EFFECT OF BIOCHAR ON THE ACTIVITY OF ENZYME IN THE NUTRIENT CYCLE OF TYPICAL SOIL AGGERGATES IN NORTHEAST CHINA

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Abstract. This study used a potted experiment to apply biochar to black soil (BCS) and saline-alkali soil (BSAS) in Northeast China, with a dosage of 0 and 300 g biochar/pot applied, respectively. The original black soil (CS) and saline-alkali soil (SAS) were used as controls to investigate the effects of different particle sizes of soil aggregates on the activity of enzymes related to carbon, nitrogen, and phosphorus cycle. Compared with CS and SAS, the soil organic matter (SOM) values for biochar added into the black soil (BCS) and saline-alkali soil (BSAS) treatments were increased significantly by 41.86 % and 31.98 %. Compared with CS, BCS significantly increased the polyphenol oxidase and β-1,4-glucosidase activity. Polyphenol oxidation activity, endo-1,4-β-D-glucanohydrolase activity, β-1,4-glucosidase activity and xylanase activity are more active in particles with a diameter of >2 mm. The alkaline phase activity and Coenzyme activity with BCS treatment was highest in soil aggregates with sizes of 1~2 mm and >5 mm, respectively. Mantel test analysis shows a highly significant effects between SOM and total nitrogen content (TN) with soil nitrogen, phosphorus, and potassium cycling. Therefore, from the perspective of soil enzyme activities, application of biochar is more effective in promoting soil bio-fertility in northeastern soil of China. Biochar to the soil is more likely to promote the improvement of soil biological fertility.

Keywords: *biochar, black soil, saline-alkali soil, soil aggregate, soil enzyme, nutrient cycle*

Introduction

The northeast region of China is a crucial grain production area in the country, known for its prime soil types that provide favorable growth conditions for major cereal crops. With abundant farmland resources and a favorable climate, the northeast region has become a significant contributor to grain production in China (He and Mao, 2023). Its significance lies in its role in ensuring national food security, promoting sustainable agricultural development, and fostering regional economic prosperity (Wu et al., 2016; Long et al., 2018).

Biochar has the potential to be applied in various ways to improve agricultural soils (Baiamonte et al., 2019). In the specific context of protecting black soil and promoting green agricultural development in Northeast China, biochar can play a crucial role (Chen et al., 2019). It can help preserve and enhance the quality of black soil, promote sustainable agricultural practices, support the growth of organic farming, reduce the environmental impact of pesticides and fertilizers, and facilitate carbon sequestration to mitigate climate change (Wen and Liang, 2001). Biochar also shows promise in improving saline-alkali soils. Its highly porous structure and negatively charged surface allow it to adsorb and immobilize salts, thereby reducing soil salinity (Liang et al., 2021). Additionally, it can neutralize soil alkalinity by reducing the content of alkaline substances, leading to an improved soil pH balance (Zhao et al., 2020; Wang et al., 2022; Shi et al., 2022). Another advantage of biochar is its ability to retain water, which

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enhances the retention capacity of the soil, reduces water evaporation, and minimizes leaching (He et al., 2020). Furthermore, biochar can adsorb and retain nutrients, providing essential plant nutrients and contributing to improved fertility in saline-alkali soils (Gong et al., 2021). Lastly, biochar creates a favorable habitat for microbial growth, increasing the quantity and diversity of soil microorganisms. These microorganisms play a crucial role in degrading harmful substances, improving soil structure, and supplying plant nutrients, thereby mitigating the adverse effects of saline-alkali soils (Shi et al., 2019).

Soil enzymes play a crucial role in soil energy metabolism and the transformation of organic and inorganic substances (Rao et al., 2014). They are involved in various biochemical processes, including nutrient cycling (Burke et al., 2011), organic matter decomposition (Štursová and Baldrian, 2011), and pollutant degradation (Wołejko et al., 2020). The activity level of soil enzymes serves as a significant indicator of soil biological characteristics and fertility status. Therefore, studying the response of soil enzyme activity to biochar can provide a better understanding of how biochar influences soil biological fertility mechanisms (Khadem and Raiesi, 2017). This knowledge can guide the application of biochar by selecting appropriate types, application rates, and methods to maximize soil fertility and improve soil quality (Awad et al., 2018). Additionally, it is crucial to investigate the application effects and suitability of biochar in protecting typical soils in Northeast China to ensure their sustainable use and healthy development.

Soil aggregates are integral to the structure of soil and have a significant impact on the soil ecosystem (Tisdall, 2020). Understanding the relationship between soil aggregate size and enzyme activity is a crucial area of research. The characteristics of soil aggregates, such as pore structure and water-holding capacity, have a direct effect on the survival and activity of soil microorganisms, which includes enzyme-producing microorganisms (Kumar et al., 2017). Coarser soil aggregates typically have larger pores and lower water-holding capacity, allowing for better oxygen permeability and faster water drainage (Qiao et al., 2019). This creates favorable conditions for aerobic microorganisms and enzymes. On the other hand, finer soil aggregates have smaller pores and higher water-holding capacity, which provide suitable conditions for moisture-dependent microbial populations and enzyme activity (Zhang et al., 2017). Therefore, the objective of this study is to investigate the impact of biochar on enzyme activity related to soil nutrient cycling at the scale of soil aggregates. The findings of this research will contribute to the theoretical foundations and provide data support for the effective utilization and enhancement of fertility in agricultural soils in Northeast China.

Materials and Methods

Site description

This study was conducted in the pot experiment area of the Modern Agricultural Demonstration Park at Harbin University in Harbin (126°50' E, 45°50' N), Heilongjiang Province in 2022. The area of soil is black soil (Chernozem). The study site is classified as a typical temperate and monsoonal climate with a maximum potential rainfall of 557 mm and mean annual temperature is ≥10 °C (Ding, 2023a).

The sampling area for saline-alkali soil testing is located within the Sifang Mountain Farm in Zhaodong City, Heilongjiang Province (125° 45’ E, 46° 12’ N), with flat terrain and a single type of landform. The area of soil is carbonate meadow alkaline soil. The average annual temperature is 2.4 °C, the annual evaporation is 1662 mm, the average annual rainfall is 396 mm. According to the USDA soil taxonomy system, the soil was predominantly loamy and saline-alkaline experimental, which is a soda saline soil dominated by sodium carbonate bicarbonate (Ding, 2023b).

Tested materials

Biochar is a stable, carbon-rich product made from agricultural waste biomass, such as crop straw and peanut shells, via pyrolysis under low temperature and anoxic conditions. The tested biochar was commercially supplied by Liao Ning Golden Future Agriculture Technology Co., Ltd. The biochar presented pH 8.69 and $N:P_2O_5:K_2O$ ratio equal to 8:11:15.

Experimental design

The experiment started on June 5 and ended on September 10, in 2022. Pot experiments were performed using polypropylene plastic pots with a height and diameter of 44 × 110 cm. Biochar and air-dried soil were well mixed and placed into the pot experiments. Four treatments were set as follows: (1) no biochar was added into the black soil (CS); (2) 40 g of biochar were added into 1 kg of black soil (BCS), i.e., 300 g biochar/pot; (3) no biochar was added into the saline-alkali soil (SAS); (4) 40 g of biochar were added into 1 kg saline-alkali soil (BSAS), i.e., 300 g biochar/pot. Each treatment is repeated three times, soil samples were collected on the 60th day of culture, a total of 12 soil samples were collected (Ding, 2023a).

Soil sampling

All collected soil samples should be stored in sterilized plastic containers, labeled, and brought back to the laboratory for storage at 4°C. In the laboratory, a portion of the soil samples will be mixed and ground, sieved through a 2 mm sieve, and used to determine the physical and chemical properties as well as soil enzyme activity. The remaining soil samples will be air-dried and stored in a 4°C refrigerator for determining the soil aggregate content using the improved dry sieving method (Zhang et al., 2021; Ding et al., 2021). The soil moisture content will be measured every 6 hours until the mass moisture content reaches approximately 10% to 15%. Once the soil reaches this moisture range, it will be sieved through an 8 mm sieve and placed into a nested set of sieves with aperture sizes of 5 mm, 2 mm, 1 mm, and 0.25 mm, from top to bottom. Each sieving process will involve 200 g of soil and will be conducted for 5 minutes. The aggregates of particle sizes > 5 mm, 5-2 mm, 2-1 mm, 1-0.25 mm, and < 0.25 mm will be obtained, and their respective masses will be measured.

Soil chemical analysis

Soil pH was determined in a 2.5:1 water/soil suspension using a pH meter. Soil exchange capacity (EC) was determined by the $BaCl_2$ compulsive exchange method. The Walkley-Black titration method was carried out to determine the soil’s organic matter (SOM) content. The methods of concentrated H_2SO_4 digestion and Kjeldahl were used to determine the total nitrogen content (TN) of the soil samples (Abujabhah et al., 2016). Total phosphorus content (TP) of the soil samples was determined by $HClO_4$ and H_2SO_4 digestion molybdenum antimony anti-colorimetry (Pan et al., 2016). Total potassium content (TK) was determined by atomic absorption spectrophotometry (Guan, 1986). The soil’s available nitrogen (AN) was measured using the Alkali-diffusion method (Deng et al., 2016). Determination of the available phosphorus (AP) in soil was measured by using

$NaHCO_3$ extraction- Mo-Sb Anti-colorimetry (Mehlich, 1984). Available potassium content (AK) was extracted with 1 $mol \cdot L^{-1}$ NH_4OAc, and then determined by flame absorption spectroscopy (Bao, 2005). According to the manufacturer's instructions, the enzyme activities of 16 different enzymes will be determined using an enzyme-linked immunosorbent assay (ELISA) kit (manufactured by Jiangsu Enzyme Immunoassay Co., Ltd.), Including Polyphenol oxidase activity, Lignin peroxidase activity, Endo-1,4-β-D-glucanohydrolase activity, Xylanase activity, β-1,4-glucosidase activity, Nitrogenase activity, Ammonia monooxygenase activity, Nitrite reductase activity, Nitrate reductase activity, Nirtric oxide reductase activity, Nitrous oxide reductase activity, N-acetyl-glucosaminidase activity, Leucine aminopeptidase activity, Alkaline phosphatase activity, Fluorescein diacetate activity and Coenzyme activity (Shi et al., 2023).

Statistical analysis

We used R v. 3.1.2 (R Core Team, 2013) and SPSS version 16.0 for Windows (SPSS Inc., USA) to generate thermographs and Venn diagrams, and for one-way analysis of variance (ANOVA). Graphs were generated using Origin 2019. We adopted a 0.05 level of probability to test main effects and interactions. Pairwise comparison of means was tested via Tukey honestly significant difference (HSD) procedure, also at $\alpha = 0.05$. Herein, “significant” is reserved to mean statistically significant; effect magnitudes are expressed with other descriptors. The geometric mean (GEA) of all enzyme activities in each soil sample was calculated as the comprehensive enzyme activity index of soil quality (García-Ruiz et al., 2008).

Results

Soil physical and chemical properties

When biochar was applied, it resulted in changes in the physical and chemical properties of the soil (*Table 1*). The BCS treatment led to an increase in SOM, TN, TP, TK, AN, AP, AK, and EC ($p < 0.05$). Additionally, the SOM content in the BCS and BSAS treatments showed a significant increase of 41.86% and 31.98% respectively, when compared to the CS and SAS treatments ($p < 0.05$). While the AN, AP, and AK levels in the SAS treatment were significantly higher than in the CS treatment, these soil indicators showed a significant increase in the BCS treatment.

Effect of biochar on soil carbon cycling related enzyme activity

The enzyme activities related to carbon cycling in soil aggregates of different particle sizes under biochar treatments are shown in *Figure 1.* Compared to CS and SAS, both BCS and BSAS treatments have a notable enhancing effect on the Polyphenol oxidation activity of soil aggregates with <0.25 mm, 0.25~1 mm, 1~2 mm, and >5 mm. There was no significant difference in the Polyphenol oxidation activity between CS and BCS treatments in soil aggregates with a particle size of 2~5 mm. However, the application of biochar increased the Polyphenol oxidation activity in soil aggregates with all particle sizes in saline-alkali soil. In the particle size range of <0.25 mm, the β-1,4-glucosidase activity in BCS treatment decreased by 9.21% compared to CS. However, in the particle size range of 0.25~1 mm, both BCS and BSAS treatments showed a significant increase in β-1,4-glucosidase activity ($p < 0.05$) compared to CS and SAS treatments, with increases of 39.17% and 14.82%, respectively.

Table 1. *Soil physical and chemical properties in different treatments (means ± S.D., n = 3)*

Index	**Sample plot**							
	CS		**BCS**		**SAS**		**BSAS**	
pH	7.31±0.09	*b*	7.38± 0.11	*b*	9.80±0.44	*a*	9.61±0.08	*a*
SOM (g·kg^{-1})	21.14±1.51	*c*	50.49±1.08	*a*	12.63±0.58	*b*	33.25±1.04	*b*
TN (g·kg^{-1})	0.41±0.08	*b*	6.12±0.57	*a*	3.44±0.10	*c*	4.64±0.70	*b*
AN (mg·kg^{-1})	95.23±5.12	*b*	111.96±4.20	*a*	48.64±5.88	*d*	70.44±8.81	*c*
TP (g·kg^{-1})	0.41±0.08	*b*	0.53±0.09	*a*	0.36±0.01	*c*	0.51±0.07	*a*
AP (mg·kg^{-1})	29.31±0.55	*c*	41.13±2.47	*a*	32.23±1.17	*b*	29.37±2.84	*c*
TK (g·kg^{-1})	1.73±0.71	*c*	5.10±0.38	*a*	3.72±0.16	*b*	3.77±0.32	*b*
AK (mg·kg^{-1})	1733.23±12.82	*d*	3097.23±18.91	*a*	2631.55±13.92	*c*	2858.35±65.38	*b*
EC (mS·cm^{-1})	7.67±0.47	*b*	14.11±3.22	*a*	6.11±1.13	*b*	12.56±4.38	*a*

Note: data represent the mean ± standard deviations. Analysis of variance (Duncan's multiple comparison test) was used to test the significance of differences. The different lowercase letters represent significant differences ($p < 0.05$), the same below. pH, the soil pH value. TN, the total nitrogen content of the soil samples. TP, the total phosphorus content of the soil samples. AN, the available nitrogen of the soil samples. AP, the available phosphorus content of the soil samples. SOM, the soil's organic matter content. EC, the exchange capacity in soils. The soil available phosphorus (AP) and TK, the total potassium content of the soil samples. AK, the available potassium content of the soil samples

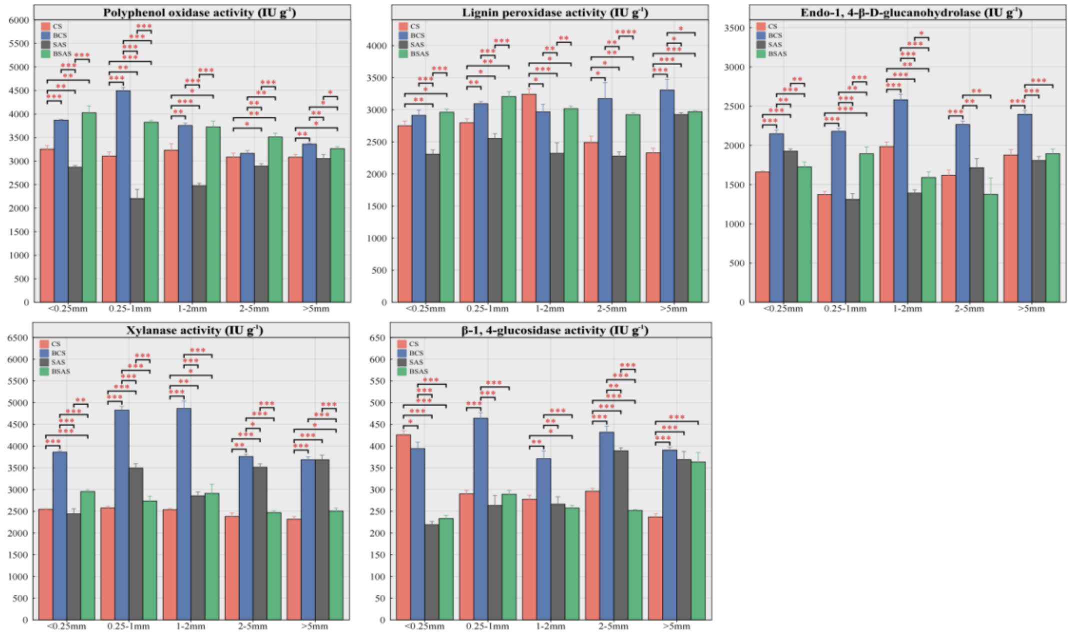

Figure 1. *Effects of biochar on soil carbon cycling enzyme activities. Note: different letters above the bars indicate statistical differences among treatments in the same size, the horizontal line indicates the difference between two groups ($p < 0.05$, n = 3), "*" indicates a significant interaction at the 0.05 level; "**" indicates a significant interaction at the 0.01 level; "***" indicates a significant interaction at the 0.001 level, the same below*

Conversely, the BSAS treatment exhibited a decrease of 5.63%, 37.27%, and 6.11% in polyphenol oxidation activity in soil aggregates with particle sizes of 1~2 mm, 2~5 mm, and >5 mm, respectively, when compared to SAS. Compared to CS and SAS treatments, both BCS and BSAS treatments significantly increased Lignin peroxidation activity with <0.25 mm, 0.25~1 mm, 1~2 mm, and >5 mm. In the 1~2 mm particle size, the activity of BCS treatment is higher than that of CS. The application of biochar (BCS) can increase Xylanase activity and Endo-1, 4-β-D-glucanohydrolase activity in soil aggregates across all particle sizes. As the soil particle size increases, the Xylanase activity gradually decreases when biochar (BSAS) is applied to saline-alkali soil.

Effect of biochar on the activity of enzymes related to the soil nitrogen cycle

Nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase are key enzymes that control soil denitrification processes. The enzyme activities related to nitrogen cycling in soil aggregates of different particle sizes under biochar treatments are shown in *Figure 2*. BCS and BSAS were significantly increased the activity of ammonia monooxygenase, nitrate reductase, nitrate oxide reductase, nitrate oxide reductase, N-acetyl-glucosaminidase, and leucine aminopeptidase in full particle size aggregates. There is no significant difference in Nitrogenase activity between SAS and BSAS treatments in saline-alkali soil. After applying biochar treatment to black soil, the enzyme activity related to nitrogen cycling in each soil was highest with a particle size of >5 mm.

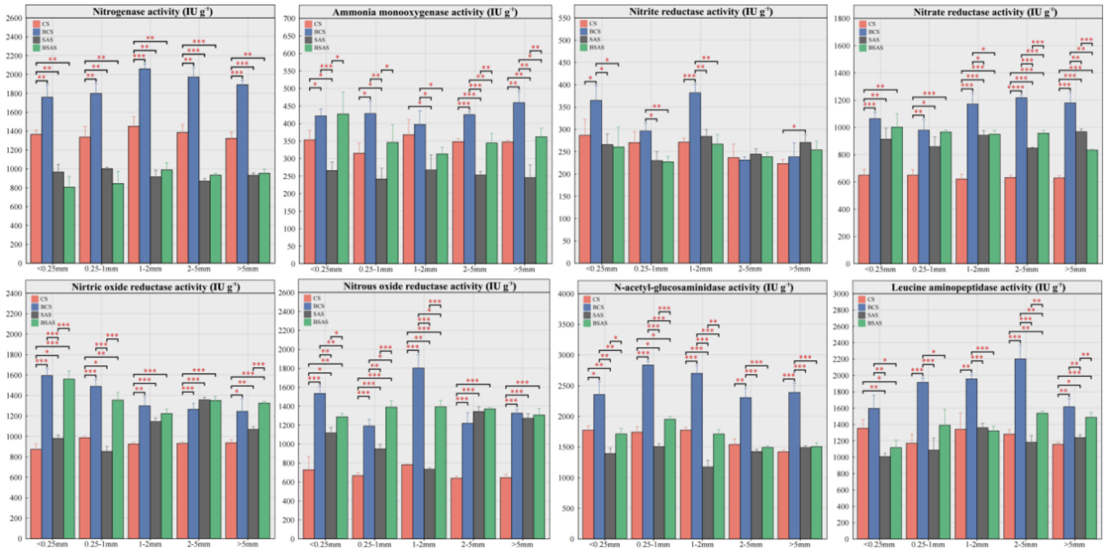

Figure 2. *Effects of biochar on soil nitrogen cycling enzyme activities*

Effect of biochar on the activity of enzymes related to the soil phosphorus cycle

As shown in *Figure 3*. Compared with CS, the application of biochar (BCS) in black soil significantly improved the Alkaline phosphatase activity, Fluorescein diacetate activity, and Coenzyme activity, and increased by 25.96%, 36.88%, and 24.25% in the 1~2mm particle size range, respectively. Application of biochar (BSAS) in saline-alkali soil resulted in significantly higher Alkaline phosphatase activity and Coenzyme activity in particles with a diameter of <0.25 mm compared to SAS. BSAS treatment increased

Coenzyme activity by 13.16% compared to SAS treatment in particle sizes >5 mm. Overall, the Alkaline phase activity and Coenzyme activity with BCS treatment were the highest in particle sizes of 1-2 mm and >5 mm, respectively.

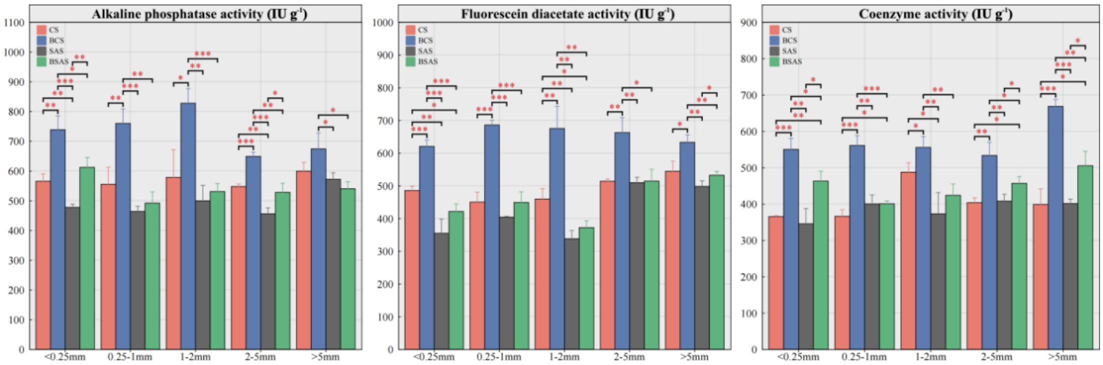

Figure 3. *Effects of biochar on the activities of alkaline phosphatase, Fluorescein diacetate activity and Coenzyme activity in soil*

Comprehensive index of soil enzyme activity in aggregates of each particle size under different treatments

Compared with CS and SAS, BCS and BSAS treatments were significantly increased GMea in all particle size aggregates (*Table 2*). The enzyme activity of particles with a diameter of<0.25 mm and >5 mm in BCS treatment increased by 24.85% and 32.96% compared to CS treatment. The enzyme activity of particles with a diameter of<0.25 mm and >5 mm in BSAS treatment increased by 18.71% and 2.73% compared to SAS treatment.

Table 2. *Comprehensive index of enzyme activities of each treatment (means ± S.D., n = 3)*

Treatment	**Comprehensive index of enzyme activities**							
	CS		**BCS**		**SAS**		**BSAS**	
<0.25 mm	904.72±11.25	*c*	1204.01±21.62	*a*	807.40±17.71	*d*	993.35±34.38	*b*
0.25~1 mm	842.04±17.55	*c*	1241.43±28.42	*a*	799.11±11.05	*d*	950.68±27.72	*b*
1~2 mm	904.24±10.04	*b*	1272.68±21.25	*a*	824.58±15.79	*c*	925.21±13.68	*b*
2~5 mm	834.95±7.61	*c*	1205.75±36.66	*a*	922.55±14.14	*b*	924.68±24.11	*b*
>5 mm	819.72±4.50	*c*	1222.86±35.61	*a*	911.28±10.71	*b*	936.91±13.34	*b*

Mantel test analysis of soil enzyme activity with soil physicochemical properties

As shown in *Figure 4*, SOM had highly significant effects on the soil carbon cycling and soil nitrogen cycling. EC, TP, TK and AK had significant effects on soil carbon cycling. SOM and TN had highly significant effects on soil phosphorous cycling and pH and AN were significant effects. EC, TN and AN on soil nitrogen cycling were significant effect, but pH and AK were not significant.

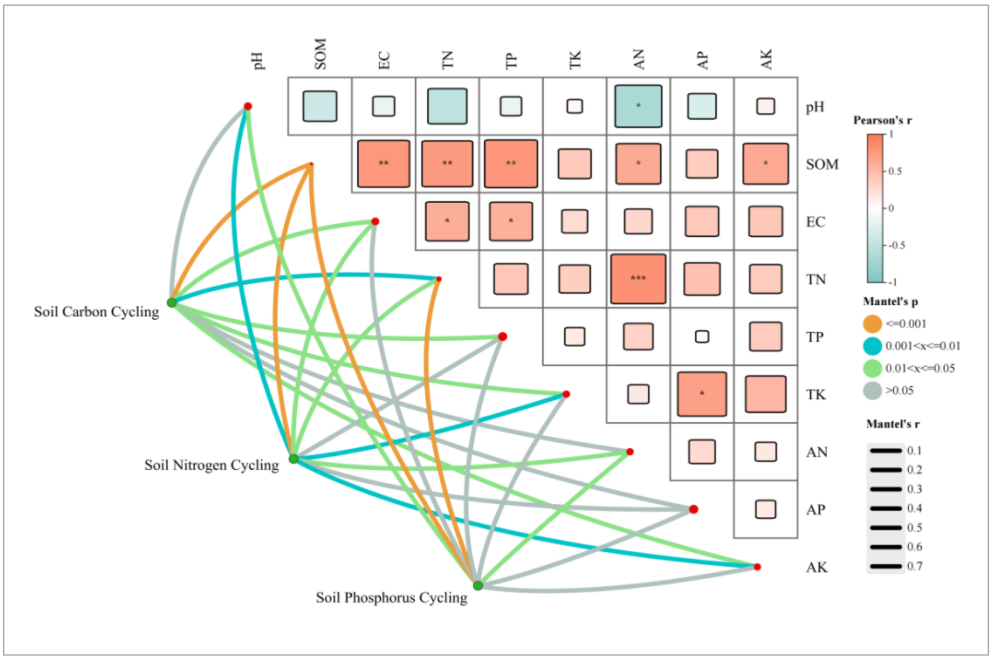

***Figure 4.** Mantel test analysis based on the relative activity of key enzymes of the soil nutrient cycling*

Discussion

Biochar may contain a certain amount of nitrogen and phosphorus during its production process. When biochar is applied to the soil, these nutrients can be released into the soil through the decomposition and degradation of biochar. Microorganisms and other soil organisms break down biochar and release nitrogen and phosphorus in forms that are available for plant absorption. Biochar has a highly porous structure and a negatively charged surface, which enables it to adsorb nitrogen and phosphorus in the soil. These nutrients exist in the form of ions in the soil solution, and biochar, through ion exchange, adsorbs these nutrients on its surface, reducing nutrient loss and leaching. Biochar forms a stable carbon structure in the soil, which can protect organic matter and nutrients from microbial decomposition and leaching. Nitrogen and phosphorus are typically bound to organic matter, and the presence of biochar can prevent the loss of these nutrients along with organic matter. In this study, the application of biochar in saline-alkali soil reduced soil pH by 8.06% compared to the CS treatment. However, some studies have suggested that biochar may not effectively improve the pH of saline-alkali soil due to the leaching of alkaline metal ions and carbonates present in biochar. Research has found that the changes in pH of saline-alkali soil are influenced by the biochar feedstock and pyrolysis temperature. For example, Sun et al. (2016) found that when wheat straw biochar (pH=6.9), corn straw biochar (pH=8.0), and peanut shell biochar (pH=7.7) were added to coastal saline soil with high chloride content (pH=8.6), only the wheat straw biochar significantly increased the soil pH, while the other two did not cause significant changes in soil pH. Further studies have confirmed that as the pyrolysis temperature increases, the ash content of biochar increases, leading to stronger alkalinity. Biochar produced at lower temperatures (<500 °C) tends to be neutral or weakly alkaline, making it more suitable for the improvement of saline-alkali soils (Tang et al., 2020).

β-1,4-glucosidase is a common enzyme that plays an important role in soil carbon cycling. It belongs to the glycosidase family and is capable of hydrolyzing β-1,4-glycosidic bonds, releasing glucose units from substrates. β-1,4-glucosidase is primarily found in various microorganisms (bacteria, fungi, etc.) as well as plant roots. β-1,4-glucosidase is involved in the breakdown of soil organic matter. It can degrade complex organic compounds such as plant residues, roots, and lignin, breaking them down into soluble glucose and other simple sugar compounds. This enables the carbon in organic matter to be utilized by microorganisms, releasing it as carbon dioxide (CO_2) and participating in soil carbon cycling. β-1,4-glucosidase hydrolyzes the glycosidic bonds in substrates, releasing glucose. This glucose can be utilized by microorganisms as an energy and carbon source, promoting microbial growth and metabolic activities, further facilitating carbon cycling in the soil. Soil aggregates, as the fundamental units of soil structure, serve as important sites for the cycling of carbon, nitrogen, phosphorus, and microbial activities. The specific microenvironments formed within soil aggregates have a significant influence on the distribution and activity of soil enzymes, consequently leading to variations in key nutrient transformation processes such as carbon, nitrogen, and phosphorus cycling (Parwada and Van Tol, 2019). This study indicates that β-1,4-glucosidase activity is higher in aggregates with a particle size of <5 mm. This could be attributed to the application of straw and biochar, which reduce the proportion of organic carbon in the intermediate-sized particles and increase the proportion of organic carbon in large and small aggregates (Guo et al., 2020). As a result, β-1,4-glucosidase activity is enhanced. Xylanase plays a crucial role in soil carbon cycling. Xylan is one of the major polysaccharides present in plant cell walls, composed of complex structures of cellulose molecules (Blonska et al., 2020). Xylanase enzymes found in soil can break down xylan into smaller xylose and glucose monomers (Wang et al., 2021). The released glucose can be utilized by soil microorganisms as a carbon source, promoting microbial growth and activity (Trivedi et al., 2016). The activity of xylanase can influence the rate and processes of carbon transformation in soil. High activity of xylanase can accelerate the degradation of xylan, providing more available carbon sources for soil microorganisms. This is essential for organic matter decomposition and carbon cycling in soil, as it can impact the stability and persistence of organic carbon (Blonska et al., 2020). Xylanase shows its main activity in aggregates with particle sizes ranging from 0.25~2 mm in black soil types. However, in saline-alkali soil types, compared to the SAS treatment, the BSAS treatment significantly reduces the xylanase activity in aggregates with particle sizes ranging from 0.25~5 mm. This indicates that the application of biochar in this experiment may significantly decrease certain soil enzyme activities related to soil carbon cycling processes (Laird et al., 2010). Polyphenol oxidase activity plays a role in promoting carbon transformation processes in the soil. It catalyzes the oxidation of polyphenolic compounds, converting them into higher molecular weight polymers (Sinsabaugh, 2010). This process alters the structure and properties of polyphenolic compounds, making them more resistant to degradation and stable in the soil (Toberman et al., 2008). Additionally, the activity of polyphenol oxidase can reduce the concentration of phenolic substances in the soil, thereby decreasing their toxicity to microorganisms. Furthermore, polyphenol oxidase is closely involved in interactions with other soil enzymes and microorganisms (Du et al., 2014). It can influence the growth and activity of soil microorganisms, thereby further regulating soil carbon cycling. The activity of polyphenol oxidase is also closely related to environmental factors such as SOM, pH, and temperature, which can affect the rate and efficacy of its catalytic reactions (Zheng et al., 2018). In this study, the addition of biochar increased the activity of polyphenol oxidase in the total particle size fraction of both black soil and saline-alkali soil. The application of biochar has a significant impact on the activity of soil hydrolytic enzymes related to carbon cycling. While the addition of biochar can enhance enzyme activity by adsorbing reactive substrates that are utilized by soil microorganisms, biochar can also adsorb various molecules in the soil and inhibit the activity of certain enzymes or their substrate reactions by affecting the reaction sites (Houssou et al., 2022). Wang et al.'s study found that the activity of polyphenol oxidase is higher in large aggregate fractions, and its activity is negatively correlated with the degree of humification of soil organic matter (Wang et al., 2000). Some studies have suggested that fungi may be the primary contributors to polyphenol oxidase in the soil (Jastrow et al., 2007). Marhan et al. (2007) found that a significant proportion of fungi is present in large particle aggregates, indicating that fungi are more abundant in coarse particle fractions. This significantly enhances the activities of cellulase and polyphenol oxidase in this fraction, which may lead to a decrease in the efficiency of lignin conversion to quinones in the soil. On the other hand, Lignin peroxidase and Endoglucanase show different responses to biochar, which could be due to the alteration of carbon distribution in soil aggregates caused by the application of biochar (Zhang et al., 2017).

Soil nitrogenase is the key enzyme involved in biological nitrogen fixation and is composed of a complex of iron protein and molybdenum-iron protein. Most microorganisms utilize the molybdenum-iron nitrogenase system for nitrogen fixation. Nitrogenase has the ability to reduce molecular nitrogen to ammonia, converting it into a nitrogen source for the synthesis of amino acids and proteins (Mikha and Rice, 2004). Research has found that high oxygen content in black soil can promote nitrogenase activity in the soil (Hao et al., 2021). In this study, the nitrogenase activity in >0.25 mm was significantly higher than in <0.25 mm. This could be attributed to the larger pore spaces and higher oxygen content in larger aggregates, which facilitate higher nitrogenase activity compared to smaller aggregates. In this study, the addition of biochar significantly increased the activity of nitrite reductase and nitrous oxide reductase in the 1~2 mm aggregate fraction, as well as the activity of nitrate reductase in the >2 mm aggregate fraction and nitric oxide reductase in the <5 mm fraction. This indicates that the application of biochar has a significant impact on soil denitrification (Yang et al., 2022). Nitrous oxide reductase is responsible for the conversion of N_2O to N_2 in the denitrification pathway, which is the final stage of soil denitrification. Therefore, increasing the activity of nitrous oxide reductase may contribute to limiting N_2O emissions from the soil. The results suggest that biochar application can influence the activity of denitrification enzymes, potentially affecting the nitrogen cycle and reducing greenhouse gas emissions from agricultural soils. However, it is important to consider the specific conditions and context of each study, as the effects of biochar on soil processes can vary depending on factors such as biochar characteristics, soil type, and environmental conditions. N-acetyl-glucosaminidase and Leucine aminopeptidase both play important roles in soil nitrogen cycling, participating in organic matter decomposition, nitrogen transformation in the soil (Piotrowska-Długosz et al., 2022). N-acetyl-glucosaminidase and Leucine aminopeptidase, both belonging to the nitrogen assimilation enzymes, exhibit completely different responses to biochar (Ma et al., 2021). This study shows that N-acetyl-glucosaminidase demonstrates higher activity in aggregates with a particle size >0.25 mm. This could be attributed to the differences in hydrolytic substrates between the two enzymes and the subsequent alteration in microbial community strategies. In other words, smaller-sized aggregates are more suitable for microorganisms capable of producing N-acetyl-glucosaminidase, while aggregates with particle sizes 2~5 mm are more favorable for microorganisms producing Leucine aminopeptidase. According to the Michaelis-Menten theory, the activity of extracellular enzymes depends on the availability of substrates, thus the differences in substrate composition within different-sized aggregates may also contribute to these divergent responses (Chen et al., 2023).

This study found that the application of biochar significantly increased alkaline phosphatase activity across all particle sizes. This is because although biochar does not contain high levels of phosphorus, it alters the soil microbial habitat, indirectly influencing enzyme activity. In this study, alkaline phosphatase exhibited higher activity in <2 mm, indicating that there is less available substrate for phosphatase utilization in medium-sized aggregates. Microorganisms need to produce more enzymes to compensate for this, suggesting that phosphorus effectiveness is higher in this particle size range. Fluorescein diacetate and Coenzyme are effective indicators for reflecting changes in soil microbial activity and are important metrics for assessing overall microbial activity in soil. Coenzyme activity shows higher activity in <2 mm, indicating an increasing trend in overall microbial activity from small to large aggregates. Although larger aggregates have larger pore sizes, allowing faster diffusion of water and nutrients, they are less stable compared to smaller aggregates and are more susceptible to external influences (Zhu et al., 2020).

Compared with CS and SAS, BCS and BSAS treatments were significantly increased GMea in all particle size aggregates. This indicates that the nature of carbon inputs into the soil has a significant impact on enzyme activity, and the mechanisms that maintain enzyme activity in soil particles vary. Organic carbon in smaller particle sizes is typically composed of aromatic and lipid compounds with a higher degree of humification, which are less readily utilized by microorganisms (Rao et al., 2019). The higher enzyme activity observed in smaller particles may be attributed to enzyme protection through adsorption by biochar or complex formation with organic carbon, indicating potential enzyme activity.

The content and quality of soil organic matter have a significant impact on soil enzyme activity. Higher organic matter content is often associated with a more abundant supply of carbon sources and nutrients, providing more substrates to support enzyme activity. Additionally, organic compounds within the organic matter can directly serve as substrates for enzymes, participating in the enzymatic reaction processes. The addition of biochar can affect the decomposition of organic matter and microbial activity in the soil, thereby influencing the cycling of phosphorus and nitrogen. Biochar can provide additional carbon sources, stimulating the decomposition and mineralization of organic matter, leading to the release of phosphorus and nitrogen. Moreover, the addition of biochar can enhance the growth environment of soil microorganisms, thereby increasing microbial activity and promoting the conversion and release of phosphorus and nitrogen (Asadyar et al., 2021). Although biochar can affect the adsorption and release of certain nutrients such as phosphorus, its impact on the availability of available potassium and nitrogen may not be significant (Barman et al., 2019). The availability of potassium is primarily influenced by soil minerals and cation exchange capacity, while nitrogen availability is influenced by various factors including microbial activity, organic matter decomposition, and the presence of nitrifying and denitrifying bacteria. Therefore, while biochar can influence soil properties and nutrient dynamics, its direct effects on nitrogen cycling and available potassium content may not be as pronounced as other soil factors. When applying biochar, it is important to combine it with other soil management practices to optimize nutrient availability and overall soil health.

Conclusions

There are significant differences in the response of enzyme activities in different particle-size aggregates to biochar. Under the experimental conditions of this study, biochar significantly increased the activity of β-1,4-glucosidase in various particle-size aggregates of both black soil and saline-alkali soil. In the denitrification enzyme system, biochar significantly increased the activity of nitrite reductase, nitrate reductase, and nitrous oxide reductase in the >2 mm particle-size aggregates. Overall, biochar significantly increased the activity of enzymes in soil aggregates of various particle sizes in the tested soils. The soil nutrient cycling processes showed significant correlations with environmental factors such as SOM and TN. Therefore, considering the enzyme activity involved in carbon, nitrogen, and phosphorus nutrient cycling in typical black soil and saline-alkali soil in Northeast China, the application of biochar is beneficial for improving soil biological fertility.

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