SAINS TANAH – Journal of Soil Science and Agroclimatology

Journal homepage: http://jurnal.uns.ac.id/tanah

Oxidized alkaline biochar and phosphate solubilizing bacteria mixture enhances direct seeded maize yield in an acid soil

Hachib Mohammad Tusar[1], Md. Kamal Uddin[1]*, Shamim Mia[2,3], Susilawati Kasim[1], Samsuri Bin Abd. Wahid[1], Tomoyuki Makino[4], Zakaria Solaiman[5]

[1] Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia
[2] School of Life and Environmental Sciences, The University of Sydney, Sydney-2015, Australia
[3] Department of Agronomy, Faculty of Agriculture, Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh
[4] Graduate School of Agricultural Science, Laboratory of Soil Science, Faculty of Agriculture, Tohoku University, Japan
[5] UWA School of Agriculture and Environment, The UWA Institute of Agriculture, M079, Perth WA 6009 Australia

ARTICLE INFO

Keywords:
Acidic soil
Maize
Microbes
Phosphorus
Pyrogenic carbon

Article history
Submitted: 2024-08-30
Revised: 2024-09-06
Accepted: 2024-09-06
Available online: 2024-12-31
Published regularly: December 2024

* *Corresponding Author*
Email address:
mkuddin@upm.edu.my

ABSTRACT

Maize is an important cereal in many developed and developing countries of the world. One of the primary challenges for maize cultivation is soil acidity. Acidic soil is a major constrain in achieving food security requiring sustainable solutions. Biochar, a pyrogenic carbon-rich material, carries reactive surfaces (i.e., high surface area and variable surface charges). Therefore, it facilitates nutrient retention in soil and gradual release to plants, thereby supporting crop growth. However, the combine effects of functionalized biochar with microbes on phosphorus (P) bioavailability and plant performance remain unclear. This study investigates the application of different oxidized biochars (i.e.,fresh rice husk biochar (RHB), pH adjusted oxidized RHB and control) and phosphate solubilizing bacteria (i.e., *Pseudomonas aeruginosa*, and control) on soil properties including phosphorus dynamics and the performance of maize grown in an acid soil. Biochar was oxidized using 10% hydrogen peroxide while the pH was adjusted to 8.5. Maize was grown in pots having 20 kg of soil or soil-biochar mixture. Overall, biochar and microbes treatments increased soil phosphorus bioavailability and maize yield with a greater effects in the oxidized biochar giving a significant biochar × microbes interactions. Specifically, oxidized biochar when applied with *Pseudomonas aeruginosa* increased P availability by 380 % which then contributed to yield increment (291%). We also observed a significant reduction in available aluminum (Al) concentration (40%) compare to the control. These improvement in yield might have occurred due to an increase soil pH, P bioavailability (r^2= 0.74), and a reduction in Al toxicity (r^2= 0.36).Findings of this study could have significant implications for crop production in acidic soil.

How to Cite: Tusar, H.M., Uddin, M.K., Mia, S., Kasim, S., Abd. Wahid, S.B., Makino, T., Solaiman, Z. (2024). Oxidized alkaline biochar and phosphate solubilizing bacteria mixture enhances direct seeded maize yield in an acid soil. Sains Tanah Journal of Soil Science and Agroclimatology, 21(2): 219-237. https://doi.org/10.20961/stjssa.v21i2.93130

1. INTRODUCTION

Maize (*Zea mays*) is considered as one of the most vital crops in numerous countries and is grown in nearly every region globally (Dragomir et al., 2022). It is regarded as a versatile crop due to its numerous uses, such as providing food for humans and feed for animals (Zaidun et al., 2019). Consequently, it can thrive in various climatic conditions and diverse soil types worldwide (Agegnehu et al., 2016). Soil pH is a major factor in agricultural productivity as it directly influences the availability of essential nutrients to plants (Barrow & Hartemink, 2023). It is one of the most noticeable abiotic factors affecting the soil microbial community (Manpoong et al., 2020). Soil pH significantly impacts enzyme production, nutrient cycling, and plant-microbe interactions (Ontman et al., 2023). However, soil acidity poses a significant challenge for maize cultivation. Tropical soils, often subject to high precipitation and temperatures, tend to lose essential cations, resulting in highly weathered, acidic conditions (Hasbullah et al., 2020). Acidic soils often exhibit low phosphorus availability due to phosphorus fixation, where

STJSSA, p-ISSN 1412-3606 e-ISSN 2356-1424
http://dx.doi.org/10.20961/stjssa.v21i2.93130

phosphorus becomes bound to soil particles and is unavailable for plant uptake (Muchoka, 2021).

Biochar a type of pyrogenic carbon-containing material generated from waste biomass, is becoming increasingly popular for controlling acidity while enhancing the fertility and health of the soil (Tusar et al., 2023). Alkaline biochar can help to raise soil pH, particularly in acidic soils, thereby mitigating soil acidity and creating more favorable conditions for plant growth (Huang et al., 2023). With its high cation exchange capacity (CEC), alkaline biochar can adsorb and retain nutrients in the soil, making them more available to plants and improving soil fertility while reducing nutrient leaching (Bedassa, 2020).

Microbes contribute to nitrogen fixation, nutrient mineralization, and the synthesis of plant growth-promoting compounds like hormones and enzymes, which enhance nutrient cycling, soil quality, and plant development (Nabi, 2023). Phosphate-solubilizing microorganisms play a crucial role in enhancing soil quality and promoting plant growth, potentially reducing the need to import phosphate from other countries. Soares et al. (2023) revealed that the *Pseudomonas aeruginosa* and *Bacillus cereus* isolates exhibited the best phosphate solubilization performance at different pH values.

Phosphorus (P) is an essential nutrient for plant growth and development, playing several crucial roles in plant nutrition (Malhotra et al., 2018). It is a key component of ATP (adenosine triphosphate), the primary energy carrier molecule in plants, providing the energy necessary for various metabolic processes, including photosynthesis, respiration, and nutrient uptake (Khan et al., 2023). Phosphorus is also involved in energy transfer and storage within the plant (Tiessen, 2008). Many enzymes require phosphate groups for their catalytic activity, serving as a cofactor or structural component for these enzymes (Malboobi et al., 2022). Phosphorus deficiency can result in stunted root growth, reduced nutrient absorption capacity, and decreased crop yield (Malhotra et al., 2018). Additionally, aluminum (Al^{3+}) uptake in acidic conditions can lead to root damage and inhibition of growth (Ofoe et al., 2023). Nitrogen loss in acidic soils occurs through pathways such as ammonium nitrogen (NH_4^+-N), and nitrate nitrogen (NO_3^--N) leaching, along with volatile ammonia loss. Alkaline biochar, with its improved adsorption capacities, is a promising soil amendment to improve nitrogen availability (Gao et al., 2023).

Studies have shown that phosphate-solubilizing microorganisms and various bacterial strains can significantly improve P release from biochar, increasing P availability in the soil and enhancing plant growth (Rossati et al., 2023). Oxidized biochar provides a stable matrix for phosphate-solubilizing bacteria (PSB) colonization and activity, enhancing their survival and persistence in the soil (Ouyang et al., 2023).

The combination of oxidized biochar and PSB can significantly improve phosphorus availability and soil acidity reclamation. One possible approach to enhance phosphorus bioavailability is the application of pH adjusted oxidized alkaline biochar which is a noble strategy to reclaim nutrient deficiencies in acidic soil. The objective of this study was to evaluate the combine effects of oxidized biochar and phosphate-solubilizing bacteria (*P. aeruginosa*) on enhancing P availability, phosphatase enzyme activity, and maize yield in acidic soil. Together, these components can create a conducive environment for plant growth, with improve nutrients availability and pH balance, leading to enhance crop productivity and soil fertility.

2. MATERIALS AND METHODS

2.1. Experimental Site

The pot trial was conducted in the new glasshouse, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor. The experimental site was located at 2°98'36.6" N (north) latitude and 101°73'81.9" E (east) longitudes with an elevation of 56.8 m from sea level at the west coast of Peninsular Malaysia. The local climate was hot, humid tropic, and the average minimum temperature was around 23 °C, and the average maximum temperature was 28 °C, relative humidity was 80% during the whole experiment.

2.2. Rice Husk Biochar Collection and Characterization

In this pot experiment, rice husk biochar (RHB) was utilized, produced from locally sourced feedstock in Malaysia. The biochar was collected from Sendi Enterprise (Sungai Burong, Selangor, Malaysia) and was produced by a pyrolysis process at 300 °C. To measure the pH of the rice husk biochar, a 1:2.5 ratio of air-dried biochar to distilled water was used, using a pH meter following the method by Ahmedna et al. (2000). The biochar was oxidized using 5%, 10%, and 15% H_2O_2. pH was adjusted to 8.5. Total nitrogen (N) and total carbon (C) in the biochar were analyzed using a CNS analyzer (TrueMac CNS Analyzer). The physico-chemical properties of the oxidized RHB are shown below (Table 1).

2.3. Soil Collection and Preparation

Surface soil (0-20 cm depth) was collected from the Bungor soil series (Typic Paleudult; Order: Ultisol) at Taman Pertanian, Universiti Putra Malaysia, Puchong, Selangor (2°58'59.7" N latitude; 101°38'47.5" E longitude). The soil sample was air-dried, ground, and sieved to less than 2 mm before undergoing chemical characterization and subsequent treatment. The properties of the soil, including texture, pH, cation exchange capacity (CEC), and exchangeable cations, are detailed in Table 2.

2.4. Pot trial

A two-factors experiment were conducted following a randomized complete block design (RCBD) with three replicates. The treatment included- a) biochar application-fresh and oxidized biochar with one control and b) microbial inoculation- *Pseudomonas aeruginosa* (Agkt 1) and with a control. Treatments were administered in plastic containers (38 cm in height, 32 cm in diameter, and 30 cm in depth) filled with 20 kg of soil. Each container had three holes drilled at the bottom to allow leachate to drain out. Moisture content was monitored and maintained using a portable moisture meter (FieldScout TDR 150 Soil Moisture Meter). Biochar was mixed into the top 15 cm of the soil two weeks before maize seeds were sown. Four seeds were planted at a depth of 2 cm

Table 1. Physical and chemical properties of oxidized RHB

Treatments	Elemental composition (%)							Specific Surface Area (SSA)	
	C	H	N	S	O	C:H	O: C	BET Surface area ($m^2 g^{-1}$)	Pore diameter Å
T1	31.18±0.66a	2.11±0.05a	2.07±0.05b	1.78±0.07b	61.74±0.21c	14.78±0.05b	1.98±0.05c	278.52±0.92a	15.82±0.12d
T2	24.67±0.83b	1.17±0.03b	3.66±0.05a	1.75±0.03b	66.81±0.33b	21.09±0.10a	2.71±0.08b	235.16±1.63b	16.97±0.03c
T3	25.05±0.53b	1.15±0.01b	4.01±0.01a	0.98±0.02c	65.04±1.66bc	21.87±0.30a	2.60±0.01b	203.78±1.58d	38.09±0.16a
T4	18.29±0.56c	1.16±0.01b	3.19±0.68ab	2.26±0.04a	71.56±0.83a	15.83±0.37b	3.92±0.08a	216.3±1.11c	24.95±0.01b
P value	<0.0001*	<0.0001*	0.0185*	<0.0001*	0.0006*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

Remarks: T1=CBC (Control biochar), T2 = 5% Oxidized biochar, T3= 10% Oxidized biochar, T4= 15% Oxidized biochar, C= Carbon, H= Hydrogen, N= Nitrogen, S= Sulpher, O= Oxyge

Table 2. The physical and chemical properties of the initial soil

Properties	Soil
Textural Class	Sand- 69.27%, Silt- 2.28% Clay-28.44% (Sandyclay loam)
pH	4.40±0.01
CEC (mmol kg^{-1})	7.11± 0.03
Available P (mg kg^{-1})	1.70± 0.05
Total C (%)	1.03± 0.02
Total N (%)	0.03± 0.02
Total S (%)	0.01±0.02
Exchangeable K (mmol kg^{-1})	0.06±0.01
Exchangeable Ca (mmol kg^{-1})	0.19±0.01
Exchangeable Mg (mmol kg^{-1})	0.32± 0.02
Exchangeable Al (mmol kg^{-1})	0.78± 0.04

in each pot, and thinned to one healthy seedling per pot after 7 days of emergence. N-P-K fertilizer was applied in each pot recommended by Kashiani (2012), application rates of urea 4.87 g pot^{-1}, triple superphosphate 3.49 g pot^{-1} , and muriate of potash 2.64 g pot^{-1}. The full dose of P and K fertilizer were applied as a basal dose one day before the seeds were sown. N fertilizer was applied in three equal splits on the 10^{th}, 40^{th} and 65^{th} day safter sowing (DAS). In this experiment, the maize seeds hydrod F1 316, Malyasia was used and obtained from a local market, was used as the test crop. Plant management included manual weeding and pesticide application as needed to maintain experimental conditions. The experiment was conducted from August 2023 to November 2023 to assess the effects of bacterial inoculation on soil and plant health.

2.5. Preparation and application of bacterial inoculums

The bacterial culture of *Pseudomonas aeruginosa* (10^{-5} cfu ml^{-1}) was utilized for soil microbial treatment. The strains were initially sub-cultured in 100 ml Erlenmeyer flasks containing LB (Luria-Bertani) broth. These cultures were then shaken continuously for 24 hours at 180 rpm and 28°C, following the method described by (Jensen, 1951). Approximately 20 ml of the bacterial suspension was applied in two equal splits: one at the time of sowing (0 DAS - Days After Sowing) and the other at 20 DAS.

2.6. Soil Analysis

2.6.1. Determination of exchangeable K, Ca, Mg, and CEC in soil

Soil cation exchange capacity (CEC) was examined using the ammonium acetate shaking method (Rowell, 2014) at pH 7. Five grams of soil sample was placed into a Falcon tube. Subsequently, 50 ml of 1M ammonium acetate (NH_4OAc buffered at pH 7) was added to each tube, followed by shaking at 180 rpm for 30 minutes to facilitate cation exchange. Upon completion of shaking, the samples were centrifuged at 4000 rpm for 10 minutes to separate the soil particles from the solution. The filtered solution was kept to determine the concentrations of potassium (K), calcium (Ca), and magnesium (Mg) using an Atomic Absorption Spectrophotometer (AAS). Following the initial analysis, the soil in each tube, now saturated with ammonium ions, passed through a three times washing step with 50 ml of 95% ethyl alcohol to remove excess ammonium acetate. The washed soil was then mixed with 50 ml of 0.1 N potassium sulfate (K_2SO_4) to facilitate the replacement of exchangeable ammonium ions by potassium ions. Subsequently, centrifugation, filtration, and analysis of ammonium were performed using a segmented flow analyzer (AA 500).

2.6.2. Determination of Inorganic nitrogen in soil

Soil inorganic N (NH_4^+-N and NO_3 -N) was analyzed by extracting with 2M KCl (soil: solution, 1:5) (Keeney & Nelson, 1982). Briefly, 10 g of soil was taken into a falcon tube, and 50 ml of 2M KCl was added while the suspension was shaken for 1 hour. Ammonium and nitrate were analyzed using a segmented flow analyzer (AA 500) as discussed before (Keeney & Nelson, 1982).

2.6.3. Determination of available phosphorus in soil

Available phosphorus in the soil was determined using the Bray II method (Bray & Kurtz, 1945). Two grams of air-dried soil (2.00 mm) were weighed into a 20 ml plastic vial and reacted with 14 ml of extracting solution (0.03 NH_4F and 0.1M HCl), then sealed with parafilm. The soil suspension was shaken for 45 seconds using the wrist inversion technique. The extract was filtered into a plastic vial through Whatman No. 42 filter paper. The final product was analyzed using a segmented flow analyzer (AA 500).

2.6.4. Leachate test

All pots were irrigated with an equivalent amount of water. Drained water was collected after each irrigation from each container and stored in plastic bottles in a refrigerator at 4°C. All the collected water samples were then analyzed for pH, phosphorus, Electrical conductivity (EC), and amount of leachate (Beqaj et al., 2016).

2.6.5. Determination of soil microbial population

Ten grams of fresh soil samples were used to determine the total microbial population using the spread dilution plate technique (Parkinson et al., 1971). Serial dilutions from 10^{-2} to 10^{-7} were prepared by sequentially transferring 1.0 ml of the sample into each test tube containing 9 ml of sterile distilled water (SDW). A sterilized bent glass rod spreads the samples over the respective media. The plates were incubated at 28 ± 2°C for 24-48 hours for bacteria. The colonies formed were counted, and populations were calculated as colony-forming units (CFU) per ml solution.

2.6.6. Determination of soil phosphatase activity

Soil phosphatase enzyme activities were determined by using testing kids from Beijing solarbio Science and Technology Co. Ltd (China). The catalog number of soil alkaline phosphatase (S-AKP/ALP) Activity Assay kit was BC0280 (Guan et al., 2023). To prepare the soil samples, 0.1 g of soil was placed in a vial. Then, 0.05 ml of toluene was added, and the mixture was shaken for 15 minutes. Next, 0.4 ml of reagent 1 was added to the vial, and the mixture was incubated at 37°C for 24 hours. After incubation, 1 ml of reagent 2 was added, and the mixture was centrifuged at 10,000 rpm for 10 minutes. The supernatant was carefully collected for further analysis. To prepare the supernatant solution, 50 µl of the collected supernatant was transferred to a new vial, and 100 µl of reagent 3 and 20 µl of reagent 4 were added. For the blank solution, 50 µl of reagent 1, 100 µl of reagent 3, and 20 µl of reagent 4 were mixed in a separate vial. For the standard solution, 50 µl of the supplied standard solution was combined with 100 µl of reagent 3 and 20 µl of reagent 4. Additionally, 830 µl of distilled water was added to the supernatant, blank, and standard solutions. All solutions were allowed to stand at room temperature for 30 minutes. After this incubation period, the absorbance of each solution was measured at 660 nm using a spectrophotometer.

2.7. Plant performance analysis

We examined the treatment effect of alkaline biochar on plant performance by measuring plant height, stem diameter,

cob length, cob diameter, and yield using a measuring tape, vernier caliper scale, and weighing balance. After harvest, the plant parts (stem, leaves, and corn) were placed into envelopes and dried in an oven at 60°C for 72 hours (Lija et al., 2017). The plant biomass was then recorded.

2.8. Measurement of SPAD value by using SPAD-502 meter

Leaf greenness, serving as an indicator of chlorophyll content, was evaluated using a portable chlorophyll meter (SPAD-502, Konica Minolta, Inc., Tokyo, Japan). To ensure precision and consistency, SPAD readings were taken from fully matured leaves of each plant, with an average of three measurements per leaf (Yuan et al., 2016).

2.9. Root measurement

After harvesting the plant from the pots were enclosed in a plastic bag immediately to prevent the dehydration, washed carefully with tap water and separated into shoot and root to the root growth. After root being washed, the root was prepared for the determination of the root length by using measuring tape.

2.10. Plant Nutrient Analysis

The dried and ground plant material (0.25 g) was used for digestion. The single wet digestion technique (Cottenie, 1980) was conducted to extract the macro elements from the plant tissues. The wet digestion technique is a widely used method for determining plant nutrient concentrations by breaking down organic material using strong acids. To begin, plant samples are first dried at 60-70°C, ground into fine powder, and then weighed (0.25 g) into digestion tubes. A mixture of concentrated sulfuric acid (H_2SO_4) was added to the sample. The sample was then pre-digested for over night. After that 2 ml 30% H_2O_2 was added and heated on a hotplate or digestion block at 285°C until the solution becomes clear, indicating complete breakdown of organic matter. Perchloric acid ($HClO_4$) may also be used for tougher samples to ensure full digestion. Once digestion is complete, the solution is cooled, diluted to a 100 ml volume with distilled water, and filtered to remove undissolved particles. The resulting clear solution was analyzed for nutrient concentrations.

N and P concentrations were analyzed using a segmented flow analyzer (AA 500). Additionally, K, Ca, and Mg concentrations were determined using atomic absorption spectroscopy (AAS, PerkinElmer). The plant nutrient uptake was calculated using Equation 1 (Rabileh et al., 2015).

$$\text{Uptake (mg plant}^{-1}) = \text{Total nutrient concentration (\%)} \times \text{biomass (g)} \quad \text{.....[1]}$$

where the nutrient concentration was found using AAS, and the biomass was the plant'srespective dry weight.

2.11. Percent Relative Data

The relative data of the values were expressed as percentages, relative to control for each element recommended by (Ashraf & Waheed, 1990), the formula are as follows where the treatment value were the biochar and microbes amendment and the control value was without amendment (Equation 2).

$$\text{Relative data (\%)} = \frac{\text{Treatment value} - \text{control value}}{\text{control value}} \times 100 \quad \text{.....[2]}$$

2.12. Statistical Analysis

All data were analyzed using the two-way analysis of variance (ANOVA) procedure, and means were separated by Tukey's Honestly Significant Difference (HSD) test at a 5% level of significance using Statistical Analysis System, JMP software (SAS incorporation).

3. RESULTS

3.1. Effect of alkaline RHB and *P. aeruginosa* on Soil pH

Rice Husk Biochar (RHB), microbes, and their combination significantly influenced soil pH at 30 and 65 days after sowing (DAS) ($P<0.05$, Fig. 1). At 30 DAS, pH ranges from 4.43 to 4.92. The application of biochar increased soil pH, with 10% oxidized biochar (T3) showing a significant difference ($p<0.0001$) compared to the control (T1), resulting in an increase of 0.4 units. Microbial treatment at T4 demonstrated a significant increase ($p=0.0027$) in soil pH, with an increment of 7% compared to the control. Among all treatments, the combination of 10% oxidized biochar and *Pseudomonas aeruginosa* (T6) exhibited the highest soil pH (4.92), significantly different ($p=0.0371$) from the control T1 (4.43), with an increase of 11.07%.

At 65 DAS, biochar application resulted in a pH increase of 0.32 units with 10% oxidized biochar (T3), significantly different ($p<0.0001$) from the control (T1). Microbial treatment at T4 (no biochar + *Pseudomonas aeruginosa*) significantly increased soil pH by 0.16 units ($p=0.0054$). The combined effect of 10% oxidized biochar and *Pseudomonas aeruginosa* (T6) resulted in the highest soil pH (4.62), significantly different ($p=0.0311$) from the control T1 (4.28), with an increment of 7.94%.

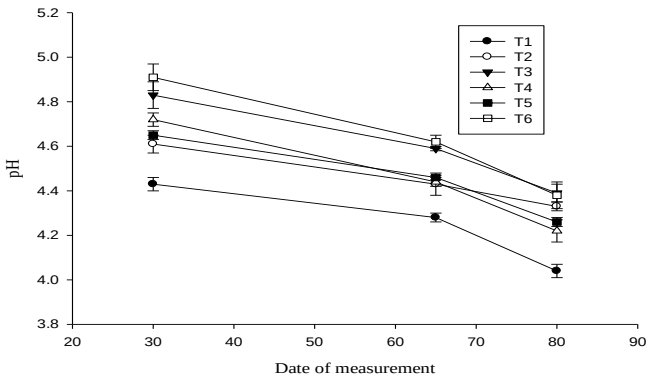

Figure 1. pH of different biochar treatments measured at a different date

Remarks: T1= No microbes and no biochar, T2= No microbes and Fresh biochar, T3= No microbes and 10% oxidized biochar, T4= *Pseudomonas aeruginosa* and no biochar, T5= *Pseudomonas aeruginosa* and Fresh biochar, T6= *Pseudomonas aeruginosa* and 10% oxidized biochar, Error bar represents standard error of mean, N=3

At 85 DAS, the lowest pH value (4.04) was obtained at T1 and the highest value (4.38) at T6. Biochar application continued to increase soil pH. On average, across microbial treatments, 10% oxidized biochar (T3) increased soil pH by 0.36 units, significantly different ($p<0.0001$) from the control (T1). However, microbial treatments showed a non-significant increase in soil pH ($p=0.3441$) compared to the

control. The interaction effect of biochar and microbes on soil pH was significant, with the 10% oxidized biochar and *Pseudomonas aeruginosa* (T6) exhibiting the highest soil pH, significantly different (p=0.020) from the control treatment T1. This treatment led to an 8.42% increase in soil pH.

3.2. Effect of alkaline RHB and *P. aeruginosa* on Post harvest soil nutrients

The application of treatments significantly increased the availability of phosphorus (P) in the soil (Table 3). The highest available P was recorded in treatment T6 (8.11 mg kg^{-1}), significantly greater than the control treatment (1.69 mg kg^{-1}). Treatment T5 (4.76 mg kg^{-1}), Biochar treatment (T3) (3.48 mg kg^{-1}), and microbes treatment T4 (1.87 mg kg^{-1}) also showed substantial increases. Statistical analysis confirmed that both biochar (p= <0.0001), microbial treatments (p= <0.0001), and their combination (p= 0.002) significantly influenced available P levels at 106%, 11%, and 380%, respectively.

In this study, Inorganic nitrogen levels varied across treatments, with the highest value observed in T3 (28.51 mg kg^{-1}) presented in Table 3. The control treatment (T1) had a lower inorganic N content (14.61 mg kg^{-1}). Biochar application significantly increased 95% inorganic N (P = 0.0125), while microbial treatments did not show a significant impact (P = 0.9542).

Table 3 demonstrates the significant effect of biochar, microbes, and their interaction on soil CEC. Applying biochar and microbes significantly increased soil CEC at 25% and 22%, respectively. However, their interaction effect was highest at T6 (11.23 mmol kg^{-1}), significantly different from control treatment T1 (6.31 mmol kg^{-1}). Biochar application had a significant positive effect on exchangeable K (P = 0.0006). Exchangeable potassium levels ranged from 0.06 to 0.16 mmol kg^{-1} and were significantly influenced by the treatments, with T6 showing the highest value (0.16 mmol kg^{-1}) and T1 (0.06 mmol kg^{-1}) showing the lowest value in Table 3. Biochar and microbes significantly affected exchangeable calcium levels. The highest values were observed in T3 (0.15 mmol kg^{-1}) and T6 (0.146 mmol kg^{-1}), both significantly different from the control (0.03 mmol kg^{-1}).

Biochar significantly increased exchangeable Ca (P = 0.0001), while the influence of microbial treatments was not significant (P = 0.0917). In this study, Table 3 revealed exchangeable magnesium (Mg) values that were highest in the combined application of 10% oxidized RHB and *Pseudomonas aeruginosa* T6 (0.07 mmol kg-1), which indicates significantly increased exchangeable Mg levels (P = 0.0023). Exchangeable aluminum ranged from 0.42 to 0.30 mmol kg^{-1}. The control treatment (0.42 mmol kg^{-1}) exhibited the highest levels of exchangeable aluminum (Al). Biochar treatment T3 significantly (P < 0.0001) reduced exchangeable Al. The combined effect of biochar and microbes also significantly decreased (P = 0.0193) exchangeable Al by 40% exchangeable Al.

3.3. Alkaline RHB and Microbes effect on maize plants nutrient concentration

The nutrient concentration of maize plants is demonstrated in Table 4. The concentration of P ranges from 0.15 to 0.49. Highest P concentration was found at T6 (10% oxidized biochar and *P.aeruginosa*) that was significantly different with control. Effect of 10% oxidized biochar and *P.aeruginosa* on P concentration was 226% higher compare to control. Biochar (p= 0.0023), microbes (p= 0.0007) and their combination (p= 0.0047) all were significantly different compare to the control. N concentration ranges from 0.03 to 0.14, and the highest value was found at 10% oxidized biochar and *P.aeruginosa* (T6) treatment. The total increment was found to be 366%. Among all treatments, biochar and microbes and their combined effect were significant (Table 4). K concentration ranges from 0.51 to 1.095%. The lowest value was found at the control treatment, T1, and the highest value was found at *P. aeruginosa* and Fresh biochar treatment, T5. Among all treatments biochar, microbes and combine effect was significant (Table 4). Ca concentration ranges from 0.03 to 0.07%. The highest value was found at T1. Among all treatments, biochar, microbes, and their combination showed no significant effect compared to control. Table 4 shows Mg concentration where the lowest value (0.01%) was found at control treatment T1 and the highest value (0.02%) was found at 10% oxidized biochar and *P.aeruginosa* treatment T6. Biochar, microbes, and the combined treatment showed no significant effect compared to control.

3.4. Alkaline RHB and Microbes' effect on maize plants total nutrient uptake

It was observed that P uptake ranges from 97.66 to 372.49 mg $plant^{-1}$. P uptake significantly increased with the application of biochar, microbes, and their combination (Table 5). On average, across microbial treatments, 10% oxidized biochar (T3) increased P uptake by 229%, which was significantly different (p=0.0008) from the control (T1). Microbial treatment at T4 showed a significant increase in P uptake (268.15 mg $plant^{-1}$), which was 275% increased compared to the control treatment (T1) (p<0.0001). Among all combinations, the highest P uptake (372.49 mg $plant^{-1}$) was observed in the treatment combining 10% oxidized biochar and *Pseudomonas aeruginosa* (T6), which was significantly (p=0.004) increased 281% from the control treatment (T1).

Similarly, nitrogen (N) uptake was significantly increased by the application of biochar, microbes, and their combination (Table 5). N uptake was ranges from 24.85 to 95.82 mg $plant^{-1}$. Biochar application at T3 resulted in a 189% increase (p=0.0003) in N uptake compared to the control (T1). Microbial application at T4 significantly increased (p<0.0001) N uptake 60.28 mg $plant^{-1}$ that was 242% increment compared to the control (T1). Among all treatments, the highest N uptake (95.82 mg $plant^{-1}$) was observed with the combined application of 10% oxidized biochar and *Pseudomonas aeruginosa* (T6), which was significantly different (p=0.0024) from the lowest N uptake (24.85 mg $plant^{-1}$) observed in the control (T1). This study revealed a significant increase in potassium (K) uptake due to the application of biochar, as shown in Table 5.

Table 3. Effect of oxidized alkaline RHB and microbes on changes in nutrients of the post-harvest soil

Treatments	Available P (Mg kg^{-1})	Inorganic N (Mg kg^{-1})	CEC (mmol kg^{-1})	Exchangeable K (mmol kg^{-1})	Exchangeable Ca (mmol kg^{-1})	Exchangeable Mg (mmol kg^{-1})	Exchangeable Al (mmol kg−1)
T1	1.69± 0.04d	14.61± 0.38b	6.31±0.30c	0.06±0.01b	0.033±0.003c	0.04±0.003bc	0.42±0.01a
T2	2.10±0.06cd	20.46±1.52ab	10.4±1.25ab	0.14±0.01a	0.14±0.01a	0.066±0.01ab	0.39±0.01ab
T3	3.48±0.49bc	28.51±2.49a	7.89±0.51bc	0.15±0.01a	0.15±0.01a	0.05±0.003abc	0.32±0.01b
T4	1.87±0.08cd	23.87± 3.07ab	7.75±0.16bc	0.13±0.01a	0.086±0.01b	0.06±0.01abc	0.48±0.04a
T5	4.76±0.42b	17.02±0.40b	9.06±0.24abc	0.12±0.01a	0.13±0.01a	0.036±0.003	0.31±0.01 b
T6	8.11±0.51a	23.01±3.19ab	11.23±0.34a	0.16±0.02a	0.146±0.01a	0.07±0.01a	0.30±0.01b
Biochar	*P*= < 0.0001*	*P*= 0.0125*	*P*= 0.0010*	*P*= 0.0006*	*P*= <0.0001*	*P*= 0.1282	*P*= <0.0001*
Microbes	*P*= < 0.0001*	*P*= 0.9542	*P*= 0.0354*	*P*= 0.0783*	*P*= 0.0917*	*P*= 0.6617	*P*= 0.4402
BC*M	*p*= 0.0002*	*p*= 0.0110*	*p*= 0.0066*	*p*= 0.0081*	*p*= 0.0033*	*p*= 0.0023*	*p*= 0.0193*

Remarks: T1= No microbes and no biochar, T2= No microbes and Fresh biochar, T3= No microbes and 10% oxidized biochar, T4= *Pseudomonas aeruginosa* and no biochar, T5 = *Pseudomonas aeruginosa* and Fresh biochar, T6= *Pseudomonas aeruginosa* and 10% oxidized biochar, N=3

Table 4. Alkaline RHB and microbes effect on maize plant nutrients concentration

Treatments	P concentration (%)	N concentration (%)	K concentration (%)	Ca concentration (%)	Mg concentration (%)
T1	0.15±0.03b	0.03±0.01c	0.51±0.09b	0.07±0.06a	0.01±0.0001a
T2	0.395±0.025a	0.08±0.01b	1.62±0.18a	0.02±0.02a	0.21±0.01a
T3	0.395±0.01a	0.1±0.01ab	1.12±0.14ab	0.02±0.01a	0.01±0.01a
T4	0.43±0.04a	0.11±0.01ab	1.16±0.16ab	0.02±0.001a	0.18±0.001a
T5	0.41±0.01a	0.12±0.01ab	1.44±0.09a	0.01±0.001a	0.02±0.003a
T6	0.49±0.0.03a	0.14±0.01a	1.095±0.04ab	0.03±0.01a	0.02±0.001a
Biochar	p= 0.0023*	0.0021*	0.0043*	0.5340	0.4345
Microbes	p=0. 0007*	0.00021*	0.1965*	0.4147	0.3317
BC*M	P= 0.0047*	0.0299*	0.0350*	0.5847	0.2287

Remarks: Means within the same column followed by the different letters are significantly different at p≤ 0.05; (Turkey's HSD test). The column represents the mean values ± standard error. T1= No microbes and no biochar, T2= No microbes and Fresh biochar, T3= No microbes and 10% oxidized biochar, T4= *Pseudomonas aeruginosa* and no biochar, , T5= *Pseudomonas aeruginosa* and Fresh biochar, T6= *Pseudomonas aeruginosa* and 10% oxidized biochar.

Table 5. Oxidized RHB and microbes effect maize plants' total nutrient uptake

Treatments	Total P (mg kg^{-1})	Total N (mg kg^{-1})	K (mg plant^{-1})	Ca (mg plant^{-1})	Mg (mg plant^{-1})
T1	97.66±15.14c	24.85±0.93d	427±28.34c	27.37±1.74ab	5.58±0.67b
T2	259.29±28.25b	58.73±5.96c	1152.89±61.71a	37.25±3.16a	14.86±1.59a
T3	322.02±18.98ab	71.80±7.22bc	869.27±36.75b	24.04±1.20b	15.68±1.76a
T4	365.81±35.54a	85.13±6.53ab	868.53±37.74b	18.27±1.35bc	12.53±1.87ab
T5	306.48±16.95ab	78.44±2.91abc	1107.85±20.99a	9.57±1.20c	13.45±1.60a
T6	372.49±5.68a	95.82±1.98a	983.79±30.12ab	26.43±3.33b	15.86±1.76a
Biochar	*P*= 0.0008*	*P*= 0.0003*	*P*= < 0.0001*	*P*= 0.5354	*P*= 0.0020*
Microbes	*P*= < 0.0001*	*P*= < 0.0001*	*P*= 0.0001*	*P*= < 0.0001*	*P*= 0.1458
BC*M	*p*= 0.0004*	*p*= 0.0024*	*p*= 0.0001*	*p*= < 0.0001*	*p*= 0.0374*

Remarks: T1= No microbes and no biochar, T2= No microbes and Fresh biochar, T3= No microbes and 10% oxidized biochar, T4= *Pseudomonas aeruginosa* and no biochar, T5= *Pseudomonas aeruginosa* and Fresh biochar, T6= *Pseudomonas aeruginosa* and 10% oxidized biochar, N=3

Uptake K ranges from 427 to 1107.85 mg plant^{-1}. The biochar application at T3 resulted in a significantly higher value of 869.27 mg plant^{-1}, compared to the control (T1) value of 427 mg plant^{-1}. The increment of K uptake was doubled compared to the control. The application of microbes led to a 103% increase in K uptake, which was significantly higher than the control. Specifically, treatment T6 (10% oxidized RHB and *Pseudomonas aeruginosa*) showed a significant increase in K uptake, with an increment of 557 mg plant^{-1} compared to T1. The highest increase in K uptake was observed in treatment T5 (fresh RHB and *Pseudomonas aeruginosa*), showing a 159% increase compared to the control treatment. This study demonstrated that the application of biochar did not showed significantly (p= 0.5354) different at calcium (Ca) uptake, as detailed in Table 5. However, the introduction of microbial treatments resulted in a 33% reduction in Ca uptake, a significant decrease compared to the control. Notably, treatment T5, which involved the use of fresh RHB combined with *Pseudomonas aeruginosa*, exhibited a substantial 66% decrease in Ca uptake compared to the control (T1). Table 5 illustrated that the application of 10% oxidized biochar significantly increased 181% Magnisium (Mg) uptake compare to no biochar and no microbes treatment (T1). Notably, among all treatments, T6 (10% oxidized RHB and *Pseudomonas aeruginosa*), exhibited 184% increase in Mg uptake compared to the control (T1).

3.5. Role of alkaline RHB and *P. aeruginosa* on SPAD value

The SPAD value was influenced by the application of biochar, microbes, and the interaction of biochar with phosphate-solubilizing bacteria (Table 6). At 30 DAS, the application of biochar and microbes alone did not show a significant effect compared to the control. However, the combination of *Pseudomonas aeruginosa* and 10% oxidized alkaline biochar resulted in the highest SPAD value (35.73), significantly different from the control T1 (34.9) with a p-value of 0.0423. At 45 DAS, the application of biochar and biochar microbes interaction effects were significant, with p-values of 0.0003 and 0.0068, respectively, while the effect of microbes alone was not significant (p=0.4717). The highest SPAD value (49.56) at this stage was observed in the biochar treatment T5, which combined *Pseudomonas aeruginosa* and fresh biochar. By 65 DAS, the highest SPAD value (51.2) was observed at *Pseudomonas aeruginosa* and fresh biochar treatment T5. The lowest SPAD value (47.06) was found in the control treatment. Biochar, microbes, and their interaction effects were not significant at 65 DAS.

3.6. Effect of Oxidized RHB and microbes on soil leachate

The application of biochar, particularly in combination with *Pseudomonas aeruginosa*, significantly influenced several soil leachate parameters that were presented in Table 7. The availability of leachate phosphorus was significantly influenced by the application of biochar (p<0.001) and the interaction between biochar and microbes (p=0.0423), although the effect of microbes alone was not significant (p=0.1284). The control treatment (no microbes and no biochar) had the highest phosphorus leaching (0.056 mg plant^{-1}), significantly different from the other treatments. Treatments T4 (*Pseudomonas aeruginosa* and no biochar) and T5 (*Pseudomonas aeruginosa* and fresh biochar) also showed higher phosphorus leaching compared to oxidized biocharT3 and T6 treatments. In this study, Soil leachate pH was significantly affected by biochar (p=0.0008), microbes (p=0.0032), and their interaction (p=0.0022). The highest pH was observed in treatment T6 (*Pseudomonas aeruginosa* and 10% oxidized biochar), with a value of 6.64, indicating a notable alkalizing effect. In contrast, the lowest pH was found in the control treatment T1 (5.42), significantly lower than in the other treatments. The combined application of biochar and microbes showed a 23% significant increment (p=0.0022) in soil pH. Besides this, the amount of collected leachate was significantly influenced by biochar (p=0.0004) and the interaction between biochar and microbes (p=0.0178) but not by microbes alone (p=0.1957). The control treatment, T1, produced the highest leachate volume (733.33 ml).

Table 6. Effect of RHB and microbes on SPAD value (mean±SE)

Treatments	30 DAS	45 DAS	65 DAS
T1	34.9 ± 0.89ab	42.76 ± 1.41ab	47.06 ± 1.76a
T2	35 ± 1.23ab	46.03 ± 0.80a	50.1 ± 0.43a
T3	33.93 ± 1.53ab	45.9± 2.40a	45.4± 3.02a
T4	28.6 ± 2.05b	33.43 ± 2.74b	38.5 ± 10.28a
T5	34.63 ± 1.82ab	49.56 ± 1.32a	51.2 ± 2.48a
T6	35.73 ± 0.56a	48.4 ± 1.41a	48.43 ± 2.99a
Biochar (BC)	*p*=0.0868	*p*=0.0003*	*p*=0.2856
Microbes(M)	*p*=0.1945	*p*=0.4717	*p*=0.7078
BC*M	*p*= 0.0423*	*p*= 0.0068*	*p*= 0.442

Remarks: T1= No microbes and no biochar, T2= No microbes and Fresh biochar, T3= No microbes and 10% oxidized biochar, T4= *Pseudomonas aeruginosa* and no biochar, T5= *Pseudomonas aeruginosa* and Fresh biochar, T6= *Pseudomonas aeruginosa* and 10% oxidized biochar, N=3

Table 7. Effect of oxidized RHB and microbes on soil leachate

Treatments	Available P (mg plant^{-1})	pH	Amount of leachate (ml)	EC (µS cm^{-1})
T1	0.056± 0.003a	5.42±0.06c	733.33±33.21a	648.67±23.25bc
T2	0.026±0.003cd	6.56±0.06ab	356.67±49.78b	769±5.13ab
T3	0.023± 0.003cd	5.43±0.20c	460±61.44b	644.67±20.79bc
T4	0.046±0.003ab	5.84±0.14bc	558.33±57.32ab	562±28.84c
T5	0.033±0.003bc	6.33±0.23ab	486.67±50.19b	886.33±3315a
T6	0.013±0.003d	6.64±0.17a	345±21.79b	818.33±56.49a
Biochar	*p*= <0.001*	*p*= 0.0008*	*p*= 0.0004*	*p*= <0.001*
Microbes	*P*= 0.1284	*P*=0.0032*	*P*=0.1957	*P*=0.0227*
BC*M	*P*=0.0423*	*P*=0.0022*	*P*=0.0178*	*P*=0.0038*

Remarks: T1= No microbes and no biochar, T2= No microbes and Fresh biochar, T3= No microbes and 10% oxidized biochar, T4= *Pseudomonas aeruginosa* and no biochar, T5= *Pseudomonas aeruginosa* and Fresh biochar, T6= *Pseudomonas aeruginosa* and 10% oxidized biochar, N=3

In comparison, the treatment T6 (*Pseudomonas aeruginosa* and 10% oxidized biochar) had the lowest volume (345 ml), indicating a 113% significant reduction in leachate with the combination of microbes and oxidized biochar. However, Electrical conductivity (EC) was significantly affected by biochar (p<0.001), microbes (p=0.0227), and their interaction (p=0.0038). The highest EC was recorded in treatment T5 (*Pseudomonas aeruginosa* and fresh biochar) with a value of 886.33 µS cm^{-1}, suggesting an increased concentration of soluble ions. Conversely, the lowest EC was observed in the control treatment T1 (648.67 µS cm^{-1}), significantly lower than those combined with biochar and microbes. Treatments T4 (*Pseudomonas aeruginosa* and no biochar) and T5 (*Pseudomonas aeruginosa* and fresh biochar) also showed higher phosphorus leaching compared to oxidized biocharT3 and T6 treatments. In this study, Soil leachate pH was significantly affected by biochar (p=0.0008), microbes (p=0.0032), and their interaction (p=0.0022). The highest pH was observed in treatment T6 (*Pseudomonas aeruginosa* and 10% oxidized biochar), with a value of 6.64, indicating a notable alkalizing effect. In contrast, the lowest pH was found in the control treatment T1 (5.42), significantly lower than in the other treatments. The combined application of biochar and microbes showed a 23% significant increment (p=0.0022) in soil pH. Besides this, the amount of collected leachate was significantly influenced by biochar (p=0.0004) and the interaction between biochar and microbes (p=0.0178) but not by microbes alone (p=0.1957). The control treatment, T1, produced the highest leachate volume (733.33 ml). In comparison, the treatment T6 (*Pseudomonas aeruginosa* and 10% oxidized biochar) had the lowest volume (345 ml), indicating a 113% significant reduction in leachate with the combination of microbes and oxidized biochar. However, Electrical conductivity (EC) was significantly affected by biochar (p<0.001), microbes (p=0.0227), and their interaction (p=0.0038). The highest EC was recorded in treatment T5 (*Pseudomonas aeruginosa* and fresh biochar) with a value of 886.33 µS cm^{-1}, suggesting an increased concentration of soluble ions. Conversely, the lowest EC was observed in the control treatment T1 (648.67 µS cm^{-1}), significantly lower than those combined with biochar and microbes.

3.7. Effect of alkaline RHB and *P. aeruginosa* on Soil phosphatase activity

Application of oxidized RHB biochar and phosphate solubilizing bacteria *Pseudomonas aeruginosa* in soil significantly improved soil phosphatase activity Figure 2. Biochar and microbes application significantly increased 47% and 48% phosphatase activity compared to the control. Among all treatments, a higher value (2.80U-gsoil-1) was recorded at T6 (10% oxidized alkaline RHB and *Pseudomonas aeruginosa*), and a lower value (1.11U-gsoil-1) was observed at T1. Inoculation of bacteria *Pseudomonas aeruginosa* on soil phosphatase activity was better than non-inoculated. Among all treatments, T6 (10% oxidized alkaline RHB and *Pseudomonas aeruginosa*) showed significantly increased152% phosphatase activity from the control treatment.

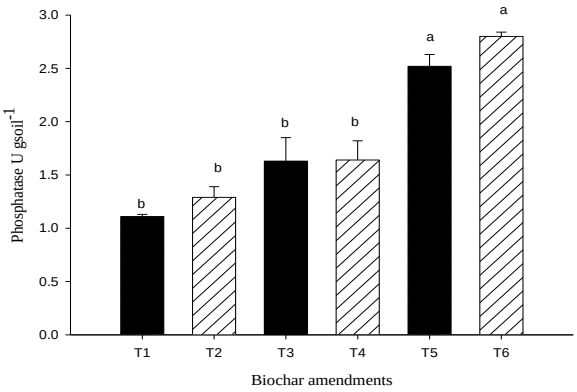

Figure 2. Alkaline RHB and microbes effect on soil phosphatase activity

Remarks: T1= No microbes and no biochar, T2= No microbes and Fresh biochar, T3= No microbes and 10% oxidized biochar, T4= *Pseudomonas aeruginosa* and no biochar, T5= *Pseudomonas aeruginosa* and Fresh biochar, T6= *Pseudomonas aeruginosa* and 10% oxidized biochar, Error bar represents standard error of mean, N=3

Table 8. Oxidized RHB and Microbes effect on soil microbial populations

Treatments	Microbial Population (cfu ml^{-1})
T1	$5.6\times10^{-5} \pm 3.5\times10^{-6}$ a
T2	$4.35\times10^{-5} \pm 5.5\times10^{-6}$ b
T3	$3.25\times10^{-5} \pm 8.7\times10^{-7}$ab
T4	$5.6\times10^{-5} \pm 4.6\times10^{-6}$ab
T5	$5.1\times10^{-5} \pm 8.6\times10^{-6}$ a
T6	$3.6\times10^{-5} \pm 1.2\times10^{-6}$ ab
Biochar (BC)	*p*=0.3891
Microbes(M)	*p*=0.7507
BC×M	*p*= 0.0028*

Remarks: T1= No microbes and no biochar, T2= No microbes and Fresh biochar, T3= No microbes and 10% oxidized biochar, T4= *Pseudomonas aeruginosa* and no biochar, T5= *Pseudomonas aeruginosa* and Fresh biochar, T6= *Pseudomonas aeruginosa* and 10% oxidized biochar, N=3

3.8. Allkaline RHB and *P. aeruginosa* effect on soil microbial populations

The influence of phosphate solubilizing and N_2-fixing bacteria and pH-adjusted alkaline oxidized RHB biochar on microbial population was presented in Table 8. There was no significant different among biochar and microbes treatments compared to unamended soil (control). However, the interaction effect of biochar and microbes was significantly different (p= 0.0028) compared to the control.

3.9. Effect of alkaline RHB and *P. aeruginosa* on plant growth characters

In this experiment, Maize plant height, stem diameter, root length, root volume, and dry biomass were significantly affected by the addition of biochar, microbes and their combination, as presented in Table 9. Plant height was significantly influenced by biochar (p=0.0044), microbes (p=0.0449), and their interaction (p=0.0169). Treatment T6 (*Pseudomonas aeruginosa* and 10% oxidized biochar) demonstrated the tallest plants with an average height of 179.33 cm, significantly higher than most other treatments. The control treatment T1 (no microbes and no biochar) resulted in the shortest plants, with an average height of 167.67 cm.The plant height increment was significantly found 7% by combine application of biochar and microbes. Stem diameter was significantly affected by the presence of microbes (p=0.0021) and the interaction between biochar and microbes (p=0.0078), though the effect of biochar alone was not significant (p=0.0552). The largest stem diameter (1.39 cm) was observed in treatment T3 (no microbes and 10% oxidized biochar), which was significantly increased by 0.46 cm from the control.

Root length was significantly influenced by biochar (p=0.0026) and the interaction between biochar and microbes (p=0.0242) but not by microbes alone (p=0.9610). The longest roots were found in treatment T4 (*Pseudomonas aeruginosa* and no biochar), with an average length of 53 cm. The shortest roots were observed in the control treatment T1 (22.33 cm). Among all treatments, a combination of biochar and microbes significantly increased root length by 138% compared to the control treatment T1. Root volume was significantly affected by biochar (p=0.0003), microbes (p<0.0001), and their interaction (p=0.0062). Treatment T6 (*Pseudomonas aeruginosa* and 10% oxidized biochar) exhibited the highest root volume (20.9 ml $plant^{-1}$), which was significantly greater than the control (T1) with a volume of 8.83 ml $plant^{-1}$.

However, Dry biomass was significantly influenced by biochar (p=0.0054) and microbes (p=0.0150), although their interaction was not significant (p=0.133). The highest dry biomass was recorded in treatment T6 (92.13 g), significantly higher than all other treatments. The control treatment, T1, had the lowest dry biomass (48.72 g). The total increment of dry biomass was 47%, found by a combination of biochar and microbes.

3.10. Effect of alkaline RHB and *P. aeruginosa* on yield contributing characters

In this study, the maize cob length, fresh cob diameter, number of grains per cob, and yield exhibited significant increment (Table 10) with the biochar, microbes and combined application of oxidized alkaline RHB and microbes compared to untreated soil (T1). Average across microbes treatments, 10% Oxidized RHB showed 19% increased cob length, 96% increased cob diameter, 159% increased number of grain and 183% increased yield respectively compare to control. Microbes treatments significantly increased 0.17 cm cob length, 6.65 cm cob diameter, 15 number of grain and 16.95 g pot^{-1} yield respectively. Among all treatments, combination of 10% oxidized alkaline RHB and *Pseudomonas aeruginosa* (T9) revealed significantly highest value on cob length (22.30 cm), cob diameter (5.33 cm), number of grain (375) and yield (56.1 g pot^{-1}) respectively. Yied increment was 291 % at T9. These improvement in yield might have occurred due to an increase soil pH, P bioavailability (r^2= 0.74), and a reduction in Al toxicity (r^2= 0.36) (Fig.3).

3.11. Correlation between plant parameters, nutrient absorption, soil pH, and nutrient concentrations

Pearson's correlation analysis was conducted to determine the relationship among the soil nutrients, plant parameters, plant nutrient uptake, and yield (Table 11). Grain yield was positively correlated with soil pH (r = 0.88), Soil available P (r = 0.86), exchangeable K, Ca, (r = 0.73 and 0.68 respectively), enzyme (r = 0.75), P uptake (r = 0.75), N uptake (r = 0.81), cob length (r= 0.72). Furthermore, available phosphorus was positively correlated with soil pH (r = 0.67), Enzyme was positively correlated with available P (r = 0.83), pH (r = 0.59), and dry biomass was positively correlated with pH (r = 0.75), available P (r = 0.57) P, N, K, Mg uptake (r = 0.86, 0.84,0.71 and 0.83 respectively).

4. DISCUSSION

4.1. Effect of oxidized RHB and microbial Amendments on Post-Harvest Soil properties

The reason for conducting this study is to address phosphorus deficiency and decrease maize yields in acidic soils. The research explores how the combined application of pH adjusted alkaline oxidized RHB and phosphate solubilizing bacteria (*P. aeruginosa*) can improve phosphorus availability, soil health, and maize productivity. It aims to provide a sustainable solution for enhancing crop yields in challenging soil conditions.

Table 9. Effect of oxidized alkaline RHB and *Pseudomonas aeruginosa* on maize plant growth characters

Treatments	Plant height (cm)	Stem diameter (cm)	Root length (cm)	Root volume (ml plant^{-1})	Dry biomass (g)
T1	167.67±1.45b	1.07± 0.02b	22.33±1.45b	8.83±0.44c	48.72±4.40b
T2	172± 3.61b	1.35±0.03ab	77.33±9.70a	15.17± 0.66b	75.16±7.89ab
T3	168± 9.07b	1.39± 0.046a	76.66±10.03a	17.17±0.44ab	80.67±4.74a
T4	158.33± 5.70b	1.52±0.05a	53±2.64ab	19.33±1.45ab	76.19±9.34ab
T5	200± 5.51a	1.34±0.11ab	62.33±8.68a	20.43±0.94a	77.14±4.02a
T6	179.33± 4.41ab	1.53±0.07a	62±11.06a	20.9± 0.95a	92.13±0.66a
Biochar	*p*= 0.0044*	*p*= 0.0552	*p*= 0.0026*	*p*= 0.0003*	*p*= 0.0054*
Microbes	*P*= 0.0449*	*P*= 0.0021*	*P*= 0.9610	*P*= <0.0001*	*P*= 0.0150*
BC*M	*P*= 0.0169*	*P*= 0.0078*	*P*= 0.0242*	*P*= 0.0062*	*P*= 0.133

Remarks: T1= No microbes and no biochar, T2= No microbes and Fresh biochar, T3= No microbes and 10% oxidized biochar, T4= *Pseudomonas aeruginosa* and no biochar, T5= *Pseudomonas aeruginosa* and Fresh biochar, T6= *Pseudomonas aeruginosa* and 10% oxidized biochar, N=3

Table 10. Effect of oxidized RHB and microbes on maize yield contributing characters

Treatments	Cob length (cm)	Cob diameter (cm)	No. of grain per cob	Yield (g pot^{-1})
T1	13.16±0.44c	2.23±0.07b	127±4.36	14.36±0.88d
T2	14.10±0.21bc	4.62±0.16a	169±8.89	25.42± 2.01c
T3	15.66±1.20bc	4.39±0.12a	328.67±5.93	40.69±1.41b
T4	13.33±1.64c	4.42±0.24a	142.33±4.33	31.31±1.00c
T5	18.66± 1.20ab	4.83±0.31a	247±21.36	33.26± 2.46bc
T6	22.30±0.7a	5.33±0.24a	375.67±10.89	56.1±2.08a
Biochar	*p*= 0.0005*	*p*= <0.0001*	*p*= <0.0001*	*p*= <0.0001*
Microbes	*P*= 0.0007*	*P*= <0.0001*	*P*= 0.0002*	*P*= <0.0001*
BC*M	*P*= 0.0238*	*P*= 0.0014*	*P*= 0.0452*	*P*= 0.0492*

Remarks: T1= No microbes and no biochar, T2= No microbes and Fresh biochar, T3= No microbes and 10% oxidized biochar, T4= *Pseudomonas aeruginosa* and no biochar, T5= *Pseudomonas aeruginosa* and Fresh biochar, T6= *Pseudomonas aeruginosa* and 10% oxidized biochar, N=3

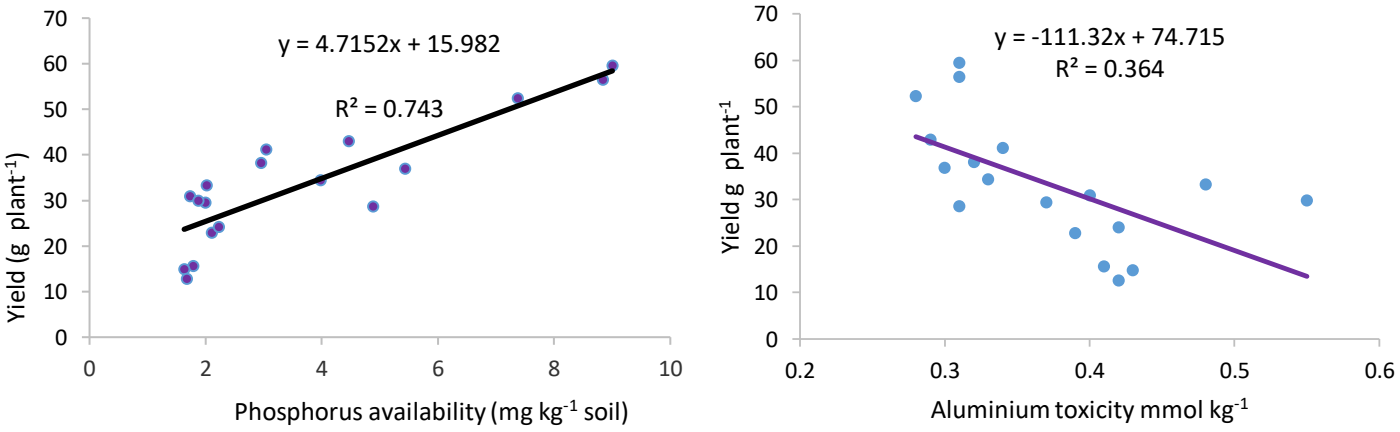

Figure 3. Regression relationship between the change of soil available P and Al toxicity against grain yield

Rice Husk Biochar (RHB) and microbes interaction significantly influenced soil pH at 30, 65, and 85 DAS ($P<0.05$, Fig. 1). At 30 DAS, the combination of 10% oxidized biochar and *Pseudomonas aeruginosa* (T6) exhibited the highest soil pH (4.92), significantly different ($p=0.0371$) from the control T1 (4.43), with an increase of 11.07%. At 65 DAS, the biochar and microbes interaction effect resulted in the highest soil pH (4.62), significantly different ($p=0.0311$) from the control T1 (4.28), with an increment of 7.94%, at 85 DAS. The interaction effect of biochar and microbes on soil pH was significant, with the 10% oxidized biochar and *Pseudomonas aeruginosa* (T6) exhibiting the highest soil pH, significantly different ($p=0.020$) from the control treatment T1. This treatment led to an 8.42% increase in soil pH.

Biochar increases soil pH has been reported by Abdulrahman et al. (2016). Biochar buffered soil pH, possibly through basic cation additions and H^+ consumption on its negative functional groups. Our result was in line with the study by Ch'ng et al. (2019), where they reported an increase of 0.99 units of soil pH after applying biochar. They found that the percent increment of soil pH was 11.06%, 7.94%, and 8.42%, respectively, supporting our result. pH buffering occurs through oxygenated functional groups of biochar. Alkaline RHB (pH = 8.15) is a huge amount of ash (~32%). During the hydrolysis process, it releases $OH^-$by its base cations, which may assist in increasing soil pH (Mosharrof et al., 2022).The notable increase in soil pH compared to the control treatment further emphasizes the effectiveness of T6 (10% oxidized biochar and *Pseudomonas aeruginosa*) in ameliorating soil acidity. Gao et al. (2023) demonstrated that biochar addition can increase soil pH levels significantly after incubation with poultry litter biochar.

The application of various soil treatments resulted in a significant increase in the availability of phosphorus (P) in the soil, as shown in Table 3. Among the treatments, T6 exhibited the highest level of available phosphorus, measuring 8.11 mg kg^{-1}, significantly greater than the control treatment (T1). This result exhibited the effectiveness of T6 in enhancing phosphorus availability compared to untreated soil. Furthermore, other treatments such as biochar treatment T3 (3.48 mg kg^{-1}), microbial treatment T4 (1.87 mg kg^{-1}), and T5 (*Pseudomonas aeruginosa* and Fresh biochar, 4.76 mg kg^{-1}) also demonstrated considerable increases in soil phosphorus levels. The statistical analysis supported these observations, revealing that both biochar ($P < 0.0001$) and microbial treatments ($P < 0.0001$), as well as their combined application ($P = 0.0002$), significantly influenced the availability of phosphorus in the soil. The pyrolysis temperature of our applied biochar was 300°C. Our result is supported by Eduah et al. (2019), who stated that Lower pyrolysis temperatures (300-450°C) reduce P sorption and increase P desorption in acid soils, potentially increasing P bioavailability. The combination of biochar and *Pseudomonas aeruginosa* had a positive effect on enhancing P availability in the soil (Heidari et al., 2020). In treatments, T5 and T6, organic material in the form of fresh rice husk biochar (RHB) and oxidized alkaline RHB are introduced into the soil. This organic material serves as a source of nutrients, including phosphorus, which becomes available to plants. *Pseudomonas aeruginosa*, a known phosphate-solubilizing bacteria, is introduced in treatments T5 and T6. These bacteria have the ability to solubilize phosphorus from organic and inorganic sources, making it more available for plant uptake (Rawat et al., 2021). The presence of both the organic material and the phosphate-solubilizing bacteria can enhance more phosphorus availability rather than individual (Timofeeva et al., 2023). Biotic factors, including soil microbes and enzyme activity are essential to the phosphorus cycle in soil (Pastore et al., 2020). Abiotic factors, such as soil pH, iron and aluminum oxides, soil organic matter content, and cation exchange, also influence phosphorus cycling processes (Fan et al., 2019). Microbes have diverse functions in the phosphorus cycle and in altering phosphorus availability. Phosphate-solubilizing microbes are vital for releasing bound phosphate, making it available for plant uptake (Li et al., 2021). This study investigated the effects of oxidized alkaline rice husk biochar (RHB) and phosphate-solubilizing bacteria, specifically *Pseudomonas aeruginosa*, on soil phosphatase activity. The findings, illustrated in Figure 2, demonstrated a significant improvement in soil phosphatase activity after applying both biochar and microbial treatments.

The application of RHB biochar and *Pseudomonas aeruginosa* markedly enhanced soil phosphatase activity. Specifically, biochar application resulted in a 47% increase, while microbial treatment led to a 48% increase in phosphatase activity compared to the control. These results suggest that both treatments independently contribute significantly to improving soil enzymatic activity, which is crucial for phosphorus cycling and availability in soil.Among all treatments, the combination of 10% oxidized alkaline RHB and *Pseudomonas aeruginosa* (T6) resulted in a remarkable 152% increase in phosphatase activity over the control, highlighting its potential as an effective soil amendment strategy.Interestingly, even a lower rate (0.5%) of oxidized RHB biochar combined with bacterial inoculation was effective in improving soil phosphatase activity.

Phosphate solubilizing bacterium can enhance available phosphorus by producing hydrolytic enzymes, such as alkaline phosphatases, acid phosphatases, and phytases, which mineralize soil organic phosphorus and release inaccessible phosphorus (Wu et al., 2021). *Pseudomonas aeruginosa* is known to produce phosphatase enzymes as part of its metabolic activities. When introduced into a system containing alkaline rice husk biochar, these bacteria may colonize the biochar surface and utilize its nutrients, releasing phosphatase enzymes in the process (Schmalenberger & Fox, 2016). *Pseudomonas aeruginosa*, as a phosphate-solubilizing bacterium, enhances phosphorus availability through the solubilization of insoluble phosphorus compounds (C. Wang et al., 2023). Phosphatase enzymes typically exhibit higher activity at alkaline pH ranges, so the increased pH provided by the biochar can enhance the enzymatic activity of phosphatase enzyme (Guan et al., 2023; Schalk & Perraud, 2023). The study examined the effects of phosphate-solubilizing and nitrogen-fixing bacteria *Pseudomonas aeruginosa*, along with oxidized rice husk biochar (RHB), on the microbial population in soil.

Table 11. The relationship between maize plant parameters, nutrient absorption, soil pH, and soil nutrient content

Parameters	pH (Av)	SPAD (Av)	AVP	Inorg.N	CEC	Ex. K	Ex. Ca	Ex. Mg	Ex. Al	Microbial population	Enzyme	P uptake	N uptake	K uptake	Ca uptake	Mg uptake	plant height	stem dia	Root length	Root volume	Dry biomass	cob dia	Cob length	No. of mature grain	yield
pH (Av)	1.00																								
SPAD (Av)	0.07	1.00																							
AVP	0.67	0.32	1.00																						
Inorg. N	0.67	-0.05	0.14	1.00																					
CEC	0.51	0.38	0.56	0.24	1.00																				
Ex. K	0.81	0.17	0.47	0.65	0.61	1.00																			
Ex. Ca	0.82	0.24	0.53	0.48	0.62	0.76	1.00																		
Ex. Mg	0.51	-0.15	0.28	0.50	0.61	0.48	0.38	1.00																	
Ex. Al	-0.51	-0.72	-0.69	-0.23	-0.44	-0.42	-0.59	0.02	1.00																
Microb.popu	-0.35	0.25	-0.23	-0.10	-0.49	-0.44	-0.43	-0.69	-0.07	1.00															
Enzyme	0.59	0.34	0.83	0.14	0.50	0.51	0.48	0.11	-0.61	-0.09	1.00														
P uptake	0.73	-0.06	0.47	0.62	0.53	0.78	0.61	0.39	-0.33	-0.31	0.59	1.00													
N uptake	0.78	-0.01	0.63	0.47	0.47	0.68	0.61	0.34	-0.39	-0.32	0.71	0.90	1.00												
K uptake	0.58	0.27	0.36	0.24	0.64	0.67	0.80	0.24	-0.38	-0.42	0.48	0.64	0.67	1.00											
Ca uptake	-0.01	0.05	-0.13	-0.01	0.09	0.07	0.04	0.37	0.17	-0.49	-0.45	-0.29	-0.33	-0.03	1.00										
Mg uptake	0.72	0.33	0.38	0.50	0.52	0.80	0.73	0.28	-0.49	-0.34	0.52	0.75	0.71	0.75	0.03	1.00									
plant height	0.11	0.64	0.39	-0.11	0.35	0.15	0.29	-0.27	-0.58	0.30	0.65	0.13	0.15	0.39	-0.42	0.27	1.00								
stem dia	0.69	-0.11	0.40	0.64	0.49	0.77	0.51	0.53	-0.15	-0.40	0.52	0.87	0.81	0.57	-0.15	0.66	0.01	1.00							
Root length	0.66	0.10	0.25	0.50	0.55	0.62	0.78	0.44	-0.37	-0.37	0.14	0.53	0.52	0.70	0.05	0.56	-0.01	0.53	1.00						
Root volume	0.67	0.05	0.58	0.37	0.48	0.66	0.60	0.13	-0.41	-0.18	0.74	0.90	0.90	0.69	-0.41	0.73	0.38	0.70	0.40	1.00					
Dry biomaass	0.75	0.27	0.57	0.59	0.58	0.74	0.71	0.40	-0.57	-0.32	0.63	0.86	0.84	0.71	-0.11	0.83	0.27	0.76	0.52	0.77	1.00				
Cob dia	0.81	0.09	0.61	0.44	0.74	0.78	0.81	0.50	-0.42	-0.47	0.66	0.78	0.84	0.81	-0.19	0.69	0.26	0.74	0.75	0.77	0.71	1.00			
Cob length	0.57	0.28	0.89	0.06	0.54	0.46	0.55	0.17	-0.62	-0.19	0.80	0.47	0.51	0.38	-0.16	0.37	0.51	0.37	0.17	0.61	0.53	0.53	1.00		
No. of mature grain	0.83	0.35	0.84	0.45	0.46	0.62	0.71	0.25	-0.78	-0.12	0.69	0.51	0.60	0.34	-0.09	0.56	0.29	0.41	0.46	0.54	0.60	0.62	0.73	1.00	
yield	0.88	0.13	0.86	0.53	0.60	0.73	0.68	0.48	-0.60	-0.32	0.75	0.75	0.81	0.44	-0.15	0.60	0.15	0.69	0.51	0.71	0.73	0.79	0.72	0.90	1.00

Remarks: Av. P: available P; Ex. K: exchangeable K; Ex. Ca: exchangeable Ca; Ex. Mg: exchangeable Mg; Ex Al: ex-changeable Al

The results, detailed in Table 8, provide insights into the interaction between these amendments and their collective influence on soil microbial dynamics. When biochar and microbial treatments were applied separately, there was no significant difference in microbial population compared to the unamended soil (control). This suggests that, individually, neither the biochar nor the microbial treatments alone were sufficient to significantly alter the microbial community structure within the time frame or conditions of this study. In contrast, the combination of biochar and microbial treatments showed a significantly different effect on the microbial population compared to the control ($p = 0.0028$). It is important to note that strong acids and bases can affect the functional activity of microorganisms (Teng et al., 2020). The presence of both biochar and microbes creates a more favorable environment for microbial growth and activity than either amendment alone (Palansooriya et al., 2019). N forms and rates influence the composition of P-solubilizing microbes and the abundance of P functional genes. Ammonia-N increased P-solubilizing bacteria, while continuous N deposition lowered soil pH and inhibited microbial activity (S. Wang et al., 2023).

4.2. Amendments of alkaline oxidized RHB and microbes on soil leachate

Biochar and *Pseudomonas aeruginosa* on various soil leachate parameters, revealing significant impacts on phosphorus availability, pH, leachate volume, and electrical conductivity (EC). Biochar application, particularly in combination with *Pseudomonas aeruginosa*, significantly influenced leachate phosphorus availability ($p=<0.001$). Treatments T4 (*Pseudomonas aeruginosa* alone) and T5 (*Pseudomonas aeruginosa* with fresh biochar), exhibited higher phosphorus leaching compared to oxidized biochar treatments (T3 and T6). Yang et al. (2021) demonstrated that biochar influences phosphorus leaching losses both directly and indirectly by adsorbing phosphorus, enhancing soil phosphorus retention, and aiding phosphorus uptake by plants. Incorporating biochar into the soil may have reduced nutrient leaching, thereby positively affecting plant growth (Sohi et al., 2009). Treatment T6, combining *Pseudomonas aeruginosa* with 10% oxidized biochar, exhibited the highest pH (6.64).The increase in pH values indicates that biochar and microbial amendments were effective in neutralizing acidity or alkalizing the medium. Biochar applications increased soil pH and reduced exchangeable acidity significantly (Chen et al., 2023). The control treatment (T1) produced the highest leachate volume (733.33 ml), whereas treatment T6 *(Pseudomonas aeruginosa* with 10% oxidized biochar) had the lowest volume (345 ml).

This reduction in leachate volume with biochar and microbial combination treatments suggests improved water retention and reduced leaching losses. The reduction in leachate production in treatments suggests improved water retention or reduced drainage compared to the control (Coats, 2014). Biochar significantly decreased the leaching volume compared with the unamended soil (Sorrenti & Toselli, 2016). Reduction in leachate production could be attributed to several factors, including enhanced soil structure, increased microbial activity, and improved nutrient retention facilitated by biochar and microbial interactions (Brtnicky et al., 2023).

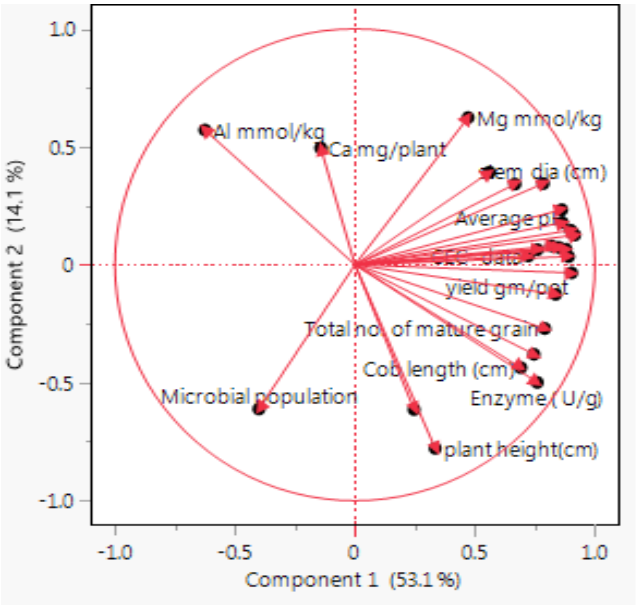

Figure 4. Analysis of the principal component of different variables

4.3. Alkaline RHB and Microbial Interactions on Maize Plant Development

This study investigated the impact of biochar, microbial inoculation, and their combined application on several growth parameters of maize plants, including plant height, stem diameter, root length, root volume, and dry biomass. Plant height was significantly affected by the addition of biochar ($p=0.0044$), microbes ($p=0.0449$), and their interaction ($p=0.0169$). The tallest plants were observed in treatment T6, which combined *Pseudomonas aeruginosa* with 10% oxidized biochar, achieving an average height of 179.33 cm. The largest stem diameter was observed in treatment T3, which involved 10% oxidized biochar without microbes, with a diameter of 1.39 cm. Treatment T4, which applied *Pseudomonas aeruginosa* without biochar, produced the longest roots at an average length of 53 cm. The highest root volume was recorded in treatment T6, with a combination of *Pseudomonas aeruginosa* and 10% oxidized biochar, reaching 20.9 ml plant^{-1}. This was significantly higher than the control treatment T1. The highest dry biomass was recorded in treatment T6 (92.13 g pot^{-1}), significantly surpassing all other treatments. The combination treatment, particularly involving *Pseudomonas aeruginosa* and 10% oxidized biochar (T6), generally produced the best results across most parameters. Biochar alone also showed beneficial effects, especially on root length and volume, while microbial inoculation independently enhanced stem diameter and dry biomass. The porosity and adsorption capacity of biochar makes it an effective carrier for immobilizing plant growth-promoting rhizobacteria (PGPR), thereby enhancing crop growth (Ajeng et al., 2020). Studies have highlighted that *Pseudomonas* isolates, including *P. aeruginosa*, possess plant growth-promoting traits like phosphate solubilization, siderophore production, and

indole-3-acetic acid (IAA) synthesis, contributing to enhanced plant growth (Gupta & Buch, 2019). Recently, numerous studies have combined PGPR and biochar to enhance soil quality and agricultural productivity. Most of these studies have reported a significant increase in agricultural productivity when both are applied together compared to the use of PGPR or biochar alone (Malik et al., 2022). Biochar application has been shown to influence plant hormone levels and biochemical processes. It can modify soil pH and nutrient availability, which in turn can affect hormone signaling pathways in plants, leading to enhanced growth and development. As a soil amendment, biochar improves soil physical and biochemical properties and increases soil fertility and productivity particularly over the long-term increasing soil aggregation, water retention, pH, and microbial activities, thus, improving overall soil quality (Gupta & Buch, 2019). The addition of RHB may have enhanced soil aeration and water infiltration, promoting root growth and nutrient uptake by maize plants (Grover et al., 2021). This improved soil structure could have facilitated better root penetration and exploration, leading to increased root length and volume. Adding spent mushroom substrate (SMS), SMS-derived biochar (SBC), and SBC immobilized PGPR (BCP) significantly enhanced soil nitrogen and potassium content. The 5% BCP treatment yielded the highest fresh weight, leaf number, chlorophyll, anthocyanin content, and the lowest root malondialdehyde content (Guan et al., 2023). Oxidized alkaline RHB may have contributed to the immobilization or detoxification of harmful substances present in the soil, reducing their negative impact on plant growth. This could have resulted in healthier, more vigorous plants with increased biomass accumulation (Rizwan et al., 2016).

4.4. Enhancing maize yield: The impact of alkaline RHB and microbial synergy

The maize cob length, fresh cob diameter, number of grains per cob, and yield showed significant increases (Table 10) with the application of biochar, microbes, and the combined application of oxidized RHB and microbes compared to untreated soil (T1). Among all treatments, the combination of 10% oxidized alkaline RHB and *Pseudomonas aeruginosa* resulted in the highest values, with cob length at 22.30 cm, cob diameter at 5.33 cm, number of grains at 375, and yield at 56.1 g pot^{-1}. The PCA (Table 11, Fig. 4) biplot suggests that certain soil properties and nutrient uptakes (such as soil pH and enzyme activity) have a strong influence on maize yield and growth parameters. Management practices focusing on these key variables can potentially optimize maize growth and yield.

The addition of oxidized alkaline RHB may have promoted root proliferation by providing a favorable environment for root elongation and branching. *Pseudomonas aeruginosa*, as a plant growth-promoting rhizobacterium, can produce phytohormones such as auxins, cytokinins, and gibberellins, which regulate root growth and development (Deng et al., 2023). The synergistic action of biochar and beneficial microbes likely facilitated the development of a well-branched root system in maize plants grown, leading to increased nutrient uptake efficiency and improved nutrient assimilation, thereby contributing to higher grain yield (Aufa Ain & Noraini, 2023; Sun et al., 2022). The number of grains per cob is a key factor influencing maize yield, as it directly correlates with the total grain yield. Treatment T6 exhibited a substantial increase in the number of grains per cob (375) compared to the control, indicating enhanced reproductive success and grain filling. This suggests that the combined application of oxidized alkaline RHB and *Pseudomonas aeruginosa* positively influences pollination, fertilization, and grain development processes, resulting in a higher grain yield. The co-inoculation of *Rhizobium and Pseudomonas* in combination with chemical fertilizers significantly improved panicle length, biological yield, grain yield, and thousand grains weight in rice crops (Imperiali et al., 2017). A significant increase in grain yield (25.77%) was noted in the treatment that combined the application of 0.2% wheat straw biochar with Bacillus sp. (Ahmad et al., 2020). The joint application of biochar and *Paraburkholderia phytofirmans* significantly boosted soybean yield by 14% under drought-stress conditions (Nawaz et al., 2023).

5. CONCLUSION

This study demonstrates that the combined application of oxidized RHB and *Pseudomonas aeruginosa* could bring desirable changes in soil properties and increase crop yield. The application of alkaline oxidized RHB and phosphate-solubilizing bacteria *Pseudomonas aeruginosa* significantly increased the availability of phosphorus 380%, phosphatase enzyme activity 152% and yield of maize 290% in acidic soils. This approach can improve crop yields and soil health, reducing the need for chemical fertilizers and contributing to more sustainable farming systems. Further research should explore the long-term effects of this combined treatment on soil health and crop performance across different soil types and environmental conditions. Additionally, the economic feasibility and practical implementation strategies for large-scale adoption should be investigated..

Funding

This research was funded by Universiti Putra Malaysia, Fundamental Research Grant Scheme (FRGS 1/2020/WAB04/Vote no 5540389).

Acknowledgments

The authors are grateful to the Universiti Putra Malaysia, Selangor Darul Ehsan, Malaysia, for the provision of the research facilities.

Declaration of Competing Interest

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

References

Abdulrahman, D. K., Othman, R., & Saud, H. M. (2016). Effects of empty fruit bunch biochar and nitrogen-fixing bacteria on soil properties and growth of sweet corn. *Malaysian Journal of Soil Science*, *20*, 177-194.

https://msss.com.my/mjss/Full%20Text/vol20/12-abdulrahman.pdf

Agegnehu, G., Bass, A. M., Nelson, P. N., & Bird, M. I. (2016). Benefits of biochar, compost and biochar–compost for soil quality, maize yield and greenhouse gas emissions in a tropical agricultural soil. *Science of The Total Environment*, *543*, 295-306. https://doi.org/10.1016/j.scitotenv.2015.11.054

Ahmad, M., Wang, X., Hilger, T. H., Luqman, M., Nazli, F., Hussain, A., . . . Mustafa, A. (2020). Evaluating Biochar-Microbe Synergies for Improved Growth, Yield of Maize, and Post-Harvest Soil Characteristics in a Semi-Arid Climate. *Agronomy*, *10*(7), 1055. https://doi.org/10.3390/agronomy10071055

Ahmedna, M., Marshall, W. E., & Rao, R. M. (2000). Production of granular activated carbons from select agricultural by-products and evaluation of their physical, chemical and adsorption properties1Louisiana Agricultural Experiment Station manuscript 99-21-0066.1. *Bioresource Technology*, *71*(2), 113-123. https://doi.org/10.1016/S0960-8524(99)00070-X

Ajeng, A. A., Abdullah, R., Ling, T. C., Ismail, S., Lau, B. F., Ong, H. C., . . . Chang, J.-S. (2020). Bioformulation of biochar as a potential inoculant carrier for sustainable agriculture. *Environmental Technology & Innovation*, *20*, 101168. https://doi.org/10.1016/j.eti.2020.101168

Ashraf, M., & Waheed, A. (1990). Screening of local/exotic accessions of lentil (Lens culinaris Medic.) for salt tolerance at two growth stages. *Plant and Soil*, *128*(2), 167-176. https://doi.org/10.1007/BF00011106

Aufa Ain, A. S., & Noraini, M. J. (2023). Effects of rice husk biochar (RHB) with combined inoculation of arbuscular mycorrhizal fungi (AMF) and phosphate solubilizing bacteria (PSB) on growth of maize (Zea mays). *IOP Conference Series: Earth and Environmental Science*, *1131*(1), 012007. https://doi.org/10.1088/1755-1315/1131/1/012007

Barrow, N. J., & Hartemink, A. E. (2023). The effects of pH on nutrient availability depend on both soils and plants. *Plant and Soil*, *487*(1), 21-37. https://doi.org/10.1007/s11104-023-05960-5

Bedassa, M. (2020). Soil acid management using biochar. *International Journal of Agricultural Science and Food Technology*, *6*(2), 211-217. https://doi.org/10.17352/2455-815X.000076

Beqaj, B., Rroço, E., & Doko, A. (2016). Evaluation of nutrient leaching in pots under greenhouse condition. *Albanian Journal of Agricultural Sciences*, *15*(1), 1-7.

Bray, R. H., & Kurtz, L. T. (1945). Determination of total, organic, and available forms of phosphorus in soils. *Soil Science*, *59*(1), 39-46. https://journals.lww.com/soilsci/fulltext/1945/01000/determination_of_total,_organic,_and_available.6.aspx

Brtnicky, M., Mustafa, A., Hammerschmiedt, T., Kintl, A., Trakal, L., Beesley, L., . . . Holatko, J. (2023). Pre-activated biochar by fertilizers mitigates nutrient leaching and stimulates soil microbial activity. *Chemical and Biological Technologies in Agriculture*, *10*(1), 57. https://doi.org/10.1186/s40538-023-00430-7

Ch'ng, H. Y., Haruna, A. O., Majid, N. M. N. A., & Jalloh, M. B. (2019). Improving soil phosphorus availability and yield of Zea mays L. using biochar and compost derived from agro-industrial wastes. *Italian Journal of Agronomy*, *14*(1), 34-42. https://doi.org/10.4081/ija.2019.1107

Chen, J., Yu, J., Li, Z., Zhou, J., & Zhan, L. (2023). Ameliorating Effects of Biochar, Sheep Manure and Chicken Manure on Acidified Purple Soil. *Agronomy*, *13*(4), 1142. https://doi.org/10.3390/agronomy13041142

Coats, W. (2014). UC COOPERATIVE EXTENSION CENTENNIAL: Improved leaching practices save water, reduce drainage problems. *California Agriculture*, *68*(3), 77-77. https://doi.org/10.3733/ca.v068n03p77

Cottenie, A. (1980). Soil and Plant Testing As a Basis of Fertilizer Recommendations.

Deng, X., Long, C., Chen, L., Du, Y., Zhang, Z., Gan, L., & Zeng, Y. (2023). Special Microbial Communities Enhanced the Role of Aged Biochar in Reducing Cd Accumulation in Rice. *Agronomy*, *13*(1), 81. https://doi.org/10.3390/agronomy13010081

Dragomir, V., Ioan Sebastian, B., Alina, B., Victor, P., Tanasă, L., & Horhocea, D. (2022). An overview of global maize market compared to Romanian production. *Romanian Agriculture Research*, *39*, 535-544. https://doi.org/10.59665/rar3951

Eduah, J. O., Nartey, E. K., Abekoe, M. K., Breuning-Madsen, H., & Andersen, M. N. (2019). Phosphorus retention and availability in three contrasting soils amended with rice husk and corn cob biochar at varying pyrolysis temperatures. *Geoderma*, *341*, 10-17. https://doi.org/10.1016/j.geoderma.2019.01.016

Fan, Y., Zhong, X., Lin, F., Liu, C., Yang, L., Wang, M., . . . Yang, Y. (2019). Responses of soil phosphorus fractions after nitrogen addition in a subtropical forest ecosystem: Insights from decreased Fe and Al oxides and increased plant roots. *Geoderma*, *337*, 246-255. https://doi.org/10.1016/j.geoderma.2018.09.028

Gao, X., Yang, J., Liu, W., Li, X., Zhang, W., & Wang, A. (2023). Effects of alkaline biochar on nitrogen transformation with fertilizer in agricultural soil. *Environmental Research*, *233*, 116084. https://doi.org/10.1016/j.envres.2023.116084

Grover, M., Bodhankar, S., Sharma, A., Sharma, P., Singh, J., & Nain, L. (2021). PGPR Mediated Alterations in Root Traits: Way Toward Sustainable Crop Production [Review]. *Frontiers in Sustainable Food Systems*, *4*. https://doi.org/10.3389/fsufs.2020.618230

Guan, T.-K., Wang, Q.-Y., Li, J.-S., Yan, H.-W., Chen, Q.-J., Sun, J., . . . Zhang, G.-Q. (2023). Biochar immobilized plant growth-promoting rhizobacteria enhanced the physicochemical properties, agronomic characters and microbial communities during lettuce seedling [Original Research]. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1218205

Gupta, V., & Buch, A. (2019). *Pseudomonas aeruginosa* predominates as multifaceted rhizospheric bacteria with combined abilities of P-solubilization and biocontrol. *J Pure Appl Microbiol*, *13*(1), 319-328. https://doi.org/10.22207/JPAM.13.1.35

Hasbullah, N. A., Ahmed, O. H., & Ab Majid, N. M. (2020). Effects of Amending Phosphatic Fertilizers with Clinoptilolite Zeolite on Phosphorus Availability and Its Fractionation in an Acid Soil. *Applied Sciences*, *10*(9), 3162. https://doi.org/10.3390/app10093162

Heidari, E., Mohammadi, K., Pasari, B., Rokhzadi, A., & Sohrabi, Y. (2020). Combining the phosphate solubilizing microorganisms with biochar types in order to improve safflower yield and soil enzyme activity. *Soil Science and Plant Nutrition*, *66*(2), 255-267. https://doi.org/10.1080/00380768.2019.1704180

Huang, K., Li, M., Li, R., Rasul, F., Shahzad, S., Wu, C., . . . Aamer, M. (2023). Soil acidification and salinity: the importance of biochar application to agricultural soils [Review]. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1206820

Imperiali, N., Chiriboga, X., Schlaeppi, K., Fesselet, M., Villacrés, D., Jaffuel, G., . . . Campos-Herrera, R. (2017). Combined Field Inoculations of Pseudomonas Bacteria, Arbuscular Mycorrhizal Fungi, and Entomopathogenic Nematodes and their Effects on Wheat Performance [Original Research]. *Frontiers in Plant Science*, *8*. https://doi.org/10.3389/fpls.2017.01809

Jensen, H. L. (1951). Notes on the biology of Azotobacter. *Proceedings of the Society for Applied Bacteriology*, *14*(1), 89-94. https://doi.org/10.1111/j.1365-2672.1951.tb01997.x

Kashiani, P. (2012). *Genetic potential of selected sweet corn inbred lines and analysis of their combining ability assisted by microsatellite DNA markers* [PhD Thesis, Universiti Putra Malaysia].

Keeney, D. R., & Nelson, D. W. (1982). Nitrogen—Inorganic Forms. In *Methods of Soil Analysis* (pp. 643-698). https://doi.org/10.2134/agronmonogr9.2.2ed.c33

Khan, F., Siddique, A. B., Shabala, S., Zhou, M., & Zhao, C. (2023). Phosphorus Plays Key Roles in Regulating Plants' Physiological Responses to Abiotic Stresses. *Plants*, *12*(15), 2861. https://doi.org/10.3390/plants12152861

Li, Y., Wang, C., Gao, S., Wang, P., Qiu, J., & Shang, S. (2021). Impacts of simulated nitrogen deposition on soil enzyme activity in a northern temperate forest ecosystem depend on the form and level of added nitrogen. *European Journal of Soil Biology*, *103*, 103287. https://doi.org/10.1016/j.ejsobi.2021.103287

Lija, M., Haruna, A. O., & Kasim, S. (2017). Maize (Zea mays L.) nutrient use efficiency as affected by formulated fertilizer with Clinoptilolite Zeolite. *Emirates Journal of Food and Agriculture*, *26*(3), 284-292. https://doi.org/10.9755/ejfa.v26i3.16183

Malboobi, M. A., Sabet, M. S., Zamani, K., Lohrasebi, T., Fathi, Z., & Zamani, J. (2022). Acid Phosphatases Roles in Plant Performance. In P. Jeschke & E. B. Starikov (Eds.), *Agricultural Biocatalysis* (pp. 117-157). Jenny Stanford Publishing. https://doi.org/10.1201/9781003313106

Malhotra, H., Vandana, Sharma, S., & Pandey, R. (2018). Phosphorus Nutrition: Plant Growth in Response to Deficiency and Excess. In M. Hasanuzzaman, M. Fujita, H. Oku, K. Nahar, & B. Hawrylak-Nowak (Eds.), *Plant Nutrients and Abiotic Stress Tolerance* (pp. 171-190). Springer Singapore. https://doi.org/10.1007/978-981-10-9044-8_7

Malik, L., Sanaullah, M., Mahmood, F., Hussain, S., Siddique, M. H., Anwar, F., & Shahzad, T. (2022). Unlocking the potential of co-applied biochar and plant growth-promoting rhizobacteria (PGPR) for sustainable agriculture under stress conditions. *Chemical and Biological Technologies in Agriculture*, *9*(1), 58. https://doi.org/10.1186/s40538-022-00327-x

Manpoong, C., De Mandal, S., Bangaruswamy, D. K., Perumal, R. C., Benny, J., Beena, P. S., . . . Tripathi, S. K. (2020). Linking rhizosphere soil biochemical and microbial community characteristics across different land use systems in mountainous region in Northeast India. *Meta Gene*, *23*, 100625. https://doi.org/10.1016/j.mgene.2019.100625

Mosharrof, M., Uddin, M. K., Mia, S., Sulaiman, M. F., Shamsuzzaman, S. M., & Haque, A. N. A. (2022). Influence of Rice Husk Biochar and Lime in Reducing Phosphorus Application Rate in Acid Soil: A Field Trial with Maize. *Sustainability*, *14*(12), 7418. https://doi.org/10.3390/su14127418

Muchoka, J. P. (2021). *Mycorrhizal Fungi Associated with Aspilia pluriseta And Phosphorus Availability on Sorghum Growth* University of Embu].

Nabi, M. (2023). Chapter eleven - Role of microorganisms in plant nutrition and soil health. In T. Aftab & K. R. Hakeem (Eds.), *Sustainable Plant Nutrition* (pp. 263-282). Academic Press. https://doi.org/10.1016/B978-0-443-18675-2.00016-X

Nawaz, F., Rafeeq, R., Majeed, S., Ismail, M. S., Ahsan, M., Ahmad, K. S., . . . Haider, G. (2023). Biochar Amendment in Combination with Endophytic Bacteria Stimulates Photosynthetic Activity and Antioxidant Enzymes to Improve Soybean Yield Under Drought Stress. *Journal of Soil Science and Plant Nutrition*, *23*(1), 746-760. https://doi.org/10.1007/s42729-022-01079-1

Ofoe, R., Thomas, R. H., Asiedu, S. K., Wang-Pruski, G., Fofana, B., & Abbey, L. (2023). Aluminum in plant: Benefits, toxicity and tolerance mechanisms [Review]. *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/fpls.2022.1085998

Ontman, R., Groffman, P. M., Driscoll, C. T., & Cheng, Z. (2023). Surprising relationships between soil pH and microbial biomass and activity in a northern hardwood forest. *Biogeochemistry*, *163*(3), 265-277. https://doi.org/10.1007/s10533-023-01031-0

Ouyang, P., Narayanan, M., Shi, X., Chen, X., Li, Z., Luo, Y., & Ma, Y. (2023). Integrating biochar and bacteria for sustainable remediation of metal-contaminated soils. *Biochar*, *5*(1), 63. https://doi.org/10.1007/s42773-023-00265-3

Palansooriya, K. N., Wong, J. T. F., Hashimoto, Y., Huang, L., Rinklebe, J., Chang, S. X., . . . Ok, Y. S. (2019). Response of microbial communities to biochar-amended soils: a critical review. *Biochar*, *1*(1), 3-22. https://doi.org/10.1007/s42773-019-00009-2

Parkinson, D., Gray, T. R. G., & Williams, S. T. (1971). *Methods for studying the ecology of soil micro-organisms* Oxford, Blackwell Scientific Publications for the International Biological Programme.

Pastore, G., Kernchen, S., & Spohn, M. (2020). Microbial solubilization of silicon and phosphorus from bedrock in relation to abundance of phosphorus-solubilizing bacteria in temperate forest soils. *Soil Biology and Biochemistry*, *151*, 108050. https://doi.org/10.1016/j.soilbio.2020.108050

Rabileh, M. A., Shamshuddin, J., Panhwar, Q. A., Rosenani, A. B., & Anuar, A. R. (2015). Effects of biochar and/or dolomitic limestone application on the properties of Ultisol cropped to maize under glasshouse conditions. *Canadian Journal of Soil Science*, *95*(1), 37-47. https://doi.org/10.4141/cjss-2014-067

Rawat, P., Das, S., Shankhdhar, D., & Shankhdhar, S. C. (2021). Phosphate-Solubilizing Microorganisms: Mechanism and Their Role in Phosphate Solubilization and Uptake. *Journal of Soil Science and Plant Nutrition*, *21*(1), 49-68. https://doi.org/10.1007/s42729-020-00342-7

Rizwan, M., Ali, S., Qayyum, M. F., Ibrahim, M., Zia-ur-Rehman, M., Abbas, T., & Ok, Y. S. (2016). Mechanisms of biochar-mediated alleviation of toxicity of trace elements in plants: a critical review. *Environmental Science and Pollution Research*, *23*(3), 2230-2248. https://doi.org/10.1007/s11356-015-5697-7

Rossati, K. F., Figueiredo, C. C. d., & Mendes, G. d. O. (2023). Aspergillus niger Enhances the Efficiency of Sewage Sludge Biochar as a Sustainable Phosphorus Source. *Sustainability*, *15*(8), 6940. https://doi.org/10.3390/su15086940

Rowell, D. L. (2014). *Soil science: Methods & applications*. Routledge. https://doi.org/10.4324/9781315844855

Schalk, I. J., & Perraud, Q. (2023). and its multiple strategies to access iron. *Environmental Microbiology*, *25*(4), 811-831. https://doi.org/10.1111/1462-2920.16328

Schmalenberger, A., & Fox, A. (2016). Chapter Three - Bacterial Mobilization of Nutrients From Biochar-Amended Soils. In S. Sariaslani & G. M. Gadd (Eds.), *Advances in Applied Microbiology* (Vol. 94, pp. 109-159). Academic Press. https://doi.org/10.1016/bs.aambs.2015.10.001

Soares, A. S., Nascimento, V. L., de Oliveira, E. E., Jumbo, L. V., dos Santos, G. R., Queiroz, L. L., . . . de Souza Aguiar, R. W. (2023). *Pseudomonas aeruginosa* and Bacillus cereus Isolated from Brazilian Cerrado Soil Act as Phosphate-Solubilizing Bacteria. *Current Microbiology*, *80*(5), 146. https://doi.org/10.1007/s00284-023-03260-w

Sohi, S., Lopez-Capel, E., Krull, E., & Bol, R. (2009). Biochar, climate change and soil: A review to guide future research. *CSIRO land and water science report 05/09*, 64 pp. https://www.build-a-gasifier.com/PDF/BiocharClimateChangeSoil_WfHC_pdf%20Standard.pdf

Sorrenti, G., & Toselli, M. (2016). Soil leaching as affected by the amendment with biochar and compost. *Agriculture, Ecosystems & Environment*, *226*, 56-64. https://doi.org/10.1016/j.agee.2016.04.024

Sun, H., Wang, Y., & Wu, Q. (2022). Synergistic Effects of Biochar and Microbes on Soil Remediation. *Highlights in Science, Engineering and Technology*, *26*, 303-311. https://doi.org/10.54097/hset.v26i.3990

Teng, Z., Shao, W., Zhang, K., Yu, F., Huo, Y., & Li, M. (2020). Enhanced passivation of lead with immobilized phosphate solubilizing bacteria beads loaded with biochar/ nanoscale zero valent iron composite. *Journal of Hazardous Materials*, *384*, 121505. https://doi.org/10.1016/j.jhazmat.2019.121505

Tiessen, H. (2008). Phosphorus in the global environment. In P. J. White & J. P. Hammond (Eds.), *The Ecophysiology of Plant-Phosphorus Interactions* (pp. 1-7). Springer Netherlands. https://doi.org/10.1007/978-1-4020-8435-5_1

Timofeeva, A. M., Galyamova, M. R., & Sedykh, S. E. (2023). Plant Growth-Promoting Soil Bacteria: Nitrogen Fixation, Phosphate Solubilization, Siderophore Production, and Other Biological Activities. *Plants*, *12*(24), 4074. https://doi.org/10.3390/plants12244074

Tusar, H. M., Uddin, M. K., Mia, S., Suhi, A. A., Wahid, S. B. A., Kasim, S., . . . Anwar, F. (2023). Biochar-Acid Soil Interactions—A Review. *Sustainability*, *15*(18), 13366. https://doi.org/10.3390/su151813366

Wang, C., Pan, G., Lu, X., & Qi, W. (2023). Phosphorus solubilizing microorganisms: potential promoters of agricultural and environmental engineering [Perspective]. *Frontiers in Bioengineering and Biotechnology*, *11*. https://doi.org/10.3389/fbioe.2023.1181078

Wang, S., Song, M., Wang, C., Dou, X., Wang, X., & Li, X. (2023). Mechanisms underlying soil microbial regulation of available phosphorus in a temperate forest exposed to long-term nitrogen addition. *Science of The Total Environment*, *904*, 166403. https://doi.org/10.1016/j.scitotenv.2023.166403

Wu, X., Peng, J., Liu, P., Bei, Q., Rensing, C., Li, Y., . . . Cui, Z. (2021). Metagenomic insights into nitrogen and phosphorus cycling at the soil aggregate scale driven by organic material amendments. *Science of The Total Environment*, *785*, 147329. https://doi.org/10.1016/j.scitotenv.2021.147329

Yang, L., Wu, Y., Wang, Y., An, W., Jin, J., Sun, K., & Wang, X. (2021). Effects of biochar addition on the abundance, speciation, availability, and leaching loss of soil phosphorus. *Science of The Total Environment*, *758*, 143657. https://doi.org/10.1016/j.scitotenv.2020.143657

Yuan, Z., Cao, Q., Zhang, K., Ata-Ul-Karim, S. T., Tian, Y., Zhu, Y., . . . Liu, X. (2016). Optimal Leaf Positions for SPAD Meter Measurement in Rice [Methods]. *Frontiers in Plant Science*, *7*. https://doi.org/10.3389/fpls.2016.00719

Zaidun, S. W., Jalloh, M. B., Awang, A., Sam, L. M., Besar, N. A., Musta, B., . . . Omar, L. (2019). Biochar and clinoptilolite zeolite on selected chemical properties of soil cultivated with maize (Zea mays L.). *EURASIAN JOURNAL OF SOIL SCIENCE (EJSS)*, *8*(1), 1-10. https://doi.org/10.18393/ejss.468100