Patel *et al. Discover Soil* (2025) 2:92
https://doi.org/10.1007/s44378-025-00121-9

Discover Soil

RESEARCH **Open Access**

Effects of varying biochar application rates on the biological properties of sandy loam soil

Maga Ram Patel[1], Narayan Lal Panwar[1*] and Ram Hari Meena[2]

*Correspondence:
Narayan Lal Panwar
nlpanwar@rediffmail.com
[1]Department of Renewable Energy Engineering, Maharana Pratap University of Agriculture and Technology, Udaipur 313001, Rajasthan, India
[2]Department of Soil Science and Agricultural Chemistry, Maharana Pratap University of Agriculture and Technology, Udaipur 313001, Rajasthan, India

Abstract
This study investigates the impact of varying biochar application rates on the biological properties of sandy loam soil, with a particular focus on its impact on soil health, microbial activity, and enzyme functions in agricultural applications. Biochar analyses indicate a high carbon content (65.83% fixed carbon and 79.38% total carbon), low O/C (0.14) and H/C (0.69) ratios, and alkaline characteristics (pH 8.28), implying possible advantages for soil carbon sequestration, fertility, and structure. Moreover, biochar exhibits low electrical conductivity (1.15 dS/m) and can enhance pH levels, mitigating soil salinity risk. Toxicant investigation revealed no detectable hazardous substances, affirming its environmental safety. The utilization of biochar markedly affected soil enzymatic activities, including dehydrogenase and alkaline phosphatase, signifying increased soil microbial activity and improved phosphorus cycling. Biochar positively influenced soil microbial biomass carbon and augmented the microbial population, encompassing bacterial, fungal, and actinomycete counts. Statistical study validated the substantial impacts of various biochar dosages on soil biological characteristics. This study highlights the efficacy of biochar as a sustainable soil amendment for enhancing soil health, microbial activity, and long-term carbon sequestration.

Keywords Biochar, Soil health, Microbial activity, Carbon sequestration, Soil enzymatic activities, Sustainable amendment

1 Introduction

Soil-related constraints significantly limit crop production in semi-arid regions. The dominant soil order faces numerous challenges in India, including weak structural integrity, low fertility, and accelerated erosion. The agriculture expansion into marginal lands, reduced fallow periods, deforestation, inappropriate farming practices, and low-input systems exacerbate these issues, resulting in adverse economic and environmental impacts [1]. Given the limited resilience of sandy loam soils, implementing improved natural resource management strategies is critical to mitigating soil degradation, enhancing agricultural productivity, ensuring food security, and reducing poverty levels in these regions.

Biochar is a prominent agricultural soil amendment known for its ability to enhance soil fertility, improve nutrient retention, and promote long-term soil sustainability. Its application has demonstrated significant potential in increasing agricultural productivity while addressing soil degradation and environmental challenges in various agroecosystems. Biochar, a carbon-dense substance produced via the pyrolysis of organic biomass, including agricultural crop residues, forest woody waste, and agro-industrial organic solid waste, in conditions of limited or absent oxygen, has attracted considerable interest for its prospective advantages in agriculture [2]. Its utilization in soil is proposed to promote soil porosity, water retention capacity, carbon content, and fertility, boost agricultural yield, and aid in carbon sequestration [3, 4]. Biochar, as a stable carbon form, persists in the soil for prolonged durations, affecting numerous soil characteristics and processes [5]. Soil biological characteristics, such as enzyme activity, microbial biomass, and soil respiration, are essential indicators of soil health [6]. Soil microorganisms are crucial for nutrient cycling, organic matter breakdown, and the preservation of soil [7]. Enzymatic activities related to nitrogen, carbon, and phosphorus cycles indicate the biochemical functioning of the soil ecosystem. Soil respiration, quantifying the emission of carbon dioxide from soil organisms, reflects microbial activity and the overall metabolic function of the soil [8]. The biological features of soil are essential for sustainable agriculture, as they directly influence nutrient availability, soil structure, and crops' resistance to diseases and pests [9, 10]. Consequently, preserving and improving these attributes is essential for sustained soil vitality and agricultural output.

Sandy loam soils, distinguished by their elevated sand content, moderate silt levels, and low to moderate clay concentration, pose distinct problems and opportunities for agricultural techniques. These soils are recognized for their excellent drainage and ease of cultivation. However, they frequently exhibit limited capabilities for water and nutrient retention. In India, applying excessive fertilizers to attain elevated agricultural yields has been common for decades, resulting in soil deterioration and environmental contamination [11]. Although several studies [12–14] have examined biochar's influence on soil properties, most have focused on single application rates, used low quality biochar or specific soil types. Limited research has systematically assessed how different biochar application rates influence the biological properties of sandy loam soils in semi-arid agriculture field. This constitutes a key research gap. The hypothesis of this study is that varying application rates of biochar will differentially influence the biological properties of sandy loam soils, with an optimal rate enhancing microbial activity, enzyme functions, and overall soil health indicators more effectively than either under or over application. This study aims to evaluate the effects of varying biochar application rates on microbial biomass, enzyme activities, and soil respiration in sandy loam soil; assess changes in soil health indicators in relation to biochar dose and application frequency; and determine the optimal biochar application rate. Given the current global emphasis on climate-resilient and sustainable agricultural practices, this study is timely. A comprehensive understanding of the impact of varying biochar application rates on soil biological characteristics in this region is essential. Biochar has the potential to simultaneously enhance soil health, improve productivity, and mitigate environmental degradation, making it a valuable intervention for sandy loam soils in semi-arid regions. The findings of this research will contribute to developing evidence-based guidelines for biochar use in Indian agriculture and similar agroecosystems worldwide.

2 Materials and methods

2.1 Experimental site

The field experiment of biochar application was carried out from October 2022 to September 2023 at the Instructional Farm of the College of Technology and Engineering (CTAE), Maharana Pratap University of Agriculture and Technology (MPUAT) in Udaipur (refer to Fig. 1). This area was categorized as agro-climatic zone IV-A in Rajasthan. Further information regarding the experimental site and crop selection was elucidated in our prior study [15].

Table 1 Delineates the attributes of the experimental site and presents essential information concerning the soil's diverse physical and chemical qualities. This data established a foundation for comprehending the environmental circumstances and soil fertility status at the study site, which was crucial for analyzing the experiment's outcomes and assessing its influence on soil biological features.

The cumulative rainfall for the trial from October 2022 to September 2023 was 805 mm. The daily average temperatures reached a maximum of 30.83 °C and a minimum of 15.20 °C, with extremes of 41.7 °C and − 4.4 °C. The daily average relative humidity (RH) at 7:30 AM was 73.92%, ranging from 25% to 95%. At 2:30 AM, the average relative humidity was 37.84%, ranging from 6% to 92%.

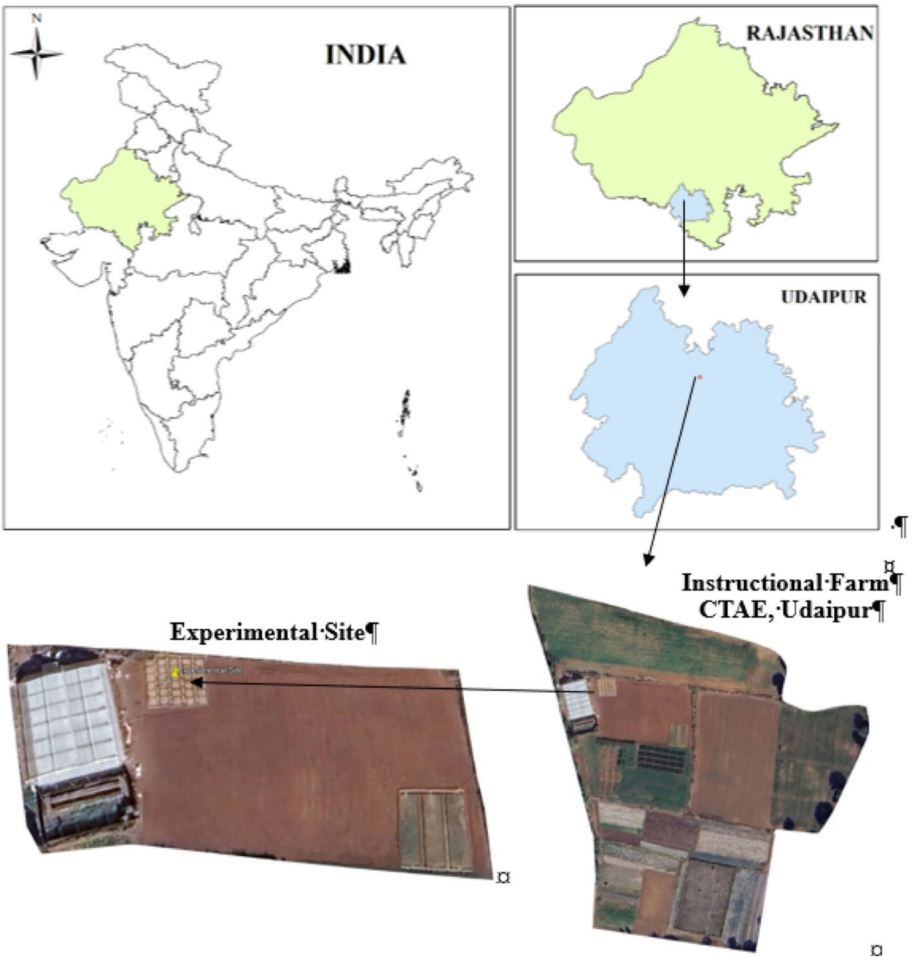
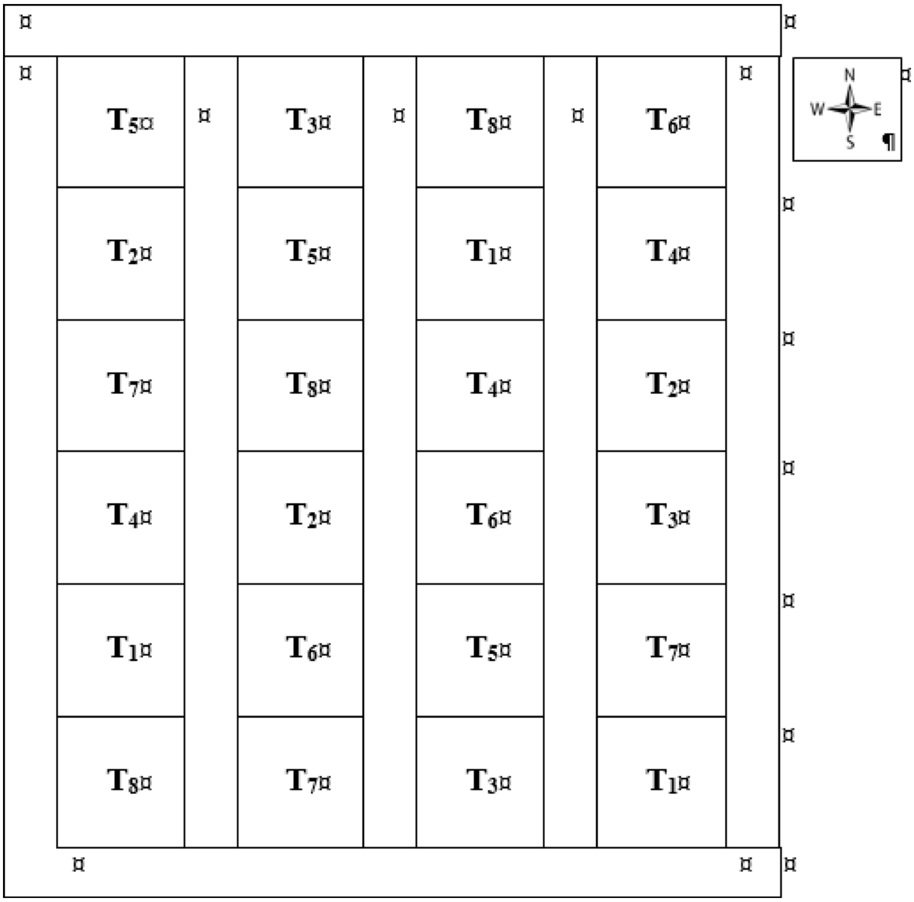


Fig. 1 Location of biochar field application site

Table 1 Preliminary physicochemical characteristics of soil

Sr. No.	Properties of Soil	
1.	Sand (%)	68.89
2.	Silt (%)	18.57
3.	Clay (%)	12.54
4.	Textural class	Sandy Loam
5.	pH	6.41
6.	EC (dSm^{-1})	1.02
7.	Bulk density (Mg/m^3)	1.44
8.	Particle density (Mg/m^3)	2.38
9.	Porosity (%)	39.88



T_5	T_3	T_8	T_6
T_2	T_5	T_1	T_4
T_7	T_8	T_4	T_2
T_4	T_2	T_6	T_3
T_1	T_6	T_5	T_7
T_8	T_7	T_3	T_1

Fig. 2 Layout of the experiment site

2.2 Experimental setup

The experimental strategy for biochar application utilized a randomized block design with eight distinct treatment combinations, each reproduced three times. Treatments were randomly assigned to plots utilizing the Fisher and Yates random number table [16]. The arrangement and treatment allocation are depicted in Fig. 2.

The soybean straw harvested from the experimental location was utilized for biochar synthesis. The methodology for biochar generation was delineated in our prior research [17, 18]. The biochar yield from soybean straw at 500 to 600 °C for 6 h in the Department of Renewable Energy Engineering, MPUAT was around 1:3 The untreated biochar (particles size < 8 mm) was applied to the experimental field at various rates. The specifics of the eight different treatments are presented in Table 2. Control treatment refers to soil or crop plots without biochar application, used for comparison in evaluating biochar effects.

2.3 Biochar characterization

2.3.1 pH

According to TMECC (2001) protocols [19], the pH assessment of biochar required the preparation of a biochar sample at a 1:20 ratio of biochar to deionized water. The mixture was agitated for 90 min to facilitate enough interaction, after which the pH was assessed using a Toshcon-Toshniwal Deluxe CL-54$^+$ pH meter.

2.3.2 Electrical conductivity (EC)

The method for EC analysis adhered to a protocol akin to that of pH measurement, employing a modified dilution and equilibration technique [20]. Following equilibration, the electrical conductivity of the solution was assessed with an ELICO CM 180 conductivity meter. This instrument delivered precise measurements of the solution's conductivity, assessing its capacity to conduct electricity.

2.3.3 Liming ability

The AOAC 955.01 method [19], as specified by the Association of Analytical Communities, was employed to assess liming capacity in samples with a pH exceeding 7. This technique utilized potentiometric titration on "as received" (wet) samples, with computations grounded in dry weight to ascertain the percentage of $CaCO_3$ and report it "per dry sample weight." During this procedure, the wet sample was titrated to evaluate its acidity or alkalinity using a standardized solution, and the quantity of ($CaCO_3$) was determined based on the volume of titrant utilized. This figure was subsequently modified to reflect the dry weight of the sample, yielding an accurate depiction of the liming capacity per unit of dry sample weight.

2.3.4 Carbon stability

The carbon stability of biochar can be assessed by determining the molar ratio of hydrogen (H) to organic carbon (C) [21]. This ratio elucidates the biochar's extent of carbonization and stability, with diminished H-to-organic carbon ratios signifying enhanced carbon stability. This evaluation assessed the appropriateness of biochar for enduring carbon sequestration and soil enhancement objectives.

2.3.5 Toxicant assessment

As specified by the International Biochar Initiative [19] criteria for biochar utilized in soil applications, this test evaluated potential toxicants. Samples for toxicant evaluation were prepared by standard laboratory protocols, which included grinding and homogenizing the biochar to guarantee consistency in testing and the acid digestion procedure.

Table 2 Details of various treatments

Treatments	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Biochar (ton/ha)	Control	4	8	12	16	20	24	28

Employ the suitable analytical method of ICP-MS (Thermofisher, iCAP RQ) at CSMCRI, Bhavnagar, to detect and quantify hazardous metals.

2.4 Soil sampling

Soil samples were collected from the same experimental plots to ensure consistency. A sanitized stainless-steel core cutter was used to extract soil from the upper 0–15 cm layer, corresponding to the primary root zone and active microbial layer. Sampling was performed in a zigzag configuration across each plot, with 5–7 subsamples per plot taken from evenly spaced points to capture spatial variability [22]. After collection, these subsamples were meticulously combined in a sterile polyethylene container. The soil samples were after that sealed in pre-labelled, airtight sampling bags and conveyed to the soil laboratory. Upon arrival, each sample was stored fresh at 4 °C for the soil biological characteristics analysis.

2.5 Determination of soil biological properties

Soil alkaline phosphatase (AlP) activity was measured using the following method given by Tabatabai and Bremner [23], and soil dehydrogenase activity was determined using the Anthrone extraction method as outlined by Casida et al. [24]. Soil microbial biomass carbon (SMBC) was analyzed through the Chloroform-Fumigation incubation technique developed by Vance et al. [25]. Bacteria, fungi, and actinomycetes were cultured on specific agar mediums, and colony counts were adjusted by the dilution factor and expressed as colonies per gram of dry soil. These methods provide critical insights into soil enzyme activities and microbial biomass, essential indicators of soil health and biological functioning.

2.6 Data analysis

The Design Expert-13 software was utilized to assess notable variations in soil biological characteristics at different biochar doses by one-way analysis of variance (ANOVA) [26]. Post-hoc comparisons were performed using Tukey's Honestly Significant Difference (HSD) test to validate the statistical significance of the results. This statistical method facilitates the identification of the effects of varying biochar levels on soil biology through mean comparison and significance determination.

3 Result and discussion

3.1 Biochar properties

The proximate and final analysis of biochar discussed in our prior study [17] underscores its appropriateness for soil application. The research indicates that the biochar possesses a fixed carbon content of 65.83%, which is advantageous for long-term soil carbon sequestration. The carbon concentration is 79.38%, suggesting its potential as a carbon-rich soil supplement. The biochar exhibits low H/C (0.69) and O/C (0.14) ratios, signifying enhanced stability and aromaticity. These attributes render it helpful in enhancing soil fertility and structure, so making it an advantageous option for agriculture. The biochar has a pH of 8.28, indicating alkalinity and the capacity to elevate soil pH when applied as a soil amendment. The biochar, with an EC of 1.15 dS/m, exhibits low salinity, indicating a reduced likelihood of soil salinity problems upon application. Literature indicates that the electrical conductivity of biochar varies from 0.04 dS/m to 54.2 dS/m

[27]. Furthermore, its liming potential of 5.14% $CaCO_3$ equivalent indicates its proficiency in neutralizing soil acidity, comparable to the impact of 5.14% calcium carbonate, hence improving soil pH and fostering soil fertility. Biochar comprises several inorganic constituents, including metal carbonates, sulfates, phosphates, silicates, and chlorides. Research indicates that the alkalinity of biochar is associated with the amount of $CaCO_3$, $MgCO_3$, $Mg(OH)_2$, and MgO [28]. These attributes collectively highlight the appropriateness of biochar for soil enhancement, providing advantages such as improved soil structure and increased nutrient availability.

ICP-MS analysis revealed that Chromium, Cobalt, Nickel, Arsenic, Selenium, Molybdenum, Cadmium, Mercury, Lead, and Boron were below the detection limits in the biochar. This absence of potentially harmful elements suggests minimal risk of soil contamination [29] or adverse environmental [30] and human health effects, supporting the suitability of the biochar for sustainable agricultural and environmental applications.

3.2 Accounting for biochar carbon added to soils

The total change in carbon stocks of soils correlated with biochar amendment varies according to the amount of biochar applied per hectare (see Fig. 3). For each increment of biochar application from 4 tons/ha to 28 tons/ha, the corresponding carbon stock change in the soil increases linearly. Specifically, at 4 tons/ha, the carbon stock change is 1.87 tons/ha. With every additional 4 tons/ha of biochar, the carbon stock change increases by approximately 1.87 tons/ha, resulting in respective carbon stock changes of 3.73 tons/ha, 5.60 tons/ha, 7.46 tons/ha, 9.33 tons/ha, 11.19 tons/ha, and 13.06 tons/ha for the treatment T_3 to T_8, respectively. This linear relationship suggests that increasing biochar application rate leads to a proportional increase in soil carbon sequestration, highlighting the biochar potential as a sustainable soil amendment for enhancing soil carbon storage and mitigating climate change. Understanding the percentage of feedstock carbon transformed into chemically stable biochar is crucial for estimating the biochar's Carbon Decay Rate (CDR) value [31, 32]. However, for national greenhouse gas

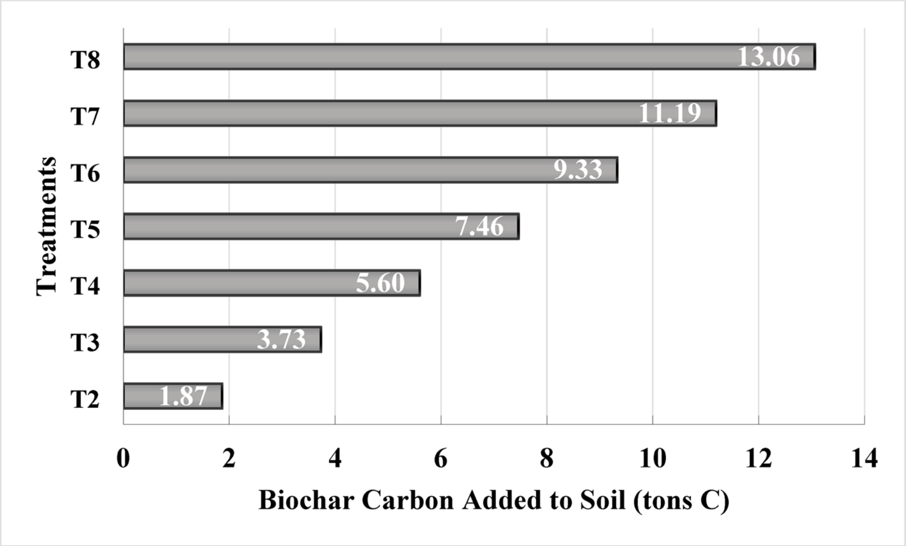


Fig. 3 The total change in carbon stocks of biochar amendment soil

accounting, it is equally important to consider the impact on other carbon stocks resulting from the utilization of biomass and waste materials in biochar production [33].

3.3 Dehydrogenase activity

Figure 4 depicts the effect of different biochar application rates on soil dehydrogenase activity. The utilization of biochar markedly enhanced soil dehydrogenase activity ($P < 0.01$), associated with elevated biochar application rates compared to control treatments. A maximum of 14.92 mg TAF per gram of soil per hour was recorded at 28 t/ha, while a minimum of 9.19 mg TAF per gram of soil was noted in the control treatment. The application rate of biochar can substantially influence soil dehydrogenase activity, an enzyme critical for organic matter decomposition and microbial activity in soil [34].

In this study, dehydrogenase activity recorded a mean value of 12.74 ± 0.05 mg TAF g^{-1} soil h-^{1}, based on three replicates per treatment. Data analysis was carried out using a one-way ANOVA, and the observed differences among treatments were found to be statistically significant at the 1% level. Biochar can function as a home and substrate for soil microbes, fostering an advantageous environment for microbial growth. The augmentation of microbial biomass and activity induced by biochar can result in elevated soil dehydrogenase activity [35]. Biochar additions can improve nutrient availability in the soil, supplying vital nutrients for microbial metabolism and enzymatic activity. Enhanced nitrogen availability may enhance soil dehydrogenase activity [36]. Biochar can affect soil pH and regulate soil acidity or alkalinity. Optimal pH levels are essential for microbial activity, particularly dehydrogenase activity. Alterations in soil pH generated by biochar may influence the activity of soil dehydrogenase enzymes. Biochar enhances water retention, soil structure, and aeration, fostering a more conducive environment for soil microbes. Improved soil physical characteristics may indirectly enhance soil dehydrogenase activity by fostering microbial development and activity [37]. Biochar comprises organic carbon and functional groups that can engage with microbial populations and soil organic matter. These interactions may affect the breakdown of organic materials and the activity of soil dehydrogenase enzymes. The impact of biochar on soil dehydrogenase activity may fluctuate over time as biochar decomposes and engages with

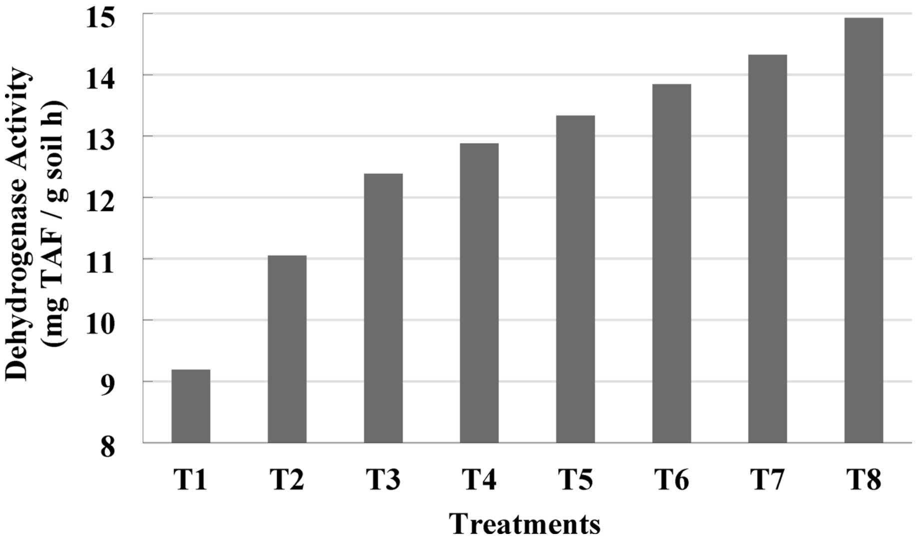


Fig. 4 Biochar effect on the dehydrogenase activity in the soil

the soil microbial community [38]. Biochar amendment improved nutrient cycling by stimulating bacterial communities associated with higher β-glucosidase and phosphatase activities [39]. The initial stimulatory effects of biochar on dehydrogenase activity may diminish or stabilize over time.

3.4 Alkaline phosphate activity

Figure 5 illustrates the effect of different biochar application rates on soil alkaline phosphatase activity. The use of biochar markedly ($p < 0.01$) enhanced soil alkaline phosphate activity relative to the treatment without biochar. The range was identified as 92.11 to 136.54 mg PNP/g soil h. With the increase in biochar application quantity, a notable improvement in soil alkaline phosphate activity was measured, with the application of 28 t/ha of biochar resulting in the peak alkaline phosphate activity of 136.54 mg PNP/g soil h. This indicates a 48.23% increase in alkaline phosphatase activity relative to soils without biochar application.

The results of the mean alkaline phosphatase activity under various biochar application rate was found 118.48 ± 0.15 mg PNP/g soil h with three replicants of each treatment, indicating high precision in measurement. Statistical analysis was conducted using a one-way ANOVA and results show a significant enhancement in alkaline phosphatase activity with increasing biochar doses. This rigorous experimental design and statistical validation ensure the reliability and reproducibility of the findings. The application of biochar can affect soil alkaline phosphatase activity, a crucial enzyme in phosphorus cycling [40]. The biochar impact on soil alkaline phosphate activity is complex. The biochar porous structure and elevated surface area can provide a habitat for soil microorganisms, potentially augmenting microbial populations that contribute to phosphorus mineralization and enzymatic activity [41, 42]. Moreover, biochar may modify soil pH, establishing conditions that enhance the activity of alkaline phosphatase enzymes. Additionally, biochar can aid in stabilizing and maintaining organic phosphorus molecules in the soil, hence affecting their availability for microbial mineralization and enzymatic hydrolysis [43, 44]. The influence of biochar on soil alkaline phosphate

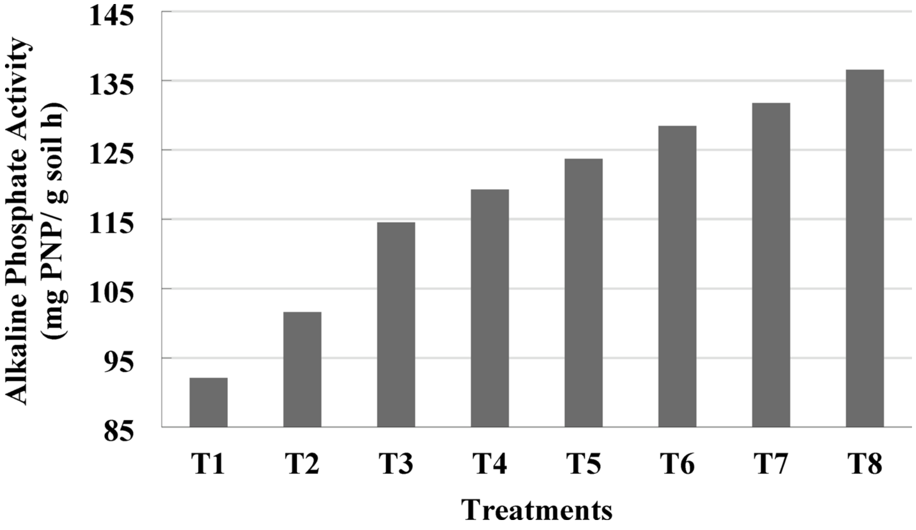


Fig. 5 Effect of biochar on the alkaline phosphate activity in the soil

activity is contingent upon the properties of the biochar and soil, requiring site-specific assessments for a thorough understanding of its impacts [45, 46].

3.5 Soil microbial biomass carbon

Figure 6 illustrates the effect of different biochar doses on the soil microbial biomass carbon (SMBC). The experimental results demonstrate a substantial impact of varying biochar dosages on the SMBC. The MBC of soil was measured at 142.38, 164.64, 180.31, 206.53, 236.25, 256.19, 268.73, and 303.07 mg/kg for Treatments T1 through T8, respectively. The maximum SMBC measured was 303.07 mg/kg with a 28 t/ha biochar application rate, while the minimum was noted in the control treatment. The use of biochar significantly influences SMBC, however its effects may fluctuate over time. Mitchell et al. [47] reported no substantial alteration in SMBC during a brief interval (20–30 days) post-biochar treatment, although showed enhancements in SMBC levels after one year. Similarly, Ameloot et al. [48] noted a 29% increase in SMBC in sandy loam soils treated with biochar produced from willow wood pyrolyzed at temperature of 700 °C. Luo et al. [49] likewise showed elevated SMBC levels in clay loam soils supplemented with biochar generated at identical temperatures. The data indicate that although biochar can improve soil microbial biomass in the long term, the integration of biochar with organic amendments may alleviate the early decline in SMBC.

The results for SMBC are presented as mean values with corresponding standard errors to ensure data clarity and reproducibility. For instance, the SMBC mean value was 220.74 ± 1.99 mg/kg, calculated from three replicates. Statistical analysis was performed using a one-way ANOVA, and the differences among treatments were found to be significant at the 1% level. Biochar positively influences SMBC by improving soil structure, enhancing nutrient availability, and increasing organic carbon content. Its porous structure provides a habitat for microorganisms, while its high surface area aids in retaining water and nutrients. These factors collectively boost MBC, contributing to enhanced overall ecosystem productivity. The use of biochar can markedly affect soil MBC, which is a favorable indicator of soil nutrient cycling and microbial activity [50]. Biochar can improve SMBC by offering a habitat and substrate for soil microorganisms,

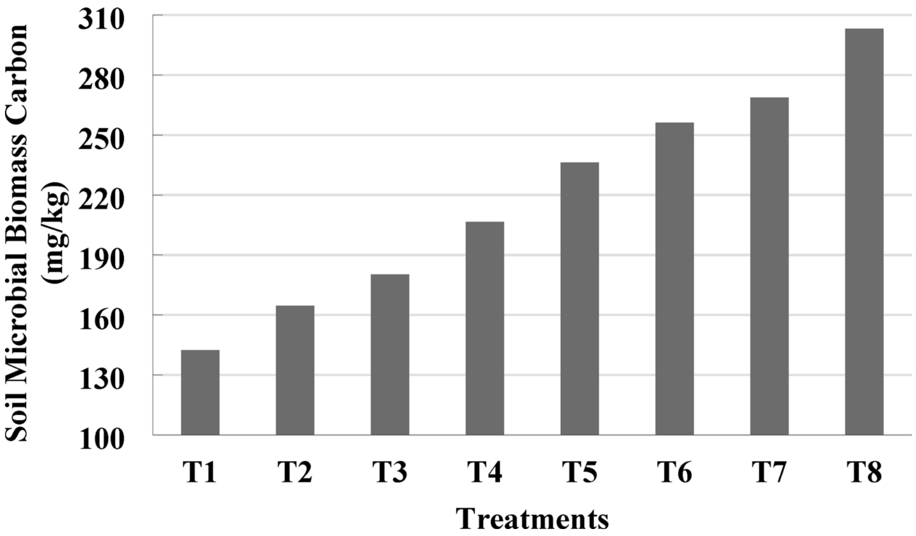


Fig. 6 Effect of biochar on the soil microbial biomass carbon in the soil

hence promoting microbial growth and activity [51, 52]. Moreover, biochar amendments can enhance soil physico-chemical characteristics, fostering a conducive environment for microbial populations. The influence of biochar on MBC may fluctuate over time and across different locations, and high application rates of biochar could inhibit microbial biomass.

3.6 Soil microbial population

The application rate of biochar markedly affects soil microbial communities, encompassing bacteria, fungi, and actinomycetes. Increased biochar applications often provide an environment that enhances the proliferation and variety of microbial populations. This improvement is due to biochar's capacity to promote soil structure, nutrient accessibility, and moisture retention, which are essential for microbial growth and activity.

Figure 7 depicts the effect of biochar application rates on the proliferation of the soil bacterial community. The use of biochar considerably ($p < 0.01$) affects soil bacterial populations, increasing both their variety and abundance. The populations of soil bacteria ranged from 53.38 to 82.50 × 10^6 CFU/g, with the maximum and minimum values recorded in treatments of 28 t/ha and the control treatment, respectively. The biochar porous structure creates an advantageous environment for bacteria, supplying both protection and vital materials for their proliferation. This habitat enhancement results from biochar's capacity to hold moisture and nutrients, essential for bacterial activity. Furthermore, biochar modifies soil pH to a more neutral state, fostering a more conducive environment for a broader spectrum of soil microbes. Consequently, augmenting the biochar application rate often fosters a more robust and diversified bacterial population, thereby enhancing soil health and nutrient cycling.

Figure 8 depicts the impact of various biochar application dosage on the proliferation of soil fungus. The application rate of biochar considerably ($p < 0.01$) affects soil fungal populations, mostly due to alterations in soil microbial habitats and physicochemical features. The populations of soil fungus ranged from 18.27 to 28.50 × 10^5 CFU/g, with the treatment of 28 t/ha producing the highest values and the control treatment yielding the lowest. Increased biochar application can alter the soil ecology, enhancing conditions for

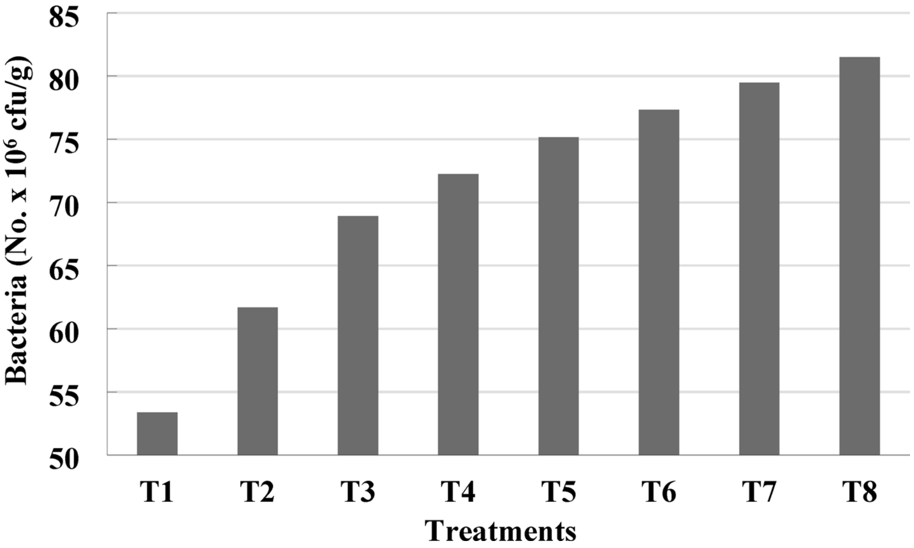


Fig. 7 Effect of biochar on the bacteria population in the soil

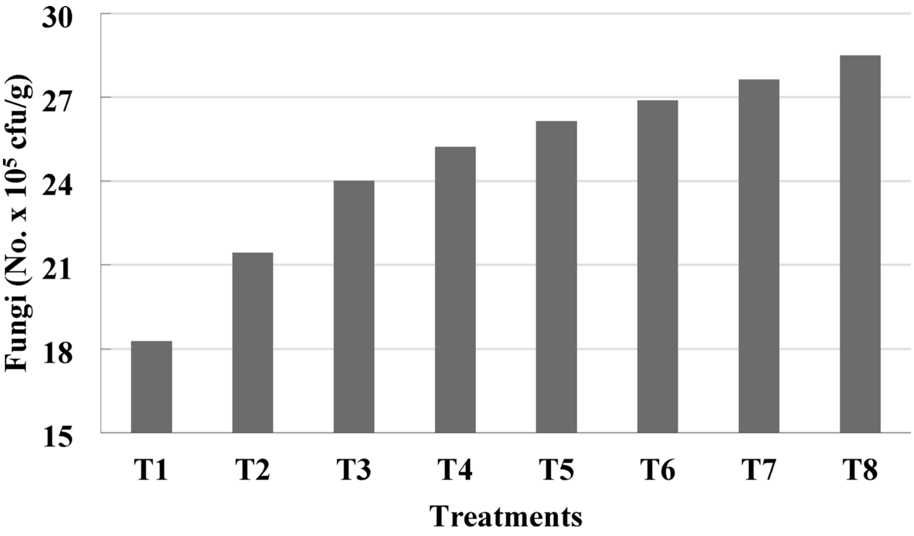


Fig. 8 Effect of biochar on the fungi population in the soil

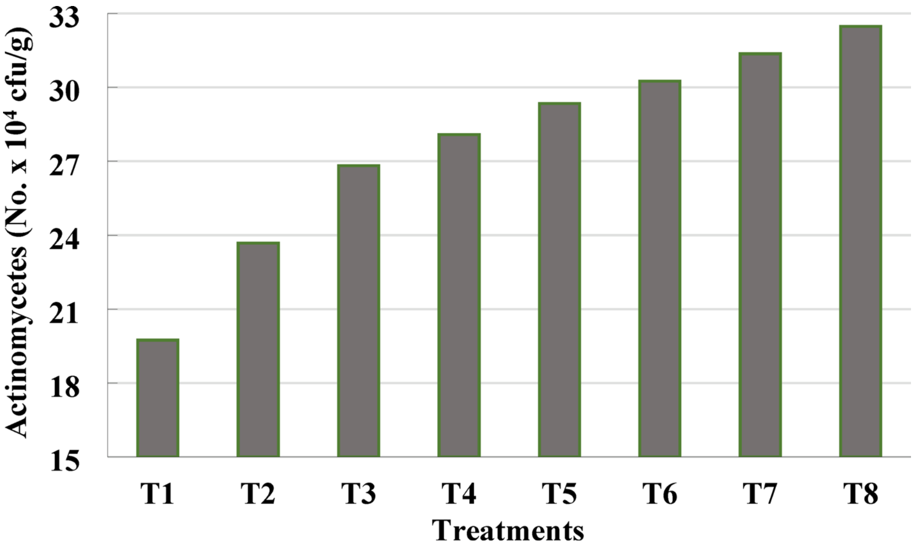


Fig. 9 Effect of biochar on the actinomycetes population in the soil

fungus proliferation. The porous structure of biochar offers a protective environment for fungus, and its alkaline characteristics can modify soil pH, hence favoring the proliferation of specific fungal populations over others. Furthermore, the enhanced soil structure and augmented water retention resulting from biochar application can facilitate fungal spore germination and mycelial proliferation. The specific impacts on soil fungal populations can differ based on the types of biochar, its application rate, and the intrinsic soil conditions.

Figure 9 illustrates the impact of different biochar application dosage on the growth of the soil actinomycetes population. The varying application rates of biochar considerably ($p < 0.01$) affect soil actinomycetes populations, acting as a crucial determinant of their activity and abundance in soil ecosystems. The population of actinomycetes was found to range from 19.74 to 32.47 × 10^4 CFU/g. Treatment T_8 yielded the highest actinomycetes populations, measuring 32.47 × 10^4 CFU/g. An observable enhancement in soil alkaline phosphate activity was identified with the increasing application of biochar. Numerous studies indicate that biochar, characterized by its extensive surface area and porous architecture, offers an optimal environment for actinomycetes, which are essential for organic matter decomposition and the enhancement of soil health [53, 54]. The incorporation of biochar into soil can improve moisture retention and nutrient accessibility, hence promoting the proliferation of beneficial bacteria. With the rising application of biochar, there is frequently a concomitant increase in actinomycetes populations, which can enhance soil structure, fertility, and promote plant health by inhibiting soilborne diseases through antibiotic synthesis.

The microbial population data presented in this study are based on three replicates, and the results are showing the mean bacterial population was 71.31 ± 0.24, the mean fungal population was 24.76 ± 0.09, and the mean actinomycetes population was 27.72 ± 0.10. The one-way ANOVA statistical analysis results show the differences among treatments were found to be significant at 1% level of significance ($p < 0.01$). The enhancements in soil aggregate formation may be attributed to the presence of organic material and organic carbon in biochar, which serve as essential binding agents [55, 56]. The porous characteristics and elevated interior surface area of biochar promote its function as a stabilizing agent and augment microbial growth. These microbes then produce transient chemicals that facilitate the formation of soil aggregates [57]. The presence of oxidized carboxylic acid groups on the biochar particles surface likely enhances the soil aggregates stability, hence improving the soil's structural integrity [58]. The integration of biochar into soil can markedly modify its physical and chemical properties, fostering a more conducive environment for microbial proliferation [59]. The appeal of biochar to microorganisms is partially attributed to its surface hydrophobicity; when combined with fertilizers, biochar can augment microbial populations and hence increase nutrient availability. The increase in microbial activity is ascribed to biochar's influence on soil properties, its capacity to neutralize toxic substances, and its provision of protection against predation [60]. Steiner et al. [61] noted that biochar's high water retention capacity further bolsters microbial activity.

The structure of biochar, characterized by holes that provide refuge for soil bacteria from predators, is essential for sustaining microbial communities [62]. Pores above 200 nm facilitate optimal environments for bacterial habitation, while smaller pores assist in the retention of water and nutrients vital for microbial existence [63]. The alkaline biochar properties promote the growth of gram-positive bacteria relative to gram-negative bacteria [65], and as biochar matures, a reduction in pH has been noted to enhance fungal proliferation [66].

An experiment adding a pine wood biochar and bacterial mixture to sandy loam soils resulted in a 16% increase in bacterial population density after four weeks [69]. However, Quilliam et al. [62] also noted instances where biochar application did not promote microbial colonization, possibly due to its lower nutrient content relative to the surrounding soil and a tendency to adsorb low-molecular-weight substances. Recent field studies in other degraded soils show microbial responses to biochar similar to ours, but with notable regional differences. For example, a landfill cover trial using peanut-shell biochar improved soil pH, organic matter, nutrient availability, plant growth, and microbial richness [67], while a restored mine study reported enhanced soil nutrients, plant performance, and more complex microbial networks [68]. Overall, biochar tends to boost microbial diversity and soil quality in disturbed sites, though effects vary with feedstock, rate, soil chemistry, and vegetation. These findings highlight the need for site-specific assessments and multi-site, long-term studies to evaluate broader applicability.

3.7 Statistical analysis

Statistical analysis of soil biological properties under varying biochar application rates involved twenty-four experimental runs utilizing parametric configurations suggested by full factorial randomized design (FFRD) using Design Expert-13 software. Dehydrogenase activity, alkaline phosphate activity, SMBC, and soil microbial populations (fungi, bacteria, and actinomycetes) were assessed as response variables. Analysis of variance and fit statistics using the FFRD technique examined soil biological property responses (see Table 3). All responses exhibited high F-values ranging from 444.26 to 29775.54, indicating significant biochar effects on the biological properties of soil. Additionally, all responses had P-values below 0.01, signifying significance at the 1% level. The P-values less than 0.01 show the significant effect of various biochar doses in soil amendment. Regression models with lower P-values and larger F-values were deemed significant [70]. Evaluation based on regression coefficient (R^2) values indicated satisfactory agreement ($R^2 = 0.99$) between experimental outcomes and calculations, meeting the criterion for an appropriate model.

The fit statistics for various biological properties of soil under different biochar application rates provide important insights into the variability and consistency of these soil biological indicators. Standard deviation, as a measure of variability, allows us to understand the spread or dispersion of values around the mean for each property [71]. Dehydrogenase activity, with a low standard deviation of 0.05, indicates very consistent results across different biochar rates, suggesting a stable microbial respiratory process. Alkaline phosphatase activity, which has a higher standard deviation of 0.15, exhibits more variability, indicating that biochar influences phosphate cycling processes in soil to a greater extent, depending on application rates. Soil microbial biomass carbon (SMBC) has a relatively large standard deviation of 1.99, reflecting significant variability in how

Table 3 ANOVA and fit statistics for various biological properties of soil

Source	Dehydrogenase Activity	Alkaline Phosphate Activity	SMBC	Bacteria	Fungi	Actinomycetes
Biological Properties						
Sum of Square	73.40	4979	69,110	1925	247.81	377.41
DF	7	7	7	7	7	7
Mean Square	10.49	697.00	9872.84	275.00	35.40	53.92
F-Value	444.26	29775.54	2498.68	4648.16	4481.28	5459.82
P-Value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Remark	S	S	S	S	S	S
Fit Statistics						
Std. Dev.	0.05	0.15	1.99	0.24	0.09	0.10
Mean	12.74	118.48	220.74	71.31	24.76	27.72
C.V. (%)	0.38	0.13	0.90	0.34	0.36	0.36
R^2	0.99	0.99	0.99	0.99	0.99	0.99
Predicted R^2	0.99	0.99	0.99	0.99	0.99	0.99
Adeq precision	204.48	502.94	140.17	200.24	199.22	221.88

DF = Degree of Freedom; C.V. = Coefficient of variation; Std. Dev.= Standard Deviation; S = Significant; R^2 = Coefficient of determination

Table 4 Post-Hoc tukey's HSD multiple comparison test for soil biological properties under different treatments

	Mean Diff	SEM	q value	Prob	Alpha	Sig	LCL	UCL
APA DTA	105.74	12.00	12.46	0	0.05	1	69.92	141.57
SMBC DTA	207.02	12.00	24.40	0	0.05	1	171.19	242.85
SMBC APA	101.28	12.00	11.94	0	0.05	1	65.45	137.10
Bacteria DTA	58.47	12.00	6.89	0	0.05	1	22.64	94.30
Bacteria APA	−47.27	12.00	5.57	0	0.05	1	−83.10	−11.45
Bacteria SMBC	−148.55	12.00	17.51	0	0.05	1	−184.38	−112.72
Fungi DTA	−12.02	12.00	1.41	0.92	0.05	0	−23.81	−47.85
Fungi APA	−93.72	12.00	11.05	0	0.05	1	−129.55	−57.90
Fungi SMBC	−195.00	12.00	22.98	0	0.05	1	−230.83	−159.17
Fungi Bacteria	−46.45	12.00	5.47	0.01	0.05	1	−82.28	−10.62
Actinomycetes DTA	14.98	12.00	1.77	0.81	0.05	0	−20.85	50.81
Actinomycetes APA	−90.77	12.00	10.70	0	0.05	1	−126.59	−54.94
Actinomycetes SMBC	−192.04	12.00	22.63	0	0.05	1	−227.87	−156.22
Actinomycetes Bacteria	−43.49	12.00	5.13	0.01	0.05	1	−79.32	−7.67
Actinomycetes Fungi	2.96	12.00	0.35	0.99	0.05	0	−32.87	38.78

Sig equal 1 indicates that the difference of the means is significant at the 0.05 level

Sig equal 0 indicates that the difference of the means is not significant at the 0.05 level

biochar affects the overall microbial community. This variability could be due to differences in microbial response to carbon-rich biochar and its influence on organic matter availability. The standard deviation for bacteria (0.24) suggests moderate variability, whereas fungi (0.09) and actinomycetes (0.10) show lower variability, indicating more uniform responses to biochar application among these microbial groups. The fit statistics analysis shows that while some soil biological properties, like dehydrogenase activity and specific microbial groups (fungi and actinomycetes), respond consistently to biochar applications, others, like SMBC and alkaline phosphatase, are more variable, reflecting the complex and differential impacts biochar has on soil biological processes.

The coefficient of variation (C.V.) is a measure of relative variability and indicates the dispersion of data points relative to the mean [72]. For biological properties of soil with varying biochar application rates, the C.V. values provide insights into the consistency of these parameters. Dehydrogenase activity, with a C.V. of 0.38%, shows a very low variability, implying high consistency across treatments. Alkaline phosphate activity has even less variability (0.13%), indicating an extremely uniform response [73]. Soil MBC, with a C.V. of 0.90%, displays higher variability, suggesting that biochar rates significantly affect microbial biomass. The C.V. values for bacteria (0.34%), fungi (0.36%), and actinomycetes (0.36%) are all moderately low, indicating a reasonably stable microbial community response, though with some variation in how different microbial groups react to biochar amendments. These fit statistics suggest that biochar application dosages have a more pronounced impact on microbial biomass carbon, while enzymatic activities and microbial populations exhibit moderate stability [38].

Tukey's HSD multiple comparison (see Table 4) analysis revealed statistically significant differences ($p < 0.05$) among treatments for most soil biological properties, including alkaline phosphatase activity (APA), dehydrogenase activity (DTA), soil microbial biomass carbon (SMBC), and microbial populations (bacteria, fungi, and actinomycetes). The largest mean differences were observed between SMBC and DTA (207.02) and between SMBC and fungi (−195.00), indicating strong treatment effects on microbial biomass. Positive mean differences indicate higher values in the first-named treatment, while negative values indicate higher values in the second. Fungi DTA and actinomycetes fungi comparisons showed no significant differences ($p > 0.05$), suggesting these parameters were less responsive to treatments. Overall, the results confirm that biochar application rates significantly influence most soil biological indicators, with clear separations between treatment means.

4 Conclusion

This study highlights the significant advantages of utilizing biochar to enhance soil fertility and structure. The produced biochar exhibits advantageous characteristics, including high carbon content, low O/C and H/C ratios, alkalinity, and minimal salt content, rendering it appropriate for improving nutrient availability, soil pH, and overall soil health. The lack of hazardous substances in the biochar renders it suitable for agricultural and environmental applications. The study demonstrated that biochar at the optimal rate of 28 t/ha markedly improved soil biological characteristics, encompassing dehydrogenase and alkaline phosphatase activity, SMBC, and microbial populations. The elevated surface area, porous architecture, and optimal pH of biochar foster conditions conducive to microbial proliferation, resulting in enhanced organic matter breakdown and nutrient cycling. The rise in populations of bacteria, fungi, and actinomycetes indicates that biochar positively influences soil microbial dynamics, leading to enhanced soil quality. The findings are significant as they provide evidence-based recommendations for the optimal application rate of biochar in sandy loam soils, supporting sustainable soil management practices in semi-arid agricultural systems. This research contributes to addressing critical issues of soil degradation, low productivity, and environmental sustainability in Indian agriculture and similar agroecosystems worldwide. Future studies should focus on the long-term impacts of optimal biochar application under different climatic and cropping conditions, its interactions with other soil amendments, and its role in mitigating climate through carbon sequestration.

Author contributions

Maga Ram Patel: Experimental study, Writing original draft; Narayan Lal Panwar and Ram Hari Meena: Edit and finalized the draft.

Funding

This research received no specific grant from any funding agency.

Data availability

The datasets generated during and/or analysed during the study have been presented in the article. **Clinical trial number: ** Not Applicable.

Declarations

Ethics approval and consent to participate

This work does not contain any studies with human participants or animals. All authors provided informed consent to participate in this study.

Competing interests

The authors declare no competing interests.

Acknowledgments

The authors are grateful to the Indian Council of Agricultural Research, Govt. of India, for providing financial aid to complete the research project under the Consortium Research Platform on Energy from Agriculture.

Received: 2 April 2025 / Accepted: 6 October 2025

References

1. Adekiya AO, Agbede TM, Olayanju A, Ejue WS, Adekanye TA, Adenusi TT, et al. Effect of Biochar on soil Properties, soil Loss, and Cocoyam yield on a tropical sandy loam Alfisol. Sci World J. 2020;2020:1–9. https://doi.org/10.1155/2020/9391630.
2. Danesh P, Niaparast P, Ghorbannezhad P, Ali I. Biochar production: recent Developments, Applications, and challenges. Fuel. 2023;337:126889. https://doi.org/10.1016/j.fuel.2022.126889.
3. Khan S, Irshad S, Mehmood K, Hasnain Z, Nawaz M, Rais A, et al. Biochar production and characteristics, its impacts on soil health, crop production, and yield enhancement: a review. Plants. 2024;13:166. https://doi.org/10.3390/plants13020166.
4. Lal R, Negassa W, Lorenz K. Carbon sequestration in soil. Curr Opin Environ Sustain. 2015;15:79–86. https://doi.org/10.1016/j.cosust.2015.09.002.
5. Yin J, Zhao L, Xu X, Li D, Qiu H, Cao X. Evaluation of long-term carbon sequestration of Biochar in soil with biogeochemical field model. Sci Total Environ. 2022;822:153576. https://doi.org/10.1016/j.scitotenv.2022.153576.
6. Cardoso EJBN, Vasconcellos RLF, Bini D, Miyauchi MYH, dos Santos CA, Alves PRL, et al. Soil health: looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? Sci Agric. 2013;70:274–89. https://doi.org/10.1590/S0103-90162013000400009.
7. Horwath W. Carbon cycling and formation of soil organic matter. Soil Microbiol Ecol. 2007, pp. 303–39. https://doi.org/10.1016/B978-0-08-047514-1.50016-0.
8. Chen J, Chen D, Xu Q, Fuhrmann JJ, Li L, Pan G, et al. Organic carbon quality, composition of main microbial groups, enzyme activities, and temperature sensitivity of soil respiration of an acid paddy soil treated with Biochar. Biol Fertil Soils. 2019;55:185–97. https://doi.org/10.1007/s00374-018-1333-2.
9. Tahat M, Alananbeh MM, Othman KA, Leskovar YI. D Soil Health Sustainable Agric Sustain. 2020;12:4859. https://doi.org/10.3390/su12124859.
10. Hartmann M, Six J. Soil structure and microbiome functions in agroecosystems. Nat Rev Earth Environ. 2022;4:4–18. https://doi.org/10.1038/s43017-022-00366-w.
11. Srivastava P, Balhara M, Giri B. Soil Health in India: Past History and Future Perspective, 2020, pp. 1–19. https://doi.org/10.1007/978-3-030-44364-1_1.
12. Yu H, Zou W, Chen J, Chen H, Yu Z, Huang J, et al. Biochar amendment improves crop production in problem soils: A review. J Environ Manage. 2019;232:8–21. https://doi.org/10.1016/j.jenvman.2018.10.117.
13. Hien TTT, Tsubota T, Taniguchi T, Shinogi Y. Enhancing soil water holding capacity and provision of a potassium source via optimization of the pyrolysis of bamboo biochar. Biochar. 2021;3:51–61. https://doi.org/10.1007/s42773-020-00071-1.
14. Liao JX, So PS, Bordoloi S, Li DN, Yuan HR, Chen Y et al. Plant performance and soil–plant carbon relationship response to different Biochar types. Biochar 2024;6. https://doi.org/10.1007/s42773-024-00355-w.
15. Patel MR, Panwar NL. Evaluating the agronomic and economic viability of Biochar in sustainable crop production. Biomass Bioenergy. 2024;188:107328. https://doi.org/10.1016/j.biombioe.2024.107328.
16. Fisher RA, Yates F. Statistical tables for biological, agricultural and medical research. Edinburgh: Oliver and Boyd; 1963.
17. Patel MR, Panwar NL. Development, process optimization and assessment of sustainable mobile Biochar kiln for agricultural use. J Clean Prod. 2024;477:143866. https://doi.org/10.1016/j.jclepro.2024.143866.
18. Patel MR, Panwar NL, Agrawal C, Gupta T, Meena KK, Meena SS. Design, Development, and optimization of sustainable pyrolyzer for Biochar production from agricultural crop residue. BioEnergy Res. 2024. https://doi.org/10.1007/s12155-024-10786-9.
19. International Biochar Initiative. Standardized product definition and product testing guidelines for Biochar that is used in soil. Int Biochar Initiat 2015:23.
20. Smith JL, Doran JW. Measurement and use of pH and electrical conductivity for soil quality analysis. Methods Assess Soil Qual. 2015;169–85. https://doi.org/10.2136/sssaspecpub49.c10.
21. Leng L, Huang H, Li H, Li J, Zhou W. Biochar stability assessment methods: A review. Sci Total Environ. 2019;647:210–22.
22. Carter MR. EGG. Soil sampling and methods of analysis. CRC; 2007. https://doi.org/10.1201/9781420005271.
23. Tabatabai MA, Bremner JM. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem. 1969;1:301–7. https://doi.org/10.1016/0038-0717(69)90012-1.
24. Casida LEJ, Klein DA, Thomas S. Soil dehydrogenase activity. Soil Sci. 1964;98:371–6.
25. Vance ED, Brookes PC, Jenkinson DS. An extraction method for measuring soil microbial biomass C. Soil Biol Biochem. 1987;19:703–7. https://doi.org/10.1016/0038-0717(87)90052-6.
26. Ajayi AE. Biochar-Induced changes in soil resilience: effects of soil texture and Biochar dosage. Pedosphere. 2017;27:236–47. https://doi.org/10.1016/S1002-0160(17)60313-8.
27. Smider B, Singh B. Agronomic performance of a high Ash Biochar in two contrasting soils. Agric Ecosyst Environ. 2014;191:99–107. https://doi.org/10.1016/j.agee.2014.01.024.
28. Wang L, Butterly CR, Wang Y, Herath HMSK, Xi YG, Xiao XJ. Effect of crop residue Biochar on soil acidity amelioration in strongly acidic tea garden soils. Soil Use Manag. 2014;30:119–28. https://doi.org/10.1111/sum.12096.
29. Bilias F, Nikoli T, Kalderis D, Gasparatos D. Towards a soil remediation strategy using biochar: effects on soil chemical properties and bioavailability of potentially toxic elements. Toxics. 2021;9. https://doi.org/10.3390/toxics9080184.
30. Natasha N, Shahid M, Khalid S, Bibi I, Naeem MA, Niazi NK, et al. Influence of Biochar on trace element uptake, toxicity and detoxification in plants and associated health risks: A critical review. Crit Rev Environ Sci Technol. 2022;52:2803–43. https://doi.org/10.1080/10643389.2021.1894064.
31. Sanei H, Rudra A, Przyswitt ZMM, Kousted S, Sindlev MB, Zheng X, et al. Assessing biochar's permanence: an inertinite benchmark. Int J Coal Geol. 2024;281:104409. https://doi.org/10.1016/j.coal.2023.104409.
32. Tisserant A, Cherubini F. Potentials, limitations, co-benefits, and trade-offs of Biochar applications to soils for climate change mitigation. Land. 2019;8. https://doi.org/10.3390/LAND8120179.
33. Hedley M, Camps-Arbestain M, Mclaren S, Jones J, Chen Q, Hedley CB et al. A review of evidence for the potential role of Biochar to reduce net GHG emissions from new Zealand agriculture 2020.
34. Pokharel P, Ma Z, Chang SX. Biochar increases soil microbial biomass with changes in extra- and intracellular enzyme activities: a global meta-analysis. Biochar. 2020;2:65–79. https://doi.org/10.1007/s42773-020-00039-1.
35. Tian X, Wang L, Hou Y, Wang H, Tsang YF. Responses of soil microbial community structure and activity to incorporation of straws and straw biochars and their effects on soil respiration and soil organic carbon turnover. Pedosphere. 2019;29:492–503. https://doi.org/10.1016/S1002-0160(19)60813-1.

36. Tesfaye M. Influence of enhanced malate dehydrogenase expression by alfalfa on diversity of rhizobacteria and soil nutrient availability. Soil Biol Biochem. 2003;35:1103–13. https://doi.org/10.1016/S0038-0717(03)00162-7.
37. Tang L, Shi Y, Zhang Y, Yang D, Guo C. Effects of plant-growth-promoting rhizobacteria on soil bacterial community, soil physicochemical properties, and soil enzyme activities in the rhizosphere of alfalfa under field conditions. Diversity. 2023;15:537. https://doi.org/10.3390/d15040537.
38. Brtnicky M, Dokulilova T, Holatko J, Pecina V, Kintl A, Latal O, et al. Long-term effects of biochar-based organic amendments on soil microbial parameters. Agronomy. 2019;9:747. https://doi.org/10.3390/agronomy9110747.
39. Liao J, Huang Y, Su Y, Guo H, Ni J, Li D et al. Biochar effects on bacterial communities in recycled concrete aggregates. Front Environ Sci Eng 2025;19. https://doi.org/10.1007/s11783-025-1967-x.
40. Gul S, Whalen JK. Biochemical cycling of nitrogen and phosphorus in biochar-amended soils. Soil Biol Biochem. 2016;103:1–15. https://doi.org/10.1016/j.soilbio.2016.08.001.
41. Palansooriya KN, Wong JTF, Hashimoto Y, Huang L, Rinklebe J, Chang SX, et al. Response of microbial communities to biochar-amended soils: a critical review. Biochar. 2019;1:3–22. https://doi.org/10.1007/s42773-019-00009-2.
42. Yang F, Sui L, Tang C, Li J, Cheng K, Xue Q. Sustainable advances on phosphorus utilization in soil via addition of Biochar and humic substances. Sci Total Environ. 2021;768:145106. https://doi.org/10.1016/j.scitotenv.2021.145106.
43. Li F, Liang X, Niyungeko C, Sun T, Liu F, Arai Y. Effects of biochar amendments on soil phosphorus transformation in agricultural soils, 2019, pp. 131–72. https://doi.org/10.1016/bs.agron.2019.07.002.
44. Zhang L, Xiang Y, Jing Y, Zhang R. Biochar amendment effects on the activities of soil carbon, nitrogen, and phosphorus hydrolytic enzymes: a meta-analysis. Environ Sci Pollut Res. 2019;26:22990–3001. https://doi.org/10.1007/s11356-019-05604-1.
45. Fang X, Lee X, Twagirayezu G, Cheng H, Lu H, Huang S, et al. A critical review of the effectiveness of Biochar coupled with arbuscular mycorrhizal fungi in soil cadmium immobilization. J Fungi. 2024;10:182. https://doi.org/10.3390/jof10030182.
46. Umar W, Ayub MA, Rehman MZ ur, Ahmad HR, Farooqi ZUR, Shahzad A et al. Nitrogen and Phosphorus Use Efficiency in Agroecosystems. Resour. Use Effic. Agric., Singapore: Springer Singapore; 2020, pp. 213–57. https://doi.org/10.1007/978-981-15-6953-1_7.
47. Mitchell PJ, Simpson AJ, Soong R, Schurman JS, Thomas SC, Simpson MJ. Biochar amendment and phosphorus fertilization altered forest soil microbial community and native soil organic matter molecular composition. Biogeochemistry. 2016;130:227–45. https://doi.org/10.1007/s10533-016-0254-0.
48. Ameloot N, De Neve S, Jegajeevagan K, Yildiz G, Buchan D, Funkuin YN, et al. Short-term CO2 and N2O emissions and microbial properties of Biochar amended sandy loam soils. Soil Biol Biochem. 2013;57:401–10. https://doi.org/10.1016/j.soilbio.2012.10.025.
49. Luo Y, Durenkamp M, De Nobili M, Lin Q, Devonshire BJ, Brookes PC. Microbial biomass growth, following incorporation of biochars produced at 350°C or 700°C, in a silty-clay loam soil of high and low pH. Soil Biol Biochem. 2013;57:513–23. https://doi.org/10.1016/j.soilbio.2012.10.033.
50. Zhou H, Zhang D, Wang P, Liu X, Cheng K, Li L, et al. Changes in microbial biomass and the metabolic quotient with Biochar addition to agricultural soils: A Meta-analysis. Agric Ecosyst Environ. 2017;239:80–9. https://doi.org/10.1016/j.agee.2017.01.006.
51. Liu S, Meng J, Jiang L, Yang X, Lan Y, Cheng X, et al. Rice husk Biochar impacts soil phosphorous availability, phosphatase activities and bacterial community characteristics in three different soil types. Appl Soil Ecol. 2017;116:12–22. https://doi.org/10.1016/j.apsoil.2017.03.020.
52. Zheng Y, Han X, Li Y, Yang J, Li N, An N. Effects of biochar and straw application on the physicochemical and biological properties of paddy soils in Northeast China. Sci Rep. 2019;9:16531. https://doi.org/10.1038/s41598-019-52978-w.
53. Devassy M, John E, Mariam Paul N. Characterization of plant growth promoting activity of actinomycetes isolated from biochar enhanced rhizosphere soil. Mater Today Proc. 2023. https://doi.org/10.1016/j.matpr.2023.11.007.
54. Gorovtsov AV, Minkina TM, Mandzhieva SS, Perelomov LV, Soja G, Zamulina IV, et al. The mechanisms of biochar interactions with microorganisms in soil. Environ Geochem Health. 2020;42:2495–518. https://doi.org/10.1007/s10653-019-00412-5.
55. Six J, Bossuyt H, Degryze S, Denef K. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res. 2004;79:7–31. https://doi.org/10.1016/j.still.2004.03.008.
56. Hati KM, Swarup A, Dwivedi AK, Misra AK, Bandyopadhyay KK. Changes in soil physical properties and organic carbon status at the topsoil horizon of a vertisol of central India after 28 years of continuous cropping, fertilization and manuring. Agric Ecosyst Environ. 2007;119:127–34. https://doi.org/10.1016/j.agee.2006.06.017.
57. Ma N, Zhang L, Zhang Y, Yang L, Yu C, Yin G, et al. Biochar improves soil aggregate stability and water availability in a Mollisol after three years of field application. PLoS ONE. 2016;11:e0154091. https://doi.org/10.1371/journal.pone.0154091.
58. Glaser B, Lehmann J, Zech W. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal - a review. Biol Fertil Soils. 2002;35:219–30. https://doi.org/10.1007/s00374-002-0466-4.
59. Xu H-J, Wang X-H, Li H, Yao H-Y, Su J-Q, Zhu Y-G. Biochar impacts soil microbial community composition and nitrogen cycling in an acidic soil planted with rape. Environ Sci Technol. 2014;48:9391–9. https://doi.org/10.1021/es5021058.
60. Warnock DD, Lehmann J, Kuyper TW, Rillig MC. Mycorrhizal responses to biochar in soil – concepts and mechanisms. Plant Soil. 2007;300:9–20. https://doi.org/10.1007/s11104-007-9391-5.
61. Steiner C, Glaser B, Geraldes Teixeira W, Lehmann J, Blum WEH, Zech W. Nitrogen retention and plant uptake on a highly weathered central Amazonian ferralsol amended with compost and charcoal. J Plant Nutr Soil Sci. 2008;171:893–9. https://doi.org/10.1002/jpln.200625199.
62. Quilliam RS, Glanville HC, Wade SC, Jones DL. Life in the 'charosphere' – Does Biochar in agricultural soil provide a significant habitat for microorganisms? Soil Biol Biochem. 2013;65:287–93. https://doi.org/10.1016/j.soilbio.2013.06.004.
63. Brewer CE, Brown RC. Biochar. Compr. Renew., Energy E. 2012, pp. 357–84. https://doi.org/10.1016/B978-0-08-087872-0.00524-2.
64. Gul S, Whalen JK, Thomas BW, Sachdeva V, Deng H. Physico-chemical properties and microbial responses in biochar-amended soils: mechanisms and future directions. Agric Ecosyst Environ. 2015;206:46–59. https://doi.org/10.1016/j.agee.2015.03.015.
65. Farrell M, Kuhn TK, Macdonald LM, Maddern TM, Murphy DV, Hall PA, et al. Microbial utilisation of biochar-derived carbon. Sci Total Environ. 2013;465:288–97. https://doi.org/10.1016/j.scitotenv.2013.03.090.

66. Zimmermann M, Bird MI, Wurster C, Saiz G, Goodrick I, Barta J, et al. Rapid degradation of pyrogenic carbon. Glob Chang Biol. 2012;18:3306–16. https://doi.org/10.1111/j.1365-2486.2012.02796.x.
67. Liao J, Hao G, Guo H, Chen H. Effects of Biochar on plant and microbial communities in landfill soil. Appl Soil Ecol. 2024;204. https://doi.org/10.1016/j.apsoil.2024.105749.
68. Liao JX, Bordoloi S, Wang YC, Hau BCH, Ng CWW. Field study of Biochar effects on vegetation and bacterial communities in a restored mine. Appl Soil Ecol 2025;211. https://doi.org/10.1016/j.apsoil.2025.106141.
69. Hale L, Luth M, Crowley D. Biochar characteristics relate to its utility as an alternative soil inoculum carrier to peat and vermiculite. Soil Biol Biochem. 2015;81:228–35. https://doi.org/10.1016/j.soilbio.2014.11.023.
70. Anupam K, Swaroop V, Deepika, Lal PS, Bist V. Turning Leucaena Leucocephala bark to Biochar for soil application via statistical modelling and optimization technique. Ecol Eng. 2015;82:26–39. https://doi.org/10.1016/j.ecoleng.2015.04.078.
71. Webster R. Statistics to support soil research and their presentation. Eur J Soil Sci. 2001;52:331–40. https://doi.org/10.1046/j.1365-2389.2001.00383.x.
72. Shechtman O. The Coefficient of Variation as an Index of Measurement Reliability, 2013, pp. 39–49. https://doi.org/10.1007/978-3-642-37131-8_4.
73. Sebastián M, Arístegui J, Montero MF, Escanez J, Xavier Niell F. Alkaline phosphatase activity and its relationship to inorganic phosphorus in the transition zone of the North-western African upwelling system. Prog Oceanogr. 2004;62:131–50. https://doi.org/10.1016/j.pocean.2004.07.007.

Publisher's note