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RETRACTED: Effects of the Application of Biochar in Four Typical Agricultural Soils in China

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Received: 6 February 2020; Accepted: 28 February 2020; Published: 4 March 2020



Abstract: The purpose of this study was to explore the effects of biochar application on soils in the main tobacco-producing areas in China. The study was conducted in four study regions in China, where the same three experimental treatments were set up in each area, including a control (CK), a treatment involving the application of chemical fertilizer (F), and a treatment involving the application of biochar (B). We analyzed the basic physical and chemical properties, microbial diversity, and root system of tobacco plants. The results show that: Biochar increased the soil pH, which was most obvious in the study site in Shaowu City, Fujian Province (FUS), where the soil pH increased by 22.64% and 27.49% compared with soil under the CK and F treatments, respectively. Biochar increased the microbial biomass carbon (MBC) content, and increased the soil content of available nitrogen, phosphorus, and potassium; this effect was most obvious in FUS. The root activity in plots treated with biochar increased by 6.95% and 13.72% compared to the CK and F plots, respectively. Similarly, the number of root tips increased by 89.76% and 21.48% compared to the CK and F plots, respectively. In short, biochar improved the physical soil structure, increased the soil pH, and promoted the effectiveness of soil nutrients. Furthermore, biochar improved the bacterial soil diversity, enriched the population structure of soil bacteria, and promoted the healthy development of flue-cured tobacco roots. However, the demand for and types of biochar suitable for use in different tobacco-planting soils need further study.

Keywords: biochar; physical and chemical properties of soil; microbial diversity; root; tobacco

1. Introduction

Biochar is a substance produced via pyrolysis at a lower carbonization temperature (350–700 °C) under conditions of complete or partial anoxia. The carbon content of biochar is high, and its chemical properties are stable [1]. Due to its highly aromatic inner structure, biochar can be used in a wide range of applications, including reducing greenhouse gas emissions, improving soil quality, and recycling waste, among others [2]. Biochar has a large specific surface area, is porous, can carry a large amount of negative charge and a high charge density, and can form an electromagnetic field [3]. Biochar can absorb water and nutrients in soil, but also adsorb organic compounds and inorganic ions [4], which can promote the absorption of nutrients by plants. Biochar can also increase the soil active organic carbon, as well as increasing the abundance of soil microflora. These effects on the soil can help promote plant growth and development, as well as improve crop quality. Biochar has been used to improve soil for thousands of years. The first reported use of biochar to improve soil was the in the

Amazon's Terra Preta black soil [2]. Biochar has recently received a lot of international attention [5], and increasingly more articles about biochar have been published in recent years [6]. Changes in soil quality have contributed to the increased interest in biochar research, as biochar can be used to achieve waste recycling and reduce greenhouse gas emissions. Furthermore, the combination of current economic and environmental benefits of using biochar is a hot topic of research. There is a wide range of raw materials that can be used to make biochar, including wood materials; agricultural waste, such as olive shells; corn cobs; peanut shells [2,7,8]; green waste, such as animal manure; and other waste [9–11]. The application of biochar has been proven to have many advantages, including improved soil quality and plant growth [9,12], and reduced soil greenhouse gas emissions [12–14].

In recent decades, due to the long-term use of excessive chemical fertilizers in China, and the lack of awareness regarding the importance of land conservation, China's agricultural development has been extremely dependent on the use of chemical fertilizers. However, over the same period, China's cultivated soil has also experienced severe acidification, compaction, and salting. In the past 8 to 10 years, the pH of farmland has dropped by an average of 0.5–1.0, and the abundance of soil microbial communities has decreased by 2.17% [15]. China is a large agricultural country, and therefore the question of how to solve the problem of soil degradation has become an important aspect of research regarding China's agricultural health, environmental protection, and rapid and sustainable development. In order to explore the application of biochar in tobacco-growing soil, we used four typical tobacco-growing soils (Ferralsol, Acrisol, Fuvisol, and Phaeozem) and tobacco as research objects in China, and set up three experimental treatments for each soil type. The three treatments consisted of a control treatment (CK), treatment with nitrogen fertilizer (F), and treatment with biochar (B). Subsequently, we analyzed the physical, chemical, and microbiological properties of soils under the different treatments, as well as the tobacco root activity and the total number of root tips. We used these data to explore the effects of biochar on the soil, as well as on the growth and development of flue-cured tobacco roots. Furthermore, our data provide theoretical support for the application of biochar in agricultural contexts.

2. Materials and Methods

2.1. Test Materials

(1) Test soil: Our study took place in the following locations: Shaowu City, Fujian Province (FUS; 27°17'48" N, 117°19'42" E), which has a mid-subtropical monsoon climate, with an average elevation of 199 m, annual average rainfall between 1500 and 2100 mm, annual average temperature of 20.6–21.5 °C, day and night temperature difference of 6–7 °C; Chenzhou City, Hunan Province (HUS; 25°50'24" N, 113°07'39" E), which has a subtropical Monsoon climate, with an average altitude of 251 m, annual average rainfall of 1600–1800 mm, annual average air temperature of 18.5–18.9 °C, day and night temperature difference of 9–11 °C; Xuchang City, Henan Province (HES; 34°06'09" N, 113°47'36" E), which has a north warm temperate monsoon climate, average elevation 96 m, the average annual rainfall is 671–736 mm, the average temperature is 14.3–14.6 °C, and the temperature difference between day and night is 8–15 °C; and Mudanjiang City, Heilongjiang Province (HEIS; 44°23'56" N, 129°28'58" E), which has a marine (semi-humid) mid-temperate monsoon climate, with an average elevation of 230 m, the annual average rainfall is 500–600 mm, the average temperature is 4.6–5.3 °C, and the temperature difference between day and night is 6–17 °C. According to Chinese soil texture standards, they are classified as a red soil (FUS), a yellow brown soil (HUS), a cinnamon soil (HES), and a dark brown forest soil (HEIS), or Ferralsol, Acrisol, Fuvisol, and Phaeozem [16], respectively. The basic physical and chemical properties of the soils in each area are shown in Table 1.

Table 1. Basic chemical properties of the four soils included in this study.

Site	Classification	N	C	S	pH	Alkaline Nitrogen (mg/kg)	Available Phosphorus (mg/kg)	Available Potassium (mg/kg)
		(g/kg)	(g/kg)	(mg/kg)				
FUS	Ferralsol	0.78	16.92	0.09	4.61	112.53	36.85	237.53
HUS	Acrisol	0.62	19.81	0.13	5.12	81.89	29.59	184.62
HES	Fuvisol	0.46	13.41	0.05	6.13	97.57	19.88	206.48
HEIS	Phaeozem	0.81	23.26	0.07	6.95	89.48	21.61	165.45

Note: The soil data was obtained in April 2016. The soil was sampled using the five-point method in the experimental field. The soils at five points in the same test plot were mixed and the physical and chemical properties were measured. The N, C, and S columns represent the total soil nitrogen, total carbon, and total sulfur. The physical and chemical properties were determined three times. Note: FUS: Shaowu City, Fujian Province; HIS: Chenzhou City, Hunan Province; HES: Xuchang City, Henan Province; HEIS: Mudanjiang City, Heilongjiang Province.

(2) Biochar: Biochar was prepared using continuous flow carbonization and pyrolysis techniques [17]. The raw material used was peanut shell, and the carbonization temperature was raised at a rate of 26 °C min⁻¹ to 450 °C for 25 min [18]. The basic properties of the biochar used in this study are shown in Table 2.

Table 2. Basic physical and chemical properties of biochar used in this study.

Variable	Limit of Detection	Biochar
Specific surface area (m ² /g)	0.7	16.72
pH	0.05	8.6
Electrical conductivity, EC (dS/m)	0.02	2.3
Total nitrogen (mg kg ⁻¹)	0.2	7.9
Total carbon (mg kg ⁻¹)	4	583.4
Ammonium-N (mg kg ⁻¹)	0.2	587
Nitrate-N (mg kg ⁻¹)	0.2	0.38
Aluminum (g kg ⁻¹)	0.004	1.7
Arsenic (mg kg ⁻¹)	4	<4
Boron (mg kg ⁻¹)	5	13
Calcium (g kg ⁻¹)	0.002	56
Cadmium (mg kg ⁻¹)	0.3	<0.15
Cobalt (mg kg ⁻¹)	0.5	4.3
Chromium (mg kg ⁻¹)	0.2	7.9
Copper (mg kg ⁻¹)	0.3	26
Iron (g kg ⁻¹)	0.0004	5
Potassium (g kg ⁻¹)	0.005	4.1
Magnesium (g kg ⁻¹)	0.0007	12.2
Manganese (mg kg ⁻¹)	0.2	1893
Molybdenum (mg kg ⁻¹)	0.4	1.8
Sodium (g kg ⁻¹)	0.006	1.6
Nickel (mg kg ⁻¹)	0.5	5.8
Phosphorus (g kg ⁻¹)	0.0002	2.3
Lead (mg kg ⁻¹)	2	3.7
Sulfur (g kg ⁻¹)	0.0007	0.39
Selenium (mg kg ⁻¹)	3	<3
Zinc (mg kg ⁻¹)	0.6	117

2.2. Experimental Design

The study used a field trial in which a field with uniform fertility was selected in each tobacco-producing area, and the crops were consistent (tobacco). Three treatments were set up in each area: a control treatment (CK, with no application of fertilizer or biochar), a fertilizer treatment (F, involving the application of 60 kg/hm² of fertilizer with ammonium nitrate: superphosphate: potassium sulfate = 2:1:1), and a biochar treatment (B, involving the application of 2400 kg/hm² biochar + 36 kg/hm² fertilizer, which is the same fertilizer used in treatment F).

2.3. Sampling Method

Soil and tobacco samples were collected in 2018. Sample harvest dates for the four locations were: FUS—May 15, HUS—June 7, HES—June 28, and HEIS—July 11. For each treatment, soil samples and tobacco samples were taken 65 days after tobacco transplanting (tobacco flourish period). We collected five tobacco plants in each treatment and preserved the rhizosphere soil. After pulling out the tobacco plants, we gently shook off the soil near the roots, and collected two such rhizosphere soils into a ziplock bag. Each of these soil samples weighed 100 g. One sample was dried in the shade and then passed through a sieve with a pore size of 0.3 mm; this sample was used for determining the physical and chemical properties of the soil. Another sample was stored in liquid nitrogen and used to determine the soil bacterial diversity. Each tobacco plant root was collected from 10 g of three to four lateral roots, stored at 4 °C, and the root activity was measured within one hour to avoid excessive root explant time.

2.4. Determination of Biochar Properties and Soil Chemical Properties

Electrical conductivity (EC), nitrate and ammonium salts, bray and pH, available phosphorus, total nitrogen and carbon, and broken biochar were determined using the methods described by Van Zwieten [19]. In short, the total carbon and nitrogen were measured using Dumas combustion with an Elementar vario MAX CN (manufactured by Shanghai Haichao Optoelectronic Technology Co., Ltd., Thuringia, Germany) in an analyzer with a combustion chamber temperature set at 900 °C. The flow rate of oxygen and carbon was 125 mL min⁻¹. The pH was determined in 0.01 M CaCl₂ (1:5) according to method 4B2 [20]. Acid extractable elements and metals were determined using inductively coupled plasma optical emission spectroscopy (ICP–OES) using a Varian 720–EC ICP–OES (Yuying Industrial Co., Ltd., Shanghai, China) according to USEPA (U.S. Environmental) 6010. The soot value of the biochar was determined to be the carbonate equivalent using method 19A1 [21]. The soil pH was determined using potentiometry. The soil microbial biomass carbon (MBC) was determined using the chloroform fumigation extraction method, where $MBC = (\text{fumigation soil organic carbon} - \text{no soil organic carbon})/0.45$ [21].

2.5. Bacterial Diversity

The 16S rRNA gene of the rhizosphere soil bacterial community was subjected to DNA extraction, PCR, and sequencing [19]. Rhizosphere soil (0.5 g) was used for DNA extraction, where the sample represented the bacterial community of the soil around the root (i.e., the soil closely attached to the root surface). The obtained microbial 16S rRNA gene sequence was subjected to extensive filtration, and its Mothur [22] were clustered into an operation taxonomic unit (OTU) [23,24]. After clustering OTUs with 97% similarity, abundance filters were used, which eliminated all OTUs with an abundance of <60 counts [24]. In addition, the OTU counts in the subsamples ($n = 5$) were graphed, and the resulting samples were rounded to integers, which simplified the data analysis and reduced the complexity of the model [19]. The soil total carbon was measured using a fully automatic carbon-nitrogen elemental analyzer (Elementar, Germany, C/N/S Vario EL/micro cube). Fast-acting nitrogen (AN) was extracted using 1 mol/L KCl solution-flow analysis. The soil NO₃⁻-N and NH₄⁺-N content was determined by the carbon-nitrogen elemental analyzer, and then the sum of the two was obtained. The available phosphorus (AP) was determined using the Olsen method (0.5 mol/L NaHCO₃ leaching molybdenum anti-colorimetric method). Rapid-acting potassium (AK) was extracted with 1 mol/L NH₄O Ac solution using flame photometry [12]. The root activity of flue-cured tobacco was determined using the TTC (2,3,5–triphenyltetrazolium chloride) method. The most vigorous part of the vitality was polished with ethyl acetate, and the apical section of 0 to 1.0 cm was cut to become the test material. The oxidation state of TTC is colorless and can be reduced by hydrogen to an insoluble red tribenzidine (TTF). The degree of staining was used to identify the vitality of the seeds. Furthermore, the total number of root tips of flue-cured tobacco was measured using the counting method [25,26].

2.6. Data Processing

Microsoft Excel 2016 was used to make the tables, and Origin2017 (Origin Lab, Los Angeles, CA, USA,) was used to make the figures. Each sample was analyzed three times using the same method, and the three data obtained were used for the analysis of variance. The data used in the manuscript was the average of these three data. SPSS19.0 was used to analyze the data via one-way analysis of variance, and multiple comparisons were performed using the least significant difference (LSD) method. Venn diagrams of soil bacterial β diversity were plotted using R software (version 3.3.1) [27].

3. Results

3.1. Effects of Biochar on Soil Physical Properties

Biochar significantly reduced the soil volumetric weight at each study site (Table 3). The variation in soil porosity was affected by each treatment and was related to the soil volumetric weight. Plots treated with biochar (treatment B) had significantly higher porosity than soils in treatments CK and F in all four study sites. In study sites FUS and HEIS, the soil conductivity of soil under treatment B was significantly higher than that of soils under CK and F treatments, while at study site HUS, there was no significant difference in conductivity between treatments. In study site HES, the conductivity of soil under treatment B was significantly higher than that of soils under treatment CK, although the difference from soils under treatment F was not significant. There was no significant difference in water holding capacity between soils under the three different treatments and in the four different study sites. In summary, we found that biochar optimized the physical structure of the soil by making the soil structure looser, which improved ventilation and increased the water retention effect. However, due to the difference in soil types and climatic conditions, biochar affected different soils in different ways.

Table 3. Effects of biochar on soil physical properties.

Index	Treatments	FUS	HUS	HES	HEIS
Pb * (g·cm ⁻³)	CK	1.28 ± 0.07 aA	1.03 ± 0.11 abB	0.89 ± 0.08 aA	0.82 ± 0.17 abB
	F	1.36 ± 0.12 aA	1.16 ± 0.52 aA	0.93 ± 0.06 aA	0.97 ± 0.08 aA
	B	1.14 ± 0.09 bB	0.89 ± 0.07 bC	0.82 ± 0.13 aB	0.74 ± 0.11 bC
Porosity (%)	CK	46.34 ± 1.36 aB	52.69 ± 2.58 bB	62.44 ± 3.04 abB	72.19 ± 1.01 bB
	F	47.25 ± 0.94 aB	48.16 ± 1.35 cC	58.19 ± 0.95 bC	70.43 ± 0.64 bB
	B	49.86 ± 2.01 aA	58.15 ± 2.19 aA	67.43 ± 1.17 aA	79.21 ± 2.92 aA
Conductivity (%)	CK	36.11 ± 3.18 bB	29.56 ± 0.48 aB	41.94 ± 1.02 bC	38.45 ± 0.69 cC
	F	34.98 ± 2.63 bB	30.54 ± 0.09 aAB	45.97 ± 0.57 aB	42.61 ± 0.18 bB
	B	42.19 ± 0.85 aA	32.94 ± 0.15 aA	47.85 ± 0.46 aA	47.15 ± 0.05 aA
Water-holding capacity (μS·cm ⁻¹)	CK	57.21 ± 0.12 aA	62.89 ± 0.36 aA	51.92 ± 0.79 aA	41.97 ± 0.08 aA
	F	57.49 ± 0.04 aA	62.17 ± 0.44 aA	51.29 ± 0.04 aA	42.18 ± 0.51 aA
	B	57.77 ± 0.19 aA	63.08 ± 1.87 aA	51.94 ± 0.11 aA	42.66 ± 0.17 aA

Note: Different lowercase letters in the table indicate that the difference was significant at the $p < 0.05$ level. Different uppercase letters indicate that the difference was significant at the $p < 0.01$ level. Data are shown as mean ± the standard deviation. Differential analysis was performed between different treatments of the same soil type. * pb represents soil volumetric weight. For study site details, see Table 1.

3.2. Effects of Biochar on Soil Chemical Properties

Biochar increased the soil pH (Table 4). In study sites FUS, HUS, and HES, the pH of soils under treatment B was significantly different from that of soils under treatments CK and F. However, in study site HEIS, the pH of soils under treatment B was not significantly different from that of soils under treatments CK and F. Compared with soils in the CK treatment, soils in the B treatment had more available nitrogen (A–N) in all four study sites, although soils under the F treatment had more available nitrogen than soils under both the B and CK treatments. Soils under treatment B had more available phosphorus (A–P) than soils under treatments CK and F, and in study site HES, there was a significant difference between the available phosphorus (A–P) content of soils under treatment B and soils in both

the CK and F treatments. Biochar also increased the soils' available potassium (A-K) and microbial biomass carbon (MBC), where these parameters in soils under treatment B were significantly different in all four study sites from those in soils under treatments CK and F. Based on these results, we can conclude that biochar effectively raised the pH value of near-acidic soil, while there was no clear effect of biochar on pH in near-neutral soil.

Table 4. Effects of biochar on the chemical properties of tobacco-planting soil.

Index	Treatment	FUS	HUS	HES	HEIS
pH	CK	4.79 ± 0.52 bB	5.31 ± 0.15 bB	6.21 ± 0.09 aB	6.88 ± 0.31 aA
	F	4.74 ± 0.07 bB	5.35 ± 0.22 bB	6.18 ± 0.35 aB	6.82 ± 0.54 aA
	B	5.02 ± 0.46 aA	5.74 ± 0.36 aA	6.45 ± 0.17 aA	6.93 ± 0.08 aA
A-N (mg kg ⁻¹)	CK	117.25 ± 1.95 aB	84.59 ± 0.79 bB	68.46 ± 1.23 bB	65.82 ± 2.64 aB
	F	136.59 ± 3.14 aA	117.46 ± 2.18 aA	81.29 ± 2.31 aA	75.49 ± 0.85 aA
	B	125.84 ± 0.99 aB	95.84 ± 1.66 bB	77.54 ± 2.11 aA	73.51 ± 2.14 aA
A-P (mg kg ⁻¹)	CK	35.94 ± 0.56 bB	34.91 ± 0.94 bB	18.64 ± 0.54 bC	25.64 ± 0.56 bB
	F	43.62 ± 0.08 aA	40.59 ± 1.31 aA	21.59 ± 1.21 abB	30.77 ± 0.79 aA
	B	45.58 ± 1.05 aA	41.66 ± 0.08 aA	24.61 ± 0.16 aA	32.19 ± 1.31 aA
A-K (g kg ⁻¹)	CK	237.48 ± 3.95 bC	178.51 ± 2.94 bB	195.84 ± 3.64 bC	148.18 ± 2.11 bB
	F	269.62 ± 1.87 aB	220.16 ± 3.05 aA	221.61 ± 2.11 aB	156.04 ± 1.65 aA
	B	283.94 ± 1.16 aA	225.47 ± 1.04 aA	230.87 ± 2.97 aA	161.84 ± 1.77 aA
MBC (mg kg ⁻¹)	CK	406.78 ± 3.65 aB	487.66 ± 5.47 bC	309.14 ± 5.88 cC	457.91 ± 1.59 bB
	F	413.97 ± 4.91 aB	519.64 ± 2.89 abB	337.84 ± 1.79 bB	467.28 ± 5.64 bB
	B	441.89 ± 1.96 aA	557.33 ± 7.01 aA	369.21 ± 2.07 aA	495.79 ± 2.91 aA

Note: A-N, A-P, and A-K represent available nitrogen, available phosphorus, and available potassium, respectively, and MBC stands for microbial biomass carbon. Different lowercase letters in the table indicate that the difference was significant at the $p < 0.05$ level. Different uppercase letters indicate that the difference was significant at the $p < 0.01$ level. Data are shown as mean ± standard deviation. Differential analysis was performed between different treatments of the same soil type.

3.3. Effects of Biochar on Bacterial Diversity

The numbers of unique bacterial communities (at the OTU classification level) in soils under treatment B in study sites FUS, HUS, and HES were less than that of soils under treatments CK and F (Figure 1A–C). In the four study sites, the core bacterial analysis community occupancy rate (that is, bacteria shared among all samples within the given group) of soils under treatment B was 97.93% (FUS), 97.42% (HUS), 98.15% (HES), and 78.76% (HEIS). In study site FUS, the core bacterial community occupancy rate in soil under treatment B was 7.86% and 5.18% higher than that of soils under CK and F treatments, respectively. In study site HUS, the core bacterial community occupancy rate in soil under treatment B was 5.79% and 3.26% higher than that of soils under treatments CK and F, respectively. In study site HES, the core bacterial community occupancy rate in soil under treatment B was 4.57% and 0.35% higher than that of soils under CK and F treatments, respectively. As shown by this analysis, biochar increased the population abundance of bacteria. Furthermore, in acidic soils (i.e., the soils at sites FUS, HUS, and HES), biochar increased the ratio of soil bacterial community abundance in soils under treatment B relative to that of soils under treatments CK and F. In neutral soil (i.e., the soil at study site HEIS), these effects of biochar were not found.

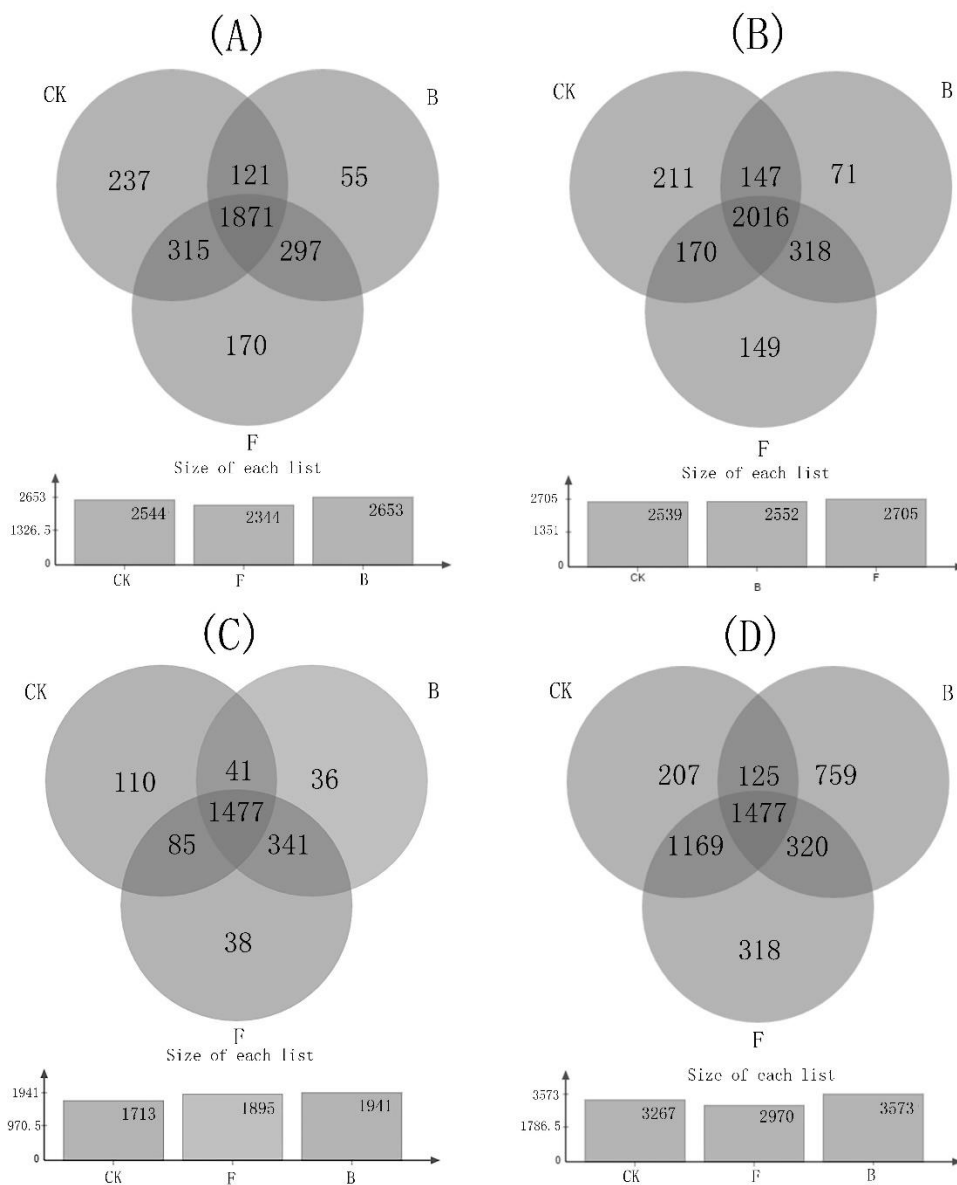


Figure 1. Analysis of β diversity of soil bacteria at the OTU level. (A–D) represent Shaowu City, Fujian Province (FUS); Chenzhou City, Hunan Province (HUS); Xuchang City, Henan Province (HES); and Mudanjiang City, Heilongjiang Province (HEIS), respectively.

3.4. Effects of Biochar on the Growth and Development of Tobacco Plant Roots

In all four study sites, the root activity of tobacco plants grown in soils under treatment B was higher than that of tobacco plants grown in soils under treatments CK and F. In study sites HES and HEIS, the root activity of tobacco plants grown in soils under treatment B treatment was significantly different from that of tobacco plants grown in soils under treatments CK and F, while in study site HEIS, the root activity of tobacco plants grown in soils under treatment F was significantly higher than that of tobacco plants grown in soils under treatment CK. In study site HUS, the root activity of tobacco plants grown in soils under treatment B was significantly higher than that of tobacco plants grown in soils under treatment CK; however, there was no significant difference between the root activity of tobacco plants grown in soils under the three treatments in study site FUS. The root activity of tobacco plants in study site HEIS was significantly higher than that of tobacco plants in the other study sites (Figure 2). The total number of root tips of tobacco plants grown in soils under treatment B in the four study sites was higher than that of tobacco plants grown in soils under treatments CK and F. In study

sites HUS and HES, there were significant differences between tobacco plants grown in soils under treatment B and tobacco plants grown in soils under the other two treatments, with a significantly higher number of root tips in tobacco plants grown in soils under treatment F compared to tobacco plants grown in soils under treatment CK. The variation of the total number of root tips of tobacco plants in study sites FUS and HEIS was similar, and the difference between treatments B and CK was significant; however, the difference between treatments B and F was not significant. The total number of root tips was highest in study site HEIS (Figure 3), where the number of root tips was significantly higher than in the three other study sites. The total number of root tips of tobacco plants grown in the four study sites was ordered as follows: HEIS > HUS > FUS > HES. However, when considering only tobacco plants grown in soils under treatment B, the total number of root tips of the four soils was ordered as follows: HEIS > HES > FUS > HUS. In short, biochar promoted the development of tobacco roots, which improved the root activity and increased the total number of root tips. When the tobacco root system is improved, the above-ground part of the plant will be promoted, and the yield of tobacco will be increased accordingly, which can ultimately increase the farmers' income.

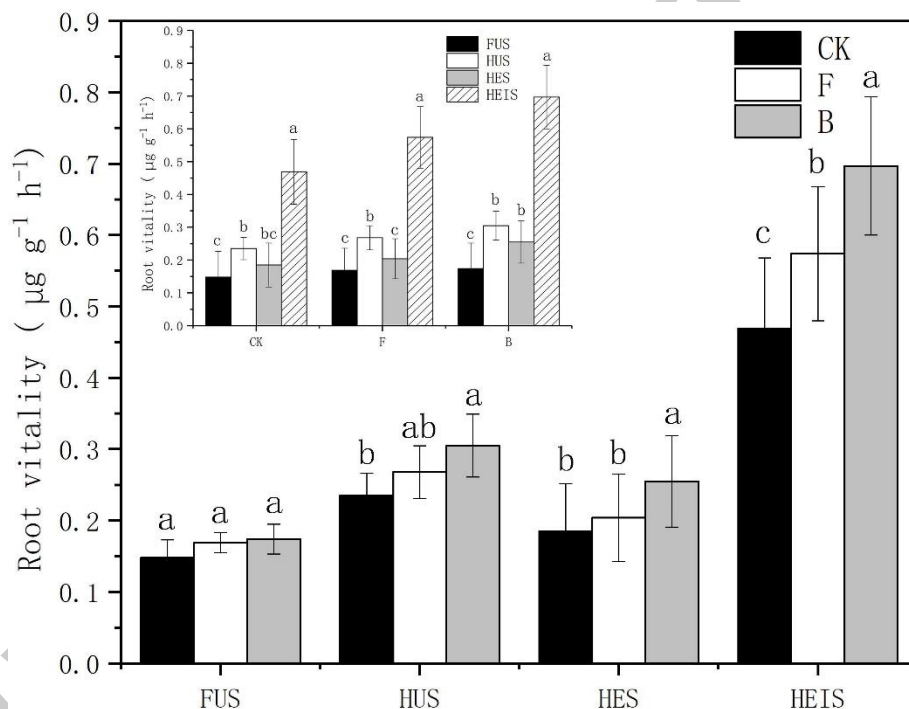


Figure 2. Effect of biochar on tobacco plant root activity. Error bars represent the standard deviation from the mean, $p > 0.05$. CK: control treatment, F: treatment with nitrogen fertilizer, B: treatment with biochar.

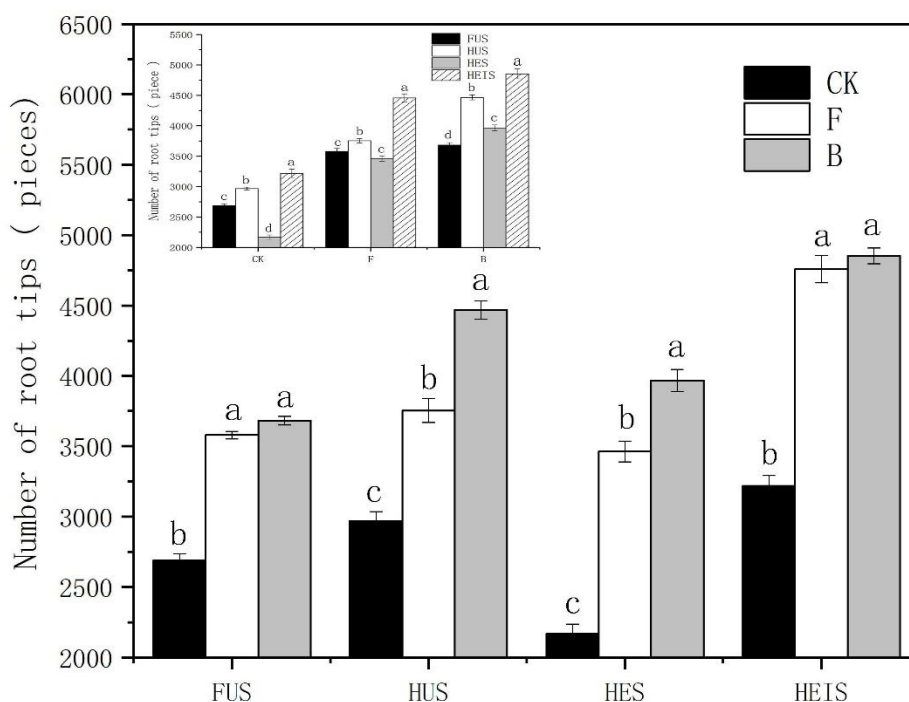


Figure 3. Effects of biochar on the number of tobacco plant root tips. Error bars represent the standard deviation from the mean, $p > 0.05$. CK: control treatment, F: treatment with nitrogen fertilizer, B: treatment with biochar.

3.5. Correlation Analysis between Soil Physical and Chemical Properties and Root Development of Flue-Cured Tobacco

Soil volumetric weight and the other soil properties were negatively correlated, and was significantly negatively correlated with MBC, AN, AK, and root activity. Furthermore, the correlation coefficients of soil volumetric weight with MBC and root activity were -0.92 and -0.94 , respectively. With the exception of soil volumetric weight, all other soil properties were positively correlated. The correlation coefficient between A–K and total root tips was 0.92 (Table 5). As demonstrated by these results, although biochar could not be directly absorbed and utilized by plants, it indirectly affected the growth and development of plant roots. Biochar directly increased the soil organic carbon and microbial biomass carbon, and these two soil properties had a significant positive correlation with tobacco root tip number and root activity. Biochar also increased the root activity and the total number of roots by improving soil aeration; increasing soil pH; and by increasing the effective nitrogen, phosphorus, and potassium levels.

Table 5. Correlation analysis of soil physicochemical indexes and root development of flue-cured tobacco.

Parameter	MBC	pH	ρ_b	A–N	A–P	A–K	Root Activity
pH	0.75 **						
ρ_b	-0.92 **	-0.18					
A–N	0.81 **	0.73 **	-0.75 **				
A–P	0.64 **	0.31 *	-0.39 *	0.93 **			
A–K	0.96 **	0.25 *	-0.81 **	0.76 **	0.56 **		
Root activity	0.61 **	0.48 *	-0.94 **	0.35 *	0.71 **	0.73 **	
Total root tips	0.56 **	0.88 **	-0.63 **	0.74 **	0.83 **	0.92 **	0.82 **

Note: * Significantly correlated at the 0.05 level, ** significantly correlated at the 0.01 level; MBC represents the soil microbial biomass carbon; A–N, A–P, and A–K represent the available nitrogen, available phosphorus, and available potassium, respectively; * ρ_b represents soil volumetric weight.

4. Discussion

4.1. Effects of Biochar on Soil Physical and Chemical Properties

Soil porosity and volumetric weight are important indicators for measuring the physical properties of soils [28]. In this study, the application of biochar on different types of soils increased the soil porosity and reduced the soil volumetric weight, which is consistent with results reported by Mehr [29]. This may be due to the low density of biochar itself [30], which is applied to the soil to reduce the soil volumetric weight and increase its porosity, or may be because biochar increases the abundance of microorganisms in the soil [31]. Soil microbes increase the soil permeability through their own metabolism, and also increase the agglomerate structure of soil [32], which ultimately leads to increased soil porosity and volumetric weight. This study also shows that biochar can increase soil conductivity, which may be due to increased soil porosity. Increased soil porosity may also affect other soil characteristics [33]. For example, when soil porosity and gas permeability increase, soil conductivity also increases [30]. As demonstrated by the studies cited above, biochar increases the moisture content of the soil; this may be due to the high total porosity of biochar, which can not only retain water in the pores, thereby increasing the water holding capacity (WHC), but also enable the water to flow from the topsoil to deeper soil layers after heavy rain [34].

4.2. Effects of Biochar on Soil Chemical Properties

After biochar was applied to tobacco-planting soil in this study, the pH value of the soil increased. This may be because the biochar itself contained a large amount of soluble base ions [35], such as potassium, calcium, sodium, magnesium, zinc, etc. These ions used their own holding action to reduce the exchange level of soil H^+ and exchangeable Al^{3+} [36]. Furthermore, biochar itself has a higher pH (Table 4), therefore biochar caused an increase in soil pH, as was also concluded by Rees [37]. However, the pH of different soil types was affected differently by biochar. The pH of Ferralsol soil (at study site FUS) was greatly affected by biochar, while the pH of Phaeozem soil (at study site HEIS) was less affected by biochar (Table 4). The soil at study site FUS was acidic, whereas the soil at study site HEIS was close to neutral (Table 1). The effect of biochar on soil pH may be caused by the fact that biochar is alkaline, and when it is applied to the soil, it increases soil pH. Soils to which biochar is applied tend to change the pH to be closer to the pH of biochar [38], and the closer the pH value of soil is to the pH value of biochar, the smaller the effect of adding biochar to the soil is. The pH of the soil at study site FUS was more likely to increase following the addition of biochar due to its lower pH. Conversely, the soil at study site HEIS had a higher pH, and thus was less affected by the addition of biochar.

The surface of biochar has a many functional groups and a large specific surface area. When biochar is applied to the soil, it adsorbs a large amount of ions, such as phosphorus, calcium, magnesium, iron, etc. [39], and this adsorption is selective. For example, biochar has a stronger adsorption effect on ammonium ions and nitrate ions [40]. This study shows that biochar increased the soil content of available nitrogen, phosphorus, and potassium. This result is inconsistent with the results of Chen [35], which may be due to the difference in the type of biochar or test crops used in these two studies. Biochar can increase the soil abundance of soil microbes [41], which promotes the soil mineralization efficiency of nitrogen, phosphorus, and potassium, and subsequently, the soil content of available nitrogen, phosphorus, and potassium increases.

Although soil has a low organic carbon content, it is an important criterion for measuring soil fertility and health. Furthermore, organic carbon plays an extremely important role in maintaining soil texture, fertility, and agricultural cycle development [42]. Biochar can effectively increase soil organic carbon content, thereby improving soil quality and health [42]. Our research shows that biochar can effectively increase the content of organic carbon in soil, which is consistent with the findings of Lu [42]. This may be because biochar increases the soil microbial community abundance, which increases the soil carbon mineralization intensity, which in turn promotes the accelerated decomposition of plant residues, thereby increasing the soil organic carbon content. In addition, biochar itself also contains a

large amount of organic carbon, which can directly and quickly increase the soil organic carbon content. Soil microbial biomass carbon accounts for a very small proportion of soil total carbon, and its content varies greatly, but it is the most active component of organic carbon [43]. Soil microbial carbon can not only reflect soil fertility, but is also an important indicator of soil biological activity [44]. In this study, we found that biochar promoted the increase of microbial biomass carbon in soil, but the treatment of chemical fertilizers reduced the content of soil microbial biomass carbon. This may be because the large specific surface area of biochar and the large amount of pore structures provided an excellent habitat for soil microorganisms, which may have increased their rates of reproduction. Furthermore, the functional groups on the surface of biochar can adsorb a variety of organic compounds and water, ensuring the nutritional needs for soil microbial growth are met. Biochar stimulates soil microbial multi-metabolism and growth and development [45], thereby increasing the soil microbial biomass carbon content.

4.3. Effects of Biochar on Soil Microbial Diversity

Biochar has a large specific surface area and a large amount of porous structures, which can effectively improve the water retention and gas permeability of the soil. As such, biochar can help provide a better growth environment for soil microorganisms, help microorganisms avoid predators, and promote their rapid propagation [46,47]. It has been reported that after the addition of biochar, the transformation of microbial communities may be related to changes in nutrient conversion and utilization [19]. Carbon-rich anthrosol has a unique microbial community and higher microbial biomass and diversity than adjacent carbon-poor soils [48], which is consistent with the conclusions of the current study. Biochar can also change the microbial community structure by changing the physical and chemical properties of the soil. For example, biochar can increase soil porosity and water content, and it can simultaneously reduce the leaching and volatilization of soil nutrients [49]. As such, biochar can help provide sufficient nutrients for soil microbial growth and metabolism. This may be because biochar changes the physical and chemical properties of the soil, is conducive to the growth and development of soil microbes, and its structural characteristics and chemical characteristics result in good living conditions for soil microbes [48], which eventually contributes to the increase in soil microbial diversity. However, the physical and chemical properties and ecological conditions of different types of soils are different; therefore, the soil responses to biochar will also be different, resulting in differences in the degrees and modes of microbial diversity change in different soils.

4.4. Effects of Biochar on the Development of Flue-Cured Tobacco Roots

Biochar can effectively promote the growth and development of crop roots, increase the nutrient utilization of crops, and increase the root activity of crops [50]. Furthermore, the addition of biochar affects the interaction between the roots and soil [51]. The results of this study show that biochar effectively increased the root activity and number of total root tips of tobacco plants, but biochar had different effects on tobacco root activity and numbers of root tips in different soils. A study by Prendergast [52] demonstrated that plant roots respond to the addition of biochar because biochar provides nutrients directly by increasing the retention of soluble phosphorus. Biochar also indirectly increases soil carbon and soil nutrients by increasing nitrogen retention in the rhizosphere and bulk soils, and biochar can increase plant root activity and root hair count [53], which is consistent with our findings. These results indicate that biochar can effectively increase soil porosity, which promotes soil gas exchange, enhances the respiration and metabolic intensity of tobacco roots [54], and increases root activity. The utilization efficiency of nutrients in the soil enhances the water-holding capacity of the soil. When the roots of tobacco plants obtain sufficient nutrients and water, the metabolic strength increases, the root differentiation ability increases accordingly [55], and the number of root tips increases. More analysis of the impact and economic value of biochar on tobacco growth needs to be studied in order to provide evidence and support for the use of biochar in agricultural production.

5. Conclusions

In summary, biochar improved the physical and chemical characteristics of tobacco-growing soil, while at the same time improved the soil bacterial community structure and promoted the growth of tobacco roots. The results show that biochar effectively increased soil pH and porosity; increased the content of available nitrogen, phosphorus, and potassium in the soil; and enriched the community structure of bacteria in the soil. Biochar had different effects on different types of soil, where the soil in Shaowu City, Fujian Province, was most affected by biochar. However, more research is needed on the effects of different types of biochar on soil and tobacco, and the effects of biochar on tobacco growth and economic indicators.

Author Contributions: Conceptualization: H.W. and H.S.; methodology: H.W., T.R., H.F., G.L., and K.L.; data collection: Y.F., H.S., H.W., and T.R.; formal analysis: H.F. and H.W.; supervision: T.R. and H.S.; funding acquisition: H.S. and T.R.; writing—original draft preparation: H.W.; writing—review and editing: H.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Topics of the National Key R&D Program: Integration and Application of Fertilizer Reduction Technology in Crop Production under High Efficiency Utilization of Green Manure, China.

Acknowledgments: The authors wish to thank Ondřej Mašek and Qiaona Pan for their analyses of the samples. We would also like to extend our appreciation to all members of the UK Biochar Research Centre for their helpful suggestions and support of this study. We would like to thank Editage (www.editage.cn) for English language editing.

Conflicts of Interest: The authors declare no conflict of interest.

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