Influence of Biochar Amendment on Soil Enzyme Activities, Carbon Storage, and Productivity of *Glycine max* (L.) Merr.

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ABSTRACT

Background: Decreasing organic carbon in soil and biochemical performance limit ultimately sustainable production of soybean (*Glycine max* (L.) Merr.) which aroused interest in organic modifications that can help improve soil functions and reduce the consequences of climate change.

Objective: The study assessed the effect of various amounts of biochar application on soil enzyme activities, soil carbon preservation ability and soybean productivity.

Methods: The field trial used randomized complete block design with four treatments (0, 5, 10 or 15 tons per hectare of soybean stover biochar produced at 450 ± 25 ˚C) replicated thrice, each treatment receiving a recommended amount of fertilizer. Soil dehydrogenase, urease, phosphatase and β-glucosidase activities, soil organic carbon stock and soybean productivity indicators were measured and analysed using ANOVA and Duncan's Multiple Range Test at $P < 0.05$.

Results: The utilization of biochar has greatly enhanced all the enzymatic activities and carbon stock in soil compared with the unamended control, recording the biggest changes at 10 and 15 t ha^{-1}. The yield of grains has increased by up to 41.7% compared to the control along with the better nodulation, root length, and harvest index. The relationship between enzyme activity and carbon storage and yield of grains has also been found.

Conclusion: Biochar application improves soil biochemical health as well as carbon sequestration process to enhance soybean productivity, thus stimulating its application in climate-friendly and sustainable soybean production systems, while there is a need for its long-term multi-site verification.

Keywords: Biochar; Soybean (*Glycine max* L. Merr.); Soil enzyme activity; Soil organic carbon sequestration; Carbon storage; Sustainable agriculture; Climate-smart agriculture; Soil fertility.

1. Introduction

The soybean (*Glycine max* (L.) Merr.) is one of the most crucial leguminous plants in economic terms, as it produces a significant part of vegetable protein and oil utilized in food, feed, and industry [1]. The increasing demand has caused an expansion of soybean production on less fertile soils. Continuous cropping, inappropriate fertilization, and insufficient return of organic materials have led to the loss of soil organic carbon and biological activity.

Soil degradation is manifested through reduced amount of organic matter, decreased microbial biomass, poor enzymatic activity, and disrupted nutrient cycling. All these factors limit root development and nodulation of leguminous plants and yield formation as well. In order to counteract the negative tendencies, such amendments are needed that will improve soil structure, biological activity, and carbon storage at the same time and will not make it too expensive for farmers.

Biochar is a carbon-rich solid, which appears during the pyrolysis of different organic materials under low oxygen conditions and has different positive effects [2]. It possesses a porous structure, as well as high specific surface area and different surface functional groups capable to retaining moisture and providing soil microorganisms with habitats.

Enzymes of the soil such as dehydrogenase, urease, phosphatase, and β-glucosidase are well-known because they serve both as biological indicators of soil health and as catalysts of crucial processes in the carbon, nitrogen, and phosphorus cycles, and they respond well to changes caused by agricultural practices affecting the microbial population and enzyme activities.

The chemical structure of biochar increases its resistance to microorganisms in comparison to regular organic materials, providing the tool for long-term soil carbon storage and greenhouse gas emissions mitigation [2, 3].

While various studies are being conducted in this regard, many discrepancies are found in terms of optimal application rates, types of feedstock, and response of crops, particularly the soybean crop to different climate regions.

The present study aims to achieve such research objectives as: (i) determining the effect of biochar application rates on soil enzyme activity, (ii) evaluating soil organic carbon storage and its sequestration potential, and (iii) assessing the effects on soybean growth and yield.

2. Related Work

Biochar production technologies range from traditional slow pyrolysis to modern fast pyrolysis and gasification systems, with feedstock type and pyrolysis temperature strongly determining the resulting carbon stability, nutrient content, and surface characteristics [7, 8]. Agricultural residues, including crop stover, husks, and woody biomass, remain the most widely studied feedstocks owing to their availability and favourable carbon-to-nitrogen ratios.

Numerous field and pot studies report significant increases in soil organic carbon following biochar incorporation, with effect magnitude depending on application rate, soil texture, and climatic regime [9, 10], while meta-analyses indicate that biochar-induced increases in microbial biomass carbon are generally accompanied by shifts in microbial community composition favouring fungal and actinobacterial groups [6].

Reported effects on soil enzyme activities are broadly positive but methodologically variable; several authors caution that assay conditions and biochar-induced sorption of substrates or products can confound enzyme activity measurements, necessitating standardized protocols [5, 13]. Improvements in nutrient availability, particularly of phosphorus and potassium, and in water-holding capacity have also been documented across diverse soil types [4, 8].

Mechanistically, biochar-induced carbon sequestration arises from the intrinsic recalcitrance of pyrogenic carbon, physical protection of native organic matter within biochar pores, and, in some cases, negative priming effects that suppress native soil organic carbon mineralization [19, 21].

Studies on legume crops, including soybean, indicate that biochar can enhance nodulation and biological nitrogen fixation, likely through improved rhizosphere aeration and micronutrient availability, translating into measurable yield gains [15, 17]. However, inconsistencies persist regarding optimal dosage, with excessive rates occasionally reported to depress yield due to nutrient immobilization or altered soil pH [18]. Future research is increasingly directed toward feedstock-specific biochar formulations, co-application with organic and inorganic fertilizers, and long-term multi-site trials to resolve these inconsistencies and support evidence-based recommendations for climate-smart agriculture.

Table 1, “Summary of Previous Studies on Biochar Application in Agricultural Soils,” consolidates representative findings from the literature reviewed above, illustrating the diversity of feedstocks, application rates, and reported effects across cropping systems.

Table 1: Summary of previous studies on biochar application in agricultural soils and their reported effects on soil properties and crop performance.

Study	Feedstock	Application Rate	Key Reported Effect
Zhang *et al.* [3]	Rice straw	10-40 t ha^{-1}	Increased grain yield; reduced N2O emissions
Chintala *et al.* [14]	Corn stover	0.5-2% w/w	Reduced soil acidity; increased cation exchange capacity
Major *et al.* [15]	Mixed wood	0-20 t ha^{-1}	Increased maize yield in years 2-4 post-application
Bhattacharjya *et al.* [17]	Rice husk	5-10 t ha^{-1}	Enhanced dehydrogenase and urease activity
Agegnehu *et al.* [12]	Corn cob (+compost)	5-10 t ha^{-1}	Improved soil quality; reduced greenhouse gas emissions
Awad *et al.* [21]	Wood biochar	Laboratory incubation	Reduced organic matter decomposition; raised enzyme activity

3. Materials and Methods

3.1 Experimental Site

The field experiment was conducted on a sandy clay loam soil under a sub-humid, sub-tropical climate characterized by a mean annual rainfall of approximately 950 mm and mean temperatures ranging from 14°C to 34°C. Pre-experimental soil properties are summarized in Table 2.

3.2 Experimental Design

The trial was laid out in a Randomized Complete Block Design comprising four treatments replicated three times, giving twelve experimental plots of 4 m × 5 m each, separated by 1 m bunds to prevent lateral movement of biochar and irrigation water.

3.3 Biochar Preparation and Treatments

Biochar was produced from soybean stover through slow pyrolysis at 450 ± 25°C for a residence time of 3 hours in a controlled-atmosphere kiln, then air-cooled, ground, and sieved to 2 mm before characterization (Table 2). Four treatments were evaluated: T1, an unamended control (0 t ha^{-1}); T2, a low biochar rate (5 t ha^{-1}); T3, a medium biochar rate (10 t ha^{-1}); and T4, a high biochar rate (15 t ha^{-1}), each combined with the recommended dose of fertilizer and thoroughly incorporated into the top 15 cm of soil two weeks before sowing. The overall experimental workflow, from feedstock collection through statistical analysis, is depicted in Figure 1, “Experimental Design and Workflow for Biochar Application in Soybean Cultivation.”

3.4 Crop Details and Agronomic Management

A locally recommended, high-yielding soybean variety was sown at a spacing of 45 cm × 5 cm following seed treatment with Rhizobium and phosphate-solubilizing bacterial inoculants. Standard agronomic practices, including irrigation, weeding, and integrated pest management, were uniformly applied across all treatments.

3.5 Soil Sampling, Enzyme Assays, and Carbon Storage

Composite soil samples were collected from a 0-15 cm depth at 30, 60, and 90 days after sowing (DAS) for determination of pH, electrical conductivity, organic carbon, available N, P, K, bulk density, and moisture content, following standard analytical protocols. Dehydrogenase, urease, phosphatase, and β-glucosidase activities were assayed colorimetrically using triphenyl tetrazolium chloride, indophenol-blue, and para-nitrophenyl substrate methods, respectively. Soil organic carbon stock was calculated from organic carbon concentration, bulk density, and sampling depth, and carbon sequestration potential was estimated as the net increase in SOC stock relative to the control.

3.6 Crop Growth, Yield Parameters, and Statistical Analysis

Plant height, branch number, root length, nodule count, pods per plant, seeds per pod, 100-seed weight, grain yield, biological yield, and harvest index were recorded at physiological maturity. Data were subjected to Analysis of Variance appropriate to the RCBD, and treatment means were separated using Duncan's Multiple Range Test at $P < 0.05$ using R statistical software.

Table 2: Physicochemical properties of the experimental soil and biochar used in the study.

Parameter	Soil Value	Parameter (Biochar)	Biochar Value
Soil texture	Sandy clay loam	Feedstock	Soybean stover
pH (1:2.5 H2O)	6.8	pH	9.4
Electrical conductivity (dS m^{-1})	0.32	Electrical conductivity (dS m^{-1})	1.85
Organic carbon (%)	0.54	Fixed carbon (%)	58.6
Available N (kg ha^{-1})	218	Total N (%)	1.12
Available P (kg ha^{-1})	16.4	Total P (%)	0.68
Available K (kg ha^{-1})	182	Total K (%)	1.34
Bulk density (Mg m^{-3})	1.42	Surface area (m^2 g^{-1})	182
Water holding capacity (%)	34.2	Ash content (%)	12.8

Fig 1: Experimental design and workflow for biochar application in soybean cultivation.

4. Results

Biochar application produced consistent and statistically significant improvements across all measured soil biochemical and crop productivity parameters relative to the unamended control, with the magnitude of response generally increasing with application rate up to 10 t ha^{-1}, beyond which gains plateaued (Table 3).

4.1 Soil Chemical Properties and Enzyme Activities

Soil pH increased modestly and organic carbon content nearly doubled at the highest biochar rate compared with the control. Dehydrogenase, urease, phosphatase, and β-glucosidase activities increased significantly with biochar rate, with the 10 and 15 t ha^{-1} treatments statistically at par but significantly superior to lower rates, indicating enhanced microbial activity and nutrient cycling capacity.

4.2 Carbon Storage

Soil organic carbon stock increased from 9.8 Mg ha⁻¹ in the control to 16.8 Mg ha⁻¹ at 15 t ha⁻¹ biochar, representing a carbon sequestration gain of approximately 71%. Carbon storage efficiency, expressed as the proportion of applied biochar-carbon retained in the soil carbon pool, was highest at intermediate application rates.

4.3 Soybean Growth and Yield

Plant height, root length, and nodulation increased significantly with biochar application, with the highest nodule counts recorded at 10 t ha⁻¹. Grain yield increased from 1842 kg ha⁻¹ in the control to 2611 kg ha⁻¹ at 15 t ha⁻¹, a 41.7% improvement, while harvest index improved from 32.4% to 37.2% across the same gradient.

4.4 Correlation Among Parameters

Grain yield was positively and significantly correlated with dehydrogenase activity ($r = 0.89$), soil organic carbon stock ($r = 0.86$), and urease activity ($r = 0.81$), suggesting that biochar-induced enhancement of enzymatic function and carbon storage jointly contributed to improved productivity rather than acting through a single independent pathway.

Table 3, "Effect of Biochar Amendment on Soil Enzyme Activities, Carbon Storage, and Soybean Productivity," presents the complete treatment-wise dataset underlying the results summarized above.

Table 3: Effect of biochar amendment on soil enzyme activities, carbon storage, and soybean productivity. Means followed by different letters within a row differ significantly at $P < 0.05$ (DMRT).

Parameter	T1 (0 t)	T2 (5 t)	T3 (10 t)	T4 (15 t)	CD (5%)
Dehydrogenase (μg TPF g^{-1} $24h^{-1}$)	18.4c	24.6b	31.2a	33.8a	2.1
Urease (μg NH4-N g^{-1} h^{-1})	42.1c	51.7b	61.4a	63.9a	3.4
Phosphatase (μg PNP g^{-1} h^{-1})	86.5c	104.2b	126.8a	131.5a	6.7
β-glucosidase (μg PNP g^{-1} h^{-1})	39.8c	48.3b	58.9a	61.2a	3.9
Soil organic carbon (%)	0.55c	0.71b	0.89a	0.94a	0.06
SOC stock (Mg ha^{-1})	9.8c	12.6b	15.9a	16.8a	1.1
Grain yield (kg ha^{-1})	1842c	2156b	2534a	2611a	142
Harvest index (%)	32.4b	34.1ab	36.8a	37.2a	2.3

The pathways linking biochar amendment to these outcomes are summarized in Figure 2, "Proposed Mechanism of Biochar Influence on Soil Enzyme Activities, Carbon Sequestration, and Soybean Productivity."

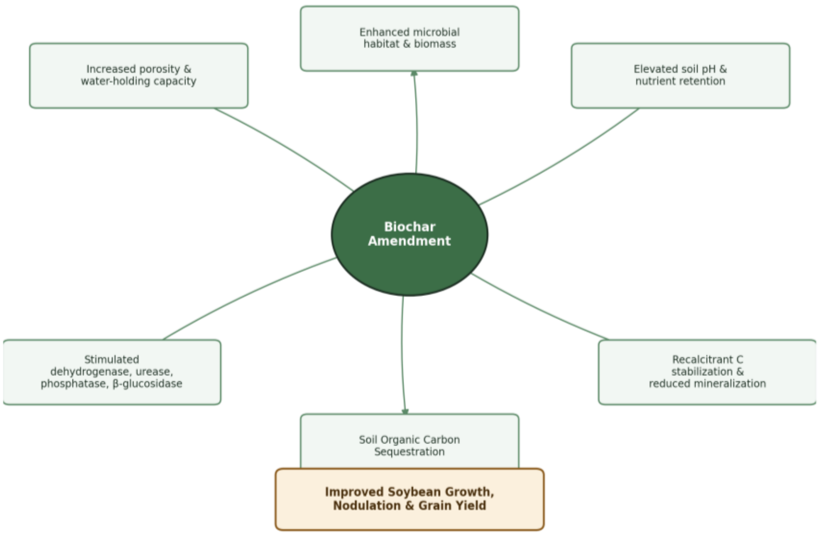

Fig 2: Proposed mechanism of biochar influence on soil enzyme activities, carbon sequestration, and soybean productivity.

5. Discussion

The observed increases in dehydrogenase, urease, phosphatase, and β-glucosidase activities are consistent with reports that biochar improves microbial habitat quality through enhanced porosity, moisture retention, and substrate availability [5, 6], supporting the interpretation that biochar acts primarily by creating a more favourable physical and chemical environment for soil microbial communities rather than through direct enzymatic contribution.

The positive correlation between enzyme activities and nutrient cycling aligns with established understanding that dehydrogenase reflects overall microbial oxidative activity while urease, phosphatase, and β-glucosidase directly govern nitrogen, phosphorus, and carbon mineralization, respectively [10], explaining the parallel improvements observed in soil nutrient status and crop nutrient uptake.

The substantial increase in soil organic carbon stock corroborates the recognized role of pyrogenic carbon in long-term carbon stabilization through its aromatic, decomposition-resistant structure [2, 19], reinforcing biochar's potential contribution to soil-based climate change mitigation strategies at the field scale.

Improved soybean nodulation and grain yield are consistent with previous findings linking biochar application to enhanced biological nitrogen fixation and legume productivity [15, 17], plausibly mediated through improved rhizosphere aeration, micronutrient availability, and reduced aluminium or manganese toxicity in acidic microsites.

From a practical standpoint, the plateauing of responses beyond 10 t ha^{-1} suggests that intermediate application rates may offer the most economically favourable balance between input cost and productivity gain, an important consideration for smallholder adoption. Environmentally, the dual benefit of enhanced soil fertility and carbon sequestration positions biochar as a genuinely climate-smart input, though its economic feasibility remains contingent on local feedstock availability and pyrolysis infrastructure costs.

This study is limited by its single-season, single-site design, which restricts extrapolation across diverse agro-climatic zones and soil types. Long-term, multi-location trials incorporating varying feedstocks, pyrolysis conditions, and co-amendment strategies with organic and inorganic fertilizers are needed to validate these findings and refine site-specific application guidelines.

6. Conclusion

The current research paper suggests that biochar application substantially improves important soil enzyme activities, promotes the storage of soil organic matter, and benefits the growth, nodulation and grain yield of soybean plants. Strong positive impact of the biochar amendment was observed at application rates within the range of 10-15 t ha^{-1}. Overall, these results provide sufficient proof that biochar should be included in sustainable soybean production practices as it helps to restore soil biological functions and at the same time contributes to mitigation of climate change through carbon capture. However, the potential advantages of use of biochar in agriculture should be validated in long-term trials conducted under different types of soils and climatic conditions before implementation of large-scale biochar application within the farming community.

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