Archives of Agriculture and Environmental Science 10(1): 59-66 (2025)
https://doi.org/10.26832/24566632.2025.100109

e-ISSN: 2456-6632

This content is available online at AESA

Archives of Agriculture and Environmental Science

Journal homepage: journals.aesacademy.org/index.php/aaes

ORIGINAL RESEARCH ARTICLE

Synergy of biochar and organic fertilizer improves soybean (*Glycine max* L.) growth by alleviation nutrient stress in strongly acidic Taiwanese soil

Marianus Evarist Ngui[1,2*], Yong-Hong Lin[2,*] and Sabrina Diana Blanco[1]

[1]Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung County, 912, Taiwan
[2]Soil and Fertilizer Laboratory, Department of Plant Industry, National Pingtung University of Science and Technology, Pingtung County, 912, Taiwan
[*]Corresponding author's E-mails: yonghong@mail.npust.edu.tw & nguimarianus@gmail.com

ARTICLE HISTORY

Received: 02 December 2024
Revised received: 11 February 2025
Accepted: 19 February 2025

Keywords

Rice husk biochar
Soil acidity
Soil degradation
Sustainable agriculture
Soybean

ABSTRACT

The potential of biochar and organic fertilizer to enhance soil fertility, promote crop growth, and increase yield is driving their growing popularity in modern agricultural practices. This research investigated the effects of applying a combination of biochar and organic fertilizer on soil physico-chemical properties and the growth attributes of soybean (*Glycine max* L.) in the strong acidic soil of Taiwan. The study was conducted in a greenhouse using a pot experiment arranged in a randomized complete block design with two factorial treatments, replicated three times. Fifteen days after soil amendment, treatment $B_{35}F_{140}$ had a significant increase in soil pH of 5.54 compared to the control group. Similar treatment resulted in higher P available in soil of 19.11 mg kg^{-1}. In addition, 45 days after soil amendment, organic matter, available potassium, calcium, magnesium, copper, and zinc increased by 19.59%, 236.36%, 38.39%, 112.76%, 7.01%, and 44.59% in the application of treatments $B_{70}F_{140}$, $B_{35}F_{140}$, $B_{35}F_{105}$, $B_{70}F_{140}$, and $B_{35}F_{70}$, respectively, compared to control. The tallest soybean plants were seen on the application of treatment $B_{70}F_{140}$ with a maximum height of 112.75 cm, while treatment $B_{35}F_{140}$ produced many soybean plant leaves — 51 leaves per plant. Moreover, root hairs extensively grew on soybean plants grown on the amended soils than those of the control treatment. Conclusively, our findings have statistically demonstrated that the combination of biochar and organic fertilizer can improve soil quality and soybean growth characteristics under conditions of strongly acidic soil, as evidenced by a short-term pot and greenhouse study.

Citation of this article: Ngui, M.E., Lin, Y. H., & Blanco, S. D. (2025). Synergy of biochar and organic fertilizer improves soybean (*Glycine max* L.) growth by alleviation nutrient stress in strongly acidic Taiwanese soil. *Archives of Agriculture and Environmental Science*, *10*(1), 59-66, https://dx.doi.org/10.26832/24566632.2025.100109

INTRODUCTION

Soybean (*Glycine max* L.) is a legume crop that is extensively cultivated for its edible beans, which are renowned for their protein and oil content. They contain approximately 40% of protein and 20% of non-cholesterol oil (Banaszkiewicz, 2011). Additionally, soybeans serve as a rich source of vitamins B1, B9, and K1 (Agyenim-Boateng *et al*., 2023). The consumption of soybean protein has been linked to various health benefits, such as renal support, blood pressure regulation, diabetes management, and reduced risk of prostate and breast cancer (Jayachandran & Xu, 2019). Moreover, soybean products can be utilized in animal feed (Janocha *et al*., 2022). Soil erosion, organic matter loss, acidification, and unbalanced nutrient composition have caused moderate to severe soil degradation in 33% of all soils around the world (Smith *et al*., 2024). The primary cause of this degradation is due to unsustainable management practices (Ferreira *et al*., 2022). Furthermore, the evident degradation of soil fertility is related to the excessive use of inorganic fertilizers (Guo & Wang, 2021; Sandrakirana & Arifin, 2021). Excessive use of chemical fertilizers over time on the same soil can lead to soil degradation, loss of beneficial soil microorganisms and organic matter, pollution of underground water, increased greenhouse gas emissions, and reduced food quality (Jote, 2023). Moreover, continuous application of inorganic fertilizers, like ammonia-based fertilizers, drastically accelerates soil acidification in agricultural soils (Singh *et al*., 2017). Strongly acid soils are characterized by the deficiency of essential plant nutrients and toxicity of metals such as aluminium (Al) and manganese (Mn) (Cárcamo *et al*., 2019).

Although biochar technology is still underutilized as a soil amendment method in agriculture, it is still an inspiring technique in the field of agriculture (Gairhe *et al*., 2024; Ibrahim, 2022; & Wu *et al*., 2022). Biochar, a stable, carbon-rich material produced by the thermochemical decomposition of biomass, is currently considered a promising method for improving soil quality—a critical factor for enhancing crop growth and yields (Torabian *et al*., 2021). Biochar exhibits several properties that make it desirable to use on agricultural lands. These include ameliorating soil acidity, retaining a high capacity of soil nutrients, promoting soil microbial activity, and enhancing soil water retention capacity (Dejene & Tilahun, 2019). We conducted this study due to the naturally strong acidic soil in our experimental area that is known to limit soybean development. Plant nutrient availability is strongly correlated with soil pH. The availability of major plant nutrients, such as N, P, K, Mg, and Ca, is highly reduced in acidic soils (Jing *et al*., 2024). Additionally, this study investigated the effect of soybean growth (*Glycine max* L.), as the crop tends to take up more cations from the soil, especially when its nitrogen source relies solely on atmospheric N_2 fixation, which further increases soil acidity. Therefore, the main objective of this study was to evaluate the effectiveness of combining biochar and organic fertilizer in alleviating soil acidity, enhancing nutrient availability, and improving soybean growth in strongly acidic soil. Several studies have recommended applying biochar in combination with inorganic fertilizers to enhance its efficiency during plant development (Ibrahim, 2022; Parker *et al*., 2021; Sikdar *et al*., 2023). However, there is limited scientific information available regarding the synergistic effects of biochar and organic fertilizer on soybean development. Therefore, our objective was to provide practical advice on the suitability of using biochar and organic fertilizer to soybean producers, particularly in Taiwan, where the negative impacts of strongly acidic soils have limited soybean growth and prevented harvests from reaching their full potential.

MATERIALS AND METHODS

Experimental area and materials

This investigation was conducted in a greenhouse belonging to the Department of Plant Industry at the National Pingtung University of Science and Technology (NPUST) located at 22.6433° N, 120.6098° E. The study was carried out from September 2023 to February 2024, with a tropical monsoon climate characterized by a temperature average of 26-32°C in the summer. The soybean seed variety (Tainan 10) was obtained from the Tainan District of Agricultural Research and Extension Station in Taiwan. This particular soybean variety was selected because of its favorable grain characteristics, widespread consumer preference, and consequent high demand in Taiwan. Rice husk biochar was prepared at Sin-Fong Rice Milling Factory under a pyrolysis temperature of 600 °C for 24 hours. The organic fertilizer was supplied by the Nancho Farmer's Association, Pingtung, Taiwan.

Soil sampling and biochar analysis

Soil samples were randomly collected on the entire soil collection area by using soil auger and spade. The depth of soil collected ranged from 0-20 cm and included both top and subsoils. Each soil portion from each site was mixed to make a composite soil sample. Thereafter, 1 kg of the collected composite soil sample was air-dried for seven days inside the laboratory. The soil sample was ground and sieved through a 2 mm sieve. According to laboratory soil analysis procedures, initial soil properties were conducted before the amending of the soil using biochar and organic fertilizer. On the 15 and 45 days after amendment (DAA) of the strong acidic soil, soil samples were sampled for analysis from each pot. The analysis was performed at the Soil and Fertilizer Laboratory of NPUST. The soil pH at 1:5 (soil: water) was measured using a glass electrode pH meter (McLean, 1982). In the EC determination, soil solution was filtered through Whatman filter paper No. 5, and then using an EC meter, the values of EC were recorded. A mixture of hydrochloric acid (HCl) and ammonium fluoride (NH_4F) was used to extract phosphorus and calcium-bound phosphorus (Bray & Kurtz, 1945), then a spectrophotometer was utilized to determine its amount in soil solution. The modified Walkley-Black method, described by (Nelson & Sommers, 1982), was applied to determine soil organic matter. This method involved oxidizing soil samples using dichromate and tetraoxosulphate (VI) acid. Using 50 ml of Mehlich 3 Extractant, soil K, Ca, Mg, Cu, and Zn were extracted from soil samples (Mehlich, 1978; Mehlich, 1984). Then, by using inductively coupled plasma atomic mass spectrometry (ICP-MS), concentrations of available K, Ca, Mg, Cu, and Zn in soil solution at wavelengths of 766.49 nm, 317.93 nm, 279.81 nm, 324.75 nm, and 213.86 nm, respectively, were determined. Available P, K, Ca, and Mg in biochar were determined by ICP-MS after digestion with HNO_3-H_2O_2 system (Piash *et al*., 2021). Table 1 and 2 show the pre-amendment physicochemical properties of experimental soil and the treatments, respectively.

Experimental setup and preparation of experimental soil

A pot experiment was designed under greenhouse conditions using a randomized complete block design (RCBD) with factorial 2 × 3 treatments (n=3). The treatment combination comprised two rates of biochar (35 and 70 g/pot) and three rates of organic fertilizer (35, 105, and 140 g/pot) (Table 3), resulting in a total of 21 experimental pots. The dimensions of each pot were composed of a top diameter of 30 cm, a bottom diameter of 20 cm, and a height of 30 cm. Conditions in the greenhouse were

Table 1. Pre-amendment soil properties.

Attributes	pH (H_2O)	EC (mS/cm)	OM (%)	Texture	Available (mg/kg)							
					P	K	Ca	Mg	Fe	Mn	Cu	Zn
Value	5.11	0.08	4.04	CL S- 30% C- 32% L- 38%	15.03	64.44	1,198.24	24.29	169.94	8.21	0.51	2.58

EC-electrical conductivity, OM-organic matter, CL-clay loam, S- sand, C- clay, L-loam.

Table 2. Physico-chemical properties of rice husk biochar and organic fertilizer

Property	Rice Husk Biochar	Property	Organic Fertilizer
pH (biochar: H_2O) 1:5	9.89	pH (OF: H_2O) 1:5	8.22
Available P (mg/kg)	4,455.47	OM (%)	61
Available K (mg/kg)	10,605.53	Total N (%)	2.5
Available Ca (mg/kg)	2,016.42	Total P (%)	2.3
Available Mg (mg/kg)	1,112.65	Total K (%)	2
		C: N	20

OF-organic fertilizer.

Table 3. Treatments design details.

Treatment code		Application rates (g/10.5 kg of soil in each pot)		
CK	=	0 g/pot Biochar	+	0 g/pot Organic fertilizer
$B_{35}F_{70}$	=	35 g/pot Biochar	+	70 g/pot Organic fertilizer
$B_{35}F_{105}$	=	35 g/pot Biochar	+	105 g/pot Organic fertilizer
$B_{35}F_{140}$	=	35 g/pot Biochar	+	140 g/pot Organic fertilizer
$B_{70}F_{70}$	=	70 g/pot Biochar	+	70 g/pot Organic fertilizer
$B_{70}F_{105}$	=	70 g/pot Biochar	+	105 g/pot Organic fertilizer
$B_{70}F_{140}$	=	70 g/pot Biochar	+	140 g/pot Organic fertilizer

CK-denotes unamended soil. The subscript numbers on B and F letters represent grams of applied biochar and organic fertilizer, respectively.

maintained using an automated rotating fan system to maintain a temperature of 25°C at night and 30°C during the day. The choice for a pot experiment under greenhouse conditions was made to avert potential open field disruptions from unstable weather conditions, such as typhoons, which frequently occur annually between September and November, coinciding with the soybean growing season in Taiwan. A total of 10.5 kg of experimental soil sample was thoroughly mixed with rice husk biochar (RHB) and organic fertilizer (OF) and deposited into each pot. The amalgamated soil was allowed to settle for 14 days before sowing soybean seeds. This method facilitated the further integration between the biochar and organic fertilizer into the experimental soil medium. Due to the dampness of the soil, no water was supplied to the amalgamated soil throughout a 14-day period. On 15 DAA, seven soybean seeds were sown in each pot, spaced 5 cm apart, and planted to a depth of 2.5 cm. Following the seeds germination and emergence of seedlings (7 –10 days later), the plants were thinned to two per pot. As a result, a total of 42 soybean plants were retained. After seed germination, soybean plants in each pot were regularly watered throughout the growing period. Weed and pest management were implemented during the growing season. By using tensiometer, the soil moisture level was monitored. The plants in each pot were irrigated with 400 mL of tap water during the whole vegetative growth phase. The frequency of irrigation was determined using the readings obtained from the tensiometer.

Observations

On the 15 and 45 DAA of strong acidic soils, soil samples (0-20 cm depth) were collected from each pot for analysis. Using a tape measure, the heights of soybean plants were measured from the soil's surface to the soybean plant's highest point; measurements were done during the vegetative growth stage. A total number of leaves on both soybean plants per pot was counted and then averaged. This was done after the 2nd, 4th, and 6th weeks. After harvest, soybean plants were uprooted from the soil. The root and stem were separated, and then, using tap water, the root part was carefully washed to detach any soil material. Thereafter, observation of root hair growth was done. As previously mentioned, two distinct time intervals were considered for soil sampling and analysis. This was due to the amalgamated soil being left to settle for 14 days following the amendment and prior to planting the soybean seeds. Accordingly, we investigated some of essential soil properties that are significant in influencing early plant vegetative growth. As a result, we analyzed soil pH, electrical conductivity (EC), and available P on the 15 DAA. In addition, when the soybean plants had reached 6 weeks since sown and were in full vegetative growth, the second soil sampling and analysis were carried out. It corresponded to 45 DAA. Analysis of soil properties on 45 DAA aimed to understand the extent to which the soil properties were changed when soybean plants completed their vegetative growth stage on 45 DAA.

Statistical analysis

The collected data were subjected to statistical analysis using SAS version 9.4 (TS1M6, Cary, NC, USA). Analysis of variance (ANOVA) was performed to test the significance of the differences among the treatments. The means were compared using the Least Significant Difference (LSD) test at a $p< 0.05$ level of significance. Microsoft Excel was used for entering, organizing data, and constructing graphs.

RESULTS AND DISCUSSION

Effect of treatments on soil properties

The synergistic effects of biochar and organic fertilizer on soil properties are shown in Tables 4 and 5. A significant change in soil pH, observed at 15 DAA, demonstrates the efficacy of combining biochar and organic fertilizer to alleviate soil acidity. Our findings, which show an increase in soil pH due to biochar addition, align with those of Acharya *et al.* (2022) & Singh *et al.* (2022). Biochar contains various functional groups, including carboxylic and hydroxyl groups, formed during the pyrolysis process. These functional groups play a significant role in metal sorption (Li *et al.*, 2017). Similarly, the organic fertilizer, with a pH of 8.22, indicated that it was alkaline. Thus, the combination of organic fertilizer and biochar (pH 9.89) results in a multitude of acid-alkali reactions, neutralizing the strong acidity of the soil. In contrast, the soil pH in the control group decreased by 0.39% compared to the pre-planting pH. This decline in soil pH can be attributed to the net efflux of H_3O^+ ions from soybean roots into the rhizosphere, which occurs when the plant relies solely on nitrogen fixation from atmospheric N_2. Additionally, soil electrical conductivity (EC) in all amended soil significantly increased compared to the control (CK) group (Table 4). Treatments $B_{35}F_{140}$ and $B_{70}F_{140}$ resulted in a significant 158.33% increase in EC compared to the CK treatment. EC is a key indicator of soil salt content and an important measure of soil health, influencing soil microbial activity, crop growth, quality, and nutrient availability. High soluble concentrations (>2.5) can create reverse osmosis pressure, leading to root desiccation. However, since the EC values in all treatments were below 2.5, this indicates a normal salt concentration, promoting a healthy root system. Organic matter content in the soil enhances water retention capacity, nutrient retention, and availability while promoting soil aggregation. At 45 DAA, treatments $B_{35}F_{105}$ and $B_{70}F_{140}$ had significantly higher soil organic matter compared to other treatments. The increase in organic matter content was definitely attributed to the addition of biochar and organic fertilizer in the strong acidic soil because the organic fertilizer used in our study was a compost mixture of cattle, chicken, pig manure, sawdust, and soybean crop residues.

The combination of biochar and organic fertilizer in our study significantly increased soil available phosphorous (Table 4), aligning with findings by Liu *et al.* (2022). Plants predominantly require phosphorous for photosynthesis, as it is a key component of the energy carrier molecule adenosine triphosphate (ATP). In addition, the growth and development of plant roots are strongly fortified by the availability of phosphorous in the soil. In contrast, the lowest phosphorus levels were found in the CK soil, likely due to phosphorus fixation caused by increased solubility of aluminium (Al) and manganese (Mn) in acidic soils.

Table 4. Soil pH, EC, available phosphorous at 15 DAA, and organic matter content at 45 DAA by using a combination of biochar and organic fertilizer.

Treatments	15 DAA			OM-45 DAA
	Soil pH (1:5 soil: water)	EC (mS/cm)	P (mg/kg)	(%)
CK	5.11^{a}	0.12^{a}	15.55^{ab}	4.39^{a}
$B_{35}F_{70}$	5.40^{bc}	0.26^{bc}	16.14^{ab}	4.79^{a}
$B_{35}F_{105}$	5.48^{bc}	0.23^{bc}	17.00^{ab}	5.19^{b}
$B_{35}F_{140}$	5.54^{c}	0.31^{c}	19.11^{b}	4.68^{a}
$B_{70}F_{70}$	5.44^{bc}	0.22^{b}	16.63^{ab}	4.64^{a}
$B_{70}F_{105}$	5.29^{ab}	0.30^{b}	15.10^{a}	4.47^{a}
$B_{70}F_{140}$	5.52^{c}	0.31^{c}	18.83^{b}	5.25^{b}

Means with the same superscript lower-case letter within the column do not significantly differ ($p < 0.05$) by the least significant difference (LSD). CK denotes unamended soils. DAA-days after amendment, EC-electrical conductivity, OM-organic matter. The subscript numbers on B and F represent grams of applied biochar and organic fertilizer, respectively.

Table 5. Soil-available potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), and zinc (Zn) on 45 DAA by using a combination of biochar and organic fertilizer.

Treatments	45 DAA				
	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
CK	144.92^{a}	$1,152.95^{b}$	76.91^{a}	1.57^{cd}	7.58^{a}
$B_{35}F_{70}$	343.77^{b}	$1,575.91^{c}$	135.02^{b}	1.68^{d}	10.96^{b}
$B_{35}F_{105}$	467.30^{de}	$1,595.51^{c}$	149.12^{bc}	1.09^{bcd}	10.03^{b}
$B_{35}F_{140}$	487.46^{e}	$1,536.84^{c}$	149.50^{bc}	0.52^{ab}	9.20^{ab}
$B_{70}F_{70}$	450.99^{c}	820.59^{a}	131.57^{b}	0.88^{abc}	9.95^{b}
$B_{70}F_{105}$	453.34^{cd}	902.52^{a}	148.91^{bc}	0.46^{ab}	9.94^{b}
$B_{70}F_{140}$	449.92^{cde}	$1,440.76^{c}$	163.63^{c}	0.32^{a}	9.26^{ab}

Means with the same superscript lower-case letter within the column do not significantly differ ($p < 0.05$) by the least significant difference (LSD). CK-denotes unamended soils. DAA- days after amendment. The subscript numbers on B and F represent grams of applied biochar and organic fertilizer, respectively.

According to Bader *et al.* (2021), the decomposition of organic matter by soil bacteria releases organic acids such as humic and fluvic acids. These organic acids aid in dissolving soil minerals, especially those of potassium (K) content (Al-Jabori *et al.*, 2011). The biochar used in the study was rich in available potassium (K), which it released directly into the soils. As a result of this release and the additional K resulted from the decomposition of organic matter due to added organic fertilizer, the amount of available K in the amended soils significantly increased. This increase aligns with findings from Du *et al.* (2024) and Gairhe *et al.* (2024). This is highly important as potassium plays a fundamental role in stomata-opening control, allowing gas exchange and water fluxes in plants (Andrés *et al.*, 2014; Tränkner *et al.*, 2018). Our findings show that the lowest soil calcium levels were in treatments CK, $B_{70}F_{70}$, and $B_{70}F_{105}$, likely due to their related soil pH of less than 5.5, which increased the solubility of aluminium and iron, competing with calcium for binding sites. This significantly reduced calcium availability by displacing calcium ions from soil particles and forming insoluble compounds. Calcium is essential for plants as it plays a major role in cell growth and development (Jing *et al.*, 2024).

The combination of biochar and organic fertilizer significantly increased soil magnesium levels. Magnesium is essential for plants as it contributes to chlorophyll synthesis (Ishfaq *et al.*, 2022), enzyme activation (e.g., Rubisco) (Shao *et al.*, 2021), and electrostatic activation of substrates, such as ATP (Nam *et al.*, 2024). Furthermore, it has numerous essential functions in plant physiology, growth, development, production, and stress tolerance (Bin *et al.*, 2023 & Hamedeh *et al.*, 2022). On 45 DAA, treatment $B_{35}F_{70}$ showed a 425% increase in available copper (Cu) compared to treatment $B_{70}F_{140}$. Additionally, the available Cu in the former treatment was significantly higher than those in treatments B35F140, B70F70, and B70F105. In the CK soil group, the concentration of available copper (Cu) was greater than that in the B70F140, which can be attributed to its lower pH level. While acidic soils increase the solubility of Cu and zinc (Zn), they also make these elements less available as soil pH rises due to the binding of soil particles. Surprisingly, treatment $B_{35}F_{70}$ resulted in a significant 44.59% increase in available Zn compared to the CK soil group. Both copper and zinc are crucial for plant growth, influencing processes like chlorophyll biosynthesis and hormone regulation (Chen *et al.*, 2022). Zn is renowned for increasing the production of cytochrome, regulating auxin hormone, stabilizing CO_2 in mesophyll, and restoring PS-II (Gupta *et al.*, 2016). Lastly, the biomass of organic residues contains all essential plant nutrients (N, P, K, Ca, Mg, Cu, and Zn) that are released into the soil during mineralization, supporting plant growth (Brempong & Addo-Danso, 2022).

Effect on plant height

The impact of treatments on plant height is highlighted in Figure 1. Our findings have illustrated poor soybean plant growth under the control soil group (CK), while those grown in amended soils showed a significant increase in plant height. By the second and fourth week, soybean plants grown in treatments $B_{35}F_{70}$, $B_{35}F_{140}$, and $B_{70}F_{140}$ resulted in the tallest plants, with heights significantly greater than CK. By the sixth week, treatments $B_{35}F_{70}$, $B_{35}F_{105}$, $B_{35}F_{140}$, $B_{70}F_{105}$, and $B_{70}F_{140}$ resulted in the tallest soybean plants compared to CK. The increase in plant height in our study is consistent with the findings of Liu *et al.* (2022) & Parker *et al.* (2021). The improved plant growth in amended soils reflects the positive influence of biochar and organic fertilizer on soil nutrients essential for soybean development. Poor plant growth in the CK treatment was likely caused by root growth inhibition due to H^+ influx under low soil pH stress. As noted by Lukin *et al*, (2019), soil acidity affects mineral nutrition and growth, with most legumes thriving in moderately acidic to neutral soils.

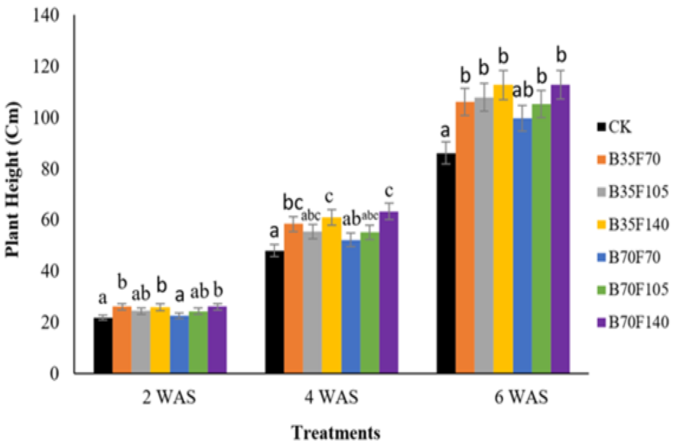

Figure 1. *Average soybean plant height on applied treatments. Error bars sharing the same lowercase letters are not significantly different as determined by Least Significant Difference (LSD) at a significance level of p< 0.05. Numbers preceding the letters B and F represent grams of biochar and organic fertilizer applied, respectively. CK represents unamended soil. WAS- weeks after sowing .*

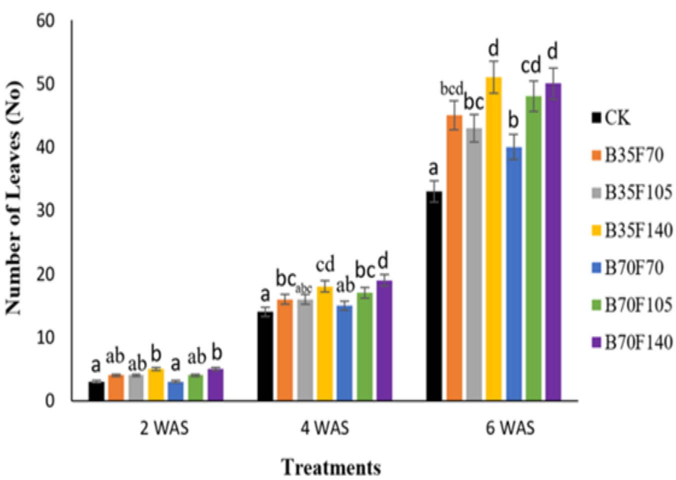

Figure 2. *Average number of soybean plant leaves on applied treatments. Error bars sharing the same lowercase letters are not significantly different as determined by Least Significant Difference (LSD) at a significance level of p< 0.05. Numbers preceding the letters B and F represent grams of biochar and organic fertilizer applied, respectively. CK represents unamended soil. WAS- weeks after sowing.*

Synergistic effects of biochar and organic fertilizer on the number of soybean plant leaves

The average number of soybean plant leaves is depicted in Figure 2. Plant leaves are essential components of plants due to their key role in photosynthesis. Given that adequate amounts of essential plant nutrients (N, P, K, Ca, and Mg) are necessary for plant growth and development (Jing *et al*., 2024), in light of the applied treatment, we confirmed its association with the number of leaves that were formed on each plant. Soybean plants grown in treatments $B_{35}F_{140}$ and $B_{70}F_{140}$ produced more leaves by the second week after sowing, compared to CK and $B_{35}F_{70}$ treatments (Figure 2). By the fourth week, treatments $B_{35}F_{70}$, $B_{35}F_{140}$, $B_{70}F_{105}$, and $B_{70}F_{140}$ showed a significant increase in leaf number compared to CK treatment. By week six, all amended treatments resulted in significantly more leaves than the CK group (Figure 2). These findings align with Nurmalasari *et al.* (2024). Overall, soybean plants grown under the CK soil group had the fewest leaves, the shortest growth, and fewer root hairs than those grown in amended soils. This is likely due to the CK soil's high acidity which limited nutrient availability for growth.

Soybean root hair growth as influenced by the synergy of biochar and organic fertilizer

The synergy of biochar and organic fertilizer increased soil phosphorus and calcium availability, promoting root growth and development (Khan *et al*., 2023). In some of the treatments, the available phosphorous and calcium were significantly increased after the amendment of strongly acidic soil using a synergy of biochar and organic fertilizer (Tables 4 & 5). Because of this, there was more extensive root hair growth in the soybean plants grown in the amended soils (Figure 3b-g) compared to those grown in the CK soil group (Figure 3a). Improved soil pH in these treatments made more nutrients available for root absorption. Wu *et al.* (2022) also reported positive effects of biochar on soybean root morphology. Root hairs are essential for a plant's ability to absorb water and nutrients from the soil, and to anchor the plant in the soil. They also increase the surface area of the root and interact with soil microorganisms. One hundred DAA, soil pH shifted from strongly acidic to moderately acidic facilitating nutrient availability, particularly P, K, Mg, and Ca (Ngui *et al*., 2024). In contrast, the high acidity in CK soil hindered root growth causing aluminum toxicity that damaged the roots. This prevented the elongation and division of root cells and limited nutrient uptake of P and Ca^{2+}.

Linear regression relationships

Figures 4(a-l) below show a linear regression relationship between various soil parameters. Following the amendment of strong acidic soil through the combination of biochar and organic fertilizer, we found a correlation of (R^2 = 0.6455), (R^2 = 0.7954), (R^2 = 0.221), (R^2 = 0.7175), (R^2 = 0.5235), and (R^2 = 0.2726) between soil pH and available phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), EC, and available zinc (Zn) (Figure 4a, 4b, 4c, 4d, 4e, and 4f respectively). This meant that the addition of biochar and organic fertilizer to strong acidic soils could give rise in soil pH, and lead to the increase of soil available P, K, Ca, Mg, EC, and Zn in the amended soils. In contrast, our results found a negative correlation between soil pH and available Cu (Figure 4g). This indicated that a rise in soil pH resulted in to decrease in soil available Cu levels. Cu is among the microelements, meaning that its solubility increases with a decrease in soil pH. Positive correlation between organic matter content and soil available Zn (R^2 = 0.1116), Ca (R^2 = 0.3959), K (R^2 = 0.3071), and Mg (R^2 = 0.4511) was determined (Figure 4h, 4i, 4j, and 4k respectively). The linear regression relationship between organic matter and soil-available copper, as it was found in soil pH, was negatively correlated (Figure 4l).

Figure.3a. Soybean roots in CK

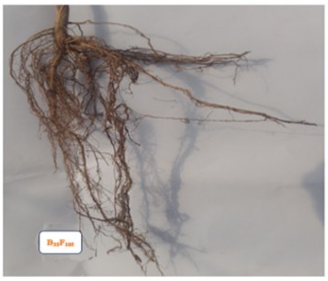

Figure.3b. Soybean roots in $B_{35}F_{105}$

Figure.3c. Soybean roots in $B_{35}F_{70}$

Figure.3d. Soybean roots in $B_{35}F_{140}$

Figure.3e. Soybean roots in $B_{70}F_{70}$

Figure.3f. Soybean roots in $B_{70}F_{140}$

Figure.3g. Soybean roots in $B_{70}F_{105}$

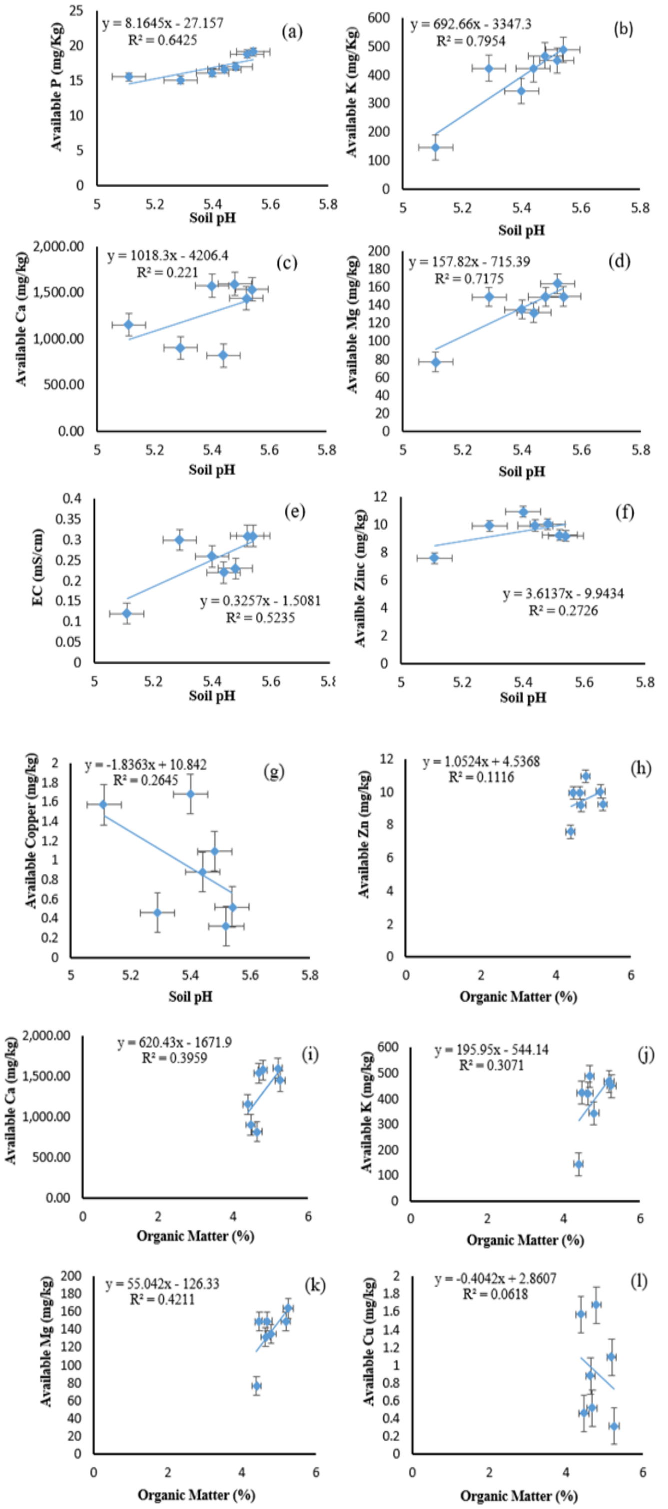

Figure 4. *Linear regression relationships.*

Conclusion and future perspectives

Statistical analysis shows that the amendment of strong acidic soil with rice husk biochar and organic fertilizer significantly improved soil physicochemical properties. Soybean plants grown in amended soils showed a notable improvement in height, leaf development, and root hairs, reflecting the enhanced soil quality. These results demonstrate that adding biochar and organic fertilizer to acidic soils can boost both soil health and soybean growth. However, our study focused on the short-term effects of the combination of biochar and organic fertilizer on soil properties and soybean growth traits in a greenhouse pot experiment. Further research is needed to evaluate the long-term impacts and cost-effectiveness of biochar application in field conditions. Therefore, we recommend that future studies should aim to optimize biochar production at a lower cost while preserving its beneficial properties.

ACKNOWLEDGEMENTS

We would like to express our heartfelt gratitude to Mr. Steven Wei and Mr. Jimmy Wang for their invaluable contributions in preparing the experimental study sites (greenhouse and laboratory) and for offering *ICP-MS* analytical support, respectively. Additionally, the first author wishes to acknowledge the support received from ICDFTaiwan during his study and to thank Mr. Christopher Kalema for his insightful comments on this work.

DECLARATIONS

Authors contribution

Conceptualization and methodology: Y-H.L. and M.E.N.; Investigation: M.E.N.; Data curation: M.E.N.; Writing -original draft preparation: M.E.N.; Writing-review and editing: M.E.N. and S.D.B.; Supervision: Y-H.L. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest: The authors declare no conflict of interest.

Ethics approval: This study did not involve any animal or human participant and thus ethical approval was not applicable.

Consent for publication: All co-authors gave their consent to publish this paper in AAES.

Data availability: The data that support the findings of this study are available on request from the corresponding author.

Supplementary data: No supplementary data is available for this paper.

REFERENCES

Acharya, N., Vista, S. P., Shrestha, S., Neupane, N., & Pandit, N. R. (2022). Potential of Biochar-Based Organic Fertilizers on Increasing Soil Fertility, Available Nutrients, and Okra Productivity in Slightly Acidic Sandy Loam Soil. *Nitrogen*, *4*(1), 1-15.

Agyenim-Boateng, K. G., Zhang, S., Shohag, M. J. I., Shaibu, A. S., Li, J., Li, B., & Sun, J. (2023). Folate biofortification in soybean: challenges and prospects. *Agronomy*, *13*(1), 241.

Al-Jabori, J., Al-Obaed, B., & Al-Amiri, A. (2011). Effect of soil gypsum content and kind of organic matter on status and behavior of potassium. *Tikrit Journal for Agricultural Sciences*, *11*(4), 299-310.

Andrés, Z., Pérez-Hormaeche, J., Leidi, E. O., Schlücking, K., Steinhorst, L., McLachlan, D. H., Schumacher, K., Hetherington, A. M., Kudla, J., & Cubero, B. (2014). Control of vacuolar dynamics and regulation of stomatal aperture by tonoplast potassium uptake. *Proceedings of the National Academy of Sciences*, *111* (17), E1806-E1814.

Bader, B. R., Taban, S. K., Fahmi, A. H., Abood, M. A., & Hamdi, G. J. (2021). Potassium availability in soil amended with organic matter and phosphorous fertilizer under water stress during maize (Zea mays L) growth. *Journal of the Saudi Society of Agricultural Sciences*, *20*(6), 390-394.

Banaszkiewicz, T. (2011). Nutritional value of soybean meal. *Soybean and nutrition*, *12*, 1-20.

Bin, M., Yi, G., & Zhang, X. (2023). Discovery and characterization of magnesium transporter (MGT) gene family in Citrus sinensis and their role in magnesium deficiency stress. *Plant Growth Regulation*, *100*(3), 733-746.

Bray, R. H., & Kurtz, L. T. (1945). Determination of total, organic, and available forms of phosphorus in soils. *Soil science*, *59*(1), 39-46.

Brempong, M. B., & Addo-Danso, A. (2022). Improving soil fertility with organic fertilizers. *New Generation of Organic Fertilizers*, 1.

Cárcamo, M. P., Reyes-Díaz, M., Rengel, Z., Alberdi, M., Omena-Garcia, R. P., Nunes-Nesi, A., & Inostroza-Blancheteau, C. (2019). Aluminum stress differentially affects physiological performance and metabolic compounds in cultivars of highbush blueberry. *Scientific reports*, *9*(1), 11275.

Chen, G., Li, J., Han, H., Du, R., & Wang, X. (2022). Physiological and molecular mechanisms of plant responses to copper stress. *International Journal of Molecular Sciences*, *23*(21), 12950.

Dejene, D., & Tilahun, E. (2019). Role of biochar on soil fertility improvement and greenhouse gases sequestration. *Horticulture International Journal*, *3*(6), 291-298.

Du, L., Zhang, Z., Chen, Y., Wang, Y., Zhou, C., Yang, H., & Zhang, W. (2024). Heterogeneous impact of soil acidification on crop yield reduction and its regulatory variables: A global meta-analysis. *Field Crops Research*, *319*, 109643.

Ferreira, C. S., Seifollahi-Aghmiuni, S., Destouni, G., Ghajarnia, N., & Kalantari, Z. (2022). Soil degradation in the European Mediterranean region: Processes, status and consequences. *Science of the Total Environment*, 805, 150106.

Gairhe, J. J., Bhattarai, P., Gyanwali, P., Khanal, R., Mainali, R., Poudel, S., Pokhrel, M., & Sharma, P. K. (2024). Effect of various biochar on selected soil properties and agronomical parameters of okra (Abelmoschus esculentus L.) at Rupandehi, Nepal. *Archives of Agriculture and Environmental Science*, *9*(1), 134-142.

Guo, Y., & Wang, J. (2021). Spatiotemporal changes of chemical fertilizer application and its environmental risks in China from 2000 to 2019. *International Journal of Environmental Research and Public Health*, *18*(22), 11911.

Gupta, N., Ram, H., & Kumar, B. (2016). Mechanism of Zinc absorption in plants: uptake, transport, translocation and accumulation. *Reviews in Environmental Science and Bio/Technology*, *15*, 89-109.

Hamedeh, H., Antoni, S., Cocciaglia, L., & Ciccolini, V. (2022). Molecular and physiological effects of magnesium–polyphenolic compound as biostimulant in drought stress mitigation in tomato. *Plants*, *11*(5), 586.

Ibrahim, I. I. (2022). Efficacy of biochar and npk fertilizer on soil properties and yield of okra (Abelmoschus esculentus L.) in Guinea Savanna region of Nigeria. *Journal of Environmental Bioremediation and Toxicology*, 5(1), 6-10.

Ishfaq, M., Wang, Y., Yan, M., Wang, Z., Wu, L., Li, C., & Li, X. (2022). Physiological essence of magnesium in plants and its widespread deficiency in the farming system of China. *Frontiers in plant science*, *13*, 802274.

Janocha, A., Milczarek, A., Pietrusiak, D., Łaski, K., & Saleh, M. (2022). Efficiency of soybean products in broiler chicken nutrition. *Animals*, *12*(3), 294.

Jayachandran, M., & Xu, B. (2019). An insight into the health benefits of fermented soy products. *Food chemistry*, *271*, 362-371.

Jing, T., Li, J., He, Y., Shankar, A., Saxena, A., Tiwari, A., Maturi, K. C., Solanki, M. K., Singh, V., & Eissa, M. A. (2024). Role of calcium nutrition in plant Physiology: Advances in research and insights into acidic soil conditions-A comprehensive review. *Plant Physiology and Biochemistry*, 108602.

Jote, C. A. (2023). The impacts of using inorganic chemical fertilizers on the environment and human health. *Organic & Medicinal Chemistry International Journal*, *13*(3), 555864.

Khan, F., Siddique, A. B., Shabala, S., Zhou, M., & Zhao, C. (2023). Phosphorus plays a key role in regulating plants' physiological responses to abiotic stresses. *Plants*, *12*(15), 2861.

Li, H., Dong, X., da Silva, E. B., de Oliveira, L. M., Chen, Y., & Ma, L. Q. (2017). Mechanisms of metal sorption by biochars: Biochar characteristics and modifications. *Chemosphere*, *178*, 466-478.

Liu, M., Linna, C., Ma, S., Ma, Q., Guo, J., Wang, F., & Wang, L. (2022). Effects of biochar with inorganic and organic fertilizers on agronomic traits and nutrient absorption of soybean and fertility and microbes in purple soil. Frontiers in Plant Science, *13*, 871021.

Lukin, S. V., Zavalin, A. A., Sokolov, O. A., & Shmyreva, N. Y. (2019). Legume reaction to soil acidity. *Amazonia Investiga*, *8*(23), 162-170.

McLean, E. (1982). Soil pH and lime requirement. *Methods of soil analysis, In part, 2*.

Mehlich, A. (1978). New extractant for soil test evaluation of phosphorus, potassium, magnesium, calcium, sodium, manganese and zinc. *Communications in Soil Science and Plant Analysis*, *9*(6), 477-492.

Mehlich, A. (1984). Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Communications in soil science and plant analysis*, *15*(12), 1409-1416.

Nam, K., Thodika, A. R. A., Tischlik, S., Phoeurk, C., Nagy, T. M., Schierholz, L., Ådén, J., Rogne, P., Drescher, M., & Sauer-Eriksson, A. E. (2024). Magnesium induced structural reorganization in the active site of adenylate kinase. *Science Advances*, *10*(32), eado5504.

Nelson, D. W., & Sommers, L. E. (1982). Total carbon, organic carbon, and organic matter. *Methods of soil analysis*: *Part 2 chemical and microbiological properties*, *9*, 539-579.

Ngui, M. E., Lin, Y. H., Wei, I. L., Wang, C. C., Xu, Y. Z., & Lin, Y. H. (2024). Effects of the combination of biochar and organic fertilizer on soil properties and agronomic attributes of soybean (*Glycine max* L.). *PloS one*, *19*(9), e0310221.

Nurmalasari, A. I., Rahayu, M., Sakya, A. T., Purnomo, D., & Rosadi, A. A. (2024). The role of biochar and organic fertilizers on the growth and yields of soybean. *Journal of Central European Agriculture*, *25*(1), 275-283.

Parker, N., Agyare, W. A., Bessah, E., & Amegbletor, L. (2021). Biochar as a substitute for inorganic fertilizer: effects on soil chemical properties and maize growth in Ghana. *Journal of Plant Nutrition*, *44*(11), 1539-1547.

Piash, M. I., Iwabuchi, K., Itoh, T., & Uemura, K. (2021). Release of essential plant nutrients from manure-and wood-based biochars. *Geoderma*, *397*, 115100.

Sandrakirana, R., & Arifin, Z. (2021). Effect of organic and chemical fertilizers on the growth and production of soybean (*Glycine max*) in dry land. *Revista Facultad Nacional de Agronomía Medellín*, *74*(3), 9643-9653.

Shao, Y., Li, S., Gao, L., Sun, C., Hu, J., Ullah, A., Gao, J., Li, X., Liu, S., & Jiang, D. (2021). Magnesium application promotes rubisco activation and contributes to high-temperature stress alleviation in wheat during the grain filling. *Frontiers in Plant Science*, *12*, 675582.

Sikdar, A., Zaman, N., Deb, S., Chakma, N., & Sharif, M. O. (2023). Effect of Co-application of Different Biochars and Inorganic Fertilizers on the Growth and Yield of Okra (*Abelmoschus esculentus* L.) in sandy soil. *Journal of Agroforestry and Environment*, *16*(2), 20-27.

Singh, H., Northup, B. K., Rice, C. W., & Prasad, P. V. (2022). Biochar applications influence soil physical and chemical properties, microbial diversity, and crop productivity: a meta-analysis. *Biochar*, *4*(1), 8.

Singh, S., Tripathi, D. K., Singh, S., Sharma, S., Dubey, N. K., Chauhan, D. K., & Vaculík, M. (2017). Toxicity of aluminium on various levels of plant cells and organism: a review. *Environmental and Experimental Botany*, 137, 177-193.

Smith, P., Poch, R. M., Lobb, D. A., Bhattacharyya, R., Alloush, G., Eudoxie, G. D., Anjos, L. H., Castellano, M., Ndzana, G. M., & Chenu, C. (2024). Status of the World's Soils. *Annual Review of Environment and Resources*, 49.

Torabian, S., Qin, R., Noulas, C., Lu, Y., & Wang, G. (2021). Biochar: an organic amendment to crops and an environmental solution. *AIMS Agriculture and Food*, *6*(1), 401-415.

Tränkner, M., Tavakol, E., & Jákli, B. (2018). Functioning of potassium and magnesium in photosynthesis, photosynthate translocation and photoprotection. *Physiologia plantarum*, *163*(3), 414-431.

Wu, D., Zhang, W., Xiu, L., Sun, Y., Gu, W., Wang, Y., Zhang, H., & Chen, W. (2022). Soybean yield response of biochar-regulated soil properties and root growth strategy. *Agronomy*, *12*(6), 1412.