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Research Article

Combined Application of Biochar and Arbuscular Mycorrhizal Fungi Enhances Soil Fertility, Antioxidant Defense, and Growth Performance of Cocoa (*Theobroma cacao* L.)

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Abstract

Cocoa (*Theobroma cacao* L.) productivity in tropical agroecosystems is constrained by declining soil fertility, nutrient limitations, and increasing environmental stress driven by climate variability. Sustainable soil management strategies that enhance soil quality and plant resilience are therefore essential for stable cocoa production. This study evaluated the effects of biochar (40 t ha^{-1}) and arbuscular mycorrhizal fungi (AMF; 10 g $plant^{-1}$), applied individually and in combination, on soil physicochemical properties, enzymatic activities, antioxidant responses, and field performance of young cocoa plants. The experimental soil was slightly acidic and nutrient-deficient with low organic matter content. Both sole and combined applications of biochar and AMF significantly improved soil chemical properties and plant growth compared with the control. Soil respiration was influenced by amendments, with basal respiration following the order Control > AMF > Biochar + AMF > Biochar, while substrate-induced respiration showed no significant treatment effects. Soil phosphatase activities (acid and alkaline phosphatase) were significantly enhanced by amendments, with the highest activities observed under the combined biochar + AMF treatment, indicating improved phosphorus cycling and nutrient availability. Biochar and AMF also altered plant biochemical and antioxidant responses. Biochar alone induced higher proline accumulation and antioxidant enzyme activities (SOD and GPx), suggesting a stress-priming effect. In contrast, the combined treatment enhanced photosynthetic pigments, soluble sugars, non-structural carbohydrates, and antioxidant enzymes (CAT, SOD, and GPx), indicating improved physiological performance and metabolic balance. Seasonal variation significantly influenced plant metabolism, with the dry season promoting accumulation of osmoprotectants and stronger antioxidant defense, while the rainy season increased chlorophyll, carotenoids, and phenolic content. Overall, the integrated application of biochar and AMF improved soil fertility, enhanced enzymatic and antioxidant functions, and promoted cocoa growth under field conditions. These findings demonstrate that biochar–AMF synergism is a promising climate-smart strategy for improving soil functionality and sustaining cocoa production in tropical agroecosystems.

Keywords: Cacao, organic, amendment, soil, enzyme, metabolities, performance .

1. Introduction

Cocoa (*Theobroma cacao* L.) is a globally important perennial crop that provides substantial economic, nutritional, and industrial benefits. It serves as a major source of raw materials for chocolate production, income for smallholder farmers, and foreign exchange earnings for producing countries [1]. Cocoa cultivation supports the livelihoods of millions of people across tropical regions in Africa, Asia, and the Americas, which contribute approximately 63.2%, 17.4%, and 14.1% of global production, respectively [2-4]. Major producing countries include Côte d'Ivoire, Ghana, Indonesia, Cameroon, Ecuador, and Nigeria, where production is largely dominated by smallholder systems [5-7]. Beyond its economic relevance, cocoa-derived products are rich in flavonoids and antioxidants, contributing to human health by reducing the risk of cardiovascular diseases, cancer, and other age-related conditions [8].

Despite its importance, cocoa productivity is highly constrained by suboptimal soil fertility and increasing environmental stresses. Cocoa is predominantly cultivated under rainfed conditions and is highly sensitive to variations in soil moisture, temperature, and nutrient availability [9-10]. In West Africa, annual rainfall is typically below 2000 mm and occurs in a bimodal distribution, exposing cocoa systems to alternating wet and dry seasons that can negatively affect

plant growth and yield [10-13]. Climate change has further intensified these challenges, increasing the frequency of drought and extreme weather events. Notably, cocoa mortality rates of up to 70% within two years of field establishment have been reported due to drought stress, posing a serious threat to sustainable production [12-14].

To address these constraints, climate-smart and sustainable soil management practices are increasingly being explored. Among these, the application of organic amendments and beneficial microorganisms has gained attention for improving soil fertility, plant resilience, and overall system productivity [15-17]. Biochar, a carbon-rich material derived from the pyrolysis of organic biomass, has been widely reported to enhance soil structure, water retention, cation exchange capacity, and nutrient availability [15, 16]. Its porous structure promotes microbial colonization and reduces nutrient leaching, thereby creating a more stable and fertile soil environment [17-20]. Similarly, arbuscular mycorrhizal fungi (AMF), belonging to the phylum Glomeromycota, form mutualistic associations with plant roots and play a critical role in nutrient acquisition as well as in enhancing plant tolerance to abiotic and biotic stresses [21-23]. AMF are increasingly recognized as effective biostimulants in sustainable agriculture due to their ability to improve nutrient cycling, soil aggregation, and plant health under adverse environmental conditions [22-24]. Recent studies suggest that the co-application of biochar and AMF may offer synergistic benefits by enhancing microbial activity, carbon and nitrogen mineralization, and nutrient uptake, ultimately improving plant growth and stress resilience [21, 22]. Biochar can also act as a carrier matrix for microbial inoculants, facilitating the establishment and activity of AMF in soil systems. Such integrated approaches are considered promising strategies for improving agricultural sustainability under climate variability [24, 25].

Under field conditions, plants are frequently exposed to multiple abiotic stresses, including drought, salinity, extreme temperatures, and soil nutrient imbalances, all of which can disrupt physiological and biochemical processes [26-28]. These stress conditions often lead to the excessive production of reactive oxygen species (ROS), which can damage cellular components such as lipids, proteins, nucleic acids, and carbohydrates [28-30]. The imbalance between ROS generation and antioxidant defense mechanisms results in oxidative stress, which adversely affects plant growth and productivity. Plants have evolved complex defense systems to mitigate oxidative stress, including enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as non-enzymatic antioxidants and osmolytes [31-32]. Osmolytes such as proline, soluble sugars, and glycine betaine play critical roles in osmotic adjustment, ROS scavenging, and stabilization of cellular structures under stress conditions [28-30]. These biochemical responses are essential for maintaining cellular homeostasis and enhancing plant tolerance to environmental stresses.

Although the individual effects of biochar and AMF on soil properties and plant performance are well documented, there remains limited understanding of their combined influence on soil biochemical processes, antioxidant enzyme activities, and metabolite dynamics in field-grown cocoa systems. In particular, the role of these amendments in modulating stress-responsive mechanisms and improving resilience under fluctuating environmental conditions requires further investigation. Therefore, this study was designed to evaluate the sole and combined effects of biochar and AMF on (i) soil physical and chemical properties, (ii) soil respiration and enzyme activities (acid and alkaline phosphatase), (iii) active metabolites including photosynthetic pigments, osmolytes, and antioxidant enzymes, and (iv) growth performance of young cocoa plants under field conditions. It is hypothesized that the integrated application of biochar and AMF will enhance soil quality, stimulate biochemical and physiological responses, and improve cocoa resilience to environmental stress.

2. Materials and Methods

2.1 Study site and conditions

The experiment was carried out at the Experiment Station of the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure (FUTA), Nigeria. Akure is located in the rainforest zone of south-western Nigeria (latitude 7°15′N, longitude 5°15′E). The site has a bimodal rainfall pattern with mean annual rainfall of approximately 1400–1500 mm, and mean daily temperatures ranging from 22 °C to 28 °C. A two-year-old cocoa field was demarcated into plots for treatment application.

2.2. Experimental materials, treatments, and design

2..2.1. Biochar production and AMF inoculum

Biochar was produced by slow pyrolysis of sawdust at 400 °C following the methods described by Adekiya et al. [15] and Oni et al. [16]. Arbuscular mycorrhizal fungi (AMF) inoculum, comprising a mixed species formulation containing Rhizophagus irregularis, Funneliformis mosseae, and Gigaspora margarita, was obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

2.2.2. Treatment application and experimental design

The treatments consisted of: (i) sole biochar, (ii) sole AMF, (iii) biochar + AMF (co-application), and (iv) non-amended control. Biochar was applied at a rate of 40 t ha^{-1} (≈3 % w/w in the top 15 cm soil), which is within the optimum range (1–4 %) recommended for humid tropical soils [15, 17]. AMF was inoculated at 10 g per plant, placed directly below the cocoa seedlings at transplanting. The treatments were arranged in a randomized complete block design (RCBD) with four replications. Each plot contained ten young cocoa trees (clone 'F3 Amazon') planted at a spacing of 3 m × 3 m.

2.1. Soil sampling and laboratory analyses

2.1.1. Soil chemical properties

Pre-experiment and post-experiment soil samples were collected from 0–20 cm depth using a soil auger. Soil pH

(water, 1:2.5 soil:water ratio) was measured with a glass electrode pH meter. Soil organic matter (SOM) was determined by the loss-on-ignition method, and total organic carbon (TOC) by the Walkley-Black method [33]. Total nitrogen (N) was analysed by the micro-Kjeldahl method [34]. Available phosphorus (P) was extracted using Bray-1 solution and quantified colorimetrically [35]. Exchangeable potassium (K), calcium (Ca), and magnesium (Mg) were extracted with 1 M ammonium acetate (pH 7.0) and measured by atomic absorption spectrophotometry. Cation exchange capacity (CEC) was determined by the ammonium acetate method.

2.1.2. Soil microbial respiration

Basal and substrate-induced respiration were measured using the gas entrapment method [36]. For basal respiration, 50 g of field-moist soil (sieved to 2 mm) was placed in 500 mL airtight glass jars. A beaker containing 10 mL of 0.5 M NaOH was placed inside each jar to trap evolved CO_2. Blank jars (without soil) served as controls. The jars were incubated at 25 °C for 21 days, and the NaOH traps were replaced weekly. For substrate-induced respiration, the same procedure was followed after adding 1 % (w/w) glucose to the soil. The trapped CO_2 was determined by titrating the excess NaOH with 0.5 M HCl after precipitating carbonates with 1 M $BaCl_2$. Respiration rates were expressed as mg CO_2-C kg^{-1} soil day^{-1}..

2.1.3. Soil enzyme activities (acid and alkaline phosphatase)

Acid phosphatase (ACP, EC 3.1.3.2) and alkaline phosphatase (ALP, EC 3.1.3.1) activities were assayed using p-nitrophenyl phosphate (pNPP) as substrate [37]. One gram of fresh soil was incubated with 0.2 mL of toluene, 4 mL of modified universal buffer (MUB, pH 6.5 for ACP and pH 11 for ALP), and 1 mL of 0.025 M pNPP at 37 °C for 1 h. After stopping the reaction with 0.5 M $CaCl_2$ and 0.5 M NaOH, the released p-nitrophenol was measured spectrophotometrically at 410 nm. Activity was expressed as μg p-nitrophenol g^{-1} soil h^{-1}.

2.2. Plant Sampling and Biochemical Analyses

2.2.1. Photosynthetic pigments

Fresh leaves (third leaf from the apex) were collected between 9:00 and 10:00 a.m. Chlorophyll a, chlorophyll b, and total carotenoids were extracted in absolute ethanol (1 h at 60 °C in a water bath). After cooling, absorbances were read at 664, 648, and 470 nm using a spectrophotometer (UV-1800, Shimadzu, Japan). Pigment concentrations were calculated according to Lichtenthaler [38] and Wellburn [39] and expressed as mg g^{-1} fresh weight.

2.2.2. Proline content

Proline was determined following Bates et al. [40]. Fresh leaf tissue (0.5 g) was homogenized in 10 mL of 3 % sulfosalicylic acid and centrifuged. The supernatant (2 mL) was reacted with 2 mL of acid ninhydrin and 2 mL of glacial acetic acid at 100 °C for 1 h. After cooling, the complex was extracted with 4 mL of toluene and the absorbance read at 520 nm. Proline concentration was calculated from a standard curve and expressed as mg g^{-1} fresh weight.

2.2.3. Lipid peroxidation (malondialdehyde, MDA)

MDA content was measured as an indicator of lipid peroxidation using the thiobarbituric acid (TBA) method [41]. Leaf samples (0.25 g) were homogenized in 5 mL of 0.1 % trichloroacetic acid (TCA) and centrifuged. The supernatant (1 mL) was mixed with 4 mL of 0.5 % TBA in 20 % TCA, heated at 95 °C for 30 min, then quickly cooled. Absorbance was read at 532 nm and corrected for non-specific turbidity at 600 nm. The MDA concentration was calculated using an extinction coefficient of 155 $mM^{-1}cm^{-1}$ and expressed as nmol g^{-1} fresh weight.

2.2.4. Antioxidant enzyme assays

Fresh leaf tissue (0.5 g) was homogenized in 5 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1 % polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12,000×g for 20 min at 4 °C, and the supernatant was used for enzyme assays.

Catalase (CAT, EC 1.11.1.6) activity was assayed by monitoring the decrease in absorbance at 240 nm due to H_2O_2 decomposition [42]. The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2, and enzyme extract. Activity was calculated using an extinction coefficient of 39.4 mM^{-1} cm^{-1} and expressed as μmol H_2O_2 decomposed min^{-1} mg^{-1} protein.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined by measuring the inhibition of nitroblue tetrazolium (NBT) photochemical reduction [43]. One unit (U) of SOD was defined as the amount of enzyme required to inhibit 50 % of NBT reduction. Activity was expressed as U mg^{-1} protein.

Glutathione peroxidase (GPx, EC 1.11.1.9) activity was assayed by following the oxidation of glutathione (GSH) in the presence of H_2O_2 using NADPH and glutathione reductase [44]. The decrease in absorbance at 340 nm was recorded. Activity was expressed as nmol NADPH oxidized min^{-1} mg^{-1} protein.

2.3. Soluble sugars and non-structural carbohydrates (NSC)

Soluble sugars (glucose, fructose, sucrose) were extracted from dried leaf powder (0.1 g) with 80 % ethanol at 80 °C. After centrifugation, the supernatant was used for sugar determination by the anthrone method [45]. Non-structural carbohydrates (NSC) were calculated as the sum of soluble sugars plus starch (starch was determined after enzymatic digestion). Results were expressed as mg g^{-1} dry weight..

2.4. Plant Growth Variables

At monthly intervals (July 2024 to June 2025), the following growth parameters were recorded: stem height (from soil surface to the apical meristem) using a measuring tape, number of primary branches, root length (after careful excavation), and fresh weights of roots, stems, and leaves using a digital balance (accuracy ±0.1 g).

2.5. Statistical analysis

The experiment was conducted in four replications, and the

collected data were analyzed using analysis of variance (ANOVA). When significant effects were detected, treatment means were separated using Tukey's Honest Significant Difference (HSD) test at the 5% probability level ($P < 0.05$). All figures were prepared using Microsoft Excel 2016.

3. Results

3.1. Soil chemical properties at commencement and termination of experiment

The experimental soil had a mean pH of 4.7, indicating nutrient deficiencies and low microbial activity (Table 1). The nitrogen level of 0.2% was also very low, as nitrogen is an essential nutrient for plant growth and cocoa plants require adequate nitrogen for optimal development. Potassium (K) was 71.5 mg/kg, which is a relatively low level for cocoa production, particularly for fruit development and disease resistance. Phosphorus (P) was 13.4 mg/kg, which is a very low level, crucial for root development, fruiting, and overall plant growth. Sodium (Na) was 27.4 mg/kg, representing a moderate level. Calcium (Ca) was 30.8 mg/kg, a relatively low level important for cell wall development, root growth, and nutrient uptake. The low potassium and phosphorus levels indicated nutrient deficiencies, while low calcium levels could impact plant growth and development.

Regarding soil biological properties, bacterial colony-forming units (cfu) showed low to moderate levels of beneficial bacteria, indicating reduced soil fertility and structure. Yeast and fungi spore-forming units suggested some fungal activity, but still at relatively low levels. The low levels of fungal activity have implications for organic matter decomposition and nutrient cycling. Nematode counts (mean of 114/275g) were relatively high, indicating some soil disturbance or stress, potentially affecting cocoa plant growth (Table 1).

Although the soil of the experimental site was slightly acidic and low in critical nutrient elements (N, P, K, Ca, and Mg) including organic matter, soil fertility status improved across all treatments at the termination of the experiment compared with the initial conditions (Table 2). Following amendment with biochar, AMF, and their combination, soil pH increased slightly, and fertility status improved as exemplified by increases in the values of organic carbon, N, P, K, and Na (Table 2). Soil pH improved best with biochar+AMF treatment, as did organic matter, organic carbon, potassium, and CEC, while values of N and P were also highest for the biochar + AMF amendment (Table 2).

Table 1. Baseline soil physiocochemical properties at the commencement of the experiment

Soil Parameters	Value
Clay (%)	8.8
Silt (%)	21.5
Sand (%)	69.7
Bacteria colonyforming unit:(cfu)	13.00×10^7
Yeast spore forming units: (sfu)	10.80×10^7
Fungi spore forming unit (sfu)	4.73×10^2
Nematodes count (100g sample)	251
Soil pH	4.7
N (g g^{-1})	0.2
P (mg kg^{-1})	13.4
K (cmol kg^{-1})	71.5
Ca (cmol kg^{-1})	30.8
NA (cmol kg^{-1})	27.4
Organic carbon (g g^{-1})	3.91

Note: N = total nitrogen; P = available phosphorus; K = exchangeable potassium; Ca = exchangeable calcium; Na = exchangeable sodium; cfu = colony forming units; sfu = spore forming units.

3.2. Effects of AMF and Biochar amendments on soil basal respiration

Across the incubation days, all treatments followed a similar respiration trend with fluctuations at different incubation periods. Peaks occurred around day 2 and day 3 (higher respiration), followed by a decline and moderate stabilization by day 21. The control treatment showed the highest mean respiration (18.27), followed by AMF (17.98), Biochar + AMF (16.12), and Biochar (15.92). Across the treatments, the differences were statistically not significant. The lack of significant variation suggests that biochar, AMF, or their combination did not alter basal respiration compared to the control. This may indicate that soil microbial activity was stable across treatments, or that the incubation period was too short or conditions too uniform to highlight treatment effects. The natural fluctuations (high activity at day 2–3) likely reflect microbial responses to substrate availability rather than treatment effects (Table 3).

Table 2. Effects of AMF and Biochar amendments on soil chemical properties.

Treatment	**pH**	**OC**	**OM**	**N**	**P**	**K**	**Na**
	(water)	**(%)**	**(%)**	**(%)**	**mg/kg**	**(cmol//kg)**	(cmol//kg)
Biochar	5.68a	1.15a	1.98b	0.46a	5.44c	0.46a	0.63ab
AMF	5.78a	1.33a	2.29a	0.50a	9.36a	0.49a	0.67a
Bio +AMF	5.80a	1.26a	2.17a	0.53a	7.86b	0.34b	0.53bc
Control	5.60a	1.19a	2.04b	0.32b	5.30c	0.31b	0.51c

Table 3. Effects of AMF and biochar amendment on soil basal respiration.

	Days After Incubation						
Treatment	**1**	**2**	**3**	**7**	**14**	**21**	**Mean**
Biochar	13.4a	24.6a	13.4a	14.8a	21.0b	14.7a	16.98
AMF	16.1a	13.5b	14.2a	14.4a	17.5b	14.7a	15.06
Bio +AMF	16.4a	19.6ab	13.3a	15.0a	19.0b	16.0a	16.55
Control	15.0a	26.3a	16.5a	15.0a	25.5a	16.2a	19.08

Table 4. Effects of AMF and biochar amendment on soil substrate-induced respiration.

Treatments	**ACP Activity**	**ALP Activity**
Biochar	38.61b	26.89b
AMF	35.22b	14.39c
Bio +AMF	46.89a	40.78a
Control	19.89c	10.50d

Table 5. AMAMF and biochar amendment effects on acid and alkaline phosphatase.

	Days After Incubation						
Treatment	**1**	**2**	**3**	**7**	**14**	**21**	**Mean**
Biochar	12.1a	17.5a	13.9a	15.3a	22.3a	14.4a	15.92a
AMF	14.8a	22.6a	21.2a	15.5a	17.4a	16.4a	17.98a
Bio +AMF	13.8a	15.4a	19.9a	14.9a	15.2a	17.5a	16.12a
Control	14.9a	23.4a	21.2a	13.6a	21.2a	15.3a	18.27a

Table 6. Effects of AMF and biochar amendments on metabolite constituents and antioxidant activity of cocoa.

Treatments	Catalase Activity (U/(uM/min)	Total Phenol mg/g	SOD Activity (U/g)	Carotenoid (mg/kg)	GPx (uM/g)
Biochar	17.07c	20.81b	0.80bc	0.062b	4347.52b
AMF	33.18b	24.76a	2.13b	0.075a	5801.42a
Bio +AMF	21.23b	18.40c	0.45c	0.048c	3475.18c
Control	23.22a	14.11d	6.13a	0.021d	2751.77d

Table 7. Effects of AMF and biochar amendments on metabolite constituents and antioxidant activity of cocoa

Treatments	Proline (mg/g)	CAT (μM/mg)	Total chlorophyll (mg/g)	Fructose (mg/g)	Sucrose (mg/g)	Glucose (mg/g)	NSC (mg/g)
Biochar	0.0145a	148.53a	31.14b	138.14bc	140.33	103.78b	1.37a
AMF	0.0128a	137.43b	37.32a	141.62a	143.4a1a	118.53a	1.40a
Bio +AMF	0.0119a	121.53b	44.93a	144.64a	148.22a	99.19b	1.41a
Control	0.0178b	177.56a	23.42b	128.55	132.42b	71.43c	1.28b

Table 8. Metabolite constituents and antioxidant activity of cocoa (rainy and dry season observation).

Season	Catalase Activity (uM/min)	Total Phenol mg/g	SOD Activity (U/g)	Carotenoid (mg/kg)	GPx (uM)	Proline (mg/g)
Rainy	17.43	19.53	2.91	0.052	4093.93	0.0138
Dry	22.04	13.63	4.15	0.013	2115.51	0.0184

Table 9. Metabolite constituents and antioxidant activity of cocoa (rainy and dry season observation).

Season	DPPH (%)	CAT (μM/mg)	Total chlorophyll (mg/g)	Fructose (mg/g)	Sucrose (mg/g)	Glucose (mg/g)	NSC (mg/g)
Rainy	71.63	168.53	31.32	133.14	141.33	116.63	1.43
Dry	84.16	197.32	22.41	176.08	184.33	175.23	0.95

3.3. Effects of AMF and Biochar amendments on substrate-induced respiration

At the initial stage (Day 1), there was no significant difference, as microbial respiration rates were similar across treatments at the start. On Day 2, control and biochar showed significantly higher respiration than AMF, suggesting that biochar and control supported a rapid microbial flush early in incubation, while AMF initially suppressed respiration. On Day 3 and Day 7, there were no significant differences, as respiration stabilized across treatments, indicating that microbial activity was balanced. On Day 14, control (25.5a) remained highest, followed by biochar, biochar + AMF, and least was AMF (17.5b). This suggests that native microbes in the control soil were most active, while amendments moderated microbial respiration. On Day 21, there were no significant differences, and respiration converged again.

The control showed the highest overall mean values, followed by biochar, biochar + AMF, and AMF, indicating that amendments generally reduced average soil respiration compared to the control. Control soil had the highest respiration, suggesting that native microbes were highly active—possibly due to limited nutrient balance, causing microbes to respire more as a stress response. Biochar and biochar+AMF moderated respiration, reflecting more efficient microbial metabolism and carbon stabilization, which reduces rapid CO_2 release. AMF alone showed the lowest mean respiration, suggesting that AMF may regulate microbial communities and reduce excessive respiration, possibly through better nutrient use efficiency (Table 4).

3.4. Effects of AMF and Biochar amendment on acid phosphatase and alkaline phosphatase

The acid phosphatase (ACP) and alkaline phosphatase (ALP) activities were lowest in the control, indicating that without amendments, soil enzyme activities were suppressed (Table 4). With biochar alone, ACP was significantly higher than the control, and ALP was also higher than the control, implying that biochar improves soil phosphate activity likely by enhancing soil organic matter and microbial habitat. With AMF, ACP was significantly higher than the control, similar to biochar. However, AMF in ALP was higher than the control but much lower than biochar, indicating that AMF mainly boosts acid phosphatase (ACP), but is less effective than biochar for ALP (Table 4).

The biochar+AMF combined treatment showed higher ACP than ALP overall activity, demonstrating a synergistic effect: combining biochar and AMF enhanced enzyme activity more than either alone. ACP is involved in organic phosphorus mineralization under acidic conditions, while ALP functions in neutral to alkaline soils. The increase in both enzymes under biochar+AMF suggests improved phosphorus cycling and availability. The ranking of effectiveness was: ACP: Bio + AMF > Biochar ≈ AMF > Control; ALP: Bio + AMF > Biochar > AMF > Control (Table 5).

3.5. Soil amendment effects on metabolite and antioxidant activity of cocoa

The leaf chlorophyll contents of cocoa differed significantly among the amendments (Table 6 and 7). Soil amendment effects differed on leaf contents of carotenoids, proline, SOD, phenol, GPx, and CAT. Effects of amendment on glutathione (GSH) and glutathione peroxidase (GPx) activity of cocoa showed similar response patterns. Catalase activity (CAT) of cocoa differed under the amendments. For biochar alone, proline, SOD, and GPx were highest, while these variables had lowest values for biochar + AMF. The combination of

biochar and AMF had the highest chlorophyll concentrations as well as sucrose and NSC contents. These combinations also produced highest proline, carotenoids, SOD, phenol, CAT, fructose, and glucose in cocoa leaves. The organic amendments enhanced the leaf contents of proline and SOD, phenol, GPx, and CAT in addition to high contents of fructose and NSC compared with the non-amendment (Table 6 and 7).

The metabolite constituents and antioxidant activity of cocoa were observed during rainy and dry seasons. Catalase activity was higher in the dry season, suggesting stronger enzymatic antioxidant defense in dry conditions. SOD (superoxide dismutase) was higher in the dry season, indicating enhanced detoxification of superoxide radicals during dry season (Table 8). Glutathione peroxidase (GPx) was much higher in the rainy season when plants rely more on GPx for peroxide detoxification. Catalase (CAT) contents in cocoa leaves were higher in the dry season, which aligns with GPx activity. The antioxidant enzyme activity shifts with season; dry season favors catalase and SOD, while rainy season favors GPx. Total phenol was higher during the rainy season (improved soil water availability enhanced accumulation of phenolic compounds and antioxidant activity). Carotenoids were also higher in the rainy season, which helps in the formation of more protective pigments (Table 8). Total chlorophyll was favored in the rainy season with positive implications for photosynthetic capacity during the wet period of the year. The rainy season also promoted other secondary metabolites (phenols, carotenoids, chlorophylls).

Despite lower phenols in the dry season, cocoa had higher antioxidant activity and possibly increased enzyme action and stress-induced metabolites. In the rainy season, non-structural carbohydrate production was enhanced compared with the dry season (Table 9). The sugars and non-structural carbohydrates (NSC) concentration in leaves were higher in the rainy season, while fructose, sucrose, and glucose were higher during the rainy season. In the dry season, cocoa accumulated more simple sugars (glucose, fructose, sucrose: the osmoprotectants). Antioxidant enzyme activities differed between the rainy and dry seasons. Proline was high in the dry season, a stress indicator which accumulates more under drought. High proline accumulation may indicate greater physiological stress for plants in the dry season. The accumulation of antioxidant compound (DPPH) was higher in the dry season period when radical-scavenging activity increased (Table 9).

3.6. 6. Amendment effects on growth variables of cocoa

3.6.1. Branching

The effects of biochar, AMF, their combination, and non-amendment (control) on branching are shown in figure 1. AMF alone had the highest effect on the number of branches of young cocoa seedlings compared with other treatments, followed by the biochar and AMF combined application. Observations were recorded during the rainy (July to November, 2024) and dry seasons (January to June, 2025). The non-amended trees had the lowest number of branches compared with the amended treatments. AMF alone significantly improved the number of branches both during rainy (wet) and dry periods of the year. The effect decreased in this order: sole AMF > biochar > AMF-biochar combination > control (least value).

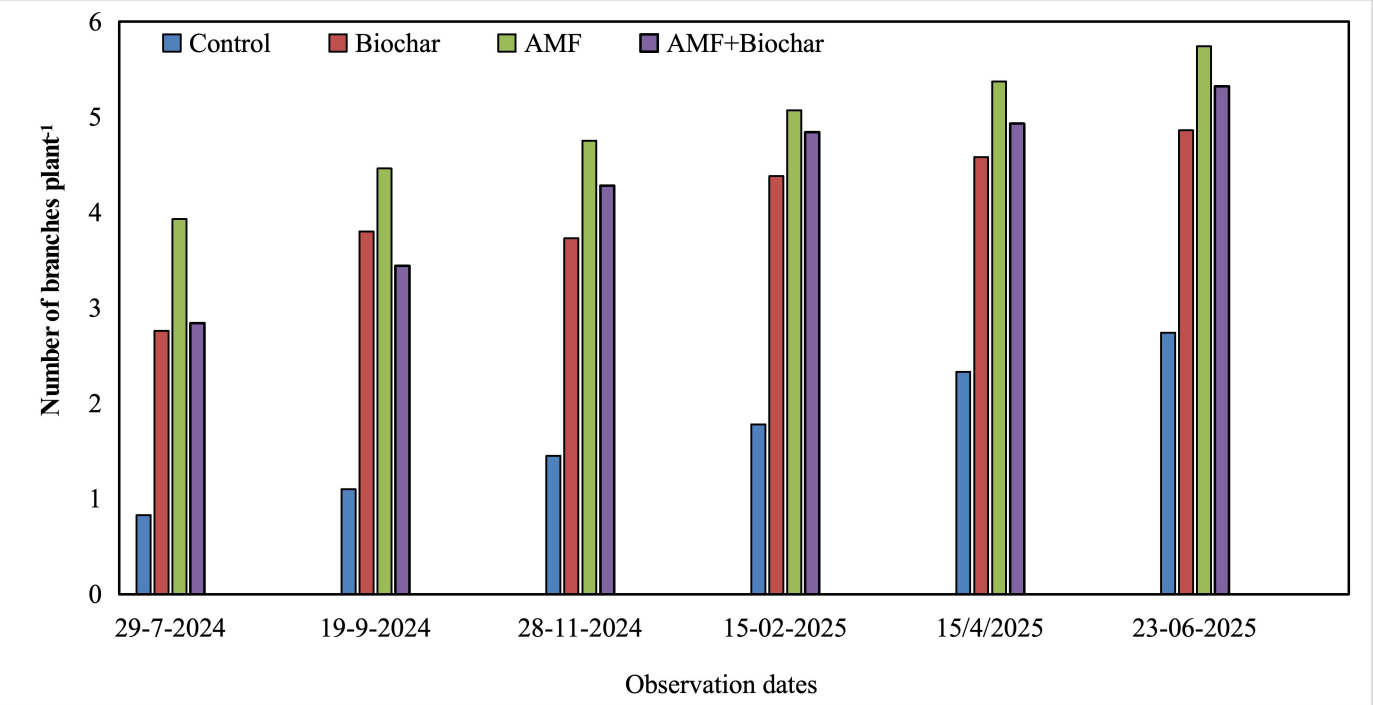

Figure 1. Biochar and AMF amendment effects on cocoa branch production.

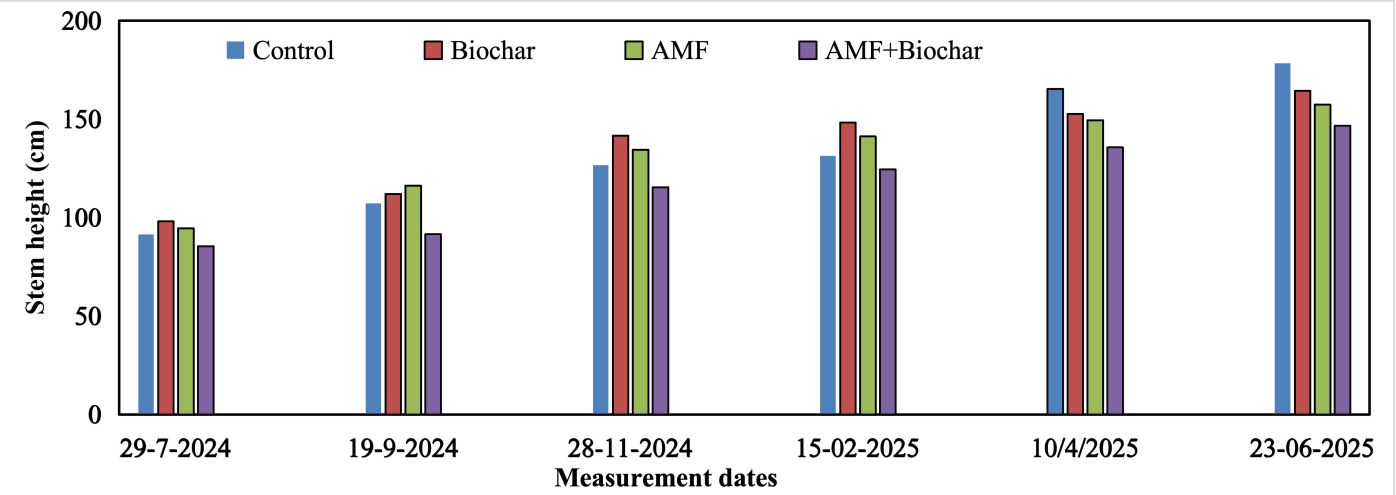

Figure 2. Biochar and AMF amendment effects on stem height of cocoa.

3.6.2. Stem height development

In July 2024, after amendment application, biochar and AMF showed higher effects on the cocoa plants' stem heights. During September 2024, AMF alone showed the highest stem height, followed by biochar, control, and least with their combination. In November 2024, biochar showed higher stem height compared to the previous measurement. In December 2024, the trends in stem height growth were similar across treatments. However, in April and June 2025, there were no significant differences in the responses of cocoa plants to the amendments compared to the control (no amendment). The control had the longest stem height of cocoa plants, while values were close for biochar and AMF compared to observations for October 2024. Observations for April and June 2025 showed that the descending order of stem height development was: control > biochar > AMF > biochar-AMF combination, which had the least stem height values (Figure 2). These results showed that there were no significant effects of amendments on stem height after a long period of sole application of biochar and AMF, which was considerably fair compared to the combination of both biochar + AMF.

4. Discussion

4.1. Soil fertility improvements

Soil fertility is a key determinant of cocoa establishment and productivity because it regulates nutrient availability, root development, microbial activity, and overall plant growth. The experimental soil was strongly constrained by low pH, poor organic matter content, and deficiencies in essential nutrients including N, P, K, and Ca, conditions commonly observed in degraded tropical cocoa-growing soils [10, 12]. Such nutrient limitations often reduce microbial abundance, suppress nutrient cycling processes, and increase vulnerability of young cocoa plants to environmental stress.

The present study demonstrated that biochar, AMF, and particularly their co-application significantly improved soil pH, organic carbon, total N, available P, exchangeable K, and exchangeable Ca compared with the initial soil condition (Table 2). The greatest improvements were generally observed in the biochar+AMF treatment, indicating a synergistic interaction between the two amendments. The increase in soil pH may be attributed to the alkaline nature of biochar and its capacity to neutralize soil acidity through the release of base cations, thereby reducing proton activity in the soil solution [15, 21, 22]. Improved soil pH subsequently enhances nutrient availability and creates a more favorable environment for microbial proliferation and root growth.

The substantial increase in soil organic carbon following biochar application is consistent with the recalcitrant carbon structure of biochar, which contributes to long-term carbon sequestration and improved soil aggregation [15, 20, 45]. Increased soil organic carbon can enhance water-holding capacity, cation exchange capacity, and nutrient retention, thereby improving soil quality. The observed increases in total N, available P, and exchangeable K and Ca may result from both direct nutrient contributions from biochar and enhanced nutrient acquisition through AMF hyphal networks [21, 22, 45]. Arbuscular mycorrhizal fungi are particularly effective in mobilizing relatively immobile nutrients such as phosphorus by extending the absorptive surface area beyond the nutrient depletion zone surrounding roots [21, 45].

The superior performance of the combined treatment suggests complementary mechanisms. Biochar provides a porous habitat that protects microbial communities and improves nutrient retention, while AMF enhance nutrient uptake efficiency and nutrient translocation to host plants [21, 22, 45]. Previous studies have similarly reported positive interactions between biochar and AMF in improving soil fertility, microbial colonization, and nutrient acquisition in tropical agricultural systems [21, 46, 47]. Therefore, the integration of biochar and AMF represents an effective strategy for restoring fertility in degraded cocoa soils while improving nutrient-use efficiency..

4.2. Soil respiration and microbial activity

Soil respiration is a widely used indicator of microbial activity and carbon cycling because it reflects the metabolic intensity of soil microorganisms and their utilization of available carbon

substrates. Changes in respiration patterns provide important insights into soil biological functioning and carbon stabilization processes. In the present study, basal respiration showed a similar temporal pattern across treatments, reaching a peak during the first few days and subsequently stabilizing (Table 3). The absence of significant treatment differences suggests that biochar and AMF did not substantially alter overall microbial maintenance respiration under relatively non-stressful field conditions. This finding indicates that the amendments maintained soil biological activity without causing excessive microbial stimulation or suppression.

In contrast, substrate-induced respiration (SIR) responded differently among treatments (Table 4). The control treatment exhibited the highest SIR values, whereas AMF-treated soil showed the lowest values. Elevated SIR in the control may indicate that microbial communities experienced nutrient limitations and responded with higher metabolic activity following substrate addition [48]. Such responses are commonly observed in nutrient-deficient soils where microorganisms rapidly utilize available carbon inputs to compensate for resource scarcity.

The reduced SIR observed in biochar- and AMF-amended soils may reflect improved microbial efficiency and enhanced carbon-use efficiency. Biochar can physically protect organic matter within its porous structure, reducing the accessibility of carbon substrates to microbial decomposition and thereby lowering CO_2 emissions [46, 47]. Similarly, AMF can improve nutrient availability and carbon allocation patterns, reducing microbial stress and the need for excessive respiratory activity. Previous studies have reported comparable reductions in soil CO_2 efflux following biochar application, attributing these effects to carbon stabilization and improved microbial metabolic efficiency [15, 18, 19]. These findings suggest that biochar and AMF contribute to sustainable soil carbon management by promoting efficient nutrient cycling while minimizing carbon losses.

4.3. Soil phosphatase activities

Soil phosphatases play a critical role in phosphorus cycling because they catalyze the hydrolysis of organic phosphorus compounds into plant-available inorganic forms. Their activity is therefore an important indicator of soil biological fertility and phosphorus availability. The present results showed that acid phosphatase (ACP) and alkaline phosphatase (ALP) activities were lowest in the control treatment and significantly increased following amendment application (Table 5). Low phosphatase activity in the control likely reflects the nutrient-poor condition of the experimental soil, where limited microbial biomass and reduced root activity suppress enzyme production [48].

Enhanced phosphatase activity following biochar application may be attributed to increased soil organic matter, improved microbial habitat, and greater microbial abundance [46, 47]. AMF application produced a particularly strong effect on ACP activity. This response is expected because AMF are known to stimulate phosphorus acquisition pathways and promote the production of phosphatase enzymes involved in organic phosphorus mineralization, especially under acidic soil conditions [21, 46].

The co-application of biochar and AMF generated the highest ACP and ALP activities, indicating enhanced phosphorus transformation and availability. The synergistic response may result from improved microbial colonization within biochar pores and greater AMF establishment in the rhizosphere [21, 22, 46, 47]. Biochar can act as a physical refuge and nutrient reservoir for beneficial microorganisms, while AMF increase root exploration and nutrient mobilization. Similar findings were reported by Wen et al. [46], who observed increased phosphatase activity and phosphorus uptake following combined biochar and AMF application in saline-alkaline soils. Collectively, these results suggest that integrated biochar-AMF management can substantially improve phosphorus cycling and nutrient-use efficiency in cocoa production systems.

4.4. Leaf metabolites and antioxidant enzymes

Plant metabolites and antioxidant enzymes are important physiological indicators because they reflect plant nutritional status, photosynthetic performance, and the ability to tolerate environmental stresses. Alterations in these biochemical parameters often precede visible growth responses and therefore provide valuable information regarding plant adaptation mechanisms. The present study revealed distinct biochemical responses among treatments. Biochar alone produced the highest concentrations of proline and the greatest activities of SOD and GPx. Proline accumulation is a well-established response to environmental stress and functions as an osmoprotectant, stabilizing cellular structures and maintaining osmotic balance [28, 29, 30]. Elevated antioxidant enzyme activities suggest that biochar may have induced a mild stress-priming effect, stimulating defensive pathways and enhancing the capacity of plants to detoxify reactive oxygen species.

In contrast, the combined biochar + AMF treatment significantly increased total chlorophyll, sucrose, and non-structural carbohydrates. Chlorophyll content is directly associated with photosynthetic efficiency, whereas soluble sugars and NSC represent important energy reserves that support growth, maintenance, and stress recovery. These findings indicate that the combined treatment improved photosynthetic performance and carbon assimilation, likely due to enhanced nutrient availability and more efficient nutrient uptake through mycorrhizal symbiosis [45-47].

During the dry season, plants accumulated higher concentrations of glucose, fructose, sucrose, proline, CAT, and SOD. Such responses are characteristic adaptations to drought stress, where osmolytes maintain cellular hydration and antioxidant enzymes mitigate oxidative damage caused by water deficit [26-28]. Soluble sugars additionally contribute to osmotic adjustment and membrane stabilization under drought conditions [26, 49]. Conversely, the rainy season was associated with higher GPx activity, chlorophyll content, carotenoid concentration, and total phenols. Increased chlorophyll and carotenoids likely reflect improved physiological conditions for photosynthesis, whereas elevated phenolic compounds may be associated with enhanced defense against pathogens and environmental stressors [46, 48].

Similar seasonal shifts in antioxidant systems have been

reported in tropical perennial crops, where CAT and SOD dominate drought responses while phenolic metabolism becomes more active under wetter conditions [28, 29, 30, 49]. These results indicate that biochar and AMF not only improve nutrient acquisition but also enhance biochemical mechanisms involved in stress tolerance.

4.5. Growth Performance of Cocoa

Growth parameters provide an integrated assessment of treatment effectiveness because they reflect the cumulative outcomes of improved nutrient availability, physiological functioning, and stress adaptation. Branch development is particularly important in cocoa because canopy architecture influences light interception, photosynthetic capacity, and future yield potential. In the present study, branch number was significantly increased by AMF application and remained high under the combined biochar + AMF treatment (Figure 1). Enhanced branching may be linked to improved phosphorus nutrition and hormonal regulation mediated by AMF colonization [18-20]. Mycorrhizal fungi are known to influence plant hormone balance, including auxins and cytokinins, which regulate shoot initiation and branching patterns.

Stem height responded differently across seasons (Figure 2). During the rainy season, biochar and AMF promoted greater stem elongation than the control, reflecting improved nutrient availability and favorable moisture conditions. However, during the subsequent dry season, treatment differences diminished and control plants exhibited slightly greater height growth. This unexpected pattern may be related to differential biomass allocation strategies under drought conditions. Plants receiving biochar and AMF generally developed larger root systems and greater vegetative biomass, which may have increased water demand during periods of limited moisture availability [15, 46, 47]. In contrast, smaller control plants may have maintained stem growth due to lower transpiration requirements.

Another possible explanation is that nutrient release from biochar and nutrient transfer through AMF become less effective under prolonged drought, temporarily limiting aboveground growth despite improvements in soil fertility [17, 27, 46, 47]. Nevertheless, the enhanced root fresh weight, root length, and leaf biomass observed in amended treatments indicate overall improvements in plant vigor and resource acquisition. Similar responses have been reported in previous studies where biochar and AMF promoted root development and biomass accumulation even when height responses were inconsistent under drought conditions [17, 46].

4.6. Implications for Sustainable Cocoa Production

Improving soil health and plant resilience is essential for sustaining cocoa production under increasing climate variability. The present findings demonstrate that the combined application of biochar and AMF improves multiple dimensions of soil quality and plant performance, including nutrient availability, phosphorus cycling, microbial efficiency, antioxidant defense, and growth. The enhancement of phosphatase activity indicates greater phosphorus turnover and nutrient availability, while reduced substrate-induced respiration suggests improved carbon stabilization and more efficient microbial metabolism.

At the plant level, enhanced photosynthetic pigments, carbohydrate accumulation, and antioxidant enzyme activities indicate greater physiological resilience to seasonal stress. These integrated responses are particularly important in tropical cocoa systems where declining soil fertility and recurrent drought episodes increasingly threaten productivity [21, 22, 45-47].

Overall, the co-application of biochar and AMF appears to be a promising climate-smart management practice capable of restoring degraded soils, improving nutrient-use efficiency, and enhancing cocoa adaptation to environmental stress [27-29, 45-49]. Future studies should evaluate the long-term persistence of these benefits across different soil types, climatic conditions, and cocoa genotypes, while also assessing the economic feasibility and cost-benefit ratio of large-scale implementation for smallholder farmers .

5. Conclusions

Sole and co-application of biochar and AMF affected soil chemical properties, basal respiration, enzymatic activities (acid and alkaline phosphatase enzymes), leaf metabolites and antioxidant enzymes and growth attributes of young cocoa plants on the field. Relative to sole biochar and AMF, biochar combined with AMF significantly improved soil properties (soil pH and nutrients), soil and plant enzyme activities and the performance of young cocoa plants on the field Soil and plant enzyme activities and leaf active metabolites appear to be relevant to stress mitigation due to unfavorable soil and weather conditions and plant growth promotion Thus, the amendments exert soil and plant health promotion benefits and possible abiotic stress mitigation The results have implications for improving cocoa establishment on the field. Biochar and AMF amendment offer high potentials for the development of sustainable agriculture system.

Research should explore the long-term effects of AMF and Biochar on soil health and crop performance, in addition to the economic feasibility of this practice for large-scale field practice and promote its adoption for tree crop cultivation. It is important to provide information and enhance understanding of the optimal application rates and combinations of AMF, biochar, for other crop species and soil types. This will help in developing precise recommendations for farmers to maximize benefits. Improved information is needed on the underlying mechanisms of AMF and biochar interaction on soil and plant performance including root morphology, nutrient uptake pathways, and microbial interactions on soil and plant. Large-scale field trials to upscale this practice to other geographic regions and varying farming practices so as to provide more robust data for practical applications.

Author contributions

Conceptualization, Ifedayo Oluwasiji; Data curation, Ifedayo Oluwasiji, Samuel Agele, Solomon Adejoro, Oluwagbotemi Fadare; Formal analysis, Samuel Agele, Oluwagbotemi Fadare;

Funding acquisition, Ifedayo Oluwasiji and Samuel Agele; Investigation, Ifedayo Oluwasiji, Solomon Adejoro; Writing–original draft, Ifedayo Oluwasiji, Samuel Agele, Oluwagbotemi Fadare. All authors have read and agreed to the published version of the manuscript.

Ethical approval

Not applicable.

Conflicts of Interest

The authors report no conflicts of interest.

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Data availability statement

The data presented in this study are available on request from the corresponding author.

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