Plant Nutrition & Soil Science International (2023)
Clausius Scientific Press, Canada

DOI: 10.23977/pnssi.2023.020101
ISSN 2616-1745 Vol. 2 Num. 1

Effects of Biochar-based Fertilizer on Soil Physicochemical Properties and Rhizosphere Bacterial Community Structure

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Keywords: Soil, Biochar, Flue-cured tobacco, Soil Enzyme Activity, Microbial Community Structure

Abstract: The application of biochar in agriculture and environmental fields has received widespread attention. In order to explore the response mechanism of flue-cured tobacco rhizosphere bacterial community and functional fungal taxa to biochar fertilizer, mass field experiment and two treatment methods were set up in this study, one is CK: conventional fertilization, and the other is T: 50 kg/mu biochar base fertilizer + conventional fertilization (nitrogen reduction by 20%). The experimental results showed that the pH value, organic matter content, alkaline hydrolyzable nitrogen, available phosphorus and available potassium content of the soil applied to biochar-based fertilizer increased significantly, and the neutral phosphatase activity of the soil decreased significantly. The bacterial Shannon index, ACE index and CHAO index were significantly increased, and the amplitudes of which were 10.16%, 37.39% and 38.76%, respectively. The bacterial Simpson index was significantly reduced; Actinobacteriota had the highest abundance, 25.88%-39.91%, followed by Proteobacteria, with abundances of 20.88%-27.75%, respectively. Firmicutes were positively correlated with soil organic matter, alkaline hydrolyzable nitrogen and soil catalase, and positively correlated with available phosphorus and available potassium. The results show that biochar-based fertilizer can effectively improve soil physicochemical properties, increase soil enzyme activity, change soil microbial community structure, and drive the dynamic change of soil bacterial community composition, which is conducive to improving soil quality.

1. Introduction

Biochar usually refers to a special solid product obtained by high-temperature cracking treatment of biomass energy raw materials under the condition of insufficient oxygen [1]. The main constituent elements of biochar are N, O, H, C, etc., of which carbon accounts for the largest proportion, the carbon content is more than 70%, and it is alkaline under natural conditions. Because it has a highly carboxylated and aromatic structure, this makes it extremely soluble, rich pores, and with large specific surface area [2], so it has the characteristics of strong stability, high carbon content, rich pore structure and large specific surface area. Several studies have shown that biochar-based fertilizers play an important role in soil remediation. Adding biochar to soil can increase the content of soil organic carbon, improve the content of soil availability nutrients, and improve soil enzyme activity, and the abundance and functional diversity of microbial communities, thereby promoting plant growth [3]. At the same time, it can also change the physical, chemical and biological properties of the soil. Therefore, the practice of biochar soil improvement has attracted more and more attention from researchers [4]. It was found that the combined fertilization of organic fertilizer and nitrogen fertilizer significantly increased the content of soil organic matter, total carbon, total nitrogen, total phosphorus and available phosphorus [5]. After people apply biochar, it will affect soil enzyme activity [6] and increase the activity of soil enzyme activity [7]. Huang Jian et al [8]. found that catalase will participate in the oxidation process of some organic matter in soil, and the application of biochar will increase soil organic matter, which is one of the main reasons for its influence on soil catalase[9-11]. Ibrahim [12] et al. found that the application of tobacco stalk charcoal-based fertilizer could significantly increase the activity of soil nitrogen cycling enzyme and the abundance of soil fungal taxa (proteobacteria, actinomycetes, etc.), and improve the structure of soil microbial communities. Therefore, the application of biochar can change the habitat environment of soil microorganisms, promote the reproduction rate of soil microorganisms, stimulate the change of soil microorganisms, and drive the cycle of soil elements.

By analyzing the changes of soil physicochemical characteristics, soil enzyme activity, flue-cured tobacco leaf enzyme activity and microbial community structure after the biochar-based fertilizer, this paper deeply explores the influence of the biochar-based fertilizer on the bacterial community structure of tobacco planting soil and the physicochemical characteristics of the soil, in addition, that correlation analysis is carry out on the bacterial community structure at the soil phylum and the physicochemical properties of the soil. The aim is to provide theoretical basis for efficient utilization of agricultural nitrogen and establishment of rhizosphere microbial community.

2. Materials and Methods

2.1. Experiment Materials

The experiment was carried out in the southern tobacco area of Shangluo City, Shanxi Province in 2021. The basic physicochemical properties of the experiment soil were pH 7.13, organic matter 20.12 g ·kg-1, alkaline nitrogen 120.45 mg ·kg-1, available phosphorus 42.36 mg ·kg-1, available potassium 224.62 mg ·kg-1. The category of flue-cured tobacco was Yunyan 87, which was provided by China Tobacco Shaanxi Company. Biochar-based fertilizer is provided by Henan Biochar Engineering Technology Center. Its main components include organic materials such as biochar, cake fertilizer and minerals. Its organic matter content is 45.20%, N content is 1.02%, mass fraction of P2O5 is 1.12% and mass fraction of K2O is 2.90%.

2.2. Experiment Design

Random block design was adopted in the experiment. Two treatments were set up, and each treatment was repeated 3 times. CK: conventional fertilization; T: 50 kg/mu of biological biochar-based fertilizer + conventional fertilization. The biochar-based fertilizer and the conventional fertilizer are used as the base fertilizer to be applied to tobacco planting soil at one time, and the fertilizing mode is strip application. The transplanting method, water and fertilizer management and farming operation of each treatment are consistent with the management of non-experimental fields.

2.3. Experiment Soil and Sample Collection

When flue-cured tobacco grows to the 75th day of mature period, the sampling point shall be determined according to the five-point sampling method. The rhizosphere soil of 5 tobacco plants for each treatment shall be collected and mixed evenly. One part of them was stored in a 10 mL sterile centrifuge tube and stored in dry ice for microbial diversity detection, and another part was stored in a refrigerator at 4 ℃ for soil microbial biomass detection. The last part was naturally air-dried in a cool place, ground, sieved by 0.85, 0.25 and 0.15 mm sieves respectively, and then stored in a cool place for analysis of physicochemical properties of the soil.

2.4. Determination of Physicochemical Indexes and Nutrients of Soil

The analysis method of soil physicochemical properties shall refer to Soil Agricultural Chemical Analysis Method [10], and make parallel group for sample points. The soil water content (SWC) is determined by oven drying method; the soil bulk density (SBD) is determined by ring knife method; the pH is determined by using ultrapure water as leaching agent, and the ratio of soil water is 1: 2.5, and directly measured by precision pH meter (model: IS128C); the soil available phosphorus (AP) is determined by sodium bicarbonate extraction method. Soil organic carbon (SOM) was determined by concentrated sulfuric acid-potassium dichromate heating method. The alkaline hydrolysis nitrogen (AN) adopts an alkaline hydrolysis dispersion method; Microbial biomass carbon and nitrogen (MBC, MBN) was determined by chloroform fumigation-potassium sulfate leaching method.

2.5. Detection of Soil Microorganism

Soil DNA extraction and PCR amplification analysis: according to the E. Z. N. A. ® soil kit (Omega Bio-tek, Norcross, GA, U. S.) instructions, total DNA extraction was carried out, the concentration and purity of DNA were detected using a NanoDrop 2000 ultra-micro spectrophotometer (Thermo Fisher Scientific Co.), and the mass of DNA extraction was detected by 1% agarose gel electrophoresis; The V3-V4 variable region of the bacterial 16SrRNA is amplified by PCR using 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTA CHVGGGT WTCTAAT-3') primers. The procedure for amplification is: pre-denaturing at 95 ℃ for 3 min, 27 cycles (denaturation at 95 ℃. for 30 s, annealing at 55 ℃ for 30 s, 72 ℃ for 30 s), at the end, 72 ℃ for 10 min (PCR apparatus: ABI GeneAmp ® model 9700). The amplification system was 20 ul, 4 ul 5*FastPfu buffer, 2 ul 2.5 mM dNTPs, 0.8 ul primer (5 Um), 0.4 ul FastPfu polymerase and 10 ngDNA template.

2.6. Data Analysis

Microsoft Excel 2021 was used to sort out the data. The Least Significant Difference method was used for analysis of variance. The data was analyzed and processed with SPSS26.0 software. The Heat map analysis is clustered based on similarity of abundance between species or samples, plotted with the vegan package of R software. Principal Component Analysis uses SPSS26.0 software. Microorganisms use the UPARSE software to cluster sequences based on 97% similarity, and Operational Taxonomic Units (OTU) clusters; Microbial sequence deduplication filtration using Qiime 1.9.1, sequence classification annotation.

3. Results and Analysis

3.1. Effect of Biochar-based Fertilizer on Physiochemical Properties of Soil

From Table 1, it can be concluded that the application of biochar-based fertilizer can effectively improve the physiochemical properties of the soil. Compared with the blank control, the soil pH value, organic matter and soil nutrient content increased significantly after the application of biochar-based fertilizer. At the same time, compared with the CK, after the application of biochar-based fertilizer, the soil organic matter content increased by 22.37%, the alkaline hydrolyzable nitrogen content increased by 14.41%, the available phosphorus content increased by 15.43%, and the organic matter content increased by 17.36%.

Table 1: Effect of Biochar-based Fertilizer on Physiochemical Properties of Soil

Treatment	pH	Organic matter (g/kg)	Alkaline hydrolysis nitrogen (mg/kg)	Available phosphorus (mg/kg)	Available potassium (mg/kg)
CK	7.21b	24.27b	134.99b	55.62b	251.58b
ST	7.82a	29.70a	154.44a	64.20a	295.26a

3.2. Effect of Biochar-based Fertilizer on Enzyme Activities in Flue-cured Tobacco Rhizosphere Soil

It can be seen from Figure. 1 that neutral phosphatase, sucrase, catalase and urease in rhizosphere soil are significantly affected after biochar application, which is closely related to soil carbon and nitrogen metabolism. After the application of biochar-based fertilizer in the soil, the activity of neutral phosphatase decreased by 33.90%, the sucrase activity increased by 28.17%, the catalase activity increased by 26.36%, and the urease activity increased by 17.27%, all reaching significant levels.

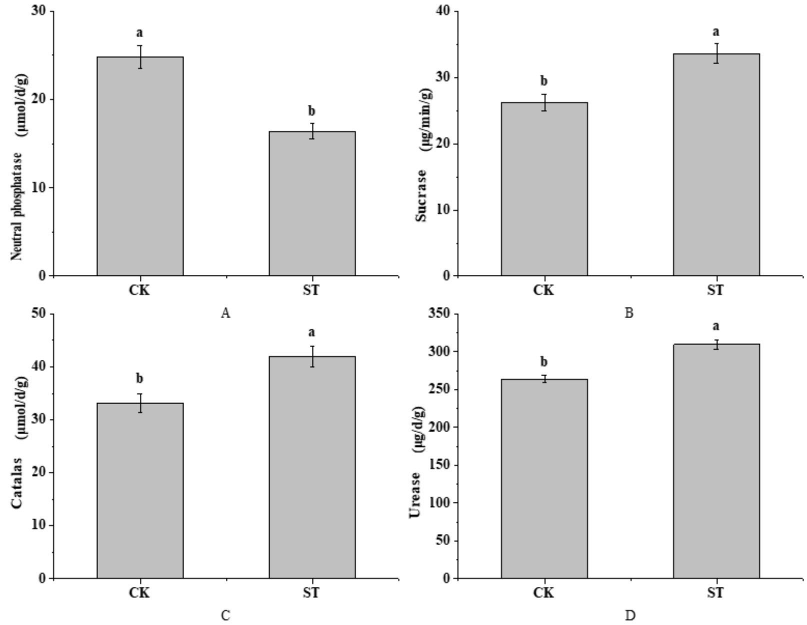

Figure 1: Effect of Biochar-based Fertilizer on Enzyme Activities of Flue-cured Tobacco Rhizosphere Soil

3.3. Effect of Biochar Fertilizer on Bacterial α Diversity in Flue-cured Tobacco Rhizosphere Soil

Six soil samples were experimented for bacteria, and a total of 344107 sequences were obtained. It can be seen from Figure. 2 that cluster analysis is carried out on the experimented sequences according to 97% sequence similarity, and 6 samples generate 1784-2724 OTUs, the average OTU number of samples is 2197. The total number of OUT of CK and T treatment is 1772, and the OUT number of rhizosphere soil with biochar-based fertilizer is increased by 42.06% compared with the blank control. It can be seen from Table 2 that the bacterial Shannon index, ace index and chao index of the soil added with biochar-based fertilizer are significantly increased by 10.16%, 37.39% and 38.76% respectively, and the bacterial simpson index is significantly reduced to 50.07%. The results showed that biochar-based fertilizer could significantly increase the α diversity of bacteria in the rhizosphere soil of flue-cured tobacco.

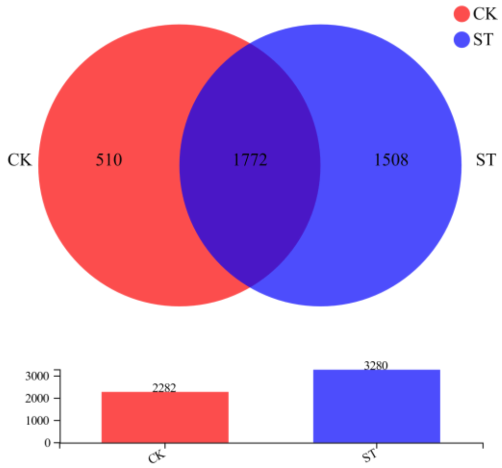

Figure 2: Soil bacterial community OUTs

Table 2: Effect of Biochar-based Fertilizer on α Diversity of Soil Bacteria

Treatment	Valid sequence	Shannon index	Simpson index	Ace index	Chao index	Coverage
CK	52356	5.98b	0.007397a	2102.15b	2088.91b	0.9921
ST	62346	6.59a	0.003694b	2888.21a	2898.56a	0.9913

3.4. Effect of Biochar-based Fertilizer on Bacterial β Diversity in Flue-cured Tobacco Rhizosphere Soil

The results of principal component analysis of soil colony structure based on OTUs abundance are shown in Figure 3 and the contribution values of PC1 axis and PC2 axis to the difference in sample composition are 74.1% and 16.87%, respectively. It can be seen from Figure. 4that after the addition of biochar-based fertilizer, the rhizosphere soil and the sample points treated with blank control showed obvious separation at the level of PC1 axis. It can be seen that the application of biochar-based fertilizer can significantly affect the change of soil bacterial community structure.

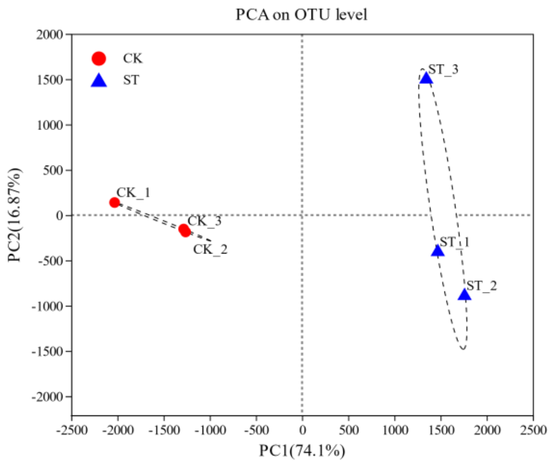

Figure 3: PCA analysis of soil bacterial community based on OTUs

3.5. Effect of Biochar Fertilizer on Bacterial Community Diversity in Flue-cured Tobacco Rhizosphere Soil

The bacterial community composition analysis was performed on the soil of the rhizosphere of flue-cured tobacco and a total of 35 bacterial flora were obtained at the phylum level in the sample. It can be seen from Figure.4 that Actinobacteriota has the highest abundance, ranging from 25.88% to 39.91%, followed by Proteobacteria, Chloroflexi, Acidobacteriota and Gemmatimonadota, with relative abundances reaching 20.88%-27.75%, 9.41%-16.90%, 7.77%-10.76% and 3.11%-6.27%, respectively. The average relative abundance was 23.49%. 12.50%, 8.86% and 5.02%. The sum of the relative abundance of these species with higher abundance accounted for more than 85% of all annotative bacteria.

According to the T-test on the relative abundance of bacterial communities in the soil samples treated with biochar and the control soil samples, it can be seen from Figure. 5 that the abundance of Chloroflexi and Firmicutes in the soil added with the biochar-based fertilizer is increased by 38.68% and 57.83%, respectively; the abundance of Actinobacteriota, Proteobacteria, Acidobacteriota and Gemmatimonadota is reduced by 20.95%, 11.02%, 9.55% and 24.49%, respectively. It can be seen that the abundance of soil bacteria after the application of biochar-based fertilizer has different degrees of influence in different phyla species.

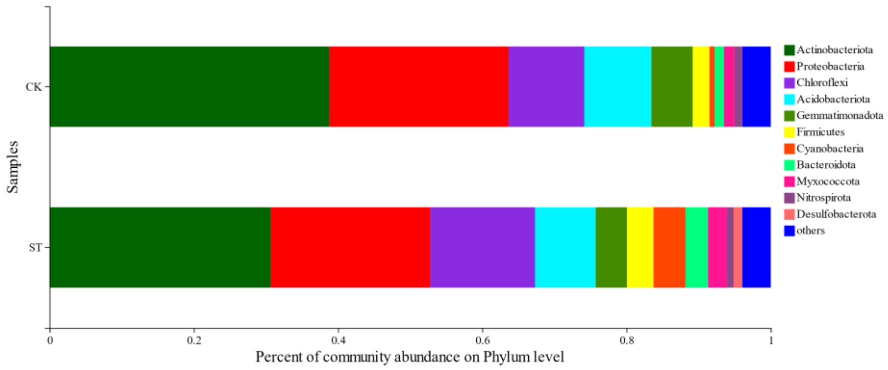

Figure 4: Relative Abundance of Bacterial Communities at Soil phylum Level

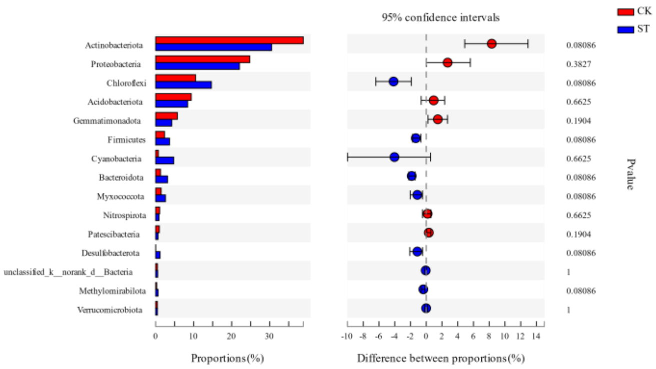

Figure 5: Difference in Abundance of Dominant Bacteria at Bacterial Phylum Level

3.7. Correlation Analysis of Bacterial Community Structure and Soil Physicochemical Properties at Soil Phylum Level

It can be seen from Figure.6 that Chloroflexi has extremely significant positive correlation with soil pH ($P<0.01$), Firmicutes and Desulfobacteria has extremely significant positive correlation with soil organic matter and soil catalase ($P<0.01$). Meanwhile, Firmicutes and alkalinelyzable nitrogen also showed a significant positive correlation, and correlated with available phosphorus and available potassium ($P<0.05$). Myxococota was positively correlated with soil organic matter and alkaline hydrolyzable nitrogen, and was significantly positively correlated with catalase and urease activities, while Bacteroidota was significantly positively correlated with available phosphorus and negatively correlated with neutral phosphatase.

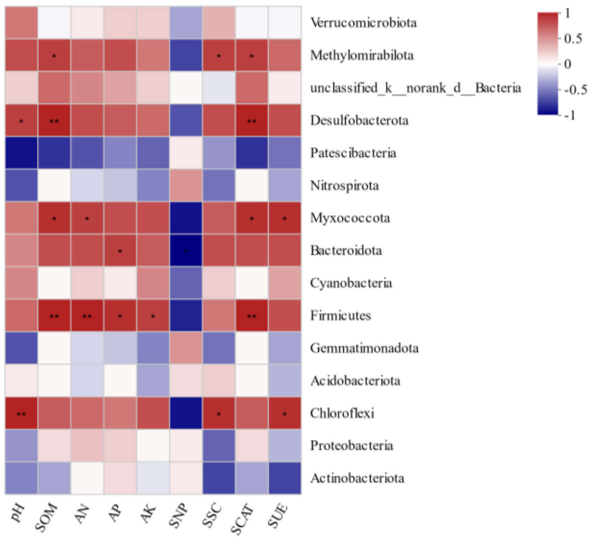

Figure 6: Correlation between dominant group of bacteria and physicochemical properties of soil

4. Conclusion and Discussion

4.1. Effect of Biochar-based Fertilizer on Physicochemical Properties and Enzyme Activity of Soil

The contents of carbon, nitrogen and mineral elements in soil are also important indexes to be considered in soil quality evaluation and land sustainable development. The results showed that the contents of pH value, organic matter, alkali-hydrolyzed nitrogen, available phosphorus and available potassium in the soil added with biochar-based fertilizer were significantly higher than those of CK treatment. This is consistent with the research results of Feng Huilin et al[13].This is due to the complex pore structure, large specific surface area, strong adsorption performance [14,15] and high cation exchange capacity [16] , which can adsorb K^{+}, Ca^{2+}, NH^{4+} and other salt-based cations [17,18] in the soil. After being applied to the soil, the soil pH and organic matter content will be significantly increased. At the same time, the addition of biochar can increase the content of alkali-hydrolyzed nitrogen, available phosphorus and available potassium in the soil, which is consistent with the research results of LIU et al.[19].

Secondly, because of the unique physicochemical characteristics of biochar, the soil aggregation structure was changed during the application of biochar to the soil, which further affected the change of soil enzyme activity. Soil enzymes can participate in soil biochemical reactions, and their activity can reflect the activity of soil organisms and biochemical reactions. Among them, urease can directly participate in the form transformation of nitrogen, and its activity can represent the nitrogen status in the soil. The catalase activity is relatively stable in the soil, which is one of the important indexes of soil fertility. Soil sucrase reflects the accumulation and decomposition rules of soil organic carbon[20]. According to the study, the soil urease, sucrase and catalase activities increased significantly after the application of biochar-based fertilizer, which is consistent with the research of Feng Huilin [9] and Zhou Zhenfeng et al.[21].

4.2. Effect of Biochar Fertilizer on Soil Bacterial Community Structure

Soil microorganism is an important component of maintaining the stability of soil ecosystem. The increase of the abundance and quantity of soil microorganism will significantly improve the stability and diversity of the whole soil ecosystem. In this study, biochar was added to increase soil bacterial diversity, consistent with previous studies[22]. Biochar promotes some soil bacterial phylum and inhibits another some at the same time[23], such as the increase of the abundance of Actinomycetes and the decrease of the abundance of Sphenomonas. Li Maosen et al. [24]think that the diversity of bacteria group participating in carbon fixation in soil is significantly related to soil type and soil environmental factors. This study results showed that the structure of soil bacterial community was driven by various environmental factors. Soil organic matter, alkali-hydrolyzed nitrogen, available phosphorus and available potassium were the common driving factors for bacterial community variation. Chloroflexi was significantly positively correlated with soil pH. Firmicutes were significantly positively correlated with soil organic matter, soil catalase, alkaline hydrolyzable nitrogen, available phosphorus and available potassium.

5. Conclusion

The application of the biochar-based fertilizer can effectively improve the physicochemical properties of the soil, increase the pH value of the soil, the content of organic matters and the available nutrients in the soil, simultaneously change the diversity and abundance of soil bacteria, improve the abundance of functional microorganisms in the rhizosphere of tobacco plants, and perfect the composition of bacterial communities. And Different dominant phylums became the driving factors of different soil physicochemical indexes, which were closely related to the changes of soil composition in the rhizosphere of flue-cured tobacco tocophy. Therefore Biochar-based fertilizer has a significant impact on cultivating soil health and promoting tobacco plant growth.

Acknowledgment

This work was supported by the Fund Project: Science and Technology Reach Project of

Guangdong Zhongyan Industry Co., Ltd. (2022440000340014); Science and Technology Reach Project of Hanzhong Company of Shaanxi Tobacco Company (2021610725200019).

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