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Assessing the synergistic effects of inorganic, organic, and biofertilizers on rhizosphere properties and yield of maize

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ABSTRACT

A single and long-term use of inorganic fertilizers harms soil quality. Therefore, it is highly recommended that inorganic fertilizers be combined with other fertilizers. This study explores the synergistic effect of inorganic, organic, and biofertilizers on maize rhizosphere properties and production. Biochar (BC) and compost (OF) were applied as organic sources, a consortium of phosphate solubilizing bacteria (PSB) was applied as phosphate biofertilizers, while urea and NPK-PONSKA fertilizers (IF) were used as inorganic fertilizers. A greenhouse experiment was designed as a completely randomized arrangement involving six treatments in triplicate, namely control (only IF), a combination of IF+BC, IF+PSB, IF+BC+PSB, IF+PSB+OF, and IF+BC+OF+PSB. The best changes in soil microbial and chemical properties, maize root dry weight, and production were observed in the IF+BC+OF+PSB combination, followed by IF+OF+PSB, IF+BC+PSB and (IF+BC; IF+PSB) and control treatment, respectively. A fertilizer combination involving the addition of BC (IF+BC, IF+BC+PSB, IF+BC+OF +PSB) significantly increased soil organic C content and soil pH compared to without biochar (IF+PSB and IF+OF+PSB). A higher root dry weight also results in higher maize production. Maize production Increased in the 4, 3, and 2 combinations compared to production in the control by 43.11%, 31.32-36.55%, and 18.57%-21.34%, respectively. In conclusion, the synergy of biochar, compost, and PSB, when integrated with fertilizer, can improve soil quality and the sustainability of maize production. This study will be useful in developing sustainable nutrient management programs to increase crop productivity with high efficiency in using inorganic fertilizers.

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1. INTRODUCTION

In Indonesia, maize (*Zea mays* L.) is one of the commodities that is encouraged in efforts to maintain food security. In 2023, the country targeted a shelled maize harvest area of 2.49 million hectares, an estimated production of 14.46 million tons (Simbolon, 2023). Indonesia has launched a program to expand the maize planting area, utilizing swamp land and dryland. However, maize cultivation in dryland faces challenges due to poor soil quality that is less favorable for plants, such as low organic matter content, low microbial activity, low N and P nutrient contents, low water retention, and sandy soil texture (Sukartono et al., 2022). These challenges are exacerbated by anthropogenic practices, especially using inorganic fertilizers such as NPK and urea. Farmers consider using inorganic fertilizers because they are more economical, affordable, easy to use, and fast-acting. They do not consider the long-term negative impact of reducing soil quality.

Although inorganic fertilizers are important inputs to increase plant productivity, their repeated and long-term use could have detrimental effects, such as degradation of soil quality (Pahalvi et al., 2021; Sharma et al., 2014) and significantly declining soil organic matter content (Pahalvi et al., 2021). In addition, the prolonged use of these fertilizers leads to chemical pollution in water, air, and soil (Pahalvi et al., 2021; Sharma et al., 2014). Furthermore, continuous application of inorganic fertilizers can disrupt microbial activity and population in the maize rhizosphere (Pahalvi et al., 2021). Therefore, to mitigate these issues, adopting advanced agricultural practices integrating inorganic fertilizers with other inputs such as biochar, organic fertilizer, and biofertilizer is essential to promoting sustainable agriculture practices (Sharma et al., 2014). Biochar, a carbon-rich material produced from biomass combustion, has been shown to enhance soil nutrient and water retention, boost maize yield, and reduce greenhouse gas emissions (Cornelissen et al., 2013; Wu et al., 2021; Zhang et al., 2016). Biochar's large surface area and negative charge improve cation adsorption and retention, fostering a conducive environment for microbial growth (Beheshti et al., 2017). This gradually releases nutrients, preventing leaching and enhancing soil fertility (Pandit et al., 2018).

Biochar, a carbon-rich material produced from biomass combustion (Santos et al., 2019), has been shown to enhance the soil's ability to retain nutrients and water (Lima et al., 2018), boosted maize yield, reduced greenhouse gas emissions (Zhang et al., 2016), and increased soil saturation base and cation exchange capacity (Cornelissen et al., 2013). The increased CEC, coupled with the large surface area and negatively charged surface of biochar, enhances the adsorption of water and retention of cations, including Ca^{2+}, Mg^{2+}, and NH^{4+} (Wu et al., 2021). The biochar surface facilitates soil bacteria and other microbes to grow and develop in a favorable environment because of its high moisture and soil nutrient content (Beheshti et al., 2017). In addition, cations bound to the biochar surface are potential for the adsorption of anions ($H_2PO_4^-$, HPO_4^{2-}) (Cornelissen et al., 2013). The adsorption and retention of these ions result in the gradual release of nutrients for plants and prevent loss through leaching, thus enhancing soil fertility (Pandit et al., 2018).

Apart from biochar, other organic fertilizers, such as compost, improve soil structure aeration and water movement. Soil with a relatively high soil organic matter content provides a suitable soil environment for microbial growth (Liu et al., 2021). Organic fertilizers also provide nutrients to soil microorganisms, thereby increasing microbial biomass and the N-fixing Azotobacter population in the soil cultivated with maize. Microorganisms that develop in soil-rich organic matter play a role in decomposition, nutrient cycling, and controlling pathogenic organisms. Therefore, organic fertilizer is essential to maintain the diversity and activity of soil microbes that contribute to plant growth.

Soil microbes such as phosphate-solubilizing bacteria (PSB) play many roles in providing and absorbing plant nutrients. Biofertilizer of phosphate-solubilizing bacteria converts insoluble soil phosphate into form by secreting organic acids such as formic, acetic, propionic, lactic, glycolate, and fumarate (Kudoyarova et al., 2017). These bacteria produce vitamins and phytohormones to improve the growth of plants and increase nutrient uptake (Kudoyarova et al., 2017; Rawat et al., 2021). Phosphate solubilizing bacteria (PSB) also assist maize growth by promoting biological N fixing, increasing the availability of phosphate, and releasing growth-promoting agents in the rhizosphere (Kudoyarova et al., 2017; Pathan et al., 2018; Rawat et al., 2021). The rhizosphere-surrounding environment is a crucial player for chemical and biological properties that build up reciprocal symbiosis to assist the growth of crops (Mohanram & Kumar, 2019).

Although the known benefit of these fertilizers individually, few studies have explored their combination with inorganic fertilizers. This integration is a new approach and can potentially solve the problem of low soil productivity in a sustainable, environmentally friendly, and cost-effective way. The study's novelty stands out by exploring the synergistic effects of a combined approach using inorganic, organic, and biofertilizers. Unlike previous studies focusing on individual amendments, our research investigates their combined impact, providing new insights into integrated fertilization strategies. This holistic approach addresses soil degradation issues more effectively, promoting sustainable maize cultivation practices that can be applied broadly to improve soil health and crop productivity. The study aims to investigate the synergistic effect of combining inorganic fertilizer with biochar, organic fertilizer, and biofertilizer (PSB) on the chemical and microbiological properties of the maize rhizosphere and yield in entisol soil. This integrated approach holds the potential to sustainably enhance soil productivity, offering an environmentally friendly and cost-effective solution to the challenges faced in maize cultivation.

2. MATERIAL AND METHODS

2.1. Greenhouse study and experimental design

The study was conducted in a greenhouse of the Faculty of Agriculture, The University of Mataram, from August to November 2022. *Zea mays* var. Lamuru was grown in each polybag containing 19 kg of air-dried soil. This experiment used entisol collected from the faculty experimental garden at Narmada village (8.583685 S, 116.201946 E), West Lombok Regency, West Nusa Tenggara, Indonesia. The soil sample was obtained by composite extraction at 0-20 cm depth. The soil was then air-dried at room temperature and sieved through a 2-mm mesh. The soil was characterized as a sandy loam texture (58% sand, 24% silt, and 18% clay), 1.18 g cm^3 of bulk density (BD), pH = 6.1; low SOM content (0.89%); total N (0.09%); available P (39.24 mg kg^{-1}); K-exchangeable (0.47 cmol kg^{-1}), cation exchange capacity (CEC) (15 cmol kg^{-1}) and bacterial population 1.35 x 10^6 colony forming unit (CFU) g^{-1} soil). The experiment was performed with a completely randomized design (CRD) with 6 different treatments (Table 1) and 3 replications for each treatment.

Each polybag was filled with an air-dried soil sample in the experimental setups involving IF, IF+PSB, and IF+OF+PSB treatments. Conversely, for IF+BC, IF+BC+PSB, and IF+BC+OF+PSB treatments, the air-dried soil sample was mixed with biochar before being introduced into the polybags. The biochar application rate was set at 10 tons per hectare, corresponding to 140 grams per polybag.

Table 1. Combination treatments used in the experiment

No	Treatment	Content
1	IF (Control)	Soil with inorganic fertilizer (2.625 g of urea and 3.150 g of PONSKA per polybag)
2	IF + BC	IF plus Biochar (140 g per polybag)
3	IF + PSB	IF plus PSB (5 mL per planting hole with a bacterial density 10^4 CFU mL^{-1})
4	IF + BC + PSB	IF plus Biochar (140 g per polybag) and PSB (5 mL per planting hole with a bacterial density 10^4 CFU.mL^{-1})
5	IF + OF+ PSB	IF plus organic fertilizer (10 g per planting hole) plus PSB (5 mL per planting hole with a bacterial density 10^4 CFU.mL^{-1})
6	IF + BC + OF+ PSB	IF plus Biochar (140 g per polybag), organic fertilizer (10 g per planting hole) and PSB (5 mL per planting hole with a bacterial density 10^4 CFU mL^{-1})

Notes: inorganic fertilizer (IF), biochar (BC), phosphate solubilizing bacteria (PSB), and Organic fertilizer (OF); one plant per polybag (equal to 71,428 plants per ha)

Each polybag was adequately watered to achieve a soil moisture level equivalent to 28.75% of field capacity. Planting was executed by placing two maize seeds in a single hole with a depth of approximately 5 centimeters. For IF+OF+PSB and IF+BC+OF+PSB treatments, each hole was additionally filled with 10 grams of compost (Susilowati & Arifin, 2020). The PSB consortium was applied around a planting hole during the planting time. Throughout the maize growth period, soil moisture levels were maintained by watering the polybags in the morning, with the daily water amount adjusted to match the loss through evapotranspiration.

As there were 2 seeds per hole, we selected only one maize plant 10 days after planting (DAP). Each polybag was then treated with inorganic fertilizer with 75% of the recommended dose for maize (Susilowati et al., 2021). Urea (46% N) was applied three times at 10, 30, and 45 days after planting (DAP) with one-third of the urea dose, and PONSKA (15% N, 15% P_2O_5, 15% K_2O, and 10% S) was applied twice at 10 and 30 DAP with half dose each (Saragih et al., 2013).

Soil samples were taken twice at 42 (the maximum vegetative growth) and 105 DAP (the harvest periods). In the first sampling, we used a PVC pipe with a diameter of 1 inch and took the sample around 10 cm from the maize stem with a depth of 10 cm. In the second sampling, polybags were dismantled to obtain the soil sample around the rhizosphere. The sample was then immediately transferred to a 4°C refrigerator for biological parameters. For chemical analysis, the sample was air-dried to reduce the water content.

2.2. Biochar, organic fertilizer, and biofertilizer PSB characteristics

Biochar (BC) was generated simply by burning rice husk on an earthen stove for 8 hours and 200-300 °C. This was done by digging the ground with 1.5 m in diameter and 1.0 m in depth (Sukartono et al., 2011). To maintain the oxygen supply during biochar production, a chimney was placed in the middle of the stove 30 cm in diameter and more than 75 cm in length. The final product of biochar contained water at 8.5%, organic C at 35.20%, total N at 0.5%, total P at 0.15%, total K at 0.76%, and ash at 38.12% with pH at 8.39.

This study's organic fertilizer (OF) was obtained from a mixture of cow manure and rice bran in a 2:1 ratio. The mixture was composed of approximately 4 weeks with a simple composting technique. The compost was characterized containing pH 6.8; water content 12.31%; organic C 16.21%; total N 0.95%; total P 0.50%; total K 0.56%; Calcium 0.16% and C/N ratio of 17.06 (Rahmawati et al., 2023).

The PSB biofertilizer used in this study was a consortium of phosphate solubilizing bacteria consisting of *Pseudomonas azotoformans*, *Acinetobacter baumannii,* and *Bacillus paramycoides and act as* a phosphate solvent, a decomposer, and an IAA producer (Susilowati et al., 2019). The potential of the bacterial combination in dissolving P-inorganic ($Ca_3(PO_4)_2$) in Pikovskaya liquid medium reached 0.5% (Arifin et al., 2021).

2.3. Soil biological and chemical properties

2.3.1. Bacterial Population

The soil bacteria was determined using a serial dilution spread plate method. A ten-gram soil sample was diluted with 90 ml water and mixed up (vortex) for 15 mins. This step was repeated until the concentration reached 10^{-9}. The sample was inoculated on the plate with Nutrient Agar (NA). The number of total bacteria was determined by the spread plate count in colony-forming units per mL after incubation of the sample for 48 hours at room temperature (Lenhart & Gorsuch, 2021). The PSB population was counted similarly to the total bacterial count. However, the sample was grown on selective Pikovskaya's agar with a dilution factor of 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. The sample was then inoculated and left at room temperature for 4 x 24 hours. PSB was counted using the formula presented in Equation 1.

$$\text{Total population of soil total bacteria (CFU.g} - 1 \text{ soil)} = (\text{number of colonies } \times \text{fp}) \div \text{soil dry weigh} \quad \text{.................[1]}$$

where fp = Dilution factor on colonized Petri dish; Soil dry weight (g) = fresh weight x (1-water content).

2.3.2. Soil respiration

Soil respiration was determined using the *Verstraete* method by Yusnaini et al. (2021). A 100 g of soil was placed in a glass with two bottles of film containing 5 mL 0.2 N KOH and 10 mL of water. The glass was then tightly sealed and left in a dark room at room temperature for a week. At the end of incubation, two drops of phenolphthalein were added to a KOH bottle and titrated with 0.1 N HCl until the reddish color disappeared. Then, it was added with two drops of methyl orange and titrated with 0.1 N HCl until the color changed from yellow to pink. The amount of HCl used in the second titration was associated with the amount of fixed CO_2. Soil respiration was calculated using the formula presented in Equation 2.

$$r = ((a - b) \times t \times 1.2 \times 100) \div n \quad [2]$$

where r = the amount of CO_2 (mg-C-CO_2 g^{-1}soil d^{-1}); a = mL of HCl for a glass containing the soil sample; b = mL of HCl for a glass without the sample (blank); t = HCl normality; 100 = 100 g of the dry soil sample; 1.2 = a constant for 0.1 N HCl and C/CO_2; n=days of incubation.

2.3.3. Soil Chemical properties

Soil pH was measured using a pH meter with a 1:2.5 ratio of soil and water, while total N was determined following the Kjeldahl method. SOC was measured using the Walkley and Black method. Available P was determined using the Bray-1 method (Sukartono et al., 2022).

2.4. Maize root growth and agronomy parameters

Maize root growth and yield parameters were observed at harvest time (105 days after planting). Root growth was determined by measuring root dry weight after ovening for 3 x 24 hours at a temperature of 65°C. Other maize parameters include 100 grains (g) weight and yield, measured on a dry grain base (14%).

2.5. Data analysis

Statistical Analysis was conducted using analysis of variance (ANOVA) using SPSS version 23 to determine the effect of treatment on all data from the soil and agronomic parameters. Fisher's LSD ($p \leq 0.05$) was also applied to determine further treatment significance. The Pearson correlation test ($p < 0.05$) and multiple regression analysis assessed the correlation among soil and growth parameters.

3. RESULTS

3.1. The synergistic effect of fertilizer combination on soil bacterial population and microbial respiration

To evaluate the effect of various combinations of fertilizer treatments to increase soil bacteria and microbial activity, we measured the population of soil bacteria and soil microbial respiration at the maximum vegetative growth and the harvest stage. Figure 1 showed that combining IF with other fertilizers markedly improved the total soil bacterial population and soil respiration in the maize rhizosphere during the maximum vegetative growth and harvest stage, compared to the IF treatment. Both IF+BC or IF+PSB combinations increased approximately one-third of the bacteria population compared to IF treatment at the maximum vegetation growth (p=0.008 and p=0.003, respectively, Fig. 1a). Only IF+PSB showed a significant difference in soil respiration in the two treatment combinations, whereas IF+BC treatment did not differ from IF treatment (Fig. 1a). Furthermore, at the harvest stage, the bacterial population and the soil respiration in the IF+BC or IF+PSB combination remains higher than in the IF single treatment (Fig. 1b).

Three or four combination treatments resulted in much higher total soil bacteria and respiration than the others. The combination of IF+BC+PSB, IF+OF+PSB, and IF+BC+OF+PSB increased the population of bacteria and soil respiration compared to those of the IF treatment at the maximum vegetative period (all with $p<0.001$, Fig. 1a). These trends of bacterial population were similar at the harvest stage (IF+BC+PSB $p<0.001$; IF+OF+PSB $p<0.001$; IF+BC+OF+PSB $p<0.001$ vs IF). However, there was no significant difference between IF+BC and IF+PSB combinations and IF+BC+PSB and IF+OF+PSB treatments at the harvest stage (Fig. 1b). These results indicate that biochar application may improve the growth of bacteria in the maize rhizosphere. Furthermore, the highest total bacterial population was found at IF+BC+OF+PSB treatment.

3.2. Population of Phosphate Solubilizing Bacteria (PSB)

Figure 2 presents the population of PSB during maximum vegetative growth and at the harvest stage at various combinations of treatments. The population of PSB at the IF+BC combination treatment was significantly higher than that at the control treatment (IF) ($p<0.001$), although there

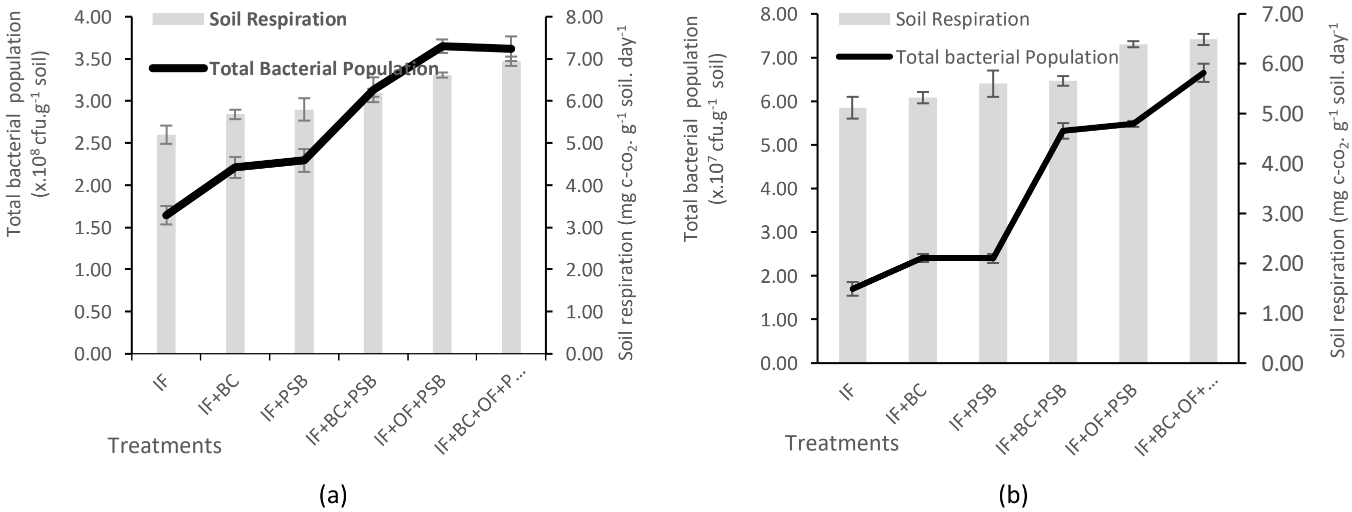


Figure 1. Total soil bacterial population and microbial respiration at the maize rhizosphere during the maximum vegetative growth (**a**) and at harvest stage (**b)** affected by fertilizer combination treatments Data represented as Mean ± SE.
Notes: IF = Inorganic fertilizer; BC = Biochar; OF = compost; PSB = phosphate solubilizing bacteria.

was no difference at the harvest stage (p=0.8). Unlike the IF+BC, the PSB bacterial population increased (about 50-60% higher) at IF+PSB treatment in both the maximum vegetation (Fig. 2a) and harvest stage (Fig. 2b) compared to a single IF treatment.

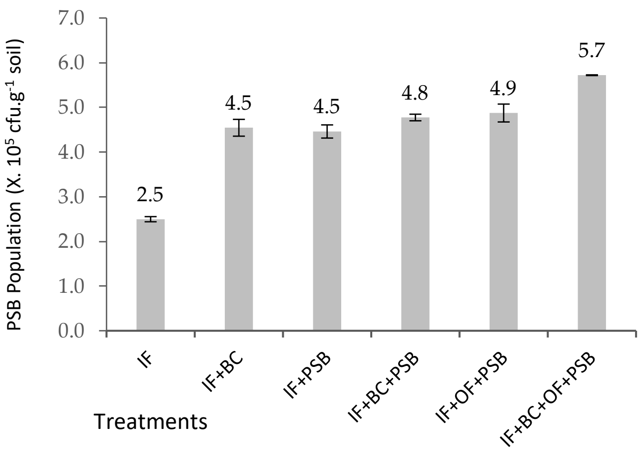
The combination of four treatments (IF+BC+OF+PSB) resulted in the highest population of PSB observed at both the maximum vegetation and harvest stage. At the same time, 3 combinations also increased the PSB population compared to IF treatment alone. However, interestingly, the IF+BC+PSB and IF+OF+PSB have no insignificant differences compared to the two treatment combinations during the maximum vegetation and harvest periods (Fig. 2).

3.3. The effect of fertilizer treatments on soil chemistry properties

The amount of available phosphorus at maximum vegetative growth of maize is presented in Table 2, Column 2. The IF treatment combinations with other fertilizer treatments intensified the available P compared to IF treatment alone (p<0.001). In the combination of 2 or 3 fertilizer treatments, the availability of P increased by approximately 4 ppm compared to the IF single treatment. In comparison, 4 treatments combination resulted in the highest increase in available P (12.21 ppm). However, the combinations of fertilizer treatments did not affect the total N content, although all 4 fertilizer treatments were applied (p = 0.11, Table 2).

Soil acidity at the harvest stage was markedly changed when exposed to the combination treatments. Table 2, Column 5 shows that soil pH at IF+BC, IF+BC+PSB, and IF+BC+OF+PSB treatments significantly increased compared to IF treatment at the harvest stage. However, these changes did not appear at the maximum vegetative growth period (Table 2, Column 4), indicating that the treatment combinations had no effect at that period (p=0.98).

Organic carbon of soil at maximum vegetative growth and harvest stage is significantly different among combinations of treatments (p=0.008 in the maximum vegetative growth and p<0.001 in the harvest time). There was no marked difference in organic C among soil treated with IF+BC, IF+BC+PSB, and IF+BC+OF+PSB as calculated in Fisher's LSD test, both in the maximum vegetation and harvest stage (Table 2).



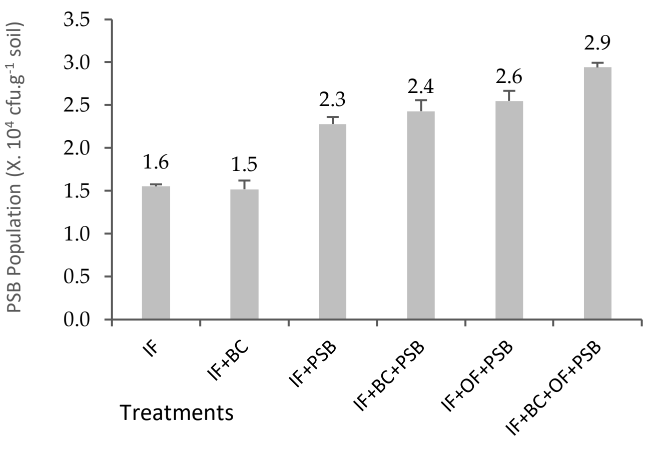


Figure 2. Effect of the combination of fertilizer treatments on phosphate solubilizing bacteria (PSB) population during the maximum vegetative growth (**a**) and at harvest stage (b). Data represented as Mean ± SE.

Notes: IF = Inorganic fertilizer; BC = Biochar; OF = compost; PSB = phosphate solubilizing bacteria

Table 2. Effect of a combination of fertilizer treatments on several soil chemical properties

Treatments	Available P$^{1)}$ (ppm)	Total N$^{1)}$ (%)	Soil pH$^{1)}$	soil pH$^{2)}$	SOC$^{1)}$ %	SOC^{2} %
IF	42.99 ± 1.29 a	0.153	6.13	6.03±0.015 a	0.93± 0.069 a	0.98± 0.030 a
IF+BC	46.95±1.25 bc	0.153	6.13	6.20±0.015 b	1.15± 0.031 c	1.25 ±0.083 b
IF+PSB	46.31± 0.34 b	0.153	6.06	6.08±0.027 a	0.99± 0.047ab	0.98 ±0.022 a
IF+BC+PSB	48.06±1.41 c	0.153	6.12	6.14±0.075 b	1.14± 0.046 c	1.22 ±0.040 b
IF+OF+PSB	48.81±0.02 c	0.153	6.05	6.10±0,027 a	1.04± 0.038 b	0.99 ±0.035 a
IF+BC+OF+PSB	55.20±0.90 d	0.153	6.06	6.20±0.025 b	1.19± 0.200 c	1.31 ±0.029 b
VC (%)	5.42	2.21	2.73	1.36	10.79	14.13
p	< 0.001	0.11	0.98	<0.05	0.008	0.001
LSD (α ≤ 5%	1.72	NS	NS	0.076	0.093	0.223

Remarks: $^{1)}$ measured at the maximum vegetative growth phase and $^{2)}$ at the harvest stage. Data represented as Mean ± SE. The same letters within the same column indicate no significant difference (p < 0.05; p < 0.01). NS = Not significant.

Notes: IF = Inorganic fertilizer; BC = Biochar; OF = compost; PSB = phosphate solubilizing bacteria.

However, when these 3 treatment combinations (IF+BC, IF+BC+PSB, and IF+BC+OF+PSB) were compared with the other 3 treatments (IF, IF+PSB, IF+OF+PSB), we found a marked difference in soil organic C at maximum vegetation and harvest stage with a similar trend (Table 2, Column 6,7).

3.4. Maize root growth and yields

The results of this research showed that the fertilizer combination treatments had a significant influence on root growth and maize yields (Table 3). The root dry weight was significantly higher in all combinations of fertilizer treatments than in the single IF treatment. The highest dry root weight (21.33 g plant^{-1}) was achieved at the IF+BC+OF+PSB combination treatment, followed by the IF+OF+PSB combination treatment (19.33 g plant^{-1}), the IF + BC and IF + BC + PSB treatments (between 16 to 18.33 g plant^{-1}) and the IF + PSB treatment. Compared to IF treatment, root dry weight increased by 60% in IF+BC+OF+PSB treatment and 45% in IF+OF+PSB treatment. In IF+BC+PSB and IF+BC treatments, it increased by approximately 25-37.5%, while in IF+PSB treatment, it increased by 20%. This indicated that applying biochar may improve maize roots' growth.

As shown in Table 3, the highest yield was observed at the IF+BC+OF+PSB combination treatments (77.05 g plant^{-1} equivalent to 5.50 tons ha^{-1}), followed by the 3 combination treatments (IF+OF+PSB; IF+BC+PSB), the 2 combination treatments (IF + BC; IF + PSB), and the control treatment, in that order. The combination treatment of IF+BC+OF+PSB resulted in a 43.11% increase in maize yields compared to the IF treatment alone. The treatments of IF+OF+PSB and IF+BC+PSB increased by approximately 31.32% - 36.55% compared to the IF treatment.

Table 3. Effect of the combination of fertilizer treatments on the root dry weight and maize yields

Treatments	Root dry weight (g plant^{-1}) ± SE	Grain yields (g plant^{-1}) ± SE	Yield* (ton ha^{-1}) ± SE	Weight of 100 grains (g)
IF	13.33 ± 1.49 (a)	53.84 ± 3.48 (a)	3.84± 0.25 (a)	23.67
IF+BC	16.67 ± 1.17 (bc)	65.33 ± 4.18 (bc)	4.67± 0.30 (bc)	25.00
IF+PSB	16.00 ± 1.24 (b)	63.84 ± 5.50 (b)	4.56± 0.39 (b)	24.67
IF+BC+PSB	18.33 ± 0.29 (bc)	70.70± 1.82 (bcd)	5.05± 0.13 (bcd)	24.33
IF+OF+PSB	19.33 ± 1.19 (cd)	73.52 ± 2.49 (cd)	5.25± 0.18 (cd)	24.00
IF+BC+OF+PSB	21.33 ± 1.17 (d)	77.05 ± 3.74 (d)	5.50± 0.27 (d)	24.67
VC (%)	17.78	13.66	13.66	3.50
P	< 0.01	< 0.05	< 0.05	
LSD	2.90	9.34	0. 67	NS

Remarks: * maize yield (ton ha^{-1}) in each treatment was calculated on a population based (71428 plants ha^{-1}). Data represented as Mean ± SE. The same letters within same column indicate no significant difference ($p< 0.05$; $p < 0.01$). NS = Not significant. IF = Inorganic fertilizer; BC = Biochar and OF = compost PSB = phosphate solubilizing bacteria

Table 4. Pearson's Correlation among soil properties, maize root dry weights and yield

Variable		TB Population	PSB population	available P	soil pH	SR	RDW	MY
TB Population	Pearson Correlation	1						
	Sig. (2-tailed)							
PSB population	Pearson Correlation	**.839***	1					
	Sig. (2-tailed)	**.037**						
available P	Pearson Correlation	.792	**.886***	1				
	Sig. (2-tailed)	.061	**.019**					
soil pH	Pearson Correlation	-.610	-.552	-.504	1			
	Sig. (2-tailed)	.199	.256	.308				
SR	Pearson Correlation	.976**	.890*	.896*	-.668	1		
	Sig. (2-tailed)	.001	.017	.016	.147			
RDW	Pearson Correlation	**.953****	**.938****	**.927****	-.555	**.982****	1	
	Sig. (2-tailed)	.003	.006	.008	.253	.000		
MY	Pearson Correlation	**.959****	**.955****	**.880***	-.561	**.970****	**.991****	1
	Sig. (2-tailed)	.003	.003	.021	.247	.001	.000	

Remarks: *. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed). TB population= total bacterial population; PSB=phosphate solubilizing bacteria; SR= soil respiration; RDW=root dry weight; MY=meize yields

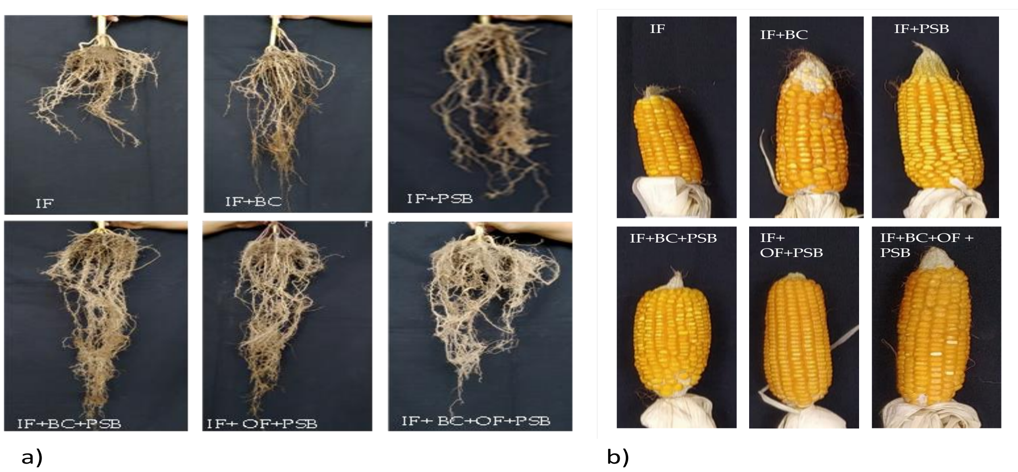


Figure 3. Performance of plant root growth (a) and corn cobs (b) in various treatments. Notes: IF = Inorganic fertilizer; BC = Biochar and OF = compost; PSB = phosphate solubilizing bacteria

Table 5. Results of multiple linear regression analysis related to the influence of several soil properties on RDW

Dependent variable		Regression coefficient	t	Sig.
1	(Constant)	-1.259	-1.60	.887
	TB Population	1.627	4.922	.039
	PSB population	.609	1.914	.196
	available P	.223	2.864	.103
2	R = 0.997	R^2=0.995	Adjusted R^2=0.987	Sig=0.008

The yields (between 63.84 to 65.33 g plant^{-1}) observed in the 2 combination treatments did not show any significant differences but were higher than that in the IF treatment, with an increase of approximately 18.57% - 21.34% compared to the IF treatment alone. Furthermore, the weight of 100 grains (Table 3) at the combination treatments was not significantly different from that in the IF treatment alone.

The performance of plant root growth and maize cobs in each treatment are presented in Figure 3. It shows that the IF+BC+OF+PSB combination treatment had the best effect on plant root growth, followed by the IF+OF+PSB combination, the combinations of IF + BC + PSB and IF+BC, the combination of IF+PSB and the IF treatment. Figure 3a confirmed that fertilizer combination with a minimal IF+ OF + PSB composition should be applied to optimize plant root growth. Figure 3b shows that the best maize cobs produced were observed at a combination treatment of IF+BC+OF+PSB.

3.5. Pearson's correlation of several rhizosphere properties on root growth and maize production

Several soil variables measured at maximum vegetative growth showed a strong correlation between one parameter and another, except soil pH. Table 4 shows the correlation between the total bacterial population and the PSB population showed a strong positive relationship pattern ($r=0.839$, $p<0.05$), as did that between the PSB population and available P ($r=0.886$, $p<0.05$). The relationship between soil respiration (SR) and the total soil bacteria population was positively correlated with a value of $r = 0.976$. TB population, PSB population, and available P significantly correlated with RDW (Table 2). RDW and maize yield are significantly correlated with a value of $r = 9.91$. Then, a multiple regression test was carried out to find out the direction and how much influence the soil variables have together on root weight (dependent variable), as independent variables are TB population, PSB population, and P available. The results of the regression test are presented in Table 5.

4. DISCUSSION

Prolonged and sole use of inorganic fertilizers could lead to reduced microbial activity and biodiversity in the cropping system, as well as alterations in soil acidity and organic matter content (Pahalvi et al., 2021). Therefore, combining inorganic fertilizers with other fertilizer treatments is necessary to maintain soil quality. Our research findings indicate that combining inorganic fertilizers with organic materials (biochar or organic fertilizer) and biological fertilizers (phosphate-solubilizing bacteria, PSB) significantly enhances various properties of the plant rhizosphere. These properties include increased total bacterial and PSB populations, higher soil respiration, greater availability of phosphorus, improved soil pH, and elevated organic carbon content compared to the use of inorganic fertilizers alone.

The significant improvements observed when combining three or four fertilizers compared to two-fertilizer combinations and the control suggest a complex interplay between the different components. With its high porosity and nutrient retention capacity, biochar likely provides a conducive environment for microbial colonization and nutrient availability. Organic fertilizers contribute essential nutrients and enhance soil organic matter, while PSB facilitates phosphorus solubilization, making this critical nutrient more accessible to plants. These components create a more fertile and biologically active soil environment, leading to improved plant growth and yield. Our research results align with several previous research. Liu et al. (2022) reported that the application of biochar combined with organic and inorganic fertilizers significantly influenced the development of bacterial populations (gram-negative and gram-positive), with population numbers ranging from high to low according to the rate of biochar addition (50 > 35 > 0 tons ha^{-1} biochar). Yan et al. (2021) reported that triple combination treatments offered superior improvements in soil biology compared to two combination treatments. The combination of IF+BC+OF+PSB produced the highest total bacterial and PSB populations and soil respiration compared to other fertilizer combination treatments. In the maximum vegetative growth phase, the total bacterial and PSB populations were 120% and 128% higher than the control treatment. The drastic increase in the total bacterial and PSB populations indicates that the four combinations of fertilizer treatments can create a favorable environment for the life of soil bacteria. Faloye et al. (2017) reported that maize's total biomass and grain yield were synergistically highest when the combination treatment of biochar and NPK fertilizer was compared with the individual application of biochar or NPK fertilizer.

As a soil quality improvement material, biochar can facilitate the growth and development of soil bacteria (Wu et al., 2021). Beheshti et al. (2017) and Lehmann et al. (2011) stated that biochar application provides a supporting niche to enhance the microbial reproduction rate. The importance of biochar as providing a suitable habitat for soil microbes is associated with the characteristics of biochar, which has high porosity, high moisture holding capacity, a large specific surface area, contains N, P, and K nutrients, and the presence of functional groups (Bolan et al., 2023; Lehmann et al., 2011). Scanning electron microscopy (SEM) shows that bacteria and fungi grow and develop in biochar pores (Bolan et al., 2023; Lehmann et al., 2011). The pore surface of biochar is filled with several functional groups that are useful for adsorbing water and soil nutrients (Lima et al., 2018; Song et al., 2018), which can then be utilized by microorganisms (Yao et al., 2017). Bolan et al. (2023) explained that functional groups such as carboxylic (COOH), active hydroxyl (OH), ketone (C=O), phenolic –OH, and amine, amide, and –CHO, which are found on the surface of biochar are effective parts of biochar for adhesion and proliferation of microbial cells. At the same time, applying compost fertilizer also positively influences the development and activities of soil microbes. The role of compost fertilizer in stimulating the growth and activity of soil microbes is mainly associated with increasing soil organic C (SOC) content and total nitrogen (Liu et al., 2021). Soil bacteria use SOC as a carbon and energy source (Lehmann et al., 2011; Liu et al., 2021). The extraordinary increase in bacterial population in the IF+BC+OF+PSB treatment can also be associated with better corn plant growth than in other treatments. Several previous research results reported that plant cultivation systems that were given integrated fertilizers such as inorganic plus organic fertilizer (biochar and organic fertilizer) had better plant growth (Glaser et al., 2015; Yan et al., 2021) and resulted in increased secretion of root exudates, which function as energy and carbon for soil bacteria (Huang et al., 2022). The results of our research confirm that creating a suitable habitat for soil bacteria requires the input of biochar and compost fertilizer, which is integrated with the application of inorganic fertilizer.

Our research results also show that applying inorganic fertilizer (IF) in combination with other fertilizers significantly affects P availability, with the highest level of availability (55.20 ppm) observed in the IF+BC+OF+PSB combination treatment. These four combination treatments can increase P availability by 28.70% compared to the P level in the control treatment. In this combination treatment, the P nutrient supply comes not only from inorganic fertilizer but also from biochar and organic fertilizer, and the availability process is played by the presence of PSB in the soil. Independently, the capacity of biochar to influence the amount of available P was demonstrated in the IF + BC treatment, where the available P content was significantly different from the P concentration in the control treatment (Table 1). Biochar can increase the amount of available P through direct input from biochar and through biochar's ability to retain P from fertilizer (Martinsen et al., 2014; Zhang et al., 2016). Biochar processed using low-temperature pyrolysis techniques (≤ 400°C) has essential nutrient content (P, K, and Ca), which is about twice the P contained in the raw material (Prayogo et al., 2012). Zhang et al. (2016) and Beheshti et al. (2017) stated that the P contained in biochar is mainly in the form of available P, which is easily dissolved and released into the soil solution. Glaser and Lehr (2019) explained that the application of biochar to acidic soil (pH <6.5) could increase the amount of available P by changing the soil environment, which influences the activity of cations that interact with phosphate anions. At the same time, adding compost to the soil also increases the available P content. Liu et al. (2022) explained that organic fertilizer contains large amounts of organic P compounds, such as phospholipids and nucleic acids, which can be released into the soil to increase the available phosphorus content through mineralization (Malhotra et al., 2018). Apart from that, the capacity of organic fertilizer to increase soil CEC, soil pH, and the activity of soil microbial life is an essential factor in increasing the availability of N, P, and K nutrients (Liu et al., 2021). Then related to the existence of PSB populations in intervening in the availability of P takes place through its role as a solvent for inorganic P compounds and as a promoter of the mineralization of P-organic compounds (Lehmann et al., 2011; Timofeeva et al., 2022). Various studies concluded that PSB induces P availability by changing soil-insoluble P into more bioavailable through solubilization mediated by organic acid secretion and mineralizing organic P by secretion of phosphatase (Beheshti et al., 2017; Timofeeva et al., 2022). Janati et al. (2023) reported a significant positive relationship between soil PSB population density and the amount of available P. This research shows that the simultaneous application of biochar, compost, and PSB can increase the amount of available P with a synergistic mechanism between the three types of fertilizer. Thus, applying these three types of fertilizer combined to IF is the best practice to reduce the use of inorganic P fertilizer but not reduce plant yields. Meanwhile, this combination fertilizer treatment did not significantly affect the total N content of the soil. Our research results differ from several previous research results, where the application of biochar combined with inorganic and organic fertilizers significantly affected the total N content of the soil. This difference in results may be due to differences in the characteristics of the biochar and compost applied and the application dose. The biochar and compost applied in our research were rice husk biochar with an N content of 0.5% and compost with an N content (0.95%). The total N content in these two organic materials is relatively low. Hossain et al. (2020) stated that the application of biochar functions to increase the total N amount of soil, especially biochar which has a high N content, for example, biochar produced from poultry manure (N 5.85%) and grass waste (N 4.9%). Martinsen et al. (2014) recommend using biochar above 10 tons ha^{-1} to significantly increase nutrient content in tropical soils with high mineral content. Another study by Liu et al. (2022) reported that biochar (dose of 0, 35, and 50 tons ha^{-1}) combined with inorganic and organic fertilizers significantly affected soil nitrogen content in rapeseed fields during the flowering stage.

Regarding soil pH, this fertilizer treatment has a significant effect ($p<0.05$) on soil pH at the harvest stage. However, it does not significantly affect soil pH during the vegetative phase of maximum growth. LSD test results ($p \leq 5\%$) at harvest showed that the soil pH in all fertilizer combination treatments containing biochar was significantly higher than in the treatment without biochar (Table 2). The results of our research confirm that as a result of administering 10 tons of rice husk biochar (pH 8.39), there was an increase in soil pH by 0.17 units from pH 6.03 (in the control) to 6.20 (in the combination IF+BC, IF+ BC+OF and IF+BC+OF +PSB). This increase in pH proves that the alkalinity characteristics of rice husk biochar have a strong limiting effect on increasing the pH of acidic soil. The results of this study align with previous findings showing that biochar can potentially increase soil pH (Lima et al., 2018; Liu et al., 2022; Martinsen et al., 2014). Martinsen et al. (2014) explained that incorporating biochar into the soil can cause a liming effect and result in acid and neutral soil pH tending to increase. However, biochar with different types (different raw materials, different pyrolysis temperatures) has different potential to reduce soil acidity problems due to different physicochemical properties (Dai et al., 2013; Martinsen et al., 2014). For example, adding 3% pig manure biochar increased the soil pH of *Psammquent* to 2.52 units, whereas alang-alang straw biochar was only 0.11 units (Dai et al., 2013). Furthermore, Dai et al. (2013) stated that the impact of applying biochar on soil pH depends on the type of biochar, biochar rate, and soil type.

Regarding changes in carbon content, the results of the LSD test ($p \leq 5\%$) show that there is a significant difference in organic C content between the combination fertilizer treatments added with biochar (IF+BC, IF+BC+PSB, and IF+BC+OF+PSB) and those without biochar (IF, IF+PSB, IF+OF+PSB) during maximum vegetation growth and harvest stages. This research shows that combining fertilizer that includes rice husk biochar (organic C= 32.2.5%) can significantly increase soil organic C content and provide long-term effects. Biochar application to soil means adding soil organic matter to the soil. Biochar is an organic material with a high carbon content, and the carbon structure in biochar is stable (challenging to decompose by microbes) (Lehmann et al., 2011; Zonayet et al., 2023). Therefore, applying biochar to the soil can quickly increase the soil's organic C content (Liu et al., 2016). At the same time, applying compost fertilizer in the IF+BC+OF+PSB treatment does not appear to contribute significantly to soil organic C content. Table 2 shows that the soil organic C content in the IF+BC+OF+PSB treatment and the IF+BC+PSB treatment is not significantly different. We suspect that soil microbes use organic C from compost in the IF+BC+OF+PSB treatment as an energy source to meet their metabolic needs, so not many organic carbon compounds from compost accumulate in the soil. According to Ibrahim et al. (2015), organic fertilizer is dominated by C-labile compounds, which quickly decompose to become a source of energy and nutrients for soil microbes and plants. These results reveal that adding biochar to an integrated fertilization system between inorganic fertilizers and other fertilizers can control the dynamics of decreasing improvements in plant rhizosphere properties due to applying combination fertilizer resulting in plant roots growing better and corn production being higher than the control treatment.

The LSD results ($p \leq 5\%$) showed that the highest root dry weight was obtained in the IF+BC+OF+PSB treatment, followed respectively by the IF+OF+PSB, IF+BC+PSB = IF+ BC, IF+ PSB treatments, and the lowest in the control treatment. The pattern of increasing maize production is in line with the increase in plant root dry weight ($r=0.99$, $p<0.001$). Plants with better root development are assumed to absorb more nutrients to stimulate plant growth and yield (Sharma et al., 2014; Susilowati & Arifin, 2020). Root dry weight and corn production observed in the IF+BC+OF+PSB combinations reached 60.02% and 43.11% higher than the control treatment (Table 3). This increase can be attributed to the synergistic effect of organic matter (OF and BC) and phosphate biofertilizer (PSB) on improving plant nutrition and soil biological properties in the plant rhizosphere. Previous research has highlighted similar trends; Susilowati and Arifin (2020) showed that soybean plants treated with a combination of inorganic and organic fertilizers containing PSB consortium were proven to increase available P, P uptake, P residue, plant growth, and soybean yield. Naeem et al. (2018) also found that the application of biochar, compost, and NPK fertilizer was proven to increase available P content, extractable K, soil pH, SOC, and electrical conductivity after harvest and produce 20.61% higher corn production compared to treatment without organic amendments. Other researchers reported that using biochar combined with organic fertilizer significantly increased the total root length, root surface area, and root volume of cotton compared to without biochar (Zhang et al., 2020). This phenomenon confirms the importance of biochar, organic fertilizer, and PSB supplementation in NPK-fertilized corn to optimize nutrient availability and improve soil biological properties, such as increasing total soil bacterial populations and respiration, positively influencing corn growth and yield.

Furthermore, Table 3 showed that the dry weight of 100 grains of corn in the combination fertilizer treatment was not significantly different from that in the NPK fertilizer treatment alone. The result of this study is in line with the results of previous research, which tested the effect of the combination of P biofertilizer (PSB) and inorganic fertilizer (SP 36 and NPK) on the dry weight of 100 corn seeds (Lovitna et al., 2021). In contrast, Naeem et al. (2018) reported a significant increase in the weight in the combination treatment of biochar, compost, and NPK fertilizer compared to the treatment alone of biochar, compost, and NPK. Asis et al. (2021) explained that plant-inherent factors mainly regulate the weight of 100 seeds. However, to achieve the optimal target weight of 100 seeds, optimal nutrient requirements are required to stimulate the plant's physiological processes in producing photosynthate (Liu et al., 2015). Gao et al. (2017) explained that the photosynthate produced after the removal period is more distributed and accumulated in the seeds and determines the weight of the seeds. This study's average weight of 100 corn grains was around 24.59 g, slightly lower than that described for corn varieties Lamuru (27 g per 100 corn). We suspect this study's combined application of inorganic and other fertilizers has not met optimal nutrient requirements to optimize the photosynthate accumulated in corn seeds.

In addition, our findings showed a strong positive correlation among soil properties measured with root dry weight (RDW) and maize yields (Table 4). The results of multiple regression show that the TB population has the most significant influence in stimulating root growth, followed by the PSB population and available P. Theoretically, each independent variable influences plant root growth and can be explained as follows. In healthy agricultural soil, various types of bacteria that are beneficial for plant growth develop well. This group of bacteria influences plant growth through various mechanisms, such as their direct role in the process of nutrient availability for plants and the synthesis of various phytohormones and enzymes, while their indirect role is in the inhibition of phytopathogens (Pii et al., 2015). Then, regarding the effect of the presence of PSB on root growth, Kudoyarova et al. (2017) and Rawat et al. (2021) explained that the critical role of PSB is not only related to increasing the solubility of inorganic phosphate but also its capacity to produce plant hormones such as indole acetic acid (IAA). This study showed that the PSB population was positively correlated with available P ($r = 0.886$; $p = 0.019$) at the maximum vegetative phase. This means that the higher the PSB population, the higher the available P content. Phosphate is the primary macronutrient in plants for various physiological and biochemical processes, especially nucleic acid synthesis, phospholipid membranes, energy metabolism, photosynthesis, and sugar transformation (Malhotra et al., 2018), so P nutrient utilization by plants plays a vital role in determining plant growth and final yield (Carstensen et al., 2019).

5. CONCLUSION

The combination of inorganic, organic, and PSB consortium fertilizers has synergistically enhanced several properties of the plant rhizosphere, such as soil microbial and chemical properties, compared to inorganic fertilizer alone, and ultimately increased maize growth and yield. Furthermore, combinations involving three or four different fertilizers resulted in more substantial improvements in rhizosphere properties than combinations of only two fertilizers or the control. Specifically, combining inorganic fertilizer with biochar, organic fertilizer, and phosphate-solubilizing bacteria (IF+BC+OF+PSB) produced the highest bacterial populations and soil respiration rates, suggesting a highly favorable environment for soil microorganisms. The maize yield in the combination of IF+BC+OF+PSB treatment reached 43.11% higher than the corn yield in the IF treatment. The synergistic effects of biochar, known for its high porosity and nutrient retention capabilities, along with the nutrient-rich compost and PSB, which enhances phosphorus availability, underscore the importance of integrated fertilization strategies. The study highlights the critical need for integrated fertilization practices that combine inorganic fertilizers with organic and biological amendments to maintain and improve soil quality, enhance microbial activity, and ultimately support sustainable agricultural productivity. Nevertheless, further research is warranted to substantiate these findings, especially in field level and to elucidate pathways the biochemical and microbial processes underlying these synergistic effects, focusing on the molecular interactions between soil amendments, microbial communities, and plant roots, as well as long-term field trials to monitor changes in soil health and crop performance.

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Declaration of Competing Interest

The authors declare that no competing financial or personal interests may appear and influence the work reported in this paper.

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