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Synergistic effect of biofilm-forming biofertilizers and ameliorants for enhancing fertilizer efficiency and maize agronomic traits on acidic soils

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ABSTRACT

Acidic soils present significant challenges to maize productivity by limiting phosphorus availability and increasing toxicity risks from aluminum (Al) and iron (Fe). This study investigates the synergistic effect of biofilm-forming biofertilizers (BF) and organic ameliorants (OA) in improving soil fertility, enhancing nutrient availability, and maximizing maize growth and yield in acidic soils. A factorial randomized block design with three replications was employed, where the first factor included four treatments: control, BF only, OA only, and a combination of BF and OA. The second factor involved the application rate of inorganic NP fertilizers (IF) at 100%, 80%, 60%, and 40% of the recommended dose. Growth parameters, grain yield, and soil fertility indicators were assessed. The results revealed that the synergistic application of BF and OA significantly improved maize height and grain yield, particularly when integrated with reduced NP fertilizer rates. The highest maize height (222.33 cm) and grain yield (10.09 t ha^{-1}) were achieved with the application of 1.200 g ha^{-1} BF and 2 t ha^{-1} OA. Furthermore, BF and OA application reduced NP fertilizer dependency by 40% without compromising yield, demonstrating enhanced fertilizer use efficiency. A strong positive correlation ($R^2 = 0.92$) was observed between soil fertility improvements and maize grain yield, with BF and OA contributing 45% to yield enhancement. These findings highlight the effectiveness of biofilm-forming biofertilizers and ameliorants as sustainable solutions for improving maize productivity and soil health in acidic-stressed environments.

Keywords: soil amendments, nutrient availability, microbial fertilizer, soil health, degraded soil, interaction effect.

INTRODUCTION

Maize is the second most important staple crop and plays a vital role in food security in Indonesia. However, the demand for maize still heavily relies on imports. To increase domestic production, both extensification and intensification on acidic soils are essential. Indonesia has an estimated 108.8 million hectares of acidic upland soils, distributed mainly in Sumatra, Kalimantan, and Papua, significantly constraining maize cultivation (Mulyani and Sarwani, 2013). The extent and potency of these acid soils in many Indonesian regions, particularly those with high rainfall and volcanic activity, are a major challenge for maize production. The primary limiting factor for maize production in these areas is the acidic nature of the soil, which affects nutrient availability and impedes healthy crop growth, requiring targeted solutions to enhance soil fertility.

Acid soils are a major constraint to sustainable agriculture, particularly in regions where soil acidity limits crop productivity and nutrient availability. Acid-stressed soils not only hinder plant growth but also reduce the efficiency of essential fertilizers, such as nitrogen (N), phosphorus (P), and potassium (K), collectively known as NPK fertilizers (Daba et al., 2021). In these conditions, nutrient leaching, fixation, and decreased microbial activity result in suboptimal fertilizer efficiency, despite the widespread use of inorganic fertilizers to correct nutrient deficiencies. Furthermore, the long-term use of inorganic fertilizers can significantly lower soil pH, leading to soil acidification, which in turn affects soil enzyme activity and reduces microbial diversity (Ren et al, 2020; Zhang et al, 2024). These challenges underscore the need for innovative and environmentally friendly approaches to enhance soil fertility and improve crop productivity in acidic soils.

Biofertilizers, particularly biofilm-forming microbial inoculants, have emerged as promising tools to enhance the performance of inorganic fertilizers in acidic conditions. These biofilm-producing microorganisms create protective layers that improve their survival and functionality under stress conditions (Kumawat et al., 2021). Additionally, they facilitate nutrient solubilization, mobilization, and uptake, thereby enhancing soil health and plant growth. When integrated with ameliorants such as biochar or organic matter, biofertilizers not only mitigate the adverse effects of soil acidity but also promote beneficial interactions between soil microbes and plants, ultimately improving fertilizer use efficiency and crop productivity (Xia et al., 2020). This study aims to assess and investigate the significant role of biofilm-forming biofertilizer inoculants and enriched ameliorants in increasing the efficiency of Inorganic fertilizers and improving maize productivity under acidic soil conditions. By exploring the synergistic effects of these eco-friendly interventions, this research seeks to address the challenges of sustainable maize cultivation in acid-stressed soils while reducing the over-reliance on chemical fertilizers.

MATERIAL AND METHOD

Experimental design

The experiment was conducted using a randomized complete block design (RCBD) with a 4 × 4 factorial arrangement and three replications. The first factor was the application of biofertilizers and ameliorants, consisting of four treatments: (1) control, (2) biofertilizer (BF) at 1,200 g ha^{-1}, (3) organic ameliorant (OA) at 2 t ha^{-1}, and (4) a combination of BF and OA. The second factor was the rate of inorganic fertilizers, expressed as percentages of the full recommended dose (300 kg ha^{-1} Urea + 200 kg ha^{-1} SP-36), with four levels: 40%, 60%, 80%, and 100%. Each treatment combination was replicated three times, resulting in 48 experimental plots.

Study site

The field trial was conducted in Pasir Banteng, Sumedang District (6°55'26" S, 107°47'19" E) at an elevation of approximately 800 meters above sea level (Figure 1). The site experiences an average monthly precipitation of 96 mm and a relative humidity of 88%. The physical and chemical properties of the soil are summarized in Table 1.

Table 1 presents the physical and chemical properties of the soil at the experimental site. The soil has a pH of 5.25, indicating that it is acidic. The organic carbon content (C-organic) is 2.30%, which is considered fair, while the C/N ratio is 11, also categorized as fair. The soil's cation exchange capacity (CEC) is 23.61 cmol.kg^{-1}, which falls under the fair category. In terms of texture, the soil consists of 8% sand, 59% silt, and 33% clay, classifying it as silty clay loam.

The soil was cultivated to a depth of 20 cm using a tractor, and 48 experimental plots (each 3 × 1.5 m with three ridges) were established, with 16

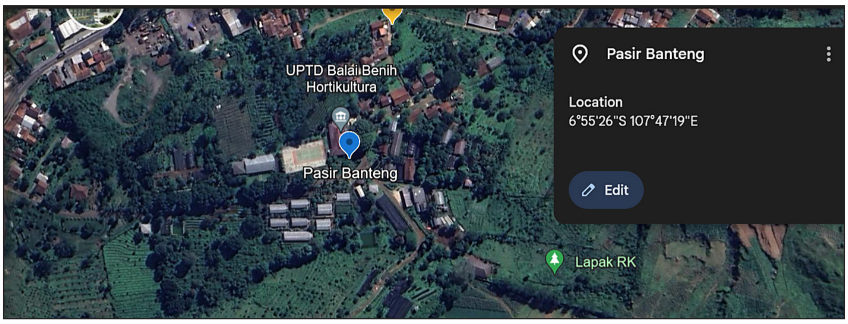

Figure 1. The location of field trial in Pasir Banteng of Sumedang District

Table 1. The soil physical and chemical properties of the experimental field

Parameter	Unit	Value	Criteria
pH : H20	-	5.25	acid
C-organic	%	2.30	fair
C/N	-	11	fair
CEC	cmol.kg^{-1}	23.61	fair
Texture			
Sand	%	8	silty clay loam
Silt	%	59	
Clay	%	33	

plots per replication and 0.5 m spacing between plots. BISI-2 maize seeds were sown at 5 cm depth with 75 × 25 cm spacing, with two seeds per hole, then covered with a soil-ameliorant mixture. After one week, thinning was performed to retain one healthy seedling per hole, followed by hilling to ensure proper soil coverage. Fertilization was applied in two stages: biofertilizers, ameliorants, and SP-36 were incorporated at planting, while Urea and KCl were applied in split doses – 50% one week after planting and 50% at four weeks, placed 5–10 cm from the plants. Maintenance included manual weeding (weekly), irrigation during two consecutive dry days, and pest and disease management, with pests controlled manually and diseases managed through chemical treatments based on plant damage assessments.

Materials used

Preparation of biofertilizer

In this study, the bacteria used are superior isolates from previous research characterized by electrophoresis. The electrophoresis results show that band 4 corresponds to *Enterobacter ludwigii* and band 7 corresponds to *Burkholderia vietnamiensis*, both of which function as nitrogen-fixing bacteria (NFB) and phosphate-solubilizing bacteria (PSB). These bacteria were selected for their proven ability to enhance nutrient availability in acidic soils and support maize plant growth, as shown in Figure 2.

The isolates of Enterobacter ludwigii and Burkholderia vietnamiensis were obtained from the maize rhizosphere in Tasikmalaya and Majalengka, West Java, Indonesia. These isolates were chosen based on their ability to solubilize phosphate and improve acidic soil conditions, which is crucial for enhancing nutrient availability for maize plants. These two bacterial strains were then enriched with other nitrogen-fixing bacteria (NFB) from the Soil Microbiology Laboratory Collection at Universitas Padjadjaran, as shown in Table 2, to enhance their overall efficacy in promoting soil fertility and maize growth. The biofertilizer consortium was prepared by inoculating bacterial strains into a carrier material consisting of peat soil, chicken manure compost, coconut shell biochar, and an additive in a 5:2:2:1 ratio. Each bacterial suspension (containing 10^8 CFU mL^{-1}) was mixed into 200 g of the carrier material using 50 mL of inoculant, ensuring uniform distribution. The inoculated mixture was then sealed in aluminum foil packaging to maintain microbial activity until application. The biofertilizer was applied using two main methods: seed treatment and soil application. For seed treatment, 20 g of the bacterial inoculant (equivalent to 0.4 kg ha^{-1}) was mixed with 1 kg of maize seeds prior to planting.

Preparation of organic ameliorant

The ameliorant used in this study is an organic ameliorant based on bagasse, which contains 50% sugarcane compost, 20% biochar, 20% dolomite, 9% guano, and 1% humic acid. Each component was first sieved using a 0.5 mm mesh and then composited before being used in the formulation.

Parameter respond

Biofilm forming test and bacterial enumeration

The biofilm-forming test was conducted using the microtiter dish assay method (Toole, 2011), where the extent of biofilm formation was measured using crystal violet dye. Bacterial enumeration was determined using the total plate count (TPC) method, which estimates the total number of bacteria in a given sample.

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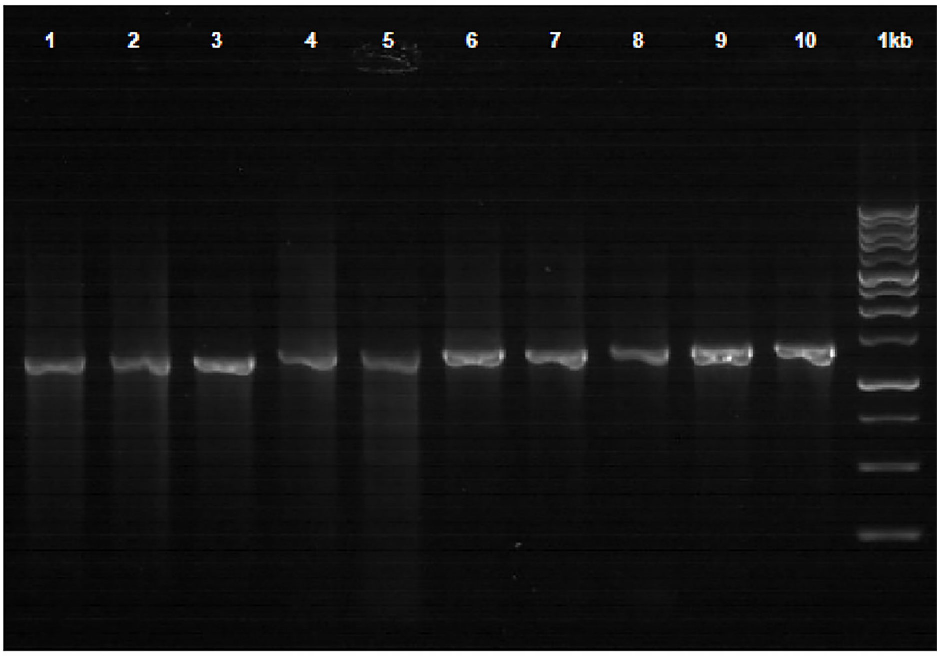

Condition: 0.8% agarose gel

Amount of DNA ladder loaded per lane: 0.2ug each

Volume of Sample loaded per lane: 1uL each

1kb DNA Ladder (bp): 250, 500, 750, **1000**, 1500, 2000, 2500, **3000**, 4000, 5000, 6000, 8000, 10000
1kb DNA Ladder (ng/0.2ug): 18, 12, 9, 37, 8, 14, 14, 37, 7, 11, 11, 11, 11
Note: The DNA ladder is not applicable for sizing comparison of non-linear DNA samples (e.g. plasmid DNA)

Figure 2. Electrophoresis analysis of DNA samples

Table 2. The traits and origin of rhizobacterial isolates

Rhizobacteria	Functional Group	Origin
Azotobacter chroococcum,	Nitrogen fixing bacteria	Collection of our laboratorium
Azospirillum sp	Nitrogen fixing bacteria	Collection of our laboratorium
Burkholderia vietnamiensi	Phosphate solubilizing bacteria	Maize Rhizosphere, Majalengka
Enterobacter ludwigii	Phosphate solubilizing bacteria	Maize Rhizosphere, Tasikmalaya

Phosphate-solubilizing bacterial enumeration was performed using Pikovskaya medium, while nitrogen-fixing bacterial enumeration was conducted using JNFB medium.

Soil chemical properties

The pH value indicates the concentration of H^+ ions in the soil solution, expressed as -log[H^+]. The concentration of H^+ extracted with water indicates pH. For the determination of cation exchange capacity (CEC) in soil, excess exchangeable cations are washed with 96% ethanol. The NH_4^+ ions adsorbed are replaced with Na^+ ions from a NaCl solution, allowing them to be measured as CEC.

In an acidic environment, carbon as an organic compound will reduce orange-colored Cr^{6+} to green-colored Cr^{3+}. The intensity of the green color formed is proportional to the carbon content and can be measured with a spectrophotometer at a wavelength of 561 nm.

Agronomic traits

The observation of dry weight is conducted by taking the plants, then pre-drying them at 70°C in an oven, and subsequently weighing them until a constant weight is achieved. The quantification of leaf chlorophyll is performed using a chlorophyll meter. The calculation of leaf count is measured visually.

The maize yield is measured by drying maize seed and weighing them.

The relative agronomic effectivenness (RAE)

RAE is calculated according to following formula:

$$RAE = \frac{tested\ yield - control}{control} \times 100\% \quad (1)$$

Statistical analysis

The observed data were analyzed using IBM SPSS Statistics version 26 (New York, USA). Analysis of variance (ANOVA) was conducted to determine the significant effects of the tested treatments. When a significant effect was detected, Duncan's multiple range test (DMRT) at $P < 0.05$ was performed to compare mean differences between treatments. Path analysis was used to interpret equations by determining the coefficient of multiple regression. Correlation and regression analyses were conducted using IBM SPSS Statistics version 26, followed by path analysis or structural equation modeling (SEM) using IBM SPSS Amos version 26.

RESULT AND DISCUSSION

Biofilm forming test and bacterial enumeration

Biofilm forming

The biofilm testing results demonstrated that all bacterial isolates used in this study were capable of producing biofilm, as indicated by the presence of a purple stain adhering to the microtiter plate walls (Figure 3). The color difference between biofilm-forming and non-biofilm-forming microtiter plates was clearly distinguishable, confirming the ability of the tested isolates to develop biofilms.

The four bacterial isolates exhibit significant potential as inoculants for plant growth-promoting rhizobacteria (PGPR) due to their ability to fix nitrogen, solubilize phosphorus, and form biofilms, which optimize nutrient availability for plants. Biofilm formation enhances bacterial stability and activity in the rhizosphere, particularly in acidic soils, ensuring a sustained supply of essential nutrients such as nitrogen and phosphorus. Additionally, biofilm-forming bacteria contribute to environmental benefits by reducing gas emissions, mitigating soil pollutants, and improving land degradation. The extracellular polymeric substances (EPS) they produce can modify soil aggregate structure, retain soil moisture, and serve as a carbon source, further enhancing soil health (Velmourougane et al, 2023). Therefore, the application of these four bacterial isolates is expected to improve maize production in acidic soils.

Population of nitrogen-fixing and phosphate-solubilizing

The role of nitrogen-fixing bacteria (NFB) and phosphate-solubilizing bacteria (PSB) in enhancing plant productivity has been widely recognized in modern agricultural research. In this study, the

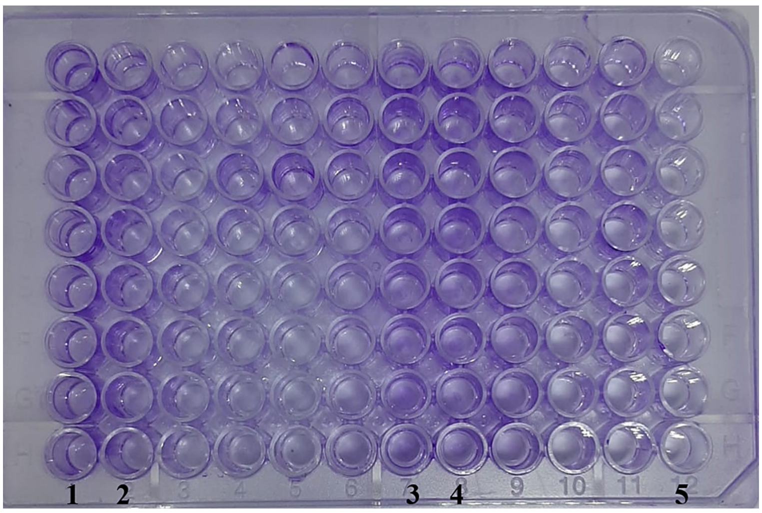

Figure 3. The appearance of the four biofilm-forming isolates characterized by a purple color on the microtiter wall (1 = *Azotobacter chroococum*, 2 = *Azos*pirillum sp., 3 = *Burkholderia vietnamiensii*, and 4 = *Enterobacter ludwigii*) was compared with the non-color-forming control (5 = control)

application of biofertilizer and organic ameliorant significantly influenced the populations of NFB and PSB, as shown in Tables 3 and 4.

Based on Table 3, it is shown that biofertilizer and ameliorant interact with NP fertilizer levels to influence NFB populations. Across all NP fertilizer levels, the control treatment exhibited the lowest NFB population. While the application of biofertilizer or organic ameliorant alone led to a slight but non-significant increase in NFB populations at lower NP fertilizer levels, both treatments significantly enhanced NFB populations at higher NP fertilizer levels. The highest NFB population was recorded in the combined treatment, particularly at 80% and 100% NP fertilizer levels, where it consistently outperformed other treatments. This trend suggests a synergistic effect between biofertilizer and organic ameliorant in promoting NFB proliferation, likely due to improved microbial habitat and nutrient availability. The interaction between biofertilizer, ameliorant, and NP fertilizer levels indicates that their combined application optimizes conditions for NFB growth, enhancing their efficacy in the soil.

The control treatment exhibited the lowest PSB populations across all NP fertilizer levels, indicating that without biofertilizer or ameliorant application, PSB populations remained relatively low and unaffected by NP fertilizer alone. The application of biofertilizer significantly increased PSB populations, while the organic ameliorant also contributed to an increase, though to a lesser extent. The highest PSB populations were observed in the combined treatment of biofertilizer and ameliorant, which consistently outperformed individual applications across all NP fertilizer levels. This suggests a synergistic effect between biofertilizer and organic ameliorant in promoting PSB proliferation, likely by enhancing microbial habitat and nutrient availability in the soil.

The significant increase in nitrogen-fixing bacteria (NFB) and phosphate-solubilizing bacteria (PSB) populations following biofertilizer and organic ameliorant application highlights their crucial role in enhancing soil microbial activity and nutrient availability. Biofertilizers enhance microbial diversity and alter the community structure in the rhizosphere, promoting beneficial bacteria and fungi that support plant growth and soil health (Gu et al, 2023). Organic ameliorants improve soil structure by increasing pore volume and surface area, which enhances water retention and aeration. They also help stabilize soil pH, creating a more conducive environment for microbial activity (Huang et al, 2023). The highest bacterial populations were observed in the combined

Table 3. Effect of biofertilizer and ameliorant on NFB population (x10^8 cfu g^{-1}) at different NP fertilizer rates

Treatment	NP Fertilizer (recommendation dosage)			
	40%	60%	80%	100%
Control	28.90 a A	30.73 b B	31.87 a C	32.83 a D
1200 g ha^{-1} BF	29.77 ab A	29.47 a A	31.80 a B	38.90 c C
2 t ha^{-1} OA	29.50 ab A	30.30 ab A	31.73 a B	35.07 b C
1200 g ha^{-1} BI + 2 t ha^{-1} OA	30.20 b A	32.20 c B	34.37 b C	39.47 c D

Note: BF – biofertilizer, OA – organic ameliorant. The mean value followed by the same letter was not significantly different according to Duncan's follow-up test at the 0.05 significance level. Lowercase letters are read vertically. Capital letters are read horizontally.

Table 4. Effect of biofertilizer and ameliorant on PSB population (x10^8 cfu g^{-1}) at different NP fertilizer rates

Treatment	NP Fertilizer (kg ha^{-1})			
	40%	60%	80%	100%
Control	2,067 a A	2,200 a A	2,167 a A	2,267 a A
1200 g ha^{-1} BF	48,817 c AB	48,500 c A	51,100 c BC	52,250 b C
2 t ha^{-1} OA	23,633 b A	24,733 b AB	26,333 b B	27,350 c B
1200 g ha^{-1} BF + 2 t ha^{-1} OA	47,183 c A	50,433 c B	55,900 d C	57,550 d C

Note: BF – biofertilizer, OA – organic ameliorant. The mean value followed by the same letter was not significantly different according to Duncan's follow-up test at the 0.05 significance level. Lowercase letters are read vertically. Capital letters are read horizontally.

treatment, indicating a synergistic effect, where biofertilizer inoculation was enhanced by the ameliorant's ability to improve soil conditions, leading to greater microbial stability and activity.

The long-term use of biofertilizers can enhance soil microbial activity and support microbial biodiversity, contributing to the balance of the soil ecosystem. Research indicates that the application of biofertilizers can increase soil microbial diversity, restructure microbial communities, and enhance enzyme activity and microbial metabolism (Shan et al., 2023; Ali et al., 2024). Additionally, the application of organic ameliorants provides a better habitat for microbes by increasing organic carbon and improving the physical and chemical properties of the soil, thus supporting the survival and growth of these microbes (Deshoux et al., 2023; Li et al., 2024). Over time, these beneficial microbes will dominate the soil, creating more stable and healthy conditions, while reducing dependence on chemical fertilizers. These positive effects naturally enhance soil fertility, improve fertilization efficiency, and reduce environmental impacts, ultimately promoting more environmentally friendly and sustainable agricultural practices.

Soil chemical properties

Application of inoculant PGPR and organic ameliorants has a significant impact on the increase in organic carbon, cation exchange capacity (CEC), and pH. However, the application of different doses of NP fertilizer does not show a significant difference (Figure 4).

Figure 4 shows that the combination of biofertilizer and organic ameliorant has a significant positive impact on organic carbon (4.31%), CEC (30.76 cmol kg^{-1}), and soil pH (6.92), all of which contribute to the improvement of soil quality and fertility. This indicates that the combined use of these treatments has a synergistic effect on enhancing the soil's chemical properties, which, in turn, can sustainably increase crop productivity. The application of biofertilizers has multiple beneficial effects on soil properties, contributing to enhanced nutrient availability and plant growth. Biofertilizers modify soil pH by introducing microorganisms that produce compounds to buffer soil acidity or alkalinity, creating a more favorable environment for plant growth (Silva et al, 2021; Jin et al, 2021). Additionally, biofertilizers increase CEC by adding organic matter and promoting microbial activity, which improves soil structure and enhances the retention of essential nutrients like calcium, magnesium, and potassium (Kassim et al, 2024). Furthermore, biofertilizers boost organic carbon levels in the soil, which improves soil structure, water retention, nutrient availability, and overall soil health, all of which contribute to better plant growth (Dlugosz et al, 2023). These synergistic effects make biofertilizers a key tool for sustainable soil management and agricultural productivity.

Agronomic character

After evaluating the effects of biofertilizers and ameliorants on soil health, the next focus of this study was to examine their impact on agronomic characteristics. Improvements in soil health, characterized by increased organic matter, enhanced nutrient retention, and higher microbial activity, are expected to positively influence plant growth and overall productivity. These agronomic data can be observed in Table 5, which provides a detailed account of the effects on key crop traits.

Table 5 demonstrates that the application of biofertilizers and ameliorants interacts with NP fertilizer rates and significantly affects stem diameter, chlorophyll content, and root dry weight, while no interaction was observed for leaf count, though both treatments still had a significant effect. This suggests that combining biofertilizers

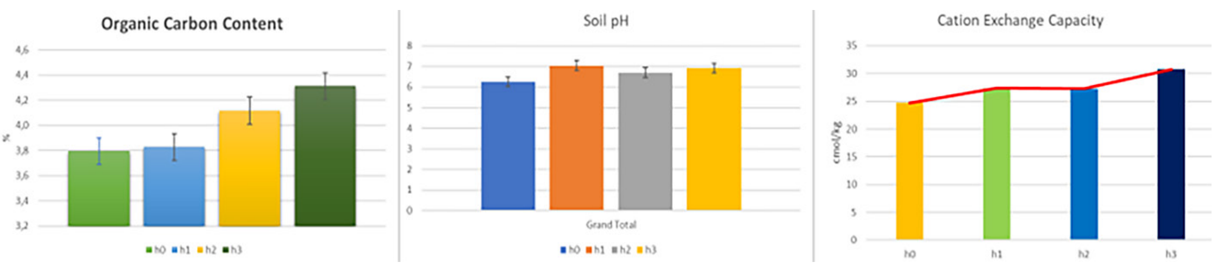

Figure 4. Effect of biofertilizer soil chemical properties

Table 5. Effect of biofertilizer and ameliorant (B) and inorganic fertilizer rates (N) and their ineraction (BxN) on Agronomic Traits (Height, Diameter, Count Leaf and Chlorophyll Meter on Maize)

Character	Treatment		Interaction
	B	N	(B x N)
Height	*	*	*
Stem diameter	*	*	*
Leaf count	*	*	ns
Chlorophyll content	*	*	*
Roots dry weight	*	*	*

Note: ns – not significant, * – significant.

and ameliorants with inorganic fertilizers can enhance various agronomic traits, contributing to improved growth and productivity of maize plants. Crop growth is essential in establishing a strong foundation for the successful generative phase of plants. High-quality growth traits, such as well-developed roots, stems, and leaves, indicate healthy plants. Optimal stem diameter and plant height are crucial factors for increased maize yield (Syafrizal et al, 2024). Plant growth, observable through traits like plant height, leaf count, and stem diameter, serves as an important indicator of overall plant health and development, reflecting the impact of soil amendments and fertilizer treatments on plant vitality (Figure 5).

Based on the images, it is evident that the control treatment (h0) exhibits limited root development, characterized by fewer and thinner roots. In contrast, the individual applications of biofertilizer (h1) and ameliorant (h2) result in more robust root systems with enhanced branching. The most significant effect, however, is observed in the treatment combining biofertilizer and ameliorant (h3), which displays a considerably denser and more developed root network. The roots in this combined treatment are thicker, longer, and exhibit increased branching, suggesting an improved capacity for nutrient and water uptake. Research has shown that the increase in root weight and distribution can enhance maize yield through improved water and nutrient absorption, water-use efficiency, and root distribution management (Feng et al, 2024). This enhanced root development indicates potential for better plant growth and overall productivity.

Maize grain yield and relative agronomic effectiveness (RAE)

The ultimate objective of agricultural practices is to enhance crop yield, with this study specifically focusing on maize yield. The application of biofertilizers and ameliorants has been shown to significantly improve soil health and agronomic characteristics in maize, including soil fertility, microbial activity, and nutrient availability. These improvements in soil properties contribute to better root development, enhanced plant growth, and increased nutrient uptake. Given the positive effects on soil health and agronomic traits, it is essential

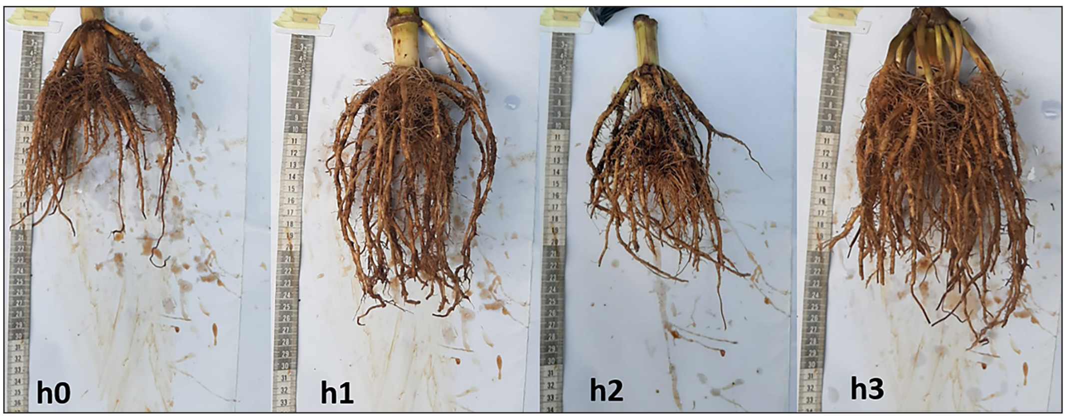

Figure 5. The effect of biofertilizer and ameliorant on root development. control (h0), biofertilizer (h1), ameliorant (h2) biofertilizer and ameliorant (h3)

to investigate how these enhancements directly influence maize grain yield and overall harvest performance. This analysis will provide a comprehensive understanding of the role of biofertilizers and ameliorants in optimizing maize productivity and inform sustainable agricultural practices.

Table 6 demonstrates that the application of both biofertilizers and ameliorants interacts synergistically and significantly improves maize seed yields compared to the control treatment. The combined use of biofertilizer and ameliorant resulted in the highest yield of 10.09 t ha^{-1}, which is a considerable increase compared to the control, where no organic fertilizers were applied, yielding only 7.6 t ha^{-1}. This indicates that the application of biofertilizers and ameliorants can effectively enhance maize productivity by improving soil conditions and supporting better nutrient uptake, ultimately leading to higher yields. The efficiency of inorganic fertilizers is calculated by comparing the treatments applied with the control treatment (Table 7). The control treatment follows the conventional method commonly used by farmers, which relies solely on the application of inorganic fertilizers without the addition of organic fertilizers (h_0p_4). The study demonstrates that the use of biofertilizers alone and ameliorants alone can improve the efficiency of inorganic fertilization by up to 20%. On the other hand, the synergy between biofertilizers and ameliorants can enhance the efficiency of inorganic fertilizer use by up to 40%.

Higher fertilizer use efficiency allows farmers to reduce their dependence on inorganic fertilizers, which are often costly and have negative environmental impacts. The use of biofertilizers and ameliorants to enhance fertilization efficiency also supports the principles of sustainable agriculture, as this practice not only reduces the environmental impact of chemical fertilizers but also improves soil health and microbial diversity (Kour et al., 2020; Nosheen et al., 2021). Furthermore, by optimizing the use of inorganic fertilizers, farmers can maximize crop yields at a lower cost, ultimately enhancing their economic resilience and promoting long-term agricultural sustainability (Yuan et al., 2022). Therefore, the application of this technology plays a critical role in achieving efficient, environmentally friendly, and sustainable agricultural practices.

One of the key long-term benefits of biofertilizer and ameliorant application is the reduction in dependence on chemical fertilizers. This reduction not only lowers fertilization costs but also mitigates negative environmental impacts, such as water pollution and soil ecosystem degradation, which are often associated with excessive chemical fertilizer use (Priya et al., 2024; Bhaskar et al., 2023). Enhancing soil quality through increased organic carbon and beneficial microbial activity plays a crucial role in sustaining soil

Table 6. Effect of biofertilizer and ameliorant on yield (ton ha^{-1} at different NP fertilizer rates)

Treatments	NP fertilizer (kg ha^{-1})			
	40%	60%	80%	100%
Control	5,97 a A	6,55 a B	7,09 a C	7,6 a D
1200 g ha^{-1} BF	6,60 b A	7,13 b B	7,91 b C	9,2 c D
2 t ha^{-1} OA	6,43 b A	6,79 ab B	7,63 b C	8,1 b D
1200 g ha^{-1} BF + 2 t ha^{-1} OA	7,24 c A	8,19 c B	9,15 c C	10,1 d D

Note: The mean value followed by the same letter was not significantly different according to Duncan's follow-up test at the 0.05 significance level. Lowercase letters are read vertically. Capital letters are read horizontally.

Table 7. Efficiency agronomic due to biofertilizer (BF) and ameliorant (OA) recommended dosage = 135 kg N + 72 kg P_2O_5 per hectare

Treatments	Percentage of NP fertilizer of recommended dosage*			
	40%	60%	80%	100%
Control	-10.12	-4.32	-1.54	Control
1200 g ha^{-1} BF	-6.13	-1.91	1.01	4.02
2 t ha^{-1} OA	-7.22	-3.33	0.13	1.33
1200 g ha^{-1} BF + 2 t ha^{-1} OA	-2.16	2.50	4.88	6.27

Note: Recommended dosage = 135 kg N + 72 kg P_2O_5 per hectare.

fertility over time. Higher organic carbon levels contribute to improved soil structure, enhanced aggregation, and better aeration and drainage, all of which support long-term soil health and productivity (Soinne et al., 2023; Saygin et al., 2023). Additionally, improved soil conditions foster greater microbial diversity and nutrient availability, ultimately strengthening plant resilience against environmental stresses such as drought and pathogen attacks. This, in turn, leads to higher and more stable maize yields, reinforcing the importance of biofertilizer and ameliorant applications in sustainable agricultural practices (Zhang et al., 2024; Akhtar et al., 2023).

Although biofertilizers and organic ameliorants have the potential to enhance soil fertility, their effectiveness varies depending on environmental conditions. In highly acidic soils, those low in organic matter, or severely degraded soils, the microorganisms in BF require a longer adaptation period, reducing their overall effectiveness (Shan et al., 2023; Macik et al., 2023). Additionally, dry climates or excessive rainfall can hinder microbial colonization in the soil, thereby decreasing the efficiency of biofertilizers in improving nutrient availability (Cuartero et al., 2024; Xiao et al., 2023). Unlike chemical fertilizers, which provide immediate nutrient availability, BF functions through biological mechanisms such as nitrogen fixation and phosphate solubilization, requiring repeated applications to establish a stable biological balance in the soil (Ali et al., 2024; Tamiru et al., 2023).

In addition to environmental factors, the effectiveness of BF is also influenced by its interaction with soil microorganisms. Competition with native microbes can hinder colonization and reduce its effectiveness (Horrocks et al., 2023). Furthermore, the microorganisms in BF require optimal storage conditions to remain viable before application to agricultural land (Fadiji et al., 2024). Therefore, the successful implementation of BF and OA must be supported by appropriate management strategies, including soil condition monitoring, adjustment of application doses, and further research to enhance their effectiveness across various agroecological conditions.

Relationship model of attributing variable to maize grain yield

To understand the factors influencing maize yield, it is essential to analyze the relationship between various agronomic variables and their contributions to overall maize productivity. From the Table 8, the following equation is obtained:

$$y = -2.436 + 0.001X1 + 0.002X2 + \\ + 0.162X3 + 0.054X4 + 0.084X5 \quad (1)$$

This regression equation represents the relationship between maize grain yield (Y) and several key factors contributing to maize productivity, namely phosphate solubilizing bacteria (PSB) population (X1), nitrogen fixation bacteria (NFB) (X2), chlorophyll content (X3), roots dry weight (X4), and 100-grain weight (X5). The negative coefficient of the constant (-2.436) indicates that, without the contribution of these factors, maize yield would tend to be low. Among the independent variables, chlorophyll content (X3) has the highest coefficient (0.162), suggesting that an increase in chlorophyll content has the most significant impact on maize yield. The 100-grain weight (X5) variable also has a significant effect with a coefficient of 0.084, indicating that 100-grain weight positively contributes to the final harvest. Although the coefficients for PSB and NFB populations are smaller, they still play an important role in increasing the availability of phosphate and nitrogen, which can enhance

Table 8. Multiple linear regression result between maize grain yield, phosphate solubilizing bacteria (PSB), nitrogen fixation bacteria (NFB), chlorophyll content, roots dry weight and 100 grain weight

Model	Unstandardized coefficients		Standardized coefficients	Zero-order
	B	Std. Error	Beta	
Constant	-2.436	.665		
PSB population	.001	.000	.128	.616
NFB population	.002	.001	.129	.726
Chlorophyll content	.162	.029	.452	.898
Roots dry weight	.054	.041	.101	.846
100 grains weight	.084	.018	.310	.863

maize productivity overall. This equation underscores the importance of these factors in achieving optimal maize productivity.

The results of correlation coefficient and determination tests are presented in Table 9. These results indicate that the correlation coefficient (R) has a value of 96,5%. This value signifies a strong linear relationship between the populations of phosphate-solubilizing bacteria and nitrogen-fixing bacteria, chlorophyll content, and root dry weight with corn yield. Additionally, the coefficient of determination (R^2) is 0.932, explaining that 93,20% of the variation in highland maize yield can be attributed to the linear relationship with PSB and NFB populations, chlorophyll content, and root dry weight. The remaining 6,8% is influenced by other factors not analyzed in this study. Further analysis to comprehend the results of the correlation and regression coefficients was conducted using path analysis. This method helps identify which variables have a greater direct impact on the dependent variable. Therefore, path analysis was performed to determine the direct effects of PSB and NFB populations, chlorophyll content, root dry weight and 100 grain weight on maize yields.

The inoculation of bacteria and organic ameliorant significantly affects various factors, including PSB, NFB, root dry weight, chlorophyll content, 100-grain weight, and maize yield. The effective contributions of these variables to grain yield, in descending order, are as follows: chlorophyll content (40.58%), 100-grain weight (26.75%), roots dry weight (8.54%), NFB population (9.36%), and PSB population (7.8%) (Table 10). The increase in chlorophyll directly influences harvest weight by enhancing photosynthesis, which prolongs the seed-filling process and increases seed weight, ultimately contributing to higher maize yield (Du et al., 2024). Nitrogen-fixing bacteria

Table 9. Correlation coefficient and determination

Model	R	R square	Adjusted R square	Std error of the estimate
1	0.965[a]	0.932	0.924	0.31148

Note: Predictors: constant, PSB and NFB population, chlorophyll content roots dry weight and 100 grain weight.

Table 10. Effective contribution for maize

Parameter	Beta	Zero-order	Effective contribution %
PSB population	0.128	0.616	7.8848
NFB population	0.129	0.726	9.3654
Chlorophyll content	0.452	0.898	40.5896
Roots dry weight	0.101	0.846	8.5446
100 grains weight	0.31	0.863	26.753

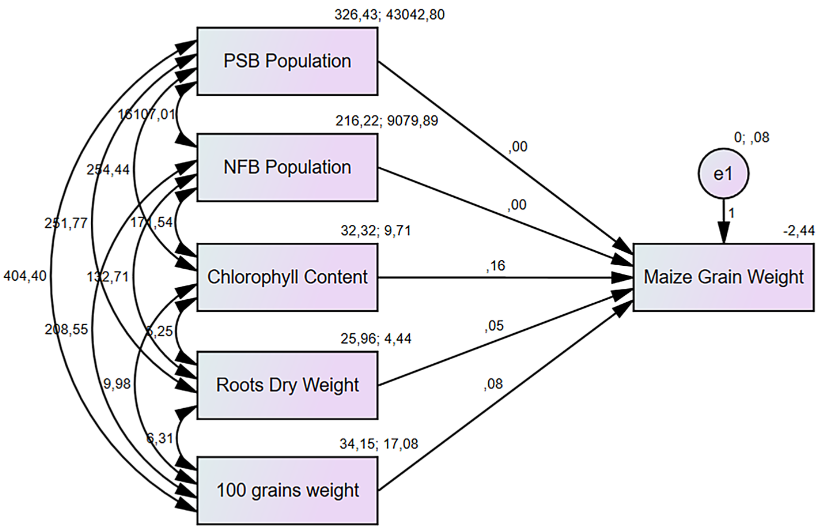

Figure 6. Contribution of attributing factors to grain yield with path coefficient value

improve nitrogen availability, promoting chlorophyll synthesis and boosting chlorophyll content, while enhancing enzyme activity, such as nitrogenase, involved in nitrogen fixation and metabolism, thereby enhancing maize growth and yield (Qin et al., 2022; Zhang et al., 2024). Additionally, PSB produces indole acetic acid (IAA) and siderophores, which stimulate plant growth by improving nutrient uptake and root development, while PSB inoculation increases soil phosphatase enzyme activity, facilitating phosphorus (P) mineralization and improving its availability for plants (Luo et al., 2024; Paiva et al., 2024) (Figure 6).

The relationship model can be used to identify key variables, such as soil health, fertilization strategies, biofertilizers, and ameliorants, that significantly influence maize yield. This model provides valuable insights into the contribution of each factor, which can be utilized to optimize agricultural practices. Furthermore, understanding the interactions between these factors and variables enables more efficient and sustainable management, ensuring higher productivity while minimizing harmful environmental impacts.

CONCLUSIONS

The application of 1200 $g \cdot ha^{-1}$ biofertilizer and ameliorant significantly improves soil health, agronomic characteristics, and fertilization efficiency, achieving up to a 40% increase in efficiency. The synergistic effect of biofertilizer and ameliorant with NP fertilizer rates notably enhances phosphate-solubilizing bacteria, nitrogen-fixing bacteria, soil physicochemical properties, chlorophyll content, root dry weight, and the weight of 100 grains across various NP fertilization doses. Additionally, the use of biofertilizer and ameliorant results in yield increases ranging from 2.55% to 6.27%. Moreover, 93.20% of the variation in highland maize yield can be attributed to the linear relationship with PSB and NFB populations, chlorophyll content, and root dry weight, highlighting the significant role of these factors in enhancing maize productivity. This finding concludes that the application of eco-friendly fertilizers, such as biofertilizers and ameliorants, provides a sustainable and environmentally friendly solution to enhance maize productivity on acidic soils.

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