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Interactive Effects of Biochar, Nitrogen, and Phosphorous on the Symbiotic Performance, Growth, and Nutrient Uptake of Soybean (*Glycine max* L.)

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Abstract: Numerous studies reported the positive effect of soil amendment with biochar on plant development. However, little is known about biochar and its interrelation with nitrogen (N) and phosphorous (P) additions and their impact on plant growth. We carried out greenhouse experiments to understand the interactive effects of nitrogen and phosphorus supply, as well as biochar amendment, on the symbiotic performance of soybean (*Glycine max* L.) with *Bradyrhizobium japonicum*, and plant growth and nutrient uptake. The biochar was produced from maize by heating at 600 °C for 30 min and used for pot experiments at an application rate of 2%. Plants were fertilized with two different concentrations of P (KH_2PO_4) and N (NH_4NO_3). Biochar application significantly increased the dry weight of soybean root and shoot biomass, by 34% and 42%, under low nitrogen and low phosphorus supply, respectively. *Bradyrhizobium japonicum* inoculation enhanced the dry weight of shoot biomass significantly, by 41% and 67%, in soil without biochar and with biochar addition, respectively. The nodule number was 19% higher in plants grown under low N combined with low or high P, than in high N combinations, while biochar application increased nodule number in roots. Moreover, biochar application increased N uptake of plants in all soil treatments with N or P supply, compared with *B. japonicum*-inoculated and uninoculated plants. A statistical difference in P uptake of plants between biochar and nutrient levels was observed with low N and high P supply in the soil. Our results show that the interactions between nitrogen, phosphorus, and biochar affect soybean growth by improving the symbiotic performance of *B. japonicum* and the growth and nutrition of soybean. We observed strong positive correlations between plant shoot biomass, root biomass, and N and P uptake. These data indicated that the combined use of biochar and low N, P application can be an effective approach in improving soybean growth with minimum nutrient input.

Keywords: maize biochar; nutrient supply and uptake; soybean; *Bradyrhizobium japonicum*; root and shoot growth

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1. Introduction

Biochar is a char produced by pyrolysis of biomass from different sources (wood chips, crop residues, dairy manure, etc.) with high carbon content [1]. It is produced at high (above 500 °C) or low (<400 °C) pyrolysis temperature under limited or in the complete absence of oxygen [2]. Several beneficial properties of biochar applications were documented, such as improved soil cation exchange capacity [3], water holding capacity [4], or soil organic matter content [5]. Moreover, biochar positively affects plant growth and development, provides nutrients, and even increases nutrient availability [6]. The improved N, P, K uptake by a plant grown in soil amended with biochar was explained by enhanced soil microbial activity involved in the decomposition of organic substances, providing nutrients readily available for plant uptake [7–10].

It has also been reported that biochar improves the symbiotic performance of legumes with rhizobia, thus enhancing N_2 fixation and nitrogen supply to the plants [11]. Increased nodulation in the roots of soybean [12], faba bean [13], and chickpea [9] in soils amended with biochar was observed, indicating the positive impact of biochar on the symbiotic performance of the plant with rhizobia. The positive effect of biochar on bacterial colonization was explained by improved habitat conditions in biochar pores and the supply of air and nutrients for bacterial growth [14–16]. Nutrient interactions in soil are complicated and paramount for nutrient uptake and plant growth [17–19]. Mineral nutrients such as N, P, and K, as well as several microelements, affect rhizobia–host symbiotic interactions in legumes such as nodule formation and root activity [20–22]. Nitrogen plays a vital role in leguminous plant physiological processes, whereas higher amounts of nitrogen inhibit the symbiotic performance of nitrogen fixation by rhizobia [23,24]. Phosphorus (P) also plays a vital role in root development and has been found to be crucial for legume–rhizobia symbiosis and nitrogen fixation. Inadequate P supply impairs nodule formation and nitrogen fixation through direct and indirect effects on the plants [25]. Moreover, low concentrations of N and P in soil result in poor plant development and productivity [26–28]. It has also been reported that P application improves nodule formation and nitrogen fixation in legumes [29,30]. Therefore, the balanced application of N and P to soil and the appropriate combination will improve soil and plant productivity in a sustainable way [31]. Several reports indicate enhanced P use efficiency by incorporation of P into compost [32], manure [33], and humic substances [34]. A synergistic effect of biochar combined with plant beneficial microbes in improving plant growth and nutrition has also been reported [35].

Although many studies reported positive effects of biochar on plant growth and nutrient acquisition, relatively few studies focus on the interrelations of biochar with N and P fertilization and microbial inoculants [36]. Shareef et al. [37] reported a positive effect of biochar derived from maize increased plant growth and improved soil properties. Similar results were found for cowpea, whereas the application of maize biochar significantly enhanced nodule number, shoot biomass, and grain yield, as well as nitrogen and phosphorus contents [38]. Mohamed et al. [13] observed increased nodule numbers and contents of N, P, and K in plant tissue of faba bean grown in soil amended with soybean straw-derived biochar, with or without amendments of N, P and K. Yusif et al. [39] found a synergistic effect of rhizobia and biochar addition on groundnut nodulation, growth and development.

Soybean (*Glycine max* L. Merr.) is one of the most important legume crops in numerous countries globally and is widely produced as a source of oil and protein [40,41]. Several studies observed improved biomass, nutrient acquisition, and soybean yield after biochar amendments [42,43]. However, the interactive effect of biochar and essential nutrients (N and P) on rhizobia–legume symbiosis, plant growth, and the prevalence of such interactions are still limited. We hypothesized that the symbiotic performance and growth of soybean are influenced by the biochar amendment in the soil and by interactions with N and P supply.

Understanding the symbiotic performance of rhizobia and plant growth responses to biochar and mineral nutrient interrelations should facilitate strategies to enhance legume production. Therefore, greenhouse experiments were carried out to understand the effect of biochar application combined with low and high N and P concentrations on shoot and root growth of soybean (*Glycine max* L.), nodule numbers, nutrient uptake (N, P), and soil nutrient contents in a loamy, sandy soil.

2. Materials and Methods

2.1. Soil Samples

The soil used in the study was a sandy loam, collected from the horizon (0–15 cm depth) of an experimental arable field under irrigation, operated by the Experimental Field Station of the Leibniz Center for Agricultural Landscape Research (ZALF), Müncheberg, Germany.

The soil had the following contents: clay and fine silt (7%), coarse and medium silt (19%), sand (74%), C org (0.6%), total N (0.07%), P (0.03%), K (1.25%), and Mg (0.18%); the pH was 6.2; CECeff (cmol (+) kg^{-1}) was 4.85 (Ca (3.8); K (0.38); Mg (0.52); Na (0.004)) [42].

2.2. Biochar Material

The biochar was supplied from the Leibniz-Institut for Agrartechnik Potsdam-Bornim e.V. (ATB) [44]. The material was produced from whole maize by heating at 600 °C for 30 min (MBC). Biochar characteristics are given in Table 1.

Table 1. Biochar characteristics.

Characteristics	Maize Biochar
DM (% FM)	92.85
Ash (% DM)	18.42
TOC (% DM)	75.47
N (% DM)	1.80
C/N ratio	41.93
pH value	9.89
Ca (g (kg DM)$^{-1}$)	9.26
Fe (g (kg DM)$^{-1}$)	11.40
Mg (g (kg DM)$^{-1}$)	4.91
K (g (kg DM)$^{-1}$)	32.26
P (g (kg DM)$^{-1}$)	5.26

FM, fresh matter; DM, dry matter; TOC, total organic carbon [44].

2.3. Plant and Bacteria

The soybean seeds (*Glycine max*. cv. Sultana) were used for pot experiments. The strain *Bradyrhizobium japonicum* (HAMBI 2314) was obtained from the Culture Collection of the University of Helsinki (HAMBI). The strain was grown on yeast extract–mannitol (YEM) medium at 28 °C for three days.

2.4. Pot Experiment

The experiment was conducted in the plant growth chamber at the Leibniz Center for Agricultural Landscape Research (ZALF), Müncheberg, Germany. The concentrations of 2% biochar were used as a soil amendment for the pot experiment. First, the soil was mixed with 2% of crushed char (particle size < 3 mm). Pots were filled with 1000 g soil-biochar mixtures. The soybean seeds were surface-sterilized using 10% *v*/*v* NaOCl for 5 min and 70% ethanol for 5 min. After that, seeds were rinsed five times with sterile distilled water and transferred to paper tissue for germination in a dark room at 2 °C for two days. To prepare the bacterial inoculant, *B. japonicum* HAMBI2314 was grown in yeast extract–mannitol (YEM) medium at 2 °C for three days and adjusted to a final concentration of approximately 10^8 CFU mL^{-1}. The germinated soybean seeds were treated with bacterial inoculants.

Three seeds were sown to each pot, and after one week, the seedlings were thinned to one plant per pot. One week later, plants were fertilized by an aqueous solution of 100 mL with different concentrations of P (KH_2PO_4) and N (NH_4NO_3) (Table 2) [19].

Table 2. The nutrient concentration used for soybean growth experiment.

Treatment	Nutrient Concentrations
high N and high P (HNHP)	NH_4NO_3—3000 µmol/L, KH_2PO_4—250 µmol/L
high N and low P (HNLP)	NH_4NO_3—3000 µmol/L, KH_2PO_4—50 µmol/L
low N and high P (LNHP)	NH_4NO_3—300 µmol/L, KH_2PO_4—250 µmol/L
low N and low P (LNLP)	NH_4NO_3—300 µmol/L, KH_2PO_4—50 µmol/L

The following treatments were set up:

1. Uninoculated control plants grown in soil with low N and low P (LNLP) and without biochar;
2. Uninoculated control plants grown in soil with high N and low P (HNLP) and without biochar;
3. Uninoculated control plants grown in soil with low N and high P (LNHP) and without biochar;
4. Uninoculated control plants grown in soil with high N and high P (HNHP) and without biochar;
5. Plants inoculated with *B. japonicum* and grown in soil with LNLP and without biochar;
6. Plants inoculated with *B. japonicum* and grown in soil with HNLP and without biochar;
7. Plants inoculated with *B. japonicum* and grown in soil with LNHP and without biochar;
8. Plants inoculated with *B. japonicum* and grown in soil with HNHP and without biochar;
9. Plants inoculated with *B. japonicum* and grown in soil with LNLP and with biochar;
10. Plants inoculated with *B. japonicum* and grown in soil with HNLP and with biochar;
11. Plants inoculated with *B. japonicum* and grown in soil with LNHP and with biochar;
12. Plants inoculated with *B. japonicum* and grown in soil with HNHP and with biochar.

A randomized, complete block design was used, four replications were set as four blocks, each block included all 12 treatments. In each block, the treatments were randomly distributed.

The plants were grown for 45 days at a temperature of 24 °C/16 °C (day/night) and in a humidity of 50–60%. At harvest, the roots were separated from the shoots, and their biomass was oven-dried at 70 °C for 48 h [45]. The dry weights of root and shoot and the number of nodules were determined from each plant.

2.5. Plant and Soil Nutrient Analyses

To determine nitrogen (N) and phosphorus (P) content, oven-dried plants were homogenized by milling, and powders of shoots and roots were combined. The N and P concentrations in plant tissues and soil were analyzed with an inductively coupled plasma optical emission spectrometer (ICP-OES; iCAP 6300 Duo ThermoFischer Scientific Inc., Waltham, MA, USA) via Mehlich-3 extraction [7]. The soil N contents were determined by the dry combustion method using a CNS elemental analyzer (TruSpec, Leco Corp., St. Joseph, MI, USA) [46]. The soil P content was analyzed by ICP-OES (iCAP 6300 Duo) via Mehlich-3 extraction [7].

2.6. Statistical Analysis

The analysis of variance and multiple comparisons between treatments were performed using Duncan's test. Linear correlation analyses were applied to characterize the relationship between various parameters, and Pearson's correlation coefficients were determined at $p < 0.05$. The correlation was visualized with a heatmap, and the correlation coefficients were displayed on each square. All statistical analyses were performed by the open-source statistical language R v1.3.1056 (R Studio, Boston, MA, USA).

3. Results

3.1. Effect of Biochar and Nutrient Amendments on Soybean Growth

The effect of biochar, N, and P concentrations on soybean root and shoot dry weight were investigated. The root and shoot growth of uninoculated soybean grown under LNLP supply without biochar amendment were lower than inoculated with *B. japonicum*. The plant inoculation with *B. japonicum* under LNLP significantly stimulated the shoot growth dry weight by 18% and the nodule numbers, but there were no significant differences in root dry weight. The results indicate that the dry weight of root and shoot of soybean grown under LNLP soil amended with biochar were significantly increased, by 34% and 42%, respectively, compared with uninoculated plants grown in soil without biochar (Figure 1).

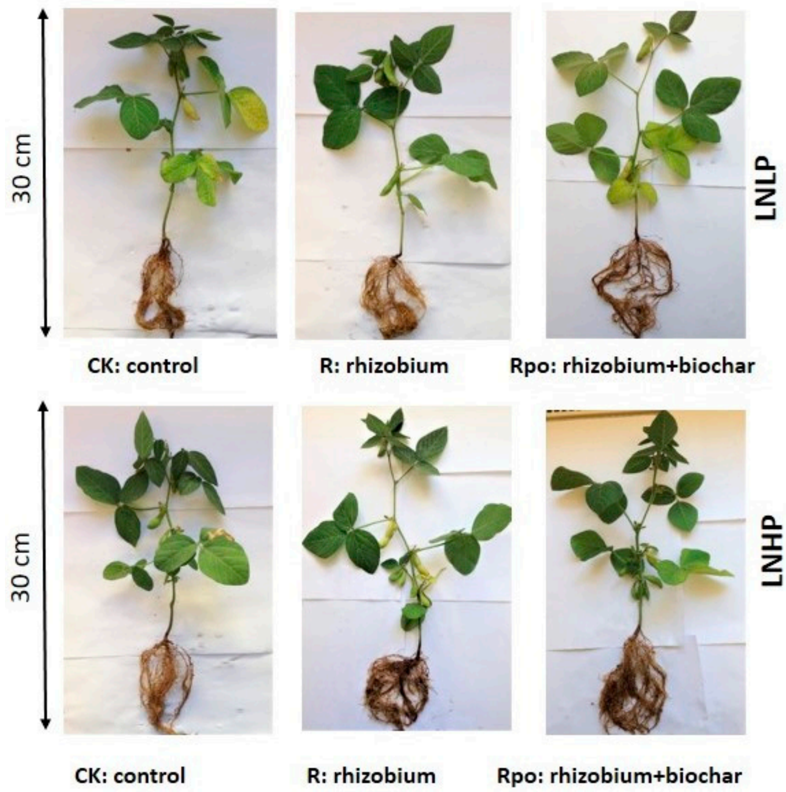

Figure 1. The root and shoot growth of soybean plants after exclusive rhizobium inoculation and rhizobium inoculation combined with biochar application under low N or low and P supply. LNLP, low N and low P; LNHP, low N and high P; CK, control; R, Rhizobium inoculation; Rpo, Rhizobium inoculation with biochar application.

A similar observation was made for plants grown on soil with LNHP supply. The dry shoot weights were, respectively, 41% and 67% significantly higher for plants inoculated with *B. japonicum* and grown in soil without biochar and with biochar addition than those in uninoculated plants (Figures 1 and 2A). No statistical differences in root growth between uninoculated plants and plants inoculated with *B. japonicum* were observed in soil without biochar. The biochar application significantly increased root dry weight (56%) of soybean grown in soil under LNHP conditions.

There were no statistical differences in plant growth parameters between uninoculated plants and inoculated plants grown in soil without biochar or biochar addition under HNLP and HNHP supply. Root and shoot dry weights of soybean inoculated with *B. japonicum* grown in soil without biochar and with biochar were 6% and 19%, and 20% and 33% higher than those in control plants under HNLP supply, respectively.

The response of the symbiotic performance of *B. japonicum* with the host plant to nutrient supply and biochar application was assessed based on nodule numbers (Figure 2C). It appears that the number of nodules in soybean roots inoculated with *B. japonicum* increased significantly, by five- to ninefold, under low N conditions combined with either low P or high P, compared with uninoculated soybean plants. Biochar application increased the nodule number of soybean roots in both nutrient conditions, compared with soybean inoculated with *B. japonicum* grown in soil without biochar amendment. In general, the nodule number was higher in plants grown under low N combined either with low or high P, as compared with high N combinations. Biochar increased nodule formation in roots under all N and P concentrations (Figure 2C). Inoculation × N input and N input × P input showed no interaction effects, but inoculation × P input provided a significant interaction effect on the nodule number (Table 3, $p < 0.05$). However, no interaction was observed on the shoot and root biomass.

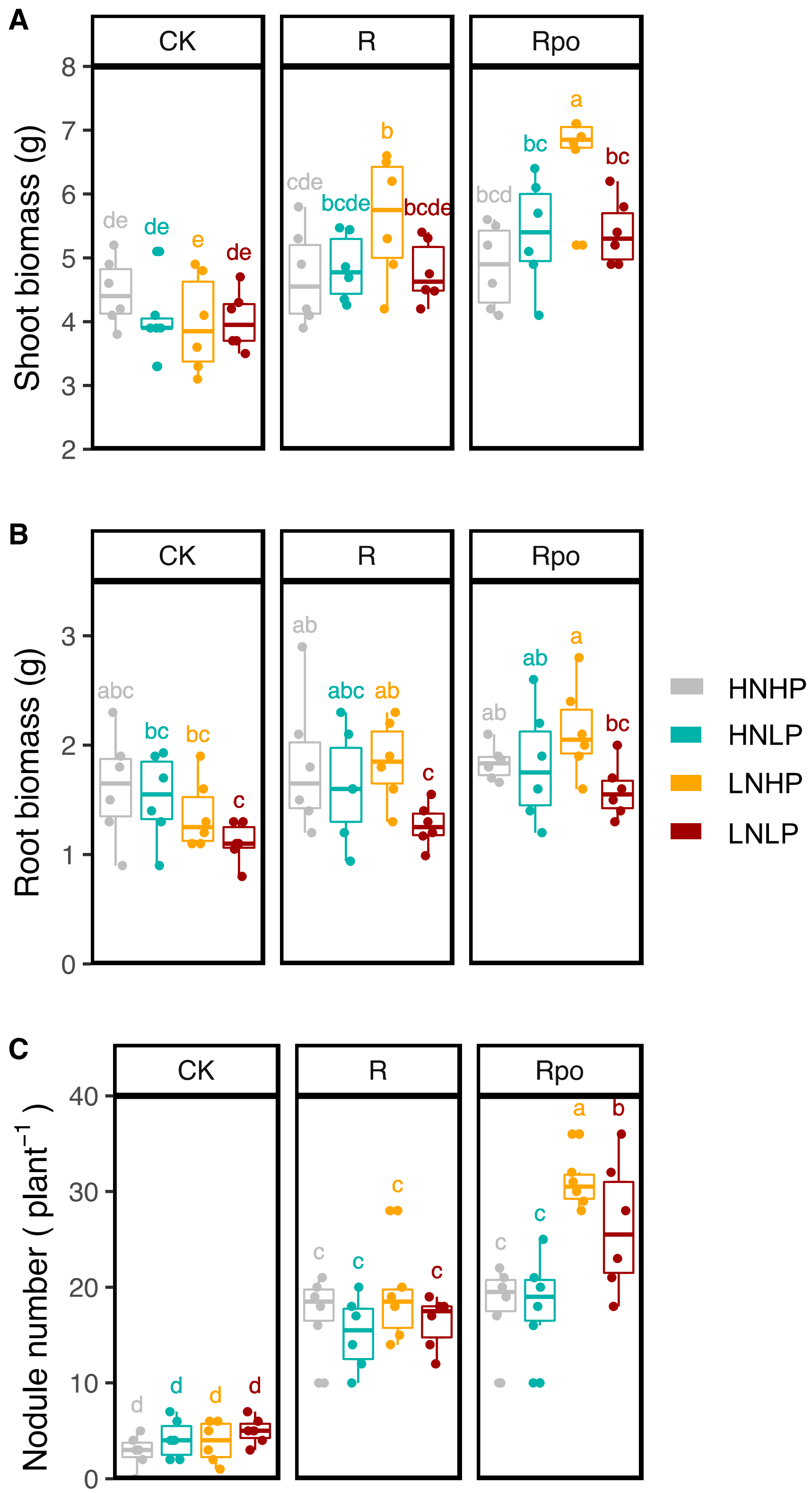

Figure 2. Shoot biomass (**A**), root biomass (**B**), and nodule number (**C**) of soybean plants after exclusive Rhizobium inoculation and Rhizobium inoculation combined with biochar application under high or low N and P supply. Letters within each column are significantly different at $p < 0.05$ based on Duncan's test. HNHP, high N and high P; HNLP, high N and low P; LNHP, low N and high P; LNLP, low N and low P; CK, control; R, rhizobium inoculation; Rpo, rhizobium inoculation with biochar application.

Table 3. Interaction effects of inoculation, N and P supplies on shoot and root biomass, nodule number and concentrations of plant and soil nutrients. Significance denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant.

Interaction Effects	Shoot Biomass	Root Biomass	Nodule Number	Plant N	Plant P	Soil N	Soil P
Inoculation × N supply	ns	ns	ns	ns	ns	***	ns
Inoculation × P supply	ns	ns	*	ns	ns	ns	ns
N input × P supply	ns	ns	ns	ns	*	ns	**
Inoculation × N supply × P supply	ns	ns	ns	ns	*	ns	ns

3.2. Effects of Biochar and Nutrients on Plant Nitrogen and Phosphorous Concentrations

N and P uptake by plants were also affected by biochar application and N and P supply. We observed that N and P content in uninoculated soybean plants grown in soil without biochar amendment was lower under LNLP. The soybean inoculation with *B. japonicum* improved N and P uptake in plant tissue, but the effect was not significant. Biochar application significantly increased P content in plants under LNLP condition, compared with inoculated plants with *B. japonicum* by 21%, whereas N uptake slightly increased (Figure 3A).

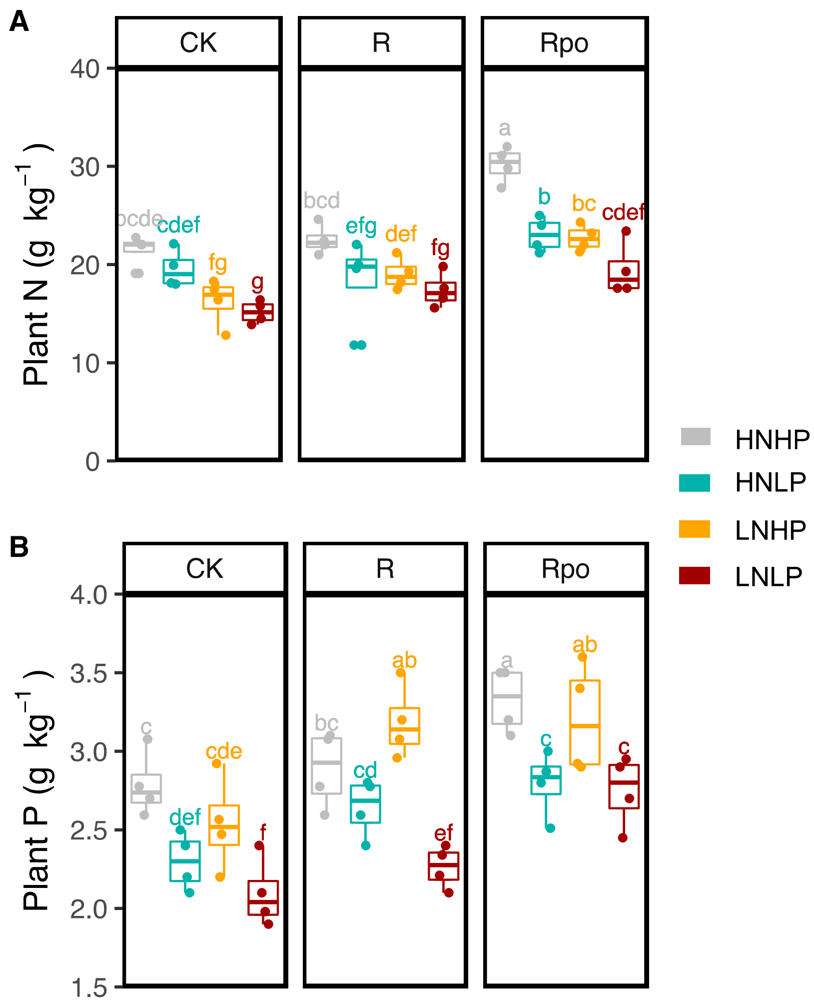

Figure 3. Contents of nitrogen (**A**) and phosphorous (**B**) in soybean plants after exclusive Rhizobium inoculation, and Rhizobium inoculation combined with biochar application under high or low N and P supply. Letters within each column are significantly different at $p < 0.05$ based on Duncan's test.

Statistical differences in P uptake by plants between biochar and nutrient levels were observed with LNHP supply in the soil. P concentration in plant tissue was 25%, higher in LNHP than in plants inoculated with *B. japonicum* and grown in soil without biochar, as compared with uninoculated plants (Figure 3B). The N concentration in plants was not affected by LNHP supply. A slight increase in N was observed under the LNHP supply, compared with the uninoculated plants. Only biochar application significantly increased N concentration by 19%, compared with inoculated plants.

No significant differences in N and P uptake were observed for plants grown under HNLP and HNHP supply comparing inoculated and uninoculated plants. Biochar application significantly increased N and P concentrations in plant tissues under both HNLP and HNHP supplies, compared with uninoculated plants. The N concentration in plants grown under HNLP and HNHP increased, by 25% and 40%, respectively, compared with uninoculated plants (Figure 3A). The P concentration in plants grown under HNHP increased by 19%, compared with uninoculated plants (Figure 3B).

Shoot biomass, root biomass, and N showed a strong positive correlation with P, and the correlation coefficients were 0.61, 0.61, and 0.58 ($p < 0.05$), respectively (Figure 4. Nodule numbers showed a significant positive correlation with shoot biomass; the correlation coefficient was 0.57 ($p < 0.05$). However, the correlation between nodule number and N was not significant.

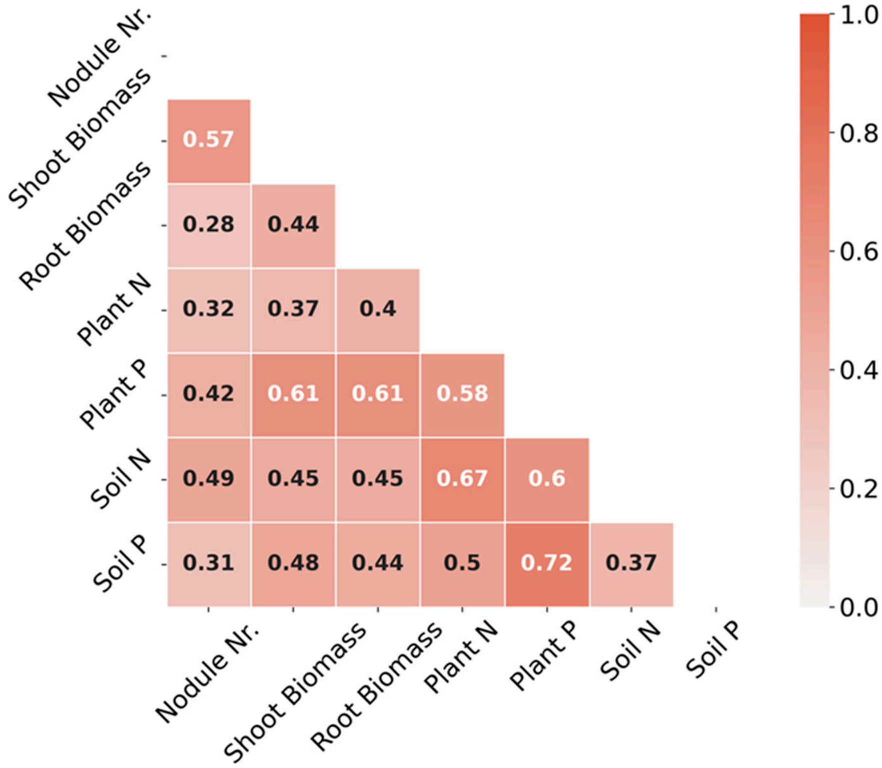

Figure 4. Heatmap of correlations between plant biomass, concentrations of plant and soil nutrients, and nodule numbers. Nodule Nr., nodule number. The color bar indicates Pearson's correlation coefficient. The white color of numbers in boxes indicates a significant correlation, while the black color indicates an insignificant correlation at $p < 0.05$.

Inoculation × N input and inoculation × P showed no interaction effects but N input × P input and inoculation × N input × P input showed significant interaction effects on plant P concentration (Table 3, $p < 0.05$). No interaction was observed on plant N.

3.3. Effects of Biochar and Nutrients on Soil Nitrogen and Phosphorous Concentration

The nutrient concentrations in soil without biochar amendment were affected by N and P supplies. The N concentrations in soil under HNLP and HNHP without inoculation were higher at 23% and 27%, compared with LNLP and LNHP, respectively (Figure 5A). Plant inoculation with *B. japonicum* did not affect soil N concentration. Soil amended with biochar showed a positive effect on N concentration in soil under LNLP, and LNHP supplies, being increased by 29%, compared with uninoculated soil.

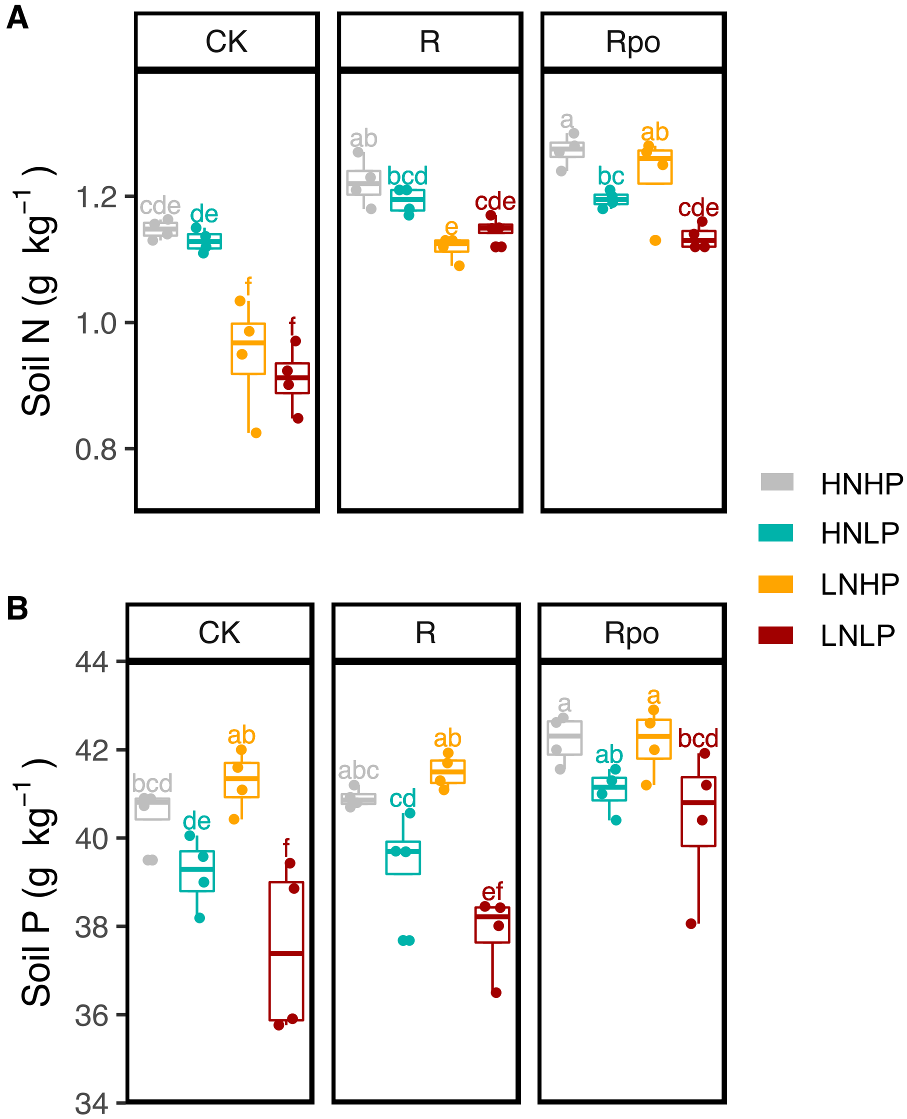

Figure 5. Contents of nitrogen (**A**) and phosphorous (**B**) in soil of soybean plantings after exclusive Rhizobium inoculation and Rhizobium inoculation combined with biochar application under high or low N and P supply. Letters within each column are significantly different at $p < 0.05$ based on Duncan's test.

The P concentrations of soil were not significantly affected by all nutrient supplies, LNLP, LHHP, HNLP, and HNHP, or by soil inoculation with rhizobia (Figure 5B). However, biochar amendment of soil slightly increased P concentration in soil inoculated with rhizobia and supplied with LNLP and HNLP. The soil N concentration showed a significant positive correlation with plant N and P concentrations; the correlation coefficients were 0.67 and 0.60 ($p < 0.05$), respectively (Figure 4).

The soil P concentration showed a strong positive correlation with plant P concentration; the correlation coefficient was 0.72 ($p < 0.05$).

Inoculation × N input showed a significant interaction effect on soil N concentration (Table 3, $p < 0.001$). In contrast, inoculation × P, N input × P input and inoculation × N input × P input showed no significant interaction effects on the soil N concentration. N input × P input indicated a significant interaction effect on soil P concentration ($p < 0.01$), whereas no interaction of inoculation × N input, inoculation × P input, and inoculation × N input × P input was observed on soil P concentration.

4. Discussion

The present study demonstrated that combined applications of N, P, and biochar have essential effects on the symbiotic performance of soybean with *B. japonicum* and on plant growth, as well as on N and P uptake of plants. The root and shoot biomass of soybean treated with *B. japonicum* were higher under all N and P levels, also in combination with biochar, compared with uninoculated soybean plants, indicating a positive effect of rhizobial inoculation. A similar observation was reported by Sun et al. [47] for *Robinia pseudoacacia*, where biochar combined with rhizobial inoculation increased plant growth, nodule formation, and N content. Moreover, an interrelation among N, P, and biochar was evident, influencing the symbiotic performance of *B. japonicum* with soybean. Biochar application increased nodule numbers of soybean roots under both low and high N and P supplies, compared with soybean inoculated with *B. japonicum* and grown in soil without biochar. The improvement of nodule formation in legumes by biochar application was also reported in several other studies [42,48]. The most likely explanation for such improvements is that biochar facilitates favorable conditions for bacterial proliferation, protects bacteria from various abiotic stresses, and provides nutrients and air to nodule bacteria [15,16]. Moreover, biochar stimulates signalling molecules such as flavonoids, which regulate root nodule development [49]. Furthermore, biochar improved root-associated microbial diversity, including Rhizobia and plant beneficial bacteria attributed to microbial production of plant growth stimulating metabolites and N_2-fixing capacity of Rhizobia [50,51].

Plants are sensitive to soil nutrient concentrations, and insufficient or suboptimal N and P suppliescause inadequate or inbalanced plant nutrition [18,52,53]. In earlier studies, O'Hara [54] and López-Bucio et al. [55] observed reduced plant root systems in soil with low P supply than under adequate P supply. The legumes require adequate P supply for nodule formation and biological nitrogen fixation, since P deficiency inhibits nodule development and plant growth [53]. Under a low nitrogen and phosphorus supply, reduced root and shoot dry biomass were observed in soil without biochar, whereas significant differences were found in soil amended with biochar. Induced changes in nutrient availability in soil amended with biochar were reported by Prendergast-Miller et al. [56], thus providing additional N and P sources for plant nutrition. Furthermore, our observation with soybean also confirmed the results of Wali et al. [57], who reported that combined application of biochar and P supply significantly improved crop growth, nodulation, and P acquisition, as compared to P and N supply alone. We have observed higher N acquisition in soybean under HNLP and HNHP, indicating that N acquisition by plants depends on forms of available nitrogen, as well as on interrelationships of N with other nutrients, such as P. Biochar application along with N fertilizers was found to improve plant growth and N uptake by reducing nitrogen mineralization and nitrification and increasing N availability to plants [58].

N uptake of soybean plants was improved by biochar application in soil regardless of the N and P supply, compared with plants either uninoculated or inoculated with *B. japonicum*. An increased N concentration in plant tissue of beans was also reported by Rondon et al. [59]. The combined effect of biochar and nutrients such as N, P, and K on total P and K uptake of faba bean was reported by Mohamed et al. [13]. The increases in nitrogen acquisition may be related to improved soybean nodulation and N_2 fixation after biochar application [49,59]. The enhanced N content in plants could be due to the supply of P with biochar, which supports nodule formation [60]. Biochar also improved the P uptake of plants, especially under low P supply; similar observations were reported by Shen et al. [61]. In general, biochar is rich in organic carbon and minerals, thus supplying additional nutrients to the soil and improving plant availability, thereby improving the nutritional status and development of plants [37,62,63]. The increase in plants' P uptake could be due to the availability of P in soil, induced by biochar addition [64], and increased microbial activity involved in soil phosphate solubilization [65].

In our study, we also observed an increase in N and P concentrations in soil amended with biochar under LNLP and LNHP. Han et al. [63] observed an increase in N concentration of soil after biochar application produced from Chinese pine, which confirms the capability of biochar improving nitrogen availability in soil [66]. The increase in soil P concentration after biochar application was explained by the retention of P content in biochar and enhanced P availability to plants [67]. Biochar enhances N immobilization by reducing N leaching and increasing N retention and bioavailability in agricultural soils. Several reasons were proposed for this, such as an increase in cation and anion exchange capacities and water holding capacity [68], and promoting NH_3 volatilization from the applied N [69].

5. Conclusions

Our results indicate that the growth, nodulation, as well as N and P uptake of soybean are significantly affected by N and P supply. The interrelationships between N, P, and biochar affect soybean growth by improving the symbiotic performance of *B. japonicum* and nodule formation. Biochar addition to soil supplied with low amounts of nitrogen and phosphorus showed a more profound effect. These interactions likely have a positive effect on plant growth and acquisition of N and P, explaining synergistic growth responses of soybean to combined N, P, and biochar additions. We observed strong positive correlations between plant shoot biomass, root biomass, and N and P uptake. Overall, these results contribute to a better understanding of the interaction between biochar and mineral nutrients (N and P) and the responses of soybean symbiosis with rhizobia to different degrees of N and P supply. These findings indicated that the combined use of biochar and low application rates of Nand P can be a practical approach in improving soybean growth with minimum expenditure of fertilizers.

Author Contributions: D.E., M.R. and S.D.B.-K. designed the experiments. D.E. and H.M. conducted the experiments. R.A.O. and H.M. analyzed the data. D.E., S.W. and H.M. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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