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Co-Inoculation of Rhizobacteria and Biochar Application Improves Growth and Nutrients in Soybean and Enriches Soil Nutrients and Enzymes

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Abstract: Gradual depletion in soil nutrients has affected soil fertility, soil nutrients, and the activities of soil enzymes. The applications of multifarious rhizobacteria can help to overcome these issues, however, the effect of co-inoculation of plant-growth promoting rhizobacteria (PGPR) and biochar on growth and nutrient levels in soybean and on the level of soil nutrients and enzymes needs in-depth study. The present study aimed to evaluate the effect of co-inoculation of multifarious *Bradyrhizobium japonicum* USDA 110 and *Pseudomonas putida* TSAU1 and different levels (1 and 3%) of biochar on growth parameters and nutrient levels in soybean and on the level of soil nutrients and enzymes. Effect of co-inoculation of rhizobacteria and biochar (1 and 3%) on the plant growth parameters and soil biochemicals were studied in pot assay experiments under greenhouse conditions. Both produced good amounts of indole-acetic acid; (22 and 16 $\mu\text{g mL}^{-1}$), siderophores (79 and 87%SU), and phosphate solubilization (0.89 and 1.02 99 g mL^{-1}). Co-inoculation of *B. japonicum* with *P. putida* and 3% biochar significantly improved the growth and nutrient content of soybean and the level of nutrients and enzymes in the soil, thus making the soil more fertile to support crop yield. The results of this research provide the basis of sustainable and chemical-free farming for improved yields and nutrients in soybean and improvement in soil biochemical properties.

Keywords: biochar; *Bradyrhizobium japonicum*; *Pseudomonas putida*; plant growth; plant nutrients; soil enzymes; soil nutrients; soybean

1. Introduction

The global climate scenario is experiencing a drastic depletion of soil nutrients due to various anthropogenic activities, burning of fossil fuel, and excess use of agrochemicals [1]. Applications of plant-growth promoting rhizobacteria (PGPR) and biochar have been advocated as an effective, cheap, and sustainable approach for the replenishment of crop health, crop nutrients, and soil nutrients and enzymes and for improving and sustaining soil fertility [2]. Furthermore, these amendments have a positive impact on the growth [3], development, and yield of several crops [4,5]. Various reports claimed that the application of plant growth-promoting rhizobacteria (PGPR) and biochar improves plant growth, plant nutrients, and physicochemical properties of soil [6–8]. Moreover, such applications of biochar also keep a check on atmospheric CO₂ levels [9] and, thus, contribute to decrease global warming effects [10], while the use of PGPR to increase soil fertility and plant nutrients will help to reduce the doses of agrochemicals in the field [11].

A wide variety of symbiotic bacteria, such as *Rhizobium* sp. and *B. japonicum*, etc., have been reported to promote seed germination, the growth of root and shoot, and the level of nutrients in soybean and also improve soil biochemical properties [4,5]. Rhizobia-legumes symbiosis plays a vital role in increasing crop yields, reducing the use of inorganic nitrogen fertilizers and improving soil fertility [12]. Rhizobial species are commonly used as inoculants in various parts of the world for improving the yield of legumes. Co-inoculation with multifarious *Bradyrhizobium* sp. and *Pseudomonas* sp. improves plant growth, plant, and soil nutrients and enzymes through the production of siderophores [13], phytohormones [14], enzymes [15], exopolysaccharide [16], stress tolerance [17], and phosphate solubilization [18–23], etc. Thus, several studies reported increases in nodules number, nodule weight, nitrogen fixed, plant growth, and yield of legumes due to co-inoculation with plant growth promoting *Bradyrhizobium* sp. and *Pseudomonas* sp. [12–14], while the combination of biochar with PGPR further increases root length, shoot length, nodule per plant, seed number, and yield of crops [5].

The activity of PGPR bioinoculants helps in improving the level of extracellular soil enzymes that facilitates the decomposition of soil organic matter and ensures the availability of nutrients in the soil [15]. Among the soil enzymes, proteases and acid and alkaline phosphomonoesterase are the major enzymes that mediate the hydrolysis of the protein and phosphate (P) into bioavailable amino acids, organic nitrogen, and soluble P [16]. However, the activities of these enzymes are governed by many factors, such as soil properties, soil organic matter level, and the presence of organic compounds [24]. We hypothesized that co-inoculation with *B. japonicum*+*P. putida* and biochar would facilitate the beneficial effects on soybean plant growth, plant nutrients, and soil nutrients and enzymes.

The present study was aimed at evaluating the effects of co-inoculation of multiple plant growth-promoting traits positive in *Bradyrhizobium japonicum* USDA 110 and *Pseudomonas putida* TSAU1 and different levels (1 and 3%) of biochar on seed germination, growth parameters, and nutrient levels in soybean and the level of nutrients and enzymes in soil. The outcome of this study may provide a better way of increasing soil fertility and increasing the growth and yield of soybean. This approach has multiple dimensions; as utilization of biochar is not only a cheaper option but will also help in solving the management issues of biochar, it is expected to minimize the doses of agrochemicals and produce chemical-free food. The consortium effect of PGPR and application of biochar provide excellent benefits to the farmers as they incur less investment and yield more crop productivity, and this organically grown crop has more demand with a good selling price.

2. Materials and Methods

2.1. Bacterial Culture, Soybean, and Biochar

B. japonicum USDA 110 and *P. putida* TSAU1 strains were collected from the culture collection of the Department of Microbiology and Biotechnology, National University of Uzbekistan, Tashkent, Uzbekistan. Soybean (*Glycine max* L. Merr.) seeds were obtained from Leibniz Centre for Agricultural Landscape Research (ZALF), Müncheberg, Germany.

The maize biochar (MBC) was collected from the Leibniz-Institute for Agriculture Engineering and Bioeconomy (ATB), Potsdam, Germany. Pyrolysis of MBC was carried out at 600 °C for 30 min and the chemical compositions of MBC were analyzed according to the method of Reibe et al. [25].

2.2. Screening for the Production of PGP Metabolites

B. japonicum USDA 110 and *P. putida* TSAU1 strains were screened for phosphate (P) solubilization on Pikovoskaya's agar and in Pikovoskaya's broth [26] for the production of indole-3-acetic acid (IAA) according to the method of Brick et al. [27], for production and estimation of siderophore according to the method of Patel et al. [28] and Payne [29], and the production and estimation of aminocyclopropane-1-carboxylate deaminase (ACCD) activity according to the method of Penrose and Glick [30]. The ACCD activity was measured as the amount of α -keto-butyrate produced per mg protein per h.

2.3. Surface Sterilization, Germination, and Bacterization of Seeds

Soybean seeds were sorted to eliminate broken, small, infected seeds and sterilized with 10% sodium hypochlorite solution for 5 min and washed three times with sterile, distilled water. Seeds were germinated in 85 mm \times 15 mm tight-fitting plastic Petri dishes with 5 mL of water. *B. japonicum* USDA 110 and *P. putida* TSAU 1 broth rich in PGP metabolites were used for the inoculation of germinated seeds. Germinated seeds were first placed with sterile forceps into bacterial suspension (5×10^6 CFU g⁻¹) for 10 min before planting, were air-dried, and then planted in plastic pots containing 400 g sandy loamy soil.

2.4. Experimental Design

The effect of rhizobacteria on the growth of soybean was studied in pot experiments in a greenhouse at ZALF, Müncheberg, Germany during July 2015. All the experiments were carried out in a randomized block design (RBD) with three replications. Experimental treatments included un-inoculated control (soil without biochar and soil with two levels of biochar (1 and 3%)), inoculation with *B. japonicum* USDA 110 (soil without biochar and soil with two levels of biochar (1 and 3%)), and co-inoculation with *B. japonicum* USDA 110 and *P. putida* TSAU 1 strains (soil without biochar and soil with two levels of biochar (1 and 3%)). The plants were grown in greenhouse conditions at 24 °C during the day and 16 °C at night for 30 days.

2.5. Measurement of Plant Growth Parameters and Plant Nutrients

Plants harvested after 30 days were subjected to the measurement of seed germination rate, root length, shoot length, root dry weight, shoot dry weight, and the number of nodules per plant of soybean. Plant nutrients, such as nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), sodium (Na), and calcium (Ca) were estimated from crushed plant tissue with an inductively coupled plasma optical emission spectrometer (ICP-OES; iCAP 6300 Duo, Thermo Fischer Scientific Inc., Waltham, MA, USA) via Mehlich-3 extraction [30]. The nitrogen and phosphorus contents of root and shoot were determined from dried powdered biomass. For nitrogen estimation, 1 g of plant biomass was digested with 10 mL concentrated H₂SO₄ and 5 g catalyst mixture in the digestion tube. The mixture was allowed to cool and then processed for distillation. The distillate was collected and titrated with H₂SO₄ blank (without leaf). Total nitrogen was calculated from the blank and sample titer reading [31]. For the estimation of P content, plant P was extracted with 0.5 N NaHCO₃ (pH8.5) and treated with ascorbic acid in an acidic medium [32]. The intensity of blue color produced was measured and the amount of P was calculated from the standard curve of P. For the estimation of potassium content of plant biomass, 25 mL of ammonium acetate solution was added in 5 g of the biomass sample, the content was shaken for 5 min and filtered, and the amount of K from the filtrate was measured [33]. For the estimation of Na, Mg, and Ca, 1 g of plant extract was mixed with 80 mL of 0.5 N HCl for 5 min at 25 °C followed by measurement of concentrations of these elements in the filtrate [34].

2.6. Analysis of Soil Nutrient and Soil Enzymes

The rootsoil (10 g) of experimental pots was air-dried soil, shaken with 100 mL ammonium acetate (0.5 M) for 30 min to effectively displace the available nutrients, and adhered to soil minerals. The soil organic carbon (SOC), nitrogen (N), phosphate (P), and potassium (K) content of soil were determined by the dry combustion method according to the method of Sims [35] and Nelson and Sommers [36] using a CNS analyzer (TruSpec, Leco Corp., St. Joseph, MI, USA). For this purpose, 10 mL of 1 N $K_2Cr_2O_7$ and 20 mL of concentrated H_2SO_4 was added in 1g soil, mixed thoroughly and diluted with 200 mL of distilled water followed by the addition of 10 mL each of H_3PO_4 and sodium fluoride. The resulting solution was used for the elemental analysis. Blank (without soil) served as control. Soil Organic Carbon (SOC) of soil sample was calculated with the help of blank and sample titer reading.

The acid and alkaline phosphomonoesterase activities were assayed according to the method of Tabatabai and Bremner [37]. Moist soil (0.5 g) was placed in a 15 mL vial, and 2 mL of modified universal buffer (MUB) (pH 6.5 for the acid phosphatase assay or pH 11 for the alkaline phosphatase assay) and 0.5 mL of p-nitrophenyl phosphate substrate solution (0.05 M) were added to the vial, sequentially. The assay and control batches were replicated 3 times. The concentration of p-nitrophenol (p-NP) produced in the assays of acid and alkaline phosphomonoesterase activities were calculated from a p-NP calibration curve after subtracting the absorbance of the control at 400 nm. Protease activity was assayed according to the method of Ladd and Butler [38]. For this, 0.5 g of soil was weighed into a glass vial, and 2.5 mL of phosphate buffer (0.2 M, pH of 7.0) and 0.5 mL of N-benzoyl-L-arginine amide (BAA) substrate solution (0.03 M) were added. The ammonium released was calculated by relating the measured absorbance at 690 nm.

2.7. Statistical Analyses

All the experiments were performed in three replicates and the average of triplicate was considered. Experimental data were analyzed with the StatView Software (SAS Institute, Cary, NC, USA, 1998) using ANOVA. The significance of the effect of treatment was determined by the magnitude of the *p*-value ($p < 0.05 < 0.001$).

3. Results

3.1. Analysis of Maize Biochar

Analysis of pyrolyzed maize biochar contained (g%) dry weight: 92.85, ash: 18.42, total C: 75.16, N: 1.65, P: 5.26, and K: 31.12 with a pH of 9.89 and electrical conductivity of 3.08.

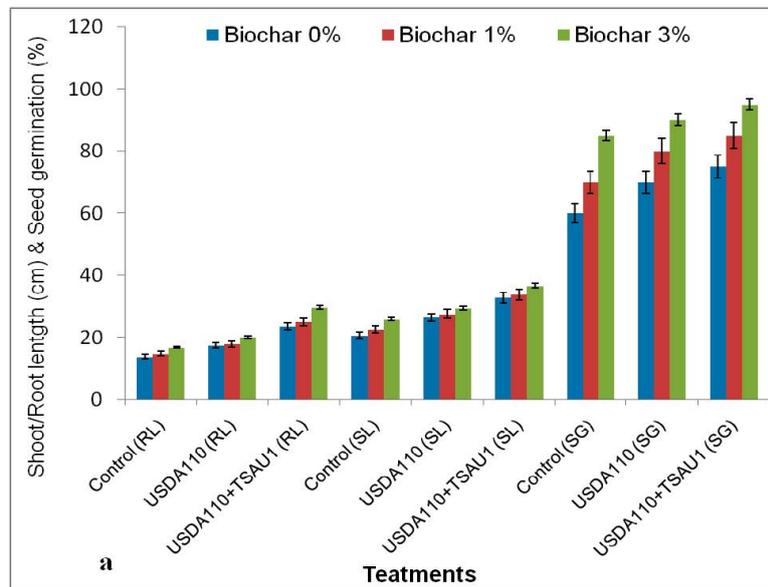
3.2. Screening for the Production of PGP Metabolites

Both the cultures under study produced a wide variety of PGP traits. *B. japonicum* USDA 110 and *P. putida* TSAU1 produced 22 and 16 $\mu\text{g mL}^{-1}$ of IAA, 79 and 87% siderophore, and 0.89 and 1.02 g mL^{-1} phosphate solubilization, respectively.

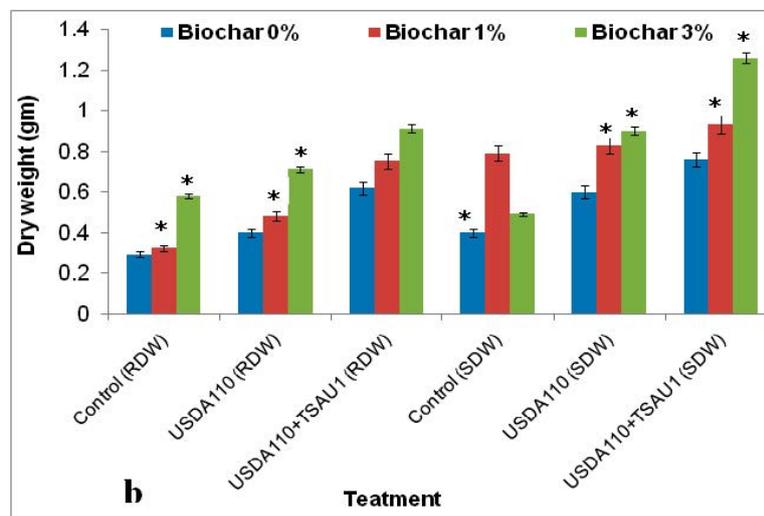
3.3. Measurement of Plant Growth Parameters and Plant Nutrients

The effect of rhizobacteria and biochar levels indicated a significant improvement in the seed germination rate and growth of the soybean plant treated with biochar and rhizobacteria over the control plant (without biochar treatment). The addition of different levels of biochar, inoculation of *B. japonicum* USDA 110, and *P. putida* strain TSAU 1 with biochar and without biochar showed variable increases in the growth parameters. Addition of 3% biochar alone enhanced the seed germination by 15%, root length by 20% (Figure 1a), shoot length by 41% (Figure 1a), root dry weight by 22% (Figure 1b), and shoot dry weight by 13% (Figure 1b), as compared to the control plant (without biochar). Individual addition of *B. japonicum* USDA 110 and *P. putida* strains TSAU 1 with varying levels of biochar (1–3%) and without biochar also promoted the growth of the plant. However, a co-inoculation

with *B. japonicum* USDA 110 and *P. putida* strains TSAU 1 with 3% biochar resulted in significant increases in seed germination and plant growth attributes. Increases in seed germination by 20%, root length by 76% (Figure 1a), shoot length by 41% (Figure 1a), root dry weight by 56% (Figure 1b), shoot dry weight by 59% (Figure 1b), and number of nodules per plant by 57% (Figure 1c) were recorded over the control plant treated with 3% biochar alone.

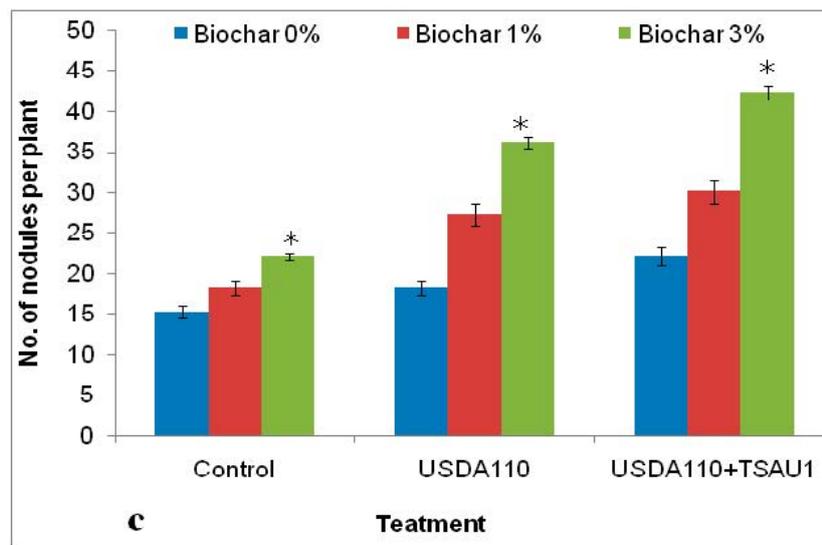


RL= Root length SL= Shoot length, SG= Seed germination *Significant at P (0.01)



RDW = Root dry weight, SDW = Shoot dry weight, *Significant at P (0.01)

Figure 1. Cont.



*Significant at P (0.01)

Figure 1. Effect of rhizobacteria and biochar concentrations on (a) root length [cm] and shoot length [cm], (b) dry weight of the root [g] and dry weight of the shoot [g], and (c) number of nodules. Plant growth parameters were measured after 30 days of growth of plant growth under greenhouse conditions.* = values significant at p 0.01.

Analysis of nutrients in a soybean plant (before sowing and after harvesting) revealed that treatments with 1 and 3% biochar improved the content of total N, P, K, Mg, Na, and Ca in the plant. The inoculation of *B. japonicum* USDA 110 alone (0% biochar) increased N content by 36%, P content by 8.3%, K content by 5.6%, Mg content by 4.8%, Na content by 30%, and Ca content by 2.88%. However, the co-inoculation of *B. japonicum* USDA 110 and *P.putida* TSAU1 with 3% biochar showed a significant improvement in N content by 62.85%, P content by 7.42, K content by 76.85%, Mg content by 5.14%, Na content by 20%, and Ca content by 28%, as compared to the control (without biochar) (Table 1).

Table 1. Effect of rhizobacteria and biochar levels on plant nutrients.

Biochar Application	Treatments	N (%)	P (%)	K (%)	Mg (%)	Na (%)	Ca (%)
0%	Control	1.75 + 0.01	0.24 + 0.01	1.40 + 0.04	0.39 + 0.10	0.02 + 0.00	0.82 + 0.03
	TSAU1	2.00 + 0.02 *	0.25 + 0.04	1.41 + 0.02	0.43 + 0.02	0.06 + 0.01 *	0.91 + 0.03
	USDA 110	2.39 + 0.02 *	0.26 + 0.04	1.49 + 0.02	0.47 + 0.02	0.08 + 0.01 *	1.07 + 0.03
	USDA110+TSAU1	2.60 + 0.02 *	0.27 + 0.02	2.09 + 0.15 *	0.62 + 0.01 *	0.09 + 0.01 *	1.17 + 0.01 *
1%	Control	1.77 + 0.02	0.27 + 0.03	2.33 + 0.02	0.66 + 0.02	0.03 + 0.01	0.95 + 0.03
	USDA 110	2.51 + 0.02 *	0.28 + 0.02	2.52 + 0.04	0.52 + 0.02	0.07 + 0.01 *	1.25 + 0.03
	USDA+TSAU 1	2.64 + 0.02 *	0.32 + 0.02	2.33 + 0.03	0.68 + 0.02	0.13 + 0.04 *	1.21 + 0.02
3%	Control	1.91 + 0.02	0.28 + 0.01	2.41 + 0.02	0.64 + 0.02	0.03 + 0.03	1.09 + 0.02
	USDA 110	2.27 + 0.01	0.37 + 0.01	3.64 * + 0.01	0.48 + 0.01	0.02 + 0.01	0.99 + 0.01
	USDA+TSAU1	2.85 * + 0.01	0.35 + 0.01 *	3.72 * + 0.01	0.39 + 0.01	0.03 + 0.01	1.04 + 0.01

Values are the average of three replicates \pm values are standard deviations. Plant nutrient contents were measured after 30 days of growth of plant under greenhouse conditions. * = values significant at p 0.01.

3.4. Estimation of Soil Nutrient Content and Soil Enzymes

Analysis of soil nutrient content revealed that the inoculation of soybean with *B. japonicum* USDA 110 alone (3% biochar) increased N content by 73%, P content by 173%, and K content by 17%, as compared to the control of 3% biochar. *B. japonicum* USDA 110 alone (3% biochar) significantly enhanced the N content by 98% and K content by 117%, as compared to the control without biochar (Table 2).

Table 2. Effect of rhizobacteria and biochar levels on soil nutrients.

Biochar Application	Treatments	SOC (%)	Total N (%)	P (mg)	K (mg)
0%	Control	21.09 ± 0.01	0.080 ± 0.01	4.29 ± 0.03	2.95 ± 0.02
	TSAU1	23.06 ± 0.01	0.082 ± 0.01	4.43 ± 0.03	3.05 ± 0.02
	USDA 110	27.08 ± 0.01	0.083 ± 0.01	4.60 ± 0.02 *	3.27 ± 0.03 *
	USDA+TSAU1	29.04 ± 0.02 *	0.094 ± 0.8 *	4.88 ± 0.02 *	5.58 ± 0.03 *
1%	Control	25.09 ± 0.01	0.091 ± 0.01	4.22 ± 0.03	4.83 ± 0.02
	USDA 110	29.06 ± 0.01	0.101 ± 0.02 *	6.14 ± 0.01 *	5.44 ± 0.01 *
	USDA+TSAU1	32.07 ± 0.8 *	0.164 ± 0.03 *	16.67 ± 0.05 *	5.68 ± 0.02 *
3%	Control	25.09 ± 0.01	0.094 ± 0.01	6.02 ± 0.01	5.35 ± 0.03
	USDA 110	33.05 ± 0.01	0.163 ± 0.01 *	16.47 ± 0.01 *	6.30 ± 0.01 *
	USDA+TSAU1	41.08 ± 0.01 *	0.170 ± 0.01 *	18.33 ± 0.01 *	8.49 ± 0.01 *

Values are the average of three replicates. ± values are standard deviations. * = values significant at $p < 0.01$. Soil nutrient contents were measured after 30 days of growth of plant under greenhouse conditions.

The lowest level of these elements was evident in the soil without biochar treatment. The highest values of SOC, N, P, and K were observed in soil amended with 3% biochar and co-inoculation with *B. japonicum* USDA 110 and *P. putida* TSAU1 vis-à-vis the lowest value found in soil with *B. japonicum* USDA 110 and *P. putida* TSAU1 alone or in combination but without biochar and soil with no bioinoculants and no biochar treatments (Table 2).

Co-inoculation of soybean with of *B. japonicum* USDA 110 and *P. Putida* TSAU 1 strains enhanced nutrient contents of soil compared to all other treatments. The combination with *B. japonicum* USDA 110 and *P. putida* TSAU 1 (3% biochar) significantly increased N content by 80%, P content by 204%, and K content by 58% compared to the control of 3% biochar. When co-inoculated with *B. japonicum* USDA 110 and *P. putida* TSAU 1 (3% biochar) the N content rose by 11% and K content by 35% compared to variants inoculated with *B. japonicum* USDA 110 alone.

The addition of biochar to soil increased the activity of soil protease and acid and alkaline phosphomonoesterase. Substantial increases of 25.05%, 21.02%, and 23.02% in the activities of protease and acid and alkaline phosphomonoesterase, respectively, were evident due to the co-inoculation of *B. japonicum* USDA 110 and *P. Putida* TSAU1 (0% biochar). A combination of this treatment with 1% biochar further improved the activities of these enzymes. However, the activities of these enzymes were significantly improved due to the co-inoculation of *B. japonicum* USDA 110 and *P. Putida* TSAU 1 with 3% biochar. 2-fold, 1.52-fold, and 1.25-fold increases in the activities of protease and acid and alkaline phosphomonoesterase, respectively, were evident due to co-inoculation with two bioinoculants and 3% biochar (Table 3).

Table 3. Effect of rhizobacteria and biochar levels on soil enzymes.

Biochar Application	Treatments	Protease Activity ($\mu\text{g NH}_4^+\text{-N g}^{-1}\text{h}^{-1}$)	Acid Phosphomonoesterase Activity ($\mu\text{g pNPG}^{-1}\text{h}^{-1}$)	Alkaline Phosphomonoesterase Activity ($\mu\text{g pNPG}^{-1}\text{r}^{-1}$)
0%	Control	19.2 \pm 0.05	650.3 \pm 30.1	300.1 \pm 16.3
	TSUA1	20.1 \pm 0.05	697.1 \pm 20.1	317.1 \pm 12.3
	USDA 110	23.5 \pm 0.10	703.3 \pm 34.5	365.6 \pm 18.1
	USDA+TSAU 1	25.8 \pm 0.19 *	780.6 \pm 38.8 *	380.2 \pm 20.4 *
1%	Control	21.4 \pm 0.07	766.3 \pm 35.7	370.5 \pm 19.5
	USDA 110	25.8 \pm 0.20 *	820.9 \pm 45.3 *	425.3 \pm 21.6 *
	USDA+TSAU 1	27.7 \pm 0.18 *	940.6 \pm 43.2 *	482.2 \pm 20.8 *
3%	Control	24.3 \pm 0.09	810.3 \pm 37.6	420.6 \pm 19.5
	USDA 110	28.5 \pm 0.11 *	911.8 \pm 46.3 *	483.5 \pm 21.2 *
	USDA+TSAU 1	30.8 \pm 0.15 *	1020.4 \pm 48.6 *	535.7 \pm 25.2 *

Values are the average of three replicates. \pm values are standard deviations. * = values significant at p 0.01. Soil enzyme levels were measured after 30 days of inoculation of PGPR and biochar.

4. Discussion

4.1. Screening for the Production of PGP Metabolites

PGPR is known to produce a wide variety of plant-beneficial metabolites that help in plant nutrition and the overall vigor of the plant [39–42]. Production of IAA, siderophore, and P solubilization have been reported in various species of *Bradyrhizobium*, including *B. japonicum* [42–45] and *P. putida* [46,47]. Sayyed et al. [48] reported the production of siderophores from *P. fluorescence* NCIM5096 isolated from the groundnut field rhizosphere. Shaikh et al. [49] reported the production of siderophore from *P. aeruginosa* isolated from the banana field rhizosphere. Pandya et al. [50] reported the production of siderophore and phytohormones, such as IAA and gibberellins in *Pseudomonas* sp., *Rhizobium* sp., and *Azotobacter* sp. isolated from the sugarcane field rhizosphere. They observed higher yields of phytohormones in *Pseudomonas* sp., as compared to the other isolates. Wani et al. [40] reported the production of siderophore in soil bacterium *P. aeruginosa* RZS9. They claimed a further increase in siderophore yield following the optimization of the process by a statistical approach. Jabborova et al. [14] reported the production of siderophore, IAA, and enzymes, such as protease, cellulase, lipase, P solubilization, and antifungal activity in nine endophytic PGPR strains. Sayyed et al. [51] reported the production of copious amounts of siderophore in *P. fluorescence* NCIM 5096 and *P. putida* NCIM2847.

4.2. Measurement of Plant Growth Parameters and Plant Nutrients

An increase in seed germination is due to the phytohormone production, while plant growth promotion during the symbiotic association is due to the nitrogen and other nutrients supplied by the bacterial symbiont. Sayyed et al. [48] reported plant growth-promoting effects of siderophore-producing *P. fluorescence* NCIM5096 in wheat and groundnut. Wani et al. [40] reported the plant growth-promoting effects and antifungal-activities production of siderophore-producing *P. aeruginosa*. Pandya et al. [50] reported that the inoculation of siderophore and phytohormone-producing *Pseudomonas* sp., *Rhizobium* sp., and *Azotobacter* sp. promoted growth in wheat. Jabborova et al. [14] found that inoculation of siderophore, IAA, and enzymes producing P-solubilizing endophytic PGPR strains promoted the growth of medicinal plants. Sayyed et al. [13] observed growth promotion in wheat due to the inoculation of siderophore-producing *P. fluorescence* NCIM 5096 and *P. putida* NCIM2847.

Masciarelli et al. [45] reported a significant increase in the number of root nodules in soybean due to inoculation with *B. japonicum*. Egamberdieva et al. [23] reported the synergistic effect of co-inoculation of *B. japonicum* and *P. putida* to be more effective in increasing nodulation in soybean. Several researchers reported that biochar increased plant growth, nodule number, and yield in different crops [3,5,47]. Pandit et al. [7] claimed that the application of 3% biochar promoted the growth of maize. Uzoma et al. [52] recorded a significant increase in the productivity of biocharized maize, as compared to a control under sandy soil conditions. Increased growth, more nodulation, and improved yield of soybean after the application of biochar were also reported by Iijima et al. [53].

The addition of organically rich biochar and inoculation with PGPR plays a vital role in increasing the soil microbial activity that provides more nutrition to the plant [54]. Egamberdieva et al. [55] reported significant ($p < 0.05$) increases in N, P, K, and Mg contents in chickpea plants treated with *Mesorhizobium ciceri* and biochar. It has been reported that the biochar amendment improves the water-holding capacity of soil [56], which increases the availability of minerals and nutrients [55]. Shen et al. [57] reported the positive effect of biochar amendment on the plant uptake of plant nutrients. Prendergast et al. [58] claimed that the addition of biochar can induce changes in nutrient availability and may provide additional N, P, K, Mg, Na, Ca. Shen et al. [57] observed an increase in P uptake in plants due to the application of biochar. Egamberdieva et al. [55] observed a significant increase in K content in chickpea roots and shoots treated with *M. ciceri* and biochar. Wang et al. [59] observed similar results and claimed an increasing level of K and Mg uptake in soybean due to the addition of bamboo biochar. Ma et al. [60] reported a positive effect of co-inoculation of *B. japonicum* and biochar on N and other nutrient contents in soybean root and shoot biomass. An increase in N content may be

due to the positive impact of biochar on the nodule number that contributes more N to the shoot and root biomass.

4.3. Estimation of Soil Nutrients and Soil Enzymes

Since biochar is an organically rich amendment, its addition is expected to increase the level of soil nutrients. Egamberdieva et al. [55] reported a two-fold rise in SOC, N, P, K, and Mg concentrations in soil amended with biochar, and a three-fold increase in these nutrients in the soil treated with biochar and inoculation with *M. ciceri*. Similar results were reported by Wang et al. [61]. An increase in the soil's organic carbon and other nutrients can also be correlated with increased mineralization due to increased enzyme activity. A linear relationship between soil nutrients and the activities of soil enzymes involved in mineralization has been proposed by Ouyang et al. [62]. Fall et al. [63] reported significant ($p < 0.05$) increases in SOC, available N, soluble P, and total nitrogen upon the application of biochar at a higher rate (12 t ha^{-1}). They also recorded an increase in rice rhizospheric carboxylate secretions. Głodowska et al. [6] suggested a combination of biochar and *B. japonicum* strain 532 C, which significantly increased the number of nodules and the growth of soybean. The combination with biochar and *B. japonicum* resulted in enhanced nodulation, nodule biomass, and shoot biomass of soybean [63]. Numerous studies have shown that biochar application increases the nutrient contents of plants and soil and improves soil fertility [7,62–64]. Egamberdieva et al. [55] found that inoculation of *B. japonicum* USDA 110 halophilic *P. putida* TSAU1 promoted growth, protein content, nitrogen, and phosphorus uptake and improved the root-system architecture of soybean. Their results indicated that the synergistic effect of co-inoculation of these two strains significantly improved plant growth, nitrogen, phosphorus contents, and contents of soluble leaf proteins as compared with the inoculation with *B. japonicum* USDA 110 alone or the control.

Masciarelli et al. [45] found that co-inoculation of soybean plants with *B. AmyloliQUEfaciens* subsp. Plantarum and *B. japonicum* showed significant improvement in plant growth parameters and nodulation. They found that inoculation of *B. amylioliQUEfaciens* subsp. Plantarum with *B. japonicum* enhanced the ability of *B. japonicum* to colonize host plant roots and increase the number of nodules. Phosphomonoesterase (E.C. 3.1.3.2) in the soil is either of plant-root or microbial origin. It plays a major role in P solubilization in soils and in making P available to plants [40]. Acid phosphomonoesterase is dominant in acidic soil, while alkaline phosphomonoesterase occurs in the alkaline soil. The presence of these enzymes and their level in the soil is directly related to the extent of P solubilization and, hence, the amount of soluble P in the soil. Non-nitrogen fixers, such as *Pseudomonas* sp. assimilate nitrogen through the decomposition of protein–nitrogen to low molecular nitrogenous compounds and increase the soil nitrogen and, thus, soil fertility. Extracellular proteases enter the soil via microbial production.

Co-inoculation of *B. japonicum* and *P. putida* along with the application of biochar has been reported to enhance the activities of a wide variety of enzymes in soil [60]. The increase in activities of soil enzyme may be due to increased microbial activity as a result of the addition of consortium of organisms and the addition of biochar that contains good amounts of carbon, nitrogen, and minerals to support cell proliferation and, therefore, enzyme activities [60]. Egamberdieva et al. [55] demonstrated a 2-fold increase in protease and a 40% increase in acid phosphomonoesterase activity due to the addition of biochar. The positive effect on the activities of the soil enzymes can be attributed to the stimulating effect of biochar on microbial activity [63]. The enhancement in the soil enzyme activities due to rhizobial inoculation was also observed by Fall et al. [63]. Ouyang et al. [62] reported that the addition of biochar increases the activities of soil enzymes and attributed this increased enzyme activity to the availability of nutrients and increased microbial activities brought by the addition of biochar to the soil. Egamberdieva et al. [55] and Ma et al. [60] also reported the positive effect of increasing the level of biochar on protease activity. Oladele [64] reported a significant ($p < 0.05$) increase in soil enzymes, such as invertase, alkaline phosphatase, urease, and catalase as a result of the higher application of biochar. It has been reported that with the amendment of more biochar, more soil proteins adhere to the surfaces of biochar pores, make the protein (substrate) unavailable in the soil, and cause a decrease

in protease activity [22]. However, we report increased protease activity with an increase in the biochar amendment to the soil.

5. Conclusions

The application of biochar positively affects the growth and nodulation of soybean by increasing nutrient contents, such as N, P, and K in soil. Inoculation with *B. japonicum* USDA 110 alone increased the number of nodules, the length and dry weight of roots, and the length of shoots of soybean, as compared to the control. *B. japonicum* enhanced the total N content, P content, and K content of the soil, as compared to controls with biochar and without biochar, respectively. Co-inoculation with *B. japonicum* USDA 110 and *P. putida* TSAU 1 significantly increased the growth of soybean, nutrient contents in soybean and soil, and activities of soil protease and acid and alkaline monophosphoesterase, as compared to the control. However, the combined application of *B. japonicum* USDA 110 and *P. putida* TSAU 1 and biochar (3%) showed pronounced positive effects on growth and vigor of soybean, nutrient levels in plant biomass and soil, and activities of soil enzymes. Thus, the co-inoculation with rhizobia and application of biochar offers the best eco-friendly and chemical-free strategy for the sustainable increase in the yield and replenishment of nutrients in soybean and soil and increase in soil biochemical properties. In general, consortia of PGPR and biochar application improves plant growth, contents of plant and soil nutrients, and soil enzyme activities, which influence soil nutrient retention, nutrient availability, and improve crop growth. The present study demonstrates that application of *B. japonicum* alone has the capacity to improve soybean growth, nutrient contents, and improve soil biochemical properties, however, the co-inoculation of this symbiont along with *P. putida* has a more positive effect on plant growth and soil biochemicals, and co-inoculation of these rhizobia in combination with biochar possesses the capacity to significantly improve the growth and nutrient contents in soybean as well as nutrients and enzyme activities in soil. However, to claim the bio-efficacy potential of the co-inoculation of rhizobacteria and application of biochar needs multiple field studies over the season and in different agro-climatic zones.

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References

1. Sarfraz, R.; Hussain, A.; Sabir, A.; Fekih, I.B.; Ditta, A.; Xing, S. Role of biochar and plant growth promoting rhizobacteria to enhance soil carbon sequestration—A review. *Environ. Monit. Assess.* **2019**, *191*, 251. [[CrossRef](#)] [[PubMed](#)]
2. Zhang, A.; Bian, R.; Pan, G.; Cui, L.; Hussain, Q.; Li, L.; Zheng, J.; Zheng, J.; Zhang, X.; Han, X. Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: A field study of 2 consecutive rice growing cycles. *Field Crops Res.* **2012**, *127*, 153–160. [[CrossRef](#)]
3. Asai, H.; Samson, B.K.; Stephan, H.M.; Songyikhangsuthor, K.; Homma, K.; Kiyono, Y.; Inoue, Y.; Shiraiwa, T.; Horie, T. Biochar amendment techniques for upland rice production in Northern Laos: 1. Soil physical properties, leaf SPAD and grain yield. *Field Crops Res.* **2009**, *111*, 81–84. [[CrossRef](#)]

4. Saxena, J.; Rana, G.; Pandey, M. Impact of addition of biochar along with *Bacillus* sp. on growth and yield of French beans. *Sci. Hortic.* **2013**, *162*, 351–356. [[CrossRef](#)]
5. Agboola, K.; Moses, S. Effect of biochar and cowdung on nodulation, growth and yield of soybean (*Glycine max* L. Merrill). *Int. J. Agric. Biosci.* **2015**, *4*, 154–160.
6. Głodowska, M.; Schwinghamer, T.; Husk, B.; Smith, D. Biochar based inoculants improve soybean growth and nodulation. *Agric. Sci.* **2017**, *8*, 1048–1064. [[CrossRef](#)]
7. Pandit, N.; Mulder, J.; Hale, S.; Martinsen, V.; Schmidt, H.; Cornelissen, G. Biochar improves maize growth by alleviation of nutrient stress in a moderately acidic low-input Nepalese soil. *Sci. Total Environ.* **2018**, *625*, 1380–1389. [[CrossRef](#)]
8. Chen, H.; Ma, J.; Wei, J.; Gong, X.; Yu, X.; Guo, H.; Zhao, Y. Biochar increases plant growth and alters microbial communities via regulating the moisture and temperature of green roof substrates. *Sci. Total Environ.* **2018**, *635*, 333–342. [[CrossRef](#)]
9. Laird, D. The charcoal vision: A win–win–win scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water quality. *Agron. J.* **2008**, *100*, 178–181. [[CrossRef](#)]
10. Hossain, M.; Strezov, V.; Chan, K.; Nelson, P. Agronomic properties of wastewater sludge biochar and bioavailability of metals in production of cherry tomato (*Lycopersicon esculentum*). *Chemosphere* **2010**, *78*, 1167–1171. [[CrossRef](#)]
11. Zhang, H.; Prithviraj, B.; Charles, T.; Driscoll, B.; Smith, D. Low-temperature tolerant *Bradyrhizobium japonicum* strains allowing improved nodulation and nitrogen fixation of soybean in a short season (cool spring) area. *Eur. J. Agron.* **2003**, *19*, 205–213. [[CrossRef](#)]
12. Egamberdieva, D.; Jabborova, D.; Wirth, S. Alleviation of salt stress in legumes by co-inoculation with *Pseudomonas* and *Rhizobium*. In *Plant-Microbe Symbiosis: Fundamentals and Advances*; Springer: Singapore, 2013; pp. 291–303. [[CrossRef](#)]
13. Sayyed, R.; Gangurde, N.; Patel, P.; Josh, S.; Chincholkar, S. Siderophore production by *Alcaligenes faecalis* and its application for growth promotion in *Arachis hypogaea*. *Indian J. Biotechnol.* **2010**, *9*, 302–307.
14. Jabborova, D.; Annapurna, K.; Fayzullaeva, M.; Sulaymonov, K.; Kadirova, D.; Jabbarov, Z.; Sayyed, R. Isolation and characterization of endophytic bacteria from ginger (*Zingiberofficinale* Rosc.). *Ann. Phytomed.* **2020**, *9*, 116–121. [[CrossRef](#)]
15. Buckley, S.; Allen, D.; Brackin, R.; Jämtgård, S.; Näsholm, T.; Schmidt, S. Microdialysis as an in situ technique for sampling soil enzymes. *Soil Biol. Biochem.* **2019**, *135*, 20–27. [[CrossRef](#)]
16. Sayyed, R.; Patel, P.; Shaikh, S. Plant growth promotion and root colonization by EPS producing *Enterobacter* sp. RZS5 under heavy metal contaminated soil. *Indian J. Exp. Biol.* **2015**, *53*, 116–123.
17. Sagar, A.; Riyazuddin, R.; Shukla, P.; Ramteke, P.; Sayyed, R. Heavy metal stress tolerance in *Enterobacter* sp. PR14 is mediated by plasmid. *Indian J. Exp. Biol.* **2020**, *58*, 115–121.
18. Elkoca, E.; Kantar, F.; Sahin, F. Influence of nitrogen-fixing and phosphorus solubilizing bacteria on the nodulation, plant growth, and yield of chickpea. *J. Plant Nutr.* **2007**, *31*, 157–171. [[CrossRef](#)]
19. Jabborova, D.; Davranov, K. Effect of phosphorus and nitrogen concentrations on root colonization of soybean (*Glycine max* L.) by *Bradyrhizobium japonicum* and *Pseudomonas putida*. *Int. J. Adv. Biotechnol. Res.* **2015**, *6*, 418–424.
20. Egamberdieva, D.; Jabborova, D.; Berg, G. Synergistic interactions between *Bradyrhizobium japonicum* and the endophyte *Stenotrophomonas rhizophila* and their effects on growth, and nodulation of soybean under salt stress. *Plant Soil* **2016**, *405*, 35–45. [[CrossRef](#)]
21. Egamberdieva, D.; Wirth, S.; Jabborova, D.; Räsänen, L.; Liao, H. Coordination between *Bradyrhizobium* and *Pseudomonas* alleviates salt stress in soybean through altering root system architecture. *J. Plant Interact.* **2017**, *12*, 100–107. [[CrossRef](#)]
22. Jabborova, D.; Enakiev, Y.; Davranov, K.; Begmatov, S. Effect of co-inoculation with *Bradyrhizobium japonicum* and *Pseudomonas putida* on root morph-architecture traits, nodulation and growth of soybean in response to phosphorus supply under hydroponic conditions. *Bulg. J. Agric. Sci.* **2018**, *24*, 1004–1011.
23. Egamberdieva, D.; Jabborova, D.; Wirth, S.; Alam, P.; Alyemeni, M.; Ahmad, P. Interaction of magnesium with nitrogen and phosphorus modulates symbiotic performance of soybean with *Bradyrhizobium japonicum* and its root architecture. *Front. Microbiol.* **2018**, *9*, 1.
24. Holik, L.; Vranová, V. Proteolytic activity in meadow soil after the Application of phytohormones. *Biomolecules* **2019**, *9*, 507. [[CrossRef](#)] [[PubMed](#)]

25. Reibe, K.; Götz, K.; Roß, C.; Döring, T.; Ellmer, F.; Ruess, L. Impact of quality and quantity of biochar and hydrochar on soil Collembola and growth of spring wheat. *Soil Biol. Biochem.* **2015**, *83*, 84–87. [[CrossRef](#)]
26. Nautiyal, C. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol. Lett.* **1999**, *170*, 265–270. [[CrossRef](#)] [[PubMed](#)]
27. Bric, J.; Bostock, R.; Silverstone, S. Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. *Appl. Environ. Microbiol.* **1991**, *57*, 535–538. [[CrossRef](#)]
28. Patel, P.; Shaikh, S.; Sayyed, R. Modified chrome azurol S method for detection and estimation of siderophores having affinity for metal ions other than iron. *Environ. Sustain.* **2018**, *1*, 81–87. [[CrossRef](#)]
29. Payne, S. Detection, isolation, and characterization of siderophores. In *Methods in Enzymol*; Elsevier: Amsterdam, The Netherlands, 1994; pp. 329–344. [[CrossRef](#)]
30. Penrose, D.; Glick, B. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol. Plant.* **2003**, *118*, 10–15. [[CrossRef](#)]
31. Labconco, C. *A Guide to Kjeldahl Nitrogen Determination Methods and Apparatus*; Labconco Corporation: Houston, TX, USA, 1998.
32. Upadhyay, A.; Sahu, R. Determination of total nitrogen in soil and plant. In *Laboratory Manual on Advances in Agro-Technologies for Improving Soil, Plant and Atmosphere Systems*; CAFT: Jabalpur, India, 2012; pp. 18–19.
33. Upadhyay, A.; Sahu, R. Determination of potassium in soil and plant. In *Laboratory Manual on Advances in Agro-Technologies for Improving Soil, Plant and Atmosphere Systems*; CAFT: Jabalpur, India, 2012; pp. 23–35.
34. Sahrawat, K. Determination of calcium, magnesium, zinc and manganese in plant tissue using a dilute HCl extraction method. *Commun. Soil Sci. Plant Anal.* **1987**, *18*, 947–962. [[CrossRef](#)]
35. Sims, J. Soil test phosphorus: Principles and methods. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals and Waters. Southern Coop. Ser. Bull.* **2009**, *408*, 9–19.
36. Nelson, D.; Sommers, L. Total carbon, organic carbon, and organic matter. *Methods Soil Anal. Part 2 Chem. Microbiol. Prop.* **1983**, *9*, 539–579. [[CrossRef](#)]
37. Tabatabai, M.; Bremner, J. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* **1969**, *1*, 301–307. [[CrossRef](#)]
38. Ladd, J.; Butler, J. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. *Soil Biol. Biochem.* **1972**, *4*, 19–30. [[CrossRef](#)]
39. Patel, P.; Shaikh, S.; Sayyed, R. Dynamism of PGPR in bioremediation and plant growth promotion in heavy metal contaminated soil. *Indian J. Exp. Biol.* **2016**, *54*, 286–290.
40. Wani, S.; Shaikh, S.; Sayyed, R. Statistical-based optimization and scale-up of siderophore production process on laboratory bioreactor. *3Biotech* **2016**, *6*, 69.
41. Saxena, B.; Rani, A.; Sayyed, R.; El-Enshasy, H.A. Analysis of Nutrients, Heavy Metals and Microbial Content In Organic and Non-Organic Agriculture Fields of Bareilly Region-Western Uttar Pradesh, India. *Biosci. Biotechnol. Res. Asia* **2020**, *17*, 399–406. [[CrossRef](#)]
42. Sagar, A.; Sayyed, R.; Ramteke, P.; Sharma, S.; Najat Marraiki Elgorban, A.; Syed, A. ACC deaminase and antioxidant enzymes producing halophilic *Enterobacter* sp. PR14 promotes the growth of rice and millets under salinity stress. *Plant Physiol. Mol. Biol.* **2020**. [[CrossRef](#)]
43. Seneviratne, M.; Gunaratne, S.; Bandara, T.; Weerasundara, L.; Rajakaruna, N.; Seneviratne, G.; Vithanage, M. Plant growth promotion by *Bradyrhizobium japonicum* under heavy metal stress. *S. Afr. J. Bot.* **2016**, *105*, 19–24. [[CrossRef](#)]
44. Mubarik, N.; Mahagiani, I.; Wahyudi, A. Production of IAA by *Bradyrhizobium* sp. *World Acad. Sci. Eng. Technol.* **2013**, 152–155. [[CrossRef](#)]
45. Masciarelli, O.; Llanes, A.; Luna, V. A new PGPR co-inoculated with *Bradyrhizobium japonicum* enhances soybean nodulation. *Microbiol. Res.* **2014**, *169*, 609–615. [[CrossRef](#)]
46. Meliani, A.; Bensoltane, A.; Benidire, L.; Oufdou, K. Plant growth-promotion and IAA secretion with *Pseudomonas fluorescens* and *Pseudomonas putida*. *Res. Rev. J. Bot. Sci.* **2017**, *6*, 16–24.
47. Genesisio, L.; Miglietta, F.; Baronti, S.; Vaccari, F.P. Biochar increases vineyard productivity without affecting grape quality: Results from a four years field experiment in Tuscany. *Agric. Ecosyst. Environ.* **2015**, *201*, 20–25. [[CrossRef](#)]
48. Sayyed, R.; Naphade, B.; Joshi, S.; Gangurde, N.; Bhamare, H.; Chincholkar, S. Consortium of *A. faecalis* and *P. fluorescens* promoted the growth of *Arachis hypogea* (Groundnut). *Asian J. Microbiol. Biotechnol. Environ. Sci.* **2009**, *48*, 83–86.

49. Shaikh, S.; Patel, P.; Patel, S.; Nikam, S.; Rane, T.; Sayyed, R. Production of biocontrol traits by banana field fluorescent *Pseudomonads* and comparison with chemical fungicide. *Indian J. Exp. Biol.* **2014**, *52*, 917–920. [[PubMed](#)]
50. Pandya, N.; Desai, P.; Sayyed, R. Antifungal, and phytohormone production ability of plant growth-promoting rhizobacteria associated with the rhizosphere of sugarcane. *World J. Microbiol. Biotechnol.* **2011**, *13*, 112–116.
51. Sayyed, R.; Badgajar, M.; Sonawane, H.; Mhaske, M.; Chincholkar, S. Production of microbial iron chelators (siderophores) by fluorescent *Pseudomonads*. *Indian J. Biotechnol.* **2005**, *4*, 484–490.
52. Uzoma, K.; Inoue, M.; Andry, H.; Fujimaki, H.; Zahoor, A.; Nishihara, E. Effect of cow manure biochar on maize productivity under sandy soil condition. *Soil Use Manag.* **2011**, *27*, 205–212. [[CrossRef](#)]
53. Iijima, M.; Yamane, K.; Izumi, Y.; Daimon, H.; Motonaga, T. Continuous application of biochar inoculated with root nodule bacteria to subsoil enhances yield of soybean by the nodulation control using crack fertilization technique. *Plant Prod. Sci.* **2015**, *18*, 197–208. [[CrossRef](#)]
54. Lehmann, J.; Rillig, M.C.; Thies, J.; Masiello, C.A.; Hockaday, W.C.; Crowley, D. Biochar effects on soil biota—a review. *Soil Biol. Biochem.* **2011**, *43*, 1812–1836. [[CrossRef](#)]
55. Egamberdieva, D.; Li, L.; Ma, H.; Wirth, S.; Bellingrath-Kimura, S. Soil amendment with different maize biochars improves chickpea growth under different moisture levels by improving symbiotic performance with *Mesorhizobium ciceri* and soil biochemical properties to varying degrees. *Front. Microbiol.* **2019**, *10*, 2423. [[CrossRef](#)]
56. Bruun, E.; Petersen, C.; Hansen, E.; Holm, J.; Hauggaard-Nielsen, H. Biochar amendment to coarse sandy subsoil improves root growth and increases water retention. *Soil Use Manag.* **2014**, *30*, 109–118. [[CrossRef](#)]
57. Shen, Q.; Hedley, M.; Camps Arbestain, M.; Kirschbaum, M. Can biochar increase the bioavailability of phosphorus? *J. Soil Sci. Plant Nutr.* **2016**, *16*, 268–286. [[CrossRef](#)]
58. Prendergast-Miller, M.T.; Duvall, M.; Sohi, S. Localisation of nitrate in the rhizosphere of biochar-amended soils. *Soil Biol. Biochem.* **2011**, *43*, 2243–2246. [[CrossRef](#)]
59. Wang, C.; Alidoust, D.; Yang, X.; Isoda, A. Effects of bamboo biochar on soybean root nodulation in multi-elements contaminated soils. *Ecotoxicol. Environ. Saf.* **2018**, *150*, 62–69. [[CrossRef](#)] [[PubMed](#)]
60. Ma, H.; Egamberdieva, D.; Wirth, S.; Bellingrath-Kimura, S.D. Effect of biochar and irrigation on soybean-*Rhizobium* symbiotic performance and soil enzymatic activity in field rhizosphere. *Agronomy* **2019**, *9*, 626. [[CrossRef](#)]
61. Wang, Y.; Yin, R.; Liu, R. Characterization of biochar from fast pyrolysis and its effect on chemical properties of the tea garden soil. *J. Anal. Appl. Pyrolysis* **2014**, *110*, 375–381. [[CrossRef](#)]
62. Ouyang, L.; Tang, Q.; Yu, L.; Zhang, R. Effects of amendment of different biochars on soil enzyme activities related to carbon mineralisation. *Soil Res.* **2014**, *52*, 706–716. [[CrossRef](#)]
63. Fall, D.; Bakhoun, N.; NourouSall, S.; Zoubeirou, A.; Sylla, S.; Diouf, D. Rhizobial inoculation increases soil microbial functioning and gum arabic production of 13-year-old *senegaliasenegal* (L.) britton, trees in the north part of Senegal. *Front. Plant Sci.* **2016**, *7*, 1355. [[CrossRef](#)]
64. Oladele, S. Effect of biochar amendment on soil enzymatic activities, carboxylate secretions and upland rice performance in a sandy clay loam Alfisol of Southwest Nigeria. *Sci. Afr.* **2019**, *4*, e00107. [[CrossRef](#)]

