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Biochar Enhances Soil–Plant–Microbe Interactions in Saline Soil

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ABSTRACT

A controlled pot experiment was carried out to examine the interactive effects of salinity stress and biochar on the growth, nutrient uptake, and soil microbial dynamics of Lablab purpureus. Results showed that wheat husk biochar significantly ($p \leq 0.05$) enhanced plant growth parameters compared to controls. Plant height increased by c. 53%, root length by 37%, fresh weight by 125%, and dry weight by 92% in wheat husk char treated soil under non-saline conditions. Wheat husk char also significantly increased pod number and node count per plant by c. 42% and 28% respectively. Nutrient analysis revealed higher concentrations of N (~6%), P (~0.3%), and K (~2%) in wheat husk biochar treatments, while salinity reduced nutrient uptake across all treatments. Although the number of flowers increased by c. 75%, the difference was not statistically significant. Although 16S rRNA gene copy numbers did not show significant changes in biochar treatments, enhanced microbial function indicated improved nutrient cycling and ecosystem functionality. Overall, the findings suggest that biochar can mitigate the adverse effects of salinity by improving plant physiological traits and stimulating microbial activity. This highlights biochar's potential as an ecological tool for sustainable agriculture, biodiversity enhancement, and ecosystem restoration in saline affected areas.

Keywords: Salinity; Biochar; Nutrient Uptake; Microbial Dynamics; Plant; Enzyme; Ecosystem; Biodiversity

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1. Introduction

Soil salinity is one of the most critical constraints to agricultural productivity in Bangladesh, affecting over one million hectares of arable land. Coastal belts such as the Ganges Delta and parts of the Chittagong coastal plain are particularly vulnerable due to tidal flooding, sea-level rise, and improper irrigation practices [1]. The Intergovernmental Panel on Climate Change (IPCC, 2023) has projected a significant escalation in salinity intrusion in low-lying coastal regions, which underscores the urgency of developing effective mitigation strategies to sustain crop yields under saline conditions [2]. Leguminous crops hold a pivotal role in enhancing soil fertility through biological nitrogen fixation, organic matter improvement, and nutrient cycling. These crops not only contribute to food security but also play a key ecological role in sustainable agriculture. However, legumes are generally sensitive to salinity stress, particularly during early development stages [3]. For example, high concentrations of Na^+ and Cl^- in saline soils disrupt water uptake, impair nutrient availability, and hinder physiological functions in plants [2,3]. Consequently, salinity stress often leads to reduced growth, yield loss, and compromised nutritional quality of crops.

In recent years, biochar has emerged as a promising soil amendment to improve soil structure, enhance nutrient availability and stimulate microbial activity leading to ecological restoration, particularly in saline affected soils [4]. Due to its porous structure and high surface area, biochar can adsorb Na^+ (dominant ion present in saline soil), reduce soil electrical conductivity, and provide habitat niches for microbial communities. Being a long-term carbon sink, biochar supports the re-establishment of plant communities and promotes agroecological sustainability in salinity-impacted ecosystems by facilitating microbial-mediated nutrient availability. The stable structure of char helps to improve soil aggregation, aeration, and water retention, which dilutes salt concentration in the rhizosphere and promotes leaching of salts beyond the root zone. The alkaline nature of char can buffer acidic conditions that may arise from salinity, thereby stabilizing nutrient availability and enhancing nutrient uptake efficiency [5]. These multifunctional benefits position biochar not merely as a soil amendment, but as a catalyst for ecological restoration and biodiversity conservation. Agricultural residues such as wheat husk and sawdust are commonly available in Bangladesh and offer a sustainable source for biochar [4,5]. However, most studies to date have examined biochar impacts under optimal soil conditions or single-variable conditions, with limited focus on its interaction with saline stress. The mechanistic understanding of how biochar influences microbial processes and nutrient cycling under saline stress is still evolving. The present study applies a multi-factorial approach assessing plant physiology, nutrient uptake, and microbial activity under a gradient of salt stress by providing mechanistic insights into how char ameliorates salinity-induced disruptions in soil biological functioning. The overarching goal of this study is to integrate microbial ecology with plant physiology and biochar chemistry. Thus, the study aims to assess the impacts of biochar on the growth and physiology of *legumes* under different salinity levels, and to evaluate their potential in mitigating salt-induced stress in soil-plant systems, offering a practical pathway for ecological restoration.

2. Materials and Methods

2.1. Plant Growth Parameters

To investigate the interaction between salinity and biochar on the development of *Lablab purpureus*, a controlled pot experiment was designed. This leguminous crop, a member of the Fabaceae family, was selected as the model species for this study. *L. purpureus* was selected due to its economic and ecological relevance in the context of saline-prone agroecosystems in Bangladesh. As a widely cultivated legume, it serves as a source of protein and minerals, contributes in nitrogen-fixing symbioses, and improves organic matter, making it resilient to environmental stressors particularly in salt-affected regions [5]. Soil samples were collected from Sonargaon Upazila in Narayanganj District, Bangladesh (23° 33’ 57’’ N, 90° 26’ 45’’ E) using the composite sampling technique [5]. The soil was then screened through a 2 mm stainless steel sieve for uniformity [6].

A total of 27 plastic pots were used for the analysis. Each 5 kg capacity plastic pot was filled with 4 kg of soil to support the growth of bean. The experimental layout was

structured as a completely randomized design (CRD) with three replications across nine treatment combinations (**Tables 1** and **2**, **Figure 1**). These treatments varied based on salt concentrations and the type of biochar amendment, either wheat husk biochar (WHB) or sawdust biochar (SDB), both applied at 5 t/ha. Simulated saline irrigation was created using sodium chloride (NaCl) at 0 mM, 75 mM, and 150 mM concentrations. Typically a concentration of > 40 mM NaCl in a soil extract is considered indicative of saline conditions [6]. We selected 75 and 150 mM saline concentrations to better understand the impact of biochar on saline soil using a range of environmentally relevant to higher than environmentally relevant concentration of char, whilst considering the relative effects of plants.

Table 1. Treatments and Their Combinations.

Symbol	Treatment
T0B0	Irrigation with water without biochar (Control)
T0B1	Irrigation with water with WHB (5 tons/ha)
T0B2	Irrigation with water with SDB (5 tons/ha)
T1B0	Irrigation with 75 mM NaCl solution without WHB/ SDB
T1B1	Irrigation with 75 mM NaCl solution with WHB (5 tons/ha)
T1B2	Irrigation with 75 mM NaCl solution with SDB (5 tons/ha)
T2B0	Irrigation with 150 mM NaCl solution without WHB/ SDB
T2B1	Irrigation with 150 mM NaCl solution with WHB (5 tons/ha)
T2B2	Irrigation with 150 mM NaCl solution with SDB (5 tons/ha)

Table 2. Experimental Pot Design Used for *Lablab purpureus*.

T0B0	T0B2	T0B1
T0B1	T0B0	T1B0
T0B2	T1B2	T1B1
T1B0	T2B0	T2B1
T1B1	T2B1	T2B2
T1B2	T1B0	T2B0
T2B0	T2B2	T0B2
T2B1	T0B1	T1B2
T2B2	T1B1	T0B0

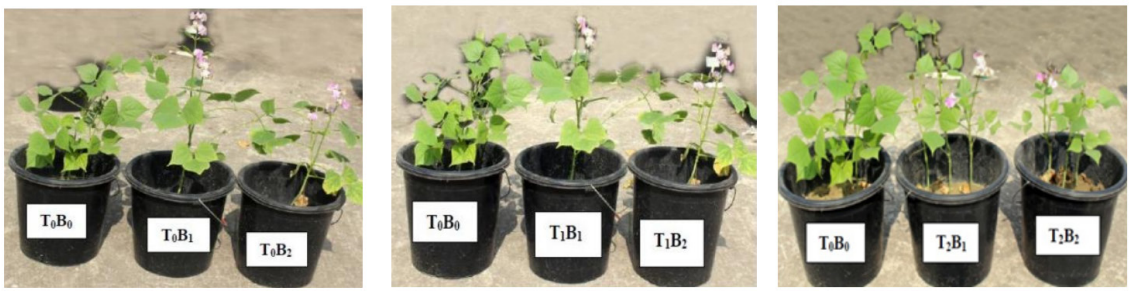

Figure 1. Experimental Set-Up.

During the experiment, evaporative losses were replenished daily to maintain consistent moisture [5]. Salt stress was induced progressively to mimic natural saline conditions. The WHB and SDB were made using pyrolysis process [6]. Wheat husk and sawdust biomass materials were first dried and then cut into small pieces. After that, these were inserted into a ceramic cooking pot which was made air tight with lid and parafilm. It was then heated using an electric furnace at 500°C pyrolysis temperature. After about 2 hours, biomass turned into biochar. The cover of the pot was not opened until it cooled down completely as it readily oxidized in contact with atmospheric air [7].

The biochar was then allowed to cool in an oxygen-free environment before sieving (0.25 mm) and storing in labeled jars. Texture, pH, cation exchange capacity (CEC) and electrical conductivity (EC) of the WHB and SDB were determined. Texture was determined manually by feel method [6,7]. pH was measured by a pH meter (Thermo Orion) after mixing the biochar with ultra-pure water at a ratio of 1:2.5 [7]. Cation exchange capacity (CEC) of biochar was determined using the ammonium acetate (1N, pH 7) saturation method, followed by displacement with 1M KCl and quantification of exchanged NH_4^+ using an ICP-OES (Thermo Scientific ICAP 7000) [6]. Electrical conductivity (EC) of biochar was measured by mixing char with ultra-pure water at a ratio of 1:10 followed by shaking and filtering. Finally the EC of the filtrate was measured using an EC meter (Orion Star) [7]. Moisture content of char was determined by oven-drying a known weight of sample at 105 °C for 24 hours until constant weight. The percentage was calculated as the weight loss relative to the initial sample weight [7]. Images of biochar were captured with a Zeiss PlanNeoFluar Microscope and ImageJ software (v1.5.4) was used to evaluate surface morphology parameters of both biochar such as surface area, perimeter, diameter, major axis and minor axis.

The seeds of *L. purpureus* were collected from Siddique Bazar, Dhaka. The seeds were submerged overnight in water mixed with malathion for pretreatment [5]. Ten seeds were sown in each pot. The seeds were germinated after 3 days and the pots were then kept surrounded with a net. Each pot contained three healthy (pest and disease free) seedlings. Throughout the experiment, plants were watered regularly and salt stress was introduced based on the respective treatment protocols. Weeds were removed manually by uprooting. To control worm infestations during the growth period, malathion was applied as an insecticide on the 30th and 37th days after germination. Plants were harvested after 60 days of sowing. Plant height was recorded using a meter scale, measuring from the soil surface to the tip of the main shoot. Root length was determined by measuring from the base to the end of the longest root using the same scale [5,6]. Node number of each plant was recorded from the day of first budding. Flower counts were taken eight weeks after sowing, and the average number of flowers was calculated to assess the variation across different treatments. Total number of pods was enumerated and then averaged to identify the pod numbers per plant. Another part of the plant samples was uprooted from the pots to conduct further experiments in the laboratory. The contents of nitrogen, phosphorus, potassium and sulphur were determined [5].

2.2. Microbiological and Biochemical Tests

Soil bacterial count was quantified via serial dilution and viable plate count [7]. Enzymatic activities—β-1,4-glucosidase (BGD), dehydrogenase (DHG), and β-1,4-N-acetylglucosaminidase (NAG)—were evaluated using commercial enzyme kits following the manufacturer's protocol, and absorbance was recorded using a UV-VIS spectrophotometer at 400 nm (BGD and NAG) and 485 nm (DHG) [8].

Soil microbial respiration was assessed by sealing soil samples in jars with NaOH traps. CO_2 absorption by NaOH was quantified via titration using barium chloride and phenolphthalein indicator after a 7-day incubation at 15°C [8]. DNA was extracted from soil for polymerase chain reaction (PCR) using a Line-Gene 9600 Plus thermal cycler (Thermo Fisher Scientific Inc.). The hypervariable V3-V4 region (~444 bp) of the 16S rRNA gene was amplified using primers 338F (50-ACTCCTACGGGAGGCAGCAG-30) and 806R (50-GGACTACHVGGGTWTCTAAT-30). Gene copy numbers were estimated through PCR using standard curves generated from *E. coli* plasmids and a regression equation [9]. The assay achieved a PCR efficiency of 90.98% with an R^2 of 0.9994.

2.3. Data Analysis and Quality Control

To ensure accuracy and reproducibility, quality control procedures were strictly followed. Detection limits were calculated using the standard deviation of ten blanks. Precision was determined from duplicate analyses of 10% of all samples, with concentrations significantly above detection limits. Accuracy was validated using an internal certified reference standard [10]. Statistical analyses were carried out using SPSS v20 and SigmaPlot v14. Normality of the data was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests, while homogeneity of variance was evaluated using Levene's test based on the mean [10].

3. Results and Discussion

3.1. Properties of Biochar

Physicochemical properties of WHB and SDB differ significantly from one another. The pH of the WHB and SDB were 7.38 and 9.08, respectively (**Table 3**). The CEC of WHB was 0.694% whereas SDB had a CEC of 1.901% (**Table 3**). Content of phosphorus did not differ between WHB and SDB. Both WHB and SDB had a black, powdery texture. The WHB had a surface area of 348 ± 0.01 m^2/g, perimeter 1.42 mm ± 0.73 mm, diameter 0.82 ± 0.50 mm, major axis 1.24 ± 0.09 mm and minor axis 1.14 ± 0.06 mm. The SDB had a surface area of 352 ± 0.08 m^2/g, perimeter 1.68 mm ± 0.21 mm, diameter 0.98 ± 0.14 mm, major axis 1.47 ± 0.05 mm and minor axis 1.62 ± 0.08 mm.

Table 3. Physico-Chemical Properties of the Biochar.

Properties	WHB	SDB
pH	7.38	9.08
CEC (%)	0.694	1.901
EC (dS/m)	0.44	0.38
Moisture content (%)	6.32	7.11
Total Nitrogen (%)	6.65	4.84
Total Phosphorus (%)	5.32	5.04
Total Potassium (%)	3.85	5.12
Total Sulphur (%)	1.86	0.47
Total Calcium (%)	2.81	1.51
Total Magnesium (%)	1.43	0.24
Total Zinc (%)	1.22	0.13
Total Manganese (%)	14.25	21.42
Total Sodium (%)	11.06	9.07

3.2. Plant Growth Parameters

Growth and yield parameters such as plant height, fresh weight and dry weight of *L. purpureus* bean in different treatments are shown in **Table 4**. The highest plant height (37.5 cm) was recorded in treatment T0B1, representing a 53.7% increase compared to the control (T0B0). The second highest plant height (37.4 cm) was noted in T0B2, which was 53.3% higher than the control. The minimum plant height (22.8 cm) was attained in T2B0. Between the two biochar types, the WHB performed slightly better than the SDB in increasing the height of the plant. It could be due to the higher CEC of the SDB (**Table 3**) leading to enhanced capacity to hold nutrient cations. Biochar promotes plant productivity and yield though several mechanisms. Dark coal color of biochar alters thermal dynamics and facilitates rapid germination, allowing more time for growth compared to control treatments [11]. Excessive salts in soil affect all major living processes such as growth, photosynthesis, protein and lipid metabolisms [12]. The results indicated reductions in plant height as a result of exposure to the salinity levels. Similar studies reported growth inhibition in soybean [13]. Salinity also reduces growth rate by reducing the uptake of water by plants [12,14]. Variations in salinity level significantly affected plant height.

Table 4. Growth Performances of Lablab purpureus in Different Treatments.

Treatment	Plant Height (cm)	Root Length (cm)	Node Count/ Plant	Flower Count/ Plant	Pod Count/ Plant	Fresh Weight of Plant (t ha^{-1})	Dry Weight of Plant (t ha^{-1})	Shoot: Root Ratio (Dry Matter Basis)
T0B0	24.4	13.2	7	12	6.9	16.3	3.8	2.46
T0B1	37.5	18.1	9	21	9.8	36.7	7.3	3.25
T0B2	37.4	17.1	9	18	9.3	35.6	6.9	3.32
T1B0	25.2	16.2	8	15	6.2	14.9	3.2	1.96
T1B1	34.3	17.7	9	19	7.2	28.3	5.4	2.62
T1B2	34.6	16.5	9	18	7.3	25.6	5.1	2.84
T2B0	22.8	12.7	7	12	6.0	11.2	2.5	1.73
T2B1	25.4	15.5	8	16	6.6	19.9	4.9	2.12
T2B2	24.6	15.1	8	15	6.8	18.7	4.6	2.06
LSD at 5%	7.87	2.51	1.28	NS	0.76	7.03	2.87	0.90

The longest root length (18.1 cm) was found in treatment T0B1, showing a 37.1% increase compared to the control (T0B0). The second longest root length (17.7 cm) was recorded in T1B1, which was 34.1% greater than the control (T0B0) (**Table 4**). Studies found that the reduction of the biomass of beans grown under saline condition was indicative of several growth limitations [14,15], and the salinity exerted negative effects not only on the biomass, but also on other morphological parameters such as plant height, number of leaves, root length and shoot/root ratio. WHB had a better contribution in increasing root length. Plant roots were significantly affected by salinity. The number of nodes per plant was increased significantly over control for treatments T0B1 and T0B2, as well as T1B1 and T1B2 (**Table 4**). Biochar contains many nutrients that help plant growth. This node number variation is due to the application of biochar, which helps to enhance node growth in bean plants, and also salinity treatment reduces node numbers in plants because of the adverse effects of salinity during plant growth.

The number of flowers per plant was increased over control for all treatments but it is not statistically significant. Pod count per plant was increased significantly over control for treatments T0B1 and T0B2 (**Table 4**). The highest pod number per plant (9.8) was recorded in T0B1 and the lowest pod number per plant (6.0) was recorded in T2B0. Our results were in agreement with previous literature. Exposure to salinity stress at any stage of plant development leads to reduced vegetative and reproductive growth constituents in most legume crops [16].

For fresh weight, T0B1 had the maximum plant weight (36.7 t/ha), which was 125.2% higher than T0B0 (control), and T0B2 had the second maximum plant fresh weight (35.6 t/ha), which was 118.4% higher than T0B0 (control). The minimum plant weight (11.2 t/ha) was attained in T2B0 (**Table 4**). Between WHB and SDB, the WHB performed slightly better than the second one in increasing the weight of the plant. It could be due to higher concentrations of N, P, S, Ca and Mg (**Table 3**), which could promote rapid vegetative growth and increased tissue hydration, resulting in greater fresh weight. A higher percentage of volatile matter in WHB might be the reason for increased fresh weight [17]. Studies demonstrated that biochar application can enhance several soil properties such as increase in soil pH, CEC, total C, total N, available P, water holding capacity, exchangeable cations, nutrient cycling and attracting more beneficial fungi and microbes [17,18], while decreasing available soil Al, soil strength, and soil bulk density. These factors provide numerous benefits to increase biomass yield and crop yield under different conditions [19]. The high level of salinity mediated by NaCl concentrations affects plant growth and development through the osmotic stress and the injurious effects of toxic levels of Na^+ and Cl^- [17,20]. It is known

to be sensitive to several environmental factors, such as salinity, which is one of the major factors limiting plant growth and productivity [15].

The dry weight of *L. purpureus* significantly increased over T0 (control) with the treatments T0B1 and T0B2 only, while the treatment T2B0 had a much lower dry weight in comparison with T0 (control). The maximum plant dry weight was observed in T0B1 (Irrigation with water with wheat-husk biochar), which was 92.1% higher than T0B0 (control), and was followed by T0B2 (irrigation with water with SDB), which was 81.6% higher than T0B0 (control). The minimum plant dry weight (2.5 t/ha) was obtained in T2B0 (Irrigation with 150 mM salt solution without biochar) (**Table 4**). Between WHB and SDB, SDB performed better than WHB in increasing dry weight of *L. purpureus*. Higher CEC (**Table 3**) could result in improved biomass partitioning into structural dry matter of *L. purpureus* leading to higher dry weight. Plant growth can also be affected by biochar-induced changes in soil nutrient conditions, particularly the cycling of P and K, facilitating biomass gain [20]. A study found that the fresh and dry weights of faba bean (*Vicia faba* cv. Misr 2) were decreased with salt treatment [21]. The highest plant shoot and root ratio (3.32) was recorded in the treatment T0B2, and the lowest plant shoot and root ratio (1.73) was recorded in T2B0. The shoot and root ratio of the plant was statistically significant, but none of the values were significantly increased over the control. This may be due to the adverse effect of salinity treatment on plants and biochar amendments in only a small amount, so that it was not significant enough.

Macronutrient Contents of Plant

Nitrogen content varied with different treatment variations. Nitrogen content in shoot of *L. purpureus* ranged from 3.15% to 5.82%. The highest N content (5.82%) was recorded in treatment T0B1 and the lowest value (3.15%) was found in T2B0 (**Figure 2**). The nitrogen content of the root of *L. purpureus* was increased significantly over control in T0B1. The highest N content (4.52%) was recorded in treatment T0B1 and the lowest value (2.37%) was found in T2B0 (**Figure 2**). During field experiment application of vermicompost to mungbean gives the highest nitrogen content (4.15%) [22].

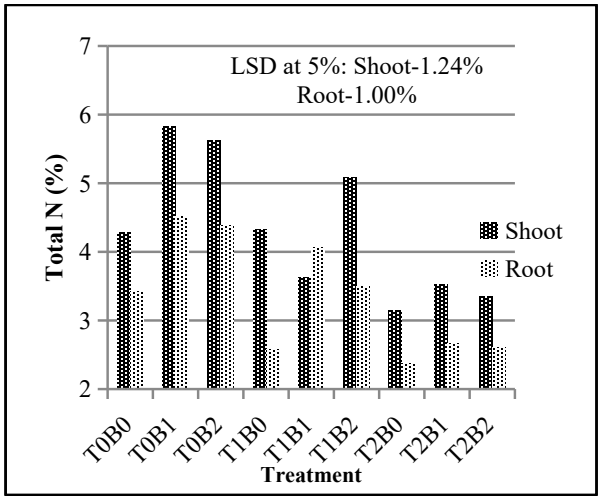

Figure 2. Total N in Plant in Response to Various Treatments.

Phosphorus content in shoot of *L. purpureus* ranged from 0.09% to 0.29% (**Figure 3**). The highest P content (0.29%) was recorded in treatment T0B1 and the lowest value (0.09%) was found in T2B0. Phosphorus content in the roots of *L. purpureus* showed a significant ($p \leq 0.05$) increase compared to the control in all treatments except T2B0, T2B1, and T2B2. The highest phosphorus concentration (0.189%) was observed in treatment T0B1, while the lowest (0.024%) occurred in T2B0. The increase in P content may be due to the direct availability of nutrients through the solubilization of ash in the solid biochar residue and other nutrients that may become available through microbial utilization of a small labile carbon component of biochar [23]. Soil salinity markedly decreases phosphorus uptake by plants due to the precipitation of phosphate ions with calcium ions [22].

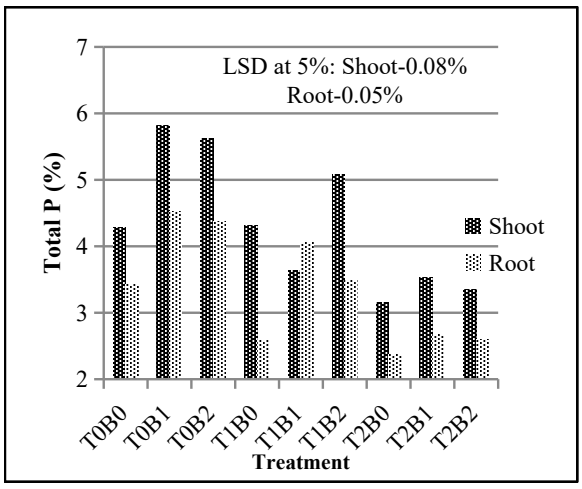

Figure 3. Total P in Plant in Response to Various Treatments.

The range of potassium content in shoot of *L. purpureus* under treatments was 0.46% to 2.37%. The maximum potassium concentration (2.37%) was observed in treatment T0B1 (irrigation with water with WHB) and the lowest value (0.46%) was found in T2B0 (irrigation with 150 mM salt solution without biochar) (**Figure 4**). The potassium content of the root of *L. purpureus* was increased significantly ($p \leq 0.05$) over control in T0B1 and T0B2. The maximum K concentration (2.59%) was detected in treatment T0B1 (Irrigation with water with WHB) and the lowest value (0.22%) was found in T2B0 (Irrigation with 150 mM salt solution without biochar) (**Figure 4**). Salinity affects nutriments assimilation and development by imposing osmotic stress on plants, causing specific ion (Na^+) toxicity and affecting activity of major cytosolic enzymes by disturbing intracellular potassium homeostasis in plant cells [24]. Also, both massive depletion of cytosolic K^+ happens in plant shoots and roots [25].

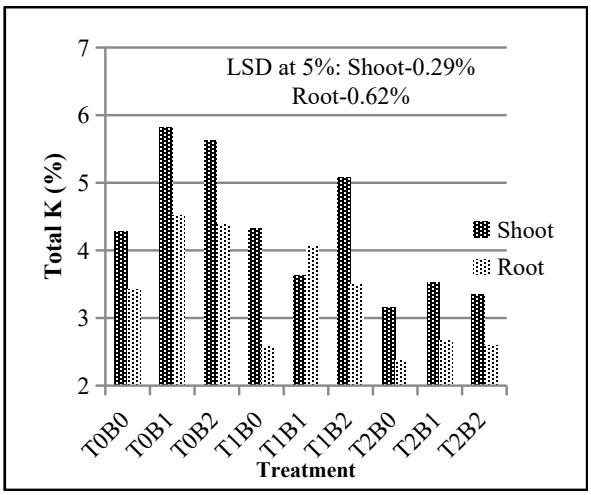

Figure 4. Total K in Plant in Response to Various Treatments.

The range of sulphur content in *L. purpureus* under treatments was 0.21% to 0.49%. The highest S content (0.49%) was recorded in treatment T0B1 (irrigation with water with WHB) and the lowest value (0.21%) was found in T2B0 (irrigation with 150 mM salt solution without biochar) (**Figure 5**). The sulphur content of the root of *L. purpureus* was increased significantly ($p \leq 0.05$) over control in T0B1 (irrigation with water with WHB), T0B2 (irrigation with water with SDB) and T1B1 (irrigation with 75 mM salt solution with WHB). The highest S content (0.465%) was recorded in treatment T0B1 (irrigation with water with WHB) and the lowest value (0.278%) was found in T2B0 (irrigation with 150 mM salt solution without biochar) (**Figure 5**).

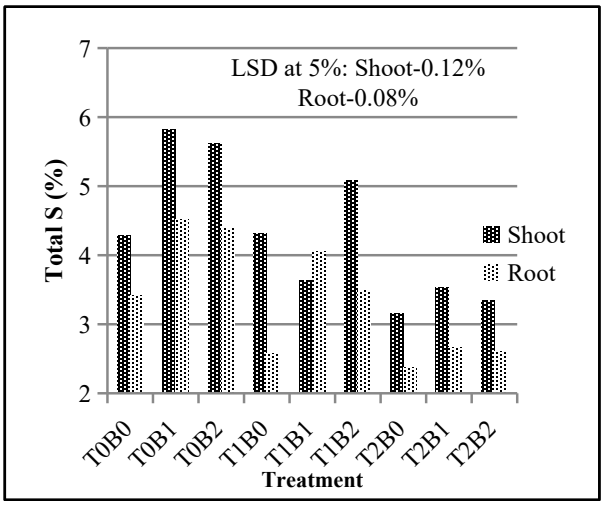

Figure 5. Total S in Plant in Response to Various Treatments.

The highest Ca content (3.91%) was recorded in treatment T0B1 (irrigation with water with WHB) and the lowest value (0.83%) was found in T2B0 (irrigation with 150 mM salt solution without biochar) (**Figure 6**). However, a significant increase in calcium content over control was observed in all treatments except T1B0 (irrigation with 75 mM salt solution without biochar), T2B0 (irrigation with 150 mM salt solution without biochar), and T2B1 (irrigation with 150 mM salt solution with WHB). The highest Ca content (2.82%) was recorded in treatment T0B2 (irrigation with water with SDB) and the lowest value (0.22%) was found in T2B0 (irrigation with 150 mM salt solution without biochar). The Ca content of plant root was increased significantly ($p \leq 0.05$) over control for T0B1 (irrigation with water with WHB), T0B2 (irrigation with water with SDB), T1B1 (irrigation with 75 mM salt solution with WHB), and T1B2 (irrigation with 75 mM salt solution with SDB).

The highest Mg content (0.34%) was recorded in treatment T0B2 (irrigation with water with SDB) and the lowest value (0.08%) was found in T2B0 (irrigation with 150 mM salt solution without biochar) (**Figure 7**). However, significant increase of magnesium content over control was in T0B1 (irrigation with water with WHB). The highest Mg content (0.31%) was recorded in treatment T0B2 (irrigation with water with SDB) and the lowest value

(0.15%) was found in T2B0 (irrigation with 150 mM salt solution without biochar). Magnesium concentration in the plant roots increased significantly ($p \leq 0.05$) compared to the control in all treatments, except for T1B0 (irrigation with 75 mM salt solution without biochar), T2B0 (irrigation with 150 mM salt solution without biochar), and T2B2 (irrigation with 150 mM salt solution with SDB). Salinity greatly affects nutrient availability in soil, movement to root surface, uptake and transport and partitioning of N, P, K, Ca, Mg and micronutrients [16–19].

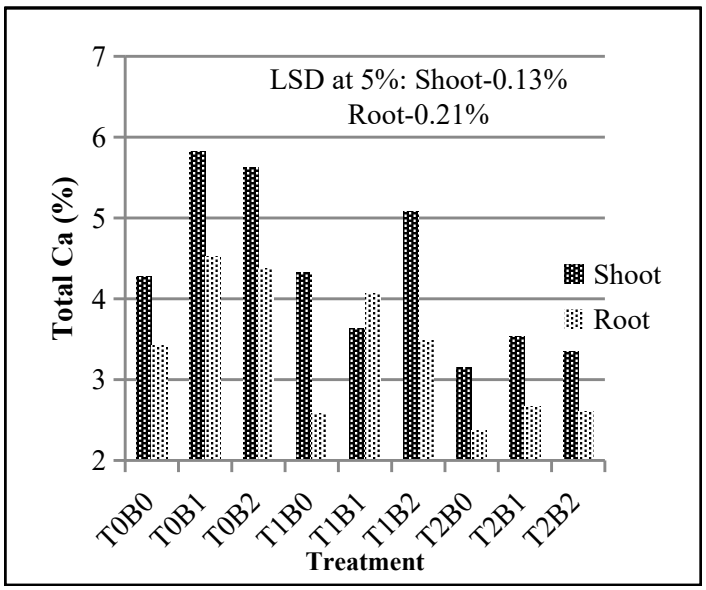

Figure 6. Total Ca in Plant in Response to Various Treatments.

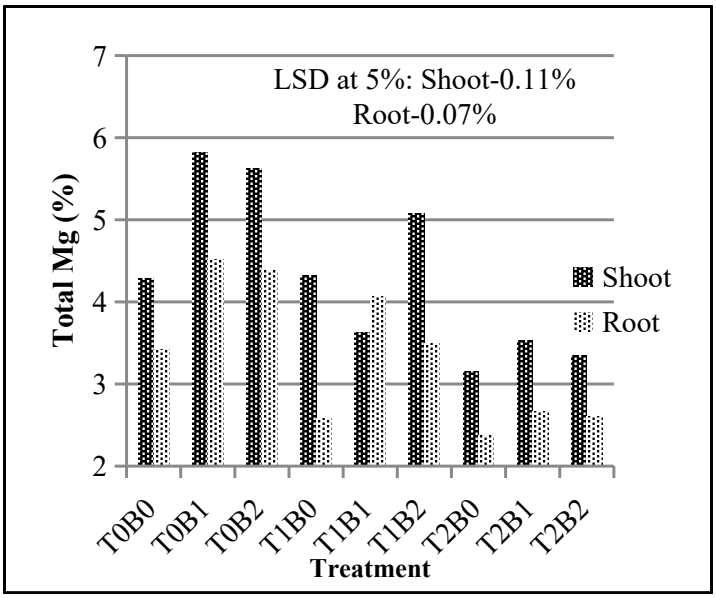

Figure 7. Total Mg in Plant in Response to Various Treatments.

3.3. Activities and Abundance of Bacteria

All microbial parameters *viz.,* bacterial count, enzyme activities (β-1,4-glucosidase, dehydrogenase and β-1,4-N-acetylglucosaminidase) and microbial respiration rate were significantly affected by the addition of biochar. Biochar significantly ($p \leq 0.05$) increased bacterial count by 54% and 59% in T0B1 and T0B2 treatments, respectively, compared to the control (**Table 5**). There was significant difference between T0B1 and T0B2. We observed significant ($p \leq 0.05$) increases in bacterial count in T1B1 (23%) and T1B2 (34%) compared to T1B0. Significant

variations were found between T1B1 and T1B2. The count was reduced by 38% in T2B0 than in T1B0. Significant increases were observed in T2B1 and T2B2 compared to those of T2B0. T2B1 and T2B2 were significantly different from each other. Activities of all three enzymes increased significantly by 18% and 19% in T0B1 and T0B2 respectively compared to the control (**Table 5**). No significant variation ($p > 0.05$) was found between T0B1 and T0B2. Significant reduction ($p \leq 0.05$) was found in T1B0 (56%) and T2B0 (54%) than in T0B1. Enzyme activities were significantly increased in T1B1 and T1B2 from T1B0, as well as T2B1 and T2B2 from T2B0. There was no significant ($p > 0.05$) difference between T1B1 and T1B2 for β-1,4-glucosidase and dehydrogenase activities whereas β-1, 4-N-acetylglucosaminidase showed significant variation.

Table 5. Bacterial Abundance, Enzyme Activity and Microbial Respiration Rate.

Treatment	Bacterial Count (CFU g^{-1})	BGD (μmol PNP $g^{-1}h^{-1}$)	DHG (μg TPF $g^{-1}h^{-1}$)	NAG (μmol PNP $g^{-1}h^{-1}$)	16S RNA Gene Copy Number	Respiration Rate
T0B0	$90 \times 10^4 \pm 0.41 \times 10^4$	3.42 ± 0.97	0.92 ± 0.64	0.89 ± 0.32	$5.27 \times 10^7 \pm 1.75 \times 10^7$	3.09 ± 0.14 g CO_2 g^{-1} soil s^{-1}
T0B1	$110 \times 10^4 \pm 0.58 \times 10^4$	4.02 ± 0.91	1.78 ± 0.61	1.68 ± 0.58	$6.48 \times 10^7 \pm 1.42 \times 10^7$	4.88 ± 0.17 g CO_2 g^{-1} soil s^{-1}
T0B2	$125 \times 10^4 \pm 0.21 \times 10^4$	4.17 ± 0.71	1.71 ± 0.42	1.93 ± 0.61	$6.77 \times 10^7 \pm 1.34 \times 10^7$	6.27 ± 0.58 g CO_2 g^{-1} soil s^{-1}
T1B0	$68 \times 10^4 \pm 0.06 \times 10^4$	1.18 ± 0.35	0.87 ± 0.29	0.65 ± 0.79	$4.27 \times 10^7 \pm 1.24 \times 10^7$	5.22 ± 0.35 g CO_2 g^{-1} soil s^{-1}
T1B1	$80 \times 10^4 \pm 0.85 \times 10^4$	3.38 ± 0.38	3.65 ± 0.48	3.86 ± 0.02	$7.84 \times 10^7 \pm 1.05 \times 10^7$	7.18 ± 0.04 g CO_2 g^{-1} soil s^{-1}
T1B2	$85 \times 10^4 \pm 0.49 \times 10^4$	3.35 ± 1.48	4.08 ± 1.31	4.55 ± 0.74	$7.97 \times 10^7 \pm 1.11 \times 10^7$	7.92 ± 0.09 g CO_2 g^{-1} soil s^{-1}
T2B0	$53 \times 10^4 \pm 0.69 \times 10^4$	1.94 ± 0.41	0.85 ± 0.44	0.67 ± 0.93	$4.07 \times 10^7 \pm 1.03 \times 10^7$	4.52 ± 0.14 g CO_2 g^{-1} soil s^{-1}
T2B1	$65 \times 10^4 \pm 0.43 \times 10^4$	2.19 ± 0.51	2.05 ± 0.89	2.36 ± 0.55	$6.58 \times 10^7 \pm 1.35 \times 10^7$	6.52 ± 0.91 g CO_2 g^{-1} soil s^{-1}
T2B2	$60 \times 10^4 \pm 0.44 \times 10^4$	2.27 ± 0.58	3.61 ± 0.82	3.45 ± 0.26	$6.28 \times 10^7 \pm 1.62 \times 10^7$	5.02 ± 0.73 g CO_2 g^{-1} soil s^{-1}

*BGD = β-1,4-glucosidase activity; DHG = dehydrogenase activity; NAG = β-1,4-N-acetylglucosaminidase activity

There were no significant differences found in the 16S RNA gene copy numbers between T0B1 and T0B2 from the control. The 16S RNA gene copy numbers were significantly reduced by 35% in T1B0 compared to the T0B2, which were then increased to 60% in T1B1 treatment. No significant ($p > 0.05$) differences were observed between T1B0 and T2B0. A similar non-significant relationship was observed between T1B1 and T1B2, as well as T2B1 and T2B2. Microbial respiration rate was significantly ($p \leq 0.05$) increased by 14% and 32% in T0B1 and T0B2 treatments respectively than in control (**Table 5**). Respiration rate was increased by 23% and 24% in T1B1 and T1B2 respectively than in T1B0. Significant variation was found between T1B1 and T1B2. We observed significant increases in T2B1 (46%) and T2B2 (32%) compared to T2B0. Significant differences ($p \leq 0.05$) were also found between T2B1 and T2B2 (**Table 5**).

Previous literature is in agreement with our present study. A large body of literature has reported that biochar can improve soil quality to create a more favorable environment for plant growth [26–28]. Under salinized conditions, biochar increases nutrient contents which is attributed to biochar's high surface area, fractures and cracks, and surface hydrophilic functional groups [28]. We observed high surface area of biochar, presence of extensive fractures and cracks, and −CO (carbonyl) functional group which is hydrophilic in nature. Increased nutrient contents together with enhanced water-holding capacity of soil was beneficial for alleviating salt stress of plant [29]. Biochar addition promotes the binding of soil particles to form channels that leads to improvement in soil permeability and leach salt-related element from soil, thereby reducing soil salini-

ty degree [27,30]. We did not measure hydrological properties in our study and we maintained a constant soil moisture throughout our experimental period (no other edaphic conditions were altered); thus, we were not able to explain the permeability and water holding capacity of soil. However, presence of fractures and cracks in the biochar evident in our study could serve as water channels leading to enhancement in soil permeability. Studies found that biochar under 30–70 t ha^{-1} application rate in moderately saline soils enhanced forage productivity while reducing soil salinity [26,27]. Although we did not apply biochar at aforementioned dose, the findings were apparent in our present study (**Table 4**).

There was a marked effect of the biochar on nutrient cycling, as indicated by the assessment of bacterial abundance. The relative abundance of bacteria and respiration rate associated with β-1,4-glucosidase, dehydrogenase and β-1,4-N-acetylglucosaminidase all increased with the application of biochar which ultimately helped to alleviate soil salinity (**Table 5**). β-1,4-glucosidase and dehydrogenase activities are positively correlated to C cycling while N cycling is correlated to β-1,4-N-acetylglucosaminidase activity [11]. Enhanced enzyme activities could result in an increase in C and N cycling. Although there was no impact on the 16S RNA gene copy number of bacteria, this is possibly due to a lack of highly labile C in the biochar [14]. Biochar significantly altered the biological and biochemical properties of saline soils [27], with direct implications for plant productivity, soil microbial health, and ecological resilience.

Data suggested that the interaction of biochar with microbes promoted plant growth in saline soil, presumably that the biochar interaction with microbes could reduce the harmful impacts of salts thereby enhancing plant yield. These outcomes confirmed previous findings that indicated the positive effects of biochar–microbe interactions after the combination of biochar and microbe application in soil on the growth, yield, and nutrient availability of different plant species such as soybean, sunflower and rice [16,26,27]. The improved growth parameters as a result of biochar–microbe interactions might be ascribed to the role of biochar in microbial growth, enzyme activity and respiration [31]. Being a component of the ecosystem, soil microbial community improves nutrient cycling by impacting on soil nutrient status [32]. Previous studies suggested that soil microbial biomass could be increased in saline soils supplemented with biochar, and that the microbial abundance and functional genes were changed over time [31,33].

Biochar increases the cation exchange capacity (CEC) of soil due to its porous structure and high surface area providing more sites for cations to bind [34]. High CEC could allow to bind salts that could reduce leaching of salts leading to reduced soil salinity. Biochar increased bacterial population (**Table 3**) in salt stressed soil that could favor microbial colonization [35]. This could in turn enhance organic matter decomposition and nutrient availability—key processes in ecosystem functioning. This would reduce the harmful impacts of salinity on nutrient cycles [32–34]. The elevated microbial activity together with enzyme activities observed in our study (**Table 5**) confirms the ecological models, where microbial communities play a mediating role in maintaining plant access to nutrients under salinity stress [36]. Osmotic and ionic stress model mentions that elevated salt concentrations in soil reduce water availability to plants leading to the accumulation of Na^+ and Cl^- ions that impair root function and limit photosynthesis [37]. Microbial suppression model suggests that saline conditions inhibit microbial abundance and respiration rate that suppresses microbial-driven processes such as mineralization and enzymatic nutrient turnover [38]. These disruptions are aggravated by feedback loop model, where reduced plant growth leads to diminished organic matter inputs, which further initiates a downward spiral of declining nutrient cycling efficiency, and soil fertility degradation [39]. These models align with our control treatment findings, where salinity caused sharp declines in plant growth, microbial populations, and enzyme activities (**Tables 4** and **5**). However, biochar treatments revealed that this degradation loop is not irreversible [40]. Biochar application reinvigorated microbial functions and supported plant physiological recovery suggesting a pathway to ecological restoration not captured in traditional salinity stress models [38–40].

Future research could explore long-term ecological effects of a wider range of biochar feedstocks with potentially different molecular compositions and surface chemistries under varying salinity regimes. Size of biochar is an important factor since previous works observed that different sizes of biochar had different uptake patterns for the

nutrients [40–42]. Nutrient uptake of the smaller/ bigger biochar particles than those investigated in the present study warrants further investigation that could provide insights into optimizing formulations for size specific biochar–microbiome interactions. Additionally, incorporating molecular techniques such as metagenomics and transcriptomics could illustrate functional roles of microbial taxa influenced by biochar under salinity stress. Surface morphology of the char using the scanning electron microscope (SEM), pore size using the surface area analyzer (SAA) and crystallinity using the X-ray diffractometer (XRD) could be determined which will provide information on the structural properties of char [41]. These information could elucidate the roles of char influencing soil–plant–microbe interactions by affecting microbial colonization, nutrient retention, and rhizosphere activity [42]. The natural progression for future work from this study would be field-scale investigations across different soil types and crop systems, integrating char into coastal land restoration strategies to ensure sustainable intensification of saline-affected agroecosystems**.**

The present study was conducted under controlled pot conditions with a limited duration of 60 days. While these allowed controls of many environmental factors and did not necessarily represent what may occur in field environment. The study was not able to assess long-term effects of biochar on plant productivity, soil properties, or microbial populations over multiple cropping cycles. Two types of biochar feedstocks were used at a single application dose, which could not explore different sources or variable rates of biochar. Different sources and variable rates of biochar are likely to influence plant–soil–microbe interactions differently. The salinity stress was induced using NaCl only, which did not represent the full spectrum of salts present in naturally saline soils. Our experiments could be better if we could collect saline soil samples from the field and study the impacts of different cations (e.g. Na^+, K^+, Ca^{2+}, Mg^{2+}) and anions (Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, NO_3^-) on biochar. The study lacks the observation of molecular structure of biochar using SEM, FTIR, etc. due to resource constraints. Future work could involve observing pores, fractures, crystallinity, and functional groups of biochar that could provide useful insights on the soil-plant-biochar interactions.

4. Conclusions

The study highlights the significant potential of biochar as a multifunctional amendment for mitigating salinity stress in legumes by restoring microbial function and improving plant productivity. The combination of biochar and salinity treatments performed better compared to the treatments with salinity but no char. The findings reinforced the ecological value of biochar as more than a soil amendment that can be strategically deployed to restore degraded soil (particularly saline soil), enhance ecosystem resilience**,** and promote biodiversity conservation. Given its environmental compatibility, biochar can be incorporated into national/ global policies on climate-resilient agriculture, sustainable soil management, and land rehabilitation. For example, biochar should be integrated into national soil fertility enhancement programs, with targeted promotion in coastal and saline-prone areas in Bangladesh. Biochar application should be proposed into coastal land rehabilitation initiatives, such as those under the Bangladesh Delta Plan 2100. Multifunctional role of biochar in restoring microbial functions, improving soil structure, and reducing salinity stress makes it a valuable tool for long-term ecological restoration and food security in vulnerable agroecosystems. These policy actions would align with broader goals of climate-smart agriculture and sustainable land management in Bangladesh. Policymakers are encouraged to support long-term studies of char into coastal restoration programs; this approach would align with the goals of sustainable development and environmental stewardship.

Author Contributions

Conceptualization, S.H.; formal analysis, T.F.K. and M.G.S.A.; investigation, T.F.K., M.G.S.A. and S.H.; writing—original draft preparation, T.F.K., M.M.G.S.A. and S.H.; writing—review & editing, T.F.K. and S.H.; funding acquisition, S.H. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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