial biomass carbon, soil enzyme activity, available N, available P and Microbial Activity Index. Hence, it is called the 'Soil Biological Fertility' factor, which is soil's capacity to jump-start nutrient cycling and biological activities vital to plant growth (Bhardwaj et al., 2014).

The second principal component (PC2) accounted for 27.5% of the variance and mainly included plant growth and crop productivity-related variables. PC2 was highly loaded by root length, plant height, leaf area index and chlorophyll, all having positive loadings.

Indices measured are content, crop yield and PGI (Plant Growth and Crop Productivity indices). This component is clearly a "Plant Performance and Yield" dimension, as the outcomes are clearly agronomic, from the microbial treatments. Soil biological variables (PC1) could be differentiated from plant performance variables (PC2), and both parameters were highly correlated, but the aspect by which they contribute to the overall system response under various levels of biofertiliser application varied. The third principal component (PC3) explained 16.1% of the total variance, and the variables related to resource-use efficiency and sustainability were correlated with PC3, such as the Nutrient-Use Efficiency Index and the Agricultural Sustainability Index. This can be considered as a "Resource Efficiency and Sustainability" dimension that is the ability of the cropping system to produce high yields with fewer external resources.

The results from PCA were summarised and displayed in Table 6, showing the percentage of variance, important variables for each component and the eigenvalues of each component. The three different components identified prove a clear conceptual understanding of the multi-faceted effects of various microbial biofertilisers and PGPR. The first component (PC1) focusses on inoculations that maximise the benefits from the soil, because of their impact on soil biological health, while the second (PC2) emphasises direct correlation between soil benefits and improved plant growth and yield. The third (PC3) will focus on the sustainability aspect of the treatments; not only is productivity increased, but the treatments are also proving to be more efficient in terms of resource use. This is a 3-D concept and a model suitable for the

holistic method of action in sustainable agriculture, where it is necessary to balance productivity and environmental protection (Altieri & Rosset, 1996).

Table 6. Results of Principal Component Analysis showing eigenvalues, variance explained, and key associated variables for the three major components.

Component	Eigenvalue	Variance Explained (%)	Cumulative Variance (%)	Key Associated Variables (Positive Loadings)
PC1	5.71	40.8	40.8	Soil microbial biomass carbon, enzyme activity, available N, available P, Microbial Activity Index
PC2	3.85	27.5	68.3	Root length, plant height, LAI, chlorophyll content, crop yield, Plant Growth Index, Crop Productivity Index
PC3	2.25	16.1	84.4	Nutrient-Use Efficiency Index, Agricultural Sustainability Index

It is concluded that the result of the PCA is consistent and can be used to complement the regression analysis and the correlation analysis. The soil microbial activity and nutrient availability were well represented in the PC1, the same as in the regression analysis, in which microbial biomass carbon proved to be the greatest determinant of crop productivity. In the same way, the distinct separation of the plant growth and yield variables in PC2 is evidence that increased soil fertility is sufficiently reflected in improved agronomic results. Significant among those are the newly added units on resource efficiency (PC3), which acknowledge that one of the drawbacks of microbial biofertilisers is that one cannot wish for yield and efficiency at the same time as it is possible with the use of biofertilisers. This outcome is significant for designing sustainable cropping systems, as soil biological activity can create a Climate Smart Agriculture win-win scenario – “sustainable agriculture and improved biological activity, both in the agricultural and the environmental dimension” (Aloo et al., 2022). Overall, the PCA provides a powerful statistical toolbox to understand the diverse benefits microbial biofertilisers and PGPR may derive and underlines their potential to play an instrumental role in harnessing the more sustainable and productive agricultural systems.

Comparative Findings

The results obtained from the treated plots were compared with the untreated or control plot to judge the effectiveness of microbial biofertilisers and PGPR versus the conventional method in terms of practical use. The overall magnitude of the improvement due to microbial inoculation was quantified by this comparison, which is an aggregate of data for all ten treatments and three cropping seasons. Results indicated that agricultural performance on all the dimensions studied is significantly increased with the addition of microbial biofertilisers and PGPRs (Table 7).

Table 7. Comparative percentage improvement of microbial biofertilizer and PGPR treatments over the conventional control.

Performance Indicator	Percentage Improvement over Control
Microbial Activity	29.8%
Nutrient-Use Efficiency	28.3%

Performance Indicator	Percentage Improvement over Control
Soil Fertility	27.4%
Crop Yield	26.7%
Plant Growth	25.9%

Conversion of biofertiliser treatment with the microbial biofertiliser and PGPR resulted in a 29.8% higher biofertiliser microbial activity index compared to the conventional fertiliser application (non-inoculated control treatment). The result corresponds to a remarkable increase in the soil biota, which is an important component of soil health and nutrient cycles (Bhardwaj et al., 2014). Nutrient-use efficiency (UPE) achieved with treatment was 28.3% higher, which means an improved nutrient-use efficiency and a higher production per unit of nutrient available, thus decreasing the potential for loss of nutrients to the environment (Vejan et al., 2016). This efficiency is one of the remarkable benefits of microbial biofertilisers compared to synthesised fertilisers, which may not be as efficient due to the problem of volatilisation, leaching and runoff.

Furthermore, the application of microbial inoculant resulted in a 27.4% improvement in the Soil Fertility Index (SFI), which is based on soil organic carbon (SOC), available nutrients and soil enzymes. An improvement in the innate fertility of the soil as such, as happened in the above experiments, is a step towards a more lasting, productive and stable soil resource base (Basu et al., 2021). Production of the treated and the check was recorded, and it was found that the production of treated plots increased by 26.7 per cent compared with the check, which shows the potential of the use of these biological inputs to address food security, the most immediate agronomic result. Lastly, the treated plants achieved 25.9% higher plant growth (root development, plant height, leaf area and chlorophyll content) and were healthier and more vigorous during the growing season (Itelima et al., 2018).

Comparative results from the use of microbial bio-fertilisers and PGPR could be useful as a robust quantitative evaluation of the advantages for their use as a part of the Sustainable Crop Production System. The consistent increases of performance in all five key KPIs (from 25.9% to 29.8%) show that the benefits are comprehensive and accumulative, instead of only in a single aspect of the soil-plant system. The greatest relative gain was for microbial activity, the underpinning to all benefits, and the other benefits to yield and nutrient use efficiency directly address the twin crises of food production and environmental impact. The results strongly argue that biofertilisers and PGPR could be a promising alternative to chemical fertilisers and may also be a viable and sustainable approach to realise the paradigm shift towards environmentally more friendly practices that reduce reliance on synthetic chemical fertilisers while maintaining soil health and crop production (Shahwar et al., 2023).

Discussion

From the biogeological concepts towards nutrient management practices in the agricultural fields, this multi-harvest and multi-location field study generated rich empirical data that indicates the microbial biofertilisers and PGPR as a good avenue to enhance the sustainability of agriculture. This may therefore alter significantly the nutrient dynamics of agricultural soils, for they result in a 29.8% increase in soil microbial biomass carbon and a 27.4% increase in soil nutrient availability and can shift soils from externally driven to a more biologically mediated and self-regulating environmental system. These results indicate the possibility of providing a real value assessment tool to the country for reducing the national subsidy of synthetic chemical fertilisers and also reducing the environmental ecological impact of such fertilisers, such as eutrophication and GHGs (Bhardwaj et al., 2014), by promoting PGPR consortia preferably along with organic manures. The results of this research can be

used by relevant practitioners like farmers and agronomists, as the PGPR consortium always showed the best result, which is in line with the concept of functional complementarity between microbial species (Basu et al., 2021) as an alternative to single-strain inoculants.

However, these conclusions have some methodological limitations that need to be noted. Only research farms were included in the study and the socio-economic and management problems that smallholder farmers encounter in these developing areas could not be fully dealt with because high quality inoculants and storage facilities are scarce. Additionally, the three-season period is longer than other similar studies, but it is too short to reflect the apparent long-term build-up of soil organic matter or the possibility of microbial community changes over time that might impact efficacy of the treatments. While the method used to determine an "outlier" (Interquartile Range) may be the norm, some "outliers" might have been considered as such that did not necessarily reflect a sub-optimal interpretation of the effects of treatments under a specific agroecological context. Also, the economic viability of the treatments was not evaluated, such as the cost-benefit of manufacturing and application of biofertiliser and synthetic fertilisers, which is an important consideration for scaling up the use of biofertilisers.

A few areas inadequately studied should, therefore, be explored to support the above findings. Long-term (5-10 years) farmer-managed long-term trials are needed to determine if the benefits mentioned are long-term and if there are cumulative benefits in soil health. Therefore, biofertilisers should be included in such studies along with economic analysis to estimate the break-even stage for the farmers and when the biofertiliser is most economical. Future research could aim to investigate possible mechanisms for synergism between the PGPR in the consortium, which may be done using metagenomics or metatranscriptomics that could provide information regarding metabolic pathways and signalling interactions involved in the enhanced plant growth promotion (Vejan et al., 2016). The result of regression shows the microbial biomass carbon can be predicted with a goodness of $\beta = 0.47$, which may be indicative that if the parameter could be measured in the field and in a quick manner, it could be a useful tool for farmers to gauge the success of inoculation activity in real time. Finally, there is a lack of information on the performance of biofertiliser treatments in response to changes in climate, such as more frequent droughts and/or greater temperatures, which means more work is required to ensure that the biological alternatives are able to function in the future climate change climate.

Conclusion

The objective of the field investigation was to evaluate the opportunity of using microbial biofertilisers along with Plant Growth-Promoting Rhizobacteria (PGPR) to improve in multi-site and multi-season agricultural production, soil fertility and nutrient-use efficiency (NUE), as well as reduce reliance on synthetic fertiliser. Results provide good evidence for this goal; in fact, the treatments resulted in a 26.7% increase in crop yield, 28.3% in nutrient use efficiency, and 29.8% in soil microbial activity in comparison to conventional management. What distinguishes us from others in the field is that we identified this trend, and we found that soil microbial biomass carbon is the single best predictor of crop productivity (standardised $\beta = 0.47$), so to understand the impact of soil biology and why it's important to sustainable intensification, it's not just about increased yield; it's about soil microbial biomass C. The overall superior performance of multi-strain (PGPR consortium) and integrated treatments (PGPR + organic amendments) on each measured index is an indication that multi-strain formulation, especially those integrated with organic amendments, is a proven approach to attain better and consistent agronomic results.

There are still a number of key knowledge gaps for future research. This three-season experiment, although longer than some other similar investigations, still is not a sufficient length of time to consider how the long-term trend of soil organic matter accumulation will change, or whether there is potential

for changes in the composition of the resident microbial community to impact efficacy at the decadal scale. Future research should be directed towards managing farmer trials over a period of at least 5-10 years to understand the limit for which biofertilisers are most cost-effective for smallholder farmers. As seen in our regression model, the interdependence of microbial biomass carbon is high, so if simple and quick field-detectable tools to measure microbial biomass carbon can be developed, they would offer farmers to assess their inoculation applications in real time (i.e. during cultivation). Moreover, there are no known studies about how biofertiliser responds to the different climate change scenarios, such as climate aridity, increased drought frequency and higher temperature, which are poorly described and a critical research area to be explored to make these biological technologies resilient to the future environment.

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