

Rice husk biochar enhances phosphorus availability and stevia leaf biomass in Ultisol under phosphorus fertilization

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ABSTRACT

Phosphorus (P) availability often limits crop growth in acidic Ultisol because a large proportion of the applied P is retained by Al- and Fe-bearing soil constituents. This study examined whether rice husk biochar could improve soil chemical conditions and modify soil P availability and stevia responses to P fertilization. A pot experiment was arranged in a 4 × 4 factorial, completely randomized design with three replicates, resulting in 48 experimental units. Data were analyzed using two-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test at $p < 0.05$. Rice husk biochar was applied at 0, 10, 20, and 30 t ha⁻¹, while P was supplied at 0, 45, 90, and 135 kg P₂O₅ ha⁻¹. Biochar increased soil pH, organic C, cation exchange capacity (CEC), leaf area, and leaf greenness. Significant biochar × P interactions were observed for available P, leaf fresh and dry weights, and leaf P accumulation. The highest available P (250.90 mg kg⁻¹) was found with 30 t ha⁻¹ biochar and 135 kg P₂O₅ ha⁻¹, which was significantly higher than with 30 t ha⁻¹ biochar and 90 kg P₂O₅ ha⁻¹ (218.34 mg kg⁻¹). However, the highest numerical leaf fresh weight (29.96 g plant⁻¹), leaf dry weight (8.66 g plant⁻¹), and leaf P accumulation (2.981 mg P plant⁻¹) occurred with 30 t ha⁻¹ biochar combined with 90 kg P₂O₅ ha⁻¹. These findings show that rice husk biochar changed how soil P availability reacted to P fertilization. Raising P from 90 to 135 kg P₂O₅ ha⁻¹ at the highest biochar level significantly increased soil available P but did not further improve leaf biomass or leaf P accumulation.

Keywords: biochar, phosphorus availability, leaf phosphorus accumulation, Ultisol, *Stevia rebaudiana*.

INTRODUCTION

Stevia (*Stevia rebaudiana* (Bertoni) Bertoni) is cultivated for its steviol glycosides found in the leaves. Phosphorus (P) is particularly important because it participates in photosynthesis and energy transfer, and its availability has been linked to biomass, leaf P concentration, and glycoside accumulation in stevia (Tavarini et al., 2018, 2020).

Much of the arable land in humid tropics, including Indonesia, is acidic, and P is a major nutrient constraint in acidic tropical soils. At low pH, phosphate reacts with iron and aluminum oxides and hydroxides to form sparingly soluble

Fe-P and Al-P compounds; therefore, much of the applied P may become sorbed or precipitated into forms with limited immediate availability to crops (Mosharrof et al., 2021). Soil pH and amendments that alter it strongly influence P availability (Glaser and Lehr, 2019).

Biochar, a carbon-rich solid produced by biomass pyrolysis under limited oxygen conditions, is commonly used to improve soil chemical conditions. Biochar can increase soil pH, CEC, and organic carbon in acidic soils (Zhang et al., 2025). By increasing the pH and lowering the activity of Al and Fe, biochar may reduce P fixation, although its effect on available P varies with

feedstock, pyrolysis temperature, and application rate (Glaser and Lehr, 2019).

Rice husks are an abundant byproduct of rice milling in Indonesia and can be converted into porous biochar suitable for acidic soils. In acidic and acid-sulfate soils, rice husk biochar increases available P and reduces exchangeable Al when applied with lime, compost, or mineral P (Mosharrof et al., 2021; Phuong et al., 2020). Combining rice husk biochar with P fertilizer has also improved P availability and crop performance in maize (Mosharrof et al., 2022).

However, the simultaneous application of rice husk biochar and P fertilizer has received limited attention in stevia. Previous studies have mainly examined P fertilization with arbuscular mycorrhizal fungi and its effects on biomass, P uptake, and secondary metabolites (Tavarini et al., 2018, 2020), whereas integrated nutrient management with compost and K fertilizer improved soil properties and stevia productivity (Fauzan et al., 2025). Therefore, this study aimed to evaluate the individual and interactive effects of rice husk biochar and P fertilization on soil pH, organic carbon, CEC, Bray-I extractable P, vegetative growth, leaf biomass, and leaf P accumulation in stevia grown in acidic Ultisol. We hypothesized that rice husk biochar would improve the soil chemical environment and P availability, thereby enhancing the response of stevia to P fertilization.

MATERIALS AND METHODS

Study site and period

The study was conducted between January and June 2026 at an experimental site located in Pakopen Village, Bandungan District, Semarang Regency, Central Java, Indonesia (7.20303° S, 110.38361° E). The soil utilized as the growing medium was sourced in January 2026 from Cepoko, Gunungpati District, Semarang City, Central Java, Indonesia (7.07983° S, 110.34772° E), at a depth of 0–20 cm. The sampling site was selected because it represents an acidic Ultisol with low P availability, providing suitable soil conditions for evaluating the effects of rice husk biochar and P fertilization. Following collection, the soil was transported to the experimental location, where it was homogenized and used as the growth medium. Prior to the treatment, representative soil subsamples were collected to ascertain the initial

chemical properties of the soil. Soil and plant analyses were performed at the Laboratory of Ecology and Plant Production, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, as well as at the BRMP Lingkungan Pertanian laboratory in Pati Regency, Central Java, Indonesia.

Soil and biochar preparation and crop management

The experimental soil was air-dried, passed through a sieve with a mesh size of < 2 mm, and weighed into 30 × 30 cm polybags at 4 kg soil per bag. The initial physical and chemical properties of the soil used as the growth medium are presented in Table 1. The soil was acidic, with a pH (H₂O) of 5.23 and a pH (KCl) of 4.21, and was characterized by low organic C, low Bray-I available P, low base saturation, and measurable exchangeable Al content.

The rice husk biochar used in this experiment was procured as a commercial product from WasteX, located in Grobogan, Central Java, Indonesia. This biochar was derived from rice husks through oxygen-limited pyrolysis conducted at approximately 500 °C, employing a continuous carbonization system. Prior to its application, the chemical properties of the biochar were independently analyzed, and the results are detailed in Table 2.

Experimental design and treatments

The treatments were arranged in a 4 × 4 factorial in a completely randomized design with three replicates, resulting in 16 treatment combinations and 48 experimental units. Each unit was a single polybag holding one plant; therefore, a total of 48 plants were grown. The first factor was rice husk biochar applied at 0 (control), 10, 20, and 30 t ha⁻¹. The second factor was phosphorus, supplied as triple superphosphate (TSP; 46% P₂O₅), at 0 (control), 45, 90, and 135 kg P₂O₅ ha⁻¹. The biochar rates were selected to span a graded range comparable with those previously used to investigate rice-husk biochar effects on P availability in acidic soils, including 0, 10, 20, and 40 t ha⁻¹ (Liu et al., 2017). The P rates were selected to provide a low-to-high gradient relevant to stevia grown under P-limiting conditions; previous studies have included 45 kg P₂O₅ ha⁻¹ as the P component of the recommended fertilizer dose (Behera et al., 2013),

Table 1. Initial physical and chemical properties of the experimental soil

Soil property	Unit	Value	Analytical method	Rating
pH (H ₂ O)	–	5.23	Electrometric (H ₂ O)	Acidic
pH (KCl)	–	4.21	Electrometric (1 M KCl)	-
Organic carbon	%	0.98	Walkley–Black	Very low
Total N	%	0.15	Kjeldahl	Low
Potential P (25% HCl-extractable P ₂ O ₅)	mg (100 g) ⁻¹	109.66	HCl 25% extraction, spectrophotometry	Very high
Available P	mg kg ⁻¹	6.61	Bray-I	Low
Potential K (25% HCl-extractable K ₂ O)	mg (100 g) ⁻¹	8.90	HCl 25% extraction, AAS	Very low
Electrical conductivity (EC)	dS m ⁻¹	0.049	Conductivity meter	Very low
CEC	cmol(+) kg ⁻¹	21.64	Titrimetric	Medium
Exchangeable cations				
K	cmol(+) kg ⁻¹	0.16	AAS	Low
Na	cmol(+) kg ⁻¹	0.11	AAS	Low
Ca	cmol(+) kg ⁻¹	3.36	AAS	Low
Mg	cmol(+) kg ⁻¹	1.81	AAS	Medium
Base saturation	%	25.14	Calculated	Low
Exchangeable acidity				
Al	cmol(+) kg ⁻¹	1.85	Titrimetric (1 M KCl)	–
H	cmol(+) kg ⁻¹	1.24	Titrimetric (1 M KCl)	–
Particle-size distribution				
Sand	%	24.96	Particle-size analysis	–
Silt	%	50.31	Particle-size analysis	–
Clay	%	24.73	Particle-size analysis	–
Textural class (USDA)	–	Silt loam	–	–

Note: AAS: Atomic absorption spectrophotometry. Soil fertility ratings were assigned according to the interpretation criteria provided in the Technical Guidelines for Chemical Analysis of Soil, Plant, Water, and Fertilizer published by the Indonesian Ministry of Agriculture (Eviati et al., 2023); “–” indicates that no rating class is defined for that parameter. The textural class was assigned to sand–silt–clay fractions using the USDA textural classification triangle.

while pot experiments with stevia in acidic soil have evaluated progressively increasing P rates up to 150 kg P ha⁻¹ (Maniruzzaman et al., 2017).

The area-equivalent application rates were calculated based on a nominal planting spacing of 25 × 25 cm. This spacing equates to 0.0625 m² plant⁻¹, resulting in a theoretical population density of 160,000 plants ha⁻¹. Because each polybag contained a single plant, the determined quantity for each plant was allocated to one polybag. Consequently, rice husk biochar application rates of 0, 10, 20, and 30 t ha⁻¹ were equivalent to 0, 62.5, 125.0, and 187.5 g of biochar per polybag, respectively. The biochar was thoroughly mixed with 4 kg of soil in each polybag a month before planting to allow interaction. The initial stem cuttings were approximately 5 cm in length. After rooting and establishment, healthy and morphologically uniform plantlets approximately 10–15 cm in height, with well-developed

roots approximately 8 cm in length, were selected for transplanting. One rooted plantlet was transplanted into each polybag at a depth of 3–5 cm. The polybags were arranged according to a completely randomized design, with 25 cm spacing between polybags. The experiment was performed outdoors at the experimental site under ambient conditions without the use of a greenhouse or rain-exclusion structure, thereby allowing natural rainfall to occur. To prevent excessive drying of the growing medium, soil moisture was supplemented by watering twice daily, in the morning and afternoon.

All experimental units received urea and potassium chloride (KCl) at area-equivalent rates of 200 and 120 kg ha⁻¹, corresponding to 1.25 and 0.75 g polybag⁻¹, respectively. Phosphorus was supplied as triple superphosphate (TSP; 46% P₂O₅) according to treatment levels of 0, 45, 90, and 135 kg P₂O₅ ha⁻¹. These levels matched 0, 97.83, 195.65,

Table 2. Selected chemical properties of the rice husk biochar used in the experiment

Property	Value	Unit
pH	8.92	–
Total N	0.53	%
Total P	0.21	%
Total K	0.35	%
Organic C	39.95	%
C/N ratio	75.38	–

Note: Values represent the chemical characteristics of the rice husk biochar used as a soil amendment. The analyses were conducted at the BRMP Laboratory, Pati Regency, Central Java, Indonesia. The C/N ratio was calculated from the organic C and total N. “–” indicates that no unit is applicable.

and 293.48 kg TSP ha⁻¹ and were about 0, 0.61, 1.22, and 1.83 g TSP polybag⁻¹, respectively.

All mineral fertilizers were applied 7 days after planting in a shallow ring approximately 5 cm from the base of each plant. Plants were watered twice a day, in the morning and afternoon, to keep the soil moist. Dead or weak cuttings were replaced within 3–5 days after planting to ensure that each experimental unit contained one healthy plant. Weeds were manually removed during the experiment. The plants were harvested 50 days after planting to assess growth, leaf biomass, and plant P variables. Soil samples were taken from each experimental unit for chemical analysis after harvest.

Soil chemical analyses

Soil samples were collected separately from each experimental unit at harvest. After the plant was removed, the soil within each polybag was thoroughly mixed to obtain a homogeneous sample, and visible roots and plant residues were manually removed. A representative subsample was collected from the homogenized soil of each polybag. The samples were air-dried at room temperature, gently crushed, and passed through a 2-mm sieve before chemical analysis. The processed soil samples were analyzed for pH, EC, organic C, CEC, and available P. Soil pH was determined electrometrically using a glass-electrode pH meter in a 1:2.5 soil-to-water suspension. The EC was measured in a 1:5 (w/v) soil-to-distilled-water suspension using a conductivity meter. Available P was determined using the Bray-I extraction method (Bray and Kurtz, 1945);

2.5 g of soil was extracted with 25 mL of Bray-I solution, shaken for 5 min, and filtered. Phosphomolybdate color was subsequently developed and measured spectrophotometrically at 889 nm. The organic C content was determined using the Walkley–Black wet-oxidation method (Walkley and Black, 1934), whereas CEC was determined after saturation with 1 M ammonium acetate buffered at pH 7. Unless otherwise stated, the analytical procedures followed the technical guidelines of Eviati et al. (2023).

Plant growth, physiological traits, leaf biomass, and phosphorus measurements

Plant height was measured at harvest, from the base of the main stem to the highest point of growth, using a ruler. The number of fully expanded leaves was counted for each plant simultaneously. Leaf area was calculated by applying a correction factor of 0.656 to the measurements of leaf length and width, as reported by Susilo (2015) for stevia. This factor was originally obtained from stevia leaves cultivated in peat-soil environments and was not independently adjusted to the Ultisol conditions of the current study. Leaf greenness was measured using a SPAD chlorophyll meter on the third or fourth fully expanded leaf from the apex with three readings per plant averaged to obtain a single value. Leaf fresh weight was determined immediately after harvest using a digital scale. The leaves were oven-dried at 70 °C for 24 h and then weighed to determine their dry weight. Dried leaves were ground, and a 1 g subsample was extracted with 10 mL of distilled water (1:10, w/v), homogenized using a magnetic stirrer, and filtered. The TSS of the resulting aqueous leaf extract was measured using a handheld refractometer and expressed as °Brix.

For tissue P analysis, dried leaf samples were ground and passed through a 0.5-mm sieve. A 0.5 g portion of the ground material underwent wet digestion with 5 mL of concentrated HNO₃ (65%) and 0.5 mL of HClO₄ (60%), following Eviati et al. (2023). The samples were left overnight for pre-digestion and then heated in a digestion block until the digest ran clear with only a small residual volume. Once cooled, the digest was diluted to 50 mL with deionized water and homogenized. The P concentration was colorimetrically determined using the molybdenum blue method. The P-color reagent was freshly prepared from ascorbic acid and a concentrated

reagent containing ammonium molybdate, sulfuric acid, and potassium antimony tartrate. A 5 mL aliquot of the digest was mixed with 0.5 mL of this reagent, homogenized, and left to develop color for 30 min before being measured spectrophotometrically at 889 nm (Eviati et al., 2023). Leaf P accumulation was calculated using Equation 1:

$$\begin{aligned} \text{Leaf P accumulation (mg P plant}^{-1}\text{)} &= \\ &= \text{Leaf P concentration (\%)} \times \\ &\times \text{Leaf dry weight (g plant}^{-1}\text{)} \times 10 \end{aligned} \quad (1)$$

Statistical analysis

Two-way analysis of variance (ANOVA) was used to analyze the data. The design was a 4×4 factorial arrangement within a completely randomized structure, and the statistical model incorporated the main effects of biochar and phosphorus rates, along with their interaction. Prior to ANOVA, the homogeneity of variance was assessed for each dependent variable using Levene's test based on the median, which is equivalent to the Brown–Forsythe modification and is less sensitive to departures from normality (Brown and Forsythe, 1974). The homogeneity of variance assumption was satisfied for all variables ($p > 0.05$). Therefore, all variables, including soil available P, were analyzed on their original measurement scales without transformation. When a significant biochar $\times$ phosphorus interaction was detected, treatment means were interpreted across the 16 treatment combinations rather than through separate main effects. For variables without a significant interaction, the main effects of biochar and phosphorus were interpreted independently. Mean comparisons were performed using Tukey's honestly significant difference (HSD) test at $p < 0.05$. All statistical analyses were performed using the IBM SPSS Statistics version 22.

RESULTS

Soil chemical properties

Biochar increased soil pH ($p < 0.001$; Table 3). The highest pH was recorded at 30 t ha^{-1} (5.27), which did not differ significantly from that at 20 t ha^{-1} (5.09), but was significantly higher than those at 0 and 10 t ha^{-1} . Phosphorus also increased pH ($p < 0.05$), with the highest value at

$135 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ (5.20), which differed significantly from that of the control (4.99), while the intermediate rates were similar to both.

EC responded to biochar alone ($p < 0.05$). The highest value occurred at 30 t ha^{-1} ($107.1 \mu\text{S cm}^{-1}$), which was significantly higher than that of the control ($86.4 \mu\text{S cm}^{-1}$); the intermediate rates did not differ from either. Soil organic carbon increased with biochar ($p < 0.01$) but was unaffected by phosphorus. The highest organic carbon was found at 30 t ha^{-1} (1.61%), which was significantly higher than that of both the control (1.37%) and the 20 t ha^{-1} treatment (1.40%).

CEC was affected by both factors ($p < 0.001$). For biochar, 30 t ha^{-1} gave the highest value ($31.62 \text{ cmol(+) kg}^{-1}$), which was significantly higher than all lower rates (24.20 – $25.56 \text{ cmol(+) kg}^{-1}$); these lower rates did not differ from one another. For phosphorus, the treatment receiving no mineral P (P0) had the lowest CEC ($22.72 \text{ cmol(+) kg}^{-1}$), significantly below the three P-fertilized treatments (27.63 – $28.42 \text{ cmol(+) kg}^{-1}$), which were statistically similar.

The interaction between biochar and phosphorus on available P was statistically significant ($p < 0.001$; Table 5). Treatment B3P3, which involved the application of 30 t ha^{-1} biochar and $135 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, resulted in the highest available P concentration ($250.90 \text{ mg kg}^{-1}$). This value was significantly greater than that observed for B3P2 ($218.34 \text{ mg kg}^{-1}$). Furthermore, B3P2 exhibited a significantly higher available P concentration than all treatment combinations that received 0 – 20 t ha^{-1} of biochar. In treatments without mineral P, available P remained low, between 6.36 and 8.10 mg kg^{-1} , with no significant differences among the biochar rates. At $45 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, the available P ranged from 36.68 to 57.43 mg kg^{-1} and showed no significant differences among the treatments. At $90 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, available P was similar among treatments with 0 – 20 t ha^{-1} biochar (58.31 – 64.47 mg kg^{-1}), but applying 30 t ha^{-1} biochar significantly increased available P to $218.34 \text{ mg kg}^{-1}$. At $135 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, available P rose from 65.23 mg kg^{-1} without biochar to 126.56 – $129.11 \text{ mg kg}^{-1}$ with 10 – 20 t ha^{-1} biochar and reached $250.90 \text{ mg kg}^{-1}$ with 30 t ha^{-1} . These patterns demonstrate that the response of available P to biochar strongly depends on the P fertilization rate. The interaction pattern between available P and associated plant responses is illustrated in Figure 1.

Table 3. Soil chemical properties at harvest as affected by rice husk biochar treatment and phosphorus rate in stevia grown on acidic Ultisol

Treatment	Soil pH	EC ($\mu\text{S cm}^{-1}$)	Organic C (%)	CEC ($\text{cmol}(+) \text{ kg}^{-1}$)
Biochar treatment (B, t ha^{-1})				
B0 (0)	4.85 ^c	86.4 ^b	1.37 ^b	25.56 ^b
B1 (10)	5.02 ^{bc}	95.9 ^{ab}	1.47 ^{ab}	24.20 ^b
B2 (20)	5.09 ^{ab}	104.2 ^{ab}	1.40 ^b	25.12 ^b
B3 (30)	5.27 ^a	107.1 ^a	1.61 ^a	31.62 ^a
Phosphorus rate (P, $\text{kg P}_2\text{O}_5 \text{ ha}^{-1}$)				
P0 (0)	4.99 ^b	94.9	1.50	22.72 ^b
P1 (45)	5.00 ^{ab}	97.5	1.43	27.73 ^a
P2 (90)	5.05 ^{ab}	103.6	1.48	28.42 ^a
P3 (135)	5.20 ^a	97.6	1.45	27.63 ^a
Significance (F-test)				
Biochar (B)	10.54 ^{***}	3.34 [*]	6.70 ^{**}	13.16 ^{***}
Phosphorus (P)	3.24 [*]	0.52 ns	0.55 ns	7.92 ^{***}
B $\times$ P	1.17 ns	1.23 ns	1.74 ns	2.01 ns
CV (%)	3.72	17.88	9.70	12.13

Note: means followed by different letters within a column and within the same treatment factor are significantly different (Tukey's HSD test, $p < 0.05$); the absence of letters indicates a non-significant treatment effect. ns = not significant; *, **, *** = significant at $p < 0.05$, 0.01, and 0.001, respectively. CV = coefficient of variation. CEC = cation exchange capacity; EC = electrical conductivity. Biochar was applied at 0, 10, 20, and 30 t ha^{-1} , and phosphorus was applied as triple superphosphate at 0, 45, 90, and 135 $\text{kg P}_2\text{O}_5 \text{ ha}^{-1}$. Soil available P is presented separately (Table 5) because the biochar $\times$ phosphorus interaction was significant.

Plant growth and physiology

Plant height remained largely stable across treatments, ranging from 22.92 to 24.58 cm. Neither biochar, phosphorus, nor their interactions had a significant effect (Table 4). In contrast, leaf number responded to phosphorus ($p < 0.01$). Plants receiving 90 and 135 $\text{kg P}_2\text{O}_5 \text{ ha}^{-1}$ produced 271.2 and 259.6 leaves plant^{-1} , respectively, and both treatments differed significantly from the treatment receiving no mineral P (209.2 leaves plant^{-1}). The intermediate rate of 45 $\text{kg P}_2\text{O}_5 \text{ ha}^{-1}$ resulted in 241.3 leaves plant^{-1} and was statistically comparable to both the P0 treatment and the higher P rates. The leaf area responded independently to biochar and phosphorus, with no significant interactions between the two factors. The application of biochar at 30 t ha^{-1} produced the largest leaf area (5.33 cm^2), significantly exceeding the values recorded at 0, 10, and 20 t ha^{-1} , which ranged from 3.48 to 3.90 cm^2 . A similar increase was observed with phosphorus application. The largest mean leaf area was obtained at 135 $\text{kg P}_2\text{O}_5 \text{ ha}^{-1}$ (4.97 cm^2); this value was significantly greater than those at 0 and 45 $\text{kg P}_2\text{O}_5 \text{ ha}^{-1}$, but did not differ from the 90 $\text{kg P}_2\text{O}_5 \text{ ha}^{-1}$ treatment. Leaf greenness was influenced

by biochar ($p < 0.01$), whereas phosphorus had no detectable effect on leaf greenness. SPAD readings reached 55.83 and 56.51 at 20 and 30 t ha^{-1} biochar, respectively, both significantly higher than the control value of 51.90. The 10 t ha^{-1} treatment was intermediate (54.22) and did not differ significantly from either the control or the two higher biochar treatments. The TSS of the aqueous leaf extract showed little variation among the treatments. The values ranged from 1.83 to 2.21 °Brix, and no significant effects of biochar, phosphorus, or their interaction were detected (Table 4).

Leaf biomass and leaf P accumulation

The leaf fresh weight depended on the combination of biochar and phosphorus, as indicated by the significant interaction between the two factors ($p < 0.01$; Table 5). The treatment responses were not strictly proportional to the input rate (Figure 1). The highest numerical leaf fresh weight was obtained with B3P2 (30 t ha^{-1} biochar + 90 $\text{kg P}_2\text{O}_5 \text{ ha}^{-1}$), reaching 29.96 g plant^{-1} . This value was statistically comparable with B2P3 (27.78 g plant^{-1}) and B2P1 (27.23 g plant^{-1}), among several other treatment combinations sharing the same

Table 4. Growth and physiological variables of stevia as affected by rice husk biochar treatment and phosphorus rate

Treatment	Plant height (cm)	Leaf number	Leaf area (cm ²)	SPAD	TSS of aqueous leaf extract (°Brix)
Biochar treatment (B, t ha ⁻¹)					
B0 (0)	23.88	232.8	3.48 ^b	51.90 ^b	1.83
B1 (10)	22.92	230.6	3.75 ^b	54.22 ^{ab}	2.21
B2 (20)	23.96	261.2	3.90 ^b	55.83 ^a	2.17
B3 (30)	24.58	256.8	5.33 ^a	56.51 ^a	2.00
Phosphorus rate (P, kg P ₂ O ₅ ha ⁻¹)					
P0 (0)	23.04	209.2 ^b	3.63 ^b	54.29	1.92
P1 (45)	23.96	241.3 ^{ab}	3.72 ^b	55.46	2.04
P2 (90)	24.25	271.2 ^a	4.13 ^{ab}	54.01	2.08
P3 (135)	24.08	259.6 ^a	4.97 ^a	54.70	2.17
Significance (F-test)					
Biochar (B)	1.37 ns	2.10 ns	8.64 ^{***}	4.56 ^{**}	2.26 ns
Phosphorus (P)	0.85 ns	6.04 ^{**}	4.63 ^{**}	0.43 ns	0.83 ns
B × P	0.95 ns	1.52 ns	0.89 ns	0.76 ns	1.54 ns
CV (%)	8.54	15.53	23.84	6.09	19.26

Note: means followed by different letters within a column and within the same treatment factor are significantly different (Tukey's HSD test, $p < 0.05$); the absence of letters indicates a non-significant treatment effect. ns = not significant; *, **, *** = significant at $p < 0.05$, 0.01, and 0.001, respectively. CV = coefficient of variation. The leaf fresh weight, dry weight, and P accumulation are shown separately (Table 5) because the interactions between biochar and phosphorus were significant.

Tukey grouping, but was significantly greater than the lowest responses recorded for B1P1 (11.75 g plant⁻¹) and B0P0 (12.55 g plant⁻¹) treatments.

A comparable interaction was detected for leaf dry weight ($p < 0.01$). Again, B3P2 produced the highest numerical value (8.66 g plant⁻¹), followed closely by B2P3 (8.33 g plant⁻¹), and these two treatments did not differ significantly from each other. In contrast, dry matter accumulation was lowest under B1P1 (3.00 g plant⁻¹), whereas B1P0 produced a relatively low value (3.94 g plant⁻¹). Therefore, the biomass response depended on the specific biochar–P combination rather than uniformly increasing with either treatment factor.

Leaf P accumulation also showed a significant biochar × phosphorus interaction ($p < 0.01$; Table 5). The highest numerical leaf P accumulation was observed in B3P2 at 2.981 mg P plant⁻¹. It was statistically similar to B1P3 (2.734 mg P plant⁻¹), B3P3 (2.485 mg P plant⁻¹), and B2P3 (2.452 mg P plant⁻¹), as well as to other combinations within overlapping Tukey groups. The lowest leaf P accumulation occurred in B0P0 (no biochar and no mineral P) (0.760 mg P plant⁻¹). The highest numerical leaf P accumulation was recorded at 90 kg P₂O₅ ha⁻¹ combined with the highest biochar rate,

whereas the highest soil available P was recorded at the higher P rate in B3P3. This difference in response is illustrated in Figure 1.

DISCUSSION

This study found that the response of stevia to phosphorus fertilization in Ultisol was greatly influenced by the chemical conditions in which the fertilizer was used. Rice husk biochar changed several soil properties related to acidity and nutrient retention. The low initial Bray-I available P (6.61 mg kg⁻¹) showed that there was limited readily available P in the experimental soil. Available P, leaf biomass, and leaf P accumulation showed significant biochar × phosphorus interactions, indicating that the response to P fertilization depended on the biochar rate rather than on either input acting independently.

At the time of harvest, the soil pH exhibited an increase corresponding to the biochar application rate, ranging from 4.85 in the absence of biochar to 5.27 at a rate of 30 t ha⁻¹. Given that the initial pH of 5.23 was measured from the bulk soil prior to the application of treatments, rather

Table 5. Interaction effects of biochar × phosphorus on soil available P, leaf fresh weight, leaf dry weight, and leaf P accumulation of stevia grown on acidic Ultisol

Biochar	Phosphorus	Available P (mg kg ⁻¹)	Leaf fresh weight (g)	Leaf dry weight (g)	Leaf P accumulation (mg P plant ⁻¹)
B0 (0)	P0 (0)	6.36 ^e	12.55 ^{de}	4.16 ^{cde}	0.760 ^e
B0 (0)	P1 (45)	48.27 ^d	17.47 ^{bde}	4.74 ^{cde}	1.417 ^{bde}
B0 (0)	P2 (90)	64.47 ^d	24.01 ^{abc}	5.95 ^{abcde}	2.366 ^{abc}
B0 (0)	P3 (135)	65.23 ^d	22.77 ^{abcd}	6.60 ^{abcd}	2.134 ^{abc}
B1 (10)	P0 (0)	6.43 ^e	16.64 ^{cde}	3.94 ^{de}	1.324 ^{cde}
B1 (10)	P1 (45)	36.68 ^{de}	11.75 ^e	3.00 ^e	0.960 ^{de}
B1 (10)	P2 (90)	58.31 ^d	22.11 ^{abcde}	6.65 ^{abcd}	1.955 ^{abcd}
B1 (10)	P3 (135)	126.56 ^c	27.03 ^{abc}	6.50 ^{abcd}	2.734 ^{ab}
B2 (20)	P0 (0)	8.10 ^e	17.69 ^{bde}	5.12 ^{bde}	1.500 ^{bde}
B2 (20)	P1 (45)	57.43 ^d	27.23 ^{abc}	7.20 ^{abc}	1.949 ^{abcd}
B2 (20)	P2 (90)	61.98 ^d	20.43 ^{abcde}	5.51 ^{abcde}	1.872 ^{abcde}
B2 (20)	P3 (135)	129.11 ^c	27.78 ^{ab}	8.33 ^{ab}	2.452 ^{ab}
B3 (30)	P0 (0)	6.93 ^e	20.91 ^{abcde}	5.38 ^{bde}	1.442 ^{bde}
B3 (30)	P1 (45)	55.53 ^d	22.30 ^{abcde}	5.84 ^{abcde}	1.868 ^{abcde}
B3 (30)	P2 (90)	218.34 ^b	29.96 ^a	8.66 ^a	2.981 ^a
B3 (30)	P3 (135)	250.90 ^a	25.88 ^{abc}	6.65 ^{abcd}	2.485 ^{ab}
Significance (F-test)					
Biochar (B)		164.98 ^{***}	7.75 ^{***}	7.04 ^{***}	4.93 ^{**}
Phosphorus (P)		376.13 ^{***}	16.44 ^{***}	13.84 ^{***}	29.67 ^{***}
B × P		54.41 ^{***}	3.84 ^{**}	3.24 ^{**}	3.13 ^{**}
CV (%)		14.12	16.08	18.12	19.42

Note: means followed by different letters within a column are significantly different (Tukey's HSD test, $p < 0.05$). ns = not significant; *, **, *** = significant at $p < 0.05$, 0.01, and 0.001, respectively. CV = coefficient of variation. Biochar was applied at 0, 10, 20, and 30 t ha⁻¹ and phosphorus was supplied as triple superphosphate at 0, 45, 90, and 135 kg P₂O₅ ha⁻¹. Available P was determined using the Bray-I method. Because the biochar × phosphorus interaction was significant for these four variables, treatment means were reported and compared as the 16 biochar × phosphorus combinations shown above, rather than as separate main-effect means.

than through repeated measurements within individual experimental units, these findings should be interpreted as indicative of treatment differences at harvest, rather than as direct evidence of temporal changes in soil pH. At the highest biochar rate, CEC increased from 25.56 to 31.62 cmol(+) kg⁻¹ and organic C from 1.37 to 1.61%. These responses were consistent with the properties of the rice husk biochar used in this study. The biochar itself was alkaline (pH 8.92) and rich in organic C (39.95%), which plausibly explains the higher soil pH and organic C measured after amendment. This fits the well-documented ameliorative role of biochar in acidic soils. Two mechanisms are commonly invoked to explain these responses: alkaline constituents in biochar can increase soil pH, whereas negatively charged surface functional groups can enhance CEC. In addition, the pyrogenic carbon added with the

biochar contributes directly to the soil organic C pool. Such responses tend to be more pronounced in acidic nutrient-constrained soils. Their magnitude varies with the feedstock, pyrolysis conditions, application rate, and initial soil properties (Aurangzeib et al., 2024; Joseph et al., 2021). The EC increase observed here, from 86.4 to 107.1 $\mu\text{S cm}^{-1}$, is consistent with this pattern and is compatible with soluble inorganic constituents contributed by the biochar amendment (Joseph et al., 2021). These values correspond to only about 0.086–0.107 dS m⁻¹, however, which points to a modest rise in soluble ionic content rather than an emerging salinity constraint.

The CEC response requires careful interpretation. Phosphorus fertilization raised CEC from 22.72 cmol(+) kg⁻¹ in the treatment without mineral P to 27.63–28.42 cmol(+) kg⁻¹ across the three P-fertilized treatments, even though TSP

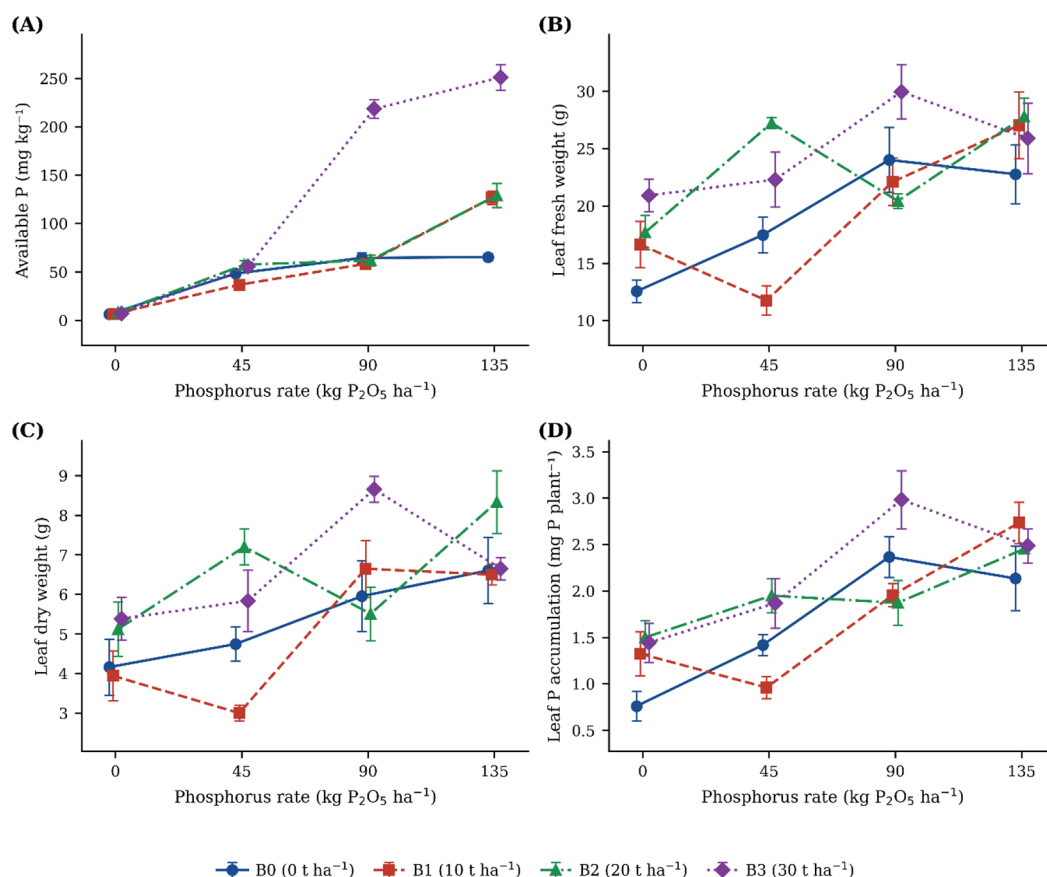


Figure 1. Interaction effects of rice husk biochar and phosphorus fertilization on (A) soil Bray-I available P, (B) leaf fresh weight, (C) leaf dry weight, and (D) leaf P accumulation in stevia grown in acidic Ultisol. Rice husk biochar was applied at 0 (B0), 10 (B1), 20 (B2), and 30 t ha⁻¹ (B3), while phosphorus was applied at 0, 45, 90, and 135 kg P₂O₅ ha⁻¹. Points represent the treatment means (n = 3)

supplies no conventional cation-exchange sites. Phosphate can modify the charge characteristics of soils with variable-charge surfaces. Specifically, adsorbed phosphate alters the surface charge and increases the negative charge, raising the soil's capacity to hold cations. Gillman and Fox (1980) found agronomically significant CEC increases after long-term superphosphate application to variable-charge soils, and Fiantis et al. (2002) reported the same pattern in variable-charge volcanic soils from West Sumatra. More recent mechanistic work by Barrow et al. (2022) showed that phosphate sorption modifies the soil surface charge, with corresponding effects on CEC and exchangeable cations. The higher CEC observed here after P application may therefore partly reflect phosphate-induced surface-charge changes, possibly alongside shifts in soil pH, rather than any direct contribution of exchange sites from the TSP itself. Surface charge characteristics were not measured directly, and the trial covered only one 50-day cropping cycle; therefore,

this mechanism remains a plausible explanation rather than a demonstrated one.

The most distinct interactive response between the two inputs was observed with available P in the soil. Initially, the soil exhibited a substantial reserve of HCl-extractable phosphorus, quantified at 109.66 mg P₂O₅ per 100 g. In contrast, the Bray-I available phosphorus was measured at only 6.61 mg kg⁻¹, which was categorized as low based on the soil-fertility interpretation criteria employed in this study. The two extraction methods measured different soil P pools, so their values could not be directly compared. Even so, the mismatch between classifications was telling: a large P reserve did not translate into a similarly high pool of readily available P. This fits the limited availability of P that is often reported in acidic soils. Available P at harvest in treatments without mineral P stayed low, between 6.36 and 8.10 mg kg⁻¹, with no significant differences among biochar rates. Biochar alone did not significantly increase Bray-I available P over the

course of the experiment. Although the biochar contained 0.21% total P, its application without TSP produced little change in the soil available P, suggesting that the P supplied by the biochar did not constitute a large readily available pool during the 50-day cropping period.

The interaction pattern indicated that the effect of rice husk biochar on Bray-I extractable P became more pronounced as the mineral P supply increased. At the relatively low P rate of 45 kg P₂O₅ ha⁻¹, much of the applied phosphate may still have been retained by the high-affinity sorption sites typical of acidic soils. At higher P inputs, however, increasing P loading together with the greater biochar-derived C input at 30 t ha⁻¹ may have shifted the balance between P sorption and desorption, allowing a larger proportion of the applied P to remain extractable. Similarly, Liu et al. (2018) showed that increasing crop-residue biochar rates decreased P sorption and increased P desorption in acidic red soils, with the strongest biochar effect occurring at higher concentrations of exogenous P. This response was associated with changes in the surface charge and increases in dissolved and humic-like organic matter. Biochar-derived dissolved organic matter can also compete with phosphate for sorption sites on Fe oxides under acidic conditions, thereby reducing phosphate retention (Schneider and Haderlein, 2016). These mechanisms provide a reasonable explanation for the marked increase in Bray-I extractable P observed at P90 and P135 when combined with 30 t ha⁻¹ biochar.

This response showed that the impact of rice husk biochar on Bray-I extractable P greatly depended on the supply of mineral P, and was especially noticeable at the highest biochar rate. Biochar alone had little detectable effect on available P. However, combined with 90 or 135 kg P₂O₅ ha⁻¹ at 30 t ha⁻¹, it substantially increased the extractable P pool. This indicates a statistical interaction between biochar and mineral P supply, although the specific soil processes behind this were not directly determined.

This response is plausible in acidic soils with exchangeable Al and low base saturation. At low pH, phosphate binds strongly to Fe- and Al-bearing minerals through adsorption and precipitation. This binding can substantially restrict the availability of P to the plant roots (Penn and Camberato, 2019). At the two highest biochar rates, soil pH changed only slightly, whereas soil organic C increased significantly at 30 t ha⁻¹ biochar. This suggests that

the pronounced increase in available P at the highest biochar rate may have been associated, at least partly, with the greater input of biochar-derived C rather than with further pH elevation. Organic matter can influence P availability by competing with phosphate for sorption sites on Fe- and Al-bearing minerals and by promoting phosphate desorption, whereas biochar-derived dissolved organic matter has been shown to reduce phosphate sorption onto Fe oxides under acidic conditions (Schneider and Haderlein, 2016; Jindo et al., 2023). However, because dissolved organic C, surface functional groups, and P-sorption characteristics were not measured in this study, this mechanism should be regarded as a plausible explanation rather than a directly demonstrated process.

However, after treatment, exchangeable Al, reactive Fe, Al fractions, P sorption, and soil P fractions were not measured. Therefore, reduced Al-related constraints and changes in P-sorption behavior should be considered as possible explanations rather than proven causes.

Glaser and Lehr (2019) found that biochar increased plant-available P, an effect that was especially pronounced in acidic soils. A broader meta-analysis by Tesfaye et al. (2021) reported substantial gains in both soil-available P and plant P uptake after biochar amendment. Evidence from rice husk biochar is consistent with this pattern. In salt-affected acidic soils, repeated rice husk biochar application increased labile and moderately labile P pools and was associated with improved P availability and crop performance (Linh et al., 2023). Mosharrof et al. (2021) similarly showed that rice husk biochar, when applied with acidity management, increased soil pH and available P, while substantially reducing exchangeable Al and Fe constraints. Previous studies reporting increased soil available P following biochar application are consistent with the pronounced response observed at the highest biochar application rate in the present study. However, the current results further show that the magnitude of the biochar response depended on the mineral P supply. The lack of a notable biochar effect at an application rate of 45 kg P₂O₅ ha⁻¹ indicates that, with this relatively low P input, a considerable portion of the added P may have been retained by P-sorption sites in the acidic soil. At 90 and 135 kg P₂O₅ ha⁻¹, however, the greater P loading combined with the stronger soil-ameliorating effect of 30 t ha⁻¹ biochar may have reduced the relative retention of additional phosphate, allowing a larger proportion to remain

Bray-I extractable. This threshold-like response may reflect combined changes in soil pH, Al- and Fe-related P retention, and P loading; however, because P sorption and post-treatment Al and Fe fractions were not measured, these mechanisms remain plausible rather than directly demonstrated.

The treatment that produced the highest soil available P differed from the treatment that produced the highest numerical plant responses. The highest numerical leaf fresh weight, leaf dry weight, and leaf P accumulation were observed with B3P2 (30 t ha⁻¹ biochar + 90 kg P₂O₅ ha⁻¹). Compared with B0P0, this treatment increased leaf fresh weight from 12.55 to 29.96 g plant⁻¹, leaf dry weight from 4.16 to 8.66 g plant⁻¹, and leaf P accumulation from 0.760 to 2.981 mg P plant⁻¹, respectively.

Increasing P from 90 to 135 kg P₂O₅ ha⁻¹ at 30 t ha⁻¹ biochar raised soil available P significantly, from 218.34 to 250.90 mg kg⁻¹. This gain in soil-extractable P, however, did not carry over into a statistically detectable increase in leaf biomass or leaf P accumulation. Leaf fresh weight moved numerically from 29.96 to 25.88 g plant⁻¹, leaf dry weight from 8.66 to 6.65 g plant⁻¹, and leaf P accumulation from 2.981 to 2.485 mg P plant⁻¹. None of these differences was significant between B3P2 and B3P3. The numerical decreases should not be read as evidence that a higher P rate hurts plant performance.

The difference between soil and plant responses indicated that the highest soil-extractable P was not required to reach the highest numerical leaf biomass or leaf P accumulation in this study. In contrast, the extra P in the Bray-I extractable pool at B3P3 did not lead to a statistically detectable improvement in plant responses during the 50-day experiment.

Plants may respond strongly to increasing P supply when P availability constrains metabolic activity and tissue production. However, once plant P demand is increasingly satisfied, further increases in soil-extractable P do not necessarily result in proportional biomass gains. Physiological P-use efficiency and the allocation of absorbed P may become increasingly important determinants of growth (Han et al., 2022). Therefore, higher Bray-I extractable P does not always lead to higher leaf P accumulation or biomass. The comparison between B3P2 and B3P3 supports this: soil available P increased significantly, but plant responses did not. Similarly, Tesfaye et al. (2021) reported that a biochar-induced increase

in plant P uptake was positively associated with soil P availability, although the relationship was not proportional. In stevia, Tavarini et al. (2018) showed that the highest nutrient use performance did not necessarily occur at the highest P input.

These vegetative responses support this interpretation. Phosphorus increased the leaf number and leaf area, with the leaf number rising from 209.2 leaves plant⁻¹ without mineral P to 271.2 leaves plant⁻¹ at 90 kg P₂O₅ ha⁻¹, while the largest mean leaf area occurred at 135 kg P₂O₅ ha⁻¹. Phosphorus is crucial for photosynthesis, cellular energy processes, nucleic acid synthesis, and membrane functions. A lack of sufficient phosphorus limits tissue growth and biomass accumulation (Lambers, 2022). When P availability is the limiting factor, increasing P supply promotes leaf initiation and expansion. Stevia follows this pattern: improved P nutrition has been shown to boost nutrient uptake and leaf biomass production (Tavarini et al., 2018). Biochar also increased leaf area, but no significant B × P interaction emerged for this variable. Leaf expansion likely drew on several improvements in the root zone environment simultaneously, rather than responding to the same interactive pathway observed for final biomass and leaf P accumulation.

Leaf greenness exhibited different patterns. SPAD increased with biochar, from 51.90 in the control to 56.51 at 30 t ha⁻¹, whereas phosphorus had no detectable effect on SPAD. SPAD readings offer a non-destructive estimate of the relative leaf chlorophyll status and often track plant N supply (Li et al., 2022). Chlorophyll formation and photosynthetic performance are also closely associated with Mg nutrition (Hauer-Jákli and Tränkle, 2019). The SPAD response may be traced to improved root-zone conditions, better nutrient retention, or a shift in the nutrient supply tied to biochar. However, the specific mechanism was not identified here. Soil pH and CEC increased alongside it, which could plausibly have contributed to the greener leaves observed under biochar, but nutrient retention and root responses were never measured directly. Read narrowly, then, the SPAD response signals higher leaf greenness or chlorophyll status under biochar. It does not, on its own, prove enhanced P nutrition.

In contrast, plant height remained statistically unchanged despite substantial shifts in leaf development, leaf P accumulation, and leaf biomass. This does not mean that the treatments failed to stimulate vegetative growth. Rather, the response was channeled into leaf development and biomass

accumulation instead of stem elongation. Tavarini et al. (2018) reported a similar pattern in stevia, where treatment-induced gains in productive growth and branching occurred without any increase in plant height. Here, the narrow height range of 22.92–24.58 cm, set against significant gains in leaf area and leaf biomass, points to the same conclusion: improved nutrition reshaped canopy development and tissue accumulation more than it drove stem elongation.

The TSS of the aqueous leaf extract showed no significant response. Soluble solids were relatively insensitive to biochar and P fertilization under these conditions. This differs from the results of Fauzan et al. (2025), who found that K fertilization raised TSS in stevia, a contrast that hints at nutrient-specific pathways for soluble solid accumulation. °Brix measured from water-extracted dried leaves, however, should not be read as the steviol-glycoside concentration. Steviol glycosides vary with nutrient supply and developmental stage, and reliable assessment requires compound-specific analysis (Tavarini et al., 2020). In other words, the unchanged °Brix here does not rule out treatment effects on stevia quality. Consequently, treatment effects on steviol-glycoside concentration cannot be inferred from the present °Brix measurements.

Therefore, maximizing soil-extractable P may not be the correct management target here. B3P3 gave the highest soil available P (250.90 mg kg⁻¹), but B3P2 produced the highest numerical values for leaf fresh weight, leaf dry weight, and leaf P accumulation. Increasing P from 90 to 135 kg P₂O₅ ha⁻¹ at 30 t ha⁻¹ biochar significantly increased soil available P from 218.34 to 250.90 mg kg⁻¹, but this additional increase was not accompanied by a statistically detectable increase in leaf biomass or leaf P accumulation. Nevertheless, B3P2 should not be interpreted as statistically superior to all other treatment combinations because several combinations produced statistically comparable plant responses. Thus, B3P2 was best regarded as the treatment that produced the highest numerical plant response among the combinations tested, rather than as the demonstrated agronomic optimum.

However, the present study did not identify an economic optimum. The biochar application rate was relatively high. The costs associated with biochar production, transport, incorporation, fertilizer inputs, and the economic value of the resulting biomass were not evaluated. The treatment rates were also discrete rather than fitted to a continuous dose–response function; therefore, the agronomic

optimum may well lie somewhere between the rates tested here. A practical biochar–P recommendation would require field-scale evaluation, complete with dose–response analysis and economic assessment.

Typical constraints associated with short-term pot experiments apply to these findings. Roots were confined to 4 kg of soil, the biochar-amended soil was incubated for only one month before planting, and treatment responses were obtained from a single 50-day vegetative cycle. Biochar does not remain chemically fixed once it enters the soil; aging, oxidation, mineral association, pore modification, and the development of oxygen-containing surface functional groups can alter its reactivity and nutrient-retention behavior over time (Joseph et al., 2021; Liu and Chen, 2022). Therefore, responses measured during the first cropping cycle may not fully represent the long-term behavior of the amendments. Field soils also impose greater spatial and temporal variability in water movement, nutrient redistribution, root exploration, and soil–biochar interactions. Mechanistic interpretation was further limited because exchangeable Al, reactive Fe and Al fractions, P sorption, and soil P fractionation were not measured after treatment. Consequently, the proposed reduction in Al/Fe-mediated phosphate fixation remains an unverified mechanistic interpretation inferred from the concurrent changes in soil pH, CEC, organic C, and Bray-I available P, and should therefore be regarded as correlational rather than causal. A further limitation is that steviol glycosides, the principal economic quality trait of stevia, were not quantified; therefore, °Brix of the aqueous leaf extract cannot substitute for compound-specific glycoside analysis or be used to infer treatment effects on steviol glycoside concentration. Future studies would benefit from longer-term field validation, greater replication, direct measurements of Al/Fe-related P-retention processes and soil P fractions, and compound-specific analysis of steviol glycosides. In addition, leaf P concentrations obtained under pot conditions should be compared with those measured in nearby field- or farm-grown stevia to determine whether the experimental values are higher or lower than representative field levels.

These findings show that rice husk biochar improved several chemical properties of the acidic Ultisol and significantly changed soil P availability in response to P fertilization. Biochar had little effect on available P without mineral P, but there were notable increases when 30 t ha⁻¹ biochar was combined with the two higher P application

rates. Raising P from 90 to 135 kg P₂O₅ ha⁻¹ at this biochar rate significantly increased soil available P from 218.34 to 250.90 mg kg⁻¹. However, this was not accompanied by an additional statistically significant increase in leaf biomass or leaf P accumulation. Therefore, the treatment that resulted in the highest soil-extractable P did not match the one that produced the highest numerical plant response.

CONCLUSIONS

Rice husk biochar enhanced the chemical properties of the acidic Ultisol by raising soil pH, organic carbon, and CEC. Phosphorus fertilization affected plant growth and P nutrition. There were notable interactions between biochar and phosphorus for soil available P, leaf fresh weight, leaf dry weight, and leaf P accumulation. The highest soil available P (250.90 mg kg⁻¹) was found with 30 t ha⁻¹ biochar and 135 kg P₂O₅ ha⁻¹, which was significantly higher than the 218.34 mg kg⁻¹ with the same biochar rate and 90 kg P₂O₅ ha⁻¹. In contrast, the highest numerical leaf fresh weight, leaf dry weight, and leaf P accumulation occurred at 30 t ha⁻¹ biochar combined with 90 kg P₂O₅ ha⁻¹. Therefore, increasing P from 90 to 135 kg P₂O₅ ha⁻¹ at the highest biochar rate significantly increased soil-extractable P but did not lead to a statistically significant increase in leaf biomass or leaf P accumulation.

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