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Effects of rice husk biochar and biochar-based fertilizer application on soil enzyme activities and bacterial communities in continuously cropped ginseng

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ABSTRACT

In field experiment, we evaluated the effects of two soil amendments biochar A10 (rice husk biochar) and B10 (mixture of biochar +organic or inorganic fertilizers) applied at 10 t·hm^{-2} on the enzymatic activities and bacterial community structures in soils under continuous ginseng cropping (CK) at various sampling dates. A10 and B10 treatments increased the activities of enzymes [soil acid phosphatase (S-ACP), soil sucrase (S-SC), soil catalase (S-CAT) and soil laccase (S-SL)], the highest increase was in S-SL activities. Short-term application of biochar and biochar-based fertilizer decreased the abundances of some soil bacterial groups (Acidobacteria, Chloroflexi, Verrucomicrobia, Myxococcota and Crenarchaeota). The soil enzyme activities had variable effects on the dynamics of different bacterial populations. A10 and B10 treatments increased the relative abundances of Firmicutes, Gemmatimonadota, Bacteroidota and Patescibacteria and decreased the relative abundances of Acidobacteria, Chloroflexi, Verrucomicrobia, Myxococcota and Crenarchaeota. Correlation analysis revealed close relationships between the soil enzyme activities and some soil bacterial populations. The changes in bacterial abundances were also associated with altered soil enzyme activities. In particular, S-ACP and S-SL were key factors associated with bacterial community structures. This study revealed the effects of rice husk biochar and biochar-based fertilizer amendment on soil enzyme activities and microbial community structures in continuous ginseng cropping.

Keywords: Bacteria, biochar fertilizer, continuous cropping problem, enzyme activity, rice husk biochar, soil microorganisms.

INTRODUCTION

Biochar contains highly aromatized refractory solid polymers produced by pyrolysis and carbonization of plant materials at 200-700 °C under anoxic or partly anoxic conditions (16,28). The physicochemical properties of biochar vary greatly depending on the raw materials and pyrolysis temperatures (6,15). The International Biochar Initiative (IBI) reported that biochar application in soil is ecofriendly and useful in agriculture (25). Its application promotes the plants growth by (i). increasing soil organic matter content, (ii). improving the soil physicochemical properties (26), (iii). increasing soil water holding capacity (4), (iv). improving nutrients availability and recycling in soil (5,30), (v). increasing soil cation exchange capacity (CEC) (12), (vi). alleviating soil acidification (7)

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and (vii). improving soil microbial activities (8). Hence recently, the biochar of corn straw, rice husk, peanut shell, walnut shell and wood chip are used to increase crop yields. However, the lack of nutrients in biochar has led to the additional use of biochar-based fertilizer (mixture of biochar +organic or inorganic fertilizers) to replace chemical fertilizers (11,33).

Ginseng is called the “king of herbs” owing to its use as traditional Chinese medicine for thousands of years and its extremely high medicinal and economic value. Ginseng plants are mainly distributed in the foothills of Changbai Mountains, Northeastern China. Despite numerous attempts to cultivate ginseng in farmland, its area is limited due to its continuous cropping problem, ecological deterioration, low yields, poor quality and high levels of pesticide residues. Thus, there is a pressing need to re-use ginseng fields in forests. The autotoxicity of ginseng and the imbalanced fungal communities in soil are key factors in continuous cropping problem of ginseng. Particularly, harmful fungal species [*Fusarium solani* (18), *Botrytis cinerea* (27), *Erwinia carotovora* (2) and *Cylindrocarpon destructans* (Zinns) Scholten (18,19)] abundances were increased in these environments. While the abundances of beneficial bacteria were decreased leading to changes in soil microbial abundances and community structures. Soil microorganisms are important sources of enzymes involved in soil biochemical reactions and affects the abundances and community structures of soil microorganisms.

Biochar contains highly aromatized refractory solid polymers produced by pyrolysis and carbonization of plant materials at 200-700°C under anoxic or partly anoxic conditions. Their application increases the soil bacterial diversity (1,31). However, the effects of biochar from different raw materials on the soil microbial community structures varies greatly and related to the compositions, structures, physicochemical properties and application rates of biochar (3,17). This study aimed to investigate the effects of rice husk biochar and biochar-based fertilizer on soil enzyme activities in addition to bacterial diversity, abundances and community structures in soils under continuous ginseng cropping.

MATERIALS AND METHODS

I. Experimental Site

The field experiment was conducted from September 2019 to October 2010 in Zuojia Town, Jilin City, Jilin Province, China (286.5 m, 125° 59′E, 43° 57′N). The experimental site has temperate continental monsoon climate with 4-distinct seasons: dry/windy spring, intensive rainfall in summer, mild/cool autumns and dry/cold winters with little snow). Annually, the mean temperature: 4.5 ℃, the mean growth period: 128 days, mean precipitation: 550 mm and mean frost-free period: 130 days. Rice husk biochar and biochar-based fertilizer were purchased from Adel Biomass Technology (Tianjin, China) and chloropicrin was used as soil fumigant from Lvfeng Chemical Co., Ltd. (Dalian, China).

II. Experimental Design

The experimental plots size were 1.8 m × 1.6 m and plots were spaced 20 cm apart. In September 2019, biochar and biochar-based fertilizer were spread on soil surface and mixed well in top 20 cm soil. The experimental treatments were: (i). Ginseng field without soil amendment (CK), (ii). Chloropicrin fumigation + biochar (A10 @ 10 $t \cdot hm^{-2}$) and (iii). Chloropicrin fumigation + biochar-based fertilizer (B10 @ 10 $t \cdot hm^{-2}$). The treatments were replicated thrice in randomized block design.

Soil samples were randomly collected after removing the 10 cm surface soils, from 10-20 cm depth, 5-times (17 June, 8 July, 28 July, 18 August and 9 October 2020) from multiple points. Fresh soil samples were brought to the laboratory and weeds and gravel were removed. A portion of the sample was air-dried and passed through a 40-mesh sieve to determine enzyme activities. Another portion of fresh soil samples was used to determine changes in the soil microbial communities.

III. Soil Enzymes Activity

Soil acid phosphatase（S-ACP）enzyme catalyzes the mineralization of soil organic phosphorus, hence, its activity directly affects the decomposition, transformation and bioavailability of soil organic phosphorus. Thus, soil acid phosphatase activity is used to evaluate the direction and intensity of soil phosphorus biotransformation. Soil sucrase (S-SC) hydrolyzes sucrose into monosaccharides, glucose and fructose that can be absorbed by the body. The enzymatic products of S-SC are closely related to the soil content of organic matter, nitrogen and phosphorus, number of microorganisms in the soil and the intensity of soil respiration. S-SC is an important indicator of soil fertility. Soil catalase (S-CAT) is important for soil microbial metabolism and plays an important role in H_2O_2 removal. Soil laccase (S-SL) is a copper-containing polyphenol oxidase with strong redox ability.

S-ACP, S-SC, S-CAT and S-SL activities were measured as previously described (13,20) and in strict accordance with Solarbio kit protocols (Beijing, China) (Catalogue numbers: BC0145, BC0245, BC0105 and BC1965).

IV. Soil Microbial Communities

The total DNA from soil samples was extracted using a DNA extraction kit. DNA quality was evaluated by 1 % agarose gel electrophoresis, while concentration and purity were measured using a NanoDrop 2000 spectrophotometer. The V3–V4 hypervariable regions of 16S rRNA genes were PCR amplified using diluted genomic DNA as template and the PCR primers 341F (5’-CCTAYGGGRBGCASCAG-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’). PCR products were identified, purified and quantified using Qubit assays. A paired-end library was then constructed for Illumina sequencing at Majorbio (Shanghai, China).

V. Data Processing

Raw sequencing data were quality-filtered using the FASTp program (https://github.com/OpenGene/fastp, version 0.20.0). The FLASH program (http://www.cbcb.umd.edu/software/flash, version 1.2.7) was used to assemble DNA sequences. UPARSE (http://drive5.com/uparse/, version 7.1) was used to cluster sequences into operational taxonomic units (OTUs) at the 97 % nucleotide similarity level. Lastly, the RDP classifier (http://rdp.cme.msu.edu/, version 2.2) was used for taxonomic annotation and alignment of 16S rRNA genes against the RDP database using an assignment confidence threshold of 70 %.

Data were analyzed using two-way ANOVA and multiple comparison tests and plotted using GraphPad Prism 8.0, with $p < 0.05$ used as threshold for statistical significance.

RESULTS AND DISCUSSION

I. Enzyme activity

S-CAT activity increased after soil amendment with biochar and biochar-based fertilizer (except in the first sample, B10). S-CAT activity was higher in A10 than in B10 at different sampling time points [Fig. 1 (a)]. S-SL activity in A10 and B10 increased with sampling time. A10 exhibited significantly higher S-SL activity than CK soils at all sampling time points [Fig. 1 (b)]. In addition, A10 and B10 exhibited higher S-SC activities than CK soils. At the second sampling point on July 8, A10 exhibited significantly higher S-SC activity ($p < 0.01$) (Fig. 1 (c)). Overall, S-ACP activity in A10 and B10 increased over time, while enzyme activity at harvest on October 7 was significantly higher in B10 than in CK (Fig. 1(d)).

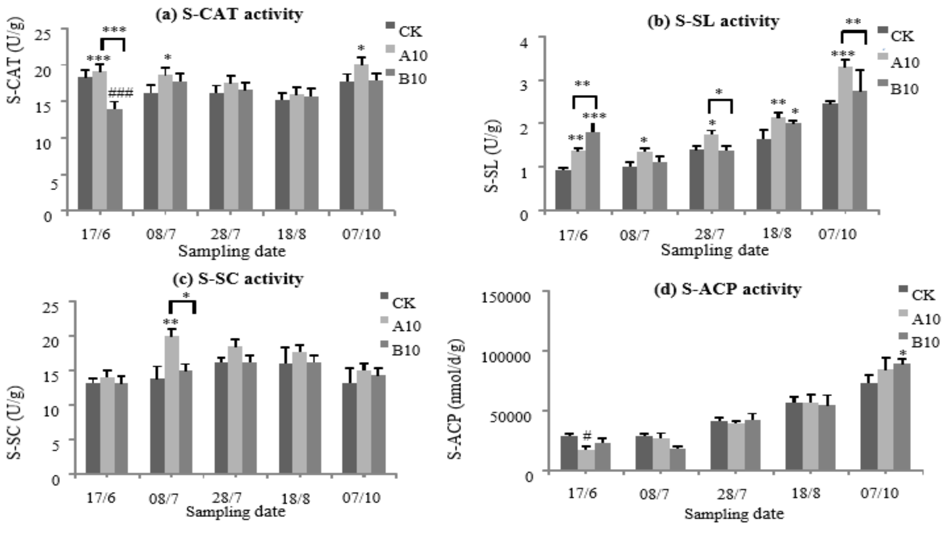

Figure 1. Effects of biochar and biochar-based fertilizer application on changes in soil enzyme activity
Note: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ indicate significantly higher values than in ginseng fields without soil amendment (CK). In addition, #: $p < 0.05$, ##: $p < 0.01$, ###: $p < 0.001$ indicates significantly lower values than in CK samples.
Sampling Dates: 17/6: 17/6/2019， 08/7: 08/7/2019, 28/7: 28/7/2019, 18/8: 18/8/2019 and 07/10: 07/10/2019

Biochar application can alter the soil physicochemical properties and also affects the soil microflora and soil enzymes activities. In this study, the biochar amendment significantly increased the activities of S-CAT and S-SLenzymes, while, biochar-based fertilizer significantly increased the activity of S-ACP in soils. Biochar adsorbs the compounds exuded from the plant roots and soils and increases the soil enzyme activity. Further, biochar also affects the enzyme-substrate binding and inhibit the soil enzyme activity (10). The structure and properties of biochar depends on the raw material used and pyrolysis temperature used for biochar production. Consequently, the activities of different enzymes influenced the biochar application rate (10,14). Soil enzyme activities are affected by the synergistic interactions of multiple factors viz., soil properties, enzyme properties, biochar properties and application rates (11). In this study, we found that soil amendment with biochar and biochar-based fertilizer increased the activities of soil enzymes. Related studies showed that soil urease, catalase and alkaline phosphatase were positively correlated with wheat yield after soil amendment with MFC (Straw charcoal + Inorganic fertilizer) (9), which is consistent with our research results.

II. Soil bacterial diversity and community structure

(i). Alpha diversity of soil bacterial communities: A total of 649,328 quality-filtered sequences were obtained from 9-soil samples and had an average sequence length of 417. The 16S rRNA gene sequences were clustered into OTUs at the 97 % nucleotide similarity threshold and subjected to bioinformatic analysis. A total of 5,025 OTUs were recovered after subsampling. Venn diagram can be used to count the number of common and unique OTU in multiple samples, which can visually show the similarity and overlap the number of OTU in environmental samples. A total of 2,047 OTUs were shared among the three treatment groups, with the number of unique OTUs being higher in control soils (CK) than in biochar treatments A10 and B10 (Fig. 2). Simpson and Shannon indices were used to evaluate community diversity levels, while the ACE and Chao1 indices were used to evaluate community richness levels and the coverage index was used to reflect coverage of community diversity. The Simpson and coverage indices of A10 communities were higher than in CK, although these differences were not significant (Table 1). Bacterial communities in B10 soil treatment exhibited significantly higher Simpson index values compared to CK communities, but significantly lower Shannon, ACE and Chao1 indices. All sample sequencing coverage levels were > 99%, indicating that the sequencing depth used for analyses was sufficient for describing the native diversity in the soils. The results showed that B10 soil treatment significantly increased the bacterial community diversity, while, A10 soil treatment had no influence. Previous studies had shown that biochar addition both at low and high doses decreased the bacterial community diversity. In this study, serious deterioration of continuously cropped ginseng soil, short application time of biochar and biochar-based fertilizer, did not significantly increase the bacterial community diversity.

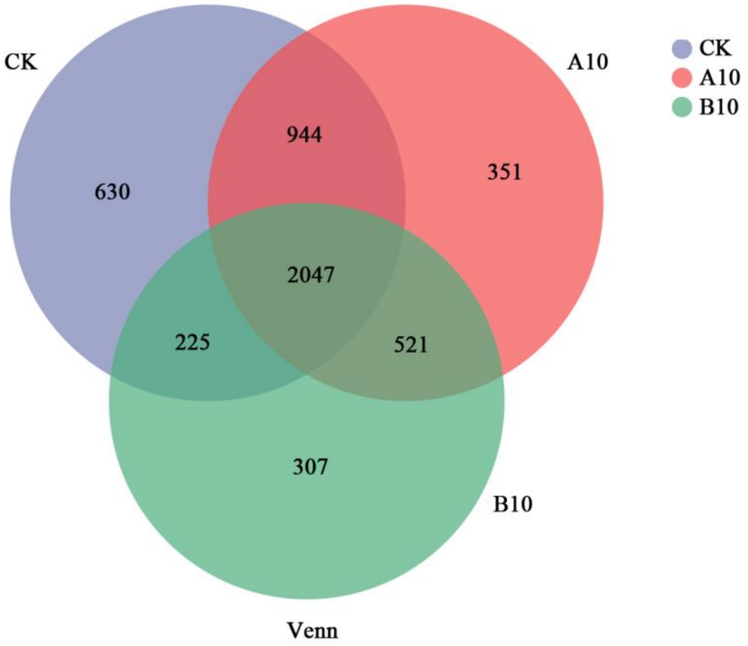

Figure 2. Number of bacterial operational taxonomic units in each treatment soil type.

Table 1. Effects of biochar and biochar-based fertilizer on the alpha diversity of soil bacterial communities.

Treatment	Simpson	Shannon	ACE	Chao1	Coverage
Control (CK)	0.00380±0.001	6.6424 ±0.06	3328.3±152. 42	3318.4 ± 130.82	0.9909 ± 0.0005
A10 Biochar	0.00737 ± 0.004	6.1183 ±0.54	2713.7 ±551.85	2694.5 ± 542.91	0.9923 ± 0.001
B10 Biochar fertilizer	0.01009±0.003$^{\#}$	5.8001±0.17$^{\#\#}$	2468.6±326.51$^{\#}$	2449.4±273.31$^{\#\#}$	0.9923± 0.0023

Note: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ indicate significantly higher values than in ginseng fields without soil amendment (CK). $^{\#}$: $p < 0.05$, $^{\#\#}$: $p < 0.01$, $^{\#\#\#}$: $p < 0.001$ indicates significantly lower values than in CK samples. A10: rice husk biochar, applied at 10 t·hm^{-2}. B10: biochar-based fertilizer, applied at 10 t·hm^{-2}.

(ii). Soil bacterial community structures: Proteobacterial abundances followed the order of B10 > A10 > CK, indicating that their abundances increased due to soil amendment. In contrast, actinobacterial abundances followed the order of CK > B10 > A10, representing decreases of 8.09 % and 9.11 % in A10 and B10 treatments compared to CK, respectively, suggesting that actinobacterial abundances decreased with soil amendment. The application of biochar and biochar-based fertilizer also resulted in increased abundances of Firmicutes, Gemmatimonadota, Bacteroidota and Patescibacteria, but decreased the relative abundances of Acidobacteria, Chloroflexi, Verrucomicrobia, Myxococcota and Crenarchaeota (Fig. 3). Communities from the A10 and B10 groups clustered together (Fig. 4), followed by clustering with CK communities. Patescibacterial abundances were inhibited in the CK treatment, while those of Crenarchaeota were inhibited in the A10 and B10 treatments.

It is known that bacterial diversity increased in biochar-amended soil and was positively correlated with the dose of biochar. The biochar application decreased the relative abundance of Acidobacteria, Chloroflexi and Gemmatimonadetes decreased but increased that of Proteobacteria, Bacteroidetes and Actinobacteria (31). Previous studies suggested that Acidobacteria (a phylum of bacteria being acidophilic) played main role in biogeochemical cycling of carbon, however, after biochar amendment the soil becomes more alkaline, which is unfavorable for Acidobacteria (21). Our results are partially inconsistent with previous studies, which may be due to the soil physico- chemical properties, type and amount of biochar applied and the crops cultivated.

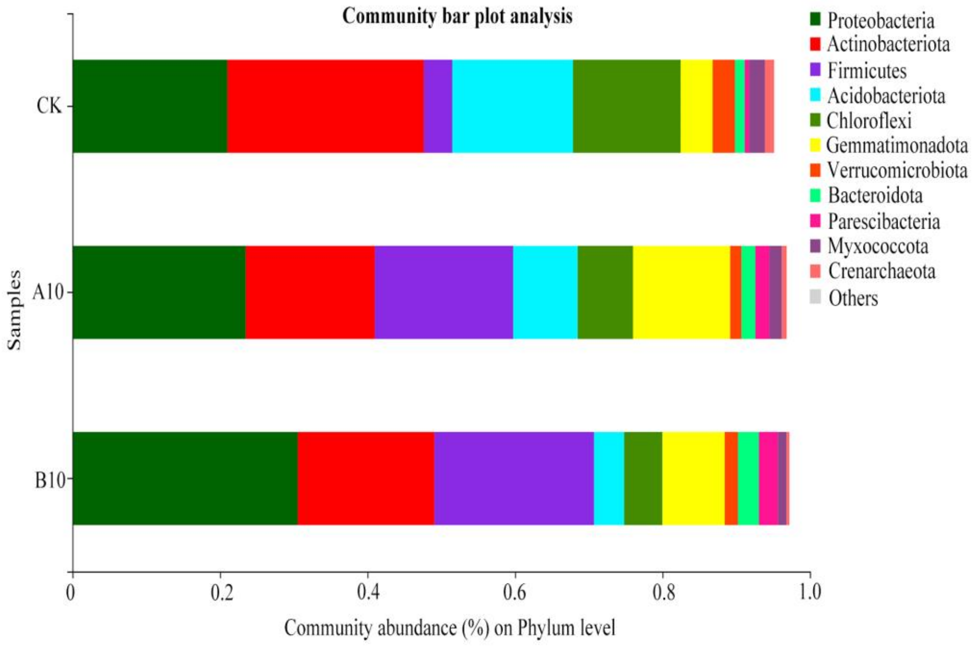

Figure 3. Relative abundances of the 11 most abundant phyla.

The two biochars, BC1 (made from peanut shell) and BC2 (wheat straw), were added to *Ralstonia solanacearum*-infected soil, BC1 and BC2 treatments significantly reduced the disease index of bacterial wilt by 28.6 % and 65.7 %, respectively (21). Biochar treatments also increased the soil neutral phosphatase and urease activity and the ratio of soil fungi/bacteria in the soil (21). The biochar sorption changes the mobility and activity of pathogens or modify signaling between pathogens and plants (17). The autotoxicity of ginseng and the imbalanced fungal communities in soil are key factors in continuous cropping problem of ginseng. While the abundances of beneficial bacteria were decreased leading to changes in soil microbial abundances and community structures. Therefore, biochar and biochar-based fertilizer can be used to (i) improve the soil beneficial microorganisms, (ii). slow down the development of soil sickness Problem and (iii) remediation of sick soil.

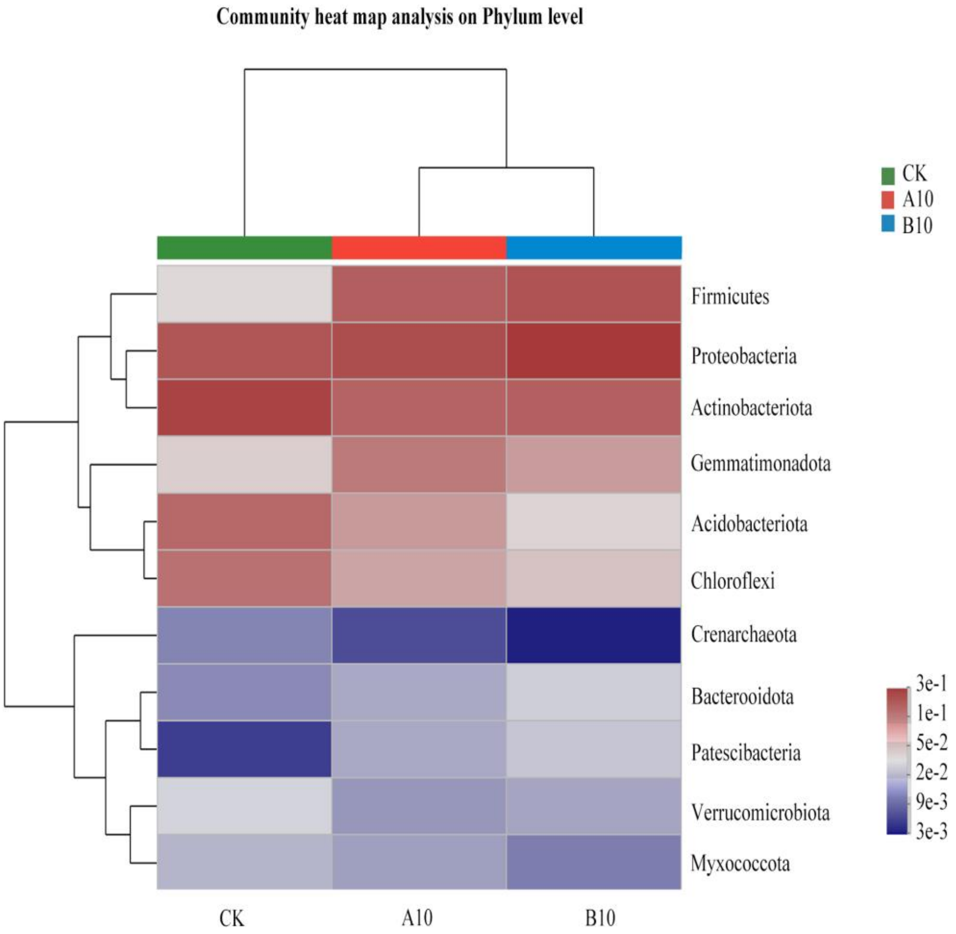

Figure. 4. Heatmap distribution of the 11 most abundant phyla.

III. Correlation of soil bacterial community structures with soil enzyme activities

The relative abundances of the 11 most abundant bacterial phyla (Fig. 4) were correlated to the activities of four different enzymes in ginseng harvested soils. The activities of the four enzymes were significantly correlated with 6 of the 11 most abundant bacterial phylum. S-ACP activity was extremely significant and positively correlated with abundances of Firmicutes, Bacteroidota, Patescibacteria and Proteobacteria ($p < 0.01$, $p < 0.05$), but significantly and negatively correlated with Verrucomicrobia and Myxococcota abundances ($p < 0.05$). In addition, S-SL activity was significantly and negatively correlated with Verrucomicrobia abundances ($p < 0.05$) (Fig. 5).

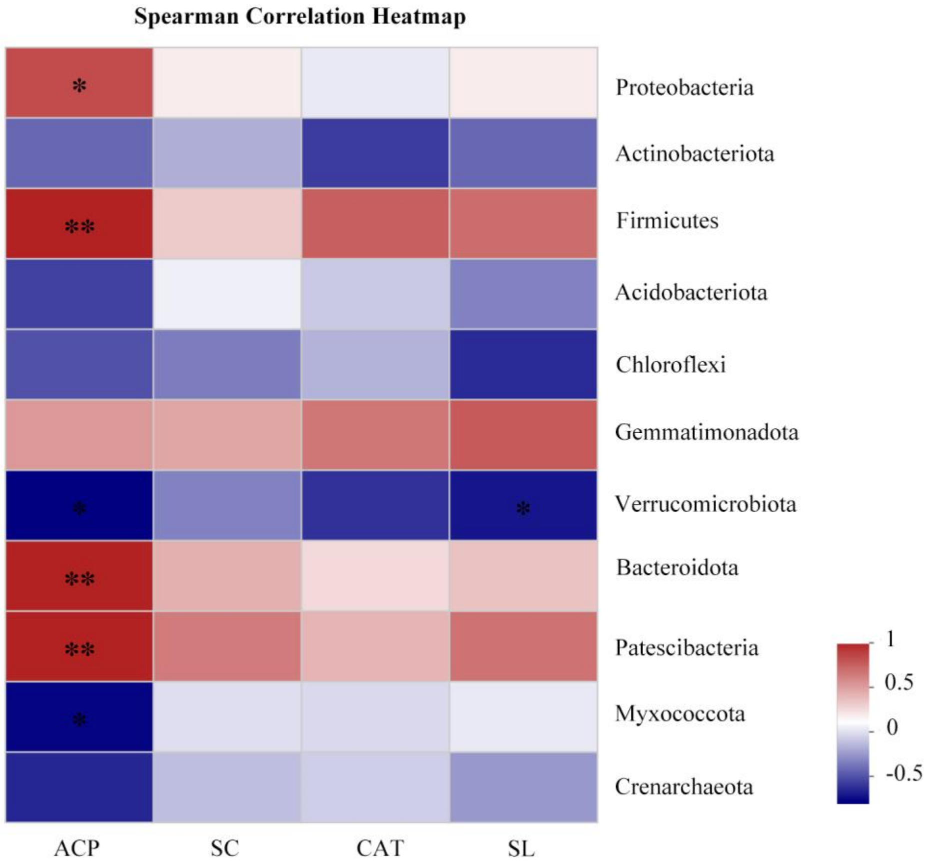

Figure 5. Correlations between dominant bacterial phyla abundances and soil enzyme activities.

The soil enzymes are derived from animal, plant and microbial residues and their activity is directly related to soil bacterial communities (23,29). Soil enzymes play an important role in maintaining soil ecology and soil health. Therefore, as an indicator of soil health, microorganisms affects the soil nutrients cycle and soil physico-chemical properties. We investigated the correlations between the abundances of 11-dominant bacterial phyla and the activities of four enzymes (S-ACP, S-SC, S-CAT and S-SL) in soils during the harvest period. S-ACP that catalyzes the mineralization of soil organic phosphorus and S-SL that is highly active in redox catalysis reactions showed significant associations with the relative abundances of soil bacterial populations (32). Specifically, S-ACP activity was positively correlated with Firmicutes, Bacteroidota, Patescibacteria and Proteobacteria abundances, but negatively correlated with Verrucomicrobia and Myxococcota abundances. Further, S-SL activity was negatively correlated with Verrucomicrobia abundances. Correlations between the enzyme activities and other bacterial phyla abundances were not significant. The biochar addition increased the abundance of Proteobacteria and Gemmatimonadetes (24), bur reduced the abundance of Actinobacteria and Acidobacteria in the soil, while, the activities of urease, S-SC, S-CAT and soil neutral phosphatase (S-NP) was increased (14). Our research showed that A10 and B10 treatments increased activities of S-ACP, S-SC, S-CAT and S-SL, with the highest increase in S-SL activities. This is consistent with the findings of Oleszczuk (31), who also reported that after the application of biochar to soil, the enzymatic activity of most enzymes increased than control.

Previous studies have shown that biochar treatment increased the abundance of Proteobacteria and Gemmatimonadetes, decreased the abundance of Actinobacteria and Acidobacteria and the activities of urease, S-SC and S-CAT in the soil (14, 24). Previous studies also indicated that bacterial community affects the S-ACP activity, available K, available P and alkali-hydrolyzale nitrogen. Our study showed that S-ACP and S-SL were the key factors associated with bacterial community structures, which is consistent with previous studies.

CONCLUSIONS

The short-term application of biochar and biochar-based fertilizer treatments increased the activities of enzymes [soil acid phosphatase (S-ACP), soil sucrase (S-SC), soil catalase (S-CAT) and soil laccase (S-SL)] and the highest increase was in S-SL activities. Biochar and biochar-based fertilizer treatments increased the relative abundances of Firmicutes, Gemmatimonadota, Bacteroidota and Patescibacteria, but decreased the relative abundances of Acidobacteria, Chloroflexi, Verrucomicrobia, Myxococcota and Crenarchaeota. Correlation analysis showed close relationships between soil enzyme activities and some soil bacterial populations. The changes in bacterial abundances were also associated with altered soil enzyme activities. We found that short-term application of biochar and biochar-based fertilizer did not increase bacterial diversity in amended soils. In addition, the relationships between soil enzyme activities and bacterial populations are irregular and dynamic due to the synergistic interactions of multiple factors. Therefore, we should monitor the changes in the soil physico-chemical properties, enzyme activities, bacterial and fungal microbial community for long time.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest : The Authors declare no conflict of Interest.

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