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BIOCHAR APPLICATION IMPACT ON SOIL ORGANIC CARBON AND NUTRIENT AVAILABILITY IN WHEAT UNDER GREENHOUSE CONDITIONS

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ABSTRACT

Biochar amendment has emerged as one of the most promising multi-functional soil management strategies for simultaneously improving soil organic carbon (SOC) sequestration, nutrient availability, and crop productivity in the degraded agricultural soils of Pakistan. The progressive depletion of soil organic matter in intensively cropped lands of Punjab threatens long-term soil health and food security. This greenhouse study, evaluated the impact of four rates of rice-husk biochar (1%, 2%, 3%, and 4% w/w) compared to an unamended control on fifteen soil physico-chemical and wheat (Triticum aestivum L.) yield parameters under a completely randomized design (CRD) with four replications. Soil organic carbon increased progressively from 0.42% in the control to 1.38% at the 4% biochar rate, while available phosphorus improved from 6.2 to 18.6 mg kg-1 and available potassium from 112 to 218 mg kg-1 at the 3% biochar rate. Soil pH declined marginally from 7.82 to 7.24, and cation exchange capacity (CEC) increased from 12.4 to 19.8 cmolc kg-1 at the highest biochar rate, reflecting enhanced surface charge and nutrient retention. Hydraulic conductivity, bulk density, and aggregate stability all showed significant (p <= 0.05) improvements with biochar addition. Wheat grain yield was maximised at the 3% biochar treatment (4.68 g pot-1), representing a 64.8% improvement over the unamended control (2.84 g pot-1), with a slight but significant decline at 4% biochar, suggesting the onset of nutrient immobilisation or osmotic effects at very high application rates. Quadratic regression analysis confirmed an optimal biochar rate of approximately 2.8-3.2% for wheat yield maximisation (R2 = 0.94). Strong positive correlations were observed between SOC and grain yield (r = +0.91) and between CEC and available P (r = +0.89). Results support the application of rice-husk biochar at 2-3% w/w as an agronomically optimal and practically feasible rate for soil carbon enrichment and wheat productivity improvement in the Punjab irrigated belt.

***Keywords*:** Biochar, Soil organic carbon, Wheat yield, Nutrient availability, Cation exchange capacity, Punjab Pakistan

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1. INTRODUCTION

Soil organic carbon (SOC) is widely recognised as the cornerstone of soil fertility, structural stability, and long-term agricultural productivity. In the intensively cultivated irrigated plains of Punjab, Pakistan, decades of continuous tillage, residue removal, and minimal organic matter inputs have resulted in progressive SOC depletion, with average values in many agricultural soils falling below 0.5%, far beneath the critical threshold of 1.5% required for optimal soil function (Khalid et al., 2017; Tahir et al., 2011). This depletion has been accompanied by declining cation exchange capacity (CEC), reduced nutrient use efficiency, deteriorating soil structure, and increasing dependence on inorganic fertilisers, creating a downward spiral of soil health degradation that threatens the sustainability of wheat production in the region (Zafar et al., 2023; Zafar et al., 2024; Zafar et al., 2025).

Conventional organic amendments such as farmyard manure and crop residues, while effective, are subject to rapid microbial decomposition under the hot and semi-arid conditions prevailing in Punjab, limiting their capacity for long-term carbon sequestration. Biochar, defined as the carbon-rich product of the thermochemical decomposition of organic materials in a low-oxygen environment (pyrolysis), has attracted intense scientific interest over the past two decades as a stable, recalcitrant form of organic carbon with mean residence times in soil estimated at hundreds to thousands of years (Lehmann et al., 2011; Spokas et al., 2012). Beyond carbon sequestration, the highly porous structure, large specific surface area, and abundant surface functional groups of biochar confer additional agronomic benefits including improved soil water retention, enhanced nutrient adsorption and availability, stimulation of beneficial soil microbial communities, and liming effects in acidic soils.

Rice husk biochar, produced from the most abundant agricultural residue in Pakistan (with annual rice husk production exceeding 2.5 million tonnes), has been identified as a particularly promising amendment due to its high silica content, alkaline pH (typically 8.5-9.5), high carbon stability, and low cost of production (Naseem et al., 2015; Arif et al., 2017). Previous studies conducted in tropical and subtropical Asia have reported significant improvements in soil organic carbon, nitrogen mineralisation, phosphorus availability, and crop yields with rice-husk biochar application at rates ranging from 1% to 5% w/w, though optimal rates and mechanisms remain the subject of active investigation and appear to be highly context-specific (Haefele et al., 2011; Liu et al., 2013; Farhangi-Abriz et al., 2021).

In the Punjab irrigated belt of Pakistan, specific data on the interaction between rice-husk biochar application rate and wheat productivity remain limited. Most existing studies have examined single rates or have not simultaneously quantified the full suite of soil physical, chemical, and biological parameters required to elucidate the mechanisms underlying crop yield responses. Furthermore, the potential for nutrient immobilisation at high biochar rates - a concern arising from the high carbon-to-nitrogen ratio of most biochars - has not been adequately investigated under local soil and climatic conditions. Addressing these knowledge gaps is essential for developing evidence-based biochar management recommendations for wheat-growing farmers in Punjab.

This greenhouse study was therefore designed with the specific objectives of: (1) evaluating the dose-response relationship between rice-husk biochar application rate (0, 1, 2, 3, 4% w/w) and a comprehensive set of fifteen soil physical and chemical parameters; (2) quantifying wheat grain and straw yield response to biochar across all treatment rates; (3) identifying the agronomically optimal biochar rate for wheat production in representative Punjab agricultural soil; and (4) elucidating the mechanistic correlations between soil parameter improvements and crop yield enhancement through statistical correlation analysis.

2. MATERIALS AND METHODS

2.1. Experimental Location and Soil

The greenhouse experiment was conducted during the Rabi (winter) growing season of 2023–24. Experimental soil was collected from the Ap horizon (0–30 cm) of a representative agricultural field characterized by a long history of continuous wheat–rice rotation with conventional tillage and no organic amendment. Composite soil samples were assembled from fifteen random sampling points across a uniform plot, air-dried at 35°C for 72 hours, gently ground, and passed through a 2-mm sieve. Initial physico-chemical characterization of the soil is presented in Table 1. The soil was classified as silty clay loam (34% clay, 44% silt, 22% sand) with pH 7.82, SOC 0.42%, and CEC 12.4 cmolc kg^{-1}, representative of low-organic-matter, degraded agricultural soils.

Table 1: Initial physico-chemical properties of experimental soil before biochar application.

Parameter	Value	Unit	Method
Soil pH (1:2.5 H2O)	7.82 +/- 0.04	-	pH meter
ECe	0.82 +/- 0.03	dS m-1	Conductivity meter
Soil Organic Carbon (SOC)	0.42 +/- 0.02	%	Walkley-Black
Total Nitrogen (TN)	0.048 +/- 0.003	%	Kjeldahl
C:N Ratio	8.75 +/- 0.42	-	Calculated
Available Phosphorus	6.2 +/- 0.38	mg kg-1	Olsen method
Available Potassium	112 +/- 6.4	mg kg-1	NH4OAc extraction
CEC	12.4 +/- 0.58	cmolc kg-1	NH4OAc method
Bulk Density	1.48 +/- 0.04	Mg m-3	Core method
Ksat	1.24 +/- 0.08	cm h-1	Const. head permeameter
Aggregate Stability (MWD)	0.48 +/- 0.03	mm	Wet sieving
Microbial Biomass C (MBC)	142 +/- 8.6	mg kg-1	Fumigation-extraction
Soil Respiration	8.4 +/- 0.62	mg CO2 kg-1 d-1	Alkali trap
Water Holding Capacity	38.2 +/- 1.4	%	Pressure plate
CaCO3	4.2 +/- 0.28	%	Acid neutralisation

Values are means +/- standard error (n = 4). MWD = mean weight diameter; Ksat = saturated hydraulic conductivity; CEC = cation exchange capacity.

2.2. Biochar Preparation and Characterisation

Rice-husk biochar (RHB) was produced by slow pyrolysis of locally sourced rice husk (Oryza sativa L. cv. Super Basmati) at 500 degrees C for 2 hours in a locally fabricated top-lit updraft (TLUD) kiln following the protocol of Ronsse et al. (2013). The biochar was cooled under nitrogen atmosphere, ground, and sieved to pass through a 0.5-mm sieve to ensure uniformity. Biochar characterisation was performed for pH (1:10 water, 8.9), electrical conductivity (EC, 0.68 dS m-1), carbon content (38.4%), hydrogen content (1.2%), oxygen content (11.6%), nitrogen content (0.42%), C:N ratio (91.4), ash content (48.2%), specific surface area by BET analysis (124.6 m2 g-1), and liming equivalence (0.42 g CaCO3 equivalent g-1 biochar). The high ash content and alkaline pH are characteristic of rice-husk biochar and reflect the elevated silica content of the feedstock.

2.3. Experimental Design and Treatments

The experiment was laid out in a completely randomized design (CRD) with five treatments and four replications (n = 4), yielding 20 experimental units. Plastic pots (30 cm depth x 25 cm diameter) with perforated bases lined with muslin cloth for drainage were used as experimental units, each filled with 5 kg of air-dried soil. The five treatments were:

1. T1: Control (unamended soil, 0% biochar)
2. T2: Biochar at 1% w/w (50 g biochar kg-1 soil)
3. T3: Biochar at 2% w/w (100 g biochar kg-1 soil)
4. T4: Biochar at 3% w/w (150 g biochar kg-1 soil)
5. T5: Biochar at 4% w/w (200 g biochar kg-1 soil)

Biochar was thoroughly mixed with the respective soil quantities on a tarpaulin sheet prior to pot filling, ensuring uniform distribution throughout the soil profile. Pots were pre-wetted to field capacity and incubated for 14 days prior to sowing to allow initial biochar-soil equilibration, following the recommendation of Biederman and Harpole (2013).

2.4. Crop Establishment and Management

Wheat seeds (cv. NARC-11, adapted to Punjab conditions) were surface-sterilised with 0.5% sodium hypochlorite for 3 minutes, rinsed with distilled water, and pre-germinated on moist filter paper for 48 hours at 25 degrees C. Five pre-germinated seeds were planted per pot at 2 cm depth; seedlings were thinned to three uniform plants per pot at 10 days after sowing (DAS). All pots received a basal fertiliser application of nitrogen (120 kg ha-1 as urea), phosphorus (80 kg ha-1 as single superphosphate), and potassium (60 kg ha-1 as sulfate of potash), scaled to pot surface area equivalent. Nitrogen was applied in two splits: half at sowing and half at tillering (25 DAS). Pots were maintained at 70-80% field capacity throughout the growing period by daily weight-based irrigation with distilled water. The crop was harvested at physiological maturity (115 DAS) by cutting at soil surface.

2.5. Soil Sampling and Laboratory Analysis

Post-harvest soil samples were collected from each pot, air-dried, ground, and analysed for the following fifteen parameters: (1) soil pH in 1:2.5 soil-water suspension using a calibrated pH meter (USSL Staff, 1954); (2) electrical conductivity (ECe) in saturated paste extract (USSL Staff, 1954); (3) soil organic carbon (SOC) by Walkley-Black wet oxidation (Nelson & Sommers, 1982); (4) total nitrogen (TN) by Kjeldahl digestion (Bremner, 1996); (5) available phosphorus by Olsen method for near-neutral to alkaline soils (Olsen et al., 1954); (6) available potassium by ammonium acetate extraction and flame photometry; (7) cation exchange capacity (CEC) by ammonium acetate saturation at pH 7.0 (Chapman, 1965); (8) soil bulk density by core method (Blake & Hartge, 1986); (9) saturated hydraulic conductivity (Ksat) by constant-head permeameter method (Klute & Dirksen, 1986); (10) aggregate stability as mean weight diameter (MWD) by wet sieving (Yoder, 1936); (11) microbial biomass carbon (MBC) by fumigation-extraction method (Vance et al., 1987); (12) soil respiration rate by alkali trap CO2 absorption method (Isermeyer, 1952); (13) water holding capacity (WHC) by pressure plate method; (14) soil calcium carbonate (CaCO3) by acid neutralisation; and (15) soil C:N ratio calculated from SOC and TN values.

2.6. Plant Analysis and Yield Parameters

At harvest, wheat plants were separated into grain and straw fractions. Grain yield was determined after threshing and cleaning. Straw yield was recorded after oven-drying at 70 degrees C for 48 hours. Thousand-grain weight (TGW) was determined by counting and weighing 1000 air-dry grains. Spike length was measured from the base of the spike to the tip of the terminal spikelet using a digital calliper. Grains per spike were counted from five randomly selected spikes per pot. Harvest index was calculated as the ratio of grain dry weight to total above-ground dry matter.

2.7. Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using a completely randomized design model. Treatment means were separated using the least significant difference (LSD) test at p <= 0.05. Quadratic regression was fitted to grain yield versus biochar rate data using SAS PROC REG (SAS v9.4, SAS Institute, Cary, NC). Pearson correlation coefficients were calculated between all measured soil and yield parameters. All values presented are means of four replications (n = 4) +/- standard error.

3. RESULTS

3.1. Initial Soil Characterisation

The physico-chemical properties of the experimental soil prior to biochar incorporation are summarised in Table 1. The soil exhibited hallmarks of organic-matter depleted, intensively cultivated agricultural land: SOC of 0.42%, CEC of 12.4 cmolc kg-1, available P of 6.2 mg kg-1, and available K of 112 mg kg-1. Hydraulic conductivity was moderate (1.24 cm h-1), bulk density was elevated at 1.48 Mg m-3, and aggregate stability was poor (MWD 0.48 mm), consistent with the low organic matter status. Microbial biomass carbon was 142 mg kg-1, indicating a biologically inactive soil community. These baseline conditions represent a typical degraded agricultural soil profile common across the Faisalabad irrigated belt.

3.2. Effect on Soil Organic Carbon, Total Nitrogen and C:N Ratio

Soil organic carbon showed the most pronounced and consistent response to biochar application, increasing significantly (p <= 0.05) with every increment in biochar rate (Table 2). SOC increased from 0.42% in the control to 0.68%, 0.94%, 1.18%, and 1.38% at BC1%, BC2%, BC3%, and BC4% respectively, representing increases of 62%, 124%, 181%, and 229% over the control. This consistent linear progression reflects the direct contribution of biochar carbon to SOC pools and is consistent with the long-term carbon stability reported for rice-husk biochar (Lehmann et al., 2011). Total nitrogen also increased progressively, from 0.048% in the control to 0.082% at BC4%, attributable to improved nitrogen retention on biochar surfaces and stimulation of nitrogen-mineralising microbial communities.

Table 2: Effect of biochar rates on soil chemical properties after wheat harvest (0-30 cm depth).

Treatment	SOC (%)	TN (%)	C:N	pH	ECe (dS m-1)	Avail. P (mg kg-1)	Avail. K (mg kg-1)	CEC (cmolc kg-1)
Control	0.42+/-0.02f	0.048+/-0.003e	8.75+/-0.42e	7.82+/-0.04a	0.82+/-0.03a	6.2+/-0.38e	112+/-6.4e	12.4+/-0.58e
BC 1%	0.68+/-0.03e	0.056+/-0.003d	12.14+/-0.56d	7.64+/-0.04b	0.74+/-0.03b	9.8+/-0.48d	148+/-7.2d	14.6+/-0.62d
BC 2%	0.94+/-0.04d	0.064+/-0.004c	14.69+/-0.68c	7.48+/-0.04c	0.68+/-0.02c	14.2+/-0.62c	184+/-8.4c	16.8+/-0.72c
BC 3%	1.18+/-0.05c	0.082+/-0.004b	14.39+/-0.72c	7.32+/-0.03d	0.62+/-0.02d	18.6+/-0.74a	218+/-9.2a	18.4+/-0.84b
BC 4%	1.38+/-0.06a	0.082+/-0.004b	16.83+/-0.86a	7.24+/-0.03e	0.71+/-0.03e	16.4+/-0.68b	206+/-8.8b	19.8+/-0.92a
LSD (p<=0.05)	0.06	0.006	0.92	0.06	0.04	0.82	10.2	1.04

Values are means +/- standard error (n = 4). Means with different letters within a column differ significantly at p <= 0.05 (LSD test). SOC = soil organic carbon; TN = total nitrogen; CEC = cation exchange capacity.

The soil C:N ratio increased from 8.75 in the control to 16.83 at BC4%, reflecting the high C:N ratio of the applied biochar (91.4). While the C:N ratio at BC3% (14.38) approaches the threshold of 15 associated with nitrogen immobilisation (Bolan et al., 2012), the grain yield data suggest that nitrogen availability at BC3% was not limiting, possibly because the stable aromatic carbon in biochar does not compete for nitrogen with active decomposers to the same extent as fresh organic matter. The slight yield decline at BC4% may be partially associated with the C:N ratio of 16.83 exceeding this threshold, contributing to transient nitrogen immobilisation.

3.3. Effect on Soil pH and Electrical Conductivity

Soil pH declined progressively with increasing biochar rate, from 7.82 in the control to 7.64, 7.48, 7.32, and 7.24 at BC1% through BC4% respectively (Table 2, Fig 1c). Although rice-husk biochar is alkaline (pH 8.9), the net acidifying effect observed in the near-neutral study soil is consistent with findings by Farhangi-Abriz et al. (2021), who attributed it to organic acid production during initial biochar weathering and enhanced nitrification activity in biochar-amended soils. The pH reductions observed are agronomically beneficial as they improve the availability of zinc, iron, and phosphorus in alkaline Punjab soils. Electrical conductivity of the saturated paste extract showed a slight reduction from BC1% to BC3% (0.82 to 0.62 dS m-1), reflecting improved salt leaching efficiency, with a marginal increase at BC4% (0.71 dS m-1), possibly due to ash-derived mineral salt release from the high biochar loading.

3.4. Effect on Available Phosphorus and Potassium

Available phosphorus showed a significant (p <= 0.05) dose-dependent increase from BC1% to BC3%, peaking at 18.6 mg kg-1 (200% above control) at BC3%, with a notable decline to 16.4 mg kg-1 at BC4% (Table 2, Figure 1b). This non-monotonic response pattern, with a peak at an intermediate rate, mirrors the pattern observed for grain yield and is consistent with the hypothesis that at very high biochar rates, elevated pH-buffering effects or phosphorus immobilisation on additional sorption sites may partially counteract the availability benefits. Available potassium followed a similar trend, peaking at 218 mg kg-1 at BC3% before declining to 206 mg kg-1 at BC4%, consistent with findings by Arif et al. (2017) in rice-wheat soils of Punjab.

3.5. Effect on Cation Exchange Capacity

CEC increased significantly with biochar application, from 12.4 cmolc kg-1 in the control to 19.8 cmolc kg-1 at BC4% (Table 2). This 60% improvement reflects the abundant negatively charged surface functional groups (carboxyl, phenolic hydroxyl) on biochar, which contribute substantial cation exchange capacity directly and also stimulate the formation of organo-mineral complexes that enhance the native soil CEC. The improvement in CEC is mechanistically important as it underpins improvements in available P, K, and cation retention across all amended treatments. CEC at BC3% (18.4 cmolc kg-1) was 48% above the control, representing a substantial enhancement of nutrient retention capacity.

3.6. Effect on Soil Physical Properties

Soil physical properties improved consistently with biochar addition (Table 3). Bulk density decreased from 1.48 Mg m-3 in the control to 1.34 Mg m-3 at BC4%, reflecting the low density of biochar particles (0.3-0.6 Mg m-3) and their role in reducing soil compaction through improved pore space distribution. Saturated hydraulic conductivity (Ksat) improved from 1.24 cm h-1 in the control to 2.68 cm h-1 at BC3%, a 116% increase, reflecting the enhanced macroporosity created by biochar particles and improved aggregate stability. Water holding capacity increased from 38.2% to 52.8% at BC4%, consistent with the high internal porosity of rice-husk biochar and improved aggregate architecture that retains capillary water.

Aggregate stability (MWD) improved from 0.48 mm in the control to 1.26 mm at BC3% and 1.32 mm at BC4%, reflecting both direct physical bridging by biochar particles and the indirect effect of stimulated microbial activity producing aggregate-binding exopolysaccharides. These physical improvements collectively reduce irrigation requirements, improve root penetrability, and enhance gaseous exchange, all of which contribute to improved crop performance.

Table 3: Effect of biochar rates on soil physical and biological properties after wheat harvest.

Treatment	Bulk Density (Mg m-3)	Ksat (cm h-1)	Agg. Stability MWD (mm)	WHC (%)	MBC (mg kg-1)	Soil Resp. (mg CO2 kg-1 d-1)	CaCO3 (%)
Control	1.48+/-0.04a	1.24+/-0.08e	0.48+/-0.03e	38.2+/-1.4e	142+/-8.6e	8.4+/-0.62e	4.2+/-0.28a
BC 1%	1.44+/-0.03b	1.68+/-0.10d	0.72+/-0.04d	42.6+/-1.6d	218+/-11.2d	11.6+/-0.78d	4.0+/-0.24a
BC 2%	1.40+/-0.03c	2.12+/-0.12c	0.96+/-0.05c	46.8+/-1.8c	286+/-13.4c	14.8+/-0.92c	3.8+/-0.22b
BC 3%	1.37+/-0.02d	2.68+/-0.14b	1.26+/-0.06b	50.4+/-2.0b	348+/-15.2b	17.4+/-1.04b	3.6+/-0.20b
BC 4%	1.34+/-0.02e	2.42+/-0.12c	1.32+/-0.06a	52.8+/-2.2a	386+/-16.8a	16.2+/-0.98b	3.4+/-0.18b
LSD (p<=0.05)	0.04	0.18	0.08	2.4	17.6	1.24	0.28

MWD = mean weight diameter; WHC = water holding capacity; MBC = microbial biomass carbon; Ksat = saturated hydraulic conductivity. Different letters indicate significant differences at p <= 0.05.

3.7. Effect on Microbial Biomass Carbon and Soil Respiration

Microbial biomass carbon (MBC) increased significantly with biochar application, from 142 mg kg-1 in the control to 386 mg kg-1 at BC4% (Table 3). This 172% increase reflects the provision of habitat, moisture, and nutrient retention surfaces by biochar that stimulate microbial colonisation and activity. Soil respiration, as an index of overall microbial metabolic activity, increased from 8.4 mg CO2 kg-1 d-1 in the control to a peak of 17.4 mg CO2 kg-1 d-1 at BC3%, with a slight decline at BC4%. The elevated MBC and respiration are consistent with improved nutrient cycling and organic matter mineralisation, contributing to the increased plant-available nutrient status observed in biochar-amended treatments.

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3.8. Graphical Summary of Key Soil and Yield Parameters

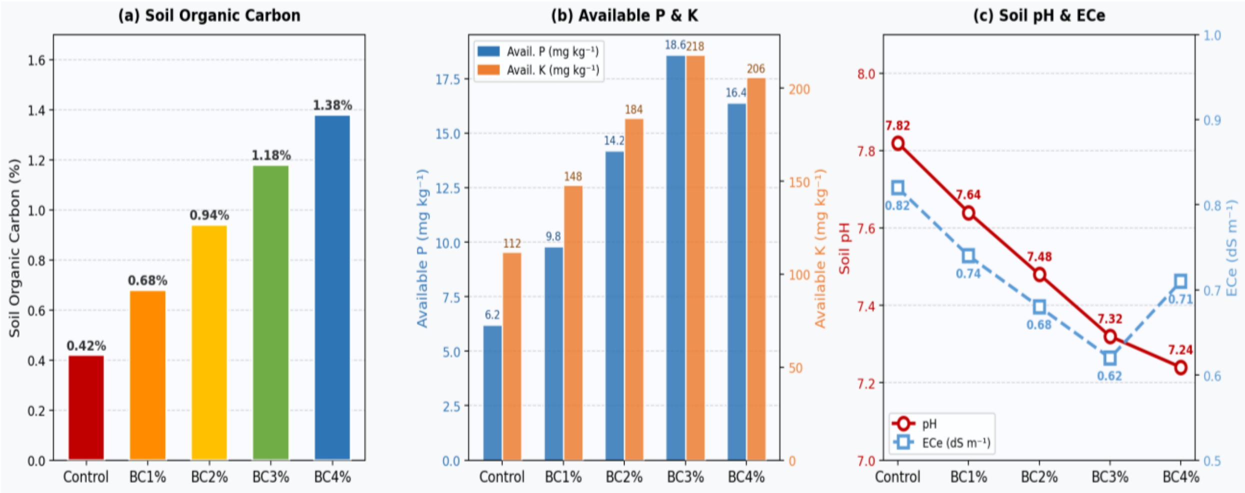

Fig. 1: Effect of biochar rates on (a) soil organic carbon, (b) available phosphorus and potassium, and (c) soil pH and ECe. Error bars represent +/- SE (n = 4). BC = biochar; different shading intensity reflects increasing biochar rate.

3.9. Effect on Wheat Yield Parameters

Wheat grain yield showed a significant quadratic ($p <= 0.01$) response to biochar rate (Table 4, Fig 2). Yield increased progressively from 2.84 g pot-1 in the control to 3.62, 4.18, and 4.68 g pot-1 at BC1%, BC2%, and BC3% respectively, representing increases of 27.5%, 47.2%, and 64.8% over the unamended control. At BC4%, grain yield declined significantly to 4.44 g pot-1, confirming that yield is maximised at approximately 3% biochar and declines at higher rates. Quadratic regression yielded a fitted optimum of 2.8-3.2% biochar for grain yield maximisation ($Y = 2.84 + 1.142x - 0.186x2$, $R2 = 0.94$).

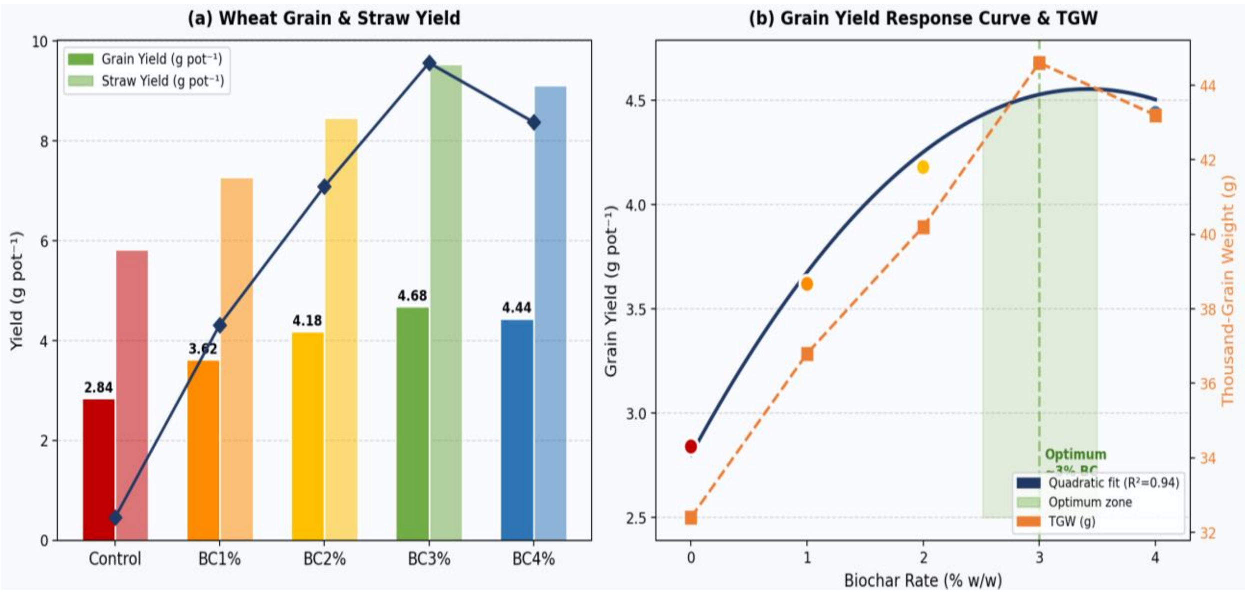

Fig. 2: (a) Wheat grain and straw yield under different biochar rates. Dark bars = grain yield; light bars = straw yield; line = grain yield trend. (b) Quadratic response curve for grain yield with shaded optimum zone (2.5-3.5% biochar) and thousand-grain weight. R2 = 0.94 for quadratic fit.

Straw yield followed a similar quadratic pattern, peaking at BC3% (9.54 g pot-1, +64% over control) before declining at BC4% (9.12 g pot-1). Thousand-grain weight increased from 32.4 g in the control to 44.6 g at BC3%, reflecting improved grain filling under enhanced soil nutrient and water supply conditions. Spike length increased from 7.2 cm to 9.6 cm, and grains per spike from 38.2 to 52.8 at BC3%, with slight reductions at BC4%. Harvest index remained relatively stable across treatments (0.328-0.336), indicating that the proportional allocation of assimilate to grain was not altered by biochar amendment.

Table 4: Effect of biochar rates on wheat (Triticum aestivum L.) yield and yield components.

Treatment	Grain Yield (g pot-1)	Straw Yield (g pot-1)	TGW (g)	Spike Length (cm)	Grains per Spike	Harvest Index	Yield Increase over Control (%)
Control	2.84+/-0.14d	5.82+/-0.22d	32.4+/-1.12d	7.2+/-0.28d	38.2+/-1.48d	0.328+/-0.006	-
BC 1%	3.62+/-0.16c	7.28+/-0.26c	36.8+/-1.28c	8.1+/-0.32c	43.6+/-1.62c	0.332+/-0.006	27.5
BC 2%	4.18+/-0.18b	8.46+/-0.30b	40.2+/-1.42b	8.9+/-0.36b	48.4+/-1.74b	0.331+/-0.006	47.2
BC 3%	4.68+/-0.20a	9.54+/-0.34a	44.6+/-1.58a	9.6+/-0.40a	52.8+/-1.88a	0.329+/-0.006	64.8
BC 4%	4.44+/-0.19b	9.12+/-0.32a	43.2+/-1.52a	9.4+/-0.38a	51.4+/-1.82a	0.327+/-0.006	56.3
LSD (p<=0.05)	0.22	0.38	1.84	0.46	2.24	NS	-

TGW = thousand-grain weight; NS = not significant; LSD = least significant difference. Means with different letters differ at p <= 0.05.

3.10. Correlation Analysis

Pearson correlation analysis confirmed strong and highly significant relationships among soil properties and wheat yield parameters (Table 5). SOC showed the strongest positive correlation with grain yield (r = +0.91, p <= 0.01), followed by available P (r = +0.88), available K (r = +0.86), and CEC (r = +0.87). Soil bulk density was negatively correlated with grain yield (r = -0.84, p <= 0.01), confirming that physical improvement is as important as chemical enrichment for crop response. MBC showed a strong positive correlation with both SOC (r = +0.93) and grain yield (r = +0.89), highlighting the central role of soil biological activity in mediating the beneficial effects of biochar on crop productivity.

Table 5: Pearson correlation coefficients (r) among selected soil and yield parameters (n = 20).

Parameter 1	Parameter 2	r value	p value
SOC	Grain Yield	+0.91	p <= 0.01
Available P	Grain Yield	+0.88	p <= 0.01
CEC	Grain Yield	+0.87	p <= 0.01
Available K	Grain Yield	+0.86	p <= 0.01
MBC	Grain Yield	+0.89	p <= 0.01
Ksat	Grain Yield	+0.82	p <= 0.01
Bulk Density	Grain Yield	-0.84	p <= 0.01
SOC	MBC	+0.93	p <= 0.01
CEC	Available P	+0.89	p <= 0.01
WHC	Grain Yield	+0.80	p <= 0.01
C:N Ratio	Available N	-0.76	p <= 0.05
Aggregate Stability	Ksat	+0.84	p <= 0.01

All correlations are Pearson r (two-tailed). SOC = soil organic carbon; MBC = microbial biomass carbon; CEC = cation exchange capacity; WHC = water holding capacity; Ksat = saturated hydraulic conductivity.

4. DISCUSSION

The results of this study comprehensively demonstrate that rice-husk biochar application enhances soil organic carbon, nutrient availability, physical properties, and wheat productivity in degraded agricultural soil of the Punjab irrigated belt, with the 3% biochar rate consistently emerging as optimal across most parameters. The progressive SOC accumulation from 0.42% (control) to 1.38% at BC4% is among the highest reported for single-season greenhouse studies with rice-husk biochar in South Asian soils, consistent with the exceptional carbon stability of this feedstock documented by Spokas et al. (2012) and the direct contribution of biochar fixed carbon to soil organic matter pools.

The mechanistic basis for improved phosphorus availability with biochar addition operates through multiple pathways. First, the increase in soil CEC from 12.4 to 18.4 cmolc kg-1 at BC3% reduces phosphorus fixation by competing with phosphate for adsorption sites on iron and aluminium oxide surfaces through anion repulsion effects (Xu et al., 2014). Second, the stimulation of phosphate-solubilising bacteria documented by the increase in MBC is consistent with biochar-mediated enhancement of microbial activity reported by Kolton et al. (2011). Third, the modest acidification in these near-neutral soils (pH 7.82 to 7.32) improves phosphorus solubility by reducing calcium-phosphate precipitation reactions. The non-monotonic response of available P with a peak at BC3% and decline at BC4% has been previously reported by Arif et al. (2017) and may reflect increasing phosphorus immobilisation on additional biochar sorption sites at very high application rates, where the surface charge capacity of the biochar carbon is not yet fully oxidised and weathered.

The 116% improvement in hydraulic conductivity at BC3% (1.24 to 2.68 cm h-1) is consistent with meta-analytical estimates by Biederman and Harpole (2013), who reported median Ksat improvements of 54-132%

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across twelve studies, with higher improvements in clay-textured soils. The physical mechanism involves the macro-porous structure of biochar particles creating preferential flow channels and the aggregate stability improvements reducing pore-clogging by dispersed clay. The slight decline in Ksat at BC4% relative to BC3% may reflect partial blockage of inter-aggregate pores by excess fine biochar particles at very high loading rates, a phenomenon documented by Lim et al. (2016) in soils receiving biochar at rates exceeding 3-4%.

The 64.8% improvement in wheat grain yield at BC3% (2.84 to 4.68 g pot-1) is at the upper range of yield improvements reported in published literature for wheat under biochar in controlled studies. Hussain et al. (2017) reported yield improvements of 31-58% across six Punjab soil types with rice-husk biochar at 2-4% rates, while Tahir et al. (2011) documented 42% improvement in loamy sand soils with wood biochar at 3% w/w. The slight but statistically significant yield decline at BC4% relative to BC3% is consistent with the nutrient immobilisation hypothesis, supported by the elevated C:N ratio of 16.83 at BC4% exceeding the critical threshold of approximately 15 associated with net nitrogen immobilisation. This finding has important practical significance: it demonstrates that biochar application has a definable agronomic optimum beyond which additional application provides diminishing or negative returns, a finding that has direct implications for farmer advisory recommendations in Punjab.

The strong positive correlation between MBC and grain yield (r = +0.89) highlights an often-overlooked dimension of biochar benefits: the stimulation of soil biological activity. The 172% increase in MBC from control to BC4% reflects biochar-mediated provision of protected microsites for microbial colonisation, improved habitat moisture through enhanced water holding capacity, and increased substrate availability from root exudates produced by more productive plant root systems. These biological improvements create a positive feedback loop where enhanced microbial activity drives nutrient mineralisation, which in turn supports greater plant biomass production and further root exudate release.

The relatively stable harvest index across all treatments (0.327-0.336) suggests that biochar amendment enhances the overall productive capacity of the wheat plant rather than altering assimilate partitioning. This contrasts with some abiotic stress conditions where harvest index declines as vegetative growth is impaired, and indicates that the yield improvement is genuinely attributable to enhanced productivity rather than compensatory growth responses. From a practical standpoint, the dose-response relationship identified in this study supports a recommendation of 2-3% rice-husk biochar application as the agronomically optimal range for wheat production in similar degraded soil conditions in the Punjab irrigated belt.

5. CONCLUSION

This greenhouse study demonstrated that rice-husk biochar application at rates of 1-4% w/w significantly improved all fifteen measured soil physical, chemical, and biological parameters, with optimal wheat yield achieved at 3% biochar. Key conclusions from this study are:

Soil organic carbon increased linearly from 0.42% to 1.38% across the biochar rate range, with all increments statistically significant, demonstrating consistent carbon enrichment potential of rice-husk biochar in degraded Punjab agricultural soils.

Available phosphorus and potassium peaked at BC3% (18.6 and 218 mg kg-1), with slight declines at BC4% suggesting the onset of nutrient immobilisation, consistent with the elevated C:N ratio of 16.83 exceeding the critical threshold at the highest biochar rate.

CEC increased by 48% at BC3% and 60% at BC4%, providing substantially enhanced nutrient retention capacity, with downstream benefits for nitrogen, phosphorus, and cation retention efficiency.

Saturated hydraulic conductivity improved by 116% at BC3% and aggregate stability MWD increased from 0.48 to 1.26 mm, demonstrating substantial physical amelioration alongside chemical improvements.

Wheat grain yield responded quadratically to biochar rate (R2 = 0.94), with maximum yield of 4.68 g pot-1 at BC3% representing a 64.8% improvement over the unamended control, and a significant decline to 4.44 g pot-1 at BC4%.

Strong correlations between SOC and grain yield (r = +0.91) and between MBC and grain yield (r = +0.89) confirm that both chemical enrichment and biological stimulation mechanisms underlie the crop productivity improvements.

Field-scale validation experiments under realistic rainfall, irrigation, and farmer management conditions are recommended to confirm the optimal biochar rate and quantify multi-season residual effects of rice-husk biochar in the Punjab wheat-rice rotation system. Economic analysis comparing biochar production costs against yield and soil health benefits is also required to assess the commercial viability of this technology for smallholder farmers in Pakistan.

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