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Biochar Additions Alter the Abundance of P-Cycling-Related Bacteria in the Rhizosphere Soil of *Portulaca oleracea* L. under Salt Stress

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Abstract: Numerous reports confirm a positive impact of biochar amendments on soil enzyme activities, nutrient cycles, and, finally, plant growth and development. However, reports explaining the process behind such diverse observations are scarce. The aim of the present study was (1) to evaluate the effect of biochar on the growth of purslane (*Portulaca oleracea* L.) and nutrients; (2) to determine the response of rhizosphere enzyme activities linked to soil phosphorus cycling after biochar amendment under non–saline and saline soil conditions. Furthermore, we investigate whether adding biochar to soil alters the abundance of P-cycling-related bacteria. Two rates of biochar (2% and 4%) were applied in pot experiments. Biochar addition of 2% significantly increased plant growth under non-saline and saline soil conditions by 21% and 40%, respectively. Moreover, applying biochar increased soil microbial activity as observed by fluorescein diacetate (FDA) hydrolase activity, as well as phosphomonoesterase activities, and the numbers of colony-forming units (CFU) of P-mobilizing bacteria. Soil amended with 2% biochar concentration increased total soil nitrogen (Nt), phosphorus (P), and total carbon (Ct) concentrations by 18%, 15%, and 90% under non-saline soil conditions and by 29%, 16%, and 90% in saline soil compared the control, respectively. The soil FDA hydrolytic activity and phosphatase strongly correlate with soil Ct, Nt, and P contents. The rhizosphere soil collected after biochar amendment showed a higher abundance of tricalcium phosphate-solubilizing bacteria than the control soil without biochar. Overall, this study demonstrated that 2% maize-derived biochar positively affects halophyte plant growth and thus could be considered for potential use in the reclamation of degraded saline soil.

Keywords: biochar; soil salinity; phosphate solubilization; bacteria; soil enzymes

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1. Introduction

Soil salinization is one of the most severe problems leading to land degradation resulting from inadequate irrigation and/or fertilization systems [1–3]. Rising sea levels due to climate change also increase the salinity of soils used for the cultivation of agricultural crops [4]. It is estimated that about 33% of irrigated agricultural land worldwide is affected by salinity, which causes significant yield losses and threatens food security [5]. Moreover, soil salinity alters the biogeochemical cycling of carbon, nitrogen, and phosphorus and leads to severe impacts on soil fertility in the long term [6]. As a further consequence, salinity exerts detrimental effects on plant physiological processes and alters soil microbial community composition and functional diversity [7].

The restoration of degraded land becomes the most critical point because soil salinity will likely increase in response to threats of global climate change. Soil is considered saline if the electrical conductivity of its saturation extract (ECe) is above 4 dS m^{-1} [8]. Urgent measurements are required to remediate degraded soils and restore vegetation. Halophytes are plants adapted to salt stress and have the ability to accumulate Na and Cl from the soil. Thus, these plants have been used for the remediation of saline soils, especially in arid and semi-arid regions, and to improve land for possible crop cultivation [9]. Several reports show halophytes are used to remove salts in an intercropping system [10]. Purslane (*Portulaca oleracea* L.), as a member of the Portulacaceae family, is a fast-growing herbaceous annual with succulent leaves and stems, tolerant to salt stress and drought, and is widely used for bioremediation of saline and arid lands [11,12]. Purslane used in an intercropping system with tomato reduced Na and Cl concentrations in tomato tissues and increased nutrient uptake and fruit yield [13]. However, even though halophytes accumulate salt and lower the salt concentration in soil, the high salt concentration lowers their biomass yield [14]. In addition, several sustainable practices are used to improve and restore salt-affected lands, including organic amendments such as sewage sludge, cattle dung, and vermicompost [15,16]. Recent findings observed an increased growth of halophytes, Sesbania (*Sesbania cannabina*) and Seashore mallow (*Kosteletzkya virginica*), improved soil nutrient contents, and soil biological activity in biochar-amended coastal soil [17].

Biochar is considered to enhance soil fertility and crop productivity and might be a valuable approach to remediating salt-affected lands [18,19]. Many studies confirmed the positive impact of biochar on soil cation exchange capacity [20], water holding capacity [21], nutrient retention [22–25], and organic matter contents [26]. Biochar application to soil was also reported to improve plant growth, nutrient acquisition, development, various physiological properties, and even tolerance to salt stress [27–29]. Such positive effects of biochar application were explained mainly by enhanced soil nutrient concentrations, especially enhanced plant-available N and P concentrations, increasing nutrient acquisition and soil biological activity [30,31]. The plants grown in soil amended with biochar support a higher rate of microorganisms with plant beneficial traits, stimulating plant development and improving nutrient uptake [29,32]. The additional input of organic carbon and nutrients increases microbial abundance, diversity, and activities involved in nitrogen, potassium, phosphorus, and carbon cycling in soil [33,34]. Biochar, is a carbon-rich material that also provides favorable conditions for the proliferation of root-associated microbes and thus increases the activities of soil enzymes such as urease, invertase, protease, and phosphatase [35–37]. However, there are considerable uncertainties regarding the effects of biochar on microbiological activities in the rhizosphere of salt-affected soils and the impacts on plant nutrient availability that need to be elucidated [38]. Soil phosphorus (P) is a critical element for plant growth, and soil salinity is known to reduce the availability of inorganic P by sorption processes and to suppress P uptake by plant roots [39]. We hypothesized that biochar addition in saline soils enhances soil enzyme activities related to P-cycling associated with an increase in microbial activities involved in P mineralization. Certain groups of soil microorganisms play a key role in increasing the contents of bioavailable P through solubilizing and mineralizing inorganic or organic P that plants can easily assimilate [40,41]. The aim of the present study was (1) to evaluate the effect of biochar at two different application rates (2% and 4%) on plant growth and soil nutrient contents and (2) to determine the response of rhizosphere soil enzyme activities linked to phosphorus cycling depending on biochar amendment under non–saline and saline soil conditions. Furthermore, we investigate whether the addition of biochar to soil alters the abundance of P-cycling-related bacteria.

2. Materials and Methods

2.1. Soil, Biochar, and Plant Seeds

The soil samples from 0 to 20 cm depth were taken from the experimental field station of the Leibniz Centre for Agricultural Landscape Research, Müncheberg, Germany. The

soil consisted of clay and fine silt (7%), coarse and medium silt (19%), and sand (74%) and was characterized by the following properties: 0.6% carbon, 0.07% nitrogen, 0.03% phosphorus, 1.25% potassium, and 0.18% magnesium. The pH was 6.2, and the EC was 0.3 dS/m [27]. The biochar was produced from maize by heating at 600 °C for 30 min (MBC) and contained 75.2% carbon, 1.6% nitrogen, 5.26% phosphorous, 31.2% potassium, and the pH was 8.9 [42]. The biochar was obtained from the Leibniz-Institute for Agricultural Engineering Potsdam-Bornim (ATB), (ATB), Germany. The seeds of purslane (*Portulaca oleracea* L.) were obtained from the University of Lille, France.

2.2. Plant Growth Experiments

The seeds of Purslane were sterilized with 70% ethanol and 10% *v*/*v* NaOCl for 5 min and washed in sterile water several times. The seeds were germinated in Petri plates (15 seeds per plate) on filter paper soaked with sterile water and placed in a dark room at 25 °C for 5–6 days.

The sieved soil was mixed with crushed char (particle size < 3 mm) at 2% and 4% (*w*/*v*) concentrations. Germinated seeds were sown in pots (two plants per pot) filled with 1000 g of soil. The treatments were as follows: (a) plants grown in soil without biochar, (b) plants grown in soil amended with 2% biochar, and (c) plants grown in soil amended with 4% biochar concentrations. We considered two soil conditions: non-salinated and salinated. Plants were irrigated with tap water containing 150 mM NaCl three times a week to obtain saline conditions. The control treatment was irrigated with tap water without NaCl. During plant growth, the electrical conductivity of non-saline soil (EC) without biochar or amended with biochar ranged between 0.5 and 0.9 dS/m and in saline soil between 10 and 15 dS/m (UMP-2 BT+ sensor, UGT GmbH, Müncheberg, Germany).

Pot experiments with four replications were designed in a randomized complete block design. Plants were grown for 35 days in a growth chamber at 26 °C/16 °C (day/night) and 50–60% humidity. The pots were carefully sampled at harvest, and soil and roots were removed. Roots were shaken gently, and soil adhering to the roots (rhizosphere soil) was collected. Whole plants, including shoot and root, were dried in a fan oven at 60 °C for 72 h, and the dry biomass of the plant was recorded.

2.3. Soil Enzyme Activities

The fluorescein diacetate (FDA) hydrolysis was determined following the method of Green et al. [43]. Briefly, 0.5 mg of soil was added to a 50 mL vial, with the subsequent addition of 25 mL of sodium phosphate (0.06 M; pH of 7.6). Then, 0.25 mL of 4.9 mM FDA substrate solution was added to all vials. The tightly capped vials were mixed and incubated in a water bath at 37 °C for 1 h. A 1 mL soil suspension was centrifuged at 8000 rpm for 5 min. The clear supernatant was measured at 490 nm against a blank reagent solution in a spectrophotometer. Controls were used according to the procedure described for the assay, but 0.25 mL of acetone was added instead of the FDA substrate solution. The concentration of fluorescein released was calculated using a standard curve with 0, 0.001, 0.005, 0.05, and 0.15 mg of fluorescein.

The method described by Tabatabai and Bremner [44] was used to determine acid (ACP) and alkaline (AKP) phosphomonoesterase activities in soil. Briefly, 0.5 g of moist soil was placed in a 15 mL vial, and 2 mL of MUB buffer (pH of 6.5 for the assay of ACP or pH of 11 for the assay of AKP) and 0.5 mL of p-nitrophenyl phosphate substrate solution (0.05 M) were added. The soil suspension was incubated in a water bath at 37 °C with 300 rpm shaking after the vial was capped. After one hour of incubation, the vial was removed from the water bath, and 2 mL of NaOH (0.5 M), 0.5 mL of CaCl2 (0.5 M), and 5 mL of distilled water were added to stop the reaction. One milliliter of soil suspension was centrifuged at 6500 rpm for 5 min. The produced p-nitrophenol (p-NP) in the assays was calculated from a p-NP calibration curve at 400 nm wavelength using a Lambda 2 UV-VIS spectrophotometer (Perkin Elmer) [45].

2.4. Soil Nutrient Contents

The soil's total nitrogen (Nt) and carbon (Ct) contents were determined by the dry combustion method using a CNS elemental analyzer (TruSpec, Leco Corp., St. Joseph, MI, USA). The soil phosphorus (P) content was analyzed by ICP-OES (iCAP 6300 Duo) via Mehlich-3 extraction.

2.5. Isolation of Root-Associated Bacteria

Three individual plants grown in non-saline and saline soil amended with 2% and 4% biochar were taken for evaluation of root-associated microbes with phosphate-solubilization activity. The rhizosphere-associated soil (1 g) was collected and mixed with 9 mL of sterile phosphate-buffered saline and shaken for 10 min using a Biosan B-1 Vortex. Then, 100 µL aliquots from dilutions (10^1–10^5) were spread on Pikovskaya [46] agar media containing tricalcium phosphate in three replications. After four days of incubation at 25 °C, colony-forming units (CFU) were recorded. The colonies that had formed a clear halo that showed activity in the solubilization of phosphate were counted. Colonies with a different color, shape, surface, or consistency were transferred to agar plates. Visually similar colonies in sizes, shapes, and colors were checked under a microscope for purity and subsequently used for DNA isolation.

2.6. Identification of Bacterial Isolates

The bacterial DNA was extracted employing a heat treatment method [47]. The supernatant was collected, and the presence of DNA was detected using horizontal gel electrophoresis. The amount of DNA was checked with the use of NanoDrop™ One (Thermo Fisher Scientific, Waltham, MA, USA). The 16S rRNA gene of the extracted DNA samples was analyzed using polymerase chain reaction (PCR) with the following primers: 27F (5′-GAGTTTGATCCTGGCTCAG-3′) (Sigma-Aldrich, St. Louis, MO, USA) and 1492R (5′-GAAAGGAGGTGATCCAGCC-3′) (Sigma-Aldrich) [48] with the use of a PTC-200 thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The PCR amplicons of the 16S rRNA gene were subjected to RFLP analysis for the determination of differences between similar isolates, according to Jinneman et al. [49]. The digested PCR amplicons fragments were subjected to gel electrophoresis and visualized with a digital gel imaging system (Gel-Doc XR TM+, Bio-Rad) to detect and eliminate identical isolates before sequencing.

The sequencing of the 16S rRNA gene was conducted with the use of ABI PRISM BigDye 3.1 Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) according to the manufacturer's protocol. The sequences were corrected with Chromas software (v. 2.6.5), merged using EMBOSS Explorer (http://emboss.bioinformatics.nl/ (accessed on 1 May 2022)), and compared with sequences from GenBank of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/ (accessed on 1 May 2022)). ClustalX 2.1 software was used for multiplying alignment of all sequences, based on which the phylogenetic tree was constructed. The Neighbor-Joining method was applied to infer the evolutionary history [50]. The branch length sum was 0.96928586. The bootstrap test (500 replicates) was used to check the frequency when the associated taxa clustered together in replicate trees and indicated next to the branches as a percentage [51]. The evolutionary distances were computed using the Maximum Composite Likelihood method [52]. Thirty-five nucleotide sequences were involved in the analysis. The final dataset contained 1278 positions. Finally, MEGA6 was used for evolutionary analyses [53], and the sequences of the 16S rRNA genes were registered in GenBank under the accession numbers MT825595–MT825614.

2.7. Statistical Analysis

The data were processed using the package "dplyr" in R 4.0.2 (R Studio, Boston, MA, USA). A two-way analysis of variance (ANOVA), interactions between factors, and Duncan's multiple comparisons of the means were conducted by the package "agricolae" and "HH". The plant biomass, activities of soil FDA hydrolase, alkaline phosphomonoesterase,

and acid phosphomonoesterase, as well as concentrations of soil Ct, Nt, and P, were analyzed by the independent factors' salinity (saline and non-saline) and biochar (0, 2%, and 4%). Figures were plotted using the package "ggplot2", and the plot panels were aligned together using the package "ggpubr".

3. Results

3.1. Plant Growth

The response of purslane growth to the biochar applications at both 2% and 4% concentrations was different under non-saline and saline soil conditions. The soil salinity increased plant growth in soil without biochar amendment (Figure 1). Biochar addition to the soil at 2% significantly increased plant growth under saline conditions by 40%. There were significant effects of biochar on the growth of purslane at 4% concentration, being 10% and 12% higher compared to plants grown in soil without biochar amendment. The interactions of biochar × salinity on the plant dry weight were significant ($p < 0.001$, Table 1).

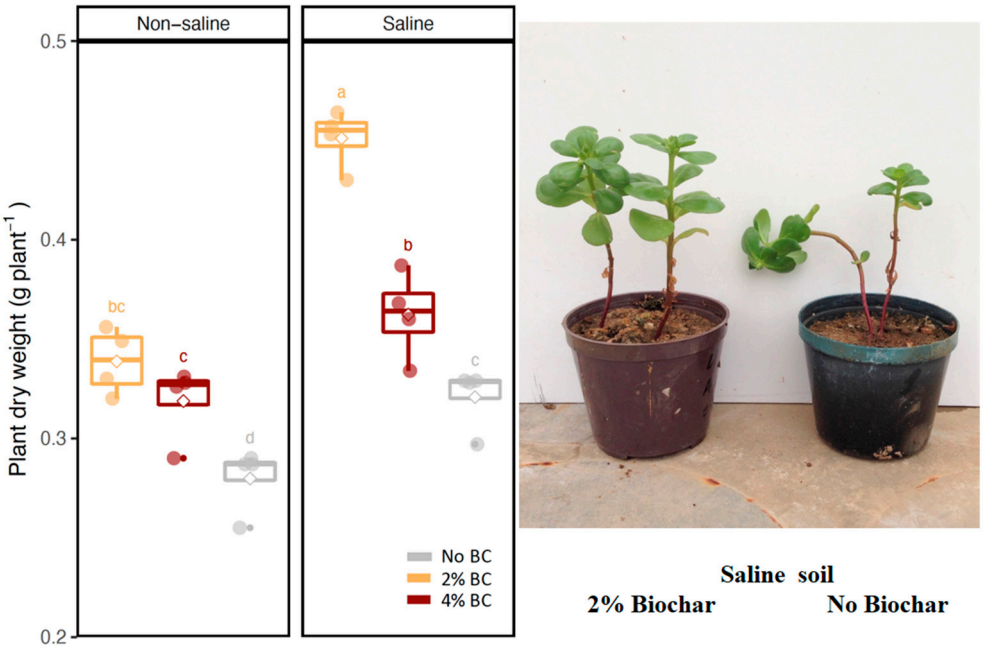

Figure 1. Effect of biochar application (2% BC, 4% BC) on dry weights of purslane biomass growing under saline or non-saline conditions. BC: biochar. Letters within each column are significantly different based on Duncan's test. The top and bottom of the box represent 75% and 25% quantiles, respectively. The bars of the box represent maximum and minimum values of observations. The line in the box represents the median. The dots represent every single observation. The rhombus in the box represents the mean.

Table 1. Main effect and two-way interactions of biochar and salinity on plant dry weight, soil enzyme activities, and soil nutrient concentrations. Soil fluorescein diacetate (FDA) hydrolase; soil alkaline phosphomonoesterase (AKP); soil acid phosphomonoesterase (ACP). Significance denoted by * $p < 0.05$, and ** $p < 0.001$.

	Plant Dry Weight	Soil FDA	Soil AKP	Soil ACP	Soil C	Soil N	Soil P
Biochar	**	**	ns	**	**	**	**
Salinity	**	*	*	*	**	**	**
Biochar × Salinity	**	ns	ns	*	ns	ns	*

3.2. Soil Enzyme Activities and Soil Nutrient Contents

Generally, salinity slightly inhibited FDA hydrolase activity in soil without biochar as well as in soil amended with biochar (Figure 2A), but the inhibition was not significant. The soil FDA hydrolase activity was significantly increased by both 2% and 4% biochar addition under non-saline and saline conditions ($p < 0.001$, Figure 2A). Salinity did not significantly affect soil alkaline phosphomonoesterase activity (Figure 2B). There was a significant difference in soil alkaline phosphomonoesterase activity between 2% biochar treatment and the control under saline conditions. Soil amendment with 2% biochar enhanced acid phosphomonoesterase activity under both non-saline and saline conditions, while saline soil decreased acid phosphomonoesterase activity compared to non-saline soil (Figure 2C). Soil amended with 4% biochar increased acid phosphomonoesterase under non-saline conditions compared to soil without biochar, and no difference was observed under saline soil.

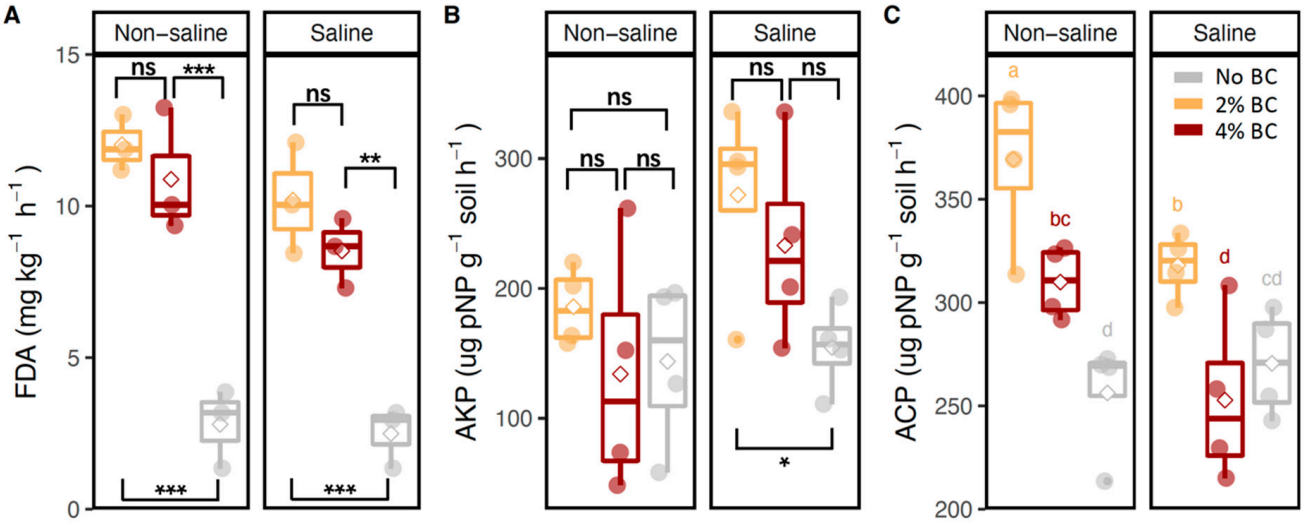

Figure 2. Effect of biochar application (2% and 4%) on soil FDA hydrolase (**A**), alkaline phosphomonoesterase, AKP (**B**), and acid phosphomonoesterase, ACP (**C**) activities in purslane planting systems under saline and non-saline conditions. The top and bottom of the box represent 75% and 25% quantiles, respectively. The bars of the box represent maximum and minimum values of observations. The line in the box represents the median. The dots represent every single observation. The rhombus in the box represents the mean. Codes (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; ns—not significant) in (**A**,**B**) indicates the significance of each paired comparison for the biochar factor. Letters within each column in (**C**) are significantly different based on Duncan's test.

The interaction of biochar × salinity on the soil ACP activity was significant ($p < 0.05$, Table 1), while soil FDA hydrolase and AKP activities indicated no interaction between biochar and salinity.

The soil Ct concentrations were significantly affected by salinity ($p < 0.05$, Figure 3A). Soil salinity decreased Ct, Nt, and P concentrations by 12%, 18%, and 8% (Figure 3A–C), respectively. Soil amended with 2% biochar concentration increased soil Ct, Nt, and P concentrations by 67%, 19%, and 16% under non-saline soil and by 99%, 30%, and 14% under saline soil compared to control soil, respectively; 4% biochar application increased the soil Ct, Nt, and P concentrations by 42%, 10%, and 5% under non-saline soil and by 60%, 23%, and 12% under saline soil compared to control soil, respectively. Interactions between biochar, salinity, and soil P concentration were observed ($p < 0.05$), but not with soil Ct and Nt concentrations (Table 1).

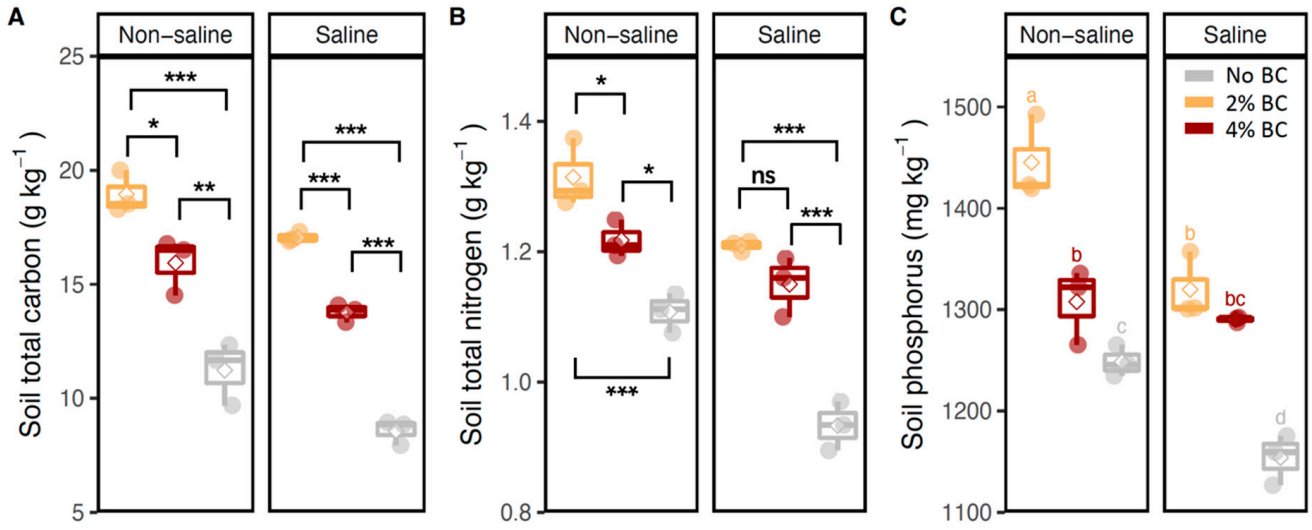

Figure 3. Effect of biochar application (2%, 4%) on soil total carbon (**A**), total nitrogen (**B**), and phosphorus (**C**) concentration in purslane planting systems under saline and non-saline conditions. The top and bottom of the box represent 75% and 25% quantiles, respectively. The bars of the box represent maximum and minimum values of observations. The line in the box represents the median. The dots represent every single observation. The rhombus in the box represents the mean. Codes (*: $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; ns—not significant) in (**A**,**B**) indicates the significance of each paired comparison for the biochar factor. Letters within each column in (**C**) are significantly different based on Duncan's test.

3.3. Abundance of Phosphate-Solubilising Bacteria

The rhizosphere soil collected from plants grown in soil without and with 2% and 4% biochar amendment were analyzed for cultivable bacterial cells with phosphate-solubilization activity. The results showed that the CFU of tricalcium phosphate-solubilizing bacteria was significantly higher in soil amended with 2% and 4% biochar amendment (5.1 ± 0.2 and 4.9 ± 0.05 Log CFU/g soil, respectively) compared to the control soil (4.0 ± 0.09 Log CFU/g soil) under non-saline conditions. The salinity slightly decreased the abundance of phosphate-solubilizing bacteria in all treatments. Soil amended with 2% and 4% biochar significantly increased the CFU of bacteria, being 4.7 ± 0.2 and 4.4 ± 0.3 (Log CFU/g soil, respectively) compared to control soil (3.9 ± 0.12 Log CFU/g soil).

3.4. Isolation and Identification of Rhizosphere Bacteria

Among all treatments, the number of CFU of bacteria was highest in soil amended with 2% biochar as compared to the other treatments. Bacterial isolates were identified to know the diversity of phosphate-solubilizing species. A total of 20 culturable bacteria were collected from the rhizosphere soil of *Portulaca oleracea* L. grown in soil amended with 2% biochar and finally left as different species after RFLP analysis. The 16S rRNA gene of the strains was analyzed and compared with related strains of bacteria registered in GenBank of NCBI, and the results are presented in Table 2.

Table 2. Sequence similarities of rhizosphere bacteria isolated from roots of *Portulaca oleracea* L. with the closest sequences registered in GenBank.

Isolated Strains Deposited to GenBank			Closest Match (16S Ribosomal RNA Genes) (GenBank)		
Strain	**Length (bp)**	**Accession Number**	**Reference Strains**	**Accession Number**	**Percent Identity**
Port 1	1439	MT825595	*Variovorax boronicumulans*	NR_041588.1	99.65
Port 2	1439	MT825596	*Pseudomonas baetica*	NR_116899.1	99.44
Port 3	1441	MT825597	*Pseudomonas helmanticensis*	NR_126220.1	99.65
Port 4	1438	MT825598	*Pseudomonas silesiensis*	NR_156815.1	99.86
Port 5	1459	MT825599	*[Brevibacterium] frigoritolerans*	NR_115064.1	99.52
Port 6	1456	MT825600	*Bacillus aryabhattai*	NR_115953.1	99.59
Port 7	1453	MT825601	*Bacillus megaterium*	NR_112636.1	99.52
Port 8	1450	MT825602	*Pseudomonas grimontii*	NR_025102.1	99.38
Port 9	1463	MT825603	*Rahnella aquatilis*	NR_025337.1	98.91
Port 10	1456	MT825604	*Pseudomonas kilonensis*	NR_028929.1	99.66
Port 11	1439	MT825605	*Pseudomonas frederiksbergensis*	NR_117177.1	99.79
Port 12	1427	MT825606	*Stenotrophomonas rhizophila*	NR_121739.1	99.65
Port 13	1424	MT825607	*Streptomyces mediolani*	NR_112465.1	99.79
Port 14	1458	MT825608	*Staphylococcus warneri*	NR_025922.1	99.38
Port 15	1399	MT825609	*Rothia endophytica*	NR_109752.1	99.50
Port 16	1439	MT825610	*Pseudomonas azotoformans*	NR_113600.1	99.65
Port 17	1446	MT825611	*Pseudomonas lini*	NR_029042.2	99.38
Port 18	1450	MT825612	*Pseudomonas reinekei*	NR_042541.1	99.10
Port 19	1457	MT825613	*Pseudomonas moraviensis*	NR_043314.1	99.11
Port 20	1446	MT825614	*Pseudomonas marginalis*	NR_117821.1	99.51

The isolated bacteria with their closest relatives are presented in a phylogenetic tree (Figure 4). The bacterial isolates are related to three phyla: Proteobacteria, Actinobacteria, and Firmicutes representing four classes: Gammaproteobacteria (13), Betaproteobacteria (1), Bacilli (4), and Actinobacteria (2). The class Gammaproteobacteria included the orders Pseudomonadales, Enterobacterales, and Xanthomonadales. Pseudomonadales was represented by the genus Pseudomonas with 11 strains, and Enterobacterales contained just one strain *Rahnella aquatilis* Port 9 from Yersiniaceae. Xanthomonadales contained one strain, *Stenotrophomonas rhizophila* Port 12, and Betaproteobacteria consisted of a single order Burkholderiales with a single strain *Variovorax boronicumulans* Port 1. The genus Bacillus was represented with one strain [Brevibacterium] *frigoritolerans* Port 5. The class of Actinobacteria presented bacterial strains of the orders Micrococcales and Streptomycetales, *Rothia endophytica* Port 15, and *Streptomyces mediolani* Port 13.

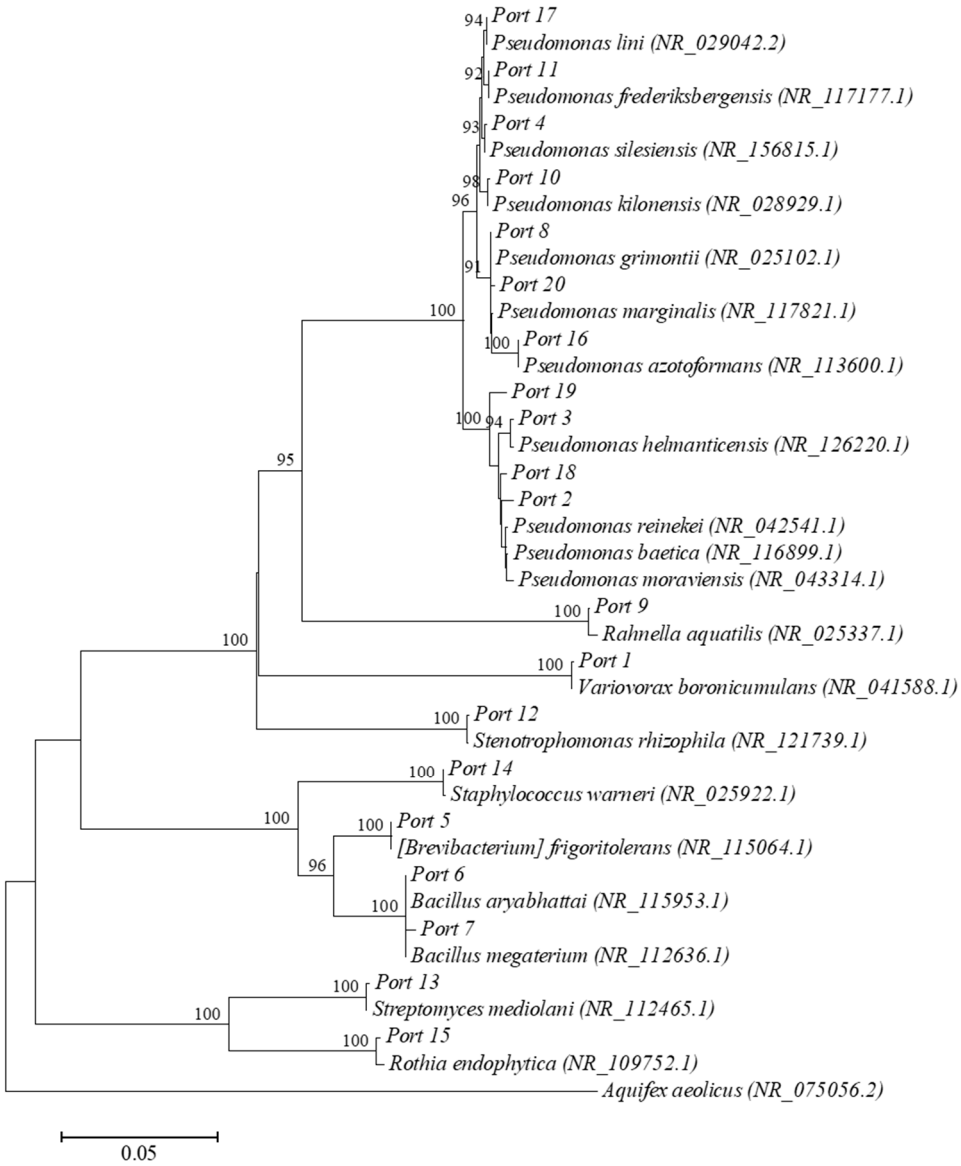

Figure 4. Phylogenetic tree of phosphate-solubilizing bacteria isolated from the rhizosphere soil of *Portulaca oleracea* L. and their closest relatives registered in GenBank (NCBI).

4. Discussion

Positive impacts of biochar amendments on the growth of purslane and soil biological activities in its rhizosphere under both saline and non-saline conditions were observed. The biomass of purslane grown in both soil conditions was higher in soil amended with biochar than plants grown in soil without biochar amendment. Several studies observed improved plant growth and stress tolerance under saline conditions after biochar application [54,55]. For example, Yang et al. [56] reported increased growth, improved plant physiological properties, and a higher quinoa yield by biochar under salt stress than control plants. The halophytes Sesbania (*Sesbania cannabina*) and Seashore mallow (*Kosteletzkya virginica*) showed higher shoot growth and biomass when grown in salinated soil amended with biochar [17]. Such positive effects were explained by enhanced microbial community, diversity, and activity involved in nutrient cycling [17,23]. Another possible cause for plant growth improvement by biochar is the increased availability of essential nutrients for plant nutrition, such as N, P, and K [57].

Soil FDA hydrolase activity indicates overall soil microbial activity [58]. In our study, the highest soil microbial activity was observed by FDA hydrolase activity in soil amended

with biochar compared to the control soil without biochar application. Our results agree with previous findings by Gonçalves Lopes et al. [59], who reported an increased FDA hydrolase activity in soil under sugarcane amended with biochar. Several explanations exist for the improved microbial activity in soil amended with biochar. One mechanism that could be enhanced is microbial activity in biochar-amended soil that provides additional carbon sources, especially for microbes associated with plant roots [60]. Biochar is a carbon-rich material and offers favorable conditions for the proliferation of rhizosphere microbes involved in soil carbon, nitrogen, and phosphorus turnover [17]. The increased enzyme activities in saline-alkaline soils amended with biochar in other studies showed an improved microbial community related to central C- and P-cycling activities [61]. Furthermore, it is well documented that biochar improved root-associated microbial diversity and activity, producing various metabolites such as phytohormones, hydrolytic enzymes, antifungal compounds, and siderophores directly involved in plant growth stimulation and improving stress tolerance [23,62].

In this study, alkaline phosphomonoesterase activity was stimulated in saline soil amended with 4% biochar; however, no significant changes were found under non-saline conditions. A similar finding was reported by Jiang et al. [63], who observed 23% increased phosphatase activity in soil amended with 2% swine-manure biochar compared to the control soil. Phosphorus (P) is vital for early plant growth and development. However, P in soils is often in a form that is not readily available for plant uptake [38]. Phosphatases play a vital role in P turnover, and it is known that root-associated microbes involved in P mineralization increase the availability of P in soil [64].

Furthermore, after biochar addition, we observed a significant increase in soil Ct, Nt, and P concentrations under non-saline and saline soil conditions. Accordingly, He et al. [22] reported a significant increase in soil organic matter and contents of N, P, and K, in saline soil. This is explained by the properties of biochar, such as its highly porous structure, strong ion exchange capacity, and large surface area [65]. In another study, the increased enzyme activities correlated with soil organic carbon contents and with the activities of soil microorganisms [66]. Qian et al. [67] explained a higher concentration of P in biochar-amended soil by an increased pH in the rhizosphere resulting in more free orthophosphates released from biochar through its cation and anion exchange capacities. Zhao et al. [25] observed higher soil nutrient concentrations of Nt, P, K, and Ca, as well as cation exchange capacity and organic matter content in saline-alkali soil layers applying corn straw biochar.

Biochar is rich in P content and can potentially increase its availability in the soil. It affects soil microbial communities, which may indirectly increase soil P availability by solubilizing inorganic P [68,69]. We found an increased number of bacteria contributing to P solubilization in the rhizosphere of biochar-amended soil under non-saline and saline conditions. Our findings were similar to a prior study reporting an increase in the abundance of tri-calcium phosphate-solubilizing bacteria in the rhizosphere of *Lolium perenne* [17], suggesting that biochar amendment enhanced microbial P mobilization. According to previous findings, rhizosphere bacteria with phosphate solubilization activity represent about 20–40% of soil microbiota [70,71]. In our study, isolates were affiliated with different bacterial species, including *Pseudomonas* spp., *Bacillus* spp., *Achromobacter* spp., *Stenotrophomonas* spp., *Saccharomyces* spp., *Azospirillum* spp., *Citrobacter* spp., *Burkholderia* spp., and *Micrococcus* spp. [72–74]. Among the identified bacterial species, *Pseudomonas* was dominant and are considered a dominant group of soil bacteria that play key roles in nutrient cycling, promoting nutrient availability and plant growth [75,76]. A recent study has observed that the bacterial inoculant *Pseudomonas* sp.—UFPI-B5-8A—significantly increased the shoot, root growth, and P acquisition of maize, and P concentration in soil amended with biochar-based phosphate fertilizers [77]. Several other studies also reported an increased P availability in soil and P uptake by plant roots based on the activity of phosphate-solubilizing bacteria in [78,79]. Furthermore, the genus Bacillus is one of the predominant bacterial genera found in rhizosphere soil. Accordingly, we identified *B. aryabhattai* and *B. megaterium* isolated from the rhizosphere of purslane.

5. Conclusions

We can conclude that biochar generally has great potential to improve soil health and productivity, increase crop stress resistance, and rehabilitate degraded agricultural soils. In particular, our results confirm the positive effects of a 2% corn biochar addition on purslane growth, enzyme activities, and rhizosphere N and P concentrations under non-saline and saline soil conditions. To our knowledge, this is the first report on the biochar-mediated promotion of halophyte growth in saline soils with particular regard to rhizosphere microbial activity. Our present study provides a detailed basis regarding phosphorus in biochar-amended saline soils, which can be the starting point for continued research in microbial community composition, especially to reveal the mechanisms in more detail that shape the rhizosphere microbiome, e.g., the metabolites involved. Lastly, the practical use of this study for the reclamation of degraded saline soils comes first from the realization that different rates of biochar addition must be considered. In addition, microbial activities in the rhizosphere must be taken into account, which is particularly critical to N and P dynamics and a key to overcoming limiting nutrient conditions in saline soils.

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