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Effects of biochar and biofertilizer on groundnut production: a perspective for environmental sustainability in Bangladesh

Fouzia Sultana Shikha^{1†}, Md Mashiur Rahman^{2*†} , Naznin Sultana³, Md Abdul Mottalib⁴ and Monira Yasmin¹

Abstract

Regular large-scale application of fertilizers, pesticides, and mulching can lead to soil health degradation and increase negative environmental impacts, contributing significantly to greenhouse gas (GHG) emissions. Considering these factors by applying biochar and biofertilizer (rhizobium inoculants) in groundnut production, a novel experiment was conducted for increasing soil fertility, groundnut productivity, and soil carbon stock in Bangladesh's Charland agro-ecosystems. The two-year experiment involved seven treatments consisting of T₁ (control), T₂ (soil test based (STB) fertilizer dose following fertilizer recommendation guide (FRG) 2018), T₃ ((T₂ minus nitrogen fertilizer) + biofertilizer), T₄ (T₃ + biochar), T₅ (T₂ + biochar), T₆ (only biofertilizer), and T₇ (only biochar). The result showed that the T₄ treatment had the highest nodule counts (78.17 plant⁻¹), nodule weights (122.97 mg plant⁻¹), root weight (1.47 g plant⁻¹) and nut yields (2.30 t ha⁻¹), all of which were statistically identical compared to the other treatments. In addition, the T₃ treatment had the highest recorded shoot weight (35.47 g plant⁻¹), whereas the control T₁ treatment had the lowest (16.50 g plant⁻¹) shoot weight. Results showed that biochar-based rhizobium inoculants increased nodulation, root weight, shoot weight, nut yield and soil nutrient uptake in plant growth at all four stages (seedling, flowering, pod formation and harvesting). The result revealed that biochar-based rhizobium inoculants modulated the abundance of functional microbes through increased soil nitrification and reduced denitrification compared to the N-use treatments. Moreover, this interactive system significantly improved soil NO₃⁻, leading to an increase in N uptake, thereby promoting plant growth and increasing nut yield. Considering all parameters, the soil amended biochar as a carrier of rhizobium inoculants had the highest soil organic carbon (SOC) stock (1.76 t ha⁻¹), about 26% higher than other treatments, which saved a considerable amount of 6.6 kg CO₂eq ha⁻¹ GHG emissions and aided in promoting environmental sustainability towards climate-smart agriculture.

Highlights

1. A novel biochar-based biofertilizer (rhizobium inoculants) application in groundnut production has been proposed.

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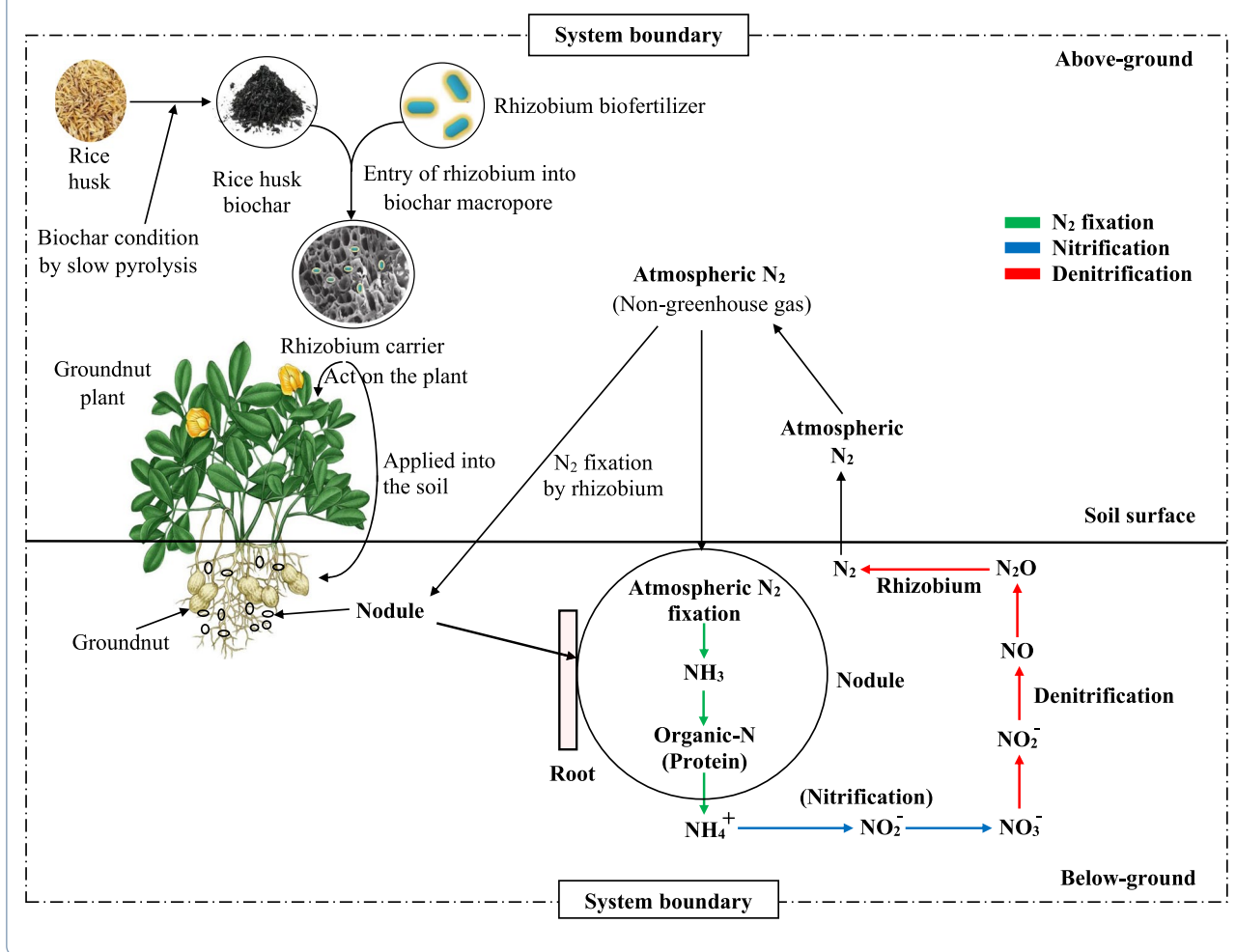
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- The combined system's impact helps uptake soil nutrients, improving plant growth, nut yield and soil organic carbon (SOC) stock.
- Interactive impact of these two reduces the need for N fertilizer while also lowering GHG emissions by sequestering SOC.

Keywords Biochar, Biofertilizer, Environmental sustainability, Groundnut production, Nitrification, Soil organic carbon accumulation

Graphical Abstract



1 Introduction

Biochar can be quickly produced from wood or biomass, which has a carbon (C) content of about 50%, whereas biochar has a carbon content of about 70% to 80% and its use in the soil may store more than 50% of the C in a highly stable way (Qambrani et al. 2017; Panwar et al. 2019; Layek et al. 2022). For this reason, biochar has been proposed as a way to increase soil fertility in agroecosystems as well as other ecosystem services and sequester C to mitigate climate change (Woolf et al. 2010; Yeboah et al. 2020; Bellè et al. 2022;

Layek et al. 2022). Moreover, biochar act as a rhizobium bacteria carrier and has also been shown to change soil biological community composition (Hardy et al. 2019); such changes might have positive impacts on nutrient cycles (Steiner et al. 2008) or soil structure (Rillig and Mummey 2006), which would then indirectly improve the plant growth, yield productivity (Warnock et al. 2010), and soil organic matter (SOC) cycling (Kuzya-kov et al. 2009; Liang et al. 2010; Tender et al. 2021; Lin et al. 2022). Nonetheless, nitrification, denitrification and methane oxidation (Yanai et al. 2007; Van Zwieten

et al. 2012), C mineralization (Kuzyakov et al. 2009; Liang et al. 2010), and nutrient transformations (Gundale and DeLuca 2006) were all found to either increase or decrease in the presence of biochar (Romero et al. 2021).

Biochar has been employed as a soil additive or an inoculant carrier, like other inoculant carriers, for example, *Azotobacter*, *Bacillus*, *Clostridium*, Blue-green algae, or *Rhizobium*, but little is understood in terms of their mode of action, even as far as the relatively well-studied rhizobia are concerned (Kumari et al. 2019; Van Beek et al. 2019; Wei et al. 2020). Biochar-type materials have long been suggested as inoculant carriers substituting for the increasingly expensive, rare, greenhouse gas-releasing and non-renewable peat (Lehmann et al. 2010). Adding biochar-type residues from vegetation fires to soil significantly increased the nodulation of plants, enhancing soil fertility and thereby led to yield productivity (Lehmann et al. 2011).

However, soil fertility decline and greenhouse gas (GHG) emissions have been perceived as widespread treating challenges globally (Hartemink 2006). According to scientific reports, crop production contributed roughly 10–12% of world GHG emissions, while land conversion from grassland and forest to croplands, soil, and biomass carbon accounted for an additional 12 to 20%. For this reason, to overcome these bottlenecks, biochar amendment has been identified as the optimum technique (Smith et al. 2015; Carlson et al. 2016; Meier et al. 2020). The International Biochar Initiative (IBI) defined biochar as a solid material obtained from the thermo-chemical conversion of biomass in an oxygen-limited environment for use in sustainable environmental and agricultural practices (International Biochar Initiative 2015; Mulabagal et al. 2021). In recent days, biochar has been gaining popularity owing to its capacity to mitigate climate change and helps to ensure environmental sustainability due to its high carbon sequestration capacity (Liang et al. 2010; Lehmann et al. 2010; Rahman et al. 2022). Besides, soil amendments that disintegrate slowly, such as compost and biochar, are another essential management approach for enhancing SOC stocks (Dignac et al. 2017), resulting in reduced greenhouse gas (GHG) emissions to combat the climate change effect. In addition, biochar may have altered other GHG chemicals, such as nitrous oxide (N_2O) or methane (CH_4), by converting them into another accessible chemical utilized by the soil and plant (Tesfahun 2018; Zhang et al. 2020).

Groundnut (*Arachis hypogaea* L.) or peanut is the sixth most important oilseed crop in the world cultivated throughout tropical and subtropical areas, followed by cereal crops. In Bangladesh, groundnut is the second most oilseed crop and has played a pivotal role in

meeting the growing oil requirements in recent years and ensuring nutritional security for a population of over 1.6 million (Miah and Mondal 2017; Shakil 2022). Though nutritionally, groundnut seeds contain about 48–50% edible oil, 22–29% protein, and 20% carbohydrate, with an average yield of 2.30 – 3.00 t ha⁻¹ (Morshed Al et al. 2002; Dun et al. 2019). Groundnut is cultivated on about 32,000 ha of land, and the total groundnut production is about 47,000 Mt in Bangladesh (Azad et al. 2020). Being a legume crop, groundnut improves soil quality by biologically fixing nitrogen without consuming non-renewable energies and disturbing agroecological balance. Furthermore, the economically vital part of the groundnut plant is the pod that encloses the seeds. From this point of view, groundnut is an unpredictable crop due to underground pod development (Zaman et al. 1970). The size and number of seeds per pod are essential criteria that determine the market value of groundnut in general.

Inoculation of legumes with biofertilizer (rhizobium bacteria) increased the nodule and nitrogen-fixing activity of the plants (Argaw 2017). When inoculated with the proper strain of bacteria, legumes can supply up to 90% of their nitrogen. Comparably, the researcher reported that inoculation with rhizobium bacteria brings about significant increases in all the growth and yield parameters than when not inoculated; biochar addition to soil increases soil nutrient concentrations and microbial activity, leading to the development of plant growth (Sajid et al. 2011; Asante et al. 2020). The symbiotic performance of legumes with rhizobia can be significantly enhanced by biochar-based rhizobial inoculants, reducing N fertilizer demand and thus promoting the sustainability of crop production in any agroecosystems (Egamberdieva et al. 2018), including Charland agroecosystems. Legumes have symbiotic relationships with rhizobia and are known as the most efficient system for biological nitrogen fixation (Reckling et al. 2016).

Ghazi and Karnwal (2017) evaluated biochar produced from rice straw as a carrier material for rhizobia and found evidence for improved colonization and survival of bacterial inoculants. The biochar-based inoculant increased root and shoot biomass, nodulation and nutrient uptake (Egamberdieva et al. 2017; Tripti et al. 2017). Hence, biochar addition for the Charland agroecosystem improvement is a hot research issue at present in Bangladesh. Biochar-based inoculant carrier with rhizobia has not been studied before in groundnut cultivation in Bangladesh, which has significantly improved the symbiotic performance of legumes with rhizobia (biofertilizer), resulting in reduced N fertilizer demand and promoted the sustainability of crop production. Therefore, the discussions mentioned above might be taken into account while this study was conducted to broaden the knowledge

of the impact of biochar amendment with biofertilizer on rhizobium nodulation, groundnut growth performance and yield productivity. Additionally, the additional focus of the current study in groundnut cultivation was on evaluating the interaction effects of biochar and biofertilizer on the growth, yield potential, and ease of GHG emissions, which contribute to environmental sustainability towards climate-smart agriculture.

2 Materials and methods

2.1 Experimental site

The experiment was conducted during the 2017–19 academic years at the Regional Agricultural Research Station (RARS) research field in Jamalpur, Bangladesh. Before beginning the tillage operation, soil samples were collected at a depth of 0–15 cm for each replicate. The chemical properties of soils in the experimental site were silt clay loam in texture belonging to the Sonalata series under Agro-Ecological Zone-9 (AEZ-9), and the research field was located at an altitude of 16.46 m, 24 56'11"N latitude, and 89 55'54"E longitude.

2.2 Experimental design and treatments

The experiment was designed as a randomized complete block (RCB) design with seven treatments each of which replicated three times. The unit plot size was 6 m² (3 m x 2 m) with a buffer distance of 1 m. A 1.5 m space was left between the experimental plots and the plot's outside border to avoid any side effects or influences from other plots. Each repetition (plot) was enclosed by bunds (*als*) 30 cm wide and 15 cm tall to prevent soil nutrient seepage. Seven treatments were adopted for this study based on the different fertilizer management packages, biochar and biofertilizer, as shown in Table 1.

2.3 Biochar incorporation and rhizobium inoculation

Biochar was used in the furrow at the rate of 10 t ha⁻¹. The tested crop was groundnut (i.e., BARI Chinabadam-8). Peat-based rhizobial inoculum (*Bradyrhizobium*

strain BARI RAh-892) containing 10⁸ cells g⁻¹ inoculum was used at the rate of 1.5 kg ha⁻¹. Groundnut seeds were mixed thoroughly with the inoculum before sowing. Seeds were used at the rate of 75 kg ha⁻¹. The rhizobium inoculant and the groundnut variety were sourced from the Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh.

2.4 Sowing, fertilization and weeding practices

The seeds were planted by dibbling, and they were sown for the first year on November 22, 2017. Here note that the uninoculated seeds were sown first to avoid contamination. Nitrogen (N), phosphorus (P), potassium (K), sulfur (S), zinc (Zn) and boron (B) were used as urea, TSP, MoP, gypsum, zinc sulfate, and boric acid, respectively. All P, K, S, Zn, B and one-third of the urea-N were applied at the time of final land preparation, and the remaining urea-N was applied in two equal installments on the 30th and 50th days of sowing. All the intercultural operations (such as irrigation, weeding, insect control) were done when necessary. The weeding operation was controlled by hand pulling.

2.5 Soil parameters and nutrients analysis

Composite soil samples were collected from the soil surface at 0–15 cm depth in pre-sowing and post-sowing stages and analyzed for their physiochemical properties. The soil's physical and chemical properties were analyzed using standard methods proposed by Olsen et al. (1954) and Page et al. (1989). Soil pH was determined in a 1:5 (w:v) soil to water ratio using a pH meter (AB150, Fisher Scientific, USA). Soil organic matter (SOM) was determined using an oxidation method with potassium dichromate. An automated azotometer (KDN-102F, Qianjian limited, Shanghai, China) was used to test soil nitrogen (N). NO₃⁻-N and NH₄⁺-N were extracted with the 2 M KCl solution at a soil/water ratio of 1:5 at 25 °C and measured using a smart continuous flow analyzer (SmartChem200, Shenzhen, China). Potential nitrification rates were measured using the chlorate inhibition

Table 1 Experimental treatments for this study

Crop	Experimental treatments	Fertilizer dose (kg ha ⁻¹)
Groundnut	T ₁ = Control	Native fertility
	T ₂ = Soil test based (STB) fertilizer dose following fertilizer recommendation guide (FRG) 2018 (Ahmmed et al. 2018)	N ₃₆ P ₃₆ K ₄₅ S ₃₆ Zn _{1.4} Mo _{0.5}
	T ₃ = (T ₂ treatment minus nitrogen fertilizer) + biofertilizer	
	T ₄ = T ₃ treatment + biochar	
	T ₅ = T ₂ treatment + biochar	
	T ₆ = only biofertilizer	
	T ₇ = only biochar	

method (He et al. 2007; Liao et al. 2020). Soil parameters of bulk density were determined using the core sampling method (Blake 2015; Rahman et al. 2021), and soil organic carbon (SOC) was determined both before the experiment started and after the two-year cropping. The SOC stock was estimated with the following equation by Milne et al. (2007); and Zeng et al. (2021).

$$SOC\ stock = SOC\ content\ of\ soil \times BD \times A \times D \quad (1)$$

$$Carbon\ accumulation\ (tha^{-1}) = Final\ C\ stock\ (tha^{-1}) - Initial\ C\ stock\ (tha^{-1}) \quad (2)$$

where, SOC stock=soil organic carbon stock (t ha⁻¹); SOC content of soil=soil organic carbon content of soil (%), BD=bulk density, A=area of a farm (m²) and D=soil sampling depth (m). The nutrient status of the initial soil prior to fertilization is presented in Table 2. Soil was sampled during the seedling stage (7 weeks), bolting stage (11 weeks), flowering stage (15 weeks), and harvest stage (24 weeks) from the beginning of the experiment in order to measure plant physiological parameters and soil properties. Each sample was measured in triplicate. The activity of the root was determined by the triphenyltetrazolium chloride method (Luo et al. 2015). N concentration was measured using a Foss Auto Analyzer Unit (Kjeldahl 8,400). At the time of harvest, grain yields were estimated. The sum of the total dry matter weight and the concentration of N in all the plant parts were used to determine the accumulation of N.

2.6 Biochar production and chemical analysis

The biochar was produced using a slow-pyrolysis with the oxygen-limited condition, and the rice husk was used as a raw

material that was locally collected. The rice husk was loaded in the iron drum covered with a metal sheet with a chimney at the top which was placed into an earthen kiln (developed by Soil Science Division, RARS, Jamalpur, Bangladesh). The rice husk was burnt in the presence of partial oxygen condition. Biochar was produced for three hours at temperatures between 490-550°C with a heating rate of 5-10°C min⁻¹ in a laboratory-scale pyrolysis unit comprising of a reactor kiln, where the pyrolysis temperature was recorded at 30-min intervals by a digital temperature recorder by placing the sensor into the kiln through an aeration hole. All preparations were carried out in duplicate. The burnt husks were then grounded and allowed to be cooled to room temperature. The final product was used as biochar. Chemical analysis was done at the Soil Science Division, BARI, Gazipur, following the standard methods. An elemental analyzer (Flash model EA-1112, Thermo Scientific) was used to determine the key analyses for the biochar characterization, including total carbon (TC) and N content. For elemental composition, 200 mg of each biochar was burnt in a muffle at 500 °C for eight hours and digestion was carried out with nitric acid and hydrogen peroxide (Enders et al. 2012). Ca, Mg, Fe, Mn, Cu and Zn contents were measured by an atomic absorption spectrometer (Analyst 200-PerkinElmer) and P content was measured by using a spectrophotometer (BEL model S05) (Murphy and Riley 1962). The chemical compositions (such as total carbon (TC), N, P, K, S, B, Cu, Fe, Mn, Zn, Mg, Ca and pH) of biochar were determined, which can be seen in Table 3.

2.7 Data Collection and statistical analysis

Data were collected on the following parameters: plant height (cm) using the meter rule, number of nut plant⁻¹, 100 nut weight (g), kernel weight of 100 nuts (g), 100 kernel weight (g), nut yield (t ha⁻¹), Shelling (%), stover yield (t ha⁻¹), nodule number plant⁻¹, nodule weight (mg) plant⁻¹, root weight (g plant⁻¹), and shoot weight (g plant⁻¹). Nodules were collected by carefully uprooting 10 (ten) sample plants selected randomly from each unit plot at the 50 percent flowering stage. Nodules were separated from the roots, counted, and then dried in the oven. After that, they were weighted by a weight meter. Yield and yield components data were collected at the maturity stage. Similarly, yield and yield contributing characters data were recorded and analyzed statistically using the statistical software STAR developed by IRRI. Then, significant differences were assessed at a 5% ($p=0.05$) significance level, and the treatment means were separated using Duncan's Multiple Range Test (DMRT).

3 Results and discussion

3.1 Biochar and Biofertilizer effects on Postharvest soil physicochemical properties

Table 4 shows the impact of biochar and biofertilizer on the post-harvest soil nutrients results following the

Table 2 Initial soil chemical properties of the experimental soils

Parameter	Unit	Value	Critical level
pH (1:5 H ₂ O)	-	6.0	-
Organic matter	(%)	0.83	-
Organic carbon (OC)	(%)	0.48	-
Bulk density	(g cm ⁻³)	1.46	-
Calcium (Ca)	meq 100 g ⁻¹	5.2	2
Magnesium (Mg)	meq 100 g ⁻¹	1.8	0.5
Potassium (K)	meq 100 g ⁻¹	0.12	0.12
Nitrogen (N)	(%)	0.044	-
Phosphorus (P)	µg g ⁻¹	15.6	10
Sulphur (S)	µg g ⁻¹	10	10
Boron (B)	µg g ⁻¹	0.3	0.2
Copper (Cu)	µg g ⁻¹	1.5	0.2
Iron (Fe)	µg g ⁻¹	28	4
Manganese (Mn)	µg g ⁻¹	2.2	1
Zinc (Zn)	µg g ⁻¹	1.42	0.6

Table 3 The chemical composition of rice husk biochar used for the experiment

Parameter	Unit	Value
pH (1:5)	-	8.7
Total carbon	(%)	39.2
Calcium (Ca)	meq 100 g ⁻¹	1.81
Magnesium (Mg)	meq 100 g ⁻¹	0.92
Potassium (K)	meq 100 g ⁻¹	0.92
Nitrogen (N)	(%)	1.1
Phosphorus (P)	µg g ⁻¹	0.73
Sulphur (S)	µg g ⁻¹	0.27
Boron (B)	µg g ⁻¹	0.011
Copper (Cu)	µg g ⁻¹	0.0012
Iron (Fe)	µg g ⁻¹	0.12
Manganese (Mn)	µg g ⁻¹	0.03
Zinc (Zn)	µg g ⁻¹	0.016

completion of the experiment, revealing that the biochar and biofertilizer application produced significant variations in the soil physiochemical characteristics. The highest pH value was observed in the treatment (T₄) of biochar-biofertilizer combined application, while the lowest values were recorded in the control (T₁) treatment. Numerous studies have examined how adding biochar to the soil might raise its pH value (Ding et al. 2016). Based on the findings of this study, the pH of the soil had been slightly raised for all treatments. The total N ranged from 0.041 to 0.067% for all the treatments except the control. In T₄ (biochar and biofertilizer) treatment, about 63% of soil's available N content was increased compared to the control (T₁). P and exchangeable K increased from 10.55 to 16.3 meq 100 g⁻¹, and 0.14 to 0.18 meq 100 g⁻¹, respectively, which were about 54.5% and 28.57% increases in the T₄ treatment in comparison to the control T₁ treatment for P and K, respectively. The addition

of biochar is capable of changing nutrient availability and might provide additional N, P, or bioavailable C sources for microbial proliferation in the rhizosphere, depending on the type of biochar (Rutigliano et al. 2014; Liao et al. 2019). The researcher observed a considerable rise in bioavailable K, Ca, and Mg contents following the application of biochar at a rate of 5 t ha⁻¹ (Karim et al. 2020). Additionally, Gundale and DeLuca (2006) observed that adding biochar at a rate of 10 t ha⁻¹ boosted the soil NH₄⁺ content and net N mineralization rate. From the analysis, it can be noted that improved soil characteristics resulted from the effects of applying biochar and biofertilizer.

Zoghi et al. (2019) reported that by adding biochar and biofertilizer to the soil, plants are given better conditions to absorb nutrients, which increases the soil's ability to store water and support plant growth. According to the findings, adding biochar to the Charland poor water-deficient soil might significantly reduce damage caused by drought stress to biomass production.

Soil bulk density (BD) is the most crucial physical indicator of soil quality and fertility, influencing plant growth and nut yield. Asadi et al. (2021) found that applying 3% rice husk biochar to clayey soil and loamy soil reduced BD by 8% and 22%, respectively. The results from Table 4 indicate that BD was decreased by a certain amount following postharvest soil analysis, compared to the original BD of 1.46 g cm⁻³. Changes in altering soil physical conditions in the rhizosphere can cause significant differences in biomass and yield observed under the various soil compaction levels. This could indirectly impact physiological processes like photosynthesis and respiration by influencing the soil's hydrological characteristics, which impact nutrient mobilization. Furthermore, this could lead to variations in the number of nuts, mass of nuts, and total biomass of groundnut. According to Dauda et al. (2019), there were also high yields in soils

Table 4 Nutrient status of post-harvest soil in groundnut production

Treatments	pH	Total N (%)	K meq 100 g ⁻¹	P µg g ⁻¹	S	B	Zn	SOC (%)	Bulk density (g cm ⁻³)
T ₁	6.53	0.041	0.14	10.55	13.62	0.25	1.12	0.46	1.46
T ₂	6.60	0.048	0.16	13.82	16.25	0.39	1.73	0.45	1.45
T ₃	6.73	0.064	0.18	13.60	18.48	0.45	2.11	0.52	1.44
T ₄	6.98	0.067	0.18	16.3	21.61	0.56	2.43	0.58	1.41
T ₅	6.91	0.057	0.17	15.6	20.17	0.44	2.00	0.54	1.42
T ₆	6.62	0.053	0.14	12.2	15.2	0.37	1.65	0.50	1.44
T ₇	6.85	0.049	0.15	11.6	16.3	0.42	1.71	0.57	1.42
Initial soil	6.50	0.044	0.12	9.6	12.6	0.30	1.42	0.48	1.46

T₁ – control; T₂– STB fertilizer dose following fertilizer recommendation guide (FRG) 2018; T₃=(T₂ treatment minus nitrogen fertilizer) + biofertilizer; T₄=T₃ treatment + biochar; T₅=T₂ treatment + biochar; T₆=only biofertilizer; T₇=only biochar

with minimal compaction and lower yields in soils with higher compaction. The same phenomenon was observed in the present study. Table 4 analysis results noted that the highest total SOC stock was obtained where biochar and biofertilizer were applied, leading to increased soil carbon stock.

3.2 Impact of biochar-biofertilizer based management practices on soil nitrification and denitrification

Figure 1 shows the amount of ammonium N ($\text{NH}_4^+\text{-N}$) and nitrate N ($\text{NO}_3^-\text{-N}$) in the soils for the different treatments under the plant growth stages of seedling, flowering, pod formation and harvesting. The highest $\text{NH}_4^+\text{-N}$ content was observed for all the stages in the T_4 (biochar and biofertilizer) treatment followed by the T_5 , T_3 and T_2 treatments, respectively. It was observed under the treatment T_5 (Urea together with biochar) that ammonium N was increased significantly rather than T_2 treatment, which belonged to only urea application. The T_4 treatments related to the biochar and biofertilizer showed the highest amount due to the presence of nitrogen-fixing rhizobium bacteria, where bacteria served as a converting agent to convert from supplied nitrogen to ammonia, resulting in nitrogen becoming available to plants. During the flowering and pod formation stages, there was no difference between these two, with the same growth happening for all the treatments. During the harvest stage, there were no differences among biochar and biofertilizer-related treatment (T_4), but all significantly increased $\text{NH}_4^+\text{-N}$ content compared to that observed with Urea treatment.

For the $\text{NO}_3^-\text{-N}$ content, urea with biochar treatment (T_5) showed the highest amount for plant growth

stages than the biofertilizer with biochar-related treatments, whereas control, only biochar, and biofertilizer treatments observed lower amounts. During the seedling stage, the $\text{NO}_3^-\text{-N}$ content under T_5 treatment (urea + biochar) significantly differed from that under Urea (T_2) and biofertilizer with biochar (T_4) treatments. The $\text{NO}_3^-\text{-N}$ contents under urea and biofertilizer with biochar treatments were not significantly different during the flowering stage, but both were significantly higher than those with control, only biofertilizer and only biochar treatments. However, during the pod formation and harvesting stage, the soil $\text{NO}_3^-\text{-N}$ content under T_5 treatment was the highest among all samples.

The combination treatment with nitrogen, biofertilizer and biochar affected the potential nitrification rates in the soil (Fig. 2). No differences were observed under the various treatments during the flowering and harvesting stages, but nitrification rates were significantly increased in the nitrogen and biochar-treated soils during the flowering and pod formation stages. The nitrification rates under the T_4 treatment were significantly greater than those under the treatments of nitrogen and biofertilizer application during the flowering and harvest stages. The nitrification rate is crucial in global N cycling by regulating ammonia-oxidizing rhizobium bacteria (Li et al. 2015). Soil potential nitrification rates describe the ability of soil-nitrifying microbes to convert NH_4^+ to NO_3^- and are regulated by the quantity of nitrifying populations. In this study, higher soil potential nitrification rate was observed in T_4 treatment than in other treatments. This might be because the soil's nitrifying bacteria converted more NH_4^+ to NO_3^- , increasing the concentration of $\text{NO}_3^-\text{-N}$ that was seen in the treatment soils. Besides soil

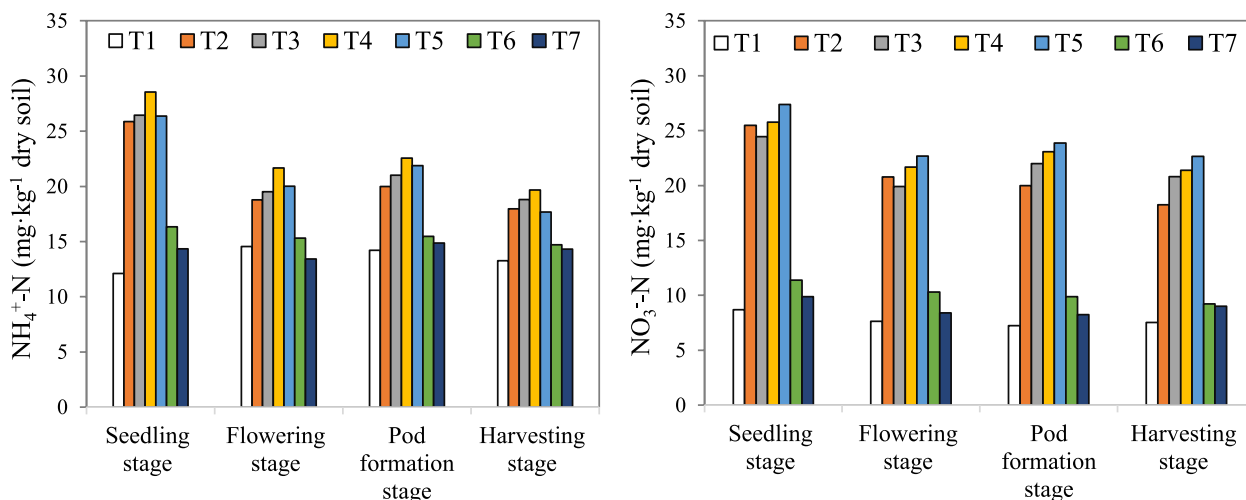


Fig. 1 Effects of the different treatments on the soil's (a) nitrate nitrogen and (b) ammonium nitrogen at different stages of plant growth in groundnut production. Here, T_1 – control; T_2 - STB fertilizer dose following fertilizer recommendation guide (FRG) 2018; T_3 = (T_2 treatment minus nitrogen fertilizer) + biofertilizer; T_4 = T_3 treatment + biochar; T_5 = T_2 treatment + biochar; T_6 = only biofertilizer; T_7 = only biochar

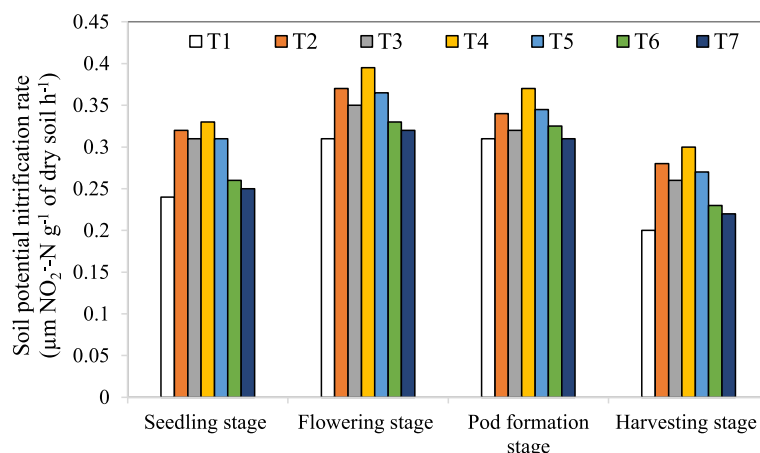


Fig. 2 Effect of different growth stages in different treatments on potential nitrification rate. Note: T₁- control; T₂- STB fertilizer dose following fertilizer recommendation guide (FRG) 2018; T₃ = (T₂ treatment minus nitrogen fertilizer) + biofertilizer; T₄ = T₃ treatment + biochar; T₅ = T₂ treatment + biochar; T₆ = only biofertilizer; T₇ = only biochar

N content, higher C input has been reported to stimulate organic matter mineralization and enhance ammonia-oxidizing bacteria growth (Simonin et al. 2015). Our finding showed that biofertilizer combined with biochar significantly increased SOC (Table 4), supporting the findings of Simonin et al. (2015).

Previous studies have reported contrasting results about the impact of biochar on microbial community composition and N nutrients in the soil (Kolton et al. 2017; Yan et al. 2022; Wang et al. 2022; Li et al. 2023). However, no previous study has reported how biochar with biofertilizer impacts soil microbes in groundnut production. This indicates that biochar combined with urea and microbes stimulates microbial activity more effectively than applying the parameters alone.

3.3 Effects of biochar and biofertilizer on nodulation, dry matter production, and plant height of groundnut cultivation

The effect of biochar and biofertilizer on the nodulation, physical characteristics and plant height was significant compared with that in the control treatment (Table 5). Table results indicate that the highest number of the nodule (avg. 78.18 plant^{-1}) and nodule weight (122.97 mg plant^{-1}) were obtained from the T₄ treatment, followed by the T₃ treatment's of nodule number (avg. 68.01 plant^{-1}) and nodule weight (115.33 mg plant^{-1}), whereas the lowest number of the nodule (43.83 plant^{-1}) and nodule weight (53.67 mg plant^{-1}) were recorded from the control (T₁) treatment. Also, the highest root weight (1.47 g plant^{-1}) was found from the T₄ treatment, and the highest shoot weight (35.47 g plant^{-1}) was recorded from the T₃ treatment, whereas the lowest root weight (0.65 g plant^{-1}) and shoot weight (16.50 g plant^{-1}) were recorded from the control

(T₁) treatment. Furthermore, the highest plant height (50.53 cm) was obtained from the T₅ (urea + Biochar) treatment (Table 5), and the lowest plant height (19.15 cm) was recorded from the control (T₁) treatment. Previous studies reported that biochar-based inoculants increased root and shoot biomass, nodulation, and nutrient uptake by groundnut plants in pot and field experiments (Egamberdieva et al. 2018). Moreover, applying biochar and rhizobium inoculation on groundnut plants increased the number of effective nodules, shoot, and root dry weights (Yusif et al. 2016). In this study, the highest nodule number performed in the T₄ treatment might be due to the effect of biochar and biofertilizer, as this application to soil helps in increasing the nutrients levels either by influencing the metabolism of the plant, which alters the composition of root exudates or influencing the solubility and availability of nutrients (Kumar et al. 2022). Additionally, the studies proved that microbes based on biochar and biofertilizer enhanced plant growth and nutrient absorption (Tripti et al. 2017).

A systematic cycle of biochar and biofertilizer in the soil-plant system within the system boundary is presented in Fig. 3. Rhizobium biofertilizers are compounds that contain symbiotic bacteria, which are the essential nitrogen-fixing organisms capable of driving atmospheric nitrogen (N_2) and delivering it to the roots of legume plants, as well as inducing nodules to grow. These nodules fix N_2 by converting it into ammonia (NH_4^+) as part of the essential process known as nitrification, which plants can then use for growth and development (Fig. 1). In this study, higher rates of soil potential nitrification were seen in the T₄ (biochar plus biofertilizer) treatment than in the other treatments (Table 4 & Fig. 2), which could explain the increased concentration of nitrate nitrogen (NO_3^- -N) in the T₄ treatment soils (Fig. 1). This was caused by the transformation

Table 5 Effects of biochar and biofertilizer on nodulation, dry matter production and plant height in groundnut production during 2017–2019

Treatments	Nodule number (plant ⁻¹)			Nodule weight (mg plant ⁻¹)	Root weight (g plant ⁻¹)	Shoot weight	Plant height (cm)
	2017–18	2018–19	Average				
T ₁	46.00d	41.67d	43.83	53.67e	0.65e	16.50e	19.15f
T ₂	55.84 cd	60.00c	57.92	75.55d	0.98bc	28.18bc	32.78 cd
T ₃	62.57bc	73.53b	68.01	115.33a	1.10b	35.47a	36.90c
T ₄	73.67a	82.67a	78.17	122.97a	1.47a	30.44b	43.97b
T ₅	62.51 bc	72.00b	67.28	106.09b	0.97bcd	25.71c	50.53a
T ₆	61.17bc	62.33c	61.75	103.25bc	0.80cde	21.44d	27.12de
T ₇	59.67bc	55.67c	57.67	96.46c	0.74de	18.37de	22.34ef
CV (%)	11.61	9.63	-	2.91	8.63	4.38	6.39
LSD (0.05)	*	*	-	*	*	*	*

Values in a column having the same letter(s) do not differ significantly at 5% level by LSD. T₁ – control, farmers practice; T₂- STB fertilizer dose following fertilizer recommendation guide (FRG) 2018; T₃ = (T₂ treatment minus nitrogen fertilizer) + biofertilizer; T₄ = T₃ treatment + biochar; T₅ = T₂ treatment + biochar; T₆ = only biofertilizer; T₇ = only biochar. * = Significant ($p \leq 0.05$)

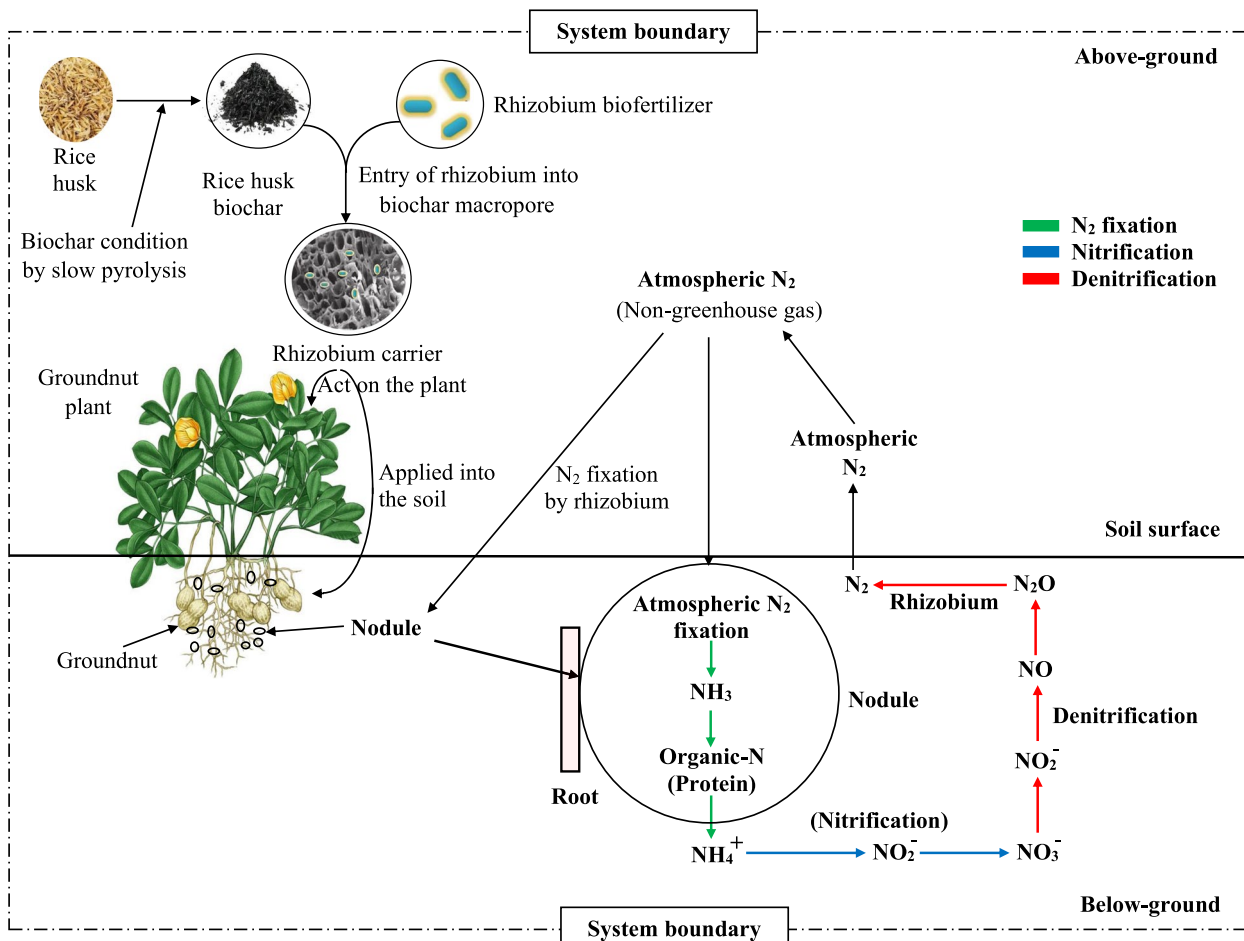


Fig. 3 Systematic conceptual cycle of the effects of biochar and biofertilizer on soil and related plant growth in the soil plant system within the system boundary in the framework of environmental sustainability

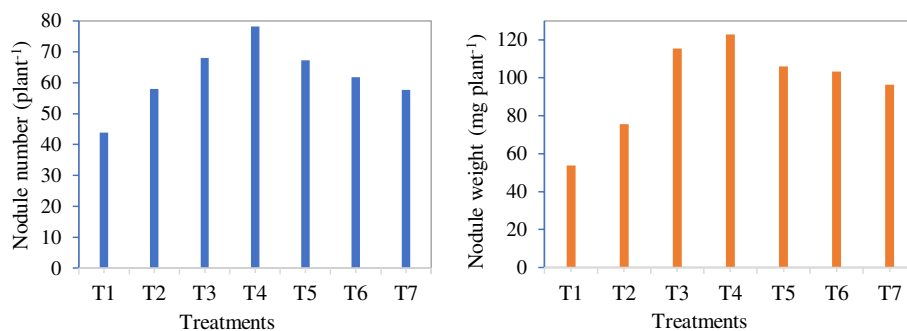


Fig. 4 Effects of rhizobium biofertilizer on the nodule number (plant⁻¹) and nodule weight in groundnut production

of a higher amount of NH_4^+ to NO_3^- due to the nitrifying rhizobium in the soil. Furthermore, increasing the copy number of the rhizobium that sped up the N nitrification process changed the rhizobium community composition and enhanced soil enzyme activities. This made more nodules form in T_4 treatment (Fig. 4), which then assured the availability of N nutrients throughout various growth stages in groundnut production. These findings show that T_4 treatment continuously ensures the supply of available N fertilizer even during the groundnut's late growth stages to support growth, thereby promoting N uptake (Fig. 2).

3.4 Biochar and biofertilizer effects on nut characters, nut yield and yield contributing characters of groundnut cultivation

The effect of biochar and biofertilizer on nut characteristics, yield productivity, and yield contributing characteristics of groundnut cultivation can be seen in Table 6. The table shows that insignificant results were obtained for nut plant⁻¹, stover output (t ha⁻¹), and shelling (%). The highest 100 nut weight (96.33 g) was obtained for the T_4 (T_3 + biochar) treatment, which was statistically similar to that in the treatments of T_3 ($(T_2 - \text{N})$ + biofertilizer) and T_5 (STB + biochar), which had (86.33 g) and (84.00 g) respectively, whereas the lowest 100 nuts weight (66.67 g) was recorded from the control (T_1) treatment. The highest 100 kernel weight (50.92 g) was obtained from the T_4 (T_3 + biochar) treatment, followed by the T_5 treatment (45.52 g), whereas the lowest 100 kernel weight (30.07 g) was recorded from the control (T_1) treatment. Likewise, the highest nut yield (2.30 t ha⁻¹) was obtained from the T_4 (T_3 + biochar) treatment, while the lowest nut yield (0.79 t ha⁻¹) was recorded from the control (T_1) treatment. The highest nut yield obtained from the T_4 treatment might be due to the nodulation effect of biochar and biofertilizer. Furthermore, Biochar-based inoculants also enhanced plant growth and nut yield, which is validated by previous research findings (Egamberdieva et al. 2018). Previously

presented tables show that inoculation with rhizobium significantly increases all the growth and yield parameters than when not inoculated; biochar addition to soil increases soil nutrient concentrations and microbial activity, leading to plant growth and yield productivity.

Potential soil nitrification rate might be altered by the treatment (T_4) using biochar and biofertilizer (Fig. 2), as it can be seen that the total nodule number and nodule weight were increased in the T_4 treatment. No changes were noticed throughout the seedling and bolting stages in the T_4 treatment; however, it was observed from the experimental field that the flowering and nut formation stages in biochar and biofertilizer-treated soils were identical with those in the other treatments. This development might be from the improved nitrification rates throughout these two stages (Rawat et al. 2019; Ramasamy et al. 2020). Eventually, the T_4 treatment successfully enhanced the highest yield by nodulating the abundance of functional rhizobium through increased soil nitrification and reduced denitrification, as compared to the other treatments, thereby promoting groundnut growth and subsequently increasing nut productivity (Fwanyanga et al. 2022).

3.5 Biochar impact on soil organic carbon accumulation

The soil organic carbon (SOC) content has increased significantly ($p < 0.05$) as a result of the addition of biochar to the soil (Table 7). Generally, most SOC is accumulated in the topsoil between 0–30 cm (Rahman et al. 2021). At this portion of the topsoil, over the two years of experimentation, the highest SOC accumulation rates were estimated for the treatments related to biochar application (T_4 & T_7), which were 1.76 and 1.49 t ha⁻¹, respectively. Previous studies also reported a similarly significant effect on SOC accumulation for soils under the biochar-related amendment (Rehman and Razzaq 2017; Jatav et al. 2020). The initial SOC (%) and soil carbon stock were 0.48% and 10.50 t ha⁻¹, respectively. Irrespective of treatments, the highest SOC was recorded in

Table 6 Effects of biochar and biofertilizers on nut characters, yield and yield contributing characteristics of groundnut during 2017-2019

Treatments	Nut (plant ⁻¹)	100 nut weight	100 kernel weight	Shelling	Stover yield	Nut yield (t ha ⁻¹)		
		(g)	(g)	(%)	(t ha ⁻¹)	2017-18	2018-19	Average
T ₁	15.93	66.67c	30.07f	62.07	13.07	0.81d	0.77d	0.79
T ₂	20.13	78.67bc	35.92d	73.76	13.37	1.73b	1.75b	1.74
T ₃	23.87	86.33ab	40.07c	65.68	13.80	1.83b	1.86b	1.85
T ₄	25.07	96.33a	50.92a	72.50	13.30	2.38a	2.23a	2.30
T ₅	22.33	84.00ab	45.52b	73.36	12.97	1.96b	1.90b	1.93
T ₆	23.33	72.33bc	30.67f	70.57	12.57	0.95d	0.91 cd	0.93
T ₇	22.20	73.06bc	33.96e	72.40	12.63	1.24c	1.08c	1.16
CV (%)	13.55	7.34	9.03	8.75	8.03	6.26	11.2	-
LSD (0.05)	-	*	*	-	-	*	*	-

Values in a column having the same letter(s) do not differ significantly at the 5% level by LSD. T₁ – control, farmers practice; T₂ - STB fertilizer dose following fertilizer recommendation guide (FRG) 2018; T₃ = (T₂ treatment minus nitrogen fertilizer) + biofertilizer; T₄ = T₃ treatment + biochar; T₅ = T₂ treatment + biochar; T₆ = only biofertilizer; T₇ = only biochar. * = Significant (p ≤ 0.05)

soil amended biochar after the experiment, about 20.08% more than the control treatment of 0.46%. It is seen from Table 7 that the soil carbon stock increased significantly from 10.07 to 12.26 t ha⁻¹ for the treatment T₄; this may be attributed to the significant carbon content of biochar which is confirmed by the report (Yang et al. 2020); similar findings were reported by Nigussie et al. (2012).

Biochar utilization may be added to soils to improve soil functions, soil fertility and reduce GHG emissions that would otherwise naturally degrade to GHGs. Without application of biochar and biofertilizer, lack of external utilization of organic inputs and microbial breakdown of absorbed carbon hinder carbon sequestration. Therefore, applying biochar with biofertilizer in the soil in groundnut cultivation helps in organic carbon accumulation to meet the ultimate goal of carbon sequestration in soil. It is clear from the reports by Blanco-Canqui et al. (2020) that introducing biochar

increased SOC content. Dejene and Tilahun (2019) also reported that significantly more SOC was accumulated when biochar was added at a rate of 5 t ha⁻¹. The main explanation for these discoveries might be the biochar’s stable carbon content, which makes it difficult to degrade in soil environments and contributes to the soil carbon pool. On the other hand, it can be predicted that other GHG of N₂O breaks down to form the atmospheric N₂, as the observed nodulation numbers increased in biochar and biofertilizer treatments (seen from the previous tables) that come from the N₂, which might then be fixation by the nodule formation, consequences the reduction of GHG emissions in the atmosphere.

3.6 Environmental benefits of biochar and biofertilizer application

Biochar can make substantial breakthroughs in reducing greenhouse gas (GHG) emissions, reducing soil nutrient

Table 7 SOC accumulation and GHG emissions reduced for related SOC accumulation under adopting different treatments in groundnut production

Treatments	Initial soil			Post-harvest soil			SOC accumulation (t ha ⁻¹)	GHG emissions saved for SOC accumulation (kg CO ₂ eq ha ⁻¹)
	SOC	BD	Soil C Stock	SOC	Bulk density	Soil C Stock		
	(%)	(g cm ⁻³)	(t ha ⁻¹)	(%)	(g cm ⁻³)	(t ha ⁻¹)		
T ₁	0.48	1.45	10.50	0.46	1.46	10.07	(-) 0.43	(-) 1.613
T ₂	0.48	1.45	10.50	0.45	1.45	10.07	0.37	1.388
T ₃	0.48	1.45	10.50	0.52	1.44	11.23	0.73	2.738
T ₄	0.48	1.45	10.50	0.58	1.41	12.26	1.76	6.60
T ₅	0.48	1.45	10.50	0.54	1.42	11.50	1.00	3.750
T ₆	0.48	1.45	10.50	0.50	1.44	10.8	0.30	1.125
T ₇	0.48	1.45	10.50	0.57	1.42	12.04	1.49	5.588

T₁ – control, farmers practice; T₂ - STB fertilizer dose following fertilizer recommendation guide (FRG) 2018; T₃ = (T₂ treatment minus nitrogen fertilizer) + biofertilizer; T₄ = T₃ treatment + biochar; T₅ = T₂ treatment + biochar; T₆ = only biofertilizer; T₇ = only biochar

leaching losses, sequestering atmospheric carbon into the soil, and thereby increasing environmental sustainability. Nevertheless, biochar can reduce the need for chemical fertilizers, resulting in reduced GHG emissions from fertilizer manufacture. Results from Table 7 show that the highest GHG emissions saved for the SOC accumulation in treatments T_4 and T_7 related to the biochar utilization were 6.6 and 5.588 kg $\text{CO}_2\text{eq ha}^{-1}$, respectively. It is noted that biochar has significantly helped the environment by saving a certain amount of GHG emissions to accumulate SOC. On the other hand, the capacity of biochar to absorb and hold ammonium in soils reduces nitrogen availability for the denitrification process, resulting in lower N_2O emissions to the atmosphere (Rehman and Razaq 2017). The study showed that the T_4 treatment significantly increased soil SOC (Tables 4 and 7), supporting the findings of Liao et al. (2020). As a result, the T_4 treatment regulated by the nitrification oxidizing rhizobium abundance during the flowering and harvest stages is an important microbiological mechanism for enhancing soil NO_3^- -N (Fig. 1); the higher NO_3^- -N concentration results in reduced denitrification. As a result, it was assumed that variables such as biochar and higher soil potential nitrification rates caused high NO_3^- -N concentrations in T_4 treatment soil while reducing N_2O emissions. According to several researchers, adding biochar typically results in a reduction in N_2O emissions of about 83 percent. Thus, our findings indicated that the T_4 treatment might have enhanced environmental benefits over the other treatments.

4 Conclusion

In this study, the effects of biochar with the rhizobium biofertilizer on plant-soil interactions in soil microbial communities in the Charland agroecosystems in Bangladesh were comprehensively evaluated for the first time. The use of biochar and biofertilizer for the growth of groundnut is the main factor underlying the elevated yield productivity and environmental sustainability, as the N nutrient affects rhizobium involved in nitrification and denitrification. The study evaluated the underlying mechanisms and found that the treatment related to biochar and biofertilizer improves soil microbial activity and shifts bacterial rhizobium community composition toward increasing N nitrification. This process improves the abundance of ammonia-oxidizing rhizobium and stimulates nitrification, accelerating the transformation of NH_4^+ to NO_3^- and reducing NO_3^- (gas) loss. Our results also indicate that N_2O emissions might be reduced by increasing the abundance of these factors in biochar and biofertilizer treatment soil.

Applying biochar and biofertilizer inoculation on groundnut plants increases the yield and yield contributing

characters. Based on the findings, it was evident that the biochar and biofertilizer treatment was the best management treatment, whereas biochar-only treatment was the second most excellent treatment. Biochar generally improves the soil's physical environment, is a measure to reduce chemical fertilizer inputs and alleviates GHG emissions because of the increase in SOC accumulation. The results of this study are based on two years of experiments; thus, these processes will be further studied to evaluate the effects of biochar and biofertilizer on N availability in plants, soil nitrification rate and environmental sustainability in the Charland agroecosystems in Bangladesh.

Abbreviations

AEZ	Agro-ecological zone
BARI	Bangladesh agricultural research institute
BD	Bulk density
CO_2	Carbon dioxide
CO_2eq	Carbon dioxide equivalent
DMRT	Duncan's multiple range test
GHG	Greenhouse gas
IBI	International biochar initiative
MoP	Muriate of potassium
NO_3^- -N	Nitrate nitrogen
NO_3^-	Nitrate
N_2O	Nitrous oxide
NH_4^+	Ammonium ion
OC	Organic carbon
RARS	Regional agricultural research station
RCB	Randomized complete block
SOC	Soil organic carbon
STAR	Statistical tool for agricultural research
TSP	Triple superphosphate

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Authors' contributions

Fouzia Sultana Shikha: investigation, funding acquisition, methodology, development or design of methodology, data curation, formal analysis, software, writing—original draft; Md Mashiur Rahman: Conceptualization, investigation, validation, formal analysis, resources, data curation, writing – review & editing, visualization, proofreading; Naznin Sultana: formal analysis, resources, data curation, writing – review & editing; Md Abdul Mottalib: formal analysis, resources, data curation, visualization, proofreading; Monira Yasmin: Investigation, provision of study material, resources. All authors read and approved the final manuscript.

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Availability of data and materials

Authors are certify that all essential data is included in the publication. The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

No.

Consent for publication

Agree.

Competing interests

All authors declare that there are no competing interests. Md Mashiur Rahman is a member of the Carbon Research youth editorial board and was not involved in the editorial review, or the decision to publish this article.

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