applied microbiology

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Synergy Between Microbial Inoculants and Mineral Fertilization to Enhance the 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 that plays 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 investigated solutions 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 demonstrated that microbial inoculation could 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

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Citation: López-Montañez, R.; Calero-Rios, E.; Quispe, K.; Huasasquiche, L.; Lastra, S.; La Torre, B.; Solórzano, R. Synergy Between Microbial Inoculants and Mineral Fertilization to Enhance the Yield and Nutritional Quality of Maize on the Peruvian Coast. *Appl. Microbiol.* **2024**, *4*, 1757–1775. https://doi.org/10.3390/applmicrobiol4040118

Academic Editor: J.H. (Han) de Winde

Received: 4 November 2024
Revised: 17 December 2024
Accepted: 19 December 2024
Published: 21 December 2024

1. Introduction

Hard yellow maize (*Zea mays* L.) is one of the most important global crops, 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 ingredient for human consumption and animal feed [4].

The 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 use of *Bacillus subtilis* in maize showed positive effects, increasing grain yield by 13–22% [20,21]. Additionally, increases in protein content, total solids, and crude fiber in the grain were also reported [21,22].

Fungi of the genus *Trichoderma* also employ various mechanisms that support plant growth and development [23]. These mechanisms include the synthesis of phytohormones like indole-3-acetic acid and other auxin analogs that promote root development, enhance nutrient uptake [24], and produce essential vitamins [25]. Trichoderma fungi also contribute to nutrient solubilization by secreting organic acids that lower soil pH, thereby increasing phosphorus availability [26]. The application of *Trichoderma* in maize was reported to increase chlorophyll content, starch, total proteins, and phytohormones when applied to the soil or seeds. [27].

Microbial inoculant application presents a promising approach to enhancing soil health and, in turn, maximizing agricultural productivity and maize quality.

In this context, the hypothesis was proposed that inoculation with *Bacillus subtilis*, *Pseudomonas putida*, and *Trichoderma viride*, combined with four levels of mineral fertilization, generates synergistic effects that enhance both yield and the nutritional quality of hard yellow maize grain. The objective of this study was to validate the hypothesis under real field conditions by evaluating two agriculturally important hard yellow maize varieties from the Peruvian coast: INIA 619 and Dekal B-7088. Currently, no prior information is available on their response in terms of yield and nutritional quality to microbial inoculation management techniques.

The expected results will not only provide a scientific foundation for the adoption of sustainable agricultural practices but also contribute an innovative approach to optimizing soil fertility and ensuring food security in the context of agricultural intensification.

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).

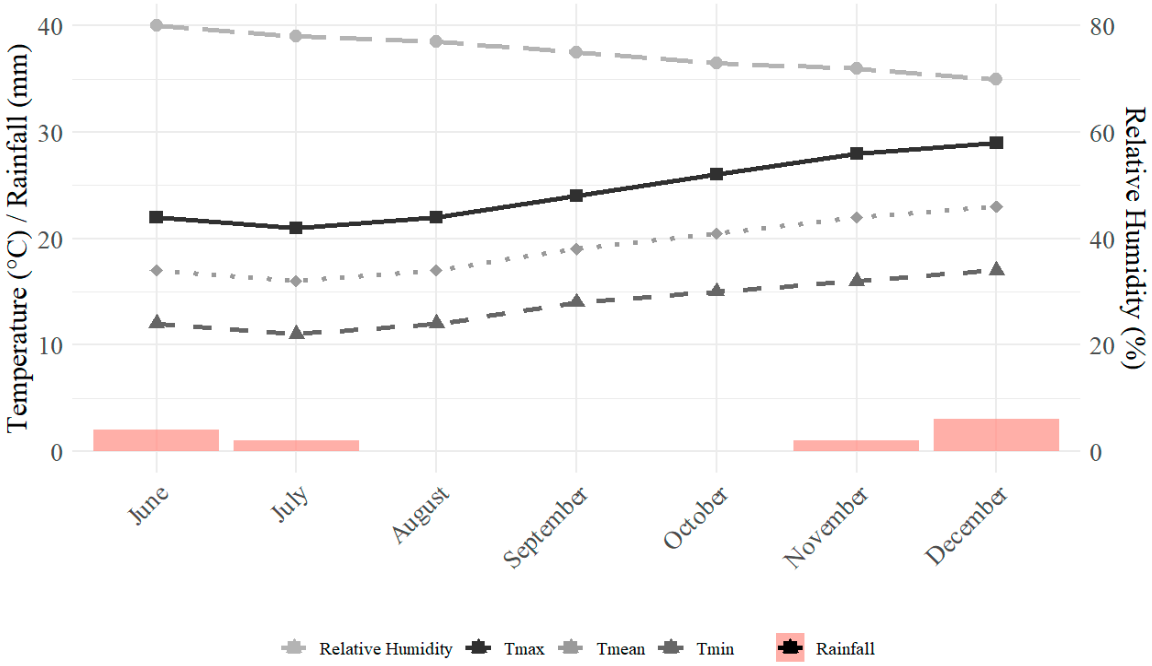

Figure 1. Climatological data of the project site on a monthly scale, including precipitation (rainfall), maximum temperature, minimum temperature, and relative humidity.

The experimental site was primarily used for agriculture purposes, 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.

The irrigation method used in the field was furrow irrigation, a system that distributes water through channels formed in the soil between crop rows. The application frequency was every 15 days.

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 m^2 area, divided into 48 plots of 30 m^2 (5 m × 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 [28]. The soil had a pH of 8.0 [29] and an electrical conductivity (EC) of 42.2 $mS{\cdot}m^{-1}$. Exchangeable cations were measured as 7 $meq{\cdot}100\ g^{-1}$ Ca^{2+}, 2.9 meq $100{\cdot}g^{-1}$ K^{+}, 2.2 $meq{\cdot}100\ g^{-1}$ Mg^{2+}, and 0.5 $meq{\cdot}100\ g^{-1}$ Na^{+}, with a cation exchange capacity (CEC) of 12.6 $meq{\cdot}100\ g^{-1}$ [28]. The total soil carbon content was 1.7%, organic carbon was 0.79%, and organic matter was 1.5% [30]. Total nitrogen content was 0.03%, and available phosphorus was measured at 28.2 $mg{\cdot}kg^{-1}$ [28].

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 set-up 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	Without inoculation (M0)	0 (0—0—0) *
2		50 (120—60—70) *
3		75 (180—90—105) *
4		100 (240—120—140) *
5	*Bacillus subtilis* (*B*)	0
6		50
7		75
8		100
9	*Trichoderma viride* (*T*)	0
10		50
11		75
12		100
13	*Pseudomonas putida* (*P*)	0
14		50
15		75
16		100

* The values in parenthesis represent the percentage of the mineral fertilizer dose of N, P_2O_5, and K_2O.

2.4. Crop Fertilization

The fertilizers used were urea, diammonium phosphate, and potassium chloride. Fertilizer rates of 420-261-233, 315-196-175, and 210-131-117 kg·ha^{-1} were applied for the 100%, 75%, and 50% doses, respectively. Fertilization was divided into two stages. The first application was one month after sowing, during the V4 phenological stage when the plants were in early vegetative development. The full dose of phosphorus, potassium, and half of the nitrogen was applied at this stage. The second application occurred one month later to complete the total nitrogen dose.

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 10^9 CFU·mL^{-1}. Then, 1% (*v*/*v*) dilutions were made for each microorganism from these cultures.

For *Trichoderma viride* (Strain SCT-11), spore suspension was prepared by washing maize inoculated with the fungus. Four 800 g bags of the product Trichomax®, containing a concentration of 1×10^{12} 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, according to Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) [31]:

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

The Physicochemical Laboratory of the Institute for Nutritional Research conducted a proximate analysis to analyze nutritional quality. The tests included protein content [32], ash [33], fat [34], fiber [35], carbohydrates [36], 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 [37].

3. Results

3.1. Ear Characteristics and Yield

3.1.1. INIA 619

The results indicated no interaction between the study factors. However, a significant effect of microbial inoculation on ear weight ($p < 0.05$) and grain weight ($p < 0.05$) was observed. 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 ($p < 0.01$), with the highest values achieved in treatments with 100%, 75%, and 50% fertilization. No significant differences were found among these fertilization levels (Table 2).

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, the 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).

Table 2. Ear characteristics of INIA 619 variety.

Factor	EL	ED	RE	GR	EW	GW	CW
	(cm)	(cm)			(g)	(g)	(g)
				Factor 1. Microorganisms			
M0	15.5 ± 1.3	4.01 ± 0.15	13.7 ± 0.4	29.2 ± 2.6	158 ± 21 b	135 ± 17 b	23.6± 4.1
B	15.5 ± 0.9	4.04 ± 0.07	13.8 ± 0.4	29.3 ± 2.3	185 ± 18 a	160 ± 17 a	25.5± 2.2
T	15.7 ± 1.2	4.02 ± 0.10	13.8 ± 0.5	29.2 ± 2.7	178 ± 18 a	152 ± 16 a	25.7 ± 3
P	15.6 ± 0.9	4.08 ± 0.14	13.9 ± 0.3	29.6 ± 2.7	186 ± 18 a	159 ± 15 a	26.7 ± 4.4
(%)				Factor 2. Fertilization Dose			
0	15 ± 1	4.04 ± 0.15	13.9 ± 0.3	27.3± 2.1 b	161 ± 20 b	137 ± 18 b	24.1± 3.5
50	16 ± 1	4.05 ± 0.11	13.9 ± 0.2	30.3 ± 2.5 a	182 ± 20 a	156 ± 17 a	26.3± 2.6
75	15.8 ± 0.7	4.04 ± 0.09	13.7 ± 0.4	29.9 ± 1.9 a	180 ± 18 a	154 ± 15 a	24.3± 3.3
100	15.1 ± 0.9	4.01 ± 0.14	13.7 ± 0.5	29.8 ± 2.5 a	186 ± 20 a	159 ± 18 a	26.7± 4.4
Interaction (p-value)	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.

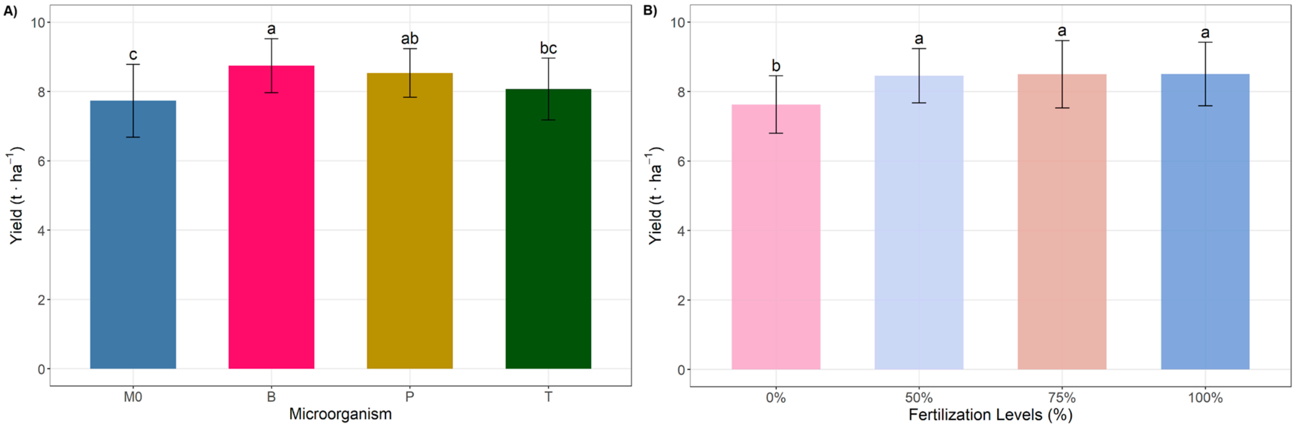

Figure 2. (**A**) Hard yellow maize INIA 619 variety yield under the effect of microbial inoculants and (**B**) mineral fertilization. Means with the same lowercase letter are statistically equivalent according to Tukey's test at a 0.05 significance level.

3.1.2. Dekal B-7088

The results indicated no interaction between study factors. 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).

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 the 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).

Table 3. Ear characteristics of Dekal B-7088 variety.

Factor	EL	ED	RE	GR	EW	GW	CW
	(cm)	(cm)			(g)	(g)	(g)
				Factor 1. Microorganisms			
M0	13.7 ± 0.7	4.39 ± 0.10	16.6 ± 0.5	31.8 ± 2.8	181 ± 15	164 ± 13.3	15.3 ± 2
B	13.7 ± 0.8	4.44 ± 0.11	16.8 ± 0.7	33.1 ± 1.8	191 ± 18	175 ± 16.4	16.4 ± 3.8
T	13.3 ± 0.6	4.42 ± 0.13	16.7 ± 0.4	32.6 ± 1.2	188 ± 18	172 ± 16.3	16.2 ± 3.6
P	13.2 ± 0.6	4.39 ± 0.12	16.7 ± 0.7	32.5 ± 1.3	187 ± 19	169 ± 10.3	16.1 ± 1.9
(%)				Factor 2. Fertilization dose			
0	13.2 ± 0.4 b	4.35 ± 0.11 b	16.4 ± 0.4	31.7 ± 1.3	176 ± 12 b	162 ± 11 c	14.7 ± 1.6
50	13.4 ± 0.7 b	4.41 ± 0.11 ab	16.7 ± 0.6	32.8 ± 2.4	184 ± 14 ab	168 ± 13 bc	16.9 ± 3.6
75	13.5 ± 0.7 ab	4.43 ± 0.10 a	16.9 ± 0.6	32.1 ± 1.7	190 ± 15 a	173 ± 14 ab	16.2 ± 3.8
100	14 ± 1 a	4.44 ± 0.11 a	17.0 ± 0.5	33.4 ± 1.7	194 ± 17 a	178 ± 16 a	16.2 ± 1.8
Interaction (p-value)	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.

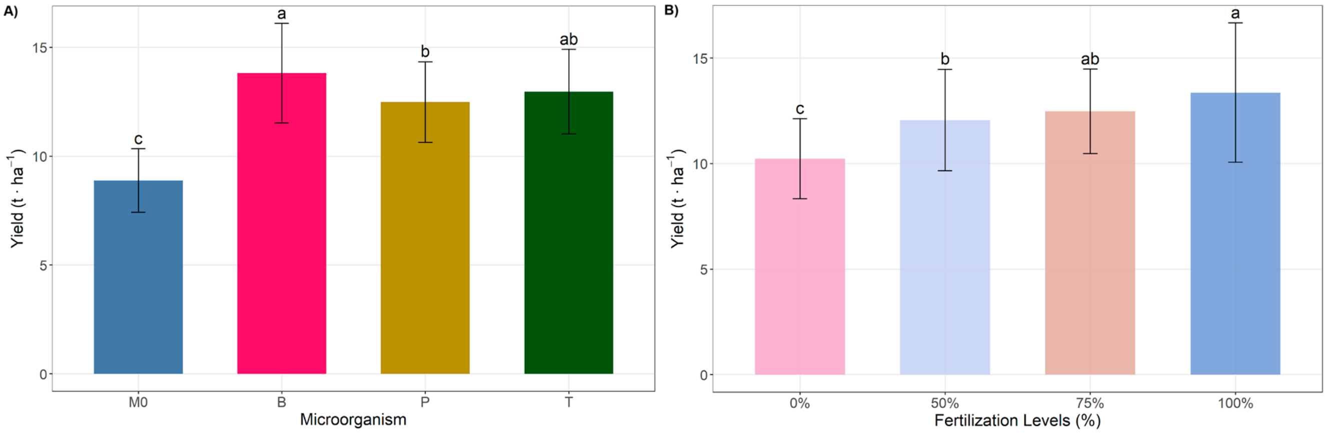

Figure 3. (**A**) Hard yellow maize Dekal B7088 variety yield under the effect of microbial inoculants and (**B**) mineral fertilization. Means with the same lowercase letter are statistically equivalent according to Tukey's test at a 0.05 significance level.

3.2. Nutritional Quality

3.2.1. INIA 619

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).

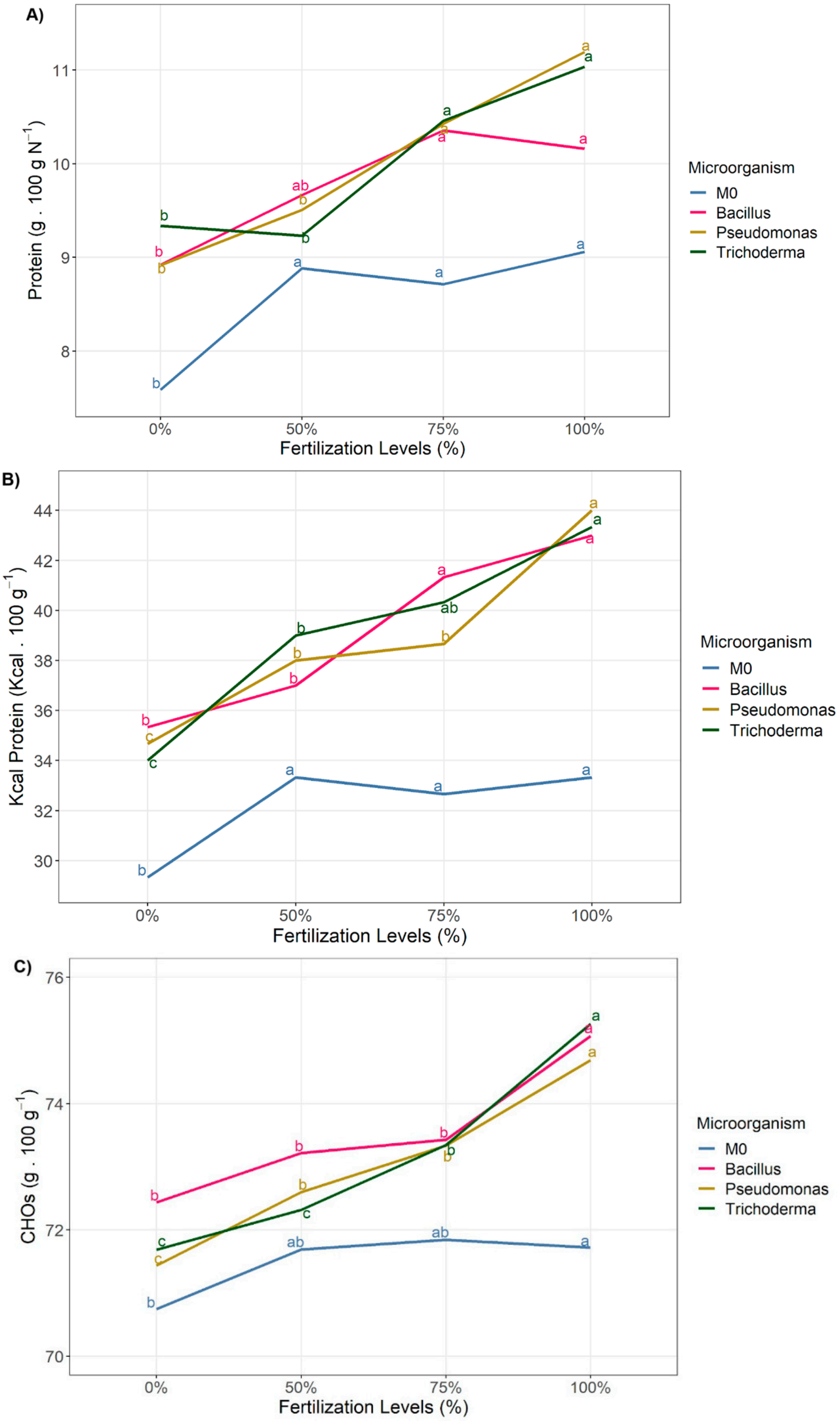

Figure 4. Interaction between mineral fertilization and microbial inoculation on (**A**) protein content; (**B**) kcal protein; and (**C**) fiber. Means with the same lowercase letter are statistically equivalent according to Tukey's test at a 0.05 significance level.

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

Figure 5 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.

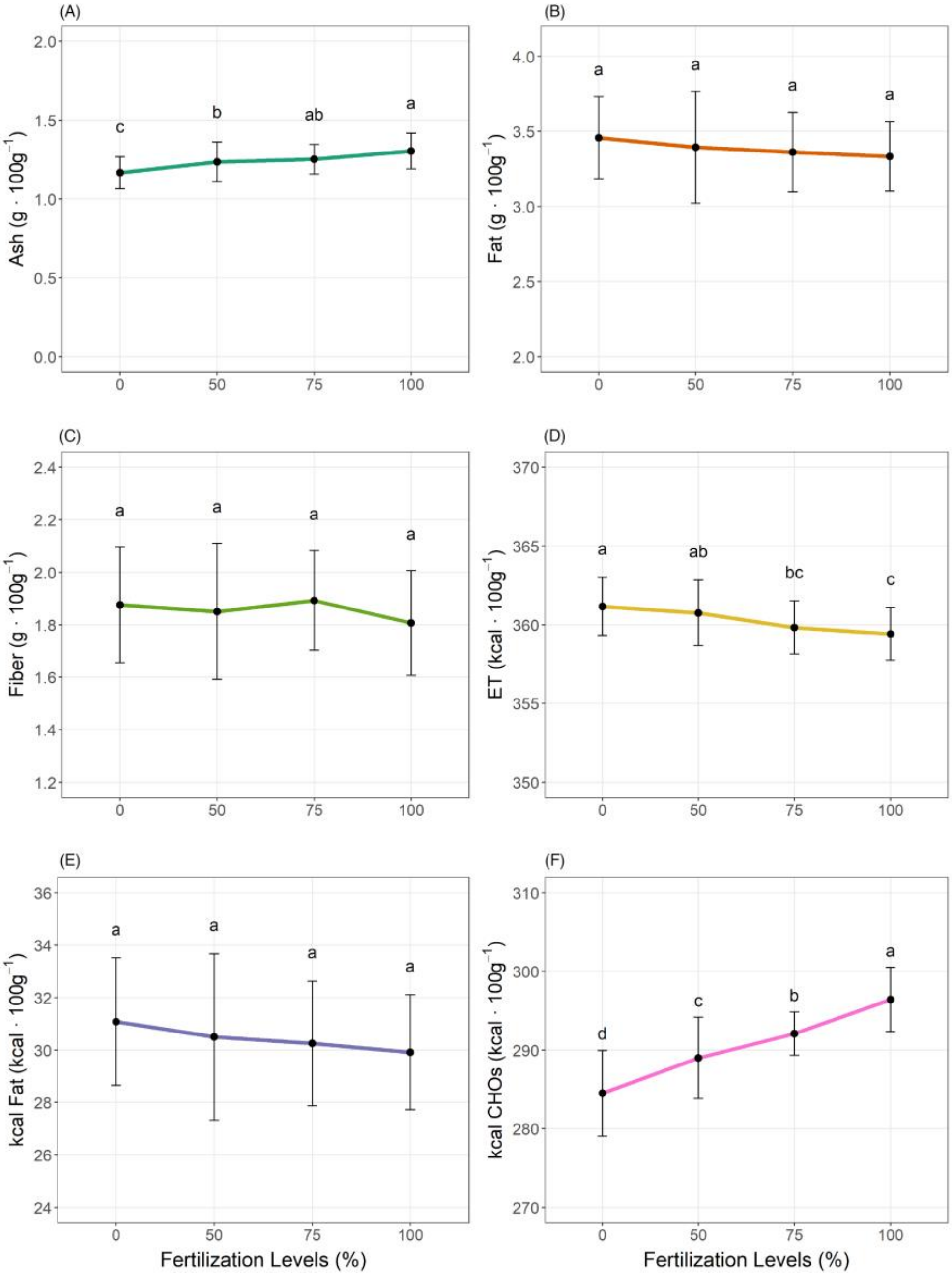

Figure 5. Nutritional quality of the INIA 619 variety according to the fertilization levels on (**A**) ash; (**B**) fat; (**C**) fiber; (**D**) total energy; (**E**) kcal fat; (**F**) kcal carbohydrate. Means with the same lowercase letter are statistically equivalent according to Tukey's test at a 0.05 significance level.

3.2.2. Dekal B-7088

The nutritional quality results 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 6A). 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 6B).

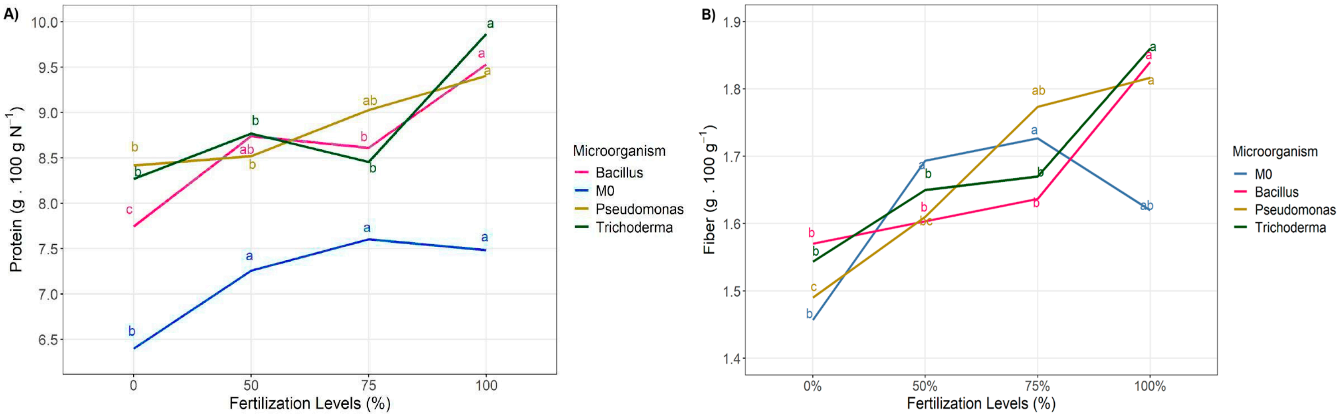

Figure 6. Interaction between mineral fertilization and microbial inoculation on (**A**) protein and (**B**) fiber content in Dekal B-7088 variety. Means with the same lowercase letter are statistically equivalent according to Tukey's test at a 0.05 significance level.

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 (Figure 7).

3.3. Heatmaps

3.3.1. INIA 619

The analysis of the heatmap graph for the INIA 619 variety showed three main clusters of treatments (Figure 8). The treatments that did not receive fertilization were grouped in Cluster 1, showing the lowest values in the evaluated parameters. Likewise, all non-inoculated treatments were grouped in this cluster, regardless of the fertilization dose they received. The worst treatment was the one that did not receive inoculation or fertilization. In contrast, Cluster 2 showed that the combined inoculated treatments at high fertilization doses (100%) had the highest values in the parameters. By decreasing the fertilization dose (50 and 75%), the effect of the inoculated treatments also decreased, which is why they were grouped in a third cluster.

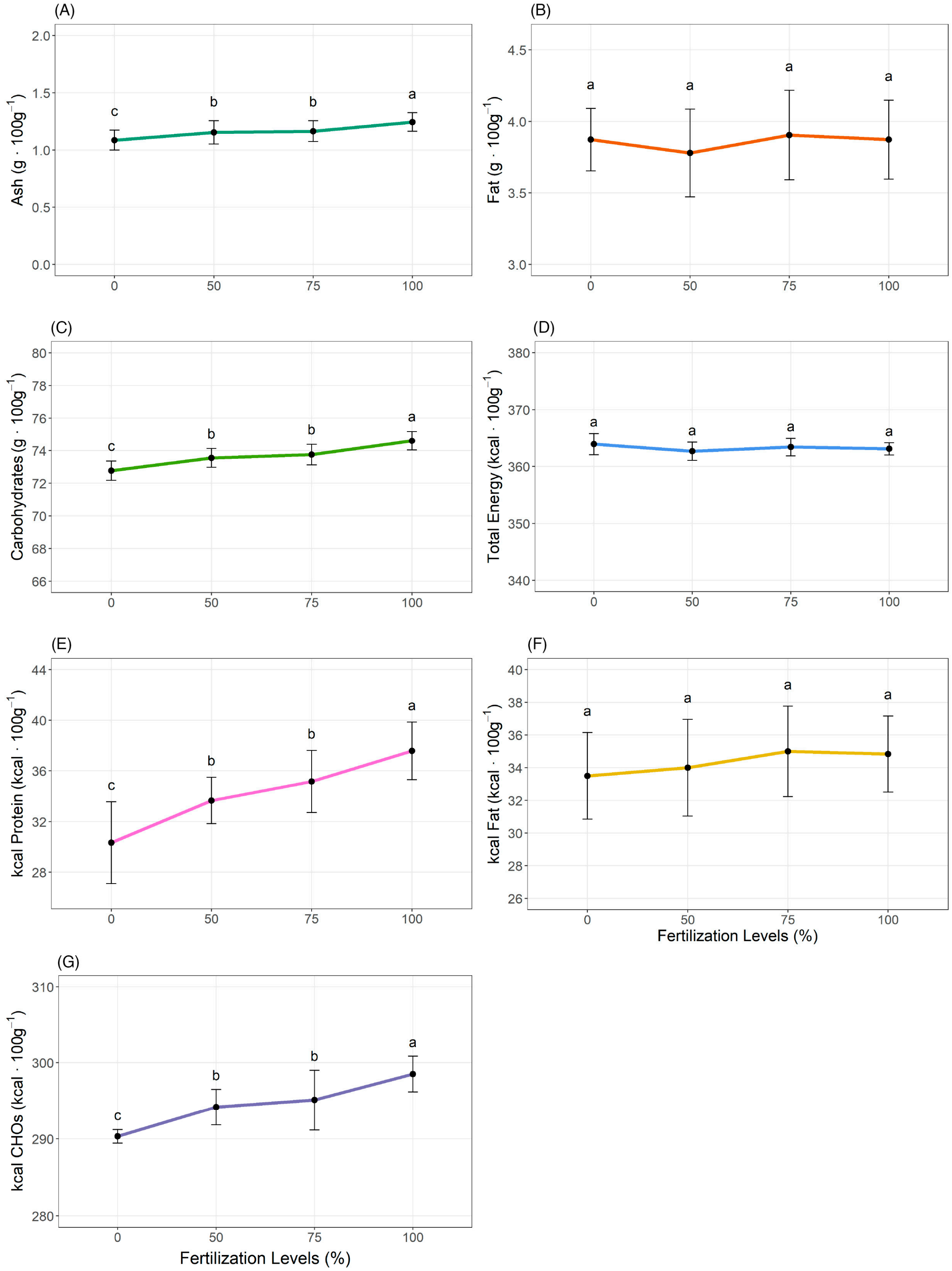

Figure 7. Nutritional quality of the Dekal B-7088 variety according to the fertilization levels (**A**) ash; (**B**) fat; (**C**) carbohydrates; (**D**) total energy; (**E**) kcal protein; (**F**) kcal fat; (**G**) kcal carbohydrate. Means with the same lowercase letter are statistically equivalent according to Tukey's test at a 0.05 significance level.

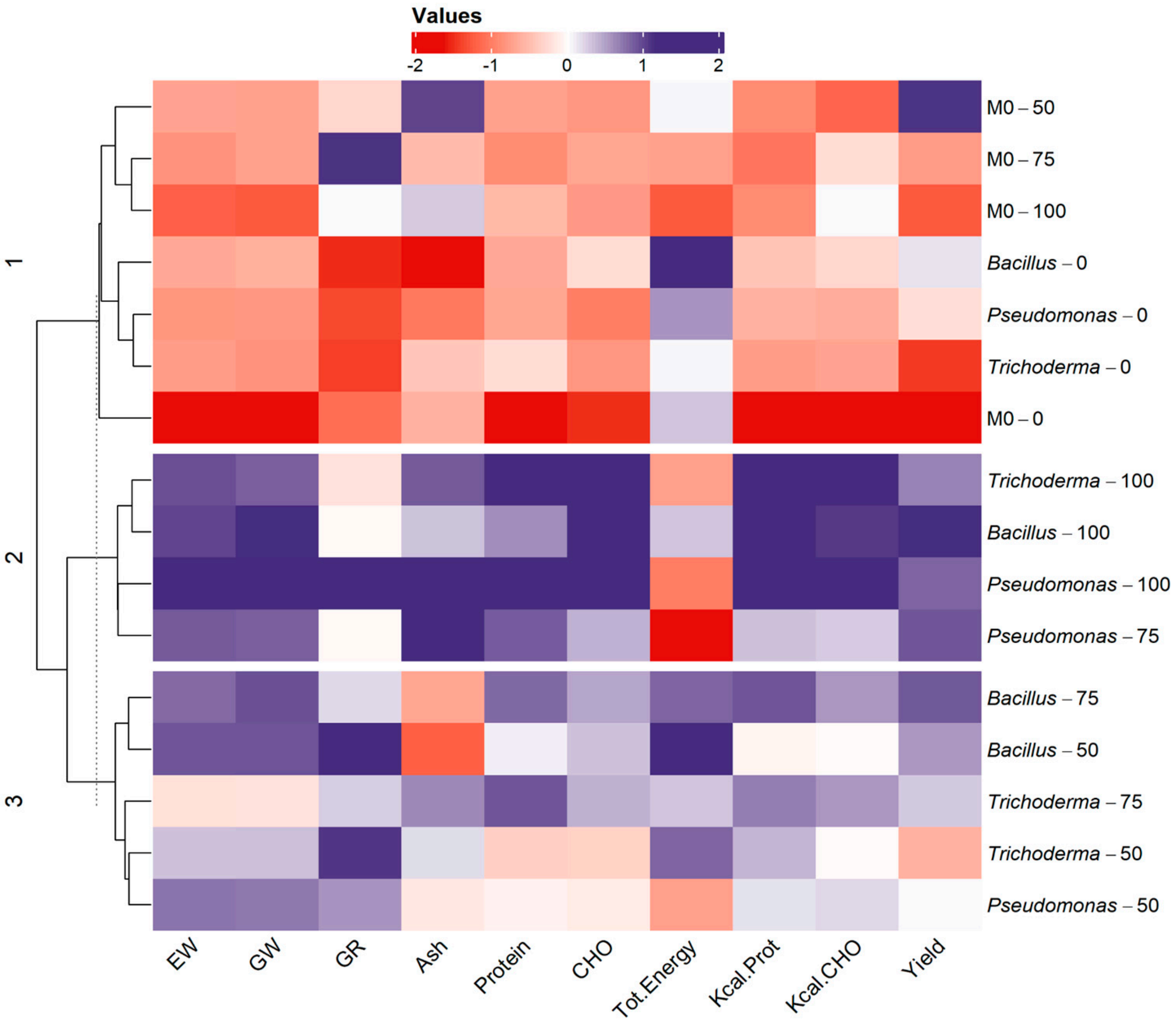

Figure 8. Heatmap with cluster analysis illustrating the combined effects of microbial inoculation and chemical fertilization on the INIA 619 variety. The $Y-$axis lists all treatments resulting from the combinations of factor levels, while the $X-$axis displays the evaluated parameters. The numbers 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

Unlike the INIA 619 variety, the heatmap analysis for the variety Dekal B-7088 showed that the inoculated treatments had a notable influence on the parameters when they were only 100% fertilized (Cluster 2). Furthermore, the 100% fertilized treatment without inoculation did not have a positive effect on the parameters. On the other hand, all treatments without fertilization showed the lowest results (Cluster 1), similar to what was observed in INIA 619. 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.

4. Discussion

The high cost of chemical fertilizers and the adverse effects of their excessive use drove the search for new technologies to support sustainable agriculture [38]. Microbial inoculants emerged as a promising alternative to enhance nutrient use efficiency [39–42] 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. [22] reported a significant increase in ear weight with the presence of PGPR in maize.

In the Dekal B-7088 variety, 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 9). 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 [43,44]. These findings highlighted the significant role of microbial inoculation in enhancing ear characteristics when combined with mineral fertilization.

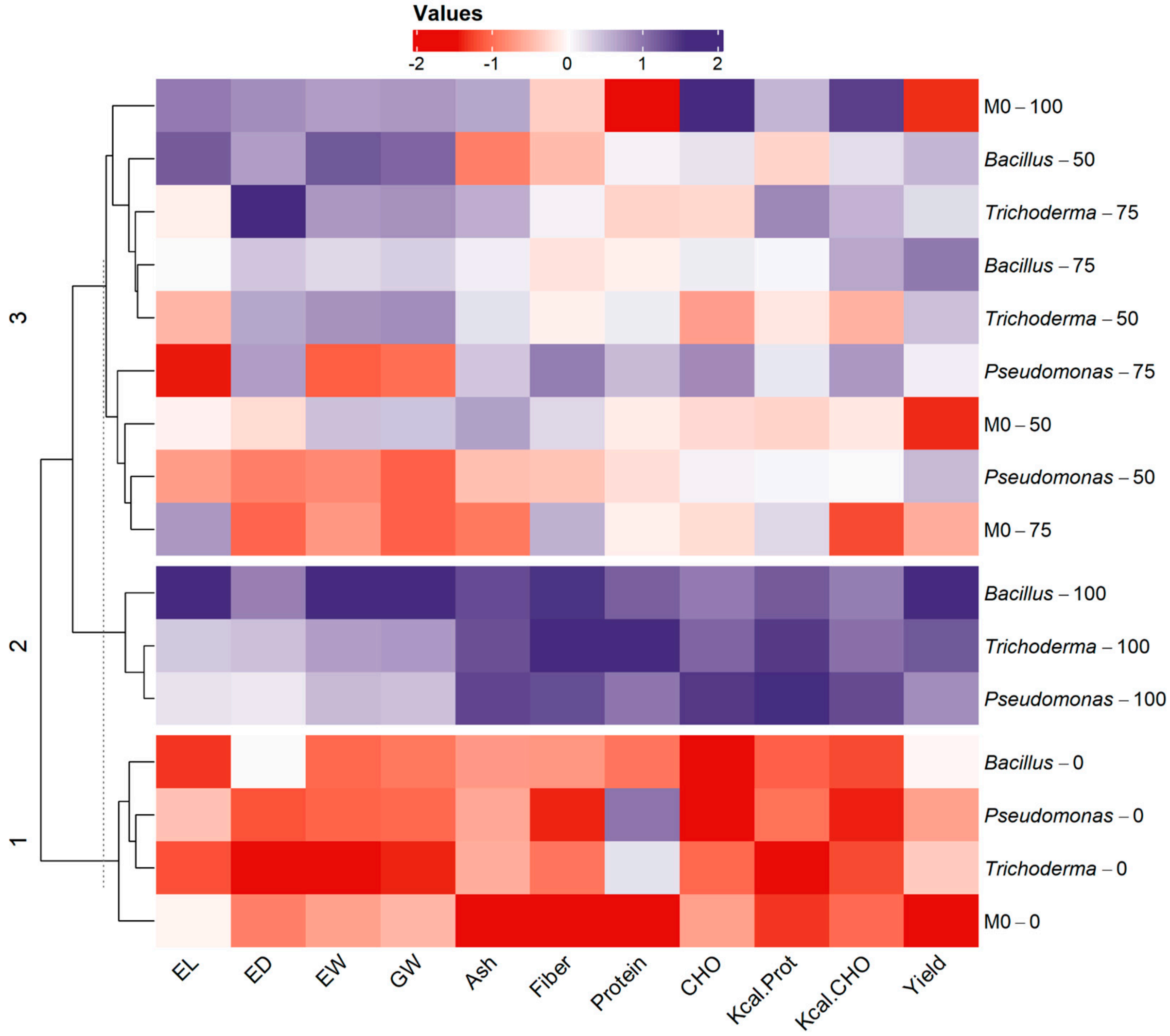

Figure 9. Heatmap with cluster analysis illustrating the combined effects of microbial inoculation and chemical fertilization on the Dekal B-7088 variety. The *Y*-axis lists all treatments resulting from the combinations of factor levels, while the *X*-axis displays the evaluated parameters. The numbers 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.

The incorporation of microbial inoculants improved both yields in two maize varieties that represent different agroecological contexts. The INIA-619 variety is a strategic option for smallholders in the highland and upper jungle regions of Peru, due to its adaptation to altitudes ranging from 1200 to 3000 m above sea level [45]. This variety was improved for low-tech farming systems with limited access to mineral fertilizers, with a potential yield of 14 t ha^{-1} [46]. In the highland and upper jungle regions of Peru, average yields of hard yellow maize range from 1.61 t ha^{-1} to 2.88 t ha^{-1}, respectively [4]. In this experiment, the grain yield was lower than both the potential yield of the variety and that of Dekal B-7088, which can be attributed to the fact that planting occurred during the winter (June), while it is recommended to plant in the summer [46]. On the other hand, the Dekal B-7088 variety was developed for low-to-medium altitude areas, characteristic of the Peruvian coast, where producers have access to more advanced technology, and fertilization practices are intensified, resulting in average yields between 6.34 and 10.43 t ha^{-1} [4]. Despite its potential yield of up to 28 t ha^{-1}, the adoption of this variety is limited by the high initial costs associated with purchasing certified seeds and the reliance on external chemical inputs [47]. Despite these differences, microbial inoculation resulted in increased productivity in both varieties, achieving yields comparable to or greater than those obtained with mineral fertilization, and acted synergistically in improving grain quality.

Numerous studies emphasized the effectiveness of PGPR and *Trichoderma* in enhancing nutrient utilization [45–48]. For instance, Oliveira-Paiva et al. [20] reported a 16% increase in grain yield in soils inoculated with *Bacillus subtilis*. Similarly, Gholami et al. [48] 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* [42,50,51], which increase the availability of nitrogen, phosphorus, and potassium, supporting better root development and more efficient nutrient utilization [21]. 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 demonstrated the biostimulant effects 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, P_2O_5, and K_2O) yielded the highest protein content in both evaluated hybrids (Figures 4A and 6A). Previous studies indicated 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 aligned 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 [22,51,52,57,58]. Solórzano and Quispe [42] reported that *Bacillus* and *Pseudomonas* enhance nitrogen uptake efficiency by promoting root growth, while Akladious and Abbas [27], 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 [59]. The cell wall is a crucial plant structure, supporting and protecting plant cells [60]. Its formation and stability rely on adequate levels of essential nutrients, particularly calcium and boron [61]. Excessive fertilization can reduce calcium availability and uptake due to ionic competition with K^+ and $NH4^+$ or precipitation with P [62]. Consequently, the total fertilization dose may have reduced grain fiber content (Figure 3B). These findings aligned 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 [63]. Therefore, higher ash content may indicate enhanced Ca assimilation, stimulating cell wall components' biosynthesis and increasing fiber content in maize grain [64]. 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 6B), likely through improved calcium assimilation and its role in cell wall component biosynthesis [65].

The fertilization effect on carbohydrate content varied between the two hybrids. Li et al. [66] reported that a nitrogen dose of 240 $kg{\cdot}ha^{-1}$—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 [67], although excessive nitrogen can degrade the grain quality of sensitive genotypes [12]. In INIA 619, microbial inoculation emerged as a beneficial alternative for carbohydrate accumulation in the INIA 619 hybrid.

These research results suggested 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 [5,68]. 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 [69]. This approach contributed 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 highlighted the synergistic effects of microbial inoculants and mineral fertilization on two maize varieties of agricultural importance in the Peruvian coastal region.

For INIA 619, the results indicated that microbial inoculation generally increased grain and ear weight and improved yield by 13.1%, with *Bacillus subtilis* showing particular effectiveness. Mineral fertilization at 75% and 50% of the recommended dose showed no significant differences compared to the full dose. Regarding nutritional quality, the interaction of *Bacillus subtilis*, *Trichoderma viridae*, and *Pseudomonas putida* with 100% mineral fertilization significantly increased protein and carbohydrate content by 47% and 6%, respectively. Notably, in INIA 619, nutritional quality was maintained even with a reduced fertilization dose of 75%.

For the Dekal B-7088 variety, microbial inoculation increased grain yield by 55.5%, with *Bacillus subtilis* again standing out. Regarding nutritional quality, the interaction of microorganisms with the full dose of mineral fertilization enhanced protein content by 54% and fiber content by 27%.

These findings could support more sustainable agricultural practices by reducing reliance on synthetic fertilizers, potentially mitigating long-term soil fertility loss. Future research should evaluate these strategies under diverse agro-climatic conditions and for various locally important crop varieties, assessing the effectiveness of microbial inoculants. Additionally, it is recommended to investigate the cumulative effects of combining mineral fertilization with long-term inoculant use on soil fertility and crop sustainability. Further studies optimizing inoculant and fertilizer dosages could provide valuable insights for developing more efficient and environmentally sustainable agricultural practices.

Author Contributions: E.C.-R., methodology, validation, writing—original draft, visualization, investigation; R.L.-M., methodology, investigation; K.Q., writing—review and editing; S.L., formal analysis, writing—review and editing; L.H., writing—review and editing, B.L.T., supervision, R.S., conceptualization, supervision, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the INIA project "Mejoramiento de los servicios de investigación y transferencia tecnológica en el manejo y recuperación de suelos agrícolas degradados y aguas para riego en la pequeña y mediana agricultura en los departamentos de Lima, Áncash, San Martín, Cajamarca, Lambayeque, Junín, Ayacucho, Arequipa, Puno y Ucayali" CUI 2487112.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: We extend our gratitude to Hector Cantaro for supplying the microbial inoculants used in this research, Sady Javier García Bendezú, the professors of the Soil Department of the National Agrarian University La Molina (UNALM) for their technical and scientific advice, and Juan Carlos Jaulis for providing the land essential to conducting this study.

Conflicts of Interest: The authors declare no conflicts of interest.

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