SAINS TANAH – Journal of Soil Science and Agroclimatology

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

Pre-Terra Preta formulation improves forage quality of *Pennisetum purpureum* cv. Mott on marginal land

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ARTICLE INFO

Keywords:
Forage biomass
Forage quality
Marginal soil
Pre-Terra Preta
Soil fertility

Article history
Submitted: 2026-01-28
Revised: 2026-06-08
Accepted: 2026-06-30
Available online: 2026-06-30
Published regularly: June 2026

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ABSTRACT

Marginal tropical soils, particularly Dystric Histosols, pose severe constraints for sustainable forage production due to their low fertility, poor nutrient retention, and low organic matter content. This study evaluated the effect of *pre-Terra Preta* formulations (biochar combined with organic matter and microbial inoculants) on biomass yield and forage quality of *Pennisetum purpureum* cv. Mott in Dystric Histosols of South Kalimantan, Indonesia. Treatments consisted of *pre-Terra Preta* formulations containing 0%, 20%, 40% and 60% biochar within the formulation matrix (w/w), applied at rates of 10, 20, and 30 tons ha^{-1}. Biomass yield was evaluated using nested ANOVA, whereas forage quality attributes (proximate composition, fiber fractions) and soil chemical properties were assessed using Pearson correlation and principal component analysis (PCA). Results demonstrated that biomass yield increased significantly only at ≥ 40% biochar, with the strongest effect observed at 60% biochar, producing nearly a fourfold increase relative to the control. Correlation analysis revealed strong internal coherence among fiber fractions and coordinated relationships among soil nutrient variables, while associations between soil chemical properties and forage quality traits were generally moderate. PCA further highlighted two dominant gradients: PC1 associated with structural fiber fractions, and PC2 reflecting soil fertility variables, underscoring the multivariate nature of soil–plant interactions. Overall, findings demonstrated that *pre-Terra Preta* formulations substantially enhanced forage biomass productivity and influenced forage quality traits in marginal tropical soils. By identifying the threshold effect of ≥40% biochar and the optimal impact at 60%, this study provides practical guidance for rehabilitating degraded landscapes and strengthening the resilience of livestock systems in tropical regions.

How to Cite: Nasution, D. P., Udiansyah, Razie, F., Syarifuddin, N. A., & Neina, D. (2026). *Pre-Terra Preta* Formulation Improves Forage Quality *of Pennisetum purpureum cv Mott* on Marginal Land. Sains Tanah Journal of Soil Science and Agroclimatology, 23(1), 120-130. https://doi.org/10.20961/stjssa.v23i1.115256

1. INTRODUCTION

Marginal tropical soils, particularly Dystric Histosols, present severe constraints for sustainable forage production due to their low fertility, poor nutrient retention, and susceptibility to degradation. These soils are characterized by low cation exchange capacity, high acidity, and low organic matter content, which collectively limit their ability to sustain perennial grasses such as *Pennisetum purpureum* cv. Mott (Kurniawan et al., 2022). This forage species, widely cultivated in Southeast Asia, is a cornerstone of livestock feeding systems, providing high biomass yields under favorable conditions. However, in degraded landscapes dominated by *Imperata cylindrica*, productivity is severely compromised, threatening both forage availability and nutritional quality. Addressing these challenges requires soil management strategies that not only enhance biomass yield but also improve forage quality to support animal nutrition and overall livestock system resilience (Siddiqui, 2025).

STJSSA, p-ISSN 1412-3606 e-ISSN 2356-1424
http://dx.doi.org/10.20961/stjssa.v23i1.115256

One promising approach is the application of biochar-enriched amendments, inspired by the ancient Amazonian Dark Earths (Terra Preta). Biochar, a carbon-rich product derived from biomass pyrolysis, has attracted global attention for its multifunctional role in soil improvement (Hoque et al., 2025). Its porous structure and chemical stability enable it to act as a long-term carbon sink while simultaneously enhancing soil nutrient retention, cation exchange capacity, and microbial activity (Ganesh et al., 2025). However, the magnitude and direction of these effects depend on biochar feedstock characteristics and production conditions, which influence its physicochemical properties. Biochar with favorable physicochemical characteristics, including alkaline pH, porous structure, and strong nutrient-retention capacity, is often considered particularly suitable for tropical soils, where nutrient leaching and poor structural stability are persistent problems. By improving soil physical and chemical properties, biochar can contribute to both immediate agronomic benefits and long-term environmental sustainability (Siddiqui, 2025).

Recent studies emphasize that biochar integration into tropical agroecosystems can significantly improve soil fertility and plant productivity. For example, biochar has been shown to increase soil organic carbon stability, enhance phosphorus availability, and reduce nutrient losses through leaching (Babu et al., 2025). These improvements directly support sustained plant growth and nutrient use efficiency. Moreover, the role of biochar in enhancing water retention is particularly relevant for marginal lands, where conventional fertilizers often fail to deliver lasting improvements due to rapid nutrient depletion and poor soil structure (Eissa et al., 2025). In tropical forage systems, these benefits translate into higher biomass yields and improved resilience against climatic variability.

Building upon this foundation, the concept of pre-Terra Preta formulations represents an innovative adaptation of traditional knowledge to modern agricultural contexts. Inspired by the processes that created Terra Preta soils in the Amazon, these formulations combine biochar with organic matter and microbial inoculants to generate synergistic effects (Evans, 2022; Lehmann & Joseph, 2024; Neina & Agyarko-Mintah, 2023). Ancient Terra Preta soils remain fertile for centuries due to their high carbon content and stable nutrient pools, offering a model for sustainable soil management. By mimicking these processes, pre-Terra Preta formulations are immature forms of biochar-rich amendments that aim to create resilient soil systems that enhance both biomass yield and forage quality (Melo & Sánchez-Monedero, 2024; Murtaza et al., 2023; Ramezanzadeh et al., 2023).

Previous studies have examined the role of biochar in improving soil fertility and plant productivity, but they have not clearly identified the threshold proportion of biochar within *pre-Terra Preta* formulations required to significantly enhance forage biomass yield and quality in marginal soils such as Dystric Histosols (Nasution et al., 2025). This gap is critical because marginal tropical soils are characterized by severe nutrient limitations, low organic matter content, and poor structural stability, which collectively constrain sustainable forage production. Addressing these constraints requires integrated *pre-Terra Preta* formulations that combine biochar, organic matter, and microbial inoculants to stabilize soil fertility and improve forage quality. The novelty of this study lies in establishing the threshold effect of biochar within pre-Terra Preta formulations for forage systems. By systematically testing different biochar proportions within *pre-Terra Preta* (0 – 60%), this research provides new empirical evidence that a specific application rate and formulation of *pre-Terra Preta* can rehabilitate marginal soil and strengthen tropical livestock systems.

This study, therefore, seeks to examine the role of pre-Terra Preta formulations in enhancing biomass yield and forage quality of Pennisetum purpureum cv. Mott cultivated on marginal soils in South Kalimantan, Indonesia. By integrating soil chemistry, proximate composition, and fiber fraction analyses, the research provides empirical evidence for the effectiveness of biochar-enriched amendments in tropical forage production. The study further employs correlation and PCA to elucidate the multivariate relationships linking soil fertility variables with forage quality traits, thereby offering a holistic perspective on soil–plant interactions. Ultimately, the findings contribute to the broader discourse on sustainable soil management and livestock system resilience in degraded tropical landscapes. By demonstrating that significant yield improvements occur only at ≥ 40% biochar, with 60% biochar offering the most pronounced benefits, the study highlights the importance of targeted amendment strategies. These insights not only advance scientific understanding but also provide practical guidance for rehabilitating marginal lands and strengthening tropical livestock systems.

2. MATERIALS AND METHODS

2.1. Site Description and Sampling

The field experiment was conducted at the Jorong Livestock Community Academy, Tanah Laut Regency, South Kalimantan, Indonesia (114°58’1.352” E; – 4°0’30.009” S) (Fig. 1). The site is located in a tropical humid climate zone with annual rainfall ranging from 2,182 to 2,454 mm. According to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2022), the soil at the study site is classified as Dystric Histosol, characterized by low fertility and poor nutrient cycling (Fig. 2). The landscape is ecologically degraded and dominated by *Imperata cylindrica*.

Prior to planting, soil samples were collected using a random composite sampling design. For each plot, five subsamples were taken (four corners and one center) at two depth layers (0–20 cm and 20–40 cm). These subsamples were homogenized to form one composite sample per depth layer, ensuring representation of spatial variability across the site. The baseline soil properties of the experimental site have been reported previously (Nasution et al., 2025), confirming that the Dystric Histosol was characterized by low fertility, acidic pH, and medium to low cation exchange capacity. These baseline data are provided in the supplementary materials.

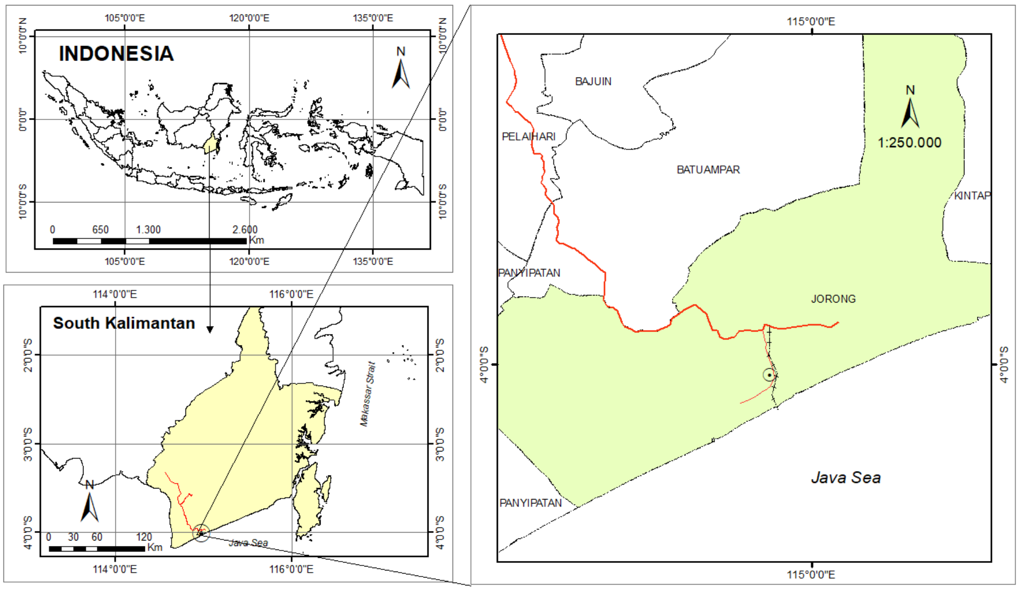

Figure 1. Map of the research location

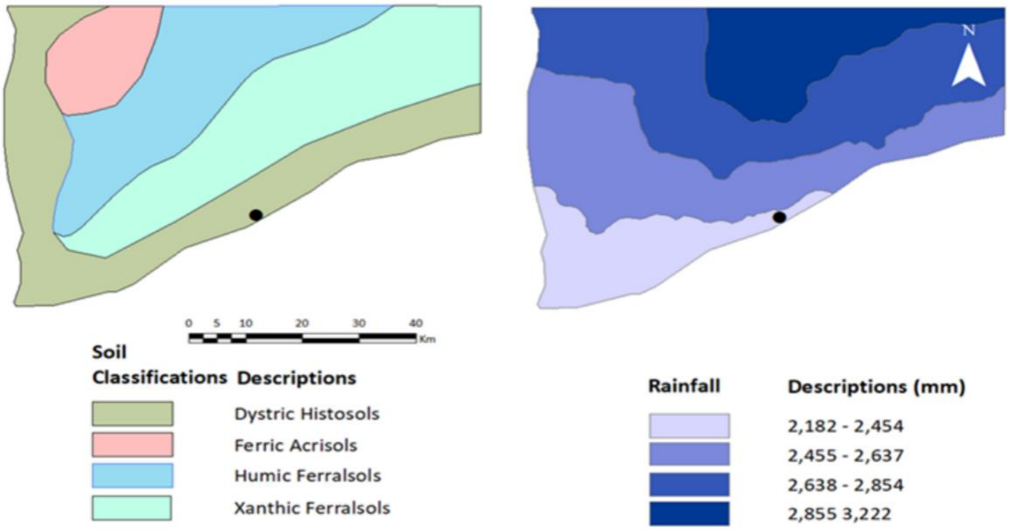

Figure 2. a) Soil map (source: Harmonized World Soil Database Viewer ver. 1.21); b) Rainfall map (source: https://www.chc.ucsb.edu/data/chirps).

2.2. Field Experiment

This study is a continuation of our previous work (Nasution et al., 2025) on the establishment of *Pennisetum purpureum* cv. Mott on marginal land using pre-Terra Preta. The same planting density and plot arrangement were maintained to ensure consistency across studies. Representative field conditions and growth performance of Pennisetum purpureum cv. Mott under *pre-Terra Preta* application during the experimental period are presented in Figure 3. *Pre-Terra Preta* used in this study was prepared following the formulation described by Nasution et al. (2025), consisting of biochar, sawdust (biomass), animal manure, and topsoil as a filler material. The formulation was anaerobically fermented for 15 days using EM4® (Effective Microorganisms 4), a commercially available microbial consortium, which was applied uniformly across all treatments to stimulate microbial activity during substrate preparation.

The field experiment was conducted for ten months (June 2024 to March 2025) on 5 × 5 m plots using a completely randomized design (CRD) in a nested pattern. Two treatment factors were applied. The main factor was the application rate of *pre-Terra Preta* formulation: a1 = 10 tons ha^{-1}, a2 = 20 tons ha^{-1}, and a3 = 30 tons ha^{-1}. The nested factor was the *pre-Terra Preta* formulations composition, consisting of k1 = 0% biochar (non–*pre-Terra Preta*), k2 = 20% biochar, k3 = 40% biochar, and k4 = 60% biochar (w/w). Thus, application rate (tons ha^{-1}) refers to the quantity of *pre-Terra Preta* applied per unit area, whereas biochar proportion (%) refers to the composition of the formulation. The composition factor (k1–k4) was nested within each application rate (a1–a3), and each combination was replicated three times, resulting in 36 experimental units.

Figure 3. Field conditions and growth performance of *Pennisetum purpureum* cv. Mott under *pre-Terra Preta* application on Dystric Histosol during the experimental period.

Fresh biomass yield was measured at three consecutive defoliation (harvest) events at 3, 6, and 9 months after planting, representing early, mid, and late regrowth stages under tropical conditions. For each plot, the mean fresh biomass across the three defoliations was calculated and used as the response variable for statistical analysis, providing a standardized comparison among treatments while avoiding bias that may arise from cumulative yield being disproportionately influenced by a single harvest. In the present study, the focus was on the post-planting phase, beginning with biomass defoliation. For forage quality analysis, subsamples of *Pennisetum purpureum* cv. Mott were collected at the first defoliation. Plant sampling followed a random approach, with five representative plants harvested per plot to capture variability in biomass and nutritional composition. Soil sampling was conducted again after planting to evaluate treatment effects. A total of 12 soil samples (3 application rates x 4 composition levels) were collected at the first defoliation. These samples were analyzed for chemical properties to assess treatment impact, following procedures described in Nasution et al. (2025).

2.3. Forage Quality Analyses

Dried forage subsamples were collected from each treatment plot at the first defoliation. Samples were submitted to the Animal Nutrition Laboratory for chemical analysis. Two analytical groups were requested: 1) Proximate analysis: crude protein (CP), crude fiber (CF), crude fat (EE), and ash; 2) Van Soest fiber fraction analysis (Van Soest, 1994; Van Soest et al., 1991): neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, cellulose, and hemicellulose. All laboratory procedures were performed independently by certified technicians following internal standard operating procedures. The research team did not conduct the analyses directly but ensured that the selected parameters aligned with the study objectives.

2.4. Statistical Analyses

Fresh biomass yield was analyzed using analysis of variance (ANOVA) under a nested completely randomized design (CRD). The main factor was the application rate of pre-Terra Preta (a1–a3), and the nested factor was the composition of pre-Terra Preta within each application rate (k1–k4). Because biomass was measured at three consecutive defoliations, the mean fresh biomass per plot (averaged across the three defoliations) was used as the response variable. When significant effects were detected, Dunnett's test was applied to compare treatment means. All analyses were performed using SPSS version 30.

Forage quality parameters (proximate composition and Van Soest fiber fractions) and soil chemical properties were analyzed using Pearson product-moment correlation. Correlations were computed between: 1) soil chemical properties: C-organic, N-total, Avail. P, Exch. K, pH, and CEC; and 2) forage quality parameters: crude protein (CP), crude fiber (CF), crude fat (EE), ash, NDF, ADF, ADL, cellulose, and hemicellulose. In addition, principal component analysis (PCA) was applied to the same dataset to identify multivariate patterns and gradients linking soil fertility variables with forage quality traits. PCA was performed using RStudio 2025.09.2, retaining components with eigenvalues >1, and results were visualized through correlation circle plots. To summarize the overall analytical workflow, the biomass yield pathway (left) was analyzed using nested ANOVA followed by Dunnett's test to compare treatments against the control (0% biochar). The forage quality and soil–plant relationship pathway (right) was analyzed using Pearson correlation and principal component analysis (PCA) to explore multivariate associations among soil chemical properties, proximate composition, and fiber fractions (Fig. 4).

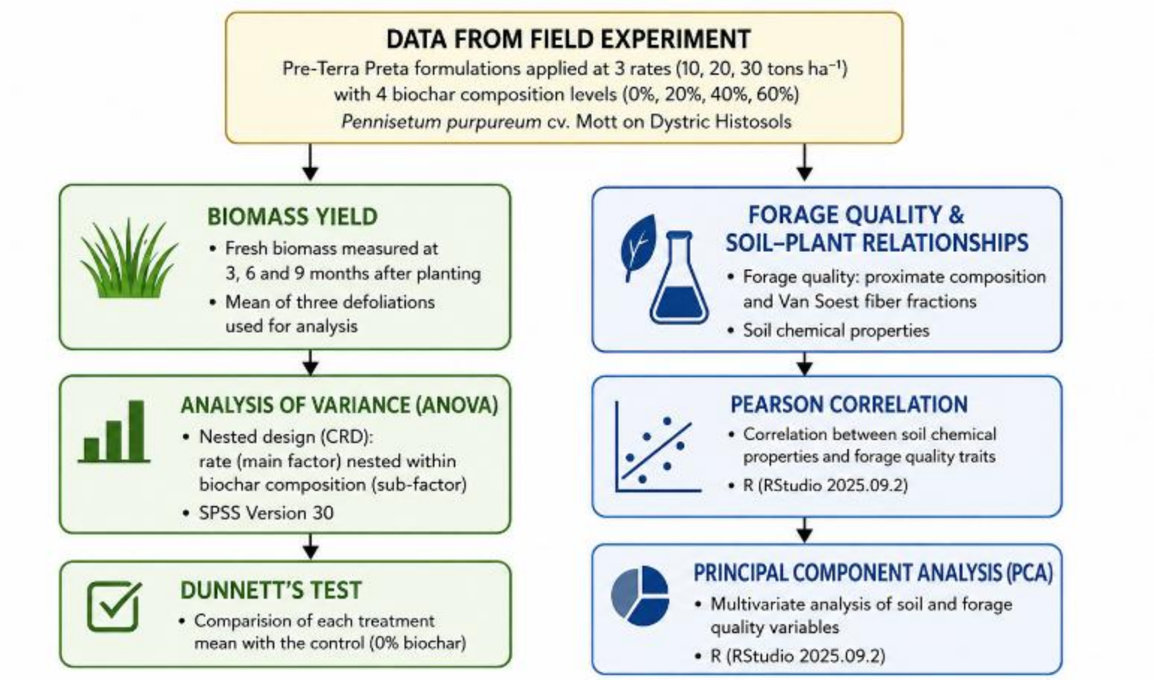

Figure 4. Workflow of the study: statistical analyses for biomass yield and for forage quality-soil-plant relationships

Table 1. Analysis of variance (nested CRD) for mean biomass yield of *Pennisetum purpureum* cv. Mott under *pre-Terra Preta* application

Source	df	Sum of Squares	Mean Square	F	p-value
Application rate (apl)	2	0.329	0.164	7.544	0.003
Composition nested in apl (apl(comp))	9	5.895	0.655	30.055	<0.001
Error	24	0.523	0.022	-	-
Corrected total	35	6.746	-	-	-

Table 2. Dunnett's test comparing *pre-Terra Preta* compositions with the control (non-*pre-Terra Preta*, biochar 0%)

Treatment (composition)	Difference vs control (tons ha^{-1}) ± SE	p-value
Pre-Terra Preta (biochar 20%)	1.153 ± 0.844	0.39 ns
Pre-Terra Preta (biochar 40%)	3.889 ± 0.844	<0.001 **
Pre-Terra Preta (biochar 60%)	10.288 ± 0.844	<0.001**

Remarks: ns = not significant; * = significant at α = 0.05; ** = highly significant at α = 0.01

3. RESULTS

3.1. Biomass Yield

Forage biomass responded positively to increasing proportions of *pre-Terra Preta*. In the control treatment (non-*pre-Terra Preta*, 0% biochar), the mean biomass yield was 3.69 tons ha^{-1}. Increasing the biochar proportion resulted in a consistent rise, with mean yields of 4.84 tons ha^{-1} at 20% biochar, 7.58 tons ha^{-1} at 40% biochar, and 13.98 tons ha^{-1} at 60% biochar. Analysis of variance (nested CRD; Table 1) indicated that *pre-Terra Preta* composition had a highly significant effect on biomass yield ($p < 0.001$).

Dunnett's test (Table 2), using the non-*pre-Terra Preta* treatment as the control, showed that the *pre-Terra Preta* (20% biochar) treatment did not differ significantly ($p = 0.39$). In contrast, the *pre-Terra Preta* (40% biochar) treatment produced a significant increase of 3.89 tons ha^{-1} above the control ($p < 0.001$), while the *pre-Terra Preta* (60% biochar) treatment resulted in a highly significant increase of 10.29 tons ha^{-1} compared with the control ($p < 0.001$). Overall, these results demonstrate that biomass yield begins to increase significantly at ≥40% biochar, with the strongest effect observed at 60% biochar, which produced nearly a fourfold increase relative to the control. These findings confirm that increasing the proportion of pre-Terra Preta substantially enhances forage biomass productivity.

3.2. Forage Quality and Soil-Plant Relationships

3.2.1. Correlation analysis

Pearson correlation analysis revealed distinct relationships among soil chemical properties, proximate composition, and fiber fractions of *Pennisetum purpureum* cv. Mott, as visualized in the correlation heat map (Fig. 5). Among soil chemical properties (Table 3), soil organic carbon (SOC) exhibited a strong positive correlation with available phosphorus ($r = 0.635; p = 0.027$), indicating a coherent association among nutrient-related variables. Cation exchange capacity (CEC) was moderately correlated with SOC ($r = 0.505; p = 0.094$), suggesting a potential linkage between organic matter content and soil nutrient-holding capacity, although this relationship was not statistically significant.

Relationships between soil properties and proximate composition parameters were generally weak to moderate (Table 4). Crude protein content showed weak correlations with most soil chemical variables ($r < 0.400$; $p > 0.050$), indicating limited direct associations. In contrast, crude fat exhibited a significant negative correlation with crude fiber ($r = -0.667$; $p = 0.018$), reflecting contrasting patterns between lipid content and structural carbohydrates. Clear and consistent correlations were observed among fiber fractions. Neutral detergent fiber (NDF) was positively correlated with acid detergent fiber ($r = 0.578$; $p = 0.049$) and cellulose ($r = 0.624$; $p = 0.030$), highlighting the structural interdependence of cell wall components. Acid detergent fiber showed a particularly strong positive association with cellulose ($r = 0.870$; $p < 0.001$). In contrast, acid detergent lignin (ADL) exhibited weak and non-significant negative correlations with cellulose ($r = -0.385$; $p = 0.216$) and hemicellulose ($r = -0.359$; $p = 0.251$), indicating variability in lignification relative to polysaccharide fractions. Overall, the correlation patterns demonstrate strong internal coherence among fiber fractions and coordinated relationships among soil nutrient variables, while associations between soil chemical properties and forage quality traits were generally moderate, suggesting that multiple interacting factors influence forage compositional variability.

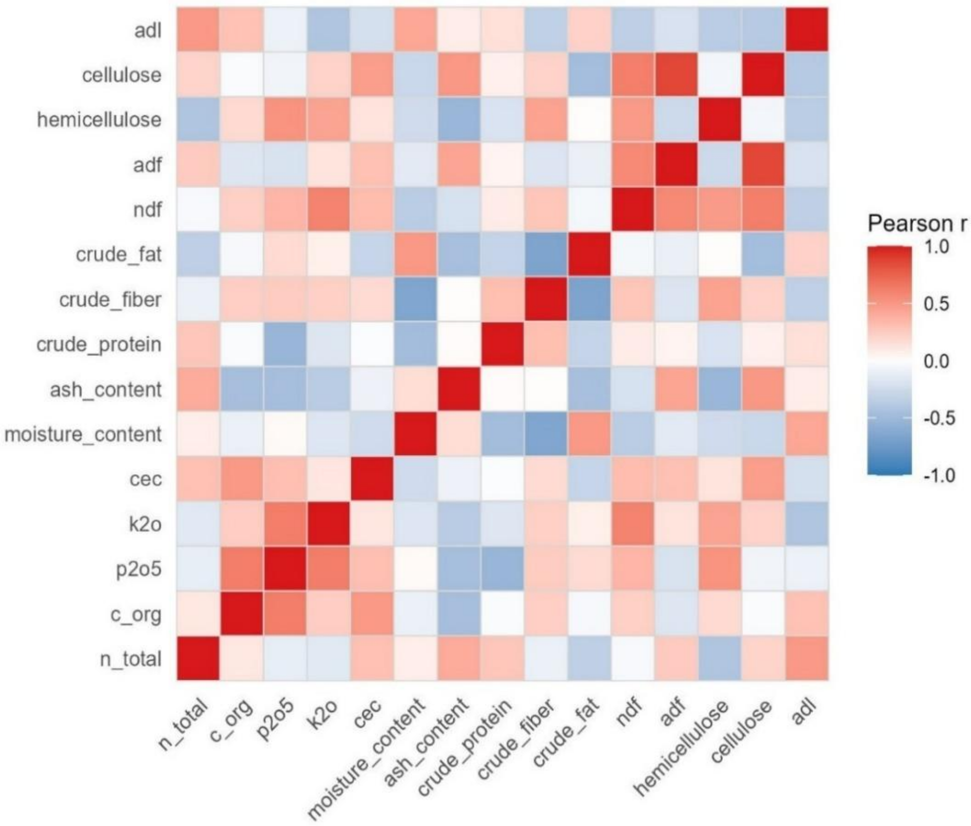

Figure 5. Correlation heat map of soil properties, proximate composition, and fiber fractions

Table 3. Results of soil sample analyses after planting

Treatments	$C_{organic}$	N_{total}	Avail. P	Exch. K	CEC	pH (H_2O)
	g kg^{-1}	g kg^{-1}	g kg^{-1}	cmolc kg^{-1}	cmolc kg^{-1}	
a_1k_1	17.20	3.20	1.65	1.65	34.66	5.60
a_1k_2	15.80	1.80	1.72	1.63	19.40	5.84
a_1k_3	15.10	2.20	0.64	1.63	25.71	6.11
a_1k_4	73.90	2.80	14.37	1.87	64.34	5.95
a_2k_1	25.10	1.60	2.31	1.65	50.66	6.15
a_2k_2	48.70	1.50	7.67	1.63	26.94	6.29
a_2k_3	24.50	1.70	8.96	4.94	33.79	6.26
a_2k_4	62.80	1.80	16.58	3.35	36.62	5.99
a_3k_1	31.60	1.80	12.57	3.67	55.52	6.17
a_3k_2	31.20	2.30	10.23	4.96	45.75	5.96
a_3k_3	76.80	2.10	17.65	5.53	39.95	6.22
a_3k_4	6.90	1.70	15.64	3.41	22.28	6.16
Average	35.80	2.04	9.16	2.93	37.97	6.06

Table 4. Proximate composition of *Pennisetum purpureum* cv. Mott under different *pre-Terra Preta*

Treatments	Moisture	Ash	Crude protein	Crude fiber	Crude fat
			(%)		
a_1k_1	81.67	17.65	15.66	24.36	2.02
a_1k_2	85.00	16.10	13.80	21.00	3.25
a_1k_3	82.67	16.92	12.41	20.16	2.55
a_1k_4	83.67	13.47	11.79	20.63	2.90
a_2k_1	80.86	13.86	14.75	25.64	1.96
a_2k_2	82.67	11.39	12.35	20.95	3.28
a_2k_3	81.00	12.70	13.63	22.90	2.94
a_2k_4	82.33	16.09	10.84	26.39	1.99
a_3k_1	82.00	15.91	11.50	23.10	2.87
a_3k_2	84.00	14.81	11.14	21.04	2.52
a_3k_3	81.67	11.33	14.71	25.03	2.74
a_3k_4	83.67	13.52	9.81	22.32	3.18

3.2.2. Principal component analysis (PCA)

Soil chemical analysis revealed substantial variation in key fertility indicators among *pre-Terra Preta* treatments. Elevated levels of soil organic carbon and available phosphorus were observed in several treatments with moderate to high biochar proportions, particularly a1k4 and a2k4 (60% biochar) and a3k3 (40% biochar). The highest soil organic carbon (76.8 g kg^{-1}) and available phosphorus (17.65 g kg^{-1}) were recorded in a3k3, whereas the highest CEC (64.34 cmolc kg^{-1}) was observed in a1k4. These findings indicate that soil chemical responses varied among treatment combinations rather than following a uniform pattern across application rates. Fiber fraction analysis (Table 5) also showed variation among treatments. NDF values ranged from 67.55 to 75.15% DM, while ADF, ADL, hemicellulose, and cellulose likewise varied across treatment combinations. The highest NDF value was recorded in a3k3, whereas the lowest was observed in a1k2, indicating that fiber responses were not consistently associated with increasing biochar proportion.

Hemicellulose remained stable across treatments, while ADL values were relatively low (2.22–2.91% DM), suggesting minimal lignification despite increased biomass. These results support the PCA findings that structural fiber traits formed a dominant gradient (PC1), reflecting coordinated cell wall synthesis under improved soil conditions.

To further explore the multivariate structure underlying soil–plant relationships, principal component analysis (PCA) was applied using RStudio to soil chemical properties, proximate composition, and fiber fractions, as visualized in the PCA correlation circle (Fig. 6).

The first two principal components explained 49.6% of the total variance, with PC1 accounting for 26.7% and PC2 for 22.9%. PC1 was primarily associated with structural fiber fractions, including neutral detergent fiber (NDF), acid detergent fiber (ADF), and cellulose, indicating a dominant fiber-related gradient. In contrast, PC2 was mainly influenced by soil fertility-related variables such as soil organic carbon (SOC), available phosphorus, exchangeable potassium, and cation exchange capacity (CEC), reflecting a soil nutrient status gradient. Proximate composition variables showed intermediate loadings between these two components, suggesting integrated soil–plant interactions influencing forage quality. The remaining 50.4% of the variance likely reflects the influence of additional factors not fully captured by the measured variables, including micro-environmental heterogeneity, plant-to-plant variability, and other biological processes affecting nutrient uptake and forage development. Such residual variation is common in field-based agroecosystem studies, where multiple interacting factors contribute to plant performance and forage quality.

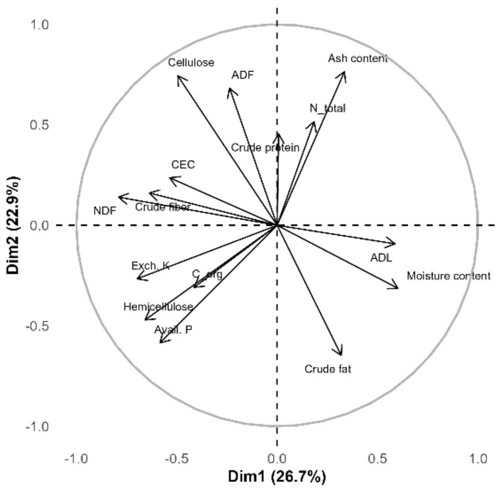

Figure 6. Principal component analysis (PCA) correlation circle showing multivariate relationships among soil chemical properties, proximate composition, and fiber fractions of *Pennisetum purpureum* cv. Mott. The first two principal components explained 49.6% of the total variance (PC1 = 26.7%, PC2 = 22.9%). Vector length and direction indicate the contribution and correlation of variables.

Table 5. Fiber fractions of *Pennisetum purpureum* cv. Mott under different *pre-Terra Preta*

Treatments	NDF	ADF	ADL	Hemicellulose	Cellulose
			% of Dry matter		
a_1k_1	70.17	32.09	2.91	38.08	30.22
a_1k_2	67.55	30.53	2.84	37.02	28.71
a_1k_3	69.33	33.74	2.60	35.59	30.70
a_1k_4	67.88	30.63	2.88	37.25	28.77
a_2k_1	70.06	30.48	2.22	39.58	30.13
a_2k_2	69.99	30.01	2.90	39.98	27.71
a_2k_3	69.32	30.87	2.41	38.45	28.76
a_2k_4	68.78	29.67	2.64	39.11	29.82
a_3k_1	74.78	35.36	2.38	39.42	32.81
a_3k_2	72.14	32.51	2.51	39.63	30.95
a_3k_3	75.15	31.46	2.64	39.69	29.98
a_3k_4	69.48	29.36	2.46	40.12	27.71

4. DISCUSSION

The present study demonstrates that variations in the composition of *pre-Terra Preta* formulations play a decisive role in enhancing forage productivity and quality on Dystric Histosols. The results revealed a clear threshold response, where significant improvements in biomass yield were observed only at ≥40% biochar proportion within the formulation, with optimal performance achieved at 60%. This finding indicates that the effectiveness of *pre-Terra Preta* depends on the balance among its constituent components and their proportional configuration within the formulation matrix. The substantial increase in biomass yield, reaching up to fourfold compared with the non-*pre-Terra Preta* treatments, suggests that higher biochar proportions within the formulation enhanced the overall effectiveness of the amendment system. These responses should be interpreted as the outcome of integrated interactions among biochar, organic materials, microbial inoculants, and soil components rather than the isolated effect of any single constituent.

The observed biomass enhancement can be attributed to the synergistic modification of soil physicochemical properties induced by variations in the composition of *pre-Terra Preta*, particularly at higher biochar proportions within the integrated system. Within this formulation, biochar contributes significantly through its highly porous structure and large specific surface area, which enhances soil organic carbon (SOC) stabilization and increases cation exchange capacity (CEC), thereby improving nutrient retention (Babu et al., 2025; Baquy et al., 2022; Gaurav et al., 2025; Haghighatnia et al., 2024; Nasution et al., 2025; Neina & Agyarko-Mintah, 2023; Neina & Glaser, 2024). In this study, the increase in SOC and available phosphorus (P) under higher biochar treatments suggests that biochar functions as both a nutrient reservoir and a buffer against nutrient leaching, which is particularly critical in highly weathered and acidic soils such as Dystric Histosols.

The sharp biomass response observed at higher biochar proportions within the *pre-Terra Preta* system is consistent with these findings and suggests that a threshold level of the formulation is required to generate a stable soil matrix capable of supporting sustained forage productivity. Notably, the absence of a statistically significant biomass increase at 20% biochar indicates that lower proportions within the amendment system may be insufficient to effectively mitigate nutrient leaching and improve cation retention in highly degraded soils. Similar nonlinear biomass responses to increasing biochar rates have been reported in tropical forage systems, where positive yield effects became evident only beyond moderate application thresholds (DeLuca et al., 2024; Hoque et al., 2025; Lehmann & Joseph, 2024; Nair et al., 2023; Yang et al., 2024). Thus, the present results support the concept that *pre-Terra Preta* acts through cumulative structural and chemical effects rather than through short-term nutrient supply alone. Beyond these physicochemical modifications, the effectiveness of the system is further reinforced by biological processes occurring within the soil matrix. Biochar, as a key component within the *pre-Terra Preta* formulation, likely contributes to improved soil biological functioning. Recent studies have highlighted that biochar provides a favorable microhabitat for soil microorganisms by offering protective niches within its pore structure, thereby enhancing microbial colonization and activity (Ramezanzadeh et al., 2023; Zhang et al., 2022). This microbial stimulation is essential for nutrient cycling processes, including mineralization and immobilization, which regulate nutrient availability for plant uptake. The enhanced microbial activity may also promote root–microbe interactions in the rhizosphere, leading to improved nutrient acquisition efficiency and root development. Consequently, the observed increase in biomass yield can be interpreted as the outcome of a coordinated improvement in soil structure, nutrient dynamics, and biological activity.

These results are further supported by multivariate analyses, where correlation patterns and principal component analysis (PCA) collectively reveal that improvements in soil chemical properties under *pre-Terra Preta* application are strongly interrelated and play a central role in shaping forage productivity and quality. The positive associations among soil organic carbon (SOC), available phosphorus, exchangeable potassium, and CEC indicate a coordinated enhancement of soil nutrient status, consistent with the known behavior of biochar-based amendments in tropical environments. Recent high-impact studies have demonstrated that biochar-rich substrates can increase soil SOC stability while simultaneously improving nutrient availability by enhancing sorption sites and reducing nutrient losses through leaching (Lehmann & Joseph, 2024; Luo et al., 2023; Ramezanzadeh et al., 2023). The moderate to strong correlations between SOC and key macronutrients observed in this study support the interpretation that *pre-Terra Preta* promotes nutrient retention through organic–mineral interactions rather than through direct nutrient release alone. CEC emerged as a central variable in both correlation and PCA analyses, reinforcing its role as a key mediator of soil fertility in marginal soils. Elevated CEC enhances the soil's capacity to retain ammonium, potassium, and calcium ions, thereby stabilizing nutrient supply to plants across growth stages. Similar findings have been reported in tropical pasture systems, where increases in CEC following biochar application were directly linked to improved forage yield and nutrient use efficiency (Antonangelo et al., 2024; Latawiec et al., 2023; Nasution et al., 2025).

One of the most robust patterns emerging from the heatmap and PCA is the strong internal coherence among fiber fractions, particularly neutral detergent fiber (NDF), acid detergent fiber (ADF), and cellulose. These components consistently loaded together along the primary PCA axis, reflecting their shared structural role in plant cell walls. The strong positive correlation between ADF and cellulose ($r = 0.87$) is consistent with established lignocellulosic frameworks, wherein cellulose constitutes the primary structural carbohydrate of the acid detergent fraction. Recent forage quality studies emphasize that such structural coherence is indicative of normal cell wall development rather than stress-induced lignification (Bhandari et al., 2023; Katoch, 2023; Main et al., 2025; Tedeschi et al., 2023). Importantly, acid detergent lignin (ADL) exhibited weaker and partially negative correlations with cellulose and hemicellulose, suggesting that lignin accumulation did not increase proportionally with polysaccharide fractions.

This pattern implies that increased biomass production under higher *pre-Terra Preta* composition did not result in excessive lignification, which would otherwise reduce forage digestibility. Similar decoupling between lignin and structural carbohydrates has been reported in biochar-amended forage systems, where improved nutrient availability promoted cell expansion rather than secondary wall thickening (Dixon et al., 2024). Consequently, the present findings suggest that *pre-Terra Preta* enhances biomass yield without compromising forage quality through excessive lignification. Although biomass production exhibited the strongest quantitative response to increasing *pre-Terra Preta* composition, forage quality should not be interpreted solely in terms of crude protein concentration. In forage systems, feeding value is determined by the combined effects of nutrient composition and structural fiber fractions, including NDF, ADF, cellulose, and lignin, which influence digestibility and nutrient utilization. The relatively low ADL values observed across treatments, together with the coordinated behavior of fiber fractions, indicate that increased biomass production was not accompanied by excessive structural deterioration. Therefore, the benefits of *pre-Terra Preta* formulations arise not only from greater forage availability and nutrient yield per unit area, but also from the preservation of favorable forage-quality characteristics that are important for livestock feeding systems. Proximate composition variables occupied intermediate positions in the PCA space, indicating that these traits respond to both soil fertility gradients and structural carbohydrate development. Crude protein showed weak to moderate correlations with soil nutrients and did not cluster strongly with any single soil chemical variable, reflecting the complex physiological controls governing nitrogen assimilation in forage crops. Recent studies highlight that crude protein content in perennial grasses is often more sensitive to plant developmental stage and nitrogen uptake dynamics than to total soil nitrogen alone (Larsen et al., 2024). The modest correlations observed here suggest that while *pre-Terra Preta* improved soil nitrogen availability, protein accumulation was regulated by multiple interacting factors, including growth rate, biomass dilution effects, and internal nitrogen partitioning.

Importantly, the proportion of variance explained by the first two PCA axes (49.6%) is consistent with expectations for complex agroecosystems and does not indicate analytical weakness. Rather, it reflects the inherent multidimensionality of soil–plant interactions, as emphasized in recent methodological syntheses on multivariate analysis in agronomy (Zhang et al., 2026). From a practical perspective, the results demonstrate that *pre-Terra Preta* formulations with higher biochar proportions can substantially enhance forage biomass while maintaining favorable forage quality characteristics on marginal tropical soils. This outcome has significant implications for sustainable livestock systems in regions where soil degradation and low fertility constrain forage availability. Recent global assessments emphasize that integrated soil amendments combining organic matter, biochar, and microbial inputs represent a promising pathway for restoring productivity on degraded lands while contributing to carbon sequestration goals (Lima & da Penha Simon, 2025). The present study provides empirical support for this approach under tropical conditions and highlights the importance of optimizing biochar proportions to achieve meaningful agronomic benefits.

Despite these promising findings, this study has limitations that should be considered. The experiment was conducted over a single growing season and at a single site, which may restrict the generalizability of results across different environments and climatic conditions. Long-term and multi-location studies are therefore required to validate the persistence and scalability of the observed threshold effects under varying tropical agroecosystems. In addition, future research integrating microbial community dynamics, greenhouse gas emissions, and animal performance is needed to fully elucidate the ecological and practical implications of *pre-Terra Preta* application. Economic feasibility assessments will also be critical to ensure that this approach can be implemented sustainably at larger scales.

5. CONCLUSION

This study showed that increasing the proportion of biochar within *pre-Terra Preta* formulations improved the biomass production of *Pennisetum purpureum* cv. Mott on marginal Dystric Histosol. A clear threshold response was observed, with significant increases occurring at biochar proportions of 40% and above, while the 60% composition gave the highest yield. The results also indicated that *pre-Terra Preta* application was associated with improved soil chemical properties and favorable forage quality characteristics. Overall, these findings suggest that optimized *pre-Terra Preta* formulations have potential as a soil amendment strategy for improving forage production on marginal tropical soils. Further studies under longer-term and broader field conditions are needed to confirm these results.

Declaration of Competing Interest

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

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