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# Effect of Biochar and Rhizobium Inoculation on Nodulation, Yield of Faba Bean (*Vicia faba* L.), and Selected Soil Properties

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## ABSTRACT

Soil fertility depletion is a major constraint in the North Gondar highlands of Ethiopia leading to the reduction of faba bean yields. Limited access organic fertilizer and heavy reliance on mineral fertilizers have degraded soil quality and crop productivity. This study investigated the effects of biochar and rhizobium inoculation on nodulation, yield of faba bean (*Vicia faba* L.), and selected soil properties in Dabat district, North Gondar. A total of six treatments were made from the factorial combination of three biochar levels (0, 5, and 10 tons ha<sup>-1</sup>) and two inoculation levels (inoculated and uninoculated) in a Randomized Complete Block Design (RCBD) with three replications. Soil samples were collected from a depth of 30 cm to determine soil properties. The effective nodule number was assessed by counting nodules per plant. Biochar application and rhizobium inoculation had a significant effect ( $p < 0.05$ ) on selected soil properties, nodulation, and grain yield of faba bean. The highest soil pH (6.66), organic carbon content (2.28%), total nitrogen (0.26%), available phosphorus (51.61 ppm), effective nodule number per plant (197.65), and yield (2617.76 kg ha<sup>-1</sup>) were recorded following the application of 10 tons ha<sup>-1</sup> of biochar combined with Rhizobium inoculation. Furthermore, this synergistic approach significantly improved faba bean nodulation by 134.9% and grain yield by 74.39% compared to the control. The application of biochar combined with rhizobium inoculation significantly improved soil nutrient levels and faba bean yields. Future research should investigate the effects of varying biochar application rates on the yields of other crops.

## 1 | Introduction

### 1.1 | Background and Justification

Soil degradation and nutrient depletion affect approximately 33% of the Earth's land surface worldwide, threatening the food security of a growing population (Boerger et al. 2021). The loss of organic carbon and soil acidification are major challenges to sustainable agricultural intensification in tropical and subtropical areas (Lehmann et al. 2021). Soil fertility decline in Ethiopia

resulting from intensive cultivation and unbalanced fertilization, has led to land degradation in the highlands (Sileshi et al. 2019; Shibabaw et al. 2018). As a result, over 50% of the country's land is at risk of critical soil carbon depletion (Asrat and Abera 2025). Furthermore, the average soil organic carbon balances below 3.7 tons ha<sup>-1</sup> year<sup>-1</sup>, which has continuously decreased over time (Van Beek et al. 2018).

Faba bean is one of the main crops produced by subsistence farmers in the highlands of Ethiopia (Woldekiros et al. 2018).

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It is a suitable rotation crop that fixes atmospheric nitrogen, reduces reliance on external fertilizer inputs, and significantly improves soil fertility. Additionally, faba bean provides a substantial source of income for farmers and generates foreign exchange for the nation (Eskezia et al. 2025). With an average productivity of 1.9 tons ha<sup>-1</sup>, it is Ethiopia's most important pulse crop in terms of both area coverage and annual production volume (Hailu et al. 2014).

In northern Highland Ethiopia, declining soil fertility, particularly nitrogen, is recognized as a significant threat to the sustainability of cereal cultivation (Blanchet et al. 2016). Due to the high cost and limited affordability, farmers have been using only small amounts of mineral fertilizer to replenish soil nutrients and improve crop yields. Managing soil fertility and maintaining food security with such limited chemical fertilizer input is becoming increasingly difficult. As an alternative, farmers are more likely to adopt locally available methods for soil fertility management and yield improvement, such as applying organic fertilizers and rotating legumes with non-legume crops (Almaz 2022).

The combined use of biochar and biofertilizers has proven to be an effective strategy for sustaining agricultural yield, enhancing soil fertility, and promoting environmental sustainability (Sreethu et al. 2024). While the application of biochar and rhizobium together to cultivate faba beans in Ethiopia shows promise, gaps remain in understanding, particularly regarding field applications and the specific conditions of the North Highland region of Ethiopia. Research indicates that *Rhizobium* inoculation increases nodulation, biomass, and grain yields, while biochar improves soil fertility (Tadesse et al. 2025). However, the effectiveness of this interaction varies depending on soil type, biochar feedstock, and environmental factors. Faba bean production is often limited by nutrient availability due to soil acidity, a common issue in Ethiopia's highlands (Tadesse and Assefa 2025). Recent studies suggest that biochar can improve soil pH and enhance populations of beneficial rhizobia, thereby increasing faba bean yields on acidic soils (Wilson 2021).

However, further information is needed to determine the optimal types of biochar and application rates for Ethiopian soil conditions and various faba bean cultivars (Tadesse et al. 2025). Research indicates that different biochar feedstocks elicit varied responses in microbial interactions, which influence nutrient dynamics and nitrogen fixation (Bolan et al. 2023). Additionally, more research is required to assess the sustainability and long-term impacts of using rhizobium and biochar in the study area. The combined effects of biochar and rhizobium inoculation on the productivity of faba bean varieties grown under different soil types in field conditions are not well documented. Therefore, this study was conducted to investigate the effects of biochar and *Rhizobium* inoculation on nodulation, yield of faba bean (*Vicia faba* L.), and selected soil properties in Nitosol of the North Gondar highlands of Ethiopia. We hypothesized that the combined application of biochar and rhizobium inoculation significantly increases nodule formation, yield of faba bean, and properties of soil through creating a porous carbonaceous environment that

encourages microbial colonization and optimising soil pH through the liming impact of biochar.

## 2 | Materials and Methods

### 2.1 | Description of the Study Area

The experiment was conducted in the Dabat district on permanent plots during the main cropping seasons at farmer sites for two consecutive years (2023–2024). Dabat district is located between latitudes 12°53'30" and 13°20'23" N, and longitudes 37°30'0" and 38°0'0" E (Figure 1). The district covers a total land area of 1187.93 km<sup>2</sup>. It lies within -cold, moist highland agro-ecology (dega) at an altitude of approximately 2596 m above sea level. The major soil types in the study area are Cambisols, Vertisols, and Nitosols (Schad 2023).

### 2.2 | Climatic Data

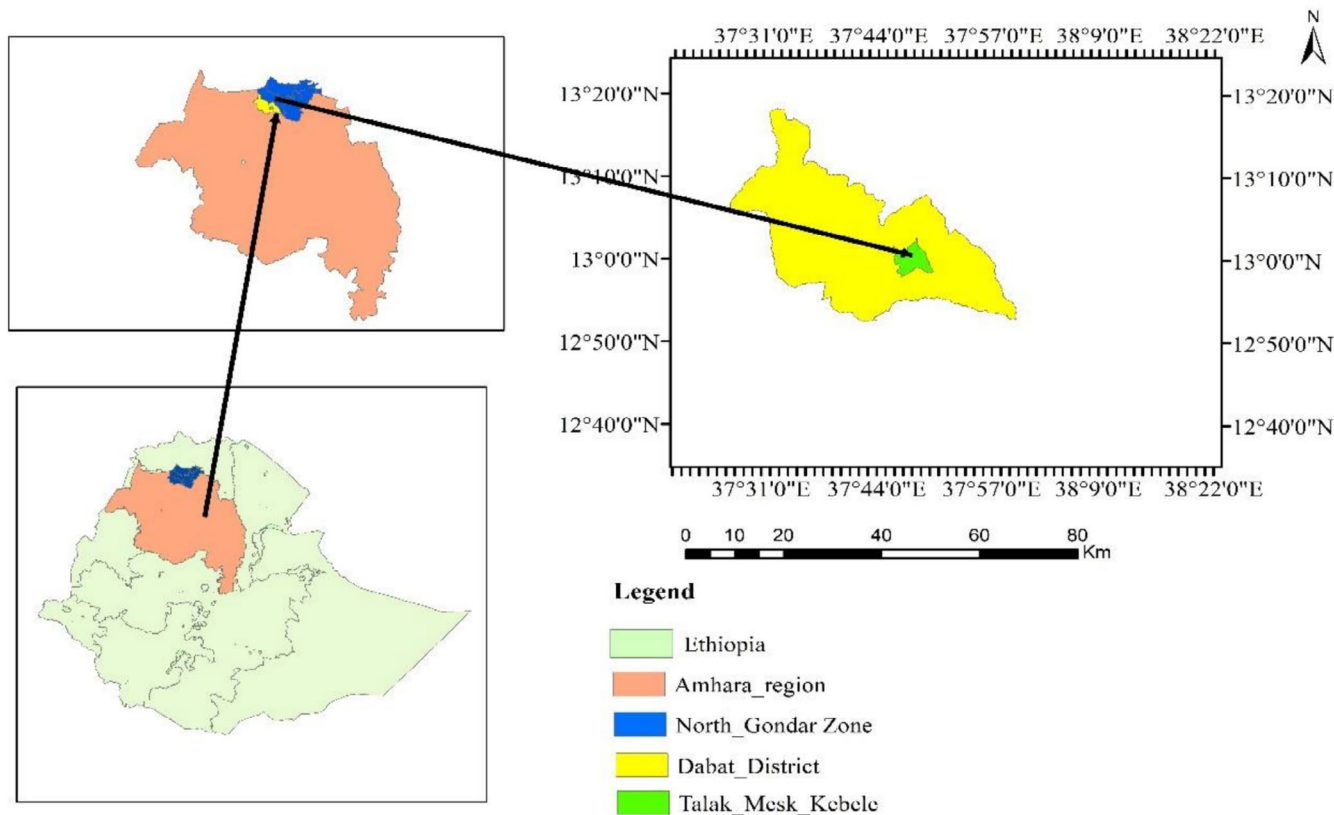
Dabat District exhibits a unimodal rainfall pattern, with the main rainy season occurring from June to September and peaking in July. The mean annual rainfall is 814.7 mm, varying between 600 and 1200 mm. The mean annual maximum temperature in Dabat District is 19.9°C, while the mean annual minimum temperature is 8.58°C.

### 2.3 | Experimental Material

Biochar was utilized as a source of organic fertilizer, composed of organic carbon, pH, available phosphorus, and nitrogen. It was prepared from the wood biomasses such as *Eucalyptus globulus* residues using a locally constructed airtight tank. Two metal barrels were inside the vessels, and the biomass was ignited inside the barrel in the absence of oxygen at high temperatures (400°C–450°C) (Tadele and Adgo 2024) (Figure 2). After an hour of cooling, 50% of the biomass was converted into biochar. For the field study, biochar was ground to pass through a 2 mm sieve. Thus, the C:N ratio of *Eucalyptus globulus* wood residues is 11.1:1. *Rhizobium leguminosarum varviciae* was selected based on its ability to enhance nodulation and grain yield under wide ecological conditions that were obtained from Holeta Agricultural Research Center.

### 2.4 | Experimental Design and Treatments

Field experiments were conducted in the Dabat district during the 2023–2024 main cropping seasons over consecutive years. The data were analysed separately in each year. The experimental trial consisted of six factors: two rhizobium inoculation treatments (with and without inoculation) and three levels of biochar application rates (0, 5, and 10 tons ha<sup>-1</sup>) (Table 1). These factors were combined in a 2 × 3 factorial arrangement and laid out in a randomized complete block design (RCBD) with three replications. Each replication (block) contained six plots, resulting in a total of 18 plots. Each plot measured 2 m × 3 m (6 m<sup>2</sup>). Faba bean was used as the test crop in the study area. The spacing between blocks and plots was 1 and 0.5 m, respectively.



**FIGURE 1** | Map of the study area.



**FIGURE 2** | This photo illustrates the biochar preparation process using metal vessels. On the left, a person monitors the setup, while on the right, another adjusts the metal barrel. The smoke indicates pyrolysis, where biomass is heated in low oxygen to create biochar, a sustainable method that enhances soil fertility and promotes carbon sequestration.

## 2.5 | Experimental Procedures

After selecting the experimental areas, the fields were cleaned and ploughed three times according to the design. Each treatment was randomly assigned to the experimental units within each block. One month before planting, biochar was applied

20 cm below the surface to enhance availability for crop growth. Before planting, seeds were inoculated using the method developed by Fatima et al. (2007) to ensure that the inoculants adhered to the seeds. Dry seeds were gently mixed with inoculants at a rate of 10 g of biofertilizers per kilogram of seeds. The recommended seed rate was 200 kg of faba bean seed per hectare.

**TABLE 1** | List of treatments utilized in the study, various biochar applications combined with rhizobium including control.

No.	Treatments	Descriptions
1	T1	Control
2	T2	RI
3	T3	5 tons ha <sup>-1</sup> biochar
4	T4	5 tons ha <sup>-1</sup> biochar
5	T5	5 tons ha <sup>-1</sup> biochar + RI
6	T6	10 tons ha <sup>-1</sup> biochar + RI

Abbreviations: ha<sup>-1</sup>, hectare; RI, Rhizobium inoculation; T, treatment.

Seeds were drilled in rows spaced 40 cm apart, with seeds placed 10 cm apart within rows and planted 5 cm deep. Two weeks after germination, the two seedlings per hill were thinned to one plant.

## 2.6 | Data Collection

### 2.6.1 | Soil Sampling

To assess the selected soil properties, samples were collected from the topsoil (0–30 cm) at the trial site using an auger at ten different points across the entire experimental area before sowing. Similarly, surface soil samples of the same depth were collected immediately after harvest from each plot by taking samples from five points within each plot. The samples were stored in plastic-coated paper bags and placed in coolers for transport from the field to the laboratory. Soil physical properties (texture) and chemical properties, such as pH, available phosphorus, organic carbon, and total nitrogen, were analysed before sowing and after harvesting faba bean.

### 2.7 | Analysis of Soil Properties and Biochar

The soil samples were analysed for selected parameters relevant to the study at the Bahir Dar Soil Laboratory. Standard laboratory procedures were followed to assess the physical and chemical properties of soil. The hydrometer method was used to determine soil texture distribution (clay, silt, and sand) in (%) (Gee and Bauder 1979). Soil textural class names were assigned based on the relative percentages of sand, silt, and clay using the USDA soil textural triangle. Soil pH was measured potentiometrically with a digital pH meter in a 1:2.5 soil-to-water suspension (Van Reeuwijk 2002). Total nitrogen was determined by the Kjeldahl method, including distillation and titration procedures of the wet digestion method (Black 1965). Available phosphorus was estimated using the Olsen extraction method (Olsen 1952). Organic carbon was determined by Walkley and Black's wet oxidation method, which oxidizes organic matter as described by Walkley (1935). The nutrient content of biochar was analysed for chemical properties such as pH, available phosphorus, total nitrogen, and organic carbon using the same methods applied to soil chemical properties.



**FIGURE 3** | Photo of Faba bean during mid flower. This photo of faba bean plants capture during a fieldwork session. The green foliage represents healthy growth, showcasing the development stage of the plants. The image highlights the dense stand of faba beans, which are an important leguminous crop, illustrating the agricultural practices and conditions under which they were cultivated.

### 2.8 | Estimation of Nodulation

At the mid-flowering stage, 10 plants were uprooted from each plot to assess the number of effective nodules per plant (Figure 3). Plant roots were randomly excavated from the border rows adjacent to each plot. Soil was manually removed from the root system after uprooting using a shovel. The roots were gently washed with water using a metal sieve to remove any remaining soil. The number of nodules was determined by counting the effective nodules on 10 plants from the border plot, and the mean value was recorded. Nodule colour was used to differentiate between effective and non-effective nodules. Cutting of nodules was made with a blade; nodules exhibiting a pink to dark-red colour were considered effective, while those with a green colour were classified as non-effective (Tadesse et al. 1991).

### 2.9 | Yield and Yield Component

Ten plants were randomly selected from the middle rows after reaching 90% maturity, and a tap meter was used to measure each plant's height from the ground to the tip. The mean number of pods per plant was determined by counting pods on 10 randomly selected plants from the net plot area. Five pods were randomly picked from these plants, excluding border rows, and the number of seeds per pod was recorded. Plants in the central row were manually harvested at ground level upon reaching physiological maturity. The weight of 10 plants from the destructive sampling rows was measured by drying the above-ground biomass in a sun dryer until a constant weight was achieved. This weight was extrapolated to the number of plants per net plot and converted to kilograms per hectare. All plants in the net plot were threshed separately, and the seeds were winnowed to separate them from debris. The yield per plot was calculated by weighing the grains. Straw yield was determined by subtracting the grain yield from the total above-ground plant biomass.

## 2.10 | Statistical Analysis

Treatment and interaction effects were analysed using the General Linear Model (GLM) procedure with SAS version 9.4. Mean values were compared using the Least Significant Difference (LSD) test at  $p=0.05$ . After verifying that the data met the assumptions of the statistical tests, the data were subjected to analysis of variance (ANOVA). Both one- and two-way ANOVA were performed to determine significant differences between treatments and interactions at  $p<0.05$ . Fisher's LSD was employed as a post hoc test to perform pairwise comparisons between treatment means. It was selected for its high statistical power in detecting differences between specific biochar rates and inoculation combinations, ensuring that the superior performance of the integrated 10 tons ha<sup>-1</sup> biochar with rhizobium treatment was mathematically distinct from the individual applications and the control.

## 3 | Results and Discussion

### 3.1 | Nutrient Content of Biochar and Selected Soil Properties Before Sowing

The pH, organic carbon (OC), total nitrogen (TN), and available phosphorus (Av. P) contents of the biochar were 8.45 (slightly alkaline), 5.67% (medium), 0.51% (low), and 52.76 ppm (high) respectively (Table 2). The CEC (12.2 Cmol(+)/kg) Eucalyptus biochar plays a specific, specialized role in soil fertility. While the CEC value is technically low by laboratory standards, due to woody materials have lower ash content, its impact on soil fertility is multifaceted acting more as a structural improver and acid neutralizer than a nutrient storage bank. Even with low CEC, Eucalyptus biochar provides excellent physical structure, aeration, and water-holding capacity in the soil. These results indicate that biochar positively influences soil

pH and nutrient status. It is a valuable amendment for mitigating soil acidity and enhancing crop yield, as it typically raises soil pH, particularly in acidic soils. As a liming agent, biochar tends to raise the pH of acidic soils, creating a more favourable environment for plant growth and helping to balance soil acidity. The moderate level of organic carbon indicates that biochar can contribute to soil carbon sequestration and improve soil structure. Peng et al. (2019) observed an increase in microbial biomass carbon in biochar-amended soils, likely due to a reduction in bulk density, as well as improvements in water-holding capacity, cation exchange capacity, and dissolved organic carbon content following the application of biochar. The low total nitrogen content indicates that this biochar can significantly contribute to nitrogen fixation in the soil. Several studies have demonstrated the positive effects of biochar amendment on nitrogen fixation in soils (Chen et al. 2017). Abujabbar et al. (2018) investigated biological nitrogen fixation as affected by biochar application rates and reported that biochar addition increased nitrogen fixation. Additionally, biochar can be a good source of phosphorus for plants. Pan et al. (2021) found that the addition of biochar improved phosphorus availability in acidic and neutral soils.

The soil analysis results indicated that the soil texture class is loam. According to Hazelton and Murphy (2025), the study soil was slightly acidic, with a pH of 6.1. A soil pH between 6.1 and 6.5 is considered slightly acidic and is acceptable for most crops because it allows for optimal nutrient availability. Additionally, the soil organic carbon (OC) content was 2.15%, which is classified as low based Landon (2014), rating scale: very low (<2%), low (2%–4%), medium (4%–10%), and high (10%–20%). Total nitrogen (TN) was 0.17%, also within the low range. Available phosphorus (Av. P) in the soil was 58.2 ppm, which may be sufficient for most crops and is important for crop growth (Table 3). Therefore, amending the soil with biochar is important to improve soil nutrients and crop yield.

**TABLE 2** | Nutrient content of biochar fertilizers.

Type of fertilizers	pH-H <sub>2</sub> O (1:2.5)	OC (%)	TN (%)	Av. P (ppm)	CEC Cmol(+)/kg
Biochar	8.45	5.67	0.51	52.76	12.20

*Note:* This table summarizes the average pH, organic carbon, total nitrogen, and available phosphorus content of biochar fertilizers, based on characterization of biochar prior to sowing, conducted in the laboratory.

Abbreviations: Av. P, available phosphorus; CEC, cation exchange capacity; OC, organic carbon; TN, total nitrogen.

**TABLE 3** | Initial nutrient contents of selected soil properties before sowing.

Parameters	Contents	Rating	References
pH-H <sub>2</sub> O (1:2.5)	6.1	Slightly acidic	Tadesse et al. (1991)
OC (%)	2.15	Low	Landon (2014)
TN (%)	0.17	Low	Landon (2014)
Av. P (ppm)	58.2	High	Landon (2014)
% Sand	48	Loam textural class	Hazelton and Murphy (2025)
% Clay	24		
% Silt	28		

*Note:* This table summarizes the average soil pH, organic carbon, total nitrogen, available phosphorus, and soil texture before sowing the crop, based on pre soil analysis conducted in soil laboratory.

Abbreviations: Av. P, available phosphorus; OC, organic carbon; TN, total nitrogen.

**TABLE 4** | The effects of biochar and *Rhizobium* inoculation on soil pH and organic carbon (OC) content (%) ( $n = 6$ ).

Treatment	pH-H <sub>2</sub> O (1:2.5)		OC (%)	
	2023	2024	2023	2024
Control	6.20 ± 0.04d	6.42 ± 0.05f	1.64 ± 0.04f	1.91 ± 0.01f
RI	6.23 ± 0.14c	6.53 ± 0.08d	1.71 ± 0.02e	2.01 ± 0.03e
5 tons ha <sup>-1</sup> Bio	6.24 ± 0.03c	6.62 ± 0.07c	1.76 ± 0.01d	2.03 ± 0.03d
10 tons ha <sup>-1</sup> Bio	6.25 ± 0.07b	6.79 ± 0.01b	1.91 ± 0.03c	2.07 ± 0.04c
5 tons ha <sup>-1</sup> Bio + RI	6.35 ± 0.03b	6.50 ± 0.07e	1.95 ± 0.01b	2.22 ± 0.02b
10 tons ha <sup>-1</sup> Bio + RI	6.45 ± 0.01a	6.88 ± 0.07a	2.28 ± 0.02a	2.29 ± 0.03a
CV (%)	1.43	1.42	2.39	2.58
<i>p</i>	0.04	0.002	0.001	0.001

Note: This table summarizes the average soil pH and soil organic carbon after harvesting faba bean crop for each treatment, based on post harvested soil analysis conducted in soil laboratory, along with their respective standard errors ( $\pm$ ). Statistically significant differences ( $p < 0.05$ ), as determined by Fisher's LSD test. Lowercase letters indicate significant differences ( $p < 0.05$ ) of treatment means. Mean within columns followed by the same letters are not significantly different at a 5% level of significance. The *p* values below 0.05 suggest strong evidence against the null hypothesis, indicating significant differences among treatment means. Abbreviations: bio, biochar; ha, hectare; *n*, number of treatments; OC, organic carbon; RI, *Rhizobium* inoculation; ha<sup>-1</sup>, hectare.

### 3.2 | The Effects of Biochar and *Rhizobium* Inoculation on Soil pH and Organic Carbon (OC)

The analysis of variance showed that significant differences among treatments in soil pH ( $p < 0.05$ ). The combined application of *Rhizobium* inoculation and 10 tons ha<sup>-1</sup> biochar produced a two-year average soil pH of 6.66, which is 5.5% higher than the control plot average of 6.31 (a decrease in acidity). The treatment's impact was noticeably stronger in the second season (pH 6.88) than in the first (pH 6.30), confirming that soil acidity was gradually neutralized over time (Table 3). This indicates that the addition of biochar raises soil pH compared to the control, both before sowing and throughout the growing seasons. The increase in soil pH may be attributed to biochar supplying and releasing essential nutrients into the soil. Additionally, basic cations such as Ca<sup>2+</sup>, Mg<sup>2+</sup>, and K<sup>+</sup> are converted into alkaline substances like hydroxides and carbonates during pyrolysis. The dissolution of these substances causes biochar to act as a liming agent (Yuan et al. 2017). Similarly, research done by Hailegnaw et al. (2019) found that the release of cations such as K, Ca, Mg, and Na from biochar contributed to the pH increase. In addition, similar research was done by Tadele and Adgo (2024) biochar production in the acacia decurrens-based taungya system significantly increases soil pH at kiln sites.

The analysis of variance showed significant differences among treatments in soil organic carbon (OC) ( $p < 0.05$ ). The combined application of 10 tons ha<sup>-1</sup> biochar and *Rhizobium* inoculation significantly enhanced soil organic carbon (OC) contents, recording a two average OC of 2.29%. This represents a 28.73% increase relative to the control average of 1.78% tons ha<sup>-1</sup> (Table 4). This might be due to biochar can improve organic matter decomposition and nutrient cycling by giving benefits for soil microorganism and rhizobium to survival. Biochar also facilitates the preservation of native soil organic matter by offering physical protection and its internal pore structure. Because biochar has a high carbon content, its application rates may cause an increase total organic carbon, water retention, increase microbial

activity, and improves nutrient availability (Lehmann and Joseph 2015). Biochar, microbes, rhizosphere breakdown, and root exudates all contribute to the increase in soil organic carbon (Pandian et al. 2016). Nutrient enriched biochar increased the soil carbon concentration which align with the finding of Sun et al. (2024) that at application rate of 10 ton ha<sup>-1</sup> biochar.

### 3.3 | The Effects of Biochar and *Rhizobium* Inoculation on Nitrogen and Phosphorus Contents

The analysis of variance showed significant differences among treatments in total nitrogen (TN) contents ( $p < 0.05$ ). The integrated application of 10 tons ha<sup>-1</sup> biochar and *Rhizobium* inoculation significantly enhanced total nitrogen contents, recording a two-year average total nitrogen (TN) content of 0.26%. This represents a 1.33% increase over the control plot average of 0.19% tons ha<sup>-1</sup> (Table 5). Particularly, the biochar amended plots showed a continuous improvement in TN from the first to the second season, while the control plots showed a decline. This indicates that biochar's high specific surface area and enhanced cation exchange capacity improve nitrogen retention and lower leaching losses.

This might be that biochar has the ability to better supply nutrients through mineralization. The control treatment showed the lowest TN contents due to the deficiencies in nutrient supply when no organic amendments were applied. *Rhizobium* and biochar combined likely enhance nitrogen fixation, particularly in leguminous plants. The higher TN levels observed in treatments that combined biochar and *Rhizobium* demonstrate a synergistic effect on soil fertility. This increase is primarily attributed to biochar's large specific surface area and porous structure, which significantly enhance its nitrogen retention capacity. The study was supported by research conducted by Zhong et al. (2025) which found that biochar preferentially enhances carbon and nitrogen concentrations in soil. This suggests improved long-term stabilization due to physical protection and chemical resistance. Additionally, biochar accelerates the mineralization of organic

**TABLE 5** | The effects of biochar and rhizobium inoculation on TN (%) and Av. P (ppm) ( $n = 6$ ).

Treatment	TN (%)		Av. P (ppm)	
	2023	2024	2023	2024
Control	0.19 ± 0.02d	0.18 ± 0.01d	30.20 ± 0.08f	37.22 ± 0.72e
RI	0.22 ± 0.04c	0.21 ± 0.06c	35.60 ± 1.71e	38.46 ± 0.056e
5 tons ha <sup>-1</sup> Bio	0.23 ± 0.02b	0.22 ± 0.01b	42.21 ± 1.15d	43.68 ± 1.62d
10 tons ha <sup>-1</sup> Bio	0.21 ± 0.02c	0.20 ± 0.03c	44.06 ± 0.54c	46.14 ± 1.13c
5 tons ha <sup>-1</sup> Bio + RI	0.24 ± 0.01b	0.23 ± 0.02b	46.24 ± 1.64b	46.28 ± 1.76b
10 tons ha <sup>-1</sup> Bio + RI	0.26 ± 0.05a	0.25 ± 0.06a	50.68 ± 1.07a	52.54 ± 0.39a
CV (%)	9.93	12.11	4.94	4.53
<i>p</i>	0.03	0.04	0.001	0.001

Note: This table summarizes the average total nitrogen and available phosphorus after harvesting faba bean crop for each treatment, based on post harvested soil analysis conducted in soil laboratory, along with their respective standard errors ( $\pm$ ). Statistically significant differences ( $p < 0.05$ ), as determined by Fisher's LSD test. Lowercase letters indicate significant differences ( $p < 0.05$ ) of treatment means. Mean within columns followed by the same letters are not significantly different at a 5% level of significance. The *p* values below 0.05 suggest strong evidence against the null hypothesis, indicating significant differences among treatment means. Abbreviations: Av. P, available phosphorus; Bio, biochar; CV, coefficient of variation; ha, hectare; *n*, number of treatments; RI, Rhizobium inoculation; TN, total nitrogen.

matter, nutrient cycling, and nitrogen fixation, all of which can enhance plant uptake of nitrogen and phosphorus, as previously noted by Solaiman et al. (2019).

The integration application of 10 tons ha<sup>-1</sup> biochar and Rhizobium inoculation significantly improved phosphorus availability, recording a two-year average available phosphorus (Av. P) content of 51.61 ppm. This represents a substantial 53.10% increase over the control plot average of 33.71 ppm. This improvement is likely mediated by biochar's ability to increase soil pH, which reduces the activity of Fe and Al oxides that typically fix phosphate ions in acidic Nitisols. Mechanistically, the oxygen-containing functional groups on the biochar surface may also compete with phosphate for sorption sites through ligand exchange, thereby increasing the concentration of bioavailable phosphorus in the soil solution for crop uptake tons ha<sup>-1</sup> (Table 5). This increase may be attributed to the organic matter, which stimulates the soil to release more phosphorus, raises soil pH, and reduces phosphorus fixation. Similarly, Forján et al. (2024) stated that biochar serves as a source of phosphorus, thereby enhancing the soil's phosphorus content compared to the control treatments. Additionally, it stimulates the growth of bacteria that solubilize phosphorus, increasing the amount of phosphorus released into the soil (Gao et al. 2016). This finding is supported by research conducted by Sun et al. (2025) which demonstrates enhanced nutrient availability through biochar amendments and bio inoculants.

### 3.4 | Effects of Rhizobium and Biochar Fertilizers on Nodulation of Faba Bean

The application of biochar integrated with Rhizobium inoculation was superior in this regard, resulting in the highest nodule numbers in both seasons. The results showed significant differences in the number of effective nodules per plant ( $p < 0.05$ ).

**TABLE 6** | Mean number of effective nodule per plant in 2023 and 2024 ( $n = 6$ ).

Treatments	Mean of effective nodules	
	2023	2024
Control	73.3 ± 10.9d	95.0 ± 14.01d
RI	125 ± 23.1c	106.3 ± 10.26cd
5 tons ha <sup>-1</sup> Bio	142.7 ± 7.3bc	105.0 ± 34.58bcd
10 tons ha <sup>-1</sup> Bio	160.7 ± 16.2abc	164.3 ± 30.42bc
5 tons ha <sup>-1</sup> Bio + RI	162.3 ± 10.7ab	179.3 ± 37.85ab
10 tons ha <sup>-1</sup> Bio + RI	185.3 ± 6.8a	211.0 ± 22.25a
CV (%)	18.2	24.6
<i>p</i>	0.003	0.007

Note: This table summarizes the average number of effective nodule for different treatments, along with their respective standard error ( $\pm$ ). Statistically significant differences ( $p < 0.05$ ), as determined by Fisher's LSD test. Lowercase letters indicate significant differences ( $p < 0.05$ ) of treatment means. Mean within columns followed by the same letters are not significantly different at a 5% level of significance. The *p* values below 0.05 suggest strong evidence against the null hypothesis, indicating significant differences among treatment means. Abbreviations: Bio, biochar; CV, coefficient of variation; ha, hectare; *n*, number of treatment; RI, Rhizobium inoculation.

Accordingly, the highest number of effective nodules per plant was recorded 10 tons ha<sup>-1</sup> biochar combined with Rhizobium inoculation achieving two-year averages, measuring 197.65, while the lowest number of effective nodules per plant was recorded in the control plot, with 84.15 (Table 6). These results represent a substantial 134.9% increase in nodulation over the control. This significant increase in nodulation indicates that the biochar provided an ideal rhizosphere environment, maybe creating protective micro-niches and enhancing the chemical properties of the soil, which facilitated better bacterial colonization and faba bean roots. The slight variation in effective nodule counts between the two seasons could be attributed to

differences in rainfall, temperature, moisture, and nutrient availability. *Rhizobium* supplies the crop with the nitrogen necessary for growth and development, while biochar improves the soil environment for *Rhizobium*, promoting better nodulation and nitrogen fixation (Shakeel et al. 2023). In addition, Mohamed et al. (2017) reported that the biochar addition and *Rhizobium* colonization increase in nitrogen derived from the atmosphere over the control. The effect of biochar additions on N<sub>2</sub> fixation by rhizobia-nodulating *Phaseolus vulgaris* in Colombia was investigated by Rondon et al. (2007). Increasing the amount of biochar (0, 30, 60, and 90 kg ha<sup>-1</sup>) raised bean yields by 46% and the percentage of nitrogen derived from fixation (percentage NdF) from 50% in the control to 72% in the 60 kg ha<sup>-1</sup> treatment.

**TABLE 7** | Mean height of per plant (cm) in 2023 and 2024 ( $n = 6$ ).

Treatment	Mean height plant <sup>-1</sup> (cm)	
	2023	2024
Control	108 ± 0.06d	95 ± 0.10d
RI	121 ± 0.04d	100 ± 0.11bcd
5 tons ha <sup>-1</sup> Bio	133 ± 0.04bc	115 ± 0.15abc
10 tons ha <sup>-1</sup> Bio	140 ± 0.09abc	116 ± 1.16abc
5 tons ha <sup>-1</sup> Bio + RI	138 ± 0.08abc	123 ± 0.07a
10 tons ha <sup>-1</sup> Bio + RI	148 ± 0.09ab	124 ± 0.10a
CV (%)	8.8	10.4
<i>p</i>	0.013	0.04

Note: This table summarizes the average yields of height per plant for different treatments, along with their respective standard error (±). Statistically significant differences ( $p < 0.05$ ), as determined by Fisher's LSD test. Lowercase letters indicate significant differences ( $p < 0.05$ ) of treatment means. Mean within columns followed by the same letters are not significantly different at a 5% level of significance. The *p* values below 0.05 suggest strong evidence against the null hypothesis, indicating significant differences among treatment means. Abbreviations: Bio, biochar; CV, coefficient of variation; ha<sup>-1</sup>, hectare; *n*, number of treatment; RI, *Rhizobium* inoculation.

**TABLE 8** | Mean of pods per plant and seeds per pod in 2023 and 2024 ( $n = 6$ ).

Treatment	Mean pods plant <sup>-1</sup>		Mean seeds pod <sup>-1</sup>	
	2023	2024	2023	2024
Control	6.30 ± 0.72d	7.33 ± 0.75c	2.33 ± 0.78 d	2.66 ± 0.65d
RI	10.0 ± 0.61cd	7.67 ± 0.52bc	2.67 ± 0.99cd	3.00 ± 0.84cd
5 tons ha <sup>-1</sup> Bio	10.7 ± 2.15bc	9.11 ± 0.92bc	3.00 ± 0.54bcd	3.66 ± 0.57abc
10 tons ha <sup>-1</sup> Bio	11.7 ± 1.76abc	10.47 ± 0.1.20ab	3.66 ± 1.35abc	3.67 ± 0.50bc
5 tons ha <sup>-1</sup> Bio + RI	11.3 ± 1.28bc	10.4 ± 1.44ab	3.67 ± 0.62abc	3.68 ± 0.12abc
10 tons ha <sup>-1</sup> Bio + RI	14.3 ± 0.89ab	11.0 ± 1.65ab	4.00 ± 1.11ab	4.11 ± 1.10ab
CV (%)	21.5	20.1	12.25	14.39
<i>p</i>	0.02	0.03	0.02	0.03

Note: This table summarizes the average pods per plant and seeds per pod for different treatments, along with their respective standard error (±). Statistically significant differences ( $p < 0.05$ ), as determined by Fisher's LSD test. Lowercase letters indicate significant differences ( $p < 0.05$ ) of treatment means. Mean within columns followed by the same letters are not significantly different at a 5% level of significance. The *p* values below 0.05 suggest strong evidence against the null hypothesis, indicating significant differences among treatment means. Abbreviations: Bio, Biochar; CV, coefficient of variation; ha<sup>-1</sup>, hectare; *n*, number of treatment; RI, *Rhizobium* inoculation.

### 3.4.1 | Plant Height

The mean plant height at 90% maturity showed a significant difference ( $p < 0.05$ ) with the application of rhizobium and biochar in both 2023 and 2024. The highest mean plant height was recorded with 10 tons ha<sup>-1</sup> of biochar combined with *Rhizobium* inoculation, measuring with a two-year average of 136 cm. This represents a 34.0% increase over the control group average of 101.5 cm (Table 7). While plant heights were greater in 2023 than in 2024 across all treatments, suggesting that climatic factors such as rainfall, temperature, and sunlight hours were more favourable in 2023. The greatest plant height observed with the application of biochar combined with *Rhizobium* inoculation can be attributed to the nutrient-rich composition of biochar, which enhances root development, nodulation, and protoplasm synthesis. As a result, the plants grow taller and produce a greater number of branches (Das et al. 2014). The present findings confirm the research conducted by Liu et al. (2025) biochar amendment significantly enhanced plant root length, plant height, and biomass of crops.

### 3.5 | Effect of Rhizobium Inoculation and Biochar Application on Yield Parameters of Faba Bean

#### 3.5.1 | Number of Pods Per Plant

The mean number of pods per plant showed a significant difference ( $p < 0.05$ ) with the application of rhizobium and biochar in both 2023 and 2024. The combined effects of *Rhizobium* and biochar at different rates significantly influenced the number of pods per plant. The highest number of pods per plant was recorded in the 10 tons ha<sup>-1</sup> biochar with *Rhizobium* treatment, achieving a two-year average of 12.65 pods per plant. Compared to the control plot average of 6.80 pods per plant, this represents a substantial 86.03% increase (Table 8). The increasing number of pods per plant indicates that *Rhizobium* inoculation combined with biochar fertilizers effectively enhances nutrient availability, aeration, and soil moisture, thereby creating more favourable physiological conditions for pod formation.

Compared to the control group, these results demonstrate that biofertilizers can increase pod numbers, although the increase is less pronounced than that achieved with *Rhizobium* inoculation alone. These findings align with previous research showing that the nutrient content and beneficial effects of organic and bio-based fertilizers on soil health can significantly improve crop yields (Mohamed et al. 2017).

### 3.5.2 | Seed Number Per Pods

The application of *Rhizobium* and biochar resulted in a significant difference ( $p < 0.05$ ) among treatments in the number of seeds per pod. The highest number of seeds per pod was observed in the treatment with 10 t ha<sup>-1</sup> biochar combined with *Rhizobium* inoculation, yielding 4.05 seeds per pod. In contrast, the control group produced 2.50 seeds per pod (Table 8). This represents a significant increase of 62% over the control plot. The number of seeds produced in each pod was statistically significantly influenced by the various fertilizer treatments. This suggests that biochar amendments and *Rhizobium* inoculation had a positive impact on the number of seeds per pod. In addition to providing the plant with an easily accessible source of nitrogen, which promotes seed formation, biochar fertilizers create an ideal environment for *Rhizobium* to grow and fix nitrogen. Compared to the control group, which produced fewer seeds, this highlights the crucial role of nitrogen in seed development. In the absence of *Rhizobium* and biochar application, the plants likely experienced a nitrogen deficiency, limiting their ability to produce seeds. The present study supported by Bhattarai et al. (2015) found that the application of biochar fertilizers can lead to increased number of seeds per pod.

### 3.5.3 | Biomass Yield

The mean aboveground biomass showed a significant difference at ( $p < 0.05$ ) with the application of *Rhizobium* and biochar fertilizers in both 2023 and 2024 years. Hence, the combined effects of *Rhizobium* with biochar application at different rates significantly influences on aboveground biomass. The highest aboveground was recorded with 10 tons ha<sup>-1</sup> biochar combined with *Rhizobium* inoculation, achieving a two-year average of 5488.90 kg ha<sup>-1</sup>. Compare to the control group average of 3266.65 kg ha<sup>-1</sup>, this represents a significant increase of 68.03% (Table 9). The result indicates that 10 tons ha<sup>-1</sup> biochar and *Rhizobium* fertilizers produced the highest biomass in both years, demonstrating their effectiveness in enhancing plant growth due to increased nutrient uptake, and improved soil fertility, which are the reasons for the higher biomass in treated plots. While, *Rhizobium* increases nitrogen availability through biological fixation, biochar improves soil structure and water retention, which benefits plant development. This indicates that the combination of *Rhizobium* and biochar application rates significantly contributes to the increment in plant growth and improved biomass yield. This may increase the synthesis of assimilates, which can result in enhanced the biomass yield (Gobarah et al. 2006). The present findings confirm the research conducted by Kalu et al. (2021) found the similar result by applying similar amount of softwood biochar on different leguminous crop that increased the biomass yield of faba bean.

**TABLE 9** | Mean of aboveground biomass yield (kg ha<sup>-1</sup>) in 2023 and 2024 ( $n = 6$ ).

Treatment	Mean of aboveground biomass (kg ha <sup>-1</sup> )	
	2023	2024
Control	3750.0 ± 220.4e	2783.3 ± 112.0d
RI	4277.8 ± 242.1de	3680.0 ± 220.5cd
5 tons ha <sup>-1</sup> Bio	4388.9 ± 311.7cd	4640.0 ± 242.2bc
10 tons ha <sup>-1</sup> Bio	5300.0 ± 134.7ab	4846.7 ± 196.7b
5 tons ha <sup>-1</sup> Bio + RI	5144.4 ± 275.3b	4813.3 ± 252.7b
10 tons ha <sup>-1</sup> Bio + RI	5511.1 ± 247.5a	5466.7 ± 347.6a
CV (%)	11.03	19.11
<i>p</i>	0.002	0.007

Note: This table summarizes the average yields of aboveground biomass for different treatments, along with their respective standard error (±). Statistically significant differences ( $p < 0.05$ ), as determined by Fisher's LSD test. Lowercase letters indicate significant differences ( $p < 0.05$ ) of treatment means. Mean within columns followed by the same letters are not significantly different at a 5% level of significance. The *p* values below 0.05 suggest strong evidence against the null hypothesis, indicating significant differences among treatment means. Abbreviations: Bio, biochar; CV, coefficient of variation; ha<sup>-1</sup>, hectare; *n*, number of treatments; RI, *Rhizobium* inoculation.

### 3.5.4 | Grain Yield

The effect of *Rhizobium* and biochar application showed a significant difference at ( $p < 0.05$ ) among the treatments on the grain yield of faba bean *Ashebeke* variety. The highest grain yields were produced in both years with 10 tons ha<sup>-1</sup> of biochar combined with *Rhizobium* inoculation, with a two-year average of 2617.76 kg ha<sup>-1</sup>. The treatment produced more than twice as much as the control in the second season. This is a significant 74.39% increase over the average of 1501.11 kg ha<sup>-1</sup> for the control plot (Table 10). This highlights the effectiveness of biochar and *Rhizobium* inoculation in improving faba bean yield. This significant variation demonstrates the crucial role of microbial inoculants and biochar amendments in improving crop performance. The increase in grain yield may be attributed to a higher number of pods per plant and an increased seed number per plant due to the influence of biochar application. Additionally, the availability of phosphorus and nitrogen to plants is enhanced through the application of biochar and *Rhizobium* inoculation (Pan et al. 2021). This positive response likely results from improved nutrient absorption from amendments and *Rhizobium* inoculation leading to enhanced reproductive parts and formation for seed produced. These findings align with previous research conducted by Ye et al. (2020) showing that biochar was a source of effective fertilizers in increasing crop yields when added in combination.

### 3.5.5 | Straw Yield

The results showed that the effect of *Rhizobium* and biochar fertilizers had a significant difference ( $p < 0.05$ ) among the treatments on straw yield. The treatment with 10 tons ha<sup>-1</sup> of biochar combined with *Rhizobium* inoculation recorded the highest

**TABLE 10** | Mean of grain yield and straw yield in the year of 2023 and 2024 ( $n = 6$ ).

Treatment	Mean of grain yield (kg ha <sup>-1</sup> )		Mean of straw yield (kg ha <sup>-1</sup> )	
	2023	2024	2023	2024
Control	1722.2 ± 94.9e	1280.0 ± 85.3d	2027.8 ± 170.1g	1503.3 ± 135.8e
RI	2088.9 ± 123.7de	1893.3 ± 108.4bcd	2188.9 ± 144.4ef	1786.7 ± 106.2de
5 tons ha <sup>-1</sup> Bio	2277.8 ± 139.2bcd	2320.0 ± 145.7bc	2111.1 ± 174.4e	2320 ± 157.9bcd
10 tons ha <sup>-1</sup> Bio	2511.1 ± 144.4ab	2368.0 ± 112.8abc	2788.9 ± 144.5bc	2446.7 ± 148.2bc
5 tons ha <sup>-1</sup> Bio + RI	2333.3 ± 69.4abc	2333.3 ± 98.4ab	2811.1 ± 250.2ab	2480.0 ± 231.7b
10 tons ha <sup>-1</sup> Bio + RI	2622.2 ± 116.0a	2613.3 ± 120.5a	2888.9 ± 139.2a	2853.3 ± 242.3a
CV (%)	9.93	21.44	15.07	20.24
<i>p</i>	0.002	0.017	0.008	0.0161

Note: This table summarizes the average yields of grain and straw for different treatments, along with their respective standard error ( $\pm$ ). Statistically significant differences ( $p < 0.05$ ), as determined by Fisher's LSD test. Lowercase letters indicate significant differences ( $p < 0.05$ ) of treatment means. Mean within columns followed by the same letters are not significantly different at a 5% level of significance. The *p* values below 0.05 suggest strong evidence against the null hypothesis, indicating significant differences among treatment means.

Abbreviations: Bio, biochar; CV, coefficient of variation; ha<sup>-1</sup>, hectare; *n*, number of treatments; RI, Rhizobium inoculation.

straw yield of 2887.1 kg ha<sup>-1</sup> during the two years respectively (Table 10). In comparison to the control treatment average of 1765.54 kg ha<sup>-1</sup>, this is a 62.62% increase. This suggests that the combination of a higher rate of biochar with rhizobium inoculation enhances nutrient availability and nitrogen fixation, having a positively impact on the increase in straw yield of faba bean. Higher seed and straw output may result from increased photosynthetic production, which could have also contributed to a rise in plant height and the number of primary, secondary, and tertiary branches per plant (Shekedar 2022). This outcome is supported by Yusif et al. (2016), who discovered that NPK fertilization, both with and without bio-inoculation, boosted faba bean production and yield components.

## 4 | Conclusions

The study investigated the combined application of biochar and rhizobium inoculation had a significant effect on selected properties of soil and yield of faba bean as compared to no fertilizer amendment. In particular, the combined application of 10 tons ha<sup>-1</sup> biochar and rhizobium inoculation significantly enhanced both yield and nodulation. These findings show a significant 134.9% increase in nodulation and a 74.4% increase in grain yield compared to the control. Additionally, compared to untreated plots, the treatment increased average organic carbon by 28.7%, total nitrogen content by 1.33%, and available phosphorus by 53.1%, demonstrating a synergistic improvement in soil health. This showed that the use of biochar combined with rhizobium is the best option to reclaim problematic acidic soil, improve microbial activity and nitrogen fixation in highland areas. Moreover, the increase in the availability of nitrogen and phosphorus as a result of these applications is continuously encouraging nodule formation, biomass growth, and yield of faba bean. In general, the use of biochar and biofertilizers improves yields and supports sustainable agricultural practices by enhancing soil health and reducing the cost of synthetic fertilizers. Future researchers should focus on different rate combinations of organic fertilizers on nitrogen fixation and yield of

different crops in various soil types. Therefore, farmers should apply biochar with rhizobium inoculation into their soil management practices to improve soil pH, enhance soil nutrients, and crop yield.

## Acknowledgements

The authors wish to express deep thankfulness to the Debark University for giving an opportunity for this study and granting my study leave. Thanks and appreciation go to the local people in Dabat District.

## Funding

All the research costs such as data collection, land rent, Biochar preparation, soil and biochar nutrient analysis were covered by Debark University.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

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