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Effect of Biochar and Microbial Inoculation on P, Fe, and Zn Bioavailability in a Calcareous Soil

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Abstract: To identify effective ways of increasing the yield of crops grown in nutrient-poor calcareous soils, the combined effects of biochar addition and inoculation with plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) on wheat growth and soil properties were investigated under rhizobox conditions. Measured soil properties included pH, electrical conductivity (EC), organic matter content (OM), the availability of P, Fe, and Zn in the rhizosphere, and the uptake of these elements by plants. Combined biochar addition and microbial inoculation were shown to significantly increase the concentration of available forms of P, Fe, and Zn in the soil when compared to non-biochar treatments. The highest soil pH (7.82) was observed following biochar addition without microbial inoculation. The EC following biochar addition and PGPR inoculation was significantly higher than the other treatments, and the soil OM content was highest when combining AMF inoculation with biochar addition. The available P content after AMF inoculation combined with biochar addition was 27.81% higher than the control conditions, and AMF inoculation increased Fe and Zn bioavailability by factors of 2.38 and 1.29, respectively, when combined with biochar addition relative to AMF inoculation alone. The simultaneous biochar addition and PGPR inoculation significantly increased P uptake by the plants. The highest shoot Fe and Zn uptake rates were observed after a simultaneous application of biochar and PGPR inoculation. Under these conditions, shoot uptake was higher than seen when combining biochar addition with AMF inoculation by factors of 1.64 and 1.21, respectively. In general, it can be concluded that combining inoculation with growth-promoting bacteria and biochar addition can effectively improve nutrient availability to plant and soil conditions.

Keywords: Organic matter; pruning waste; nutrient availability; microorganism; rhizobox

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1. Introduction

Soils in the arid and semi-arid regions of Iran are alkaline, deficient in organic matter, and mostly calcareous. Consequently, most plants in these soils suffer from deficiencies of key nutrients, including phosphorous (P), iron (Fe), and zinc (Zn) [1]. These nutrient deficiencies are common in arable lands around the world [2–7] and have negative impacts on many aspects of human health, including growth, reproduction, immune responses, and the development of neural behaviors [8–12]. It has been documented that the ability of plants to acquire sufficient nutrients depends in part on the soil's content of organic matter, the production of root exudates, and the root-microorganism relationship [13–15]. Organic matter incorporated in the soil competes with P at adsorption sites [16,17], and thus improves P availability in the soil. Furthermore, organic matter has been reported to interact with Fe and Al oxides, inhibiting their crystallization in a way that improves P uptake [18]. Moreover, organic matter increases the solubility and reduces the fixation of these elements in the soils by forming sustainable chelates with Fe and Zn. Every year, millions of tons of tree pruning waste are produced in Iran, which could be used to increase the organic matter content of nutrient-poor soils. The conversion of pruning waste into biochar [19] is one of the most important strategies for maintaining the organic carbon budget of soil, increasing Fe and Zn bioavailability, stimulating natural cycling of materials and nutrients, and improving soil quality and health in the agricultural systems. Carbon-enriched biochar, which is formed by the pyrolysis of biomass in the presence of little or no oxygen [20], can store carbon in soils for thousands of years. Biochar has the potential to increase nutrient availability to plants. However, the mechanisms responsible for this increase are largely unknown [21]. Both increases and reductions in nutrient availability have been reported in soils treated with biochar [22]. For example, Lehmann et al. [22] reported higher P bioavailability in biochar-amended soils. Adding biochar to alkaline soils increases P uptake and decreases P availability [23], due to the presence of high concentrations of oxides of alkaline elements (Ca^{2+} and Mg^{2+}) and low levels of soluble Al^{3+} in these soils [24]. Of note, biochar contains a high amount of P and may thus increase P availability in the soil, especially in the short-term, due to the direct P release from the biochar [25]. Atkinson et al. [26] observed that biochar influenced soil P availability and plant P uptake indirectly by changing the habitats of microorganisms. The biochar-induced improvements in the bioavailability of nutrients, such as Fe and Zn in soils and their influence on plant growth and yield may also be partly related to an increase in cation exchange capacity, changes in soil pH, stimulation of soil microorganisms' activity, improvements in soil qualitative traits, and the direct release of nutrients from the biochar [27]. It has been reported that biochar treatment can increase the Fe concentration in beans [28] and the Zn concentration in plants, such as broccoli and spinach [29]. Little is known regarding the response of microorganisms to biochar application [30], but the presence of microorganisms in the rhizosphere is crucial for the nutrient cycle in soil–root systems. The bioavailability of Fe and Zn could be improved by exploiting the potential of P-solubilizing microorganisms. Arbuscular mycorrhizal fungi (AMF) increase the solubility of soil nutrients, especially less mobile elements, such as P, Fe, and Zn that are normally poorly available to plants, through various mechanisms that extend the absorbing area and rate of plant roots by expanding their hyphal network [31]. In addition, plant-growth promoting rhizobacteria (PGPR) enhance plant growth via mechanisms, including the production of metabolites that are important for plant growth, such as plant hormones (auxin, cytokinin, and gibberellin), N fixation in the rhizosphere, increasing the solubility of insoluble or sparingly soluble nutrients, and thereby improving their availability by releasing organic and inorganic acids, producing phosphatases, synthesizing siderophores, and regulating ethylene synthesis in roots [32]. Inoculating plant seeds with PGPR can increase the population of these bacteria in the rhizosphere to an optimal level, resulting in soil quality improvements. Ashford and Cairney [33] stated that the mycorrhizal roots can reduce ferric EDTA, and thereby increase Fe solubility and bioavailability in soils, but non-mycorrhizal roots lack this capability. Biochar enrichment can also influence the relationship between plants and soil microorganisms by adsorbing and deactivating growth inhibitors, increasing nutrient levels, changing nutrient bioavailability, protecting microorganisms directly and physically against predators in biochar pores, and/or changing signaling processes between plants and microbes by changing the dynamics of the microorganisms [34]. In addition to the role of organic matter and microorganisms in enhancing P, Fe, and Zn bioavailability by affecting their complexation and increasing their solubility, the impact of the rhizosphere should not be neglected. The rhizosphere is the volume of soil surrounding the roots, which influence the soil's chemical and biological characteristics in ways that can alter nutrient availability. Plants secrete a range of compounds via their roots, depending on the presence of different kinds of organic matter and microorganisms in the rhizosphere. For example, Hacisalihoglu and Kochian [35] reported that grains could release phytosiderophores from their roots into the rhizosphere soil, thereby increasing Zn and Fe solubility and their availability for uptake by plants. A rhizobox is an instrument that is used to closely examine rhizosphere processes by limiting root growth to a fixed volume of soil. This increases root density and facilitates the sampling of rhizosphere soil [36]. Despite extensive research on the effect of organic matter on the bioavailability of P, Fe, and Zn, we do not yet comprehensively understand the interactions of roots and biochar or their interactions with microbial inoculation and the resulting effects on the bioavailability of key nutrients in calcareous soils, due to the complex relationships between these factors under rhizobox conditions. Due to the strategic importance of wheat in human nutrition and the common deficiency of P, Fe, and Zn in wheat farms, this work investigates the effect of treatment with growth-promoting microorganisms and biochar derived from tree-pruning waste on P, Fe, and Zn bioavailability in the wheat rhizosphere under rhizobox conditions.

2. Materials and Methods

2.1. Soil Sampling

The study was carried out in a research greenhouse using a factorial randomized complete block experimental design with four replicates under rhizobox conditions. Two factors were included in the experimental design: (i) The addition of biochar (B), with two levels (B1.5, corresponding to the addition of pruning waste biochar, and B0, corresponding to not adding biochar), and (ii) microbial inoculation, with three levels (inoculation with AMF, inoculation with PGPR, and non-inoculated control). Soil samples were collected from the surface layer (0–30 cm) of non-arable land in Salmas, West Azerbaijan Province, Iran. The soil was air-dried and passed through a 10 mesh sieve before sterilization in an autoclave at 121 °C and 1.5 atm for 2 h. Some of the soil's physicochemical properties are presented in Table 1 [37].

Table 1. Physicochemical properties of loamy sand soil.

Parameters	Unit	Soil
pH	-	7.53
EC	dS m^{-1}	0.47
OC	%	0.25
$CaCO_3$	%	14.25
N	%	0.08
P	%	7.64
K	%	98
Fe	mg kg^{-1}	1.44
Zn	mg kg^{-1}	0.62

OC: Organic carbon.

2.2. Preparation of Pruning Waste Biochar of Apple and Grape Trees

To prepare the biochar, pruning waste was collected from orchards in Urmia, West Azerbaijan Province, Iran. Then, the waste was oven-dried at 65 °C for 48 h and the dried samples were placed in a reactor (a steel cylinder with a diameter of 7 cm and height of 31 cm) and heated in an electric furnace for biochar production at 350 °C. The heating rate was 12 °C min^{-1} (slow pyrolysis) and the feedstocks were kept in the furnace for 2 h after reaching the final temperature. No ash was observed on the biochar surface, implying that oxygen had been removed and produced correctly. Finally, the biochar was ground and passed through a 35-mesh sieve (Figure 1). The characteristics of the biochar [38] are summarized in Table 2.

Figure 1. Schematic illustration of the preparation of biochar.

Table 2. Characteristics of the pruning waste biochar.

Characteristics	Unit	Pruning Waste Biochar
pH (1:10)	-	7.29
EC	dS m^{-1}	0.08
N	%	0.54
C	%	67.53
C/N	-	125
P (Total)	mg kg^{-1}	2748.07
Fe	mg kg^{-1}	303.45
Zn	mg kg^{-1}	40.88

2.3. *Greenhouse Experiment*

2.3.1. Rhizobox Experiment

Lab-built rhizoboxes with dimensions of 20 × 15 × 20 cm (length× width× height) were used [39]. The internal volume of the boxes was partitioned into two zones with 325-mesh nylon reticulated plates: (i) A rhizosphere zone with a thickness of 2 cm, and (ii) a non-rhizosphere zone with a thickness of 5.8 cm. For the greenhouse experiment, a mixture of biochar (1.5% *w*/*w*) from apple and grape trees was incorporated into the soil. In B0 treatments, sterilized inoculated soil was used. In control treatments without microbial inoculation, sterilized soil containing organic matter was used.

Inoculation was performed using microbial strains, including Pseudomonads fluorescent species (a mixture of *P. aeruginosa*, *P. fluorescens*, and *P. putida*) and mycorrhizal fungi from the genus Glomus (*G. fasciulatum*). To inoculate the seeds with the bacteria, a bacterial suspension (cell density of 10^7 cfu mL^{-1}, 1 mL of suspension per seed) was added to the soil around the seeds concurrent with their sowing. A mycorrhizal inoculum of 70 g was added to each box, uniformly dispersed under the seeds with a 0.5-cm spacing. After inoculation, the wheat seeds (*Triticum aestivum* L. cv. Pishtaz) were disinfected with 0.5% sodium hypochlorite and sown in the rhizosphere zone of the rhizoboxes. After the seeds germinated, they were thinned to four plants. During the growth periods, the plants were irrigated with distilled water and fed with P-free and Fe-free Rorison nutrient solution to satisfy their nutrient requirements [40].

2.3.2. Soil and Plant Analysis

The rhizoboxes were opened after 65 days. After harvesting, the plants' shoots were washed with tap water and then with distilled water. Thereafter, they were oven-dried at 65 °C for 48 h to determine the shoot dry weight (SDW). Following this step, the rhizoboxes were first opened from the non-rhizosphere zone and the non-rhizosphere soil was harvested. To harvest the rhizosphere soil, the plexiglass framework was removed (Figure 2). Then, the soil was removed from the rhizobox and the main and secondary roots were carefully removed from the soil. Finally, the rhizosphere soil was collected. The roots were transferred to the laboratory, where they were oven-dried at 65 °C for 48 h to determine the root dry weight (RDW). Their P content was measured using the vanadate/molybdate method (yellow method) after extraction and dry digestion. Moreover, the Fe and Zn contents were measured by the dry digestion method using an atomic absorption device (Shimadzu-AA330). The pH, electrical conductivity (EC), organic carbon content [37], P [41], and Fe and Zn [42] content of the rhizosphere soil were also determined using the previously reported methods.

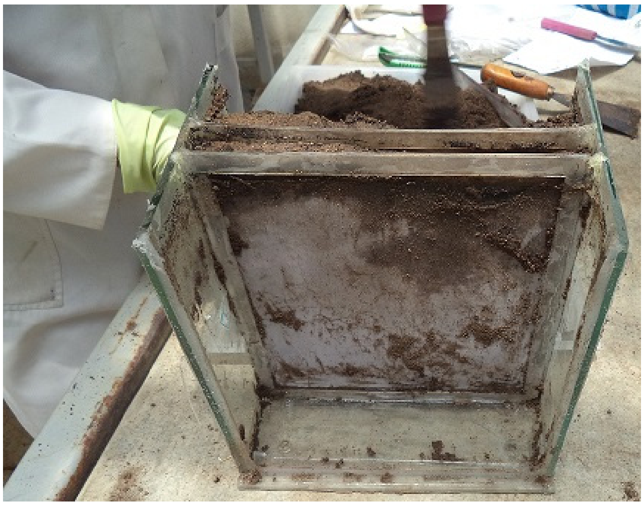

Figure 2. Schematic of rhizosphere soil harvest.

2.3.3. Statistical Analysis

Statistical analyses, including the analysis of variance and means comparison by Duncan's multiple range test with a significance threshold of $p < 0.05$ were performed using the SAS program (version 9.2, SAS Institute Inc., SAS Campus Drive, Cary, NC, USA).

3. Results and Discussion

3.1. Soil pH, EC, and Organic Carbon

The highest and lowest pH values were observed in the control treatment with 1.5% biochar and the PGPR treatment without biochar, respectively (Table 3). The relatively high content of alkaline metals in biochar may explain the higher pH in the biochar treatments [43]. The high responsiveness of biochar in soil is associated with active pH-dependent functional groups, such as OH and COOH that can be deprotonated in the soil, raising its pH [44–46]. A significant increase in soil pH was also observed in soils treated with 1%, 2% or 3% rice straw biochar, but biochar inoculation with phosphate solubilizing bacteria reduced the pH, due to the synthesis of organic exudates resulting from organic matter decomposition [47]. In addition to the role of microbial inoculation in pH decline, wheat root exudation may increase the soil's organic carbon content, leading to increased soil microbial activity and a reduced rhizosphere pH, due to the release of protons (H^+), and/or organic acids in the rhizobox experiment [48,49].

Table 3. Interaction effects of biochar amendment and microbial inoculation on pH, EC, organic matter content, and levels of P, Fe, and Zn in rhizosphere soil.

	AMF		PGPR		Control	
	B1.5	**B0**	**B1.5**	**B0**	**B1.5**	**B0**
pH	7.68 b	7.42 c	7.65 b	7.32 d	7.82 a	7.64 b
EC (dS m^{-2})	0.53 b	0.49 b c	0.65 a	0.47 c	0.53 b	0.36 d
SOM (%)	1.81 a	1.67 d e	1.65 b	0.76 d	1.14 c	0.59 e
P_{Olsen} (mg kg^{-1})	27.81 a	10.91 d	24.58 a	9.60 d	14.02 c	6.13 e
Fe (mg kg^{-1})	4.27 a	2.52 c	3.38 b	2.73 c	1.97 d	1.38 e
Zn (mg kg^{-1})	1.31 a	1.11 c	1.32 a	1.17 b	1.01 d	0.93 e

Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test at the $p < 0.05$ level. SOM: Soil organic matter. B1.5 and B0 are biochar 1.5% and no biochar, respectively.

The soil EC was highest under the PGPR+B1.5 treatment (Table 3), for which the EC was 1.37 times higher than the soil not treated with biochar (PGPR+B0). Overall, microbial inoculation increased EC in all of the treatments. However, there were no significant differences between the biochar-treated mycorrhizal-inoculated soils and the soils not treated with biochar or the uninoculated biochar-treated rhizosphere soils. However, in control treatments, the biochar application increased the EC of the rhizosphere soil 1.47-fold relative to the B0 treatment. Microorganisms can increase the water uptake capacity by increasing root hydraulic conductivity and releasing hydraulic compounds leading to osmotic adjustment. This can in turn improve the plant growth and dilute dissolved ions [50,51]. PGPRs increase the EC of the rhizosphere soil solution by reducing soil pH as a result of higher proton activity. The application of organic fertilizers increases the concentration of ions in soil solutions, and thus raises the rhizosphere EC. EC was also reported to increase with the pyrolysis temperature of added biochar, due to the separation and accumulation of alkaline salts at higher temperatures [52]. Soumare et al. [53] reported that a salinity of 3–4 dS m^{-1} represented a threshold of tolerability for moderately sensitive plants. In all of the cases, the EC values determined in this work were below this range.

A comparison of means (Table 3) indicated that the soil organic matter content after AMF inoculation and biochar addition was 8.38% higher than after AMF inoculation alone. A point of interest in Table 3 is that biochar increased the organic content of the rhizosphere soil relative to the control, and that microbial inoculation strengthened this effect. Jordan et al. [54] showed that when organic matter was incorporated in the soil, mycorrhiza consumed some of the organic matter to improve nutrient availability, while the remaining improved soil texture via interactions with hyphae, thereby contributing to the storage of organic matter in the soil. Mycorrhizal glycoproteins, such as glomalin are also involved in carbon storage, which may partly explain the increase in soil organic matter in AMF treatments. PGPRs are known to be highly active organic particles in soil, with active charged surfaces and the ability to synthesize many organic compounds, carbohydrates, and enzymes [55]. It seems that the active carbon components of biochar decompose after application to the soil. In addition, some of the biochar's carbon content is added to the soil's carbon reserve, increasing its organic matter content. Biochar decomposes relatively slowly due to its high initial C/N ratio (Table 2), and thus causes a gradual addition of organic matter to the soil. Accordingly, Ippolito et al. [56] found that applying biochar increased the organic carbon content of the soil since biochar consists mainly of carbon. In addition, root exudates, such as phenolic compounds stimulate microbial growth in the rhizosphere soil and can enhance soil organic matter and nutrients after decomposing in the soil. The high content of organic compounds in the rhizosphere promotes microbial growth since these compounds serve as a source of energy for microorganisms. This process is known as the rhizosphere effect and is estimated to increase the population of microorganisms by a factor of 5–50 depending on the soil and plant characteristics [57].

3.2. P, Fe, and Zn Bioavailability in Soil

The combined treatment with biochar and PGPR significantly increased the bioavailability of P ($p < 0.001$), Fe ($p < 0.01$), and Zn ($p < 0.01$) in the soil when compared to the B0 and control treatment (Table 3). A larger increase in nutrient availability was seen in treatments combining biochar addition with AMF inoculation. However, there was no significant difference in Zn content between PGPR and AMF inoculations. Under AMF inoculation conditions, the biochar treatment increased the P, Fe, and Zn levels 2.54-, 1.89-, and 1.80-fold compared to the case without biochar addition. As shown in Table 3, under control conditions, biochar addition increased the bioavailability of Fe and Zn (when compared to the B0 treatment). However, the levels of these elements remained below the critical level of the soil. Moreover, unlike P, the content of Fe and Zn was reduced by microbial inoculation. This is probably related to the loss of Fe and Zn upon biochar application due to the improved plant growth, leading to greater nutrient uptake from the soil.

Khalil and Hassan [58] observed that PSM exuded organic acids into the rhizosphere by decomposing organic matter. This can both reduce soil pH and dissolve mineral phosphates, such as Ca^3 $(PO_4)_2$ in soils with high pH, which could explain why the soil pH was lower when combining the biochar treatment with PSM inoculation than in the case without inoculation (Table 3). Biochar incorporation has been reported to increase P availability and stimulate AMF, which plays a vital role in nutrient cycles, especially for P uptake, since the added organic matter causes humate ions to replace adsorbed phosphates. Other factors affecting the P availability, include the release of phosphate ions, changes in the levels of dissolved organic compounds, such as sugars and organic acids in the soil solution, the formation of complexes with Ca, Fe, and Al ions, and changes in anion exclusion [59]. AMFs penetrate into the root cortex, allowing them to obtain carbon from the host plant in exchange for P and other nutrients that they absorb from the soil via their extensive hyphae. The hyphae of mycorrhizal fungi dissolve soil P by synthesizing organic acids and phosphatase enzymes. As a result, they increase the host plant's P uptake per unit root length and unit time by extending the P depletion zone around the plant roots and increasing the P uptake rate.

Improvements in the bioavailability of Fe and Zn following microbial inoculation can also be partly due to the synthesis of microbial siderophores, which are low molecular weight organic compounds with high affinities for various cations. Under conditions of Fe deficiency, soil microorganisms produce siderophores that form stable and soluble Fe complexes, leading to increased Fe availability [60]. Several PGPRs, including Pseudomonas species and mycorrhizal fungi have been reported to synthesize siderophores [61]. Siderophores of plant origin or phytosiderophores also increase Fe and Zn bioavailability in the rhizosphere. The application of biochar as a source of carbon stimulates mycorrhizal fungi, which is vital for nutrient cycles [59]. In addition to P, mycorrhizal fungi can absorb trace elements, increasing their availabilities in the soil. Among the studied treatments, AMF+B1.5 caused the greatest increase in the availability of P, Fe, and Zn, as well as the largest increase in organic carbon content (Table 3). This suggests a positive correlation between the soil's organic matter content and the bioavailability of key nutrient elements. Although several factors, including the activity of microorganisms, the composition of the rhizosphere, and chemical processes govern changes in nutrient retention in soil, it has been established that biochar incorporation influences the availability of nutrient ions by affecting ion exchange capacity and microbial activity [26]. Biochars have an amorphous and porous structure with a high surface area and the ability to absorb moisture, dissolve organic matter, gases, and minerals, in order to provide good habitats for microorganisms, especially AMFs. The pores in biochars and their size distribution protect microorganisms against predators and droughts. In a study on the ability of biochar to promote microbial colonization, Jin [62] found that biochars interacted with AMFs to increase the population of fungi and enhance nutrient availabilities in soils.

3.3. P, Fe, and Zn Uptake of Plants

P, Fe, and Zn uptake by the plant roots and shoots were significantly affected by the combined microbial inoculation and biochar application (Figure 3). PSM significantly increased the root P content relative to the B0 and control + B0 treatments (Figure 3a). The highest root P content was observed in plants exposed to the AMF + B1.5 treatment, while the P content for this treatment was not significantly different from the PGPR inoculation alone, it was 3.24 times for the B0 + AMF treatment and differed significantly from the values obtained for all of the other treatments. A comparison of means showed that similar to the roots, microbial inoculation enhanced the P content of plant shoots when compared to the other treatments (Figure 3b). However, AMF and PGPR inoculation did not have significantly different effects on shoot P content. The highest shoot P content was observed in AMF-inoculated plants exposed to B1.5. The shoot P content for this treatment was 2.14 times greater than for the control + B0 treatment and differed significantly from the shoot P contents for the B0 treatment in both inoculated and control forms. PGPR inoculation also significantly affected the Fe and Zn contents of the plants when combined with the biochar treatment (Figure 3). The highest root ($p < 0.01$) and shoot ($p < 0.001$) Fe contents were observed in PGPR-inoculated plants treated with biochar, for which the Fe and Zn contents were 1.64 and 1.21 times higher, respectively than those for AMF-inoculated plants treated with biochar (Figure 3c,d). When biochar was not applied, AMF inoculation increased the shoot Zn ($p < 0.01$) content 1.2-fold. However, the Zn content in roots ($p < 0.01$) did not differ significantly between AMF and PGPR inoculation treatments. Moreover, the biochar application increased the root and shoot Zn content of PGPR-inoculated plants by factors of 2.25 and 3 when compared to plants not treated with biochar (Figure 3e,f).

The uptake of nutrients by plants is influenced by their concentrations in the soil. The uptake is expected to increase as nutrient levels in the soil increase, subsequently leading the soil nutrient levels to fall [31]. We obtained similar results: Biochar treatment combined with PGPR inoculation reduced nutrient levels in the soil to a greater degree than the biochar treatment combined with AMF inoculation, implying a greater uptake of nutrients by plants in the former case (Figure 3). Budania et al. [63] reported that the application of 60 kg ha^{-1} P2O5 with micronutrients mixed with biochar + PGPR increased the N and P content by 4% and 0.5% in chickpea grains (*Cicer arietinum* L.), respectively, and this increase was higher than seen under control conditions. The improvement in soil characteristics and nutrient uptake by plants in biochar-treated soils can be attributed to the presence of plant nutrients and ash in biochar, its high specific area and porous nature, and its potential to create a favorable environment for bacteria. In a study on the effects of interactions between mycorrhizal fungi and biochar on P availability to beans, Vanek and Lehmann [64] found that the combined application of biochar with AMF increased the plants' P content, and that the increase in shoots was considerably higher than in the control treatment (soil). Our results showed that roots contained more Fe and Zn than shoots. The precipitation of these elements on the root surface, where they occupy the active sites for Fe and Zn uptake [65], may explain why they were more concentrated in wheat roots than in shoots. This binding could also hinder the uptake and mobilization of these nutrients to plant shoots. Singh et al. [66] observed that the interaction of rice husk biochar and PGPR influenced the Fe uptake by plants positively and significantly, which is probably related to the ability of PGPR to dissolve Fe in the presence of biochar. Adejumo et al. [67] also reported that the treatment with rice husk biochar significantly increased the Zn and Fe uptake by corn plants.

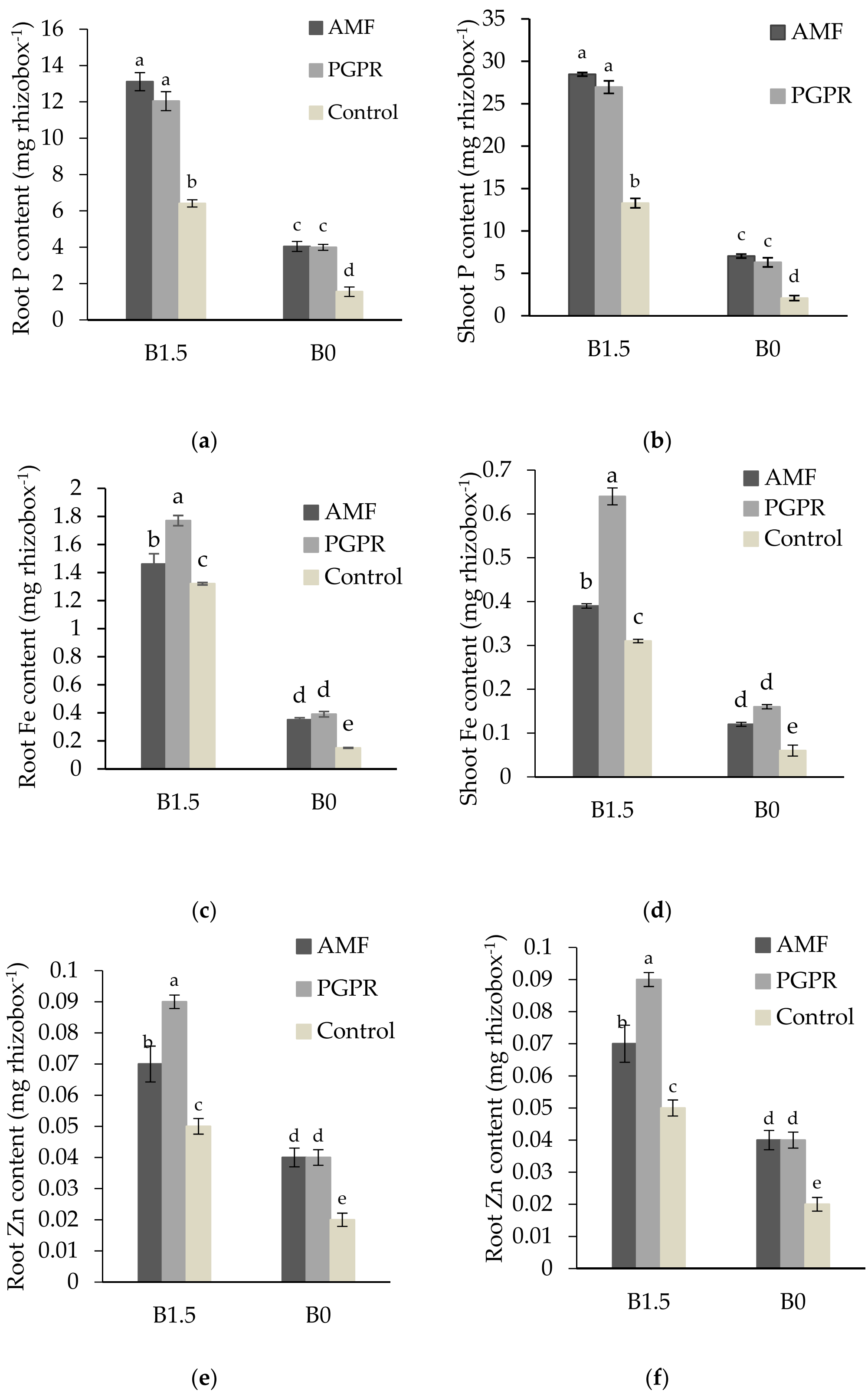

Figure 3. The effect of the organic source and microbial inoculation on the uptake of P (**a**,**b**), Fe (**c**,**d**), and Zn (**e**,**f**) in plants. Different letters indicate significantly different values at $p \leq 0.05$. B1.5 and B0 are biochar 1.5% and no biochar, respectively.

3.4. SDW and RDW

A comparison of means showed that the combined treatment with biochar and microbial inoculation significantly increased the root dry weight when compared to the controls (Figure 4a). The highest RDW of 1.8 g per rhizobox was obtained under the PGPR inoculation–biochar treatment, which was significantly different to the results obtained under the other treatments. The lowest RDW was 0.29 g per rhizobox, obtained from control + B0 treatment. The highest SDW of 4.00 g per rhizobox was observed for the PGPR inoculation–biochar treatment and was significantly different from the results obtained under the other treatments (Figure 4b). As shown in Figure 4, the combined AMF inoculation and biochar treatment also increased RDW and SDW in wheat, but to a lesser degree than the combined PGPR inoculation and biochar addition. As with RDW, the lowest SDW (0.91 g per rhizobox) was observed in the control + B0 treatment. The SDW for both inoculation levels differed significantly from the control treatment (Figure 4b).

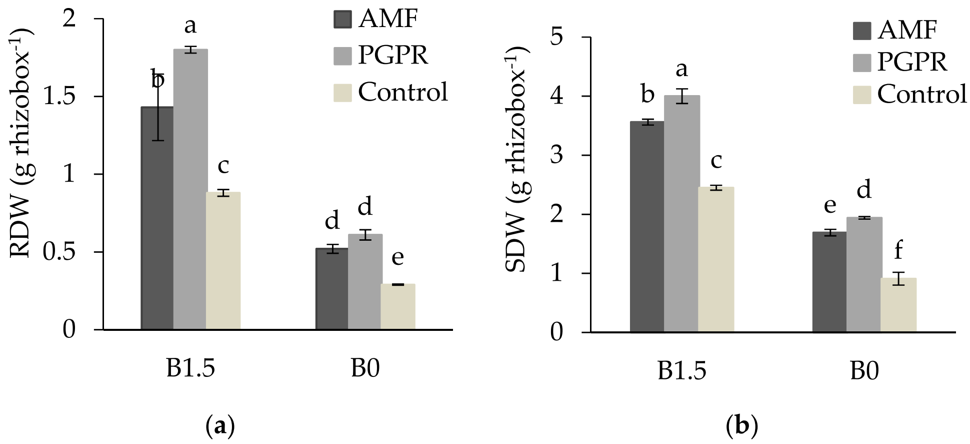

Figure 4. The effect of the organic source and microbial inoculation on RDW (**a**) and SDW (**b**). B1.5 and B0 are biochar 1.5% and no biochar, respectively. Different letters indicate significantly different values at $p \leq 0.05$.

The effects of microbial inoculation and biochar addition on plant yields depend on the soil type. The poorer the initial soil quality (i.e., the lower its organic matter content and its capacity for nutrient retention), the more likely it is that these treatments will increase the plant yields [68]. In many studies, biochar has improved root growth since its characteristics differ markedly from those of the soil surrounding the roots. Roots penetrate into the pores of biochar, and biochar changes the physical, chemical, and biological characteristics of the soil, leading to improved nutrient availability, greater aeration, and the uptake of beneficial microbes, thereby improving soil fertility and enhancing plant growth and yields [30]. Ahmad et al. [69] reported that the combined application of biochar and PGPR inoculation improved the growth and physiological traits of corn remarkably. With regards to the favorable impact of biochar and PGPR inoculation on dry weight, it should be noted the Pseudomonas bacteria used in this work have several growth-stimulating traits, including indoleacetic acid biosynthesis, ACC-deaminase and siderophore secretion, high capacity to dissolve insoluble phosphates, and high N fixing efficiency. All of these factors would tend to increase the nutrient uptake by plants. The interaction between PGPR inoculation and biochar addition increased the dry weight and yield of wheat plants since biochar increased the uptake of water and nutrients and provided a favorable habitat for the beneficial bacteria on its surface.

4. Conclusions

In the present study, our findings demonstrate that the combined biochar application and PGPR inoculation had beneficial effects on the chemical characteristics of alkaline Iranian soil. This treatment improved the availability and uptake of P, Fe, and Zn in calcareous soil, leading to increases in plant dry weight. Biochar addition can increase the activity of microorganisms, especially mycorrhizal fungi, while also increasing the bioavailability of nutrients (P, Fe, and Zn) in the soil by changing the pH, EC, and organic matter content of the rhizosphere zone, promoting the exudation of organic acids from microorganisms and plant roots, and changing the chemical balance and physical conditions in the root zone. Interestingly, combining PGPR inoculation with biochar addition had a more favorable impact on soil properties than AMF inoculation with biochar addition. Therefore, combining the amendment with organic matter, such as biochar with the biological capabilities of microorganisms can be a highly effective way of maintaining the soil's organic carbon budget, increasing soil fertility and biological activity, and enhancing the bioavailability of nutrients, such as P, Fe, and Zn, leading to significant improvements in wheat yield. Further studies on the preparation of biochar from different organic precursors and the effects of the resulting biochar on the growth of wheat and other crops in calcareous soils under field conditions are needed to confirm these findings and fully evaluate the economic benefits of applying biochar to soil.

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