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Article

Synergy Between Microbial Inoculants and Mineral Fertilization to Enhance Yield and Nutritional Quality of Maize on the Peruvian Coast

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Abstract: Hard yellow maize is a crucial crop in Peruvian agriculture, playing a significant role in food security and livestock production. However, intensive fertilization practices in agronomic management have negatively impacted soil health. To explore more sustainable agricultural technologies, researchers have investigated using microorganisms to enhance plant growth. This study assessed the synergistic effects of microbial inoculants and mineral fertilization on INIA 619 and Dekal B-7088 maize varieties' yield and nutritional quality. A split-plot design was employed, incorporating four inoculation treatments—no inoculant, *Bacillus subtilis*, *Trichoderma viride*, and *Pseudomonas putida*—combined with fertilization levels of 0%, 50%, 75%, and 100%. The findings revealed that *Bacillus subtilis* boosted yields by 13.1% in INIA 619 and 55.5% in Dekal B-7088. Additionally, combined with 100% fertilization, microbial inoculation increased protein content by 47% and carbohydrates by 6% in INIA 619, while maintaining nutritional quality with 75% fertilization. Similarly, in Dekal B-7088, inoculation with total fertilization enhanced protein content by 54% and fiber by 27%. These results demonstrate that microbial inoculation can reduce mineral fertilization by up to 25% while sustaining high yields and improving the nutritional quality of maize.

Keywords: hard yellow maize; *Trichoderma*; *Bacillus*; *Pseudomonas*; biofertilizers; grain protein

1. Introduction

Hard yellow maize (*Zea mays* L.) is one of the most important crops globally, known for its high productivity per unit area compared to other cereals [1]. Peru accounts for 14% of the total agricultural area [2], with over 275,000 hectares sown last year, resulting in 883,000 tonnes of production [3]. This crop is crucial in Peru's agricultural economy, serving as a significant input for human consumption and animal feed [4].

Agronomic management of maize typically involves conventional fertilization aimed at optimizing soil nutrient availability to promote vigorous and rapid plant development [5]. In global agriculture, inorganic fertilizers play a critical role in increasing the yield of crops like maize [6]. However, intensive fertilizer use negatively impacts soil health by reducing organic matter, impairing water infiltration, and leading to nutrient imbalances, soil acidity, and contamination [7]. Consequently, there is an ongoing search for innovative and sustainable agricultural practices to enhance maize's yield and nutritional quality [8].

Microbial soil inoculant implementation offers a novel, promising, and environmentally friendly approach [9,10]. Microorganisms play a vital role in soil biogeochemical processes, supporting

ecological balance [11]. Microbial inoculant technology, which involves the artificial incorporation of beneficial microorganisms, aims to improve soil quality by enhancing enzymatic activity in the rhizosphere and facilitating nutrient release [12], positively impacting the nutritional quality of crops [13]. Additionally, inoculants can protect plants by inducing resistance to diseases caused by pathogens and environmental stresses [9]. As a result, their use has become widespread in both natural and organic agriculture [14].

Among the most extensively studied microorganisms are rhizobacteria, specifically the genera *Bacillus* and *Pseudomonas*, which inhabit the plant rhizosphere [15]. Known as Plant Growth-Promoting Rhizobacteria (PGPR) [16], these bacteria biostimulate crop growth by producing plant hormones such as indole-3-acetic acid (IAA), gibberellins, and cytokinins [17]. Additionally, they contribute to the solubilization of nutrients like phosphorus and potassium by releasing siderophores [18] and secreting antibiotic substances that inhibit pathogenic fungi and bacteria [19]. The application of PGPR in maize cultivation has positively affected grain quality and overall yield [20].

Fungi of the genus *Trichoderma* also employ various mechanisms that support plant growth and development [21]. These mechanisms include the synthesis of phytohormones like indole-3-acetic acid and other auxin analogs that promote root development, enhance nutrient uptake [22], and produce essential vitamins [23]. *Trichoderma* fungi also contribute to nutrient solubilization by secreting organic acids that lower soil pH, thereby increasing phosphorus availability [24].

Microbial inoculant application presents a promising approach to enhancing soil health and, in turn, maximizing agricultural productivity and maize quality. This study evaluates the effects of microbial inoculation with *Bacillus subtilis*, *Pseudomonas putida*, and *Trichoderma viride*, complemented with four levels of mineral fertilization (0%, 50%, 75%, and 100% of the recommended dose of N, P₂O₅, and K₂O at 240-120-140), on the yield and nutritional quality of the grain in two commercially significant hard yellow maize varieties in Peru. This research contributes to pursuing sustainable and innovative agricultural practices that optimize soil fertility.

2. Materials and Methods

2.1. Trial Set-Up

The research was conducted at the Universidad Nacional Agraria La Molina (UNALM), located in La Molina district, Lima Province, Lima Department, Peru, on an experimental plot situated at 76° 56' 21" W and 12° 04' 55" S, at an elevation of 247 masl. The site experiences no rainfall (0 mm) and has an average temperature of 19.89°C (June-December) and relative humidity of 79.43%. These climatic data were obtained from the Alexander Von Humboldt Meteorological Station at UNALM (Figure 1).

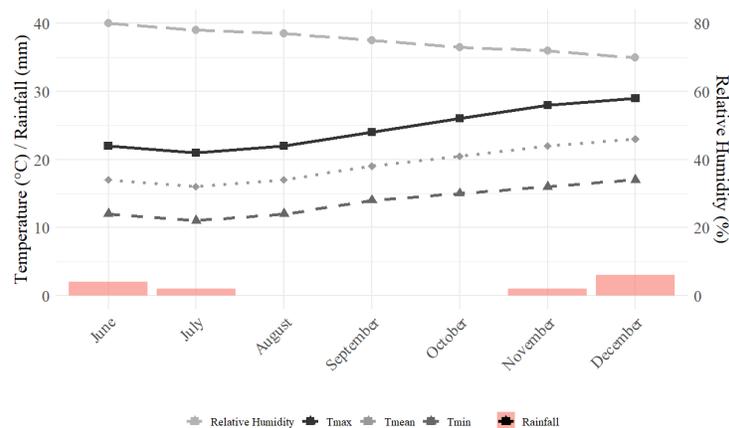


Figure 1. Climatological data of the project site on a monthly scale. Includes precipitation (rainfall), maximum temperature, minimum temperature, and relative humidity.

The experimental site had been primarily used for agriculture, with hard yellow maize cultivation over the past three years. It is worth mentioning that before soil preparation, the corn residues from the previous season were incorporated. Soil preparation included initial irrigation,

plowing, harrowing, and furrow formation. Notably, the first irrigation of the season method was applied.

The hard yellow maize hybrids used were the INIA 619 Simple Megahybrid, developed by the National Institute for Agrarian Innovation (INIA) at the Vista Florida Agricultural Experimental Station in Chiclayo between 2006 and 2009, and the Dekal B-7088 Simple Hybrid, developed by Bayer®.

The sowing occurred in the first week of June 2023, outside the regular season, with a spacing of 0.30 m between hills and 0.8 m between furrows. The trial was conducted over a 1440 m² area, divided into 48 plots of 30 m² (5 m x 6 m), each containing 7 furrows per variety.

2.2. Soil Characteristics

A soil characterization analysis revealed a sandy loam texture with 56.1% sand, 21.3% silt, and 22.6% clay [25]. The soil had a pH of 8.0 [26] and an electrical conductivity (EC) of 42.2 mS·m⁻¹. Exchangeable cations were measured as 7 meq·100 g⁻¹ Ca²⁺, 2.9 meq 100·g⁻¹ K⁺, 2.2 meq·100 g⁻¹ Mg²⁺, and 0.5 meq·100 g⁻¹ Na⁺, with a cation exchange capacity (CEC) of 12.6 meq·100 g⁻¹ [25]. The total soil carbon content was 1.7%, organic carbon was 0.79%, and organic matter was 1.5% [27]. Total nitrogen content was 0.03%, and available phosphorus was measured at 28.2 mg·kg⁻¹ [25].

2.3. Experimental Design

A randomized complete block design with a split-plot arrangement was employed. Three microbial species and a control without microorganisms were assigned to the main plots, while four levels of complete mineral fertilization were applied to the sub-plots. This setup resulted in 16 treatments with three replicates, generating 48 experimental units for each maize variety (INIA 619 and Dekal B-7088). Each variety was treated as an independent experiment. The specific treatment descriptions are presented in Table 1.

Table 1. Microorganisms and fertilization doses for the different treatments for maize hybrids.

Treatment	Main Plot Microorganisms	Subplot Fertilization (%)
1		0 (0-0-0) *
2		50 (120-60-70) *
3	Without inoculation (M0)	75 (180-90-105) *
4		100 (240-120-140) *
5		0
6		50
7	<i>Bacillus subtilis</i> (B)	75
8		100
9		0
10		50
11	<i>Trichoderma viride</i> (T)	75
12		100
13		0
14		50
15	<i>Pseudomonas putida</i> (P)	75
16		100

* The values in parenthesis represent the percentage of the mineral fertilizer dose of N, P₂O₅, and K₂O.

2.4. Crop Fertilization

The fertilizers used were diammonium phosphate, urea, and potassium chloride, serving as sources of N, P₂O₅, and K₂O, respectively. They were applied one month after sowing, when the plants were at the V4 phenological stage, and adjusted according to the specific treatments for each plot. Notably, nitrogen was applied in two stages, with the second application conducted one month after the first.

2.5. Microorganism Inoculation

Microorganism inoculation was performed twice during the trial: the first ten days after sowing and the second fifty days after sowing. The methodology involved applying each microorganism using a specialized fumigation backpack to prevent contamination. Inoculation was conducted in a ring around the base of the plant, positioned 5 cm from the neck.

For *Pseudomonas putida* and *Bacillus subtilis* inoculation, pure cultures of each strain were prepared and incubated in nutrient broth for three days at 28 °C, achieving a concentration of 10⁹ CFU·mL⁻¹. 1% (v/v) dilutions were made for each microorganism from these cultures.

For *Trichoderma viride* (Strain SCT-11), a spore suspension was prepared by washing maize inoculated with the fungus. Four 800 g bags of the product Trichomax®, containing a concentration of 1 × 10¹² conidia per kg, were used.

Subsequently, 2 L of concentrated suspensions of *Pseudomonas*, *Bacillus*, and *Trichoderma viride* were each diluted in three separate cylinders containing 200 L of non-chlorinated water, with pH adjusted to 7.02 and electrical conductivity (EC) set to 60.97 mS·m⁻¹.

2.6. Phytosanitary Management

A pre-emergent broadleaf herbicide, atrazine 50% suspension concentrate, was applied alongside the fungicide combination of azoxystrobin (250 g·kg⁻¹) and tebuconazole (500 g·kg⁻¹), as well as the insecticides spinosad (12% soluble concentrate) and emamectin benzoate (19 g·L⁻¹ concentrate).

2.7. Evaluated Parameters

Harvesting was conducted once the maize reached full physiological maturity, with grain moisture falling below 14%, approximately seven months after planting. Ear characteristics were assessed, including length, diameter, number of rows per ear, number of grains per row, ear weight, total grain weight, and cob weight. The yield was calculated as follows Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) [28]:

$$Yield \left(\frac{kg}{ha} \right) = \frac{total\ grain\ weight - Moisture\ content}{Area} \times 10 \quad (1)$$

The Physicochemical Laboratory of the Institute for Nutritional Research conducted a proximate analysis to analyze nutritional quality. The tests included protein content [29], ash [30], fat [31], fiber [32], carbohydrates [33], total energy, and energy contributions from fat, carbohydrates, and protein (in kcal).

2.8. Statistical Analysis

The factors of different treatments were analyzed using block complete randomized design in Split-Plot array ANOVA (alpha = 0.05) from R software version 4.3.1, following verification of data normality assumptions and homogeneity of variances. Multiple comparisons of means were made with the Least Significant Difference Test (alpha = 0.05) using the LSD.test function from the agricolae library in R [34].

3. Results

3.1. Ear Characteristics and Yield

The results indicate no interaction between study factors for the INIA 619 and Dekal B-7088 varieties. However, a significant effect of microbial inoculation on ear weight ($p < 0.05$) and grain weight ($p < 0.05$) was observed in the INIA 619 variety. Inoculated treatments produced heavier ears, with increases of 17.5%, 16.8%, and 12.2% compared to the control when inoculated with *Pseudomonas putida*, *Bacillus subtilis*, and *Trichoderma viride*, respectively. However, differences among these treatments were not significant. Similarly, grain weight increased by 17.6% with *Pseudomonas putida* inoculation, 12.4% with *Trichoderma viride* inoculation, and 18.23% with *Bacillus subtilis* inoculation relative to the control, with no significant differences observed between microorganisms.

Regarding mineral fertilization, significant effects were observed on the number of grains per row ($p < 0.05$), ear weight ($p < 0.01$), and grain weight ($p < 0.01$) in the INIA 619 variety, with the highest values achieved in treatments with 100%, 75%, and 50% fertilization. No significant differences were found among these fertilization levels.

Table 2. Ear Characteristics of INIA 619 Variety.

Factor	EL (cm)	ED (cm)	RE	GR	EW (g)	GW (g)	CW (g)
Factor 1. Microorganisms							
M0	15.48 ± 1.31	4.01 ± 0.15	13.65 ± 0.35	29.22 ± 2.55	158.72 ± 20.6 b	135.15 ± 17.14 b	23.57 ± 4.06
B	15.5 ± 0.88	4.04 ± 0.07	13.75 ± 0.4	29.33 ± 2.29	185.31 ± 18.16 a	159.79 ± 16.79 a	25.52 ± 2.15
T	15.63 ± 1.21	4.02 ± 0.1	13.77 ± 0.45	29.21 ± 2.74	178.19 ± 18.44 a	151.86 ± 16.1 a	25.66 ± 3.01
P	15.6 ± 0.89	4.08 ± 0.14	13.9 ± 0.34	29.57 ± 2.73	186.48 ± 17.66 a	158.96 ± 15.03 a	26.69 ± 4.44
Factor 2. Fertilization Dose							
(%)							
0	14.99 ± 1.33	4.04 ± 0.15	13.86 ± 0.33	27.34 ± 2.11 b	160.85 ± 20.42 b	136.75 ± 17.47 b	24.1 ± 3.48
50	15.97 ± 1.07	4.05 ± 0.1	13.91 ± 0.22	30.31 ± 2.53 a	182.11 ± 19.57 a	155.78 ± 17.32 a	26.33 ± 2.62
75	15.77 ± 0.69	4.04 ± 0.09	13.65 ± 0.38	29.88 ± 1.93 a	179.53 ± 17.87 a	154.39 ± 15.27 a	24.31 ± 3.33
100	15.47 ± 0.9	4.01 ± 0.14	13.65 ± 0.52	29.8 ± 2.51 a	186.2 ± 20.35 a	158.82 ± 18.02 a	26.7 ± 4.42
Interaction	0.95	0.61	0.51	0.86	0.88	0.92	0.66

Note: Ear length = EL, Ear diameter = ED, number of rows per ear = RE, number of grains per row = GR, ear weight = EW, grain weight = GW, and cob weight = CW. Husk weight was not considered in the ear weight (only cob + grains weight). Means with the same lowercase letter are statistically equal according to Tukey's test 0.05. ± Standard deviation.

In the Dekal B-7088 variety, significant differences were observed in ear length ($p < 0.05$), ear diameter ($p < 0.05$), ear weight ($p < 0.01$), and grain weight ($p < 0.01$) due to the effect of mineral fertilization. The highest values for these traits were achieved with 100% fertilization, although these results were statistically similar to those obtained with the 75% dose. Conversely, in the absence of fertilization, the lowest values for these variables were recorded.

Table 3. Ear Characteristics of Dekal B-7088 Variety.

Factor	EL (cm)	ED (cm)	RE	GR	EW (g)	GW (g)	CW (g)
Factor 1. Microorganisms							
M0	11.1 ± 4.01	4.39 ± 0.1	16.64 ± 0.47	31.83 ± 2.79	180.53 ± 14.52	164.3 ± 13.25	15.33 ± 2.03

B	11.47 ± 4.31	4.44 ± 0.1	16.81 ± 0.7	33.11 ± 1.76	191.14 ± 18.02	175.09 ± 16.4	16.42 ± 3.76
T	11.29 ± 4.22	4.42 ± 0.13	16.65 ± 0.37	32.55 ± 1.19	187.55 ± 18.13	172.22 ± 16.29	16.22 ± 3.64
P	11.13 ± 4.06	4.39 ± 0.12	16.73 ± 0.71	32.46 ± 1.27	185.66 ± 11.65	169.24 ± 10.3	16.05 ± 1.89
(%)	Factor 2. Fertilization dose						
0	13.16 ± 0.42 b	4.35 ± 0.11 b	16.36 ± 0.35	31.71 ± 1.31	176.38 ± 12.29 b	161.65 ± 10.97 c	14.73 ± 1.61
50	13.4 ± 0.69 b	4.41 ± 0.11 ab	16.64 ± 0.64	32.84 ± 2.37	184.32 ± 13.81 ab	168.09 ± 12.6 bc	16.86 ± 3.6
75	13.48 ± 0.71 ab	4.43 ± 0.1 a	16.86 ± 0.61	32.05 ± 1.65	189.8 ± 14.99 a	172.94 ± 13.9 ab	16.22 ± 3.79
100	13.92 ± 0.64 a	4.44 ± 0.11 a	16.98 ± 0.49	33.36 ± 1.7	194.38 ± 17.36 a	178.17 ± 15.85 a	16.21 ± 1.8
Interaction	0.55	0.39	0.22	0.95	0.83	0.66	0.85

Note: Ear length= EL, Ear diameter = ED, number of rows per ear = RE, number of grains per row = GR, ear weight = EW, grain weight = GW, and cob weight = CW. Husk weight was not considered in the ear weight (only cob + grains weight). Means with the same lowercase letter are statistically equal according to Tukey's test 0.05. ± Standard deviation.

The yield of the INIA 619 variety was significantly affected by microbial inoculation ($p < 0.05$). *Bacillus subtilis* inoculation resulted in the highest yield, with a 13.1% increase compared to the control. Similarly, *Pseudomonas putida* also improved yield, with a 10.3% increase relative to the control. In contrast, *Trichoderma viride* inoculation did not produce significant differences from the non-inoculated treatment (Figure 2A). Regarding mineral fertilization, results indicated that fertilizers significantly increased yields compared to treatments without fertilization ($p < 0.01$). However, no significant differences were found between the different fertilizer doses, with the 100%, 75%, and 50% doses all resulting in comparable yields (Figure 2B).

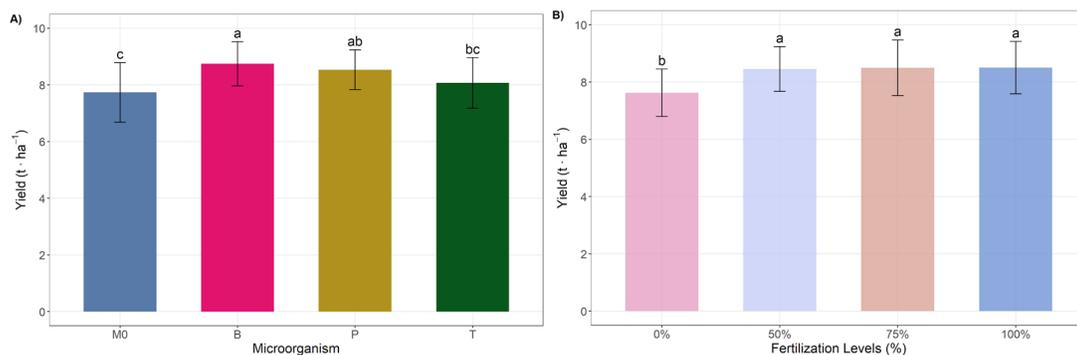


Figure 2. Hard yellow maize INIA 619 variety yield under the effect of microbial inoculants(A) and mineral fertilization (B). Means with the same lowercase letter are statistically equivalent according to Tukey's test at a 0.05 significance level.

For maize yield, no significant interaction was observed between the two study factors for either variety. However, inoculation with *Bacillus subtilis*, *Trichoderma viride*, and *Pseudomonas putida* significantly increased yield by 55.5%, 45.9%, and 40.5%, respectively, compared to the control in the Dekal B-7088 variety ($p < 0.01$) (Figure 3A). Furthermore, yield increased with the application of the total fertilizer dose, with a decreasing trend observed as the dose was reduced (Figure 3B).

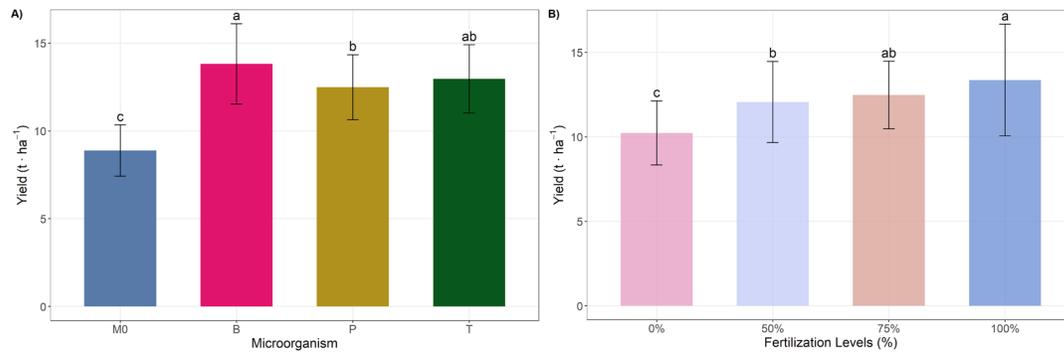


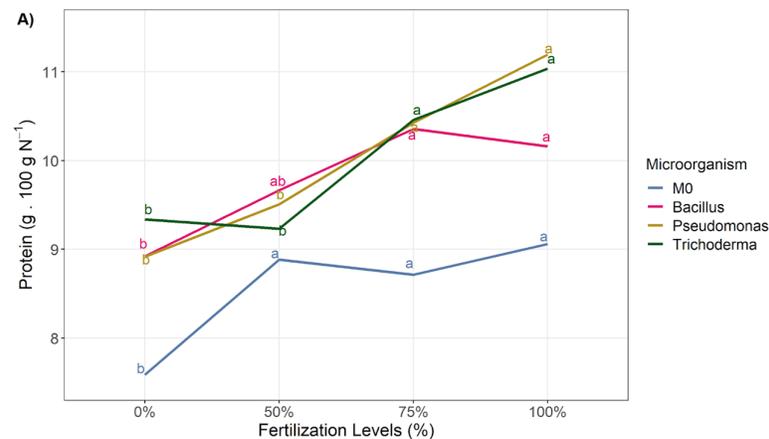
Figure 3. Hard yellow maize Dekal B-7088 variety yield under the effect of microbial inoculants (A) and mineral fertilization (B). Means with the same lowercase letter are statistically equivalent according to Tukey's test at a 0.05 significance level.

3.2. Nutritional Quality

A significant interaction between factors was observed for protein concentration ($p = 0.01$), protein energy (kcal) ($p = 0.01$), and carbohydrate concentration ($p < 0.01$) in the INIA 619 variety (Figure 4). Soil inoculation with microorganisms significantly increased protein content, protein energy (kcal), and carbohydrate levels compared to non-inoculated treatments. The combined treatments of *Pseudomonas putida* + 100% fertilization and *Trichoderma viride* + 100% fertilization showed the highest protein concentrations. However, when the fertilization dose was reduced to 75%, no significant differences were found between inoculated treatments and the control (Figure 4A).

All three microbial species significantly increased their protein energy (kcal) at the 100% fertilization dose. However, *Bacillus subtilis* produced a statistically comparable response even at the 75% fertilization rate (Figure 4B).

Additionally, carbohydrate levels declined when fertilization was reduced to 25% of the total dose in the presence of microorganisms (Figure 4C).



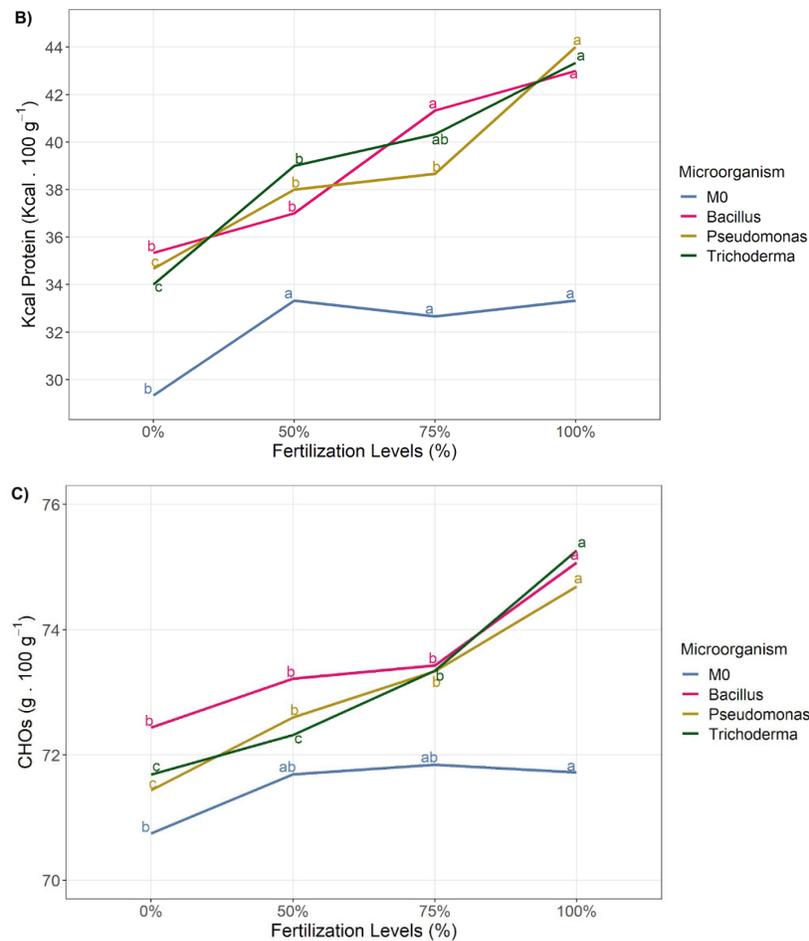


Figure 4. Interaction between mineral fertilization and microbial inoculation on (a) protein content, (b) kcal protein, and (c) fiber. Means with the same lowercase letter are statistically equivalent according to Tukey's test at a 0.05 significance level.

Table 4 presents significant differences in ash and total energy content, with the highest values recorded in grains treated with 100% fertilization. Both variables display an upward trend, where increasing fertilization levels correspond to significant increases in ash and total energy content. Carbohydrate energy (kcal) was significantly influenced by both microbial inoculation ($p < 0.01$) and mineral fertilization ($p < 0.001$), with inoculated treatments showing a 2.6% improvement over the control. Additionally, carbohydrate energy increased progressively with higher fertilization doses.

Table 4. Nutritional quality of the INIA 619 Variety.

Fertilization Dose (%)	Ash (g·100 g ⁻¹)	Fat (g·100 g ⁻¹)	Fiber (g·100 g ⁻¹)	Total Energy (kcal·100 g ⁻¹)	kcal Fat (kcal·100 g ⁻¹)	kcal Carbohydrate (kcal·100 g ⁻¹)
0	1.17 ± 0.1 c	3.46 ± 0.27	1.88 ± 0.22	359.42 ± 1.68 c	31.08 ± 2.43	284.5±5.5
50	1.24 ± 0.13 b	3.39 ± 0.37	1.85 ± 0.26	359.83 ± 1.7 bc	30.5 ± 3.18	289±5.19
75	1.25 ± 0.09 ab	3.36 ± 0.26	1.89 ± 0.19	360.75 ± 2.09 ab	30.25 ± 2.38	292.08±2.75
100	1.3 ± 0.11 a	3.33 ± 0.23	1.81 ± 0.2	361.17 ± 1.85 a	29.92 ± 2.19	296.42±4.1
Interaction	0.21	0.28	0.09	0.85	0.36	0.47

Note: Ear length= EL, Ear diameter = ED, number of rows per ear = RE, number of grains per row = GR, ear weight = EW, grain weight = GW, and cob weight = CW. Husk weight was not considered in the ear weight (only

cob + grains weight). Means with the same lowercase letter are statistically equal according to Tukey's test 0.05. \pm Standard deviation.

Nutritional quality results for the Dekal B-7088 variety showed a significant interaction between factors for protein ($p = 0.001$) and fiber ($p < 0.05$) content. Microbial inoculation improved grain protein quality, with *Trichoderma viride*, *Bacillus subtilis*, and *Pseudomonas putida* applied alongside total fertilization, increasing protein by 31%, 25%, and 26%, respectively, compared to the non-inoculated treatment (Figure 5A). Inoculated treatments combined with 100% fertilization showed a significant effect relative to the non-inoculated treatment at the same fertilization level for grain fiber. The combined inoculation treatment with *Pseudomonas putida* + 75% fertilization yielded superior fiber content to the other microorganisms at this level. Additionally, without mineral fertilization, microbial inoculation alone significantly affected fiber content (Figure 5B).

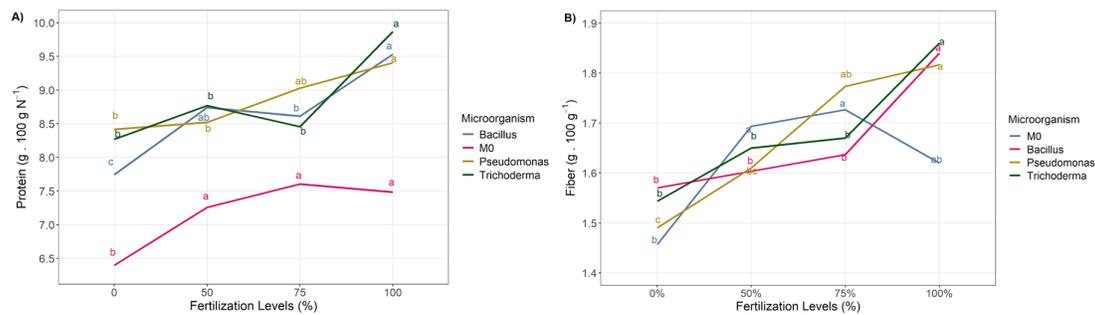


Figure 5. Interaction between mineral fertilization and microbial inoculation on (a) protein and (b) fiber content in Dekal B-7088 variety. Means with the same lowercase letter are statistically equivalent according to Tukey's test at a 0.05 significance level.

Regarding the effect of mineral fertilization, a gradual increase in ash concentration ($p < 0.01$), carbohydrate ($p < 0.01$), protein energy (kcal) ($p < 0.01$), and carbohydrate energy (kcal) ($p < 0.01$) was observed with increasing fertilization doses. The 100% dose (240-120-140 kg·ha⁻¹ of N-P-K) yielded the highest values for these variables (Table 5).

Table 5. Nutritional quality of the Dekal B-7088 Variety.

Fertilization Dose (%)	Ash (g·100 g ⁻¹)	Fat (g·100 g ⁻¹)	Carbohydrates (g·100 g ⁻¹)	Total Energy (kcal·100 g ⁻¹)	kcal Protein (kcal·100 g ⁻¹)	kcal Fat (kcal·100 g ⁻¹)	Kcal Carbohydrate (kcal·100 g ⁻¹)
0	1.09 \pm 0.09 c	3.87 \pm 0.22	72.77 \pm 0.59 d	363.92 \pm 1.88	30.33 \pm 3.23 c	33.5 \pm 2.65	290.33 \pm 2.23 d
50	1.15 \pm 0.1 b	3.78 \pm 0.31	73.55 \pm 0.58 c	362.67 \pm 1.61	33.67 \pm 1.83 b	34 \pm 2.95	294.17 \pm 2.33 c
75	1.16 \pm 0.09 b	3.9 \pm 0.31	73.75 \pm 0.63 b	363.42 \pm 1.56	35.17 \pm 2.44 b	35 \pm 2.76	295.08 \pm 8.36 b
100	1.24 \pm 0.08 a	3.87 \pm 0.28	74.6 \pm 0.57 a	363.08 \pm 1.08	37.58 \pm 2.27 a	34.83 \pm 2.33	298.5 \pm 2.35 a
Interacción	0.18	0.92	0.19	0.86	0.85	0.90	0.09

Note: Ear length= EL, Ear diameter = ED, number of rows per ear = RE, number of grains per row = GR, ear weight = EW, grain weight = GW, and cob weight = CW. Husk weight was not considered in the ear weight (only cob + grains weight). Means with the same lowercase letter are statistically equal according to Tukey's test 0.05. \pm Standard deviation.

3.3. Heatmaps

3.3.1. INIA 619

The presented heatmap for the INIA 619 variety provides an overview of treatments with microorganisms under varying doses of mineral fertilization. Significant variables were analyzed, resulting in three main clusters of treatments (Figure 6). Inoculation with *Bacillus subtilis*, *Trichoderma viride*, and *Pseudomonas putida* in combination with 100% and 75% fertilization positively influenced nutritional and yield characteristics, as shown in Clusters 2 and 3. Inoculated treatments without fertilization positively affected total energy, particularly with *Bacillus subtilis*, although performance varied across other variables (Cluster 1). Additionally, regardless of NPK presence, all treatments without inoculation were grouped in Cluster 1, where adverse effects were observed. This cluster also included inoculated treatments without fertilization.

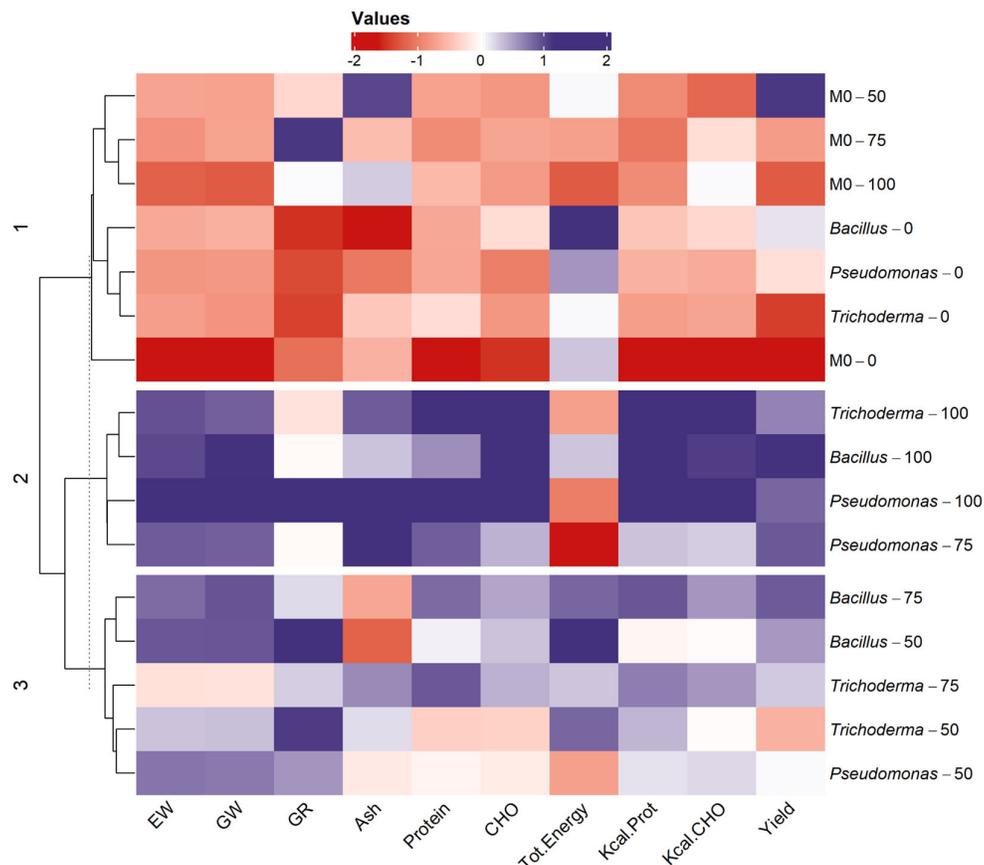


Figure 6. Heatmap with cluster analysis illustrating the combined effects of microbial inoculation and chemical fertilization on the INIA 619 variety. The Y-axis lists all treatments resulting from the combinations of factor levels, while the X-axis displays the evaluated parameters. The numbers 1, 2, and 3 denote the three identified clusters. The vertical dotted line in the dendrogram represents the cut-off level for determining these groups.

3.3.2. DEKAL B-7088

The heatmap (Figure 7) for the Dekal B-7088 variety highlights a notably positive impact of inoculated treatments with 100% fertilization on ear characteristics, yield, and protein quality (Cluster 2). Treatments without fertilization showed the poorest results across the evaluated parameters, with *Pseudomonas putida* inoculation being the only exception, as it positively influenced grain protein content (Cluster 1). However, it did not stand out in other variables. Cluster 3 displayed a wide variability in treatment effects. Non-inoculated treatments generally showed no positive impact on grain quality or yield; however, the M0 + 100% fertilization treatment achieved the highest

carbohydrate content and kcal values within this cluster. Additionally, the *Trichoderma viride* inoculation combined with 75% fertilization demonstrated a favorable effect on ear diameter, while *Pseudomonas putida* at the same fertilization level had a negative impact on ear length.

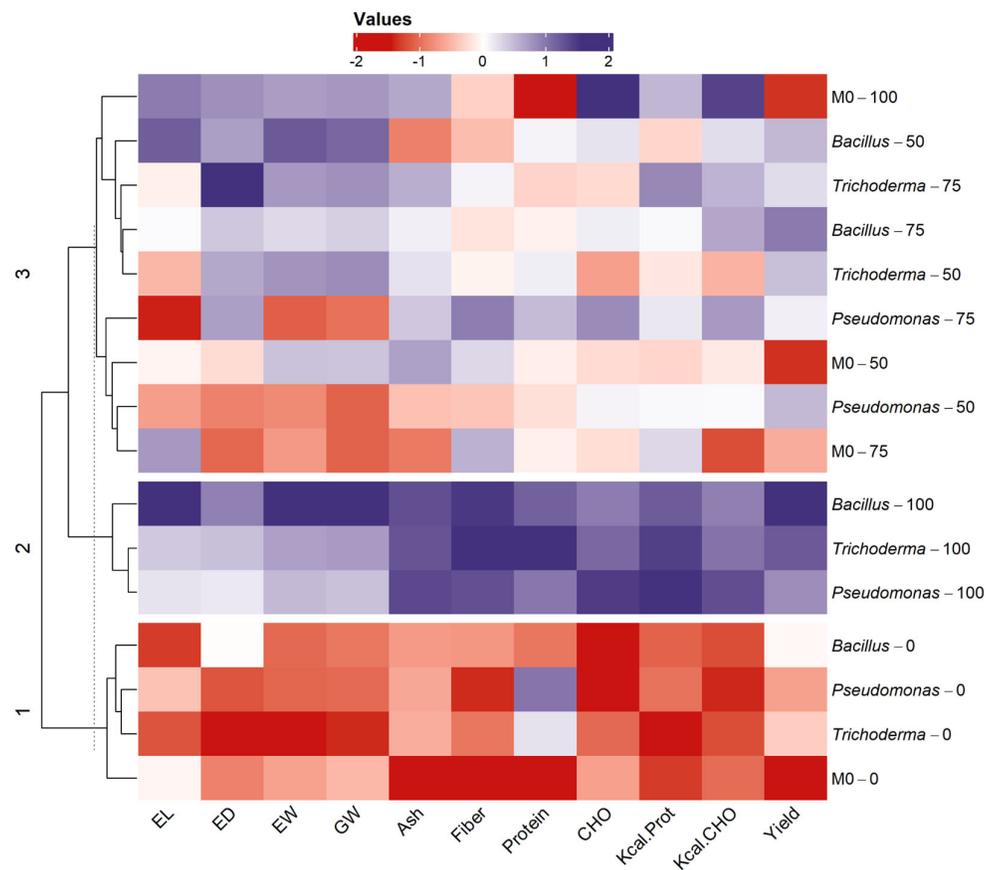


Figure 7. Heatmap with cluster analysis illustrating the combined effects of microbial inoculation and chemical fertilization on the Dekal B-7088 variety. The Y-axis lists all treatments resulting from the combinations of factor levels, while the X-axis displays the evaluated parameters. The numbers 1, 2, and 3 denote the three identified clusters. The vertical dotted line in the dendrogram represents the cut-off level for determining these groups.

4. Discussion

The high cost of chemical fertilizers and the adverse effects of their excessive use have driven the search for new technologies to support sustainable agriculture [35]. Microbial inoculants have emerged as a promising alternative to enhance nutrient use efficiency [36–39] and, consequently, to stimulate crop growth.

The results indicated that individual inoculation with *Bacillus subtilis*, *Trichoderma viride*, and *Pseudomonas putida* significantly increased ear and grain weight. Additionally, the presence of mineral fertilizer in the soil positively impacted these variables and the number of rows in the INIA 619 hybrid (Table 2). Similarly, Araujo et al. [40] reported a significant increase in ear weight with the presence of PGPR in maize. In the Dekal B-7088 variety, no statistical differences were observed between inoculated and control treatments; however, a synergistic effect was noted between the microorganisms and 100% fertilization, with favorable results trending in the inoculated + 100% fertilization treatments, grouped within a single cluster (Figure 7). Furthermore, 100% and 75% mineral fertilization levels positively influenced ear length, ear diameter, ear weight, and grain weight, underscoring the importance of adequate nutrient availability to ensure effective nutrient transfer to the grains, promoting their optimal development [41,42]. These findings highlight the

significant role of microbial inoculation in enhancing ear characteristics when combined with mineral fertilization.

In the Dekal B-7088 hybrid, yield surpassed the variety's average of 11 t·ha⁻¹ [43], with higher fertilizer doses demonstrating a positive effect. In contrast, INIA 619 did not reach its expected yield of 14 t·ha⁻¹, as indicated in its datasheet [44]. However, applying microorganisms along with 100%, 75%, and 50% mineral fertilization brought the yield significantly closer to the optimum than the control treatment.

Numerous studies emphasize the effectiveness of PGPR and *Trichoderma* in enhancing nutrient utilization [45–48]. For instance, Oliveira-Paiva et al. [46] reported a 16% increase in grain yield in soils inoculated with *Bacillus subtilis*. Similarly, Gholami et al. [47] found that inoculation with various *Pseudomonas* species increased maize grain weight, demonstrating the capacity of these bacteria to improve crop yield.

Chemical fertilization in soil provides rapid nutrient availability for plant uptake [49], a process further enhanced by the solubilizing activity of *Bacillus subtilis* and *Pseudomonas putida* [39,45,50], which increase the availability of nitrogen, phosphorus, and potassium, supporting better root development and more efficient nutrient utilization [48]. Additionally, *Trichoderma viride* plays a key role in protecting plants from adverse conditions, contributing to increased crop productivity, as reported by Syamsiyah et al. [52]. However, Khalid et al. [53] observed that the effectiveness of rhizobacteria and fungal inoculation varies with plant genotype and environmental conditions, underscoring the importance of tailoring microbial applications to specific contexts.

Regarding grain nutritional quality, this study demonstrates the biostimulant effect of *Bacillus subtilis*, *Pseudomonas putida*, and *Trichoderma viride* in enhancing grain protein, fiber, and carbohydrate concentrations. The heatmap illustrates the synergistic effect between plant growth-promoting microorganisms (PGPMs) and high fertilization doses in improving grain quality in both hybrids. Specifically, inoculation with PGPMs alongside the highest fertilization dose (240-120-140 kg·ha⁻¹ of N, P₂O₅, and K₂O) yielded the highest protein content in both evaluated hybrids (Figures 3A and 4A). Previous studies indicate that increased protein content in maize grain is closely linked to nitrogen fertilization, with nitrogen sources playing a critical role [54,55]. For wheat, Monostori et al. [56] found that higher nitrogen fertilization rates led to increased total protein in grains. In this study, however, microbial inoculation without fertilization achieved protein content values similar to complete fertilization without inoculation. Moreover, the combined effect of microbial inoculation and fertilization produced higher protein content than fertilization alone, underscoring the complementary benefits of this approach.

These findings align with previous studies suggesting that *Bacillus*, *Pseudomonas*, and *Trichoderma* release organic acids with auxinic activity, which stimulate root growth, enhance nutrient availability, and reduce fertilizer requirements [40,50,52,57–59]. Solórzano & Quispe [39] reported that *Bacillus* and *Pseudomonas* enhance nitrogen uptake efficiency by promoting root growth, while Akladios & Abbas [60] found that *Trichoderma* inoculation increases protein content in maize grain. Consequently, *Bacillus subtilis*, *Pseudomonas putida*, and *Trichoderma viride* likely improve the efficiency of nitrogen fertilizers and soil organic nitrogen use.

Conversely, fertilization at 50% and 75% of the total dose increased fiber content independently of microbial inoculation. The fiber content in maize grain corresponds to the structural components of the plant cell wall, specifically cellulose, hemicellulose, lignin, and pectin [61]. The cell wall is a crucial plant structure, supporting and protecting plant cells [62]. Its formation and stability rely on adequate levels of essential nutrients, particularly calcium and boron [63]. Excessive fertilization can reduce calcium availability and uptake due to ionic competition with K⁺ and NH₄⁺ or precipitation with P [64]. Consequently, the total fertilization dose may have reduced grain fiber content (Figure 3B). These findings align with the observed positive effect of fertilization on increased ash content in both maize hybrids. Ash is composed of mineral residues such as Ca, Mg, P, K, Na, and Fe, remaining after the incineration of organic substances like proteins, carbohydrates, and lipids [65]. Therefore, higher ash content may indicate enhanced Ca assimilation, stimulating cell wall components' biosynthesis and increasing fiber content in maize grain [66]. However, the highest fiber content was only achieved with the combined treatment of microbial inoculation and full-dose fertilization. This suggests that complete fertilization with nitrogen, phosphorus, and potassium promotes the

biostimulant activity of PGPMs in enhancing fiber content (Figure 3B), likely through improved calcium assimilation and its role in cell wall component biosynthesis [67].

The fertilization effect on carbohydrate content varied between the two hybrids. In INIA 619, significant differences were observed at 50% of the total fertilization dose, while higher rates showed no additional impact. In contrast, Dekal B-7088 demonstrated a stronger response to fertilization, with the total dose (100%) proving most effective for carbohydrate accumulation, yielding 74.6 ± 0.57 g·100 g⁻¹. Li et al. [68] reported that a nitrogen dose of 240 kg·ha⁻¹—similar to the used dose in this study—increased starch concentration in wheat grains. This supports Feng et al. [55] suggestion that higher soil nitrogen concentrations stimulate carbohydrate metabolism. Consequently, nitrogen fertilization is crucial for optimizing the nutritional quality of maize grain [69], although excessive nitrogen can degrade the grain quality of sensitive genotypes [70].

Both hybrids, sown off-season and exposed to temperatures below the optimal range during the winter in Lima (Figure 1), exhibited differing responses to fertilization regarding carbohydrate accumulation. INIA 619 showed the lowest response to fertilization in yield and grain quality, likely due to reduced nutrient uptake [71]. However, microbial inoculation emerged as a beneficial alternative for carbohydrate accumulation in the INIA 619 hybrid, achieving values between 73.22 ± 0.10 and 75.07 ± 0.50 g·100 g⁻¹ when applied at 50% and 100% of the fertilization dose, respectively.

This research results suggest that the fertilization system significantly affects yield and nutritional quality, which must be tailored to each maize variety's specific soil characteristics and unique requirements [5,72]. Furthermore, microorganism inoculation has proven to be an efficient strategy for enhancing both average crop yield and the nutritional quality of hard yellow maize grain. While microbial inoculants cannot fully replace chemical fertilization, they serve as a valuable complement by enhancing nutrient uptake [73]. This approach contributes to more sustainable agricultural practices by optimizing input use and promoting soil health, which is essential for the sustainability of maize production in the livestock industry.

5. Conclusions

This study highlights the synergistic effects of microbial inoculants and mineral fertilization on two maize varieties. The findings indicate that microbial inoculation generally enhanced grain and ear weight in INIA 619, with yield increases of 13.1% for INIA 619 and 55.5% for Dekal B-7088, with *Bacillus subtilis* showing notable effectiveness in both varieties. Mineral fertilization at 75% and 50% doses does not differ from the total dose for INIA 619. Regarding nutritional quality, the interaction of *Bacillus subtilis*, *Trichoderma viridae*, and *Pseudomonas putida* with 100% fertilization significantly boosted protein and carbohydrate content in INIA 619 by 47% and 6%, respectively, while in Dekal B-7088, protein increased by 54% and fiber by 27%. Notably, in INIA 619, nutritional quality was sustained even with a reduced fertilization dose of 75%.

These findings may support more sustainable agricultural practices by reducing reliance on synthetic fertilizers, potentially mitigating long-term soil fertility loss. Future research should examine these strategies across diverse agro-climatic conditions and crops, assessing the effectiveness of microbial inoculants on various varieties. Additionally, it is recommended that the cumulative effects of combined mineral fertilization and long-term inoculant use on soil fertility and crop sustainability should be investigated. Further studies optimizing inoculant and fertilizer dosages could provide valuable insights for developing more efficient and environmentally sustainable farming practices.

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References

- Shah, T.R., T.; Prasad, K.; Kumar, P. Maize — A Potential Source of Human Nutrition and Health: A Review. *Cogent Food Agric.* 2016, 2, 1166995, doi:10.1080/23311932.2016.1166995.
- Acosta, L.; Barreda, C.; Becerra, J.; Galarreta, L.; Huaman, O.; Moreyra, J.; Romero, C.; Rospigliosi, J. Marco Orientador de Cultivos, Campaña 2024/2025 2024.
- Perfil Productivo y Competitivo de los Principales Cultivos del Sector Available online: <https://app.powerbi.com/view?r=eyJrJoiYjYwYTk5MDgtM2M0MS00NDMyLTgzNDEtMjNhNjEzYWQyOTNliiwidCI6IjdmMDg0NjI3LTdmNDAtNDg3OS04OTE3LTk0Yjg2ZmQzNWYzZiJ9> (accessed on 4 October 2024).
- Barandiarán, M. Manual Técnico Del Cultivo de Maíz Amarillo Duro; 1; First.; Instituto Nacional de Innovación Agraria: Perú, 2020; ISBN 978-9972-44-051-9.
- Yan, X.; Chen, X.; Ma, C.; Cai, Y.; Cui, Z.; Chen, X.; Wu, L.; Zhang, F. What Are the Key Factors Affecting Maize Yield Response to and Agronomic Efficiency of Phosphorus Fertilizer in China? *Field Crops Res.* 2021, 270, 108221, doi:10.1016/j.fcr.2021.108221.
- Samaniego, T.; Pérez, W.E.; Lastra-Paúcar, S.; Verme-Mustiga, E.; Solórzano-Acosta, R. The Fermented Liquid Biofertilizer Use Derived from Slaughterhouse Waste Improves Maize Crop Yield. *Trop. Subtrop. Agroecosystems* 2024, 27, doi:10.56369/tsaes.5469.
- Mulyati; Baharuddin, A.B.; Tejowulan, R.S. Improving Maize (*Zea Mays* L.) Growth and Yield by the Application of Inorganic and Organic Fertilizers Plus. *IOP Conf. Ser. Earth Environ. Sci.* 2021, 712, 012027, doi:10.1088/1755-1315/712/1/012027.
- FAO. El estado de los recursos de tierras y aguas del mundo para la alimentación y la agricultura. La gestión de los sistemas en situación de riesgo; 2011;
- Bhattacharyya, P.N.; Goswami, M.P.; Bhattacharyya, L.H. Perspective of Beneficial Microbes in Agriculture under Changing Climatic Scenario: A Review. *J. Phytol.* 2016, 8, 26, doi:10.19071/jp.2016.v8.3022.
- Parra-Cota, F.I.; Coronel-Acosta, C.-B.; Amézquita-Avilés, C.F.; Santos-Villalobos, S. de los; Escalante-Martínez, D.I. Diversidad metabólica de microorganismos edáficos asociados al cultivo de maíz en el Valle del Yaqui, Sonora. *Rev. Mex. Cienc. Agríc.* 9, 431–442.
- Gilbert, J.A.; Neufeld, J.D. Life in a World without Microbes. *PLOS Biol.* 2014, 12, e1002020, doi:10.1371/journal.pbio.1002020.
- Wu, Q.; Chen, Y.; Dou, X.; Liao, D.; Li, K.; An, C.; Li, G.; Dong, Z. Microbial Fertilizers Improve Soil Quality and Crop Yield in Coastal Saline Soils by Regulating Soil Bacterial and Fungal Community Structure. *Sci. Total Environ.* 2024, 949, 175127, doi:10.1016/j.scitotenv.2024.175127.
- Todeschini, V.; AitLahmidi, N.; Mazzucco, E.; Marsano, F.; Gosetti, F.; Robotti, E.; Bona, E.; Massa, N.; Bonneau, L.; Marengo, E.; et al. Impact of Beneficial Microorganisms on Strawberry Growth, Fruit Production, Nutritional Quality, and Volatilome. *Front. Plant Sci.* 2018, 9, 1611, doi:10.3389/fpls.2018.01611.
- Vassilev, N.; Malusà, E. Special Issue: Microorganisms and Plant Nutrition. *Microorganisms* 2021, 9, 2571, doi:10.3390/microorganisms9122571.
- Widnyana, I.K.; Javandira, C. Activities *Pseudomonas* Spp. and *Bacillus* Sp. to Stimulate Germination and Seedling Growth of Tomato Plants. *Agric. Agric. Sci. Procedia* 2016, 9, 419–423, doi:10.1016/j.aaspro.2016.02.158.
- Vejan, P.; Abdullah, R.; Khadiran, T.; Ismail, S.; Nasrullah Boyce, A. Role of Plant Growth Promoting Rhizobacteria in Agricultural Sustainability—A Review. *Molecules* 2016, 21, 573, doi:10.3390/molecules21050573.
- Tsukanova, K.A.; Chebotar, V.K.; Meyer, J.; Bibikova, T. Effect of Plant Growth-Promoting Rhizobacteria on Plant Hormone Homeostasis. *South Afr. J. Bot.* 2017, 113, 91–102, doi:10.1016/j.sajb.2017.07.007.
- Etesami, H.; Glick, B.R. Halotolerant Plant Growth-Promoting Bacteria: Prospects for Alleviating Salinity Stress in Plants. *Environ. Exp. Bot.* 2020, 178, 104124, doi:10.1016/j.envexpbot.2020.104124.
- Sah, S.; Krishnani, S.; Singh, R. *Pseudomonas* Mediated Nutritional and Growth Promotional Activities for Sustainable Food Security. *Curr. Res. Microb. Sci.* 2021, 2, 100084, doi:10.1016/j.crmicr.2021.100084.
- Efthimiadou, A.; Katsenios, N.; Chanioti, S.; Giannoglou, M.; Djordjevic, N.; Katsaros, G. Effect of Foliar and Soil Application of Plant Growth Promoting Bacteria on Growth, Physiology, Yield and Seed Quality of Maize under Mediterranean Conditions. *Sci. Rep.* 2020, 10, 21060, doi:10.1038/s41598-020-78034-6.

21. Konieczna, W.; Turkan, S.; Warchoń, M.; Skrzypek, E.; Dąbrowska, G.B.; Mierek-Adamska, A. The Contribution of *Trichoderma Viride* and Metallothioneins in Enhancing the Seed Quality of *Avena Sativa* L. in Cd-Contaminated Soil. *Foods* 2024, 13, 2469 doi:10.3390/foods13152469.
22. Vinale, F.; Sivasithamparam, K.; Ghisalberti, E.L.; Woo, S.L.; Nigro, M.; Marra, R.; Lombardi, N.; Pascale, A.; Ruocco, M.; Lanzuise, S.; et al. *Trichoderma* Secondary Metabolites Active on Plants and Fungal Pathogens. *The Open Mycology Journal* 2014, 8,127-139, doi:10.2174/1874437001408010127
23. Rodríguez-García, D.; Vargas-Rojas, J. Efecto de la inoculación con *Trichoderma* sobre el crecimiento vegetativo del tomate (*Solanum lycopersicum*). *Agron. Costarric.* 2022.
24. Tyśkiewicz, R.; Nowak, A.; Ozimek, E.; Jaroszuk-Ścisiel, J. *Trichoderma*: The Current Status of Its Application in Agriculture for the Biocontrol of Fungal Phytopathogens and Stimulation of Plant Growth. *Int. J. Mol. Sci.* 2022, 23, 2329, doi:10.3390/ijms23042329.
25. NOM-021-RECNAT-2000; Norma Oficial Mexicana Que Establece Las Especificaciones de Fertilidad, Salinidad y Clasificación de Suelos. Estudios, Muestreo y Análisis. 2002. Available online: <https://faolex.fao.org/docs/pdf/mex50674.pdf> (accessed on 08 August 2024).
26. USEPA. METHOD 9045D. SOIL AND WASTE pH 2004; USEPA: Washington, DC, USA, 2004. Available online: <https://www.epa.gov/sites/default/files/2015-12/documents/9045d.pdf> (accessed on 14 August 2024)
27. ISO 11265:1994; Soil Quality—Determination of the Specific Electrical Conductivity. ISO (International Organization for Standardization): Geneva, Switzerland, 1994. Available online: <https://www.iso.org/standard/19243.html> (accessed on 08 August 2024).
28. Verhulst, Nele; Sayre, Ken; Govaerts, Bram *Manual de determinación de rendimiento*; 1er ed.; Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT): México, 2012; ISBN 978-607-95844-7-4.
29. AACC-Method 46-11 American Association of Cereal Chemists. Crude Protein – Improved Kjeldahl Method, Copper Catalyst Modification; St. Paul, USA, 2009.
30. INACAL. Instituto Nacional de Calidad. Norma Técnica Peruana. NTP 205.004:2022. Cereales y Leguminosas. Determinación de Cenizas; 2022.
31. INACAL. Instituto Nacional de Calidad. Norma Técnica Peruana. NTP 209.019:1976 . ALIMENTOS BALANCEADOS PARA ANIMALES. (Revisada El 2014); Perú, 2014.
32. AOCS (American Oil Chemists' Society) American Oil Chemists' Society. Ba 6-84 Crude Fiber in Oilseed By-Products; USA, 2017.
33. Collazos, C; Phlip, W.; Viñas, E; Alvistur, J; Urquieta, A; Vásquez, J Ministerio de Salud, Instituto Nacional de Nutrición. Metodología Para Carbohidratos, Por Diferencia de Materia Seca (MS-INN); Lima, Perú, 1993.
34. Mendiburu, F. de *Agricolae: Statistical Procedures for Agricultural Research* 2023. Available online: <https://cran.r-project.org/web/packages/agricolae/index.html> (accessed on 10 August 2024)
35. Paliwoda, D.; Mikiciuk, G. Use of Rhizosphere Microorganisms in Plant Production – A Review Study. *Journal of Ecological Engineering* 2020, 21, 292-310, doi: 10.12911/22998993/126597
36. dos Santos, R.M.; Diaz, P.A.E.; Lobo, L.L.B.; Rigobelo, E.C. Use of Plant Growth-Promoting Rhizobacteria in Maize and Sugarcane: Characteristics and Applications. *Front. Sustain. Food Syst.* 2020, 4, doi:10.3389/fsufs.2020.00136.
37. Pereira, S.I.A.; Abreu, D.; Moreira, H.; Vega, A.; Castro, P.M.L. Plant Growth-Promoting Rhizobacteria (PGPR) Improve the Growth and Nutrient Use Efficiency in Maize (*Zea Mays* L.) under Water Deficit Conditions. *Heliyon* 2020, 6, doi:10.1016/j.heliyon.2020.e05106.
38. Huasasquiche, L.; Alejandro, L.; Ccori, T.; Cántaro-Segura, H.; Samaniego, T.; Quispe, K.; Solórzano, R. *Bacillus Subtilis* and *Rhizophagus Intraradices* Improve Vegetative Growth, Yield, and Fruit Quality of *Fragaria* × *Ananassa* Var. San Andreas. *Microorganisms* 2024, 12, 1816, doi:10.3390/microorganisms12091816.
39. Solórzano-Acosta, R.A.; Quispe, K.R. Assessing the Role of Field Isolated *Pseudomonas* and *Bacillus* as Growth-promoting Rizobacteria on Avocado (*Persea Americana*) Seedlings. *J. Sustain. Agric. Environ.* 2024, 3, e12114, doi:10.1002/sae2.12114.
40. Araujo, J.L.; de Mesquita Alves, J.; Rocha, R.H.C.; Santos, J.Z.L.; dos Santos Barbosa, R.; da Costa, F.M.N.; de Lima, G.S.; de Freitas, L.N.; Lima, A.S.; Nogueira, A.E.P.; et al. Beneficial Microorganisms Affect Soil Microbiological Activity and Corn Yield under Deficit Irrigation. *Agriculture* 2023, 13, 1169, doi:10.3390/agriculture13061169.
41. Ning, P.; Li, S.; Yu, P.; Zhang, Y.; Li, C. Post-Silking Accumulation and Partitioning of Dry Matter, Nitrogen, Phosphorus and Potassium in Maize Varieties Differing in Leaf Longevity. *Field Crops Res.* 2013, 144, 19–27, doi:10.1016/j.fcr.2013.01.020.
42. Wang, J.; Zhao, S.; Zhang, Y.; Lu, X.; Du, J.; Wang, C.; Wen, W.; Guo, X.; Zhao, C. Investigating the Genetic Basis of Maize Ear Characteristics: A Comprehensive Genome-Wide Study Utilizing High-Throughput Phenotypic Measurement Method and System. *Front. Plant Sci.* 2023, 14, 1248446, doi:10.3389/fpls.2023.1248446.

43. Bayer. DEKALB - 7088 Available online: https://www.agro.bayer.pe/es-pe/dekalb/details.html/dekalb_-_7088.html (accessed on 14 October 2024).
44. Instituto Nacional de Innovación Agraria Híbrido Simple de Maíz Amarillo Duro INIA 619 - Megahíbrido 2012.
45. Ribeiro, V.P.; Gomes, E.A.; De Sousa, S.M.; De Paula Lana, U.G.; Coelho, A.M.; Marriel, I.E.; De Oliveira-Paiva, C.A. Co-Inoculation with Tropical Strains of *Azospirillum* and *Bacillus* Is More Efficient than Single Inoculation for Improving Plant Growth and Nutrient Uptake in Maize. *Arch. Microbiol.* 2022, 204, 143, doi:10.1007/s00203-022-02759-3.
46. de Oliveira-Paiva, C.A.; Bini, D.; de Sousa, S.M.; Ribeiro, V.P.; dos Santos, F.C.; de Paula Lana, U.G.; de Souza, F.F.; Gomes, E.A.; Marriel, I.E. Inoculation with *Bacillus Megaterium* CNPMS B119 and *Bacillus Subtilis* CNPMS B2084 Improve P-Acquisition and Maize Yield in Brazil. *Front. Microbiol.* 2024, 15, 1426166, doi:10.3389/fmicb.2024.1426166.
47. Gholami, A.; Shahsavani, S.; Nezarat, S. The Effect of Plant Growth Promoting Rhizobacteria (PGPR) on Germination, Seedling Growth and Yield of Maize. *Engineering and Technology*, 2008, 49, 19-24.
48. Elhassoufi, W.; Ghoulam, C.; Barakat, A.; Zeroual, Y.; Bargaz, A. Phosphate Bacterial Solubilization: A Key Rhizosphere Driving Force Enabling Higher P Use Efficiency and Crop Productivity. *J. Adv. Res.* 2022, 38, 13–28, doi:10.1016/j.jare.2021.08.014.
49. Sosa-Rodrigues, B.A.; García-Vivas, Y.S. Eficiencia de uso del nitrógeno en maíz fertilizado de forma orgánica y mineral. *Agron. Mesoam.* 2018, 29, 207, doi:10.15517/ma.v29i1.27127.
50. Mpanga, I.K.; Nkebiwe, P.M.; Kuhlmann, M.; Cozzolino, V.; Piccolo, A.; Geistlinger, J.; Berger, N.; Ludewig, U.; Neumann, G. The Form of N Supply Determines Plant Growth Promotion by P-Solubilizing Microorganisms in Maize. *Microorganisms* 2019, 7, 38, doi:10.3390/microorganisms7020038.
51. Nepali, B.; Subedi, S.; Bhattarai, S.; Marahatta, S.; Bhandari, D.; Shrestha, J. Bio-Fertilizer Activity of *Trichoderma Viride* and *Pseudomonas Fluorescens* as Growth and Yield Promoter for Maize. 2020,191-195, doi:10.15159/JAS.20.17.
52. Syamsiyah, J.; Herdiansyah, G.; Hartati, S. Use of *Trichoderma* as an Effort to Increase Growth and Productivity of Maize Plants. *IOP Conf. Ser. Earth Environ. Sci.* 2023, 1165, 012020, doi:10.1088/1755-1315/1165/1/012020.
53. Khalid, A.; Arshad, M.; Zahir, Z.A. Screening Plant Growth-Promoting Rhizobacteria for Improving Growth and Yield of Wheat. *J. Appl. Microbiol.* 2004, 96, 473–480, doi:10.1046/j.1365-2672.2003.02161.x.
54. Ochieng', I.O.; Gitari, H.I.; Mochoge, B.; Rezaei-Chiyaneh, E.; Gweyi-Onyango, J.P. Optimizing Maize Yield, Nitrogen Efficacy and Grain Protein Content under Different N Forms and Rates. *J. Soil Sci. Plant Nutr.* 2021, 21, 1867–1880, doi:10.1007/s42729-021-00486-0.
55. Feng, W.; Xue, W.; Zhao, Z.; Shi, Z.; Wang, W.; Bai, Y.; Wang, H.; Qiu, P.; Xue, J.; Chen, B. Nitrogen Fertilizer Application Rate Affects the Dynamic Metabolism of Nitrogen and Carbohydrates in Kernels of Waxy Maize. *Front. Plant Sci.* 2024, 15, doi:10.3389/fpls.2024.1416397.
56. Monostori, I.; Szira, F.; Tondelli, A.; Árendás, T.; Gierczik, K.; Cattivelli, L.; Galiba, G.; Vágújfalvi, A. Genome-Wide Association Study and Genetic Diversity Analysis on Nitrogen Use Efficiency in a Central European Winter Wheat (*Triticum Aestivum* L.) Collection. *PLoS ONE* 2017, 12, e0189265, doi:10.1371/journal.pone.0189265.
57. Aquino, J.P.A. de; Macedo Junior, F.B. de; Antunes, J.E.L.; Figueiredo, M. do V.B.; Alcântara Neto, F. de; Araujo, A.S.F. de Plant Growth-Promoting Endophytic Bacteria on Maize and Sorghum. *Pesqui. Agropecuária Trop.* 2019, 49, e56241, doi:10.1590/1983-40632019v49i56241.
58. Numan, M.; Bashir, S.; Khan, Y.; Mumtaz, R.; Shinwari, Z.K.; Khan, A.L.; Khan, A.; AL-Harrasi, A. Plant Growth Promoting Bacteria as an Alternative Strategy for Salt Tolerance in Plants: A Review. *Microbiol. Res.* 2018, 209, 21–32, doi:10.1016/j.micres.2018.02.003.
59. Lastochkina, O.; Pusenkova, L.; Yuldashev, R.; Babaev, M.; Garipova, S.; Blagova, D.; Khairullin, R.; Aliniaiefard, S. Effects of *Bacillus Subtilis* on Some Physiological and Biochemical Parameters of *Triticum Aestivum* L. (Wheat) under Salinity. *Plant Physiol. Biochem.* 2017, 121, 80–88, doi:10.1016/j.plaphy.2017.10.020.
60. Akladios, S.A.; Abbas, S.M. Application of *Trichoderma Harzianum* T22 as a Biofertilizer Potential in Maize Growth. *J. Plant Nutr.* 2014, 37, 30–49, doi:10.1080/01904167.2013.829100.
61. Saeed, F.; Hussain, M.; Arshad, M.S.; Afzaal, M.; Munir, H.; Imran, M.; Tufail, T.; Anjum, F. Functional and Nutraceutical Properties of Maize Bran Cell Wall Non-Starch Polysaccharides. *Int. J. Food Prop.* 2021, 24, 233–248, doi:10.1080/10942912.2020.1858864.
62. Mutungi, C.; Tungu, J.; Amri, J.; Gaspar, A.; Abass, A. Nutritional Benefits of Improved Post-Harvest Handling Practices for Maize and Common Beans in Northern Tanzania: A Quantitative Farm-Level Assessment. *J. Stored Prod. Res.* 2022, 95, 101918, doi:10.1016/j.jspr.2021.101918.
63. Hawkesford, M.J.; Horst, W.; Kichey, T.; Lambers, H.; Schjoerring, J.; Skrumsager Moller, I.; White, P. Functions of Macronutrients. In: Marschner, P., Ed.; Academic Press Amsterdam, Netherlands, 2012; pp. 135–189 ISBN 978-0-12-384905-2.

64. Havlin, J.L. Soil: Fertility and Nutrient Management. In Landscape and Land Capacity; CRC Press, 2020 ISBN 978-0-429-44555-2.
65. Ndukwe, O.K.; Edeoga, H.O.; Omosun, G. Varietal Differences in Some Nutritional Composition of Ten Maize (*Zea Mays* L.) Varieties Grown in Nigeria. *Int. J. Acad. Res. Reflect.* 2015, 3, 1–11.
66. P, Nirmala Prasadi V.; Joye, I.J. Dietary Fibre from Whole Grains and Their Benefits on Metabolic Health. *Nutrients* 2020, 12, 3045, doi:10.3390/nu12103045.
67. Khosravi, A.; Zarei, M.; Ronaghi, A. Effect of PGPR, Phosphate Sources and Vermicompost on Growth and Nutrients Uptake by Lettuce in a Calcareous Soil. *J. Plant Nutr.* 2018, 41, 80–89, doi:10.1080/01904167.2017.1381727.
68. Li, Q.; Ren, Y.; Fu, H.; Li, Z.; Kong, F.; Yuan, J. Cultivar Differences in Carbon and Nitrogen Accumulation, Balance, and Grain Yield in Maize. *Front. Plant Sci.* 2022, 13, 992041, doi:10.3389/fpls.2022.992041.
69. Wei, Z.; Ying, H.; Guo, X.; Zhuang, M.; Cui, Z.; Zhang, F. Substitution of Mineral Fertilizer with Organic Fertilizer in Maize Systems: A Meta-Analysis of Reduced Nitrogen and Carbon Emissions. *Agronomy* 2020, 10, 1149, doi:10.3390/agronomy10081149.
70. Wu, X.; Tong, L.; Kang, S.; Du, T.; Ding, R.; Li, S.; Chen, Y. Combination of Suitable Planting Density and Nitrogen Rate for High Yield Maize and Their Source–Sink Relationship in Northwest China. *J. Sci. Food Agric.* 2023, 103, 5300–5311, doi:10.1002/jsfa.12602.
71. Ma, R.; Jiang, C.; Shou, N.; Gao, W.; Yang, X. An Optimized Nitrogen Application Rate and Basal Topdressing Ratio Improves Yield, Quality, and Water- and N-Use Efficiencies for Forage Maize (*Zea Mays* L.). *Agronomy* 2023, 13, 181, doi:10.3390/agronomy13010181.
72. Cvijanović, V.; Cvijanović, G.; Rajčić, V.; Marinković, J.; Đukić, V.; Bajagić, M.; Đurić, N. Influence of Different Methods of Application of Effective Microorganisms in Nutrition of Wheat on Weight by 1000 Grains, Yield, and Content of Crude Wheat Proteins (*TRITICUM SP.*). *Cereal Res. Commun.* 2022, 50, 1259–1268, doi:10.1007/s42976-021-00226-1.
73. Fathollahi, Z.; Gharavi, K.B.; Gasemi, A. Effects of Combined Use of Bio-Fertilizers and Chemical Fertilizers at Different Stages on Physiological and Morphological Characters of Navy Bean. *Indian J. Fundam. Appl. Life Sci.* 2014, 4, 423–427.

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