Effects of Adding Pyrochar and Hydrochar to Calcareous Soil on Nutrient Uptake by Maize

Yuxiao Cai,[a] Luyu Liu,[b] Wei Zhang,[c] Sen Xing,[d] Xiaohui Liang,[a] Mingjie Gao,[a] Hewei Yu,[a] Zhaoxia Jiang,[a] Kenji Ogino,[e] Xiuxiu Chen,[a,]* Bing Wang,[a,e,]* and Hongyu Si [a,]*

The patterns and mechanisms underlying the effects of different types of biochar on crop dry matter accumulation and uptake of major soil minerals are uncertain. This study demonstrated the positive effects of adding pyrochar (PC) and hydrochar (HC) to calcareous soils on nutrient uptake by maize seedlings and revealed the important role of mycorrhizal colonization. The effects depended on the type of biochar added and the type of nutrient evaluated. The dry weights of maize seedlings were higher in the HC and PC groups than in the control group, and the P accumulation was 17% higher than that of the control. Adding PC significantly increased Zn accumulation and the concentration and accumulation of Cu in maize seedlings, whereas adding HC increased the Fe concentration. Applying PC and HC also promoted mycorrhizal colonization of maize roots, and P, Zn, and Cu accumulations in the plant were positively correlated with the mycorrhizal colonization rate. This study found that applying PC and HC to calcareous soil at the tested application rate promotes the uptake of some mineral nutrients by maize at the seedling stage, and this effect was at least partially influenced by an increased mycorrhizal colonization rate in the plant root system.

DOI: 10.15376/biores.18.2.2981-2997

Keywords: Hydrochar; Pyrochar; Maize; Mineral nutrients; Mycorrhizal colonization rate

*Contact information: a: Shandong Provincial Key Laboratory of Biomass Gasification Technology, Energy Research Institute, Qilu University of Technology (Shandong Academy of Sciences), Jinan, 250014, China; b: College of Engineering Ocean University of China, Qingdao, 266000,China; c: College of Resources and Environment, Southwest University, Chongqing, 400715, China; d: Shandong 963 Agricultural Science and Technology Co, Ltd, Jinan, 250014, China; e: Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, 2-24-16 Nakacho, Koganei-shi, Tokyo, 184-8588, Japan; *Corresponding authors: chenxx@sderi.cn; s205024v@st.go.tuat.ac.jp; sihy@sderi.cn*

INTRODUCTION

The rational disposal of agricultural waste is a common problem, and improper disposal leads to the waste of resources and environmental pollution. It is estimated that the annual global production of lignocellulosic biomass is 181.5 billion tons and that the energy value of this biomass is equivalent to 4.8 times the global energy consumption in 2020 (Deng *et al*. 2022). Carbonization of carbon (C)-rich biomass is an important method for the utilization of agricultural waste. As a biological material with a rich carbon content and strong adsorption capacity, biochar is characterized by a large specific surface area, rich pore structure, and high nutrient content, and it has gradually begun to be used in agricultural production, ecological environmental protection, and other applications. In particular, biochar has shown great potential for improving soil and thus crop growth and may be an important method for achieving the goals of sustainable agriculture in the future.

Biochar has a significant impact on the growth of crops, but the effects of different types and amounts of biochar on crops are not completely consistent. Pyrolysis carbonization and hydrothermal carbonization are the main pathways for converting biomass into biochar. Some studies have shown that the production cost of biochar/hydrothermal charcoal is mainly related to from the raw material, equipment energy consumption, and labour cost within a single treatment process (Zhang *et al.* 2020; Yang *et al.* 2016; Kung *et al.* 2015). The pyrolytic carbonization process has the advantages of large processing capacity per unit volume and low equipment requirements, and it typically has lower unit production costs, but the outcome will be affected by specific conditions. Hydrothermal carbonization has low energy consumption and high yield and is more economical in handling high-moisture-content raw materials and small-scale production. The effects of pyrochar on soil quality and crop growth have been studied extensively. However, due to the diversity of crops and growth conditions, excessive biochar addition to the soil can inhibit the growth of crops such as maize (Bai *et al*. 2022). The addition of 1 to 2% biochar generally has a positive effect on crop growth by increasing nutrient uptake (Li *et al*. 2022). Biochar may directly supplement soil nutrients through the introduction of exogenous sources of nutrients or affect plant nutrient uptake through its effects on soil structure and soil microbes (Hou *et al*. 2022).

Biochar prepared by pyrolysis carbonization or hydrothermal carbonization is rich in various forms of nitrogen. Biochar with a high ash content generally contains more phosphorus (P), and the content of potassium (K) is closely related to the pyrolysis temperature at which the biochar was prepared and to the selection of raw materials. Studies have demonstrated that hydrochar prepared at 220 °C has good pore structure, moderate pore size, good diffusion and sorption properties with more active sites for sorption (features that are conducive to sequestration and to the slow release of nutrients), and a high number of oxygen-containing functional groups that permit the adsorption of metal ions (Khan *et al*. 2019). During pyrochar preparation, as pyrolysis temperature increases, the volatile content of the biomass decreases; the ash content increases, the specific surface area increases. The total pore volume, the pH, and the basic functional group content increase, reaching their maximum levels at 550 °C; and the content of acidic functional groups decreases, making the pyrochar more favourable as an acidic soil amendment (Liao *et al*. 2022). Thus, hydrochar prepared at 220 °C and pyrochar prepared at 550 °C may aid in supplementing soil nutrients and promoting nutrient sequestration.

Micronutrients such as zinc (Zn), copper (Cu), and iron (Fe) are necessary components of various enzymes that play irreplaceable roles in plant growth and human development. It is of great importance to seek effective measures that promote the absorption of micronutrients, particularly by the roots of cereal crops, and to increase the nutrient content of grains to improve the diet of consumers. Studies have shown that biochar can significantly promote plant and grain nutrient accumulation, but the mechanism remains unclear (Abbas *et al*. 2017; Torabian *et al*. 2021; Li *et al*. 2022). Mycorrhizae are a consortium of mycorrhizal fungi in the soil and vegetative roots of higher plants. Symbiotic fungi take essential carbohydrates from the plant, and the plant obtains necessary nutrients from the fungus, thus forming a mutually beneficial symbiotic relationship. The mycorrhizal colonization rate is one of the most important indices used to evaluate the symbiosis between mycorrhizal fungi and host plants as well as the physiological function of the symbiosis. The mycorrhizal colonization rate represents the level of mycorrhizae in the root system and is typically used as an important index to judge the growth and developmental status of mycorrhizal fungi. Mycorrhizal fungi play an important role in the uptake of nutrient elements by crops. Arbuscular mycorrhizae (AM) are widely reported to promote the uptake of nutrients by crop roots by expanding the area of contact between roots and nutrients, secreting organic acids that activate soil nutrients, and interacting with rhizosphere bacteria (Baum *et al*. 2015). Studies have shown that most nutrients such as phosphorus and Zn are taken up by direct root absorption and mycorrhizae, of which AM fungi mediated by mycelium activity account for 30 to 70% of the total nutrient accumulation of plants (Cavagnaro 2008; Zhang *et al*. 2017; Yu *et al*. 2020). The mycorrhizal pathway also plays an important role in the absorption of elements such as copper and iron (Kabir *et al*. 2020). The addition of biochar increases the diversity of soil microorganisms, increases the abundance of soil microbial communities, and improves the utilization of carbon sources by rhizosphere microorganisms (Li *et al*. 2022). Biochar is characterized by a high dissolved organic carbon (DOC) content, high porosity, and high specific surface area. In addition, specific amounts of nutrients, include P, K, Ca, Mg, Fe, Mn, Cu, and Zn. These characteristics indicate that biochar has high potential for promoting mycorrhizal fungal growth, which in turn may affect the uptake of elements such as P, Zn, and Fe by plants. Studies suggest that biochar application may have a positive effect on mycorrhizal fungi by changing the physical and chemical properties of the soil, interacting with bacteria or other soil microorganisms, and regulating signalling between plants and mycorrhizae (Warnock *et al*. 2007). However, the mechanism by which biochar regulates mycorrhizal fungal community characteristics and thus promotes nutrient uptake by crop roots remains unclear. In fact, studies on the effect of biochar on Zn, Cu, and Fe levels in plants have typically focused on the removal of heavy metals from contaminated soils (Gong *et al*. 2022). No report has previously associated mycorrhizal fungi affected by biochar application with plant growth and nutrition absorption in regular agricultural fields. Therefore, it is necessary to explore the potential of biochar for promoting nutrient uptake through the mycorrhizal pathway.

Pyrolysis carbonization and HTC are the main pathways for converting biomass into biochar. In this study, a pot experiment was conducted to determine 1) the effects of hydrochar and pyrochar on the growth and development of maize at the seedling stage; 2) the effects of hydrochar and pyrochar on the accumulation and uptake of major mineral nutrients by maize at the seedling stage; and 3) the role of mycorrhizal colonization in the effects of hydrochar and pyrochar on nutrient uptake by maize.

EXPERIMENTAL

Biochar Preparation and Characteristics

The pyrochar and hydrochar used in this study were prepared under laboratory conditions. The pyrochar was prepared by heating the material to 550 °C at a rate of 10 °C/min and holding it under a nitrogen atmosphere for 7 h. The hydrochar was prepared at 220 °C for 4 h at a liquid/solid ratio of 5:1. The biochar was soaked in deionized (DI) water for 15 min, and the supernatant was removed. This step was performed five times to remove impurities from the surface of the biochar. The washed biochar was boiled again in DI water for 5 min, and the supernatant was removed. This operation was repeated three times to remove harmful substances adsorbed onto the pore structure of the biochar. Elemental analysis, specific surface area by the Brunauer–Emmett–Teller (BET) method, inductively coupled plasma (ICP) optical emission spectroscopy, pH tests, DOC measurement, and scanning electron microscope (SEM) analysis were performed on the two biochars.

Experimental Setup

Three treatments were applied, with four replicates for each treatment: a blank (CK), 1.7% pyrochar (PC), and 1.7% hydrochar (HC). Soil taken from farmland around Jinan, China, was dried, sieved, and placed in 5.0 L pots (3 kg of soil per pot). The ammonium nitrogen content of the tested soil was 4.22 $mg.kg^{-1}$ (determined by NaCl extraction and the indophenol blue spectrophotometric method), the soil nitrate-N content was 6.82 $mg.kg^{-1}$ (determined by NaCl extraction and UV spectrophotometry), the soil available P content was 17.47 $mg.kg^{-1}$ (determined by $NaHCO_3$ extraction and molybdenum-antimony anti-absorption spectrophotometry), the soil available K content was 260.46 $mg.kg^{-1}$ (determined by $NaNO_3$ extraction and the turbidimetric method using sodium tetraphenylborate); and the soil organic matter (SOM) content was 15.6 $g.kg^{-1}$ (by the potassium dichromate method and thermodilution). The Lufeng 510 maize seeds used in the experiments were sterilized in a 10% H_2O_2 solution for 30 min, rinsed with DI water, and then soaked in a saturated calcium sulfate solution for 8 h. The seeds were placed on moist filter paper on a tray and covered with filter paper to keep them moist and protected from light. Sowing was performed after the radicles had just appeared. Nitrogen (N), P (P_2O_5), and K (K_2O) fertilizers were applied before sowing, and $NaNO_3$, $NaHPO_4$, and K_2SO_4 were applied at 200 mg N, 100 mg P, and 200 mg K per kg of soil, respectively.

Sample Collection and Analysis

In this study, a 28-day pot experiment of maize seedling was conducted to study potential effects of biochar application on nutrition uptake and corresponding mechanism associated with mycorrhizal colonization. In the growth process of maize, 28 days was enough for the plants to enter into the period of jointing stage (with more than 6 leaves). Among all growth stages of maize such as seeding, jointing, and anthesis stages, especially the jointing stage of maize, represents a rapidly develop of plant and actively interact period between root and soil microorganism (Hu *et al.* 2015; Liu *et al.* 2014). That means the mycorrhizal colonization rate of root would be significantly changed and thereby making great effect on nutrition absorption of maize. The aboveground parts and underground roots of maize seedlings were collected 28 days after planting. After rapid washing, first with tap water and then with deionized water, the plant samples were dried at 60 to 65 °C, and their dry weights were recorded. The plant samples were ground in a stainless steel grinder to prepare them for nutrient analysis. A microwave-accelerated reaction system (CEM, Matthews, NC, USA) was used to digest plant samples with HNO_3-H_2O_2. The concentrations of P, Ca, Mg, Fe, Mn, Cu, and Zn in the digested solutions were determined by inductively coupled plasma optical emission spectroscopy (ICPOES, OPTIMA 3300 DV, Perkin–Elmer, Waltham, MA, USA). The root samples were cut into 1 cm segments for determination of AM colonization, stained with trypan blue and evaluated according to the methods of Feng *et al*. (2003).

Statistical Analysis

To evaluate the effects of biochar application on shoot dry weight, AM colonization, and nutritional parameters, the data obtained from the maize plants were subjected to a one-way analysis of variance (ANOVA). Linear regression was used to analyse the relationships between AM colonization and nutrient accumulation. SAS software (SAS 8.0, USA) and SPSS software (SPSS 20.0, China) were used for statistical analysis. When ANOVA revealed significant effects, the treatments were compared using the least significant difference (LSD) test at $P < 0.05$.

RESULTS AND DISCUSSION

Characteristics of Hydrochar and Pyrochar

Table 1 shows the results of elemental analysis, ICP, and BET data for the two biochars. Figure 1 shows the pore size distribution, isothermal adsorption, and SEM of the hydrochar and pyrochar.

Table 1. Physicochemical Properties of Hydrochar and Pyrochar

Elemental analysis	**Hydrochar**	**Pyrochar**
C (%)	70.15	90.78
H (%)	5.38	2.26
O (%)	23.20	5.14
N (%)	1.20	1.48
S (%)	0.06	0.34
H/C	0.92	0.025
O/C	0.25	0.056
Ph	4.41	8.67
DOC	2970	219
ICP		
P ($mg.kg^{-1}$)	$1.93×10^3$	$3.47×10^3$
K ($mg.kg^{-1}$)	$1.39×10^4$	$2.25×10^4$
Ca ($mg.kg^{-1}$)	$2.61×10^3$	$1.56×10^3$
Mg ($mg.kg^{-1}$)	$1.09×10^3$	$7.06×10^3$
Fe ($mg.kg^{-1}$)	184	$1.5×10^4$
Mn ($mg.kg^{-1}$)	24.0	453
Cu ($mg.kg^{-1}$)	21.1	70
Zn ($mg.kg^{-1}$)	13.1	133
BET		
SSA ($m^2.g^{-1}$)	3.66	30.12
TPV ($cm^3.g^{-1}$)	0.0198	0.0371
MPV ($cm^3.g^{-1}$)	0.00068	0.00726

There were obvious differences between hydrochar and pyrochar due to the different production methods. It can be seen from the elemental analysis data in Table 1 that the contents of H and O in hydrochar were higher than those in pyrochar, whereas the contents of C, N, and S were lower in hydrochar compared with those in pyrochar. Correspondingly, the H/C and O/C ratios of hydrochar were higher than those of pyrochar. The hydrochar was acidic, the pyrochar was alkaline, and the DOC content of hydrochar was much higher than that of pyrochar. It can be seen from the ICP data in Table 1 that except for Ca, the contents of P, K, Mg, Fe, Mn, Cu, and Zn in hydrochar were all lower than those in pyrochar. According to the BET data in Table 1, compared with hydrochar, pyrochar had a larger specific surface area and pore volume, indicating that the internal structure of pyrochar was more complex. The hydrochar was mainly composed of a carbon microsphere structure (Fig. 1c), whereas the pyrochar was mainly composed of a micropore structure (Fig. 1d). The pyrochar exhibited a type IV adsorption curve, and the adsorption capacity in the low pressure area increased rapidly, indicating that there were more micropores (Fig. 1b). With increasing relative pressure, capillary condensation and macroporous adsorption gradually occurred. Unlike the adsorption curve of pyrochar, the adsorption curve of hydrochar was close to a type III adsorption curve (Fig. 1a). Combined with the pore size distribution curve (Fig. 1b), this finding suggests that a small number of medium and large pores are filled in hydrochar.

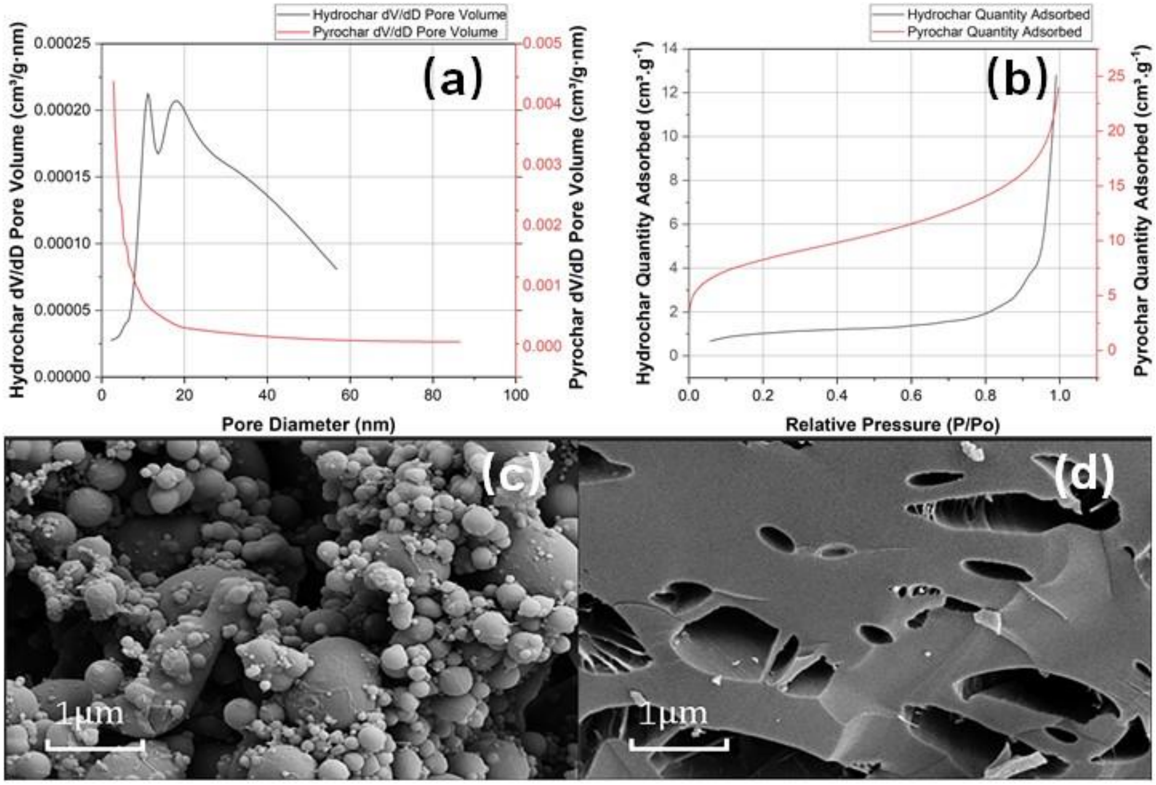

Fig. 1. Pore size distribution diagram (a) and isothermal adsorption diagram (b) of hydrochar and pyrochar, and electron microscopy diagram (c) of hydrochar and electron microscopy diagram (d) of pyrochar

Effects of Hydrochar and Pyrochar on Maize Dry Weight

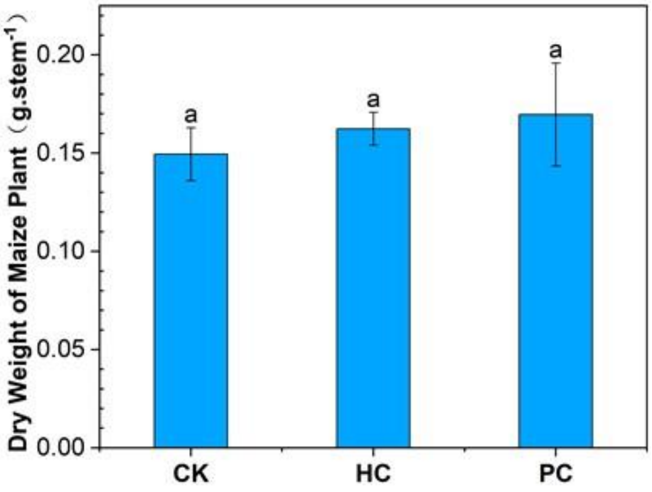

Fig. 2. Dry matter yield of maize plants (28-day growth) as affected by hydrochar and pyrochar. Values are the mean of four replications ± SE. Within each treatment, means with different lowercase letters were significantly different at $P < 0.05$.

The dry weights of the maize seedlings treated with hydrochar and pyrochar did not differ significantly from those in the CK group; however, as shown in Fig. 2, the biomass of the maize in the PC (group treated with 1.7% pyrochar) was 13.5% higher and the biomass of the maize in the HC group (treated with 1.7% hydrochar) was 8.6% higher at the seedling stage than the biomass of the seedlings in the CK group, indicating the high potential of biochar to promote maize dry weight accumulation.

Effects of Pyrochar and Hydrochar on P, Ca, and Mg Accumulation

Adding biochar had varying effects on the concentration, absorption, and accumulation of P, Ca, and Mg in the maize seedlings. Applying pyrochar significantly increased the P concentration in maize at the seedling stage, but applying hydrochar had no effect on the P concentration in the plants. Application of these biochars at 1.7% had no effect on the Ca or Mg concentrations in the plants (Table 2). The addition of pyrochar or hydrochar increased the accumulation of P in the maize plants by 17% but did not increase the accumulation or the concentration of Ca or Mg in the plants (Table 2).

Table 2. Effects of Different Treatments on the Concentrations and Accumulations of Phosphorus, Calcium, and Magnesium in Maize Seedlings

Treatment	**Nutrient Concentration (g.kg^{-1})**			**Nutrient Accumulation (mg.stem^{-1})**		
	P	Ca	Mg	P	Ca	Mg
CK	4.28±0.16^{b}	3.09±0.35^{a}	2.71±0.15^{a}	0.64±0.03^{a}	0.46±0.05^{a}	0.41±0.04^{a}
HC	4.34±0.04^{b}	2.67±0.66^{a}	2.45±0.28^{a}	0.71±0.02^{a}	0.43±0.10^{a}	0.40±0.04^{a}
PC	4.76±0.06^{a}	3.36±0.26^{a}	2.66±0.18^{a}	0.74±0.02^{a}	0.57±0.06^{a}	0.45±0.02^{a}

Values are means of four replications ± SE. Within each treatment, means with different lowercase letters are significantly different at $P < 0.05$. Values with different lowercase letters in the same column differ statistically in mean using the ANOVA test ($p < 0.05$).

Effect of Pyrochar and Hydrochar on Micronutrient Accumulation

The addition of biochar had varying effects on the concentrations, uptake, and accumulations of Zn, Cu, Fe, and Mn in the maize seedlings. The addition of pyrochar addition significantly increased the Cu concentration and the accumulation of Zn and Cu in the maize seedlings (Fig. 3c, b, and d); these elements were 68%, 28%, and 38% higher, respectively, compared with the CK group.

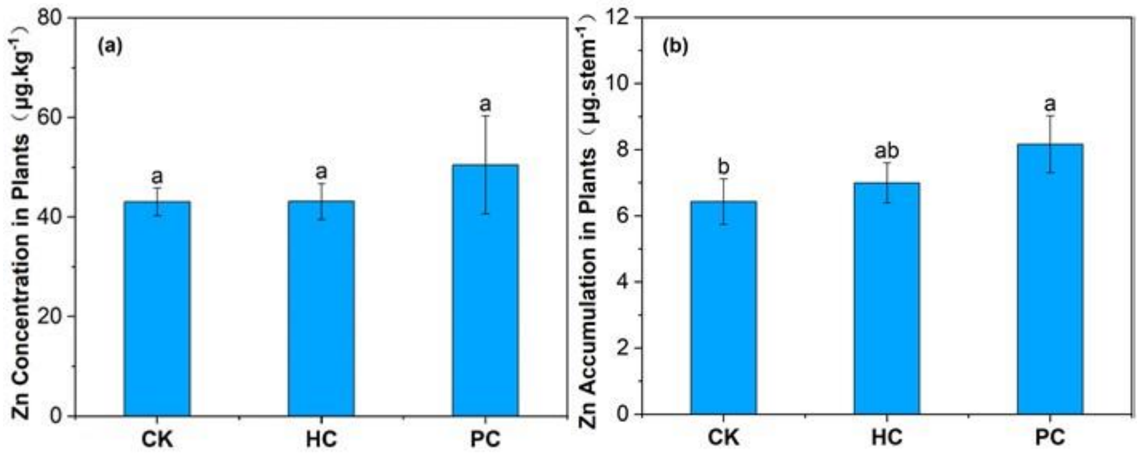

Fig. 3 (a-b). Zn concentration (a) and accumulation (b), Cu concentration (c) and accumulation (d), Fe concentration (e) and accumulation (f), and Mn concentration (g) and accumulation (h) under different treatments. Values are the mean of four replications ± SE. Within each treatment, means with different lowercase letters were significantly different at P< 0.05.

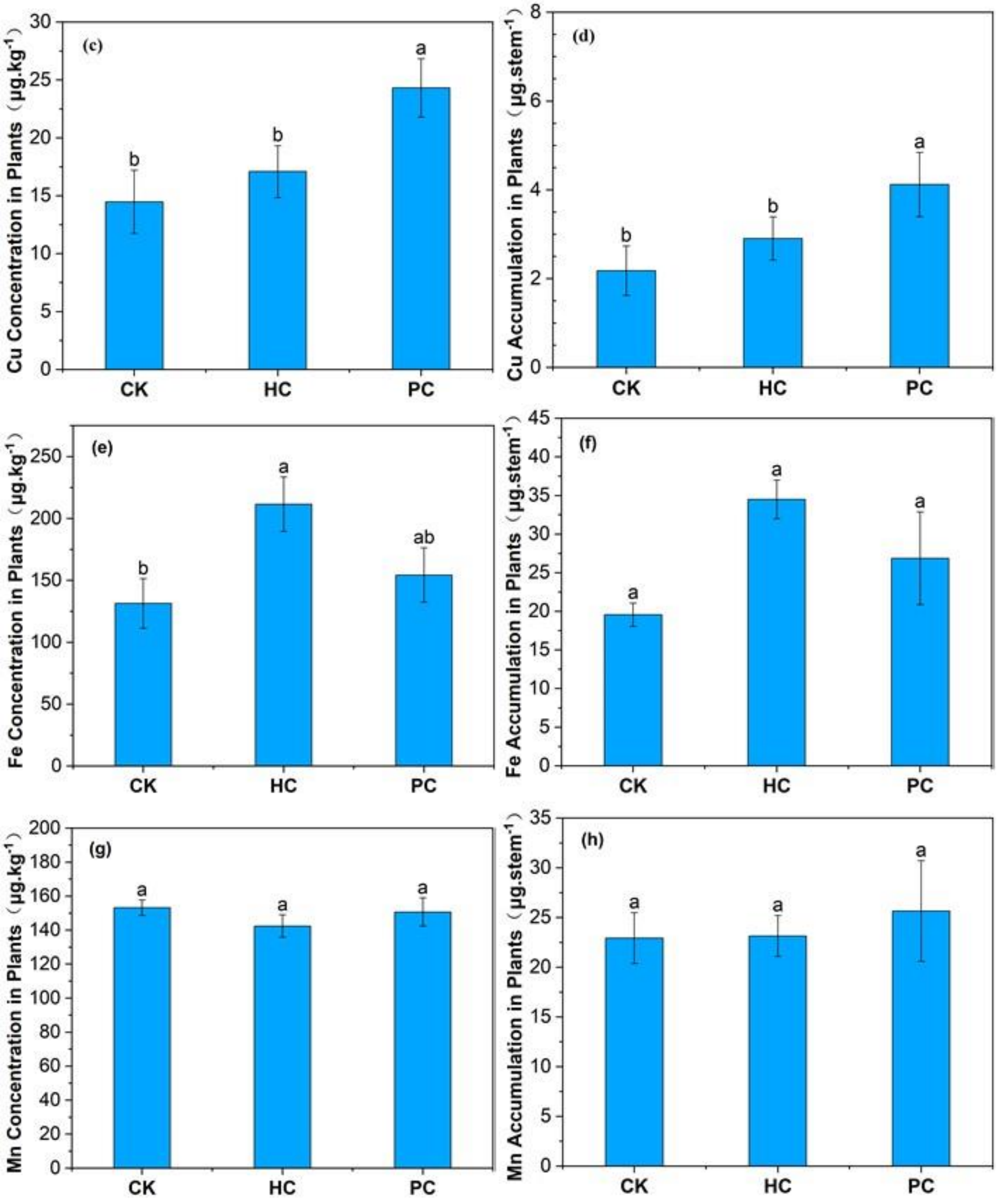

Fig. 3 (c-h). Zn concentration (a) and accumulation (b), Cu concentration (c) and accumulation (d), Fe concentration (e) and accumulation (f), and Mn concentration (g) and accumulation (h) under different treatments. Values are the mean of four replications ± SE. Within each treatment, means with different lowercase letters were significantly different at P< 0.05.

The application of hydrochar significantly increased the Fe concentration in the maize seedlings (Fig. 3e) by 61% compared with the CK group. Simultaneous application of the two biochars had no significant effect on the concentration or accumulation of Mn, the concentration of Zn, or the accumulation of Fe in the plants (Fig. 3g, h, a, and f).

Correlation Between Vesicular-Arbuscular Mycorrhizae (VAM,%) and Nutrient Absorption

The mycorrhizal colonization rates of the roots of the maize plants that received the hydrochar and pyrochar treatments increased significantly compared with that of the CK group with increases of 19% and 23%, respectively (Fig. 4). This finding indicates that adding biochar had a significant effect on the promotion of mycorrhizal colonization in maize at the seedling stage and on the development of AM fungi.

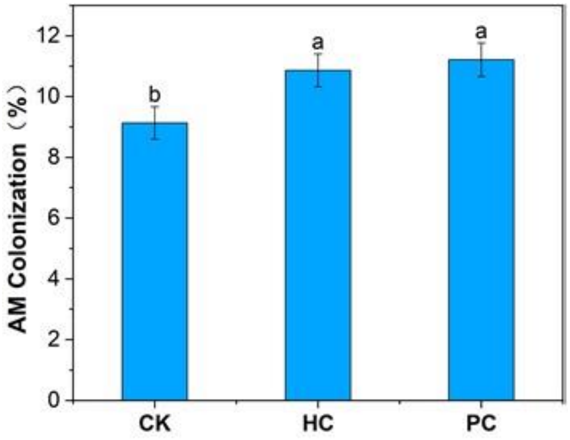

Fig. 4. Effects of different treatments on the mycorrhizal colonization rate of maize seedlings. Values are the mean of four replications ± SE. Within each treatment, means with different lowercase letters were significantly different at P< 0.05

A correlation analysis between the mycorrhizal colonization rate and the nutrient uptake of each group showed that the mycorrhizal colonization rate was significantly positively correlated with the accumulation of P in the aboveground parts of the maize plants (Fig. 5a) but not with the accumulation of Ca or Mg (Fig. 5b and c).

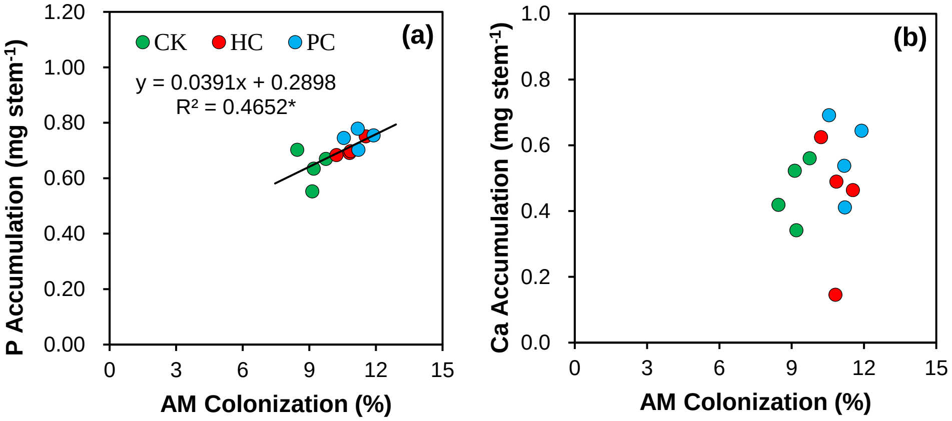

Fig. 5 (a-b). Correlation analysis of mycorrhizal colonization rate with P (a), Ca (b), Mg (c), Zn (d), Cu (e), Fe (f) and Mn (g) accumulated amounts. * Indicates that the model is significant at P<0.005

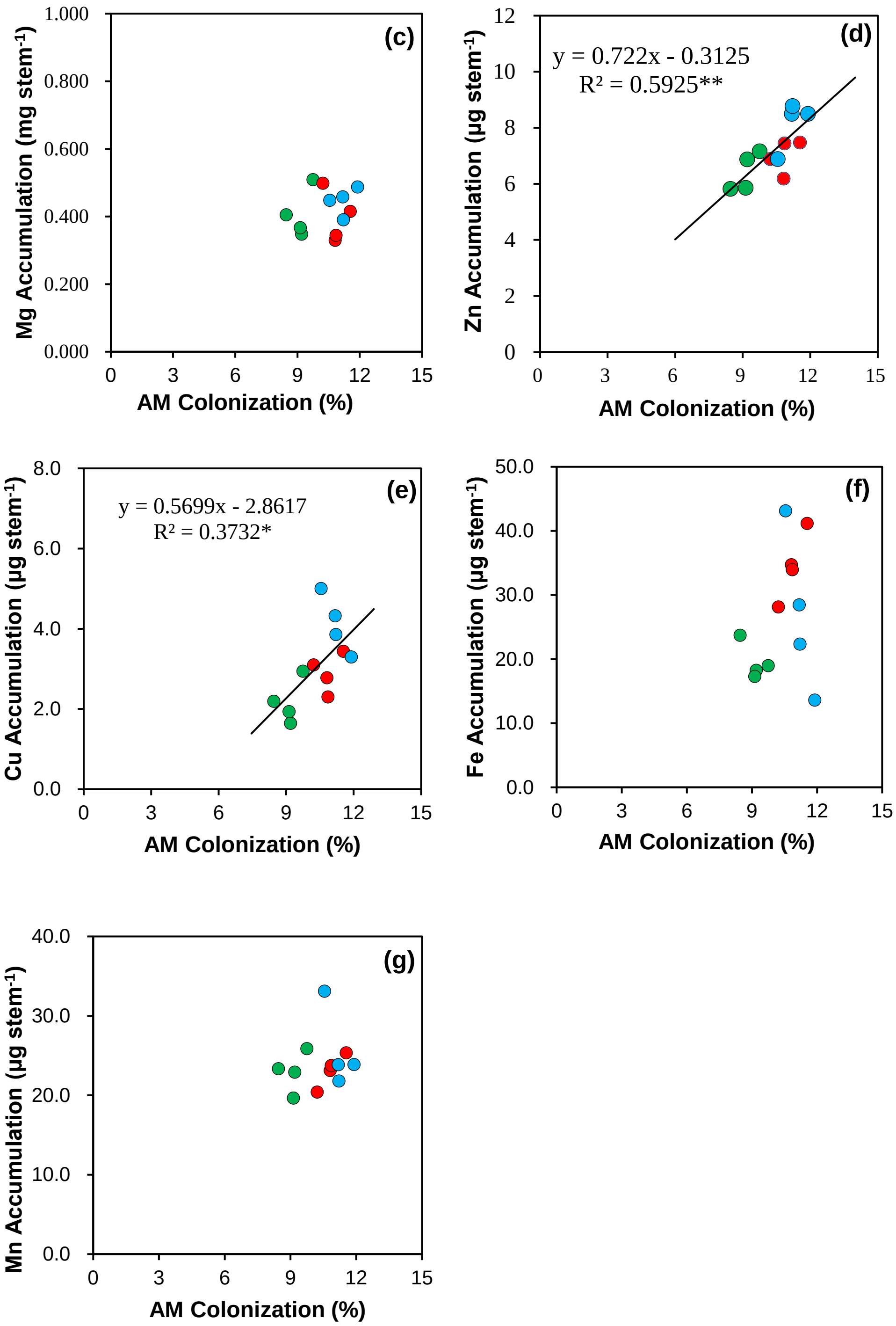

Fig. 5 (c-g). Correlation analysis of mycorrhizal colonization rate with P (a), Ca (b), Mg (c), Zn (d), Cu (e), Fe (f) and Mn (g) accumulated amounts. * Indicates that the model is significant at $P<0.005$

The mycorrhizal colonization rate of maize roots and accumulation of the trace elements Zn and Cu in the aboveground parts of the plants were significantly positively correlated (Fig. 5d and e); the correlation with Zn accumulation was stronger. The mycorrhizal colonization rate was not correlated with the accumulation of Fe or Mn (Fig.

5f and g). Overall, the increase in mycorrhizal colonization rate may be an important pathway through which biochar application promotes the uptake of P, Zn, and Cu by maize.

Biochar Characteristics

Differences in the biomass carbonization methods used, the temperature, and the retention time resulted in completely different physicochemical properties for the hydrochar and pyrochar used in the experiments (Rodriguez Correa *et al*. 2019). These differences in physicochemical properties directly affect the potential application of these biochars as soil additives. During the low-temperature HTC process, the decomposition of biomass is incomplete, and cellulose and hemicellulose are decomposed into oligomers through a hydrolysis reaction; this process promotes the formation and growth of spherical coke nuclei, resulting in the eventual formation of many microsphere structures and yielding primarily hydrochar with a small number of mesopores and macropores (Fig. 1c). In general, applying biochar with a macropore structure to the soil can effectively increase the porosity of the soil, improve soil structure, and affect the activity of microorganisms. During pyrolysis, the production of many inorganic carbonates and organic functional groups, such as -COO- and -O- makes the pyrochar alkaline (Yin *et al*. 2022). During the HTC process, organic acids such as formic acid, acetic acid, and propionic acid generated from the decomposition of fibre components make hydrochar acidic (Rillig *et al*. 2010). In this study, the application of hydrochar did not significantly increase maize dry weight accumulation compared with that of pyrochar, possibly because of the other advantages of pyrochar that promote crop growth.

Both hydrochar and pyrochar contain large amounts of mineral nutrients and DOC. However, the mineral nutrient content of hydrochar was lower than that of pyrochar, and the DOC content of hydrochar was higher than that of pyrochar (Table 1). This pattern occurred because, during the HTC process, the macromolecules are decomposed into monomers, which remain in the liquid phase and then attach to the solid hydrochar; thus, the hydrochar has a higher DOC content. Also during the HTC process, minerals are decomposed into water-soluble salts, which are then lost to the liquid phase. Therefore, the mineral nutrient content of hydrochar is low. The pore characteristics and the DOC and ash content of hydrochar and pyrochar may have a profound impact on soil microbes and on the soil structure that promotes crop growth by supplementing or slowing the release of soil mineral nutrients such as N, P, Mg, and Zn.

Nutrient Accumulation Was Affected by Pyrochar and Hydrochar Application

In this study, the dry weight of the maize seedlings in the PC and HC groups was higher than that of the seedlings in the CK group (Fig. 2), and the concentrations or accumulations of P, Zn, Cu, and Fe all increased to varying degrees (Table 2, Fig. 3b, d, and f). Biochar contains mineral nutrients and organic carbon compounds that improve soil fertility and promote crop growth. In this study, both hydrochar and pyrochar were found to contain high levels of nutrients, particularly P; the concentrations reached 1.93×10^3 and 3.47×10^3 $mg.kg^{-1}$, respectively (Table 1). Both hydrochar and pyrochar promoted the uptake of P by maize plants by increasing the available P level in the soil, thereby significantly increasing the P concentration in the plants. Additionally, at the same level of application, pyrochar provided more Zn and Cu to the soil than hydrochar (Table 1). Therefore, the accumulation of Zn and Cu in the plants that received the PC treatment was significantly higher than that in the plants that received the CK treatment.

The abundant negative charges, cation exchange capacity, high and numerous oxygen-containing functional groups (-COOH, -COH, and -OH) on the surface of biochar can cause electrostatic adsorption, exchange or formation of complexes with nutrient ions, and thereby promote the retention of nutrients such as P, Zn, Cu, and Fe on the biochar surface. These nutrients are desorbed from the biochar surface and become available for crop uptake when the nutrient concentration in the soil solution falls below the equilibrium concentration, thus preventing the loss of active soil nutrients by leaching. In addition, the DOC present at high levels in pyrochar and hydrochar (Table 1) may combine with Zn and Cu in the soil through complexation or chelation to form organic chelates with high solubility, thus increasing nutrient availability and promoting nutrient uptake by crop roots (Wang *et al.* 2017). Thus, the characteristics of biochar indicate that it has the potential to directly promote nutrient accumulation in crops. In addition, these characteristics of biochar may also be important for promoting mycorrhizal colonization. However, the mechanisms through which indirect regulation of root nutrient uptake by pyrochar and hydrochar prepared under different processing conditions occurs must still be explored from different perspectives.

Mycorrhizal colonization and nutrient accumulation

In this study, the addition of pyrochar and hydrochar significantly promoted mycorrhizal colonization of maize roots (Fig. 4). Studies have demonstrated that applying biochar has a significant impact on the characteristics of the soil AM fungal community, promotes the proliferation and development of native AM fungal populations, and has a positive impact on AM fungal abundance and the crop root colonization rate (Hammer *et al*. 2015; Liu *et al*. 2018b). Mycorrhizal fungi use biochar as a growth substrate by adhering to the surface and as a nutrient source by absorbing elements via mycelia, thus facilitating colonization (Figueiredo *et al*. 2019). Due to its high mineral nutrient content and large specific surface area, pyrochar can provide excellent nutrition and a colonization microenvironment for AM fungi, thereby promoting their growth (Hammer *et al*. 2015; Qu *et al*. 2022). The high DOC and acidity of hydrochar indicate that it has the potential to promote mycelial growth in calcareous soil. Biochar may also promote fungal spore germination and AM mycelial extension by influencing the growth of microorganisms such as phosphorus-solubilizing bacteria that interact with AM colonies (Atkinson *et al*. 2010).

The mycorrhizal pathway mediated by soil AM fungi is an important process through which crops absorb nutrients. Mycorrhizal fungi promote nutrient uptake by plant roots by secreting organic acids, extending mycelia that enlarge the absorption area, and interacting with soil bacteria. Thus, they play an important role in the uptake of P, Zn, and Cu by crops. The increase in mycorrhizal colonization may be an important reason why biochar application promoted the accumulation of a variety of nutrients in maize in this study. Previous studies have indicated that AM fungi have a positive effect on the uptake of P, Zn, and Cu by plants. AM fungi promote the uptake of P by crops under both low-P and high-P conditions, and they promote the mineralization of organic P and the activation of inorganic P in the soil by secreting phosphatases and enzymes that act on organic acids. After AM fungi colonize plant roots, the phosphate absorbed by extraroot hyphae is degraded to orthophosphate, thus promoting the transformation of soil P and the uptake of P by plants (Li *et al*. 2022; Sui *et al*. 2022). The promotional effect of AM fungi on P uptake by plants is particularly significant after the application of organic matter (Huo *et al*. 2022). The biochar used in this study contained a large amount of DOC (Table 1), which may also have indirectly enhanced the capacity for P uptake *via* the mycorrhizal pathway.

In this study, the mycorrhizal colonization rate was significantly positively correlated with the accumulation of Zn and Cu in maize (Fig. 5d and 5e). In a low-Zn soil environment, up to 20-50% of the Zn found in plants is absorbed through the mycelial pathway (Watts-Williams *et al*. 2015), and increased AM fungal colonization can significantly promote the uptake of Zn by plants. Other studies have reported that AM fungi activate soil-immobilized Zn by secreting malic acid and succinic acid (Liu *et al*. 2018a), and they may also directly affect the uptake of Zn by roots by specifically inducing the expression of specific Zn transporter proteins in plants. Lehmann and Rillig (2015) conducted a statistical analysis of 233 studies and found that AM fungi have a positive effect on the uptake of Cu by crops. The hyphae of AM fungi can penetrate deep into the micron-scale pore structure of biochar to indirectly connect the interior of the biochar to the plant rhizomes, and the fungi also transfer nutrients, such as Zn and Cu, which are then retained or stored in the pores inside the biochar and become available to the plant through the symbiotic relationship between the fungus and the plant root system, further promoting nutrient uptake by the plant. In addition, AM fungi have the ability to increase the activity of ferric chelate reductase in roots, which can promote the activation of ferric ions in the soil that are difficult to use, an effect that in turn is conducive to the absorption of Fe by maize (Li *et al*. 2015; Kabir *et al*. 2020). However, in this study, although the application of biochar increased the Fe concentration in the plants, the increase in Fe accumulation was not correlated with the increase in mycorrhizal colonization rate. Thus, the effect may be limited by the growth cycle of the crop.

CONCLUSIONS

1. Adding hydrochar or pyrochar to the soil increases dry matter accumulation and mineral nutrient uptake in maize at the seedling stage and significantly increases the mycorrhizal colonization rate in the root system.
2. The effects of the two biochars on mineral nutrient uptake were not completely consistent. Both biochars have the potential to promote P uptake by maize at the seedling stage. Pyrochar has the potential to promote Zn and Cu uptake by maize, whereas hydrochar has the potential to promote Fe uptake.
3. Adding biochar increased the mycorrhizal colonization rate of maize roots to some extent, indicating that the addition of hydrochar or pyrochar may impact the soil microbial community. The accumulation of P, Zn, and Cu in maize at the seedling stage was significantly positively correlated with the mycorrhizal colonization rate, suggesting that mycorrhizal colonization may be an important mechanism through which biochar application promotes the accumulation of nutrients such as P, Zn, and Cu.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China Youth Foundation (No. 32202606); Youth Foundation of Shandong Natural Science Foundation (No. ZR2022QC056); Foundation (No. BG-KFX-04) of Shandong Provincial Key Laboratory of Biomass Gasification Technology, Qilu University of Technology

(Shandong Academy of Sciences); Qilu University of Technology (Shandong Academy of Sciences) Science, Education, Industry Integration and Innovation Pilot Project (international cooperation project) (2022GH028); Key Research and Development Program of Shandong Province (Major Innovation Project) (No. 2021CXGC010803).

REFERENCES CITED

Abbas, T., Rizwan, M., Ali, S., Zia-ur-Rehman, M., Farooq Qayyum, M., Abbas, F., Hannan, F., Rinklebe, J., and Sik Ok, Y. (2017). "Effect of biochar on cadmium bioavailability and uptake in wheat (*Triticum aestivum* L.) grown in a soil with aged contamination," *Ecotoxicology and Environmental Safety* 140, 37-47. DOI: 10.1016/j.scienta.2015.03.002

Atkinson, C. J., Fitzgerald, J. D., and Hipps, N. A. (2010). "Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: A review," *Plant and Soil* 337(1-2), 1-18. DOI: 10.1007/s11104-010-0464-5

Bai, X., Zhang, S., Shao, J., Chen, A., Jiang, J., Chen, A., and Luo, S. (2022). "Exploring the negative effects of biochars on the germination, growth, and antioxidant system of rice and corn," *Journal of Environmental Chemical Engineering* 10(3). DOI: 10.1016/j.jece.2022.107398

Baum, C., El-Tohamy, W., and Gruda, N. (2015). "Increasing the productivity and product quality of vegetable crops using arbuscular mycorrhizal fungi: A review," *Scientia Horticulturae* 187, 131-141. DOI: 10.1016/j.scienta.2015.03.002

Cavagnaro, T. R. (2008). "The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: A review," *Plant and Soil* 304, 315-325. DOI: 10.1007/s11104-008-9559-7

Deng, Z., Xia, A., Huang, Y., Zhu, X., Zhu, X., and Liao, Q. (2022). "The correlation between the physicochemical properties and enzymatic hydrolyzability of hydrothermal pretreated wheat straw: A quantitative analysis," *Bioresource Technol.* 359, article 127475. DOI: 10.1016/j.biortech.2022.127475

Figueiredo, C. C. D., Farias, W. M., Coser,T. R., Paula, A. M.D., Silva, M. R.S.D., and Paz-Ferreiro, J. (2019). "Sewage sludge biochar alters root colonization of mycorrhizal fungi in a soil cultivated with corn," *European Journal of Soil Biology* 93, 1164-5563. DOI: 10.1016/j.ejsobi.2019.103092

Gong, H., Zhao, L., Rui, X., Hu, J., and Zhu, N. (2022). "A review of pristine and modified biochar immobilizing typical heavy metals in soil: Applications and challenges," *J. Hazard Mater.* 432, article 128668. DOI: 10.1016/j.jhazmat.2022.128668

Hammer, E. C., Forstreuter, M., Rillig, M. C., and Kohler, J. (2015). "Biochar increases arbuscular mycorrhizal plant growth enhancement and ameliorates salinity stress," *Applied Soil Ecology* 96, 114-121. DOI: 10.1016/j.apsoil.2015.07.014

Hou, J., Pugazhendhi, A., Sindhu, R., Vinayak, V., Thanh, N. C., Brindhadevi, K., Chi, N. T. L., and Yuan, D. (2022). "An assessment of biochar as a potential amendment to enhance plant nutrient uptake," *Environ Res.* 214(Pt 2), article 113909. DOI: 10.1016/j.envres.2022.113909

Hu, J., Yang, A., Zhu, A., Wang, J., Dai, J., Wong, M, H., and Lin, X. (2015). "Arbuscular mycorrhizal fungal diversity, root colonization, and soil alkaline phosphatase activity in response to maize-wheat rotation and no-tillage in North China," *Journal of Microbiology* 53, 454-61. DOI: 10.1007/s12275-015-5108-2

Huo, W.-g., Chai, X.-f., Wang, X.-h., Batchelor, W. D., Kafle, A., and Feng, G. (2022). "Indigenous arbuscular mycorrhizal fungi play a role in phosphorus depletion in organic manure amended high fertility soil," *Journal of Integrative Agriculture* 21(10), 3051-3066. DOI: 10.1016/j.jia.2022.07.045

Kabir, A. H., Debnath, T., Das, U., Prity, S. A., Haque, A., Rahman, M. M., and Parvez, M. S. (2020). "Arbuscular mycorrhizal fungi alleviate Fe-deficiency symptoms in sunflower by increasing iron uptake and its availability along with antioxidant defense," *Plant Physiol. Biochem.* 150, 254-262. DOI: 10.1016/j.plaphy.2020.03.010

Khan, T. A., Saud, A. S., Jamari, S. S., Rahim, M. H. A., Park, J.-W., and Kim, H.-J. (2019). "Hydrothermal carbonization of lignocellulosic biomass for carbon rich material preparation: A review," *Biomass and Bioenergy* 130. DOI: 10.1016/j.biombioe.2019.105384

Kung, C. C., Kong, F., and Choi, Y. (2015). "Pyrolysis and biochar potential using crop residues and agricultural wastes in China," *Ecological Indicators* 51, 139-145. DOI: 10.1016/j.ecolind.2014.06.043

Lehmann, A., and Rillig, M. C. (2015). "Arbuscular mycorrhizal contribution to copper, manganese and iron nutrient concentrations in crops – A meta-analysis," *Soil Biology and Biochemistry* 81, 147-158. DOI: 10.1016/j.soilbio.2014.11.013

Li, C., Ahmed, W., Li, D., Yu, L., Xu, L., Xu, T., and Zhao, Z. (2022). "Biochar suppresses bacterial wilt disease of flue-cured tobacco by improving soil health and functional diversity of rhizosphere microorganisms," *Applied Soil Ecology* 171. DOI: 10.1016/j.apsoil.2021.104314

Li, J.-F., He, X.-H., Li, H., Zheng, W.-J., Liu, J.-F., and Wang, M.-Y. (2015). "Arbuscular mycorrhizal fungi increase growth and phenolics synthesis in *Poncirus trifoliata* under iron deficiency," *Scientia Horticulturae* 183, 87-92. DOI: 10.1016/j.scienta.2014.12.015

Li, X., Cao, Y., Xiao, J., Salam, MMA., and Chen, G. (2022). "Bamboo biochar greater enhanced Cd/Zn accumulation in Salix psammophila under non-flooded soil compared with flooded," *International Journal of Phytoremediation* 23, 658-668. DOI: 10.1007/s42773-022-00139-0

Li, X., Romanya, J., Li, N., Xiang, Y., Yang, J., and Han, X. (2022). "Biochar fertilization effects on soil bacterial community and soil phosphorus forms depends on the application rate," *Sci. Total Environ.* 843, article 157022. DOI: 10.1016/j.scitotenv.2022.157022

Liao, W., Zhang, X., Ke, S., Shao, J., Yang, H., Zhang, S., and Chen, H. (2022). "Effect of different biomass species and pyrolysis temperatures on heavy metal adsorption, stability and economy of biochar," *Industrial Crops and Products* 186. DOI: 10.1016/j.indcrop.2022.115238

Liu, H., Zhao, P., Qin, S., and Nie, Z. (2018a). "Chemical fractions and availability of zinc in winter wheat soil in response to nitrogen and zinc combinations," *Front Plant Sci. 9*, 1489. DOI: 10.3389/fpls.2018.01489

Liu, L., Li, J., Yue, F., Yan, X., Wang, F., Bloszies, S., and Wang, Y. (2018b). "Effects of arbuscular mycorrhizal inoculation and biochar amendment on maize growth, cadmium uptake and soil cadmium speciation in Cd-contaminated soil," *Chemosphere* 194, 495-503. DOI: 10.1016/j.chemosphere.2017.12.025

Liu, W., Zheng, C., Fu, Z., Gai, J., Zhang, J., Christie, P., and Li, X. (2014). "Facilitation of seedling growth and nutrient uptake by indigenous arbuscular mycorrhizal fungi in intensive agroecosytems," *Biology and Fertility of Soils* 50, 381-94. DOI: 10.1007/s00374-013-0859-6

Qu, J., Shi, J., Wang, Y., Tong, H., Zhu, Y., Xu, L., Wang, Y., Zhang, B., Tao, Y., Dai, X., Zhang, H., and Zhang, Y. (2022). "Applications of functionalized magnetic biochar in environmental remediation: A review," *J. Hazard Mater.* 434, article 128841. DOI: 10.1016/j.jhazmat.2022.128841

Rillig, M. C., Wagner, M., Salem, M., Antunes, P. M., George, C., Ramke, H.-G., Titirici, M. M, and Antonietti, M. (2010). "Material derived from hydrothermal carbonization: Effects on plant growth and arbuscular mycorrhiza," *Applied Soil Ecology* 45(3), 238-242. DOI: 10.1016/j.apsoil.2010.04.011

Rodriguez Correa, C., Hehr, T., Voglhuber-Slavinsky, A., Rauscher, Y., and Kruse, A. (2019). "Pyrolysis vs. hydrothermal carbonization: Understanding the effect of biomass structural components and inorganic compounds on the char properties," *Journal of Analytical and Applied Pyrolysis* 140, 137-147. DOI: 10.1016/j.jaap.2019.03.007

Sui, L., Tang, C., Cheng, K., and Yang, F. (2022). "Biochar addition regulates soil phosphorus fractions and improves release of available phosphorus under freezing-thawing cycles," *Sci. Total Environ.* 848, article 157748. DOI: 10.1016/j.scitotenv.2022.157748

Torabian, S., Farhangi-Abriz, S., and Alaee, T. (2021). "Hydrochar mitigates salt toxicity and oxidative stress in maize plants," *Archives of Agronomy and Soil Science* 67, 1104-1118. DOI: 10.1080/03650340.2020.1779227

Wang, Z., Cao, M., Cai, W., and Zeng, H. (2017). "The effect of humic acid and fulvic acid on adsorption-desorption behavior of copper and zinc in the yellow soil," *AIP Conference Proceedings* 1820(1), article 040027. DOI: 10.1063/1.4977299

Warnock, D. D., Lehmann, J., and Kuyper, T. W. (2007). "Mycorrhizal responses to biochar in soil – concepts and mechanisms," *Plant Soil* 300, 9-20. DOI: 10.1007/s11104-007-9391-5

Watts-Williams, S. J., Smith, F. A., McLaughlin, M. J., Patti, A. F., and Cavagnaro, T. R. (2015). "How important is the mycorrhizal pathway for plant Zn uptake?" *Plant and Soil* 390(1-2), 157-166. DOI: 10.1007/s11104-014-2374-4

Qing, Y., Han, F., Chen, Y., Yang, H., and Chen, H. (2016). "Greenhouse gas emissions of a biomass-based pyrolysis plant in China," *Renewable and Sustainable Energy Reviews* 53, 1580-90. DOI: 10.1016/j.rser.2015.09.049

Yin, S., Zhang, X., Suo, F., You, X., Yuan, Y., Cheng, Y., Zhang, C., and Li, Y. (2022). "Effect of biochar and hydrochar from cow manure and reed straw on lettuce growth in an acidified soil," *Chemosphere* 298, article 134191. DOI: 10.1016/j.chemosphere.2022.134191

Yu, B., Chen, X., Cao, W., Liu, Y., and Zou, C. (2020). "Responses in zinc uptake of different mycorrhizal and non-mycorrhizal crops to varied levels of phosphorus and zinc applications," *Frontiers in Plant Science* 11, article 606472. DOI: 10.3389/fpls.2020.606472

Zhang, W., Chen, X., Liu, Y., Liu, D., Chen, X., and Zou, C. (2017). "Zinc uptake by roots and accumulation in maize plants as affected by phosphorus application and arbuscular mycorrhizal colonization," *Plant Soil* 413, 59-71. DOI: 10.1007/s11104-017-3213-1

Zhang, X., Zhang, P., Yuan, X., Li, Y., and Han, L. (2020). "Effect of pyrolysis temperature and correlation analysis on the yield and physicochemical properties of crop residue biochar," *Bioresource Technology* 296, article 122318. DOI: 10.1016/j.biortech.2019.122318

Article submitted: November 29, 2022; Peer review completed: January 21, 2023; Revised version received and accepted: February 23, 2023; Published: March 1, 2023.
DOI: 10.15376/biores.18.2.2981-2997