EFFECTS OF BIOCHAR ON MICROBIAL COMMUNITY DIVERSITY IN RHIZOSPHERE SOIL OF FARMLANDS IN NORTHEAST CHINA

DING, J.[1*] – LI, X.[2]

[1]*Heilongjiang Province Key Laboratory of Cold Region Wetland Ecology and Environment Research, Harbin University, Harbin 150086, Heilongjiang Province, PR China*

[2]*College of Resources and Environment, Northeast Agricultural University, Harbin 150030, Heilongjiang Province, PR China*

**Corresponding author*
e-mail: ding.junnan@163.com

(Received 12th Jan 2022; accepted 25th Mar 2022)

Abstract. The present study sought to study the effects of biochar application on the functional diversity of rhizosphere soil in the black and saline-alkaline rhizosphere soil. The results showed that biochar application to the black and saline-alkali soil could increase physical properties and enzymatic activity in the soil of northeast China. Biochar affected the bacterial community composition in the black and saline-alkali soil, of which Proteus, Bacteroides, Acidobacteria, and Actinobacteria were the dominant bacteria. Additionally, biochar inhibited the relative abundance of some bacteria. The relative abundance of Archaea and plant pathogenic functional bacteria and microorganisms affected the abundance of functional genes related to nitrification and the growth of various beneficial microorganisms, effectively inhibiting plant diseases. The canonical correspondence analysis results showed that saline alkaline soil and carbon application conditions were related to available phosphorus indicating a significant correlation between available phosphorus and the soil pH environmental factors. These results confirmed that the change in soil characteristics might indirectly affect the influence of biochar application. Overall, biochar is beneficial to relative abundance of the rhizosphere soil nutrient retention in the root system of black and saline-alkali soil of farmlands in northern China and could increase the growth-promoting bacterial community.
Keywords: *black soil, saline-alkali soil, biochar, bacterial community, functional diversity*

Introduction

Biochar is a carbon-rich material which can be prepared from various organic waste feedstock, such as agricultural wastes and municipal sewage sludge (Wang and Wang, 2019). Biochar has received increasing attention due to its unique feature such as high carbon content and cation exchange capacity, large specific surface area and stable structure (Chen et al., 2018; Yi et al., 2020). Application of biochar to soils changes soil physicochemical properties and stimulates the activities of soil microorganisms that influence soil quality and plant performance. Studying the response of soil microbial communities to biochar amendments is important for better understanding interactions of biochar with soil (Palansooriya et al., 2019). Soil microbes are key drivers of soil biological and chemical processes and critical for maintaining terrestrial ecosystem stability and ecological function, soil microbial community is the most important functional component in soil biota, soil microbial community is in the "plant-soil-soil microorganism" system (Nkongolo and Narendrula-Kotha., 2020). The interaction among plant, nutrients and carbon source supply (He et al., 2018). The formation of an effective feedback system has attracted the attention of scholars at home and abroad.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28012816

Black soil is one of the most precious soil types, with the characteristics of high fertility that is suitable for plant growth (Ou et al., 2017). Hence, as an essential land resource, its protection has become highly necessitated. Northeast China is one of the major grain producing areas in China due to its typical back soil (Jiao et al., 2018). Unfortunately, the yearning for effective agricultural products, which requires excessive use of fertilizers for prolonged periods, has led to a gradual decrease in the quality of the black soil chernozem (Delang, 2018). Recently, the farmland soils of Northeast China are facing major problems, such as soil fertility decline, severe soil and water loss, soil acidification, drought and flood, salinization, desertification, physical and chemical properties deterioration, etc. (Gu et al., 2018). Generally, all types of saline soil, alkaline soil, and different degrees of saline and alkaline soils are well known as saline-alkali land (Wu et al., 2021). It has the characteristics of more saline and alkaline components and poor physical and chemical properties, resulting in the growth of most plants being inhibited to varying degrees, or even unable to survive (Kaltas and Javidoglu, 2019). Nowadays, with the increasing shortage of cultivated land resources, the sustainable utilization of saline-alkali land as a potential reserve resource of cultivated land has been highly concerned by researchers (Liu et al., 2019). As for the black soil of Northeast China, the location of the black soil area and the importance of black soil for agricultural production has limited the research on its distribution and its relationship with the physical and chemical properties of the farmland black and saline-alkali soil in terms of the changes in soil physical and chemical properties, enzyme activity, and biochar application-mediated microbial communities' diversity. Therefore, this study discussed the effect of biochar application on soil physiochemical properties, soil enzymes activities and soil bacterial community in two different types of soils in the northeast of China (Black and Saline-alkali soil), and provide a reference for the application of biochar in agriculture.

Materials and methods

Site description

This study was conducted in the pot experiment area of the Modern Agricultural Demonstration Park at Heilongjiang Academy of Agricultural Sciences in Harbin (126°50' E, 45°50' N), Heilongjiang Province in 2020. The geographical distribution of this region is as follows: the average temperature in the coldest month is -22 °C, while the hottest month has an average. Temperature of 20 °C; the annual ≥ 10 °C accumulated temperature is 2,000-2,800 °C and the frost-free period is 115-130 d; the annual average rainfall is 450-550 mm, of which more than 59% of the rainfall occurs between July-September; the soil type is typical black soil. During the study period, the soil condition was as follows: the organic nitrogen content (SOC) was 0.58 $g \cdot kg^{-1}$, the available nitrogen (A-N) content was 89.2 $mg \cdot kg^{-1}$, the available phosphorus (A-P) content was 128.2 $mg \cdot kg^{-1}$, the available kalium (A-K) content was 106.2 $mg \cdot kg^{-1}$, and its pH was 6.8.

Test materials

Test biochar: The test biochar is a kind of highly aromatic carbon rich material, which is transformed from rice husk by pyrolysis and carbonization at high temperature. Biochar was commercially supplied by Liao Ning Golden Future Agriculture

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28012816

Technology Co., Ltd. with the properties of pH 8.69, ≧34% of total nutrient content and N: P_2O_5: K_2O = 8:11:15.

Test soybean: the chosen soybean variety was Suinong 35 (*Glycine max* (L.) Merr.). The physiological characteristics of soybean are unlimited pod setting habit. The plant is about 90 cm high, with branches, white flowers, long leaves, gray hairs, slightly curved pods, sickle shape, and brown at maturity. The seeds are round, the seed coat is yellow, the navel is light yellow, matte, and the weight of 100 seeds is about 22 g. The protein content was 39.42%, and the fat content was 21.77% (Ma, 2018; Yu et al., 2017). Resistance to gray spot in inoculation identification. In the adaptive area, the number of days from emergence to maturity is about 120 days, which needs to be ≥ 10 °C and the active accumulated temperature is about 2450 °C, which was commercially provided by the Soybean Laboratory in the Institute of Tillage and Cultivation, Heilongjiang Academy of Agricultural Sciences. Routine water and fertilizer management and timely prevention and elimination of diseases, insects and grass.

Test soil: the black control soil (BC) samples were collected from the Modern Agricultural Demonstration Park at Heilongjiang Academy of Agricultural Sciences, and the soil type was typical black soil. The saline-alkali control (SAC) soils were collected from the Fanrong village in Zhaodong City, Heilongjiang Province (125°34’34″ E~46°23’58″ N), and the crops planted in the farmland were soybeans, collected saline-alkali test soil and brought it back to the demonstration park, the soil type was typical saline-alkali soil.

Experimental design

This study was based on the pot experiment. Pot experiments were performed using polypropylene plastic pots with a height and diameter of 30 cm. The biochar and air-dried black soil were well mixed and placed into the pots for soybean pot experiments. Four treatments were set as follows: (1) treatment 1 (BC): no biochar was added into the black soil; (2) treatment 2 (BB): 40 g of biochar was added into 1 kg of black soil, i.e., 160 g biochar/pot; (3) treatment 3 (SAC): no biochar was added into the saline-alkali soil; (4) treatment 4 (SAB): 40 g of biochar was added into 1 kg saline-alkali soil, i.e., 160 g biochar/pot.

In mid-May 2020, mature soybean seeding with relatively uniform size was selected and planted evenly into the pots containing different amounts of biochar. Each pot contained 6 seeds and each treatment was performed in triplicate.

The rhizosphere soil was collected at the maturity stage of soybean after different biochar treatments. The rhizosphere soil was obtained as follows: first the whole soybean plant was carefully excavated. Then the surface soil (0–5 cm) was gently shaken off of the roots, and the root surface was gently brushed to collect the soil attached to the root surface, which is the rhizosphere soil of soybean. After being sealed in sterile bags and returned to the laboratory in an ice box, fresh rhizosphere soil was prepared by grinding and sieving (2 mm) for subsequent tests.

Determination of soybean rhizospheric soil physical and chemical properties

The Walkley-Black titration method was carried out to determine the soil organic carbon content (Cha et al., 2019). The available nitrogen in the soil was measured by using the Alkali-diffusion method (Hurisso et al., 2018). Determination of the available phosphorus in soil was measured by using $NaHCO_3$ Extraction- Mo-Sb Anti-

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28012816

colorimetry (Ghorbanzadeh, 2020). Determination of available kalium in soil was measured by using NaOH melting--flame photometric method. Determination of soil pH, the soil samples were taken at mature soybean stages and all the samples were air dried for further determination. The water-soil leaching method with water: soil = 2.5:1 was used to determine soil samples using a table pH meter (Minkina et al., 2018).

Determination of soybean rhizospheric soil enzyme activity

The test soil was collected from the potted soybeans during the mature period. Urease was treated with sodium phenate colorimetry. Catalase was titrated by potassium permanganate. Saccharase was determined by 3, 5-dinitrosalicylic acid colorimetry. Phosphatase was measured by disodium phenyl phosphate colorimetry (Nannipieri, 2018).

Preparation of soybean rhizospheric microbial samples

The control and biochar treated soil samples were placed in a dry ice-box, then brought back to the laboratory for preservation, and stored in a refrigerator at - 80 °C to detect the soil microbial diversity. Herein, the soil microbial genomic DNA was extracted using the Omega e.z.n.a DNA kit (Omega Bio-Tek, Norcross, GA, U.S.).

Statistical analysis

Data were statistically analyzed using Excel 2003. One-way ANOVA analysis and PCA (α- = 0.05) were performed using SPSS 16.0 software. The differences in the soil microbial community composition among the soil samples were mapped using R language software.

Results

Effects of biochar on soybean rhizospheric soil physical and chemical properties

The effect of biochar on the physical and chemical properties of soybean rhizosphere soil is summarized in *Table 1*. The physical and chemical properties of the soybean rhizosphere soil were significantly influenced by biochar, which were all higher than BC and SAC.

Table 1. *Effects of biochar on soil physical and chemical properties*

Treatment	pH	A-N ($mg \cdot kg^{-1}$)	A-P ($mg \cdot kg^{-1}$)	A-K ($mg \cdot kg^{-1}$)	SOC ($g \cdot kg^{-1}$)
BC	6.52 ± 0.08 c	118.54 ± 3.04 b	25.46 ± 0.52 a	228 ± 7.22 b	11.26 ± 1.86 b
BB	6.85 ± 0.19 c	122.67 ± 5.98 a	25.98 ± 1.14 a	242 ± 8.56 a	13.68 ± 1.92 a
SAC	8.89 ± 0.15 b	99.41 ± 4.34 d	21.49 ± 1.08 b	204 ± 5.19 c	9.83 ± 1.53 d
SAB	9.05 ± 0.26 a	105.78 ± 5.44 c	22.65 ± 1.73 b	207 ± 4.23 c	10.92 ± 1.26 c

Note: Different lower-cases in the same column for differences at 0.05 level ($P < 0.05$), the same below

Effect of biochar on enzyme activity of soybean rhizospheric soil

As summarized in *Table 2*, the activities of urease and phosphatase in the soybean rhizosphere soil treated with biochar were significantly higher than the BC and SAC

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28012816

treatments. The change of catalase activity in the biochar treated soil was consistent with urease, of which the catalase activity in the BB and SAB treated rhizosphere soil was 7.85% and 3.46%, which were all higher than the control treatments. Additionally, the difference between the biochar and control treatments was significant ($P < 0.05$). With the increase of biochar application, the saccharase and phosphatase activity increased, showing significant differences between different treatments ($P > 0.05$).

Table 2. *Enzyme activity in rhizosphere soil treated with different amounts of biochar*

Treatment	Urease (NH_3-Nmg·100 g^{-1}·d^{-1})	Catalase (0.002 mol·L^{-1} $KMnO_4$·g^{-1})	Saccharase (mg·g^{-1})	Phosphatase (mg·g^{-1} ± ·24 h)
BC	24.08 ± 2.43 b	11.97 ± 1.45 a	26.47 ± 4.10 a	0.23 ± 0.02 b
BB	30.73 ± 1.18 a	12.99 ± 1.72 a	31.53 ± 8.22 a	0.27 ± 0.01 a
SAC	17.63 ± 3.65 d	7.79 ± 1.23 b	18.07 ± 2.01 d	0.14 ± 0.02 d
SAB	20.15 ± 2.13 c	8.07 ± 1.31 b	20.11 ± 2.02 c	0.16 ± 0.01 c

Effects of biochar on bacterial diversity in soybean rhizosphere soil

As depicted in *Figure 1,* the levels of bacterial OTUs in different soil samples were significantly different. A total of 5698 OTUs were obtained from all the samples. Of them, BB and BC contained 1414 and 1276 soil bacterial OTUs, respectively. SAB and SAC contained 1484 and 1524 bacterial OTUs, respectively. OTU401 accounted for 64.55% of all OTU species in the BC treatment, 48 species of bacterial unique to BC, others, and OTU487 accounted for 63.79% of all OTU species in BB, and 131 species unique to BB. OTU2214 and OTU2085 accounted for 53.46% of all OTU species in SAC, and there were 16 species unique to SAC. OTU1257, OTU1587, and OTU1717 accounted for 47.12% of all OTUs species in SAB, and there were 16 species unique to SAB.

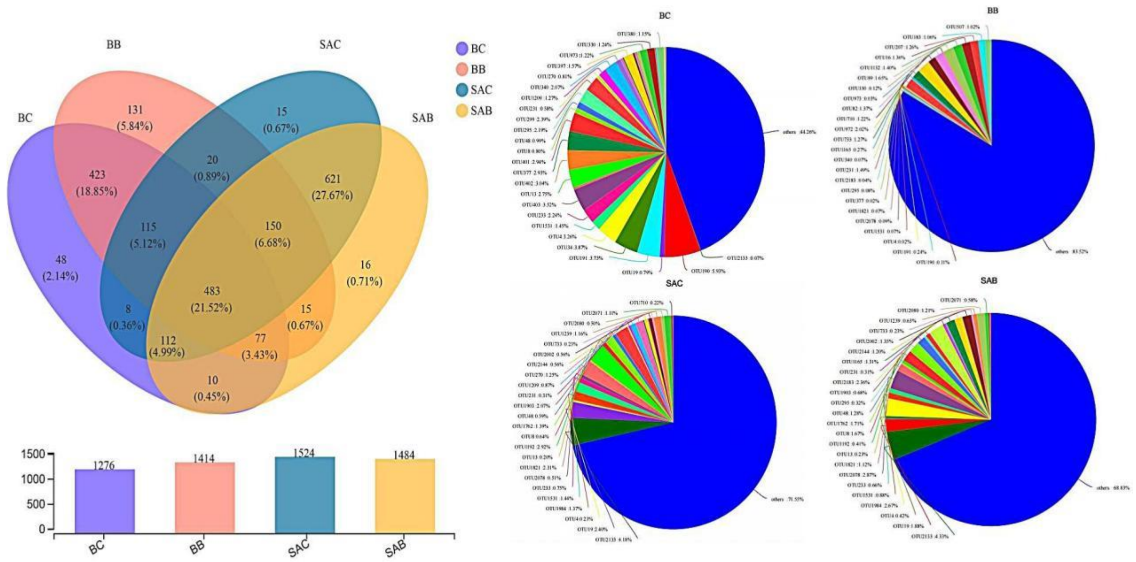

Figure 1. *OTUs numbers of bacterial communities in all samples*

As depicted in *Figure 2,* all the 23047 sequences belonged to 27 bacterial phyla, of which the primary bacterial microbial populations included *Proteobacteria*,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28012816

Bacteroidetes, *Acidobacteria*, *Actinobacteria*, *Chloroflex*, *Gemmatimonades*, *Firmicutes*, *Saccharibacteria*, *Nitrospirae*, *Verrucomicrobia*, and others. The average proportion of flora structures in the BC and BB treatments was 42.91%, 22.41%, 9.99%, 6.11%, 7.24%, 2.97%, 2.25%, 1.98%, 0.59%, 0.82%, and 2.81%, respectively. Of which, the total number of sequences belonging to *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Actinobacteria*, and *Chloroflexi* accounted for 88.66% of all sequences, and the average proportion of structures in the saline-alkali soil was 39.32%, 15.97%, 16.42%, 10.76%, 3.59%, 3.34%, 2.25%, 2.45%, 1.97%, 2.95%, and 1.78%, respectively. The total number of sequences belonging to *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Actinobacteria*, and *Chloroflex* accounted for 86.06% of all sequences. *Armimondetes*, *Chlamydiae*, *Deferribacters*, and *Thermotogae* were the inferior flora, accounting for less than 0.5% of all sequences.

Effects of biochar on bacterial community composition in soybean rhizosphere soil

Moreover, the differences in bacterial community structure between the black and saline-alkali soil were analyzed, showing similar taxonomic profiles among various species. *Proteobacteria* accounted for a high bacterial proportion in all the samples (the proportion was between 35.81% and 48.08%), indicating *Proteobacteria* to be the dominant bacterial species in all the samples. Comparing the bacterial community structures of different soil types with biochar, *Proteobacteria* and *Bacteroidetes* were the dominant bacterial species in the BC treatment, while *Proteobacteria* and *Acidobacteria* were the dominant bacterial species in the BB treatment. After biochar application, the bacterial species of *Proteobacteria* and *Bacteroidetes* were decreased by 10.35% and 26.22%, respectively. Similarly, the bacteria species of *Acidobacteria* and *Chloroflexi* were increased by 16.05% and 10.41%. The bacterial species of *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, and *Actinobacteria* were the dominant bacteria species in the saline-alkali soil. After biochar application, the bacterial species of *Proteobacteria* and *Actinobacteria* were decreased by 6.98% and 1.2%, respectively, while the bacteria species of *Bacteroidetes* and *Acidobacteria* were increased by 0.92% and 9.98%, respectively.

Effects of biochar on community structure of soybean rhizosphere soil microorganisms

As depicted in *Figure 3*, the impact of biochar application on soil microorganisms in the black and saline-alkali soil was mainly manifested by two aspects. Firstly, biochar application promoted the abundance of soil bacteria, such as *Gemmatimonadetes*, *Nitrospirae*, *Chloroflexi*, *Acidobacteria*, *Aminicenantes*, *Parcubacteria*, *Ignavibacteriae*, *Planctomycetes*, *Latescibacteria*, *Deinococcus-Thermus*, *Caldiserica*, *Gracilibacteria*, *FCPU426*, *Armatimonadetes*, *Cyanobacteria*, *Spirochaetae* than the control. Secondly, biochar application inhibited the growth of *Bacteroidetes* and *FBP* in the black soil and *Saccharibacteria* and *Deinococcus-Thermus* in the saline-alkali soil. The bacterial richness in the biochar treated soil was lower than control.

Correlation analysis between soil bacterial community structure and environmental factors

The PCA results of soil bacterial community structure based on OTUs abundance are depicted in *Figure 4*. The contribution values of the PC1 axis and PC2 axis to the

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28012816

difference in sample composition were 41.69% and 17.81%, respectively. The sample points of biochar treatment and control treatment were significantly separated on the PC1 axis, indicating that biochar had a certain impact on the soil bacterial community structure. BB and SAB treatments had a significant correlation with the SOC and A-P environmental factors in the rhizosphere soil.

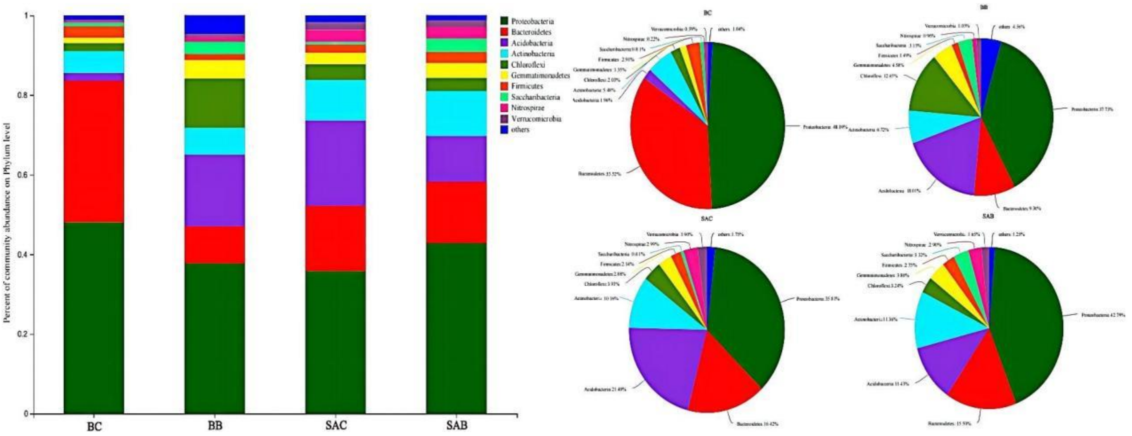

Figure 2. *Relative abundance of species at phylum level in all samples*

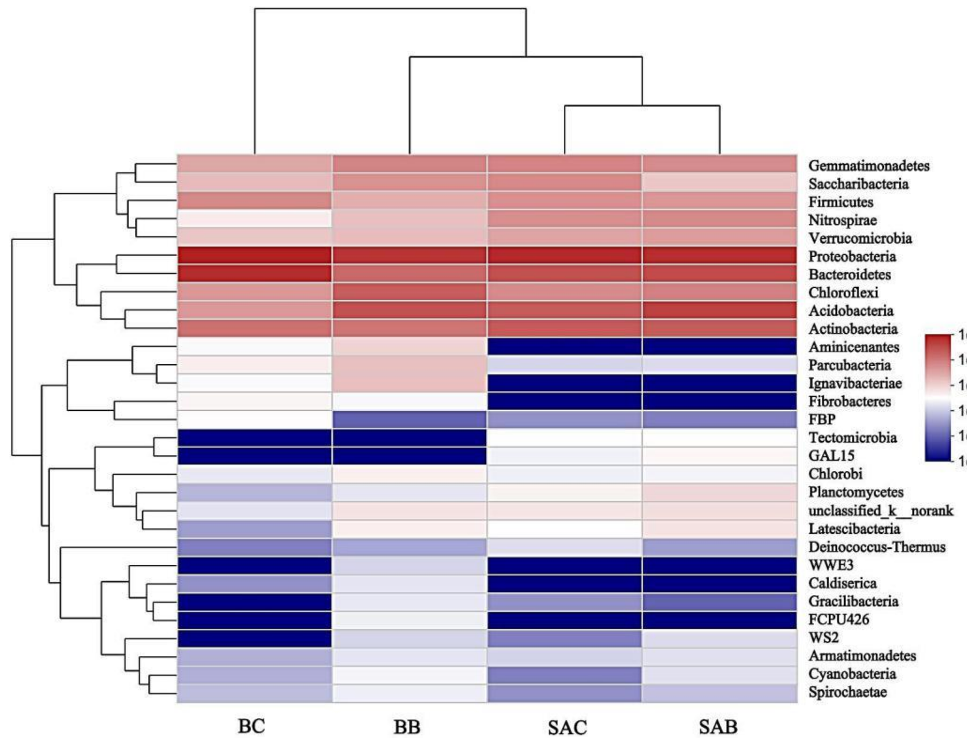

Figure 3*. Analysis of relative abundance of biochar of soil microbial community composition at the phylum level*

Effect of biochar on functional taxa of soil bacterial

As depicted in *Figure 5,* biochar application changed the abundance of functional microorganisms in the black and saline-alkali soil. While biochar application in the

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28012816

black soil increased the abundance of bacteria, such as sulfur_respiration, nitrogen_fixation, nitrite_oxidation, ammonia_oxidation, and nitrification. Compared with BC, the abundance of ammonia_oxidation and nitrification was increased by 77.03% and 76.19%, respectively. Biochar application in the saline-alkali soil increased the abundance of aromatic_compound_degradation, methanol_oxidation, methylotrophy, ammonia_oxidation, and nitrification. Compared with the saline-alkaline soil, biochar application in the black soil significantly changed the abundance of functional microorganisms.

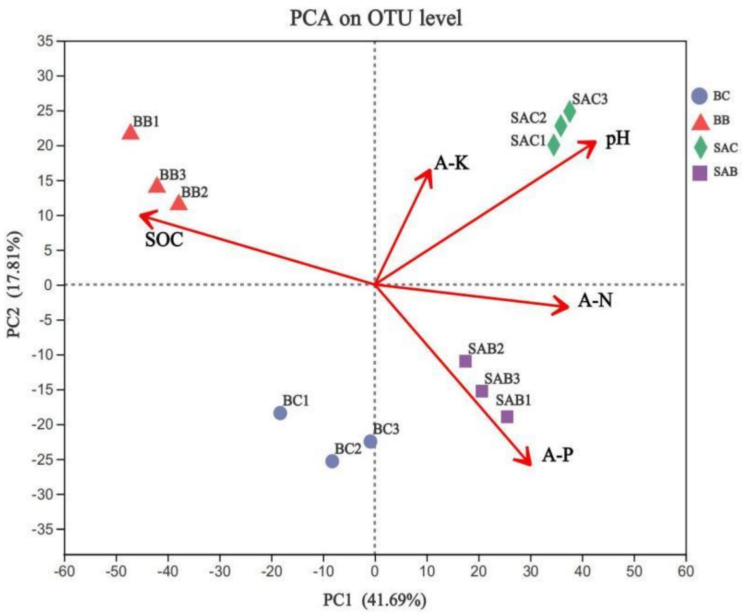

Figure 4. *PCA analysis of biochar on soybean rhizosphere soil*

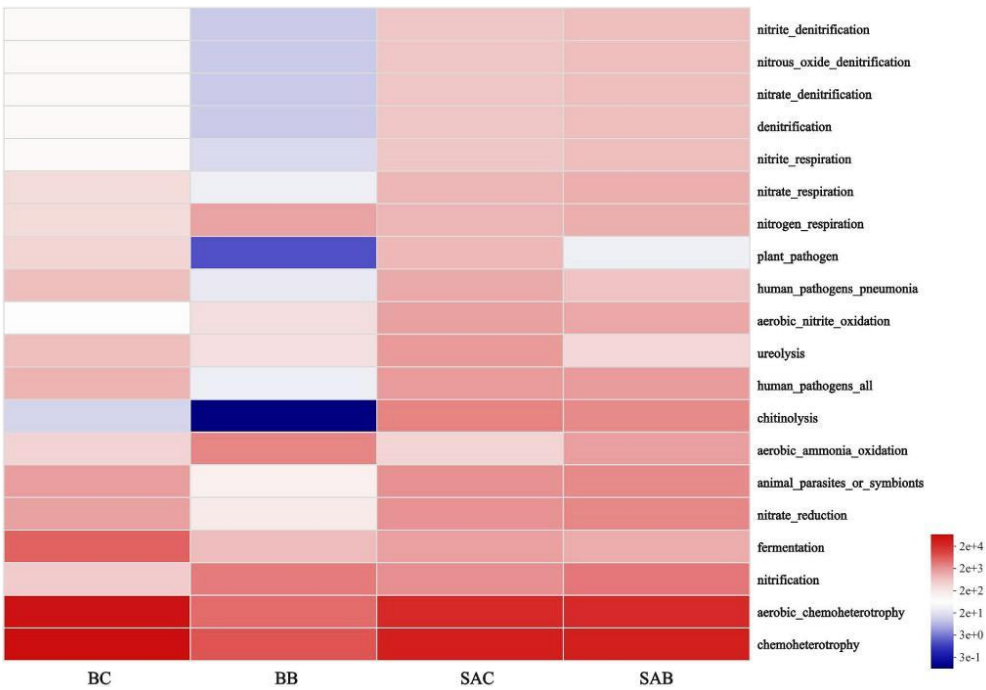

Figure 5. *Effect of biochar on functional taxa of soil bacterial*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28012816

Discussion

Based on its physical characteristics and previous studies, biochar is characterized by porous structures and weak alkalinity, and has a strong ability to absorb water, store nutrients, and increase the soil organic carbon content (Panahi et al., 2020). The pores and surfaces of the biochar can also become a microenvironment suitable for soil microorganisms, which increases the number and activity of soil microorganisms and promotes the circulation of various elements in the soil (Palansooriya et al., 2019). Animal, plant, and microorganism residues are the main sources of soil organic matter, which are derived from a complex physical and chemical transformation processes of microorganisms and soil (Indoria et al., 2018). Organic carbon is an important soil component, despite the small proportion it takes in total soil weight, the quantity and quality of the soil organic carbon content is an important indicator of soil quality (Ondrasek et al., 2019). The results in this study showed that biochar can increase the accumulation of the soil organic carbon content, and the application of greater amounts of biochar had a significant effect on the organic carbon content. After the application of the biochar in the soil, the biochar has easily volatile substances and the oxidation of the surface functional group of biochar at the initial stage, but it was passivated with the prolonging retention of the biochar in soil after biochar application (El-Naggar et al., 2019). The interaction between biochar and soil produced a protective mechanism, which not only increased the oxidation stability of soil organic carbon but also promoted its accumulation in soil (Feng et al., 2021; Yang et al., 2019).

In modern agricultural management, successive chemical fertilizer application causes soil acidification and continuous loss of base ions, resulting in poor soil, thus affecting crop growth (Dumortier et al., 2020). In the presence of more base ions, such as potassium, sodium, calcium, and magnesium, the content of exchangeable hydrogen and aluminum ions in soil were reduced by the adsorption of biochar, so the soil pH value can be improved by the biochar (Leng et al., 2019). The soil pH was determined by the base ions, since the biochar has a higher number of base ions, it was considered to be a better modifier to improve soil than lime, but it had no significant effect on alkaline soil (Fan et al., 2018).

As a biocatalyst in the soil, the main function of soil enzymes is to promote the biochemical reaction and the physicochemical properties of soil, as well maintain ecological balance (Wołejko et al., 2020). The application of biochar is able to affect this activity (Jabborova et al., 2021). The soil enzymes are mainly derived from the cell secretions of animals, plant roots, and microorganisms in soil, and the activity of the soil enzymes can reflect the intensity and direction of various biochemical processes in soil (Awet et al., 2018). Two effects have been observed on aspects of biochar on the soil enzyme activity, which might be involved in the reaction between the biochar and target substrates (Gorovtsov et al., 2020). First, the adsorption of the biochar on the reaction substrate contributed to enzyme catalysis and therefore improved the soil enzyme activity (Ameur et al., 2018). Second, the adsorption of biochar on enzymes protected the binding sites of enzyme catalysis, thus preventing enzyme catalysis (Lopes et al., 2021). In this study revealed that biochar can increase the content of the related enzymes in the chernozem, and also significantly enhanced the contents of urease, catalase and phosphatase in soil, but had no significant effect on the activity of invertase. Urease, an important hydrolase involved in the circulation of soil nitrogen, functions to catalyze the hydrolysis of urea in soil, which characterizes the intensity of nitrogen supply in the soil (Monge et al., 2018). The increase of urease activity may be

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28012816

due to the increasing demand of soybeans on nitrogen, which thereby stimulate nitrogen fixation in rhizobia (Laroca et al., 2018). The increasing activity of hydrogen peroxide may be related to the improved soil environment after the application of the biochar, which provides good conditions for the growth and reproduction of microorganisms and is beneficial to their metabolism, thus improving the soil enzyme activity (Lehmann et al., 2011). The soil pH value increased significantly with the application of biochar, which indirectly led to an enhancement of invertase activity (Sheng and Zhu., 2018).

Previous studies have reported a significant change in the community composition and bacterial and archaeal diversity both in the Amazon black soil and biochar improved soil (Zhang et al., 2021). Compared with the unmodified soil, the bacterial diversity of soil increased by 25%, which was reflected at the level of genera, species, and families (Otsuka et al., 2008). The nitrogen-fixing bacteria, possessing nitrogen-fixing enzymes, are a special type of bacteria, which can convert molecular nitrogen in the atmosphere into nitrate through nitrification for easy absorption by the plants (Igiehon and Babalola, 2018). Biochar could provide a favorable niche for nitrogen fixation due to its low oxygen partial pressure and decreased oxygen concentration (Wu et al., 2020). Meanwhile, its low inorganic nitrogen content could also provide favorable conditions for the implantation of nitrogen-fixing bacteria on the surface of biochar, making it a dominant population (Zhou et al., 2016). In these studies, a total of 23047 sequences belonging to 27 bacterial phyla, including Proteobacteria, Bacteroidetes, Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Firmicutes, Saccharibacteria, Nitrospirae, Verrucomicrobia, were detected in all the samples. While in this study, these bacteria were most dominant in the black and saline-alkali soil, and the primary microbial groups in the northern farmland soil (Yao et al., 2017; Sun et al., 2014).

According to the phylum, genus, and species-level classification of species difference analysis, Heatmap sequence analysis, and functional prediction analysis, biochar application could increase or inhibit the abundance of some microorganisms. It is mainly manifested by the following aspects: firstly, biochar application could improve the abundance of nitrifying bacteria in the black and saline-alkali soil. It could also improve soil aeration, reduce soil denitrification rate, and nitrite nitrogen production due to its porous nature (George et al., 2016). Secondly, soil nitrification is affected by several factors, such as soil type, aeration conditions, water content, temperature, soil pH, and substrate nitrogen concentration (Hu et al., 2021). Therefore, the addition of biochar to soil might change the living environment of nitrifying bacteria related to N_2O production by changing the soil properties, thereby promoting or inhibiting N_2O production through nitrification (Novak et al., 2016). Biochar could promote the abundance of Archaea (AOA). AOA, and nitrite-oxidizing bacteria, which are the primary synergists of soil nitrification (Wang et al., 2021a). In the soil nitrification process, the major completion stage includes the oxidation of ammonia to hydroxylamine under the action of ammonia monooxygenase produced by *amoA*, gene of AOA and ammonia-oxidizing bacteria (AOB), then further oxidation to NO_2^- under the action of hydroxylamine oxidoreductase, and finally, the catalysis of nitrite oxidoreductase by *nor* gene of nitrite-oxidizing bacteria oxidizing $NO_2^-$to NO_3^- (Beeckman et al., 2018). Evidence shows that the addition of biochar could increase the soil pH, which might exert a certain impact on the growth and reproduction of AOA and AOB, thus affecting nitrification (Li et al., 2021). As for the soil with biochar, the AOA diversity decreased, but the gene copy number significantly increased, while the

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28012816

diversity and gene copy number of AOB significantly increased (Cao et al., 2021). Furthermore, the porous surface of biochar could adsorb and store water and nutrients, providing excellent habitat conditions for soil microorganisms, especially bacteria, to improve the abundance of AOB, AOA, and other flora and accelerate the corresponding soil nitrification process (Wang et al., 2021b).

In this study, biochar application in the black and saline-alkali soil significantly inhibited the abundance of plant pathogenic. Soil-borne diseases are mostly caused by the pathogens living in the soil with plant residues and infect the plants from the root or stem under appropriate conditions (de Medeiros et al., 2021). The emerging global warming and strong promotion of protected land planting have increased the occurrence of soil-borne diseases in protected cultivation, resulting in huge economic losses (Bai et al., 2018). Although a lot of research has been carried out on the prevention and control of soil borne diseases, there is still a lack of cost-effective, efficient, wide adaptability, and practical technology that can be popularized in a large area (Elad et al., 2011). Recently, biochar as a new material integrating fertilizer, adsorbent, and modifier, with great potential and development prospects, has been widely adopted to prevent and control the soil-borne diseases (Ren et al., 2022). Accumulating studies have proved that biochar mainly affects the soil and plant root environment through the following mechanisms to inhibit the plant diseases occurrence: improving the physical and chemical properties of soil is conducive to plant growth but not conducive to the growth of pathogens (Gu et al., 2017); improve the available nutrients content in the soil, promote plant growth and improve disease resistance (Viger et al., 2015). Inhibit pathogenic bacterial growth through competition, heavy parasitism, and antibiotic secretion; direct inhibition of pathogens; induced plant disease resistance; strong adsorption of organic matters by biochar, and affecting the signal substances secreted by the plant roots attract pathogens and the infectious substances secreted by pathogens (Rasool et al., 2021). Overall, promoting the growth of beneficial microorganisms in the soil is one of the primary reasons for the control effect of biochar on plant pathogenic (Jaiswal et al., 2018). The 16S rRNA homology analysis results showed that most bacteria had more than 98% homology with *Pseudomonas*, *Bacillus*, *Rhizobium,* and *Bacillus brevis*, which promoted plant growth and had biological control function. Furthermore, the antibiotics producing *Pseudomonas fluorescens*, *Pseudomonas aeruginosa,* and *Pseudomonas Mendoza* were isolated and identified. It can be seen that the application of biochar in soil could promote the growth of various beneficial microorganisms and effectively inhibit the occurrence of plant diseases.

Conclusion

In this study, the application of biochar increased the content of soil organic carbon, pH value, available nitrogen, available kalium and available phosphorus under the concentration range of biochar. It also promoted the enzyme activity of rhizosphere soil in the black and saline-alkali of soybean farmland and increased the abundance of bacteria and microorganisms in the black and saline-alkali soil. Biochar affected the composition of the soil bacterial community in the black and saline-alkali soil, of which Proteus, Bacteroides, and Acidobacteria were the dominant bacteria. It also inhibited the relative abundance of bacteria, such as solibacterales, clostridiales, and rhodocyclales. Biochar application promoted the abundance of nitrification, AOA, and functional bacteria of plant pathogenic in the tested soybean rhizosphere soil, the abundance of

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28012816

functional genes related to nitrification, and the growth of various beneficial microorganisms, effectively inhibiting the plant diseases. The CCA at the OTU level showed that SAC and BB had a significant correlation with total phosphorus, organic carbon, and soil pH environmental factors, indicating that the change in soil characteristics might indirectly affect biochar application on soil bacterial community structure. In order to maximize the effective of biochar in the prevention and control of soil-borne diseases, it is necessary to comprehensively consider the preliminary treatment of biochar, supporting management measures in the field and collaborative technology. At present, there is a lack of research in this area, so it is also the focus of future research.

Acknowledgements. This article was supported by the Natural Science Foundation of Heilongjiang Province China (Grant No. LH2021D014).

Conflict of interests. The author confirms that this article content has no conflict of interests.

REFERENCES

[1] Ameur, D., Zehetner, F., Johnen, S., Jöchlinger, L., Pardeller, G., Wimmer, B., Keiblinger, K. M. (2018): Activated biochar alters activities of carbon and nitrogen acquiring soil enzymes. – Pedobiologia 69: 1-10.

[2] Awet, T. T., Kohl, Y., Meier, F., Straskraba, S., Grün, A. L., Ruf, T., Emmerling, C. (2018): Effects of polystyrene nanoparticles on the microbiota and functional diversity of enzymes in soil. – Environmental Sciences Europe 30(1): 1-10.

[3] Bai, Z. G., Caspari, T., Gonzalez, M. R., Batjes, N. H., Mäder, P., Bünemann, E. K., Goede, R. D., Brussaard, L., Xu, M. G., Ferreira, C. S. S., Reintam, E., Fan, H. Z., Mihelič, R., Glavan, M., Tóth, Z. (2018): Effects of agricultural management practices on soil quality: a review of long-term experiments for Europe and China. – Agriculture, Ecosystems & Environment 265: 1-7.

[4] Beeckman, F., Motte, H., Beeckman, T. (2018): Nitrification in agricultural soils: impact, actors and mitigation. – Current Opinion in Biotechnology 50: 166-173.

[5] Cao, H., Jia, M., Xun, M., Wang, X., Chen, K., Yang, H. (2021): Nitrogen transformation and microbial community structure varied in apple rhizosphere and rhizoplane soils under biochar amendment. – Journal of Soils and Sediments 21(2): 853-868.

[6] Cha, J. Y., Cha, Y., Oh, N. H. (2019): The effects of tree species on soil organic carbon content in South Korea. – Journal of Geophysical Research: Biogeosciences 124(3): 708-716.

[7] Chen, H. M., Ma, J. Y., Wang, X. J., Xu, P. P., Zhang, S., Zhao, Y. W. (2019): Effects of biochar and sludge on carbon storage of urban green roofs. – Forest 9(7): 413.

[8] de Medeiros, E. V., Lima, N. T., de Sousa Lima, J. R., Pinto, K. M. S., da Costa, D. P., Franco Junior, C. L., Hammecker, C. (2021): Biochar as a strategy to manage plant diseases caused by pathogens inhabiting the soil: a critical review. – Phytoparasitica 49(4): 713-726.

[9] Delang, C. O. (2018): The consequences of soil degradation in China: a review. – GeoScape 12(2): 92-103.

[10] Dominic, W., Amonette, J. E., Street-Perrott, F. A., Lehmann, J., Joseph, S. (2018): Sustainable biochar to mitigate global climate change. – Nature Communications 8: 1-9.

[11] Dumortier, J., Dokoohaki, H., Elobeid, A., Hayes, D. J., Laird, D., Miguez, F. E. (2020): Global land-use and carbon emission implications from biochar application to cropland in the United States. – Journal of Cleaner Production 258: 120684.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28012816

[12] Elad, Y., Cytryn, E., Meller Harel, Y., Lew, B., Graber, E. R. (2011): The biochar effect: plant resistance to biotic stresses. – Phytopathologia Mediterranea 50: 335-349.
[13] El-Naggar, A., Lee, S. S., Rinklebe, J., Farooq, M., Song, H., Sarmah, A. K., Ok, Y. S. (2019): Biochar application to low fertility soils: a review of current status, and future prospects. – Geoderma 337: 536-554.
[14] Fan, Q., Cui, L., Quan, G., Wang, S., Sun, J., Han, X., Yan, J. (2018): Effects of wet oxidation process on biochar surface in acid and alkaline soil environments. – Materials 11(12): 2362.
[15] Feng, W., Yang, F., Cen, R., Liu, J., Qu, Z., Miao, Q., Chen, H. (2021): Effects of straw biochar application on soil temperature, available nitrogen and growth of corn. – Journal of Environmental Management 277: 111331.
[16] George, C., Kohler, J., Rillig, M. C. (2016): Biochars reduce infection rates of the root-lesion nematode *Pratylenchus penetrans* and associated biomass loss in carrot. – Soil Biology and Biochemistry 95: 11-18.
[17] Ghorbanzadeh, N., Mahsefat, M., Farhangi, M. B., Rad, M. K., Proietti, P. (2020): Short-term impacts of pomace application and Pseudomonas bacteria on soil available phosphorus. – Biocatalysis and Agricultural Biotechnology 28: 101742.
[18] Gorovtsov, A. V., Minkina, T. M., Mandzhieva, S. S., Perelomov, L. V., Soja, G., Zamulina, I. V., Yao, J. (2020): The mechanisms of biochar interactions with microorganisms in soil. – Environmental Geochemistry and Health 42(8): 2495-2518.
[19] Gu, Y., Hou, Y. G., Huang, D. P., Hao, Z. X., Wang, X. F., Wei, Z., Jousset, A., Tan, S. Y., Xu, D. B., Shen, Q. R., Xu, Y. C., Friman, V. P. (2017): Application of biochar reduces *Ralstonia solanacearum* infection via effects on pathogen chemotaxis, swarming motility, and root exudate adsorption. – Plant and Soil 415(1/2): 269-281.
[20] Gu, Z., Xie, Y., Gao, Y., Ren, X., Cheng, C., Wang, S. (2018): Quantitative assessment of soil productivity and predicted impacts of water erosion in the black soil region of northeastern China. – Science of the Total Environment 637: 706-716.
[21] He, T. H., Liu, D. Y., Yuan, J. J., Luo, J. F., Lindsey, S., Bolan, N., Ding, W. X. (2018): Effects of application of inhibitors and biochar to fertilizer on gaseous nitrogen emissions from an intensively managed wheat field. – Science of the Total Environment 1: 628-629.
[22] Hu, J., Zhao, Y., Yao, X., Wang, J., Zheng, P., Xi, C., Hu, B. (2021): Dominance of comammox Nitrospira in soil nitrification. – Science of the Total Environment 780: 146558.
[23] Hurisso, T. T., Moebius-Clune, D. J., Culman, S. W., Moebius-Clune, B. N., Thies, J. E., Van Es, H. M. (2018): Soil protein as a rapid soil health indicator of potentially available organic nitrogen. – Agricultural & Environmental Letters 3(1): 180006.
[24] Igiehon, N. O., Babalola, O. O. (2018): Rhizosphere microbiome modulators: contributions of nitrogen fixing bacteria towards sustainable agriculture. – International Journal of Environmental Research and Public Health 15(4): 574.
[25] Indoria, A. K., Sharma, K. L., Reddy, K. S., Srinivasarao, C., Srinivas, K., Balloli, S. S., Raju, N. S. (2018): Alternative sources of soil organic amendments for sustaining soil health and crop productivity in India–impacts, potential availability, constraints and future strategies. – Current Science 115(11): 2052-2062.
[26] Jabborova, D., Ma, H., Bellingrath-Kimura, S. D., Wirth, S. (2021): Impacts of biochar on basil (*Ocimum basilicum*) growth, root morphological traits, plant biochemical and physiological properties and soil enzymatic activities. – Scientia Horticulturae 290: 110518.
[27] Jaiswal, A. K., Frenkel, O., Tsechansky, L., Elad, Y., Graber, E. R. (2018): Immobilization and deactivation of pathogenic enzymes and toxic metabolites by biochar: a possible mechanism involved in soilborne disease suppression. – Soil Biology and Biochemistry 121: 59-66.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28012816

[28] Jiao, X. Q., Mongol, N., Zhang, F. S. (2018): The transformation of agriculture in China: looking back and looking forward. – Journal of Integrative Agriculture 17(4): 755-764.
[29] Kaltas, M., Javidoglu, L. (2019): Comparison of enzyme production rate of extracted native bacteria from saline and alkaline soil. – Medbiotech Journal 3(3): 84-87.
[30] Laroca, J. V. D. S., Souza, J. M. A. D., Pires, G. C., Pires, G. J. C., Pacheco, L. P., Silva, F. D. D., Souza, E. D. D. (2018): Soil quality and soybean productivity in crop-livestock integrated system in no-tillage. – Pesquisa Agropecuária Brasileira 53: 1248-1258.
[31] Lehmann, J., Rillig, M. C., Thies, J., Masiello, C. A., Hockaday, W. C., Crowley, D. (2011): Biochar effects on soil biota - a review. – Soil Biology and Biochemistry 43(9): 1812-1836.
[32] Leng, L., Huang, H., Li, H., Li, J., Zhou, W. (2019): Biochar stability assessment methods: a review. – Science of the Total Environment 647: 210-222.
[33] Li, M., Zhang, J., Yang, X., Zhou, Y., Zhang, L., Yang, Y., Yan, Q. (2021): Responses of ammonia-oxidizing microorganisms to biochar and compost amendments of heavy metals-polluted soil. – Journal of Environmental Sciences 102: 263-272.
[34] Liu, Q., Tang, J., Liu, X., Song, B., Zhen, M., Ashbolt, N. J. (2019): Vertical response of microbial community and degrading genes to petroleum hydrocarbon contamination in saline alkaline soil. – Journal of Environmental Sciences 81: 80-92.
[35] Lopes, É. M. G., Reis, M. M., Frazão, L. A., da Mata Terra, L. E., Lopes, E. F., dos Santos, M. M., Fernandes, L. A. (2021): Biochar increases enzyme activity and total microbial quality of soil grown with sugarcane. – Environmental Technology & Innovation 21: 101270.
[36] Ma, D. D. (2018): New soybean variety Suinong 35 and its high yield cultivation techniques. – Agricultural Development & Equipments 4: 154 (in Chinese).
[37] Minkina, T. M., Mandzhieva, S. S., Burachevskaya, M. V., Bauer, T. V., Sushkova, S. N. (2018): Method of determining loosely bound compounds of heavy metals in the soil. – MethodsX 5: 217-226.
[38] Monge, E. C., Tuveng, T. R., Vaaje-Kolstad, G., Eijsink, V. G., Gardner, J. G. (2018): Systems analysis of the glycoside hydrolase family 18 enzymes from Cellvibrio japonicus characterizes essential chitin degradation functions. – Journal of Biological Chemistry 293(10): 3849-3859.
[39] Nannipieri, P., Trasar-Cepeda, C., Dick, R. P. (2018): Soil enzyme activity: a brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. – Biology and Fertility of Soils 54(1): 11-19.
[40] Nkongolo, K. K., Narendrula-Kotha, R. (2020): Advances in monitoring soil microbial community dynamic and function. – Journal of Applied Genetics 61: 249-263.
[41] Novak, J. M., Ippolito, J. A., Lentz, R. D., Spokas, K. A., Bolster, C. H., Sistani, K., Trippe, K. M., Phillips, C. L., Johnson, M. G. (2016): Soil health, crop productivity, microbial transport, and mine spoil response to biochars. – BioEnergy Research 9(2): 454-464.
[42] Ondrasek, G., Begić, H. B., Zovko, M., Filipović, L., Meriño-Gergichevich, C., Savić, R., Rengel, Z. (2019): Biogeochemistry of soil organic matter in agroecosystems & environmental implications. – Science of The Total Environment 658: 1559-1573.
[43] Otsuka, S., Sudiana, I., Komori, A., Isobe, K., Deguchi, S., Nishiuama, M., Shimizu, H., Senoo, K. (2008): Community structure of soil bacteria in a tropical rainforest several years after fire. – Microbes and Environments 23: 49-56.
[44] Ou, Y., Rousseau, A. N., Wang, L. X., Yang, B. X. (2017): Spatio-temproal patterns of soil organic carbin pHin relation to environmental factors: a case study of the black soil region of northeastern China. – Agriculture Ecosystems ＆ Environment 245: 22-31.
[45] Palansooriya, K. N., Wong, J. T. F., Hashimoto, Y., Huang, L., Rinklebe, J., Chang, S. X., Ok, Y. S. (2019): Response of microbial communities to biochar-amended soils: a critical review. – Biochar 1(1): 3-22.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28012816

[46] Panahi, H. K. S., Dehhaghi, M., Ok, Y. S., Nizami, A. S., Khoshnevisan, B., Mussatto, S. I., Lam, S. S. (2020): A comprehensive review of engineered biochar: production, characteristics, and environmental applications. – Journal of Cleaner Production 270: 122462.

[47] Rasool, M., Akhter, A., Soja, G., Haider, M. S. (2021): Role of biochar, compost and plant growth promoting rhizobacteria in the management of tomato early blight disease. – Scientific reports 11(1): 1-16.

[48] Ren, L., Hao, B., Fang, W., Zhang, D., Cheng, H., Li, Q., Cao, A. (2022): Combination of modified biochar and polyurea microcapsules to co-encapsulate a fumigant via interface polymerization for controlled release and enhanced bioactivity. – Pest Management Science 78(1): 73-85.

[49] Sheng, Y., Zhu, L. (2018): Biochar alters microbial community and carbon sequestration potential across different soil pH. – Science of the Total Environment 622: 1391-1399.

[50] Sun, D. Q., Meng, J., Liang, H., Yang, E., Huang, Y. W., Chen, W. F., Jiang, L. L., Lan, Y., Zhang, W. M., Gao, J. P. (2014): Effect of volatile organic compounds absorbed to fresh biochar on survival of Bacillus mucilaginosus and structure of soil microbial communities. – Journal of Soils and Sediments 15: 271-281.

[51] Viger, M., Hancock, R. D., Miglietta, F., Taylor, G. (2015): More plant growth but less plant defence? First global gene expression data for plants grown in soil amended with biochar. – GCB Bioenergy 7(4): 658-672.

[52] Wang, J. L., Wang, S. Z. (2019): Preparation, modification and environmental application of biochar: a review. – Journal of Cleaner Production 227(1): 1002-1022.

[53] Wang, C., Chen, D., Shen, J., Yuan, Q., Fan, F., Wei, W., Wu, J. (2021a): Biochar alters soil microbial communities and potential functions 3–4 years after amendment in a double rice cropping system. – Agriculture, Ecosystems & Environment 311: 107291.

[54] Wang, G., Li, Q., Yuwen, C., Gong, K., Sheng, L., Li, Y., Chen, R. (2021b): Biochar triggers methanogenesis recovery of a severely acidified anaerobic digestion system via hydrogen-based syntrophic pathway inhibition. – International Journal of Hydrogen Energy 46(15): 9666-9677.

[55] Wołejko, E., Jabłońska-Trypuć, A., Wydro, U., Butarewicz, A., Łozowicka, B. (2020): Soil biological activity as an indicator of soil pollution with pesticides - a review. – Applied Soil Ecology 147: 103356.

[56] Wu, X., Sun, Y., Deng, L., Meng, Q., Jiang, X., Bello, A., Xu, X. (2020): Insight to key diazotrophic community during composting of dairy manure with biochar and its role in nitrogen transformation. – Waste Management 105: 190-197.

[57] Wu, G. Q., Li, H., Zhu, Y. H., Li, S. J. (2021): Comparative physiological response of sainfoin (Onobrtchis viciaefolia) seedings to alkaline and alkaline and saline-alkaline stress. – Journal of Animal & Plant Sciences 31(4): 1028-1035.

[58] Yang, X., Tsibart, A., Nam, H., Hur, J., El-Naggar, A., Tack, F. M., Ok, Y. S. (2019): Effect of gasification biochar application on soil quality: trace metal behavior, microbial community, and soil dissolved organic matter. – Journal of Hazardous Materials 365: 684-694.

[59] Yao, Q., Liu, J., Yu, Z., Li, Y., Jin, J., Liu, X., Wang, G. (2017): Changes of bacterial community compositions after three years of biochar application in a black soil of northeast China. – Applied Soil Ecology 113: 11-21.

[60] Yi, Y., Huang, Z., Lu, B., Xian, J., Tsang, E. P., Cheng, W., Fang, Z. (2020): Magnetic biochar for environmental remediation: a review. – Bioresource Technology 298: 122468.

[61] Yu, H., Liu, R., Hu, Y., Xu, B. (2017): Flavor profiles of soymilk processed with four different processing technologies and 26 soybean cultivars grown in China. – International Journal of Food Properties 20(sup3): S2887-S2898.

[62] Zhang, H., Wang, S., Zhang, J., Tian, C., Luo, S. (2021): Biochar application enhances microbial interactions in mega-aggregates of farmland black soil. – Soil and Tillage Research 213: 105145.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28012816

[63] Zhou, J., Jiang, X., Zhou, B. K., Zhao, B. S., Ma, M. C., Guan, D. W., Li, J., Chen, S. F., Cao, F. M., Shen, D. L., Qin, J. (2016): Thirty-four years of nitrogen fertilization decreases fungal diversity and alters fungal community composition in black soil in northeast China. – Soil Biology and Biochemistry 95: 135-143.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28012816