Zhang and Shen *Annals of Microbiology* (2022) 72:35
https://doi.org/10.1186/s13213-022-01689-1

Annals of Microbiology

UNIVERSITÀ
DEGLI STUDI
DI MILANO

ORIGINAL ARTICLE

Open Access

Effects of biochar on soil microbial diversity and community structure in clay soil

Jing Zhang[1,2,3,4*] and Jiang-Long Shen[1,2,3,4]

Abstract

Purpose: We determined the microbial community diversity and structure in soil samples under different amounts of biochar added. Meanwhile, we also researched the relationships between soil microbial and soil physicochemical properties.

Method: In this study, a field experiment was set up, with a total of three experimental treatments: no biochar application, 10 t/m^3 biochar application, and 20 t/m^3 application. High-throughput sequencing technologies were used for soil samples of different treatment groups to understand soil microbial diversity and community structure.

Results: We found that the soil physicochemical properties after biochar addition were better than those without biochar addition, and the alpha diversity was higher in biochar addition level of 20 t/m^3 than other processing groups. *Proteobacteria*, *Cyanobacteria*, and *Actinobacteria* were the dominant phyla of this study. The dominant genera were *Skermanella*, *Nostoc*, *Frankia*, and *Unclassified-p-protecbacteria*. At the gate level, *Actinobacteria* had significant differences among the three groups with different addition amounts. The microbial community structure was mainly influenced by soil porosity, soil moisture content, nitrogen fertilizer, and potassium fertilizer other than soil phosphate fertilizer and organic matter.

Conclusions: The results suggested that changes under different amounts of biochar added generate changes in soil physicochemical properties and control the soil composition of microbial communities. This provides a new basis for soil improvement.

Keywords: Biochar, MiSeq sequencing, Alpha diversity, Bacterial phyla, Bacterial community structure

Introduction

Soil is an important carbon "source" and "sink" in terrestrial ecosystems. Soil carbon pools are mainly divided into soil organic carbon pools and inorganic carbon pools (Atkinson et al. 2010). The main way to mitigate climate change in the short term is to increase the soil organic carbon pool and maintain the stability of the soil organic carbon pool (Liang et al. 2010. Agricultural land accounts for 35 to 37% of the global land area and is the land most affected by human activities. The decline of organic carbon in farmland soil is the most serious degradation factor (Bronick and Lal 2005). Therefore, the change of soil organic carbon in agricultural land has been widely concerned by scholars.

Biochar has high stability and cannot be decomposed well by soil microorganisms. The impact of biochar on soil microorganisms is mainly through changes to the soil environment (Wu et al. 2017).

Biochar has a wide range of carbonization raw materials and low price. As a renewable recycling resource, it plays an important role to affect the change of soil organic carbon (Chen et al. 2013). Biochar has highly developed pore structure, huge specific surface area, and strong ion adsorption and exchange capacity. This characteristic can

*Correspondence: jingzhangzhj@126.com

[4] Shaanxi Provincial Land Consolidation Engineering Technology Research Center, Xi'an, China

Full list of author information is available at the end of the article

change the indexes of soil surface area, porosity, aggregate, and density (Steiner et al. 2010); provide niches for colonization of soil microorganisms (Zackrisson and Wardle 1996; Warnock et al. 2007; Richard et al. 2013); and affect soil aeration, water content, root movement, microbial habitat (Wildman and Derbyshire 1991), and C and N cycling in the terrestrial ecosystem (Nguyen et al. 2017; Zhang et al. 2017a; Zhang et al. 2018b).

Soil microorganism is an important participant in biochemical process. It promotes the microcirculation of vegetation soil ecosystem and has a significant effect on improving soil fertility. The diversity and community structure of soil microorganism can significantly affect soil quality and are important factor to evaluate soil quality. The diversity and community structure of soil microorganism play an important role in influencing the soil fertility, soil health, ecosystem's function, and productivity (Zou et al. 2017).

Clayey raw soil has the characteristics of poor permeability, small gap, poor ventilation and drainage performance, and slow fertilizer release and nutrient transformation (Li et al. 2012; Pang et al. 2021) and cannot meet the nutrients and water required for crop growth. Under natural conditions, the natural maturation process of raw soil is slow, which seriously hinders the rapid development of agriculture. Therefore, it is a certain trend of current agricultural development to realize the rapid improvement of the quality of new cultivated land and degraded land through biochar.

At present, biochar has been widely studied to improve soil health (Keya 2016; Yuan et al. 2018). It is mainly reflected in the effects of adding biochar on soil physical property (Wang et al. 2016b; Stéphanie et al. 2005), chemical property (Wang et al. 2021a; Zhang et al. 2018a, b), and soil microbial diversity (Gundale and Deluca 2007; Ahmad et al. 2014; Cheng et al. 2019; Ding et al. 2019). Grossman et al. (2010) study found that biochar in carbon-rich soils in the Amazon Basin can increase the number and diversity of soil bacterial communities. Khodadad et al. (2011) found that the relative abundance of actinomycetes and chlortetracyclines in soils supplemented with biochar increased significantly, indicating that inert biochar can affect bacterial community composition. Rondon et al. (2007) found that the application of biochar can significantly increase the biomass of fungi and gram-negative bacteria and can promote the biological nitrogen fixation ability of rhizobia and improve the activity of soil nitrifying microbial flora. Numerous studies have shown that biochar addition has an effect on soil microorganisms. However, most studies focus on the effect of biochar addition on multi-year degraded soil and different soil types (Wang et al. 2013; Wang et al. 2016a, b; Zhang et al. 2019). There are few studies on the effects of biochar application on soil microorganisms in clayey raw soil, and the optimal amount of biochar addition has not been determined. Clay soil has poor permeability, small voids, and low degree of maturity, which seriously affects soil quality and crop yield. Therefore, this paper adds biochar to clayey raw soil and studies the sample plots with different gradient biochar addition, in order to achieve the following goals: (1) which biochar addition has the best effect on the improvement of clayey raw soil; (2) what is the mechanism or principle of the effect of different addition amounts on different microorganisms; and (3) which soil physicochemical properties have a significant impact on soil microorganisms.

Materials and methods

Experimental field

The experiment was carried out in Qinling field monitoring center station, which is located in Shangwang village, Tangyu Town, Mei County, Baoji City, Shaanxi Province, China (33° 59′–34° 19′ N and 107° 39′–108° 00′ E). This area was characterized by a warm temperate semi-humid continental climate, and its altitude was ranged from 442 to 3767 m. The mean annual precipitation was 609.5 mm, and the annual mean temperature was 12.9°C. The soil texture was clayey soil.

Experimental design and treatments

The raw material of biochar comes from fruit tree residues (were manufactured by Shaanxi Yixin Bio-energy Technology Development Co., Ltd.). These biochar were dried in a continuous pyrolysis plant to <5% moisture content before carbonization. The production process was slow pyrolysis, at a highest treatment temperature of 550 °C and a heating rate of 5–10 °C min^{-1} (Zwieten et al. 2010). The feedstock was kept in the reactor for 30 min on average, then directly sieved (2 mm mesh). The properties of biochar were as follows: pH was 9.42, EC was 0.15 dS m^{-1}, the content of total C was 794 g kg^{-1}, the content of total N was 9.82 g kg^{-1}, the content of total H was 16.7 g kg^{-1}, and the organic carbon was 763 g kg^{-1}.

In September 2020, this experiment started to implement. This experiment adopted the method of field experiment. In this experiment, 9 test plots were set up, and the size of them was 1.5 m × 3 m. The biochar application amount was 0, 10, and 20 t/hm^2, and 3 treatments were set. The plot adopted the random block design, and each treatment was set for three repetitions. The biochar was sprinkled evenly on the soil surface, and it was mixed with the plough layer soil (20 cm) by manual stirring, so that the color of the soil was uniform everywhere, and ridges were left to stand. The same N, P, and K fertilization schemes were adopted in the experimental plots, which were basically consistent with the

fertilization habits of local farmers, which were N: 150 kg/hm^2 respectively; P_2O_5: 120 kg/hm^2; and K_2O: 90 kg/hm^2. The crops planted in the experimental plot are the same as the local crops. Wheat is planted in winter and spring, and corn is planted in summer and autumn.

Sample collection and analysis

In June 2021, soil samples were collected. The plant residues and stones were moved away from the plots. Then, samples were collected from three different regions of the plot by using a core sampler (20 mm internal diameter). The sampling depth was 20 cm. The soil samples were directly sieved (2 mm mesh), and subsamples were mixed to avoid heterogeneity and yield a soil sample for each plot. All soil samples were divided into two parts: one part was naturally air dried for the determination of soil physical and chemical properties, and the other part was frozen in refrigerator of −20 °C for the extraction of soil macrogenomic DNA.

Chemical analysis

The soil moisture content (SMC) was measured by the drying and weighing method (105°C for 24 h). Soil porosity (SP) was determined by the ring knife method. Ammonium nitrogen (AN) and nitrate nitrogen (NN) were extracted with 0.01mol/l calcium chloride and then determined by AA3 flow injection analyzer. Available phosphorus (AP) was extracted with 0.5mol/l sodium bicarbonate (pH 8.5) and then determined by Smartchem 200 continuous flow injection analyzer. Available K (AK) was extracted with 1mol/l ammonium acetate (pH 7) and determined by flame photometer. Organic matter content (OMC) was determined by heating oxidation of potassium dichromate sulfuric acid and titration of ferrous sulfate. The required index measurement methods referred to Soil Agrochemical Analysis (Third Edition) written by Shidan Bao (2000). Each analysis was performed in three replicates, and the data were presented as the averages.

DNA extraction and high-throughput Miseq sequencing

The total genomic DNA in each soil sample was extracted using the MoBio Powersoil® DNA Isolation Kit (MoBio Laboratories, USA). This method performed equally well over a range of different soils (Wüst et al. 2016). The quality and concentration of DNA were verified by 1% agarose gel electrophoresis and a NanoDrop™ 1000 spectrophotometer (Thermo Scientific, USA).

The V3-V4 region of the bacterial 16S rRNA gene was amplified using the PCR primers 338F (5′-ACT CCTACGGGAGGCAGCAG-3′) and 806R (5′-GGA CTACHVGGGTWTCTAAT-3′) and a sample tagging approach; the size of amplicon was 468bp (Caporaso et al. 2012). The formal PCR test used TransGen AP221-02: TransStart Fastpfu DNA Polymerase, 20 μl reaction system: 5×FastPfu buffer 4 μl, 2.5 mM dNTPs 2 μl, forward primer (5 μM) 0.8 μl, reverse primer (5 μM) 0.8 μl, FastPfu polymerase 0.4 μl, BSA 0.2 μl, template DNA 10 ng, and supplement ddH_2O to 20 μl. The following thermal cycling scheme was used: 30 cycles of initial denaturation at 95 °C for 3 min, denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s, followed by a final extension at 72 °C for 10 min. Amplicons were extracted from 2% agarose gels, purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and quantified using a QuantiFluor™ (Promega, USA). Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina MiSeq platform (Majorbio, Shanghai) according to standard protocols.

Sequencing data processing

The data of each sample was distinguished according to the index sequence, and the extracted data was saved in a fastq format. According to the overlap relationship between paired-end reads, the paired reads were merged into a sequence by using Fastp and Flash software. At the same time, the quality of reads and the effect of merge were quality controlled and filtered. The samples were distinguished according to the barcode and primer sequence at the beginning and end of the sequence, the effective sequence was obtained, and the sequence direction was corrected. Using Uparse Software (version 7.0.1090) n.d., the biological information of OTU at 97% similar level was statistically analyzed. According to the Silva Database (lease138) (n.d.), 97% OTU representative sequences with similar level were classified by RDP classifier Bayesian algorithm. The OTU or other taxonomic levels with 97% similarity were selected, and Mother (version v.1.30.2) (n.d.) was used to calculate the alpha diversity index (Chao, Ace, Shannon, Smith-Wilson) under different random sampling.

Statistical analysis

Differences in the soil physicochemical properties at these plots were compared using one-way ANOVA with Tukey's test. The Student test was used to analyze the differences between alpha diversity indexes. The species composition of different samples at the phylum level and genus level was analyzed by R. The evolutionary tree was constructed according to the maximum likelihood method, and then the distance matrix between samples was obtained by FastUniFrac n.d.. Finally, the sample distance Heatmap diagram was made in R (version 3.3.1) that was a programming language for statistical calculation and plotting. The beta diversity distance matrix was

Table 1 Soil physical and chemical properties between different amounts of biochar added

Treatments	SMC (%)	SP (%)	AN (mg/kg)	NN (mg/kg)	AP (mg/kg)	AK (mg/kg)	OMC (g/kg)
MC	18 ± 1.3a	47 ± 00.3a	8.13 ± 1.012	21.46 ± 1.318a	21.50 ± 5.200	131.67 ± 3.786a	5.62 ± 1.650a
BS	18 ± 0.3a	51 ± 00.1b	11.74 ± 4.839	36.62 ± 1.519b	25.87 ± 3.092	291.00 ± 84.894b	34.73 ± 7.870b
MCS	20 ± 0.5b	52 ± 00.7c	10.15 ± 2.121	42.04 ± 2.090c	24.00 ± 2.835	420.67 ± 66.606b	45.33 ± 10.957b

Different lowercase letters in the same column indicate a significant difference at the 0.05 level. MC, BS, and MCS represent the biochar addition amount of 0 t/m^3, 10 t/m^3, and 20 t/m^3 respectively

SMC soil moisture content, *SP* soil porosity, *AN* ammonium nitrogen, *NN* nitrate nitrogen, *AP* available phosphorus, *AK* available K, *OMC* organic matter content

calculated with Qiime, and the NMDS analysis was carried out with vegan packages of R. ANOSIM and PERMANOVA were calculated with vegan package of R language. The Kruskal-Wallis *H* test was used to test the significant difference between groups at the phylum level, and the stats package of R was used to plot. The Lefse Software n.d. was used to carry out linear discriminant analysis (LDA) on samples according to different groups to find out the species that have significant differences in sample division. The relationships between soil physical and chemical properties and soil microbial diversity and community structure were determined using the RDA function in redundancy analysis (RDA) in the vegan package in R. The correlation heatmap analysis was carried out with a pheatmap package of R language to calculate the correlation coefficient between soil physical and chemical properties and selected species.

Results

Soil physical and chemical properties

With the increase of biochar added, there was no significant difference between ammonium nitrogen and available phosphorus. There was also no significant difference in soil water content between this field with the addition amount of 10 t/hm^2 (BS) and the control group, but there was a significant difference in soil water content between this field with the addition amount of 20 t/hm^2 (MCS) and the control group. Porosity and nitrate

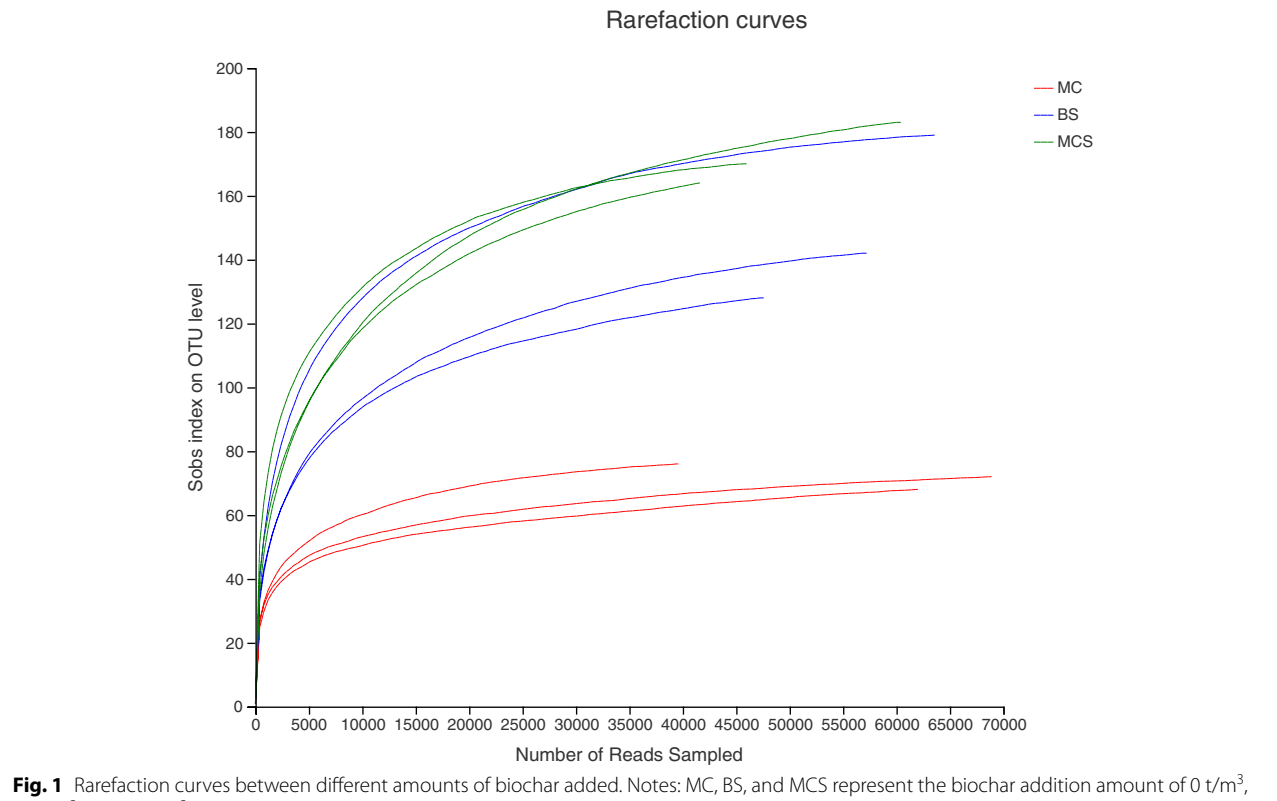

Fig. 1 Rarefaction curves between different amounts of biochar added. Notes: MC, BS, and MCS represent the biochar addition amount of 0 t/m^3, 10 t/m^3, and 20 t/m^3 respectively

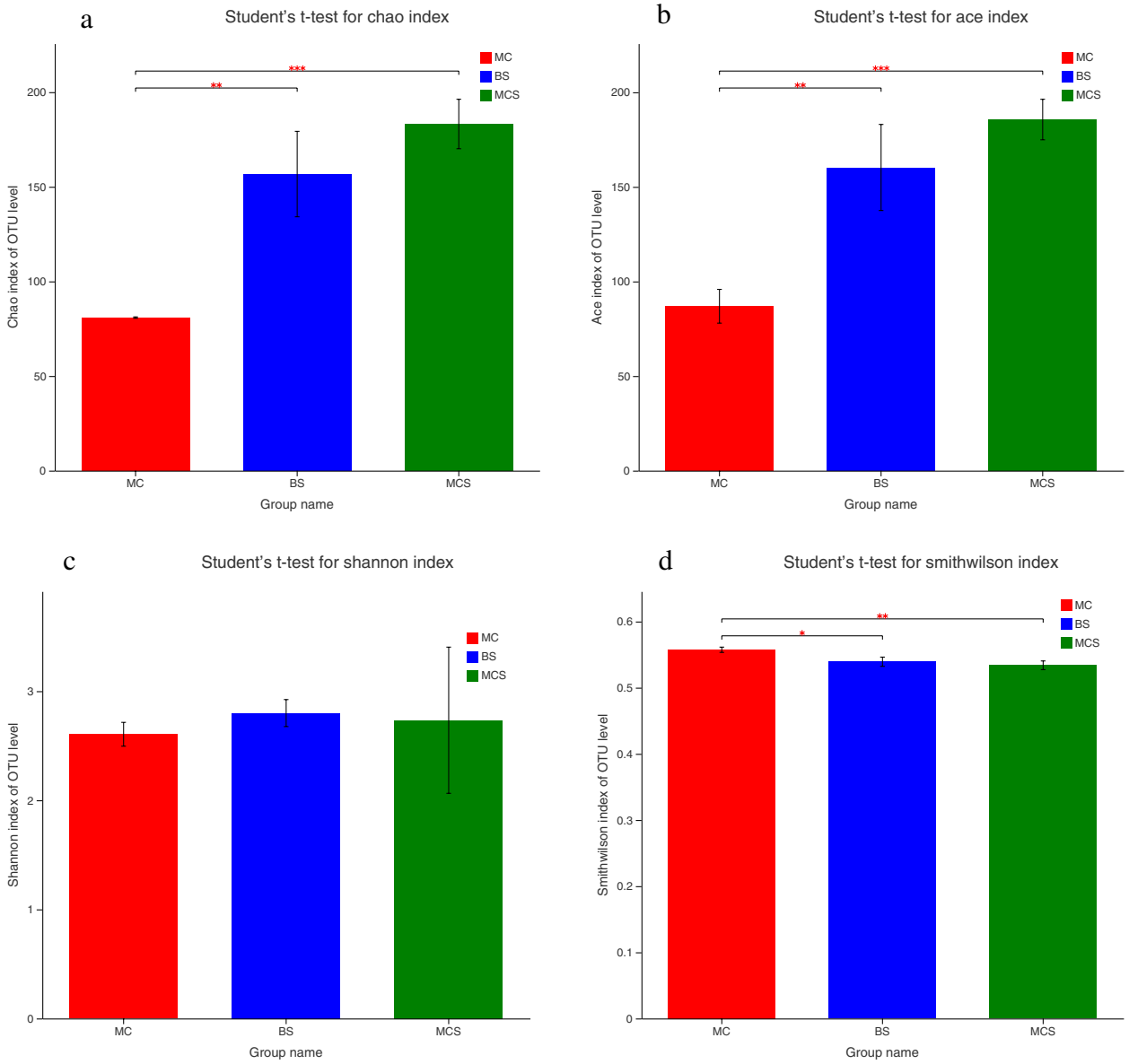

Fig. 2 Histogram of **a** Chao, **b** Ace, **c** Shannon, and **d** Smith-Wilson between different amounts of biochar added. Notes: MC, BS, and MCS are the same as in Fig. 1. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

nitrogen were significantly different among the three treatments and showed a gradual increasing trend. Compared with the control group, there were significant differences between BS and MCS in available potassium and organic matter, and the content increased with the increase of dosage (Table 1).

The composition of the microbial community among different treatments was assessed by MiSeq sequencing, which produced 49,122 to 56,739 sequences with different numbers of phylogenetic operational taxonomic units (OTUs). All rarefaction curves approached the saturation plateau, indicating that the data volume of sequenced reads was reasonable and that increasing the number of reads made only a small contribution to the total number of OTUs. However, there were significant differences in the rarefaction curves obtained from the samples, that the higher the amount of biochar addition, the higher richness (Fig. 1).

Effect of biochar addition on soil microbial community composition and overall diversity

The listed alpha diversity indices of soil bacterial were calculated based on the relative abundance of OTUs at

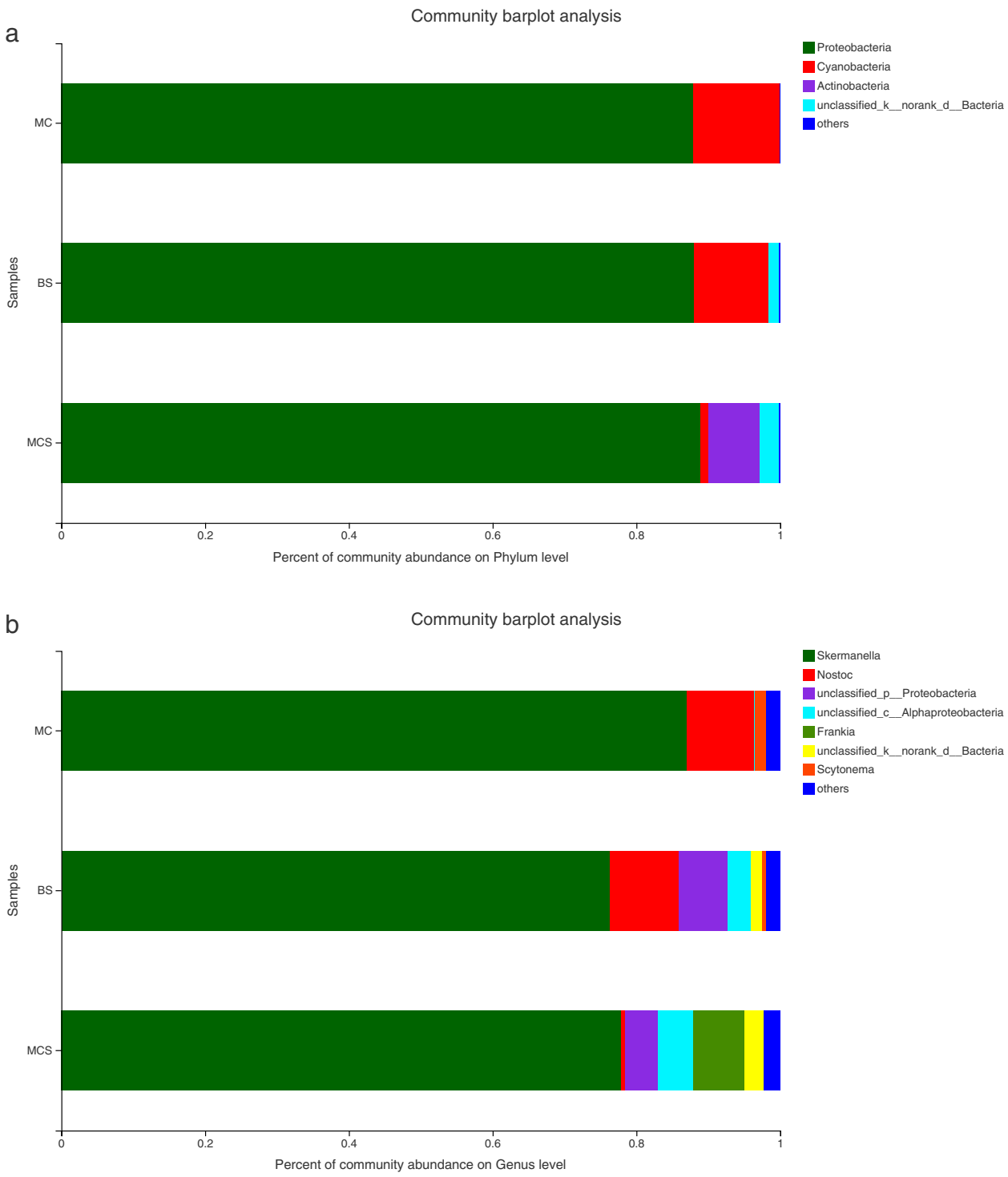

Fig. 3 Relative abundances of major taxonomic groups at the phylum and genus levels for bacteria (**a** phylum level; **b** genus level). Notes: MC, BS, and MCS are the same as in Fig. 1. Columns of different colors represent different species, and the length of the column represents the proportion of the species

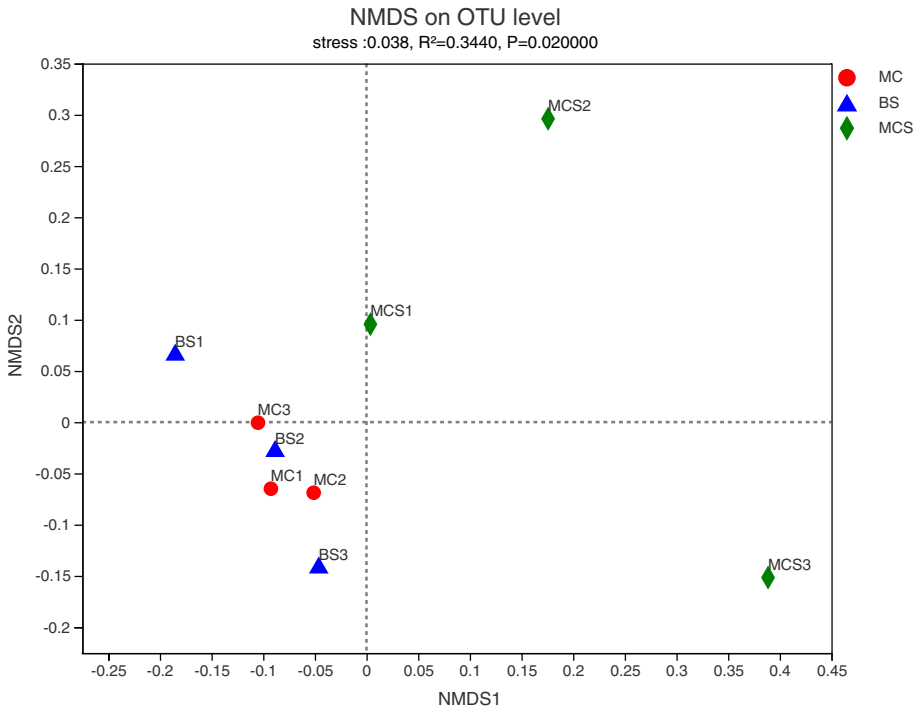

Fig. 4 NMDS plots based on Bray-Curtis dissimilarities of OTUs. Notes: MC, BS, and MCS are the same as in Fig. 1

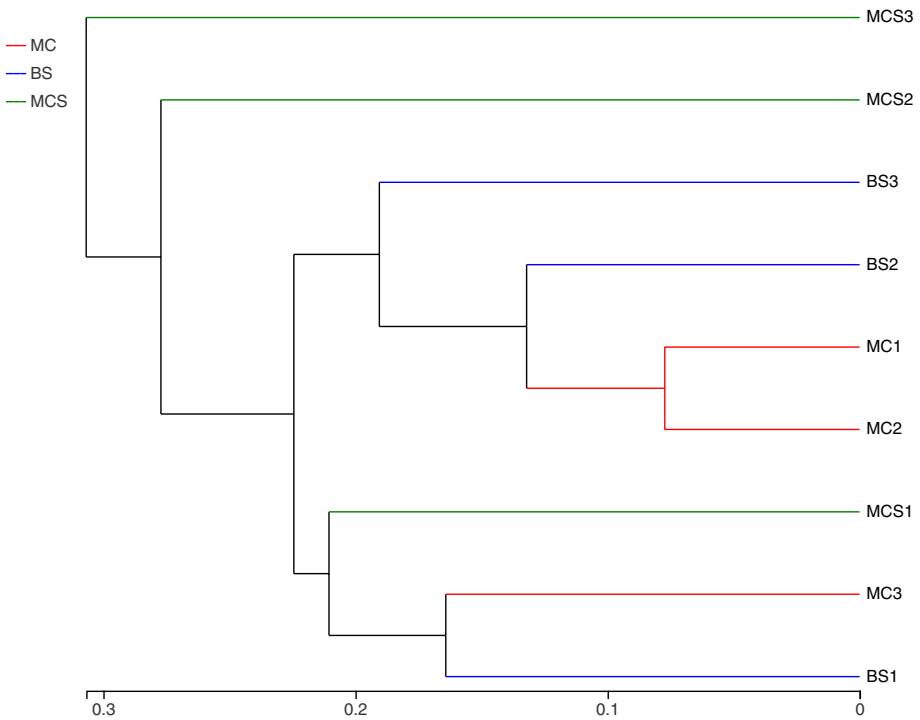

Fig. 5 Plots of hierarchical clustering based on OUT's level. Notes: MC, BS, and MCS are the same as in Fig. 1

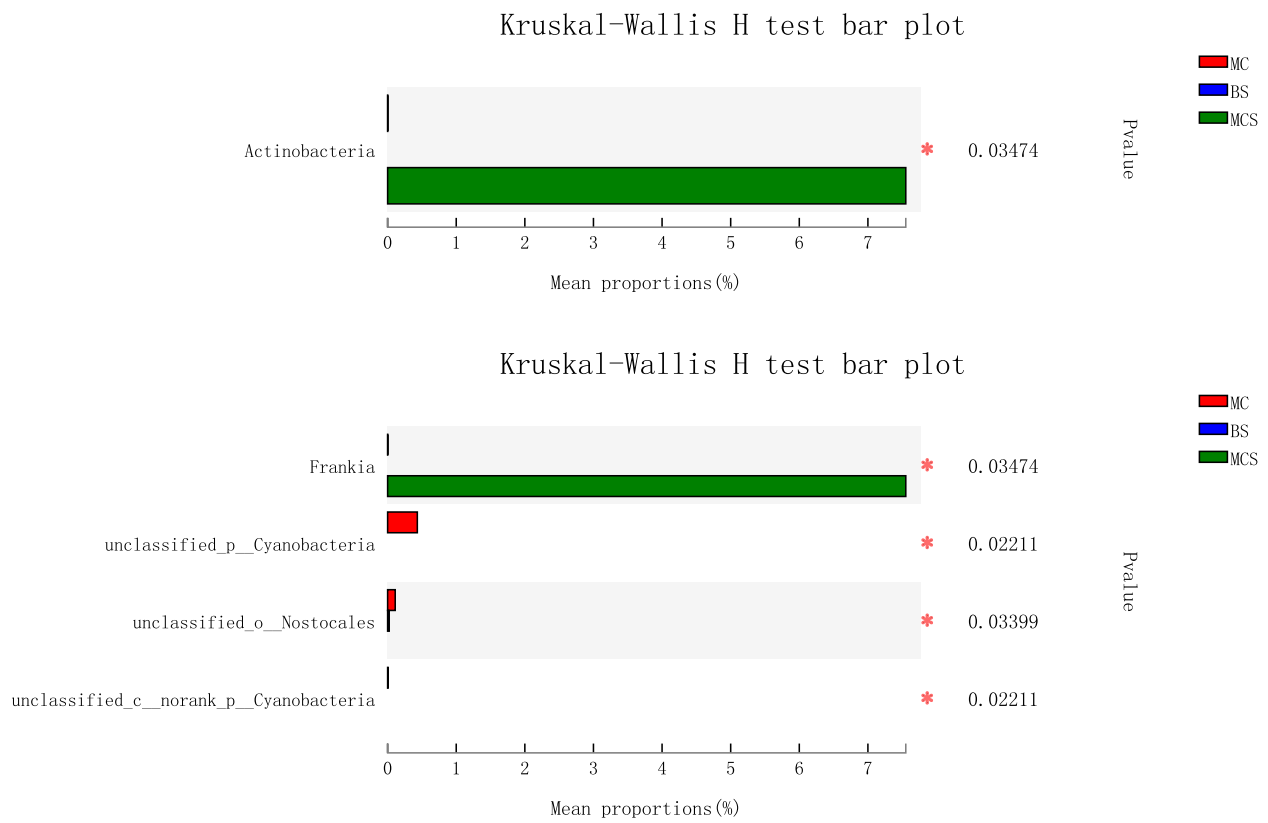

Fig. 6 The Kruskal-Wallis *H* test plot at the phylum and genus levels for bacteria. Notes: MC, BS, and MCS are the same as in Fig. 1. Columns of different colors represent different groups. The rightmost is the *P* value and the corresponding symbol. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

97% sequence similarity level and are shown in Fig. 2. Chao index and Ace index were used to describe community richness, Shannon index was used to describe community diversity, and Smith-Wilson index was used to describe community evenness. BS and MCS had significantly higher Chao and Ace compared with MC, and Smith-Wilson in BS and MCS also was significantly different but lower compared with MC, suggesting the biochar has been reported to be related to the richness and evenness. Shannon was not significantly different among these treatments, but Shannon in BS and MCS was higher than MC.

The relative abundances of major taxonomic groups have been showed in Fig. 3. OTUs were assigned into 6 bacterial phyla, 25 families, and 31 genera. The taxonomic classification of bacterial community composition showed that the dominant phyla, which accounted for more than 98% of the abundance of all species, were *Proteobacteria*, *Cyanobacteria*, and *Actinobacteria*. All soils were dominated by the phylum *Proteobacteria*, accounting for 87.8–88.9% of all sequences among treatments. *Cyanobacteria* (1.1–12.1%) was the second most abundant phyla. It was worth noting that the content of *Actinobacteria* in MCS was significantly higher than that in BS and MC, and the content of *unclassified_k_norank_d_Bacteria* was higher in BS and MCS, which was almost absent in MC.

The dominant genera were *Skermanella*, *Nostoc*, *unclassified_p_Proteobacteria*, *unclassified_c_Alphaproteobacteria*, and *Frankia*. *Skermanella* was the dominant genus accounting for 76.4–87.1%, and its content in MC was higher than BS and MCS. *Nostoc* (0.55–9.5%) was the second most abundant genera, which was almost absent in MCS. *Unclassified_p_Proteobacteria* and *unclassified_c_Alphaproteobacteria* appeared in BS and MCS. *Frankia* was unique in MCS.

Non-metric multidimensional scaling (NMDS) ordinations based on the Bray-Curtis similarity matrices was representative (stress = 0.038 < 0.05) and indicated that experimental grouping was meaningful (ANOSIM, $P = 0.013 < 0.05$; PERMANOVA, $P = 0.004 < 0.01$). NMDS showed a clear separation of the bacterial community structure in MCS from the other treatments,

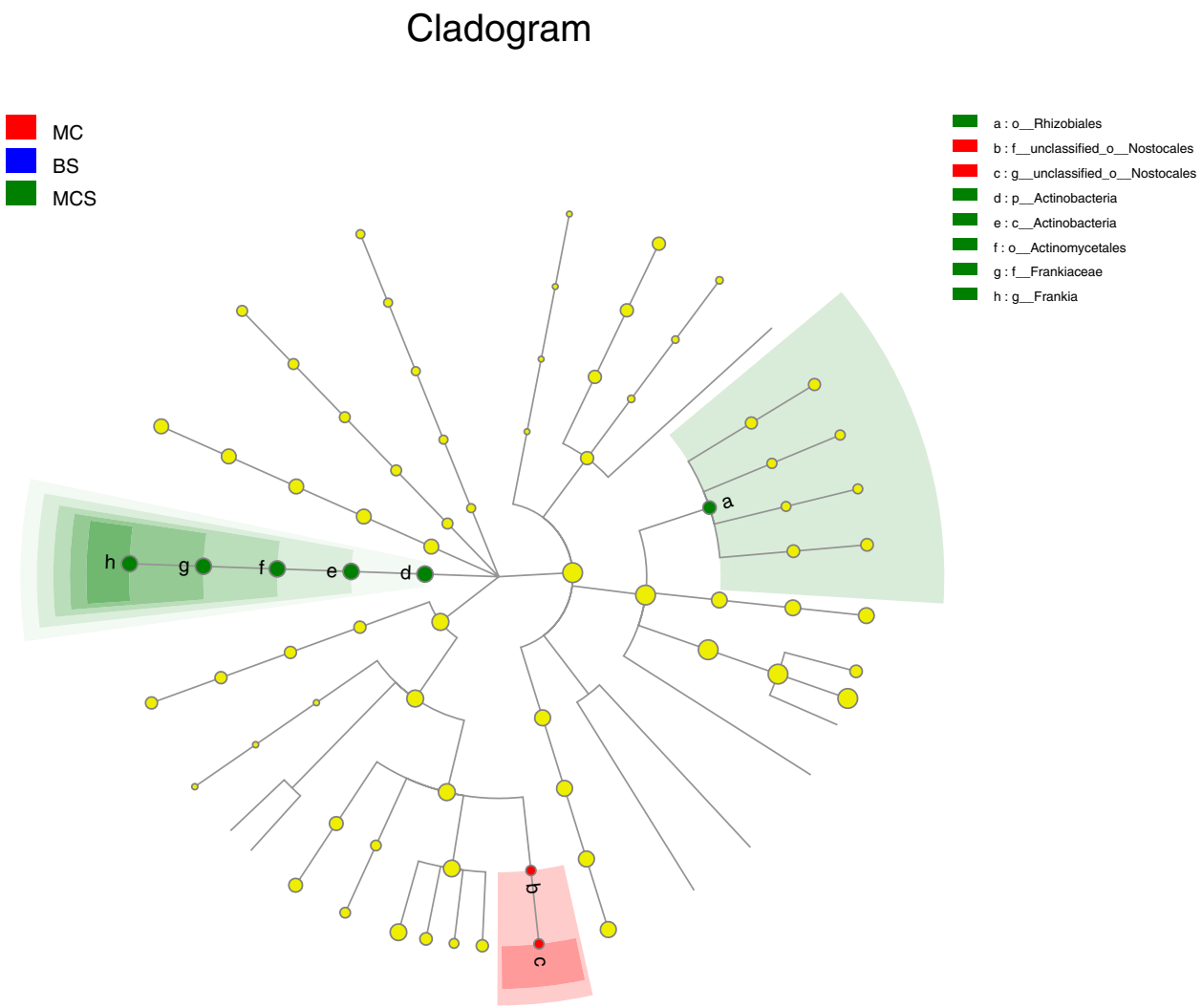

Fig. 7 LEfSe cladogram comparing communities from phylum to genus between treatment groups. Notes: MC, BS, and MCS are the same as in Fig. 1. Different color nodes represent microorganisms that are significantly enriched in the corresponding groups and have a significant impact on the differences between groups. Light yellow nodes indicate that there is no significant difference in different groups

and MC was included in BS, which was more concentrated; the community structure of MC and BS was more similar (Fig. 4). The hierarchical clustering also indicated that MCS was separated from other treatments (Fig. 5). MC and BS were located in the same or similar branches, and their community structure was basically similar; the close distance of MCS1, BS1, and BS3 indicates that they had similar effects on bacterial community structure.

Comparison of bacterial community structures in groups

The significance test of group differences at the gate level showed that *Actinobacteria* had significant differences among the three groups (Fig. 6, $P = 0.035 < 0.05$), and the average relative abundance was 7.55% in MCS. At the genus level, *Frankia*, *unclassified_p_Cyanobacteria*, *unclassified_o_Nostocales*, and *unclassified_c_norank_p_Cyanobacteria* were significantly different among the three groups. *Frankia* had a higher average relative abundance in MCS, and *unclassified_p_Cyanobacteria*, *unclassified_o_Nostocales*, and *unclassified_c_norank_p_Cyanobacteria* had a higher average relative abundance in MC. Lefse multistage species difference discriminant analysis showed that *F__unclassified_o__Nostocales*, *g__unclassified_o__Nostocales* is the marker of MC and *O__ Rhizobiales*, *p__Actinobacteria*, *c__Actinobacteria*, *o__Actinomycetales*, *f__Frankiaceae* is the marker of MCS (Fig. 7).

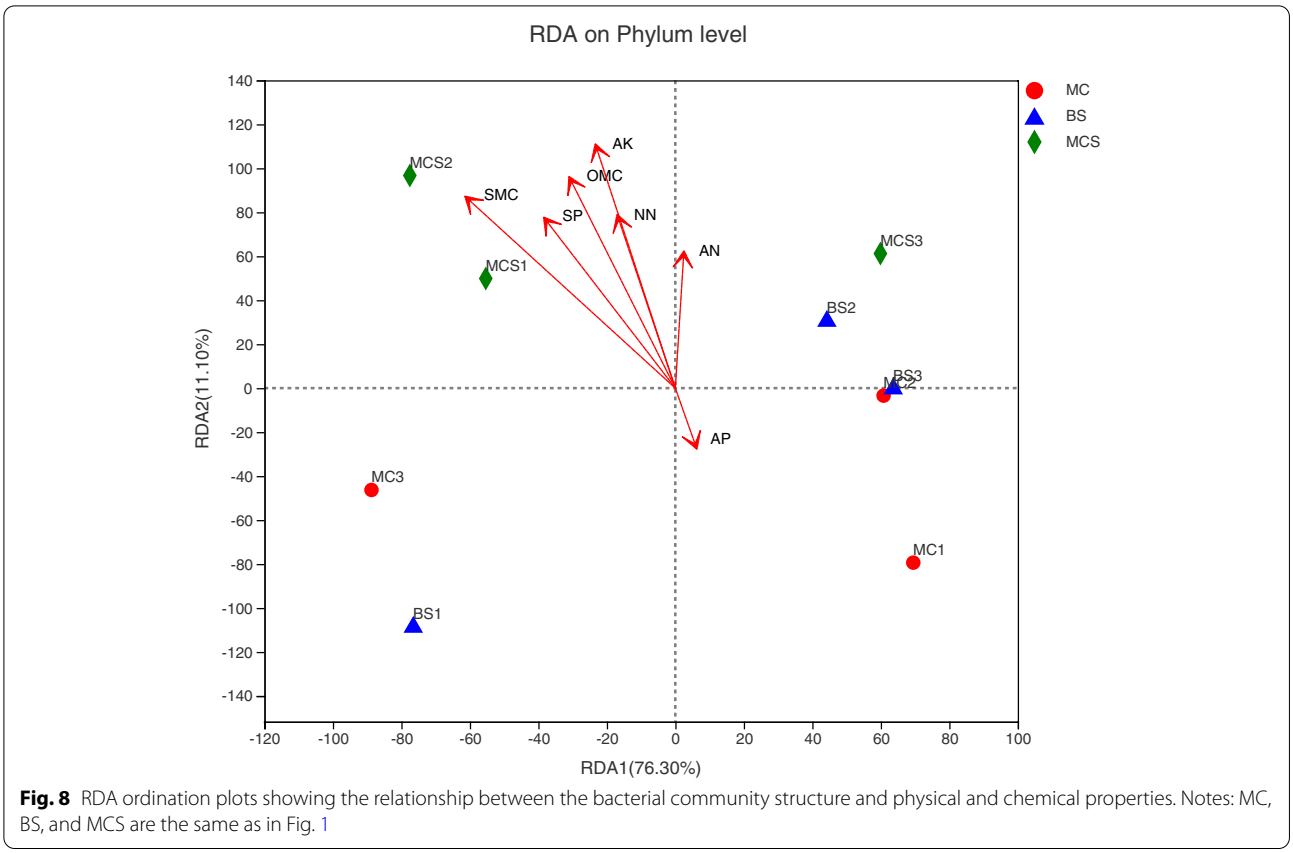

Fig. 8 RDA ordination plots showing the relationship between the bacterial community structure and physical and chemical properties. Notes: MC, BS, and MCS are the same as in Fig. 1

Correlations between soil microbial community composition and soil physical and chemical properties

PERMANOVA analysis showed that there was a significant positive correlation between SMC, AK, and community structure. RDA results demonstrated that MCS were positively associated with SMC, SP, OMC, AK, and NN and negatively associated with AP (Fig. 8). MC and BS had no significant effect on soil physical and chemical properties. Spearman correlation heatmap results are shown in Fig. 9. In addition to the negative correlation between AP and *Actinobacteria*, the influence of physical and chemical properties on Actinobacteria, unclassified_d__ Unclassified, *unclassified_k__norank_d__Bacteria*, and *Firmicutes* was positively correlated. Among them, *Actinobacteria* was significantly positively correlated with SP, SNC, and AK; *Firmicutes* was significantly positively correlated with NN; and *unclassified_ k__ norank_ d__ Bacteria* was positively correlated with NN and sp. The influence of physical and chemical properties on *Proteobacteria* and *Cyanobacteria* was negatively correlated. Among them, *Cyanobacteria* was significantly negatively correlated with NN, SMC, and AK. It is worth noting that NN has little effect on *Proteobacteria*, AN had little effect on *Firmicutes*, and AP also had little effect on *Cyanobacteria*.

Discussion

Biochar is mainly composed of carbon molecules. The addition of biochar can effectively change the physicochemical properties of soil. This study found that compared with the control, the treatment groups with different amounts of biochar were larger in the seven indexes of soil moisture content, soil porosity, ammonium nitrogen, nitrate nitrogen, available phosphorus, available potassium, and organic matter. With the increase of addition, soil moisture content, soil porosity, available potassium, nitrate nitrogen, and organic matter also increased. The results of this study might support some result previously obtained. Yin et al. (2021) also found that the addition of biochar would change the physicochemical properties of soil and increase available phosphorus, total nitrogen, nitrate nitrogen, ammonium nitrogen, and water content. Chen et al. (2018) found that the content of organic matter increased after the addition of biochar. Li et al. (2020a) found that the soil porosity increased after the addition of biochar. This may be due to the porosity and composition of biochar, which increases the soil surface area, enhances the soil

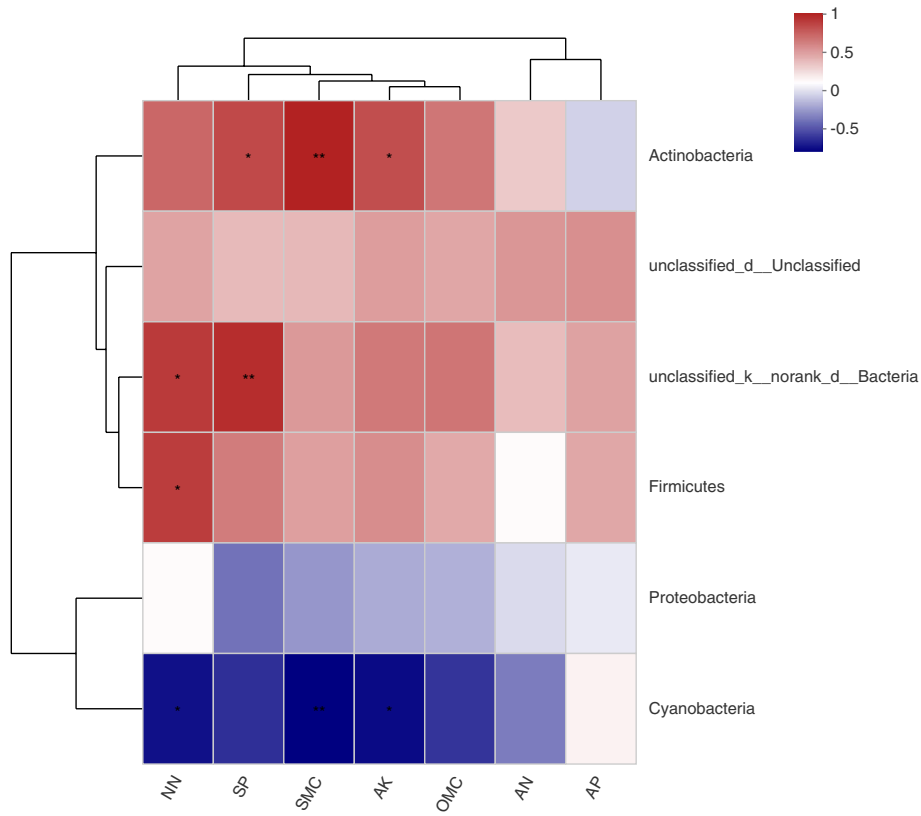

Fig. 9 Spearman correlation heatmap between bacteria at the phylum level and physical and chemical properties. Notes: NN, SP, SMC, AK, OMC, AN, and AP are the same as in Fig. 1. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$

porosity, and improves the micro-ecological environment (Agusalim et al. 2010, PiaK et al. 2016; Wang et al. 2019; Wu et al. 2014).

The biochar addition affected the physicochemical properties of soil, affected the living space of bacteria, and then affected the diversity of soil. This study found that the biochar addition increased the diversity of bacterial community and reduced the uniformity of bacterial community, and the species diversity showed an increasing trend with the increase of the amount of biochar, it is possible that the addition of biochar will change the soil microenvironment and cause the difference of bacterial community and biodiversity (Zhang et al. 2017), and this was consistent with many research (Nan et al. 2016; Wu et al. 2019; Hu et al. 2014; Thuy et al. 2014; Nguyen et al. 2018).

Studies have confirmed that biochar addition has an impact on microbial community composition (Hu et al. 2014). In this study, *Proteobacteria* and *Actinobacteria* are the dominant bacteria; this is consistent with the previous research results (Wu et al. 2019; Yin et al. 2021; Yao et al. 2017). Compared with the control, biochar addition significantly increased the relative abundance of *Actinobacteria* (Wu et al. 2019); it may be that after biochar was added, the soil nutrition was richer, and *Actinomycetes* was a eutrophic group, which can use the available carbon source to grow rapidly (Zeng et al. 2016); this showed that the addition of biochar to the soil makes *Actinomycetes* grow and reproduce better and had a significant impact on the structure of soil bacterial community, which was consistent with the previous research results (Zhang 2014).

The porosity of biochar will create an aerobic environment, which was conducive to the growth and reproduction of soil microbial community. This study found that NN, SP, SMC, and AK had a significant effect on bacteria at the phylum level and were the main factors affecting the community structure. It is worth noting that the physical and chemical properties of soil had no significant effect on *Proteobacteria*, but *Actinobacteria* was positively correlated with SP, SMC, and AK and *Cyanobacteria* was negatively correlated with NN, SMC, and AK. This could be caused by *Proteobacteria* being the largest phylum in bacteria, with large intraphylum variability. *Proteobacteria* existed in large numbers in the

study area, and the difference was not obvious. Therefore, the soil physicochemical properties had no significant effect on its abundance. The importance of soil physical and chemical properties in shaping microbial communities had been proved by several studies. As an important part of soil structure, porosity has a positive effect on the conduction of water and air in the soil (Luo et al. 2019); this is conducive to the growth and reproduction of aerobic bacteria. Deng et al. (2013) also found that soil porosity has an impact on soil microbial communities. Soil water content is one of the leading factors to maintain the life activities of soil microorganisms (Clark et al. 2009) and has a significant impact on soil microbial community, which was also confirmed by Li et al. (2020a, b). Available potassium can be decomposed and utilized by microorganisms, and its content affected microbial diversity. The study by Wang et al. (2021a, b) found that available potassium was negatively correlated with *Proteobacteria* and positively correlated with *Actinobacteria*; this was consistent with the research in this paper. Nitrate nitrogen is a kind of soil nitrogen fertilizer, and its nutrient content affects the abundance and diversity of soil microorganisms (Lan et al. 2017). Song et al. (2021) also found that nitrate nitrogen has a significant effect on microbial community structure.

Conclusions

Our study provides new basis for the rapid maturing technology of clayey raw soil using biochar. Our results indicated that the addition of biochar significantly improved the lack of fertility and low soil microbial diversity of clayey raw soil. The present study, using high-throughput sequencing technologies, provided a detailed picture of bacterial community variations on the phylum level among different biochar additions and showed the relationship between physical and chemical properties and soil microbial communities. Sequencing results and diversity indices indicated that the alpha diversity was higher in biochar addition level of 20 t/m^3 than other processing groups. The dominant phyla were *Proteobacteria*, *Cyanobacteria*, and *Actinobacteria*. At the gate level, *Actinobacteria* had significant differences among the three groups with different addition amounts, and the content was the highest in the treatment group with 20 t/m^3 addition amount. The microbial community structure was mainly influenced by soil porosity, soil moisture content, nitrogen fertilizer, and potassium fertilizer other than soil phosphate fertilizer and organic matter. This experiment shows that high addition amount of biochar has better effect on soil improvement, but the range of biochar addition in this study is small, and it is necessary to continue to expand the range of biochar addition for further research.

Acknowledgements
This research was funded by the Technology Innovation Center for Land Engineering and Human Settlements, Shaanxi Land Engineering Construction Group Co., Ltd and Xi'an Jiaotong University (2021WHZ0089), and Shaanxi Provincial Land Engineering Construction Group (DJNY2022-53).

Authors' contributions
The corresponding author is the first author: guide the completion of this experiment and revise their papers. The second author: performed experiments and recorded data. Both authors read and approved the final manuscript.

Funding
This research was funded by the Technology Innovation Center for Land Engineering and Human Settlements, Shaanxi Land Engineering Construction Group Co., Ltd and Xi'an Jiaotong University (2021WHZ0089), and Shaanxi Provincial Land Engineering Construction Group (DJNY2022-53).

Availability of data and materials
The data were obtained by the authors.

Declarations

Ethics approval and consent to participate
The study did not violate ethics, and all participants agreed to publish the paper.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Shaanxi Provincial Land Engineering Construction Group Co., Ltd., Xi'an 710000, China. [2]Institute of Land Engineering and Technology, Shaanxi Provincial Land Engineering Construction Group Co., Ltd., Xi'an 710000, China. [3]Key Laboratory of Degraded and Unused Land Consolidation Engineering, the Ministry of Natural Resources, Xi'an 710000, China. [4]Shaanxi Provincial Land Consolidation Engineering Technology Research Center, Xi'an, China.

Received: 11 April 2022 Accepted: 4 August 2022
Published online: 18 September 2022

References

Agusalim M, Hadi UW, Syechfani MS (2010) Rice husk biochar for rice based cropping system in acid soil. the characteristics of rice husk biochar and its influence on the properties of acid sulfate soils and rice growth in West Kalimantan, Indonesia. J Agric Sci (1916-9752) 2(1):39–47. https://doi.org/10.5539/jas.v2n1p39

Ahmad M, Rajapaksha AU, Lim JE et al (2014) Biochar as a sorbent for contaminant management in soil and water: a review. Chemosphere 99:19–33. https://doi.org/10.1016/j.chemosphere.2013.10.071

Atkinson CJ, Fitzgerald JD, Hipps NA (2010) Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. Plant and Soil 337(1-2):1–18. https://doi.org/10.1007/s11104-010-0464-5

Bao S (2000) Soil agrochemical analysis, 3rd edn. China Agricultural Press, Beijing

Bronick CJ, Lal R (2005) Soil structure and management: a review. Geoderma 124:3–22. https://doi.org/10.1016/j.geoderma.2004.03.005

Caporaso JG, Lauber CL, Walters WA et al (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. Isme

J Multidiscipl J Microbial Ecol 6(8):1621–1624. https://doi.org/10.1038/ismej.2012.8
Chen K, Xu X, Peng J, Feng X, Han X (2018) Effects of biochar and biochar-based fertilizer on soil microbial community structure. Scientia Agricult Sinica 51:1920–1930. https://doi.org/10.3864/j.issn.0578-1752.2018.10.011
Chen WF, Zhang WM, Meng J (2013) Advances and prospects in research of biochar utilization in agriculture. Scientia Agricult Sinica 46(16):3324–3333
Cheng J, Lee X, Tang Y, Zhang Q (2019) Long-term effects of biochar amendment on rhizosphere and bulk soil microbial communities in a karst region, southwest China. Appl Soil Ecol 140:126–134. https://doi.org/10.1016/j.apsoil.2019.04.017
Clark JS, Campbell JH, Grizzle H, Acosta-Martìnez V, Zak JC (2009) Soil microbial community response to drought and precipitation variability in the Chihuahuan Desert. Microb Ecol 57(2):248–260. https://doi.org/10.1109/82.532010
Deng C, Dong BL, Qin JT et al (2013) Effects of long-term fertilization on soil property changes and soil microbial biomass. Soils 45(05):888–893. https://doi.org/10.13758/j.cnki.tr.2013.05.019
Ding WS, Wei ZC, Meng LQ et al (2019) Effects of biochar on soil bacterial diversity in Chinese fir plantations. J Forest Environ 39(06):584–592. https://doi.org/10.13324/j.cnki.jfcf.2019.06.004
FastUniFrac. http://UniFrac.colorado.edu. Accessed 20 May 2022.
Grossman JM et al (2010) Amazonian anthrosols support similar microbial communities that differ distinctly from those extant in adjacent, unmodified soils of the same mineralogy. Microb Ecol 60(1):192–205
Gundale MJ, Deluca TH (2007) Charcoal effects on soil solution chemistry and growth of Koeleria macrantha in the ponderosa pine/Douglas-fir ecosystem. Biol Fertil Soils 43(3):303–311. https://doi.org/10.1007/s00374-006-0106-5
Hu L, Cao L, Zhang R (2014) Bacterial and fungal taxon changes in soil microbial community composition induced by short-term biochar amendment in red oxidized loam soil. World J Microbiol Biotechnol 30(3):1085–1092. https://doi.org/10.1007/s11274-013-1528-5
Keya Z. (2016) Effects of soil amendment application on the soil quality and the flue-cured tobacco growth based on biochar. Master, Nanjing Agricultural University.
Khodadad CLM et al (2011) Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. Soil Biol Biochem 43(2):385–392
Lan G, Li Y, Wu Z, Xie G (2017) Impact of tropical forest conversion on soil bacterial diversity in tropical region of China. Eur J Soil Biol 83:91–97. https://doi.org/10.1016/j.ejsobi.2017.10.007
Lefse Software. http://huttenhower.sph.harvard.edu/galaxy/root?tool_id=lefse_upload. Accessed 20 May 2022.
Li CZ, Zhang H, Yao WJ et al (2020a) Effects of biochar application combined with nitrogen fertilizer on soil physicochemical property and winter wheat yield in the typical ancient region of Yellow River, China. Chin J Appl Ecol 31(10):3424–3432. https://doi.org/10.13287/j.1001-9332.202010.028
Li QM, Zhang LL, Liu HM et al (2020b) Effects of cover crop diversity on soil microbial community functions in a kiwifruit orchard. JAES 39(02):351–359. https://doi.org/10.13254/j.jare.2019.0627
Li WB, Li XP, Li HY, Ying Y et al (2012) Effects on micro-ecological characteristics in clayey soil of tobacco area under different sand adding proportions. J Northwest A F Univ 40(11):85-90+96. https://doi.org/10.13207/j.cnki.jnwafu.2012.11.012
Liang BQ, Lehmann J, Sohi SP et al (2010) Black carbon affects the cycling of non-black carbon in soil. Org Geochem 41(2):206–213. https://doi.org/10.1016/j.orggeochem.2009.09.007
Luo J, Li LZ, Que YX et al (2019) Effect of subsoiling depths on soil physical characters and sugarcane yield. Chin J Appl Ecol 30(02):405–412. https://doi.org/10.13287/j.1001-9332.201902.010
Mother (version v.1.30.2). https://mothur.org/wiki/calculators. Accessed 20 May 2022.
Nan X, Tan G, Wang H, Gai X (2016) Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. Eur J Soil Biol 74:1–8. https://doi.org/10.1016/j.ejsobi.2016.02.004
Nguyen T, Wallace HM, Xu CY et al (2017) Short-term effects of organo-mineral biochar and organic fertilisers on nitrogen cycling, plant photosynthesis, and nitrogen use efficiency. J Soil Sediment 17(12):1–12. https://doi.org/10.1007/s11368-017-1839-5
Nguyen TTN, Wallace HM, Xu CY et al (2018) The effects of short term, long term and reapplication of biochar on soil bacteria. Sci Total Environ 636:142–151. https://doi.org/10.1016/j.scitotenv.2018.04.278
Pang CM, Guo XF, Pang YN et al (2021) A dataset of grain yield and soil nutrient of wheat and maize in fluvo-aquic clayey soil region of Southwest Shandong Province based on the annual straw returning to the field from 2010 to 2020. China Sci Data 6(04):187–195. https://doi.org/10.11922/11-6035.nasdc.2021.0016.zh
PiaK W, Nacke H, Kaiser K et al (2016) Estimates of soil bacterial ribosome content and diversity are significantly affected by the nucleic acid extraction method employed. Appl Environ Microbiol 82(9). https://doi.org/10.1128/AEM.00019-16
Richard S, Quilliam HC et al (2013) Life in the 'charosphere' – does biochar in agricultural soil provide a significant habitat for microorganisms? Soil Biol Biochem 65(1):287–293. https://doi.org/10.1016/j.soilbio.2013.06.004
Rondon MA et al (2007) Biological nitrogen fixation by common beans (Phaseolus vulgaris L.) increases with bio-char additions. Biol Fertil Soils 43(6):699–708
Silva Database (lease138). http://www.arb-silva.de. Accessed 20 May 2022.
Song JS, Zhang XL, Kong FL et al (2021) Effects of biomass conditioner on soil nutrient and microbial community characteristics of alpine desertified grassland in northwest Sichuan, China. Chin J Appl Ecol 32(06):2217–2226. https://doi.org/10.13287/j.1001-9332.202106.036
Steiner C, Glaser B, Geraldes Teixeira W, Lehmann J, Blum WEH, Zech W (2010) Nitrogen retention and plant uptake on a highly weathered central Amazonian Ferralsol amended with compost and charcoal. J Plant Nutr Soil Sci 171(6):893–899. https://doi.org/10.1002/jpln.200625199
Stéphanie T, Jean-François P, Ballof S (2005) Manioc peel and charcoal: a potential organic amendment for sustainable soil fertility in the tropics. Biol Fertil Soils 41(1):15–21. https://doi.org/10.1007/s00374-004-0804-9
Thuy TD, Corinne B, Yvan B, Thierry B, Thierry HT, Jean LJ, Patrice L, Bo VN, Pascal J (2014) Influence of buffalo manure, compost, vermicompost and biochar amendments on bacterial and viral communities in soil and adjacent aquatic systems. Appl Soil Ecol 73(2):78–86. https://doi.org/10.1016/j.apsoil.2013.08.016
Uparse Software (version 7.0.1090). http://drive5.com/uparse. Accessed 20 May 2022.
Wang J, Shi Y, Ziyuan LI et al (2016a) Effects of biochar application on N_2O emission in degraded vegetable soil and in remediation process of the soil. Acta Pedolog Sinica 53(03):713–723. https://doi.org/10.11766/trxb201509170443
Wang J, Xiong Z, Kuzyakov Y (2016b) Biochar stability in soil: meta-analysis of decomposition and priming effects. GCB Bioenerg 8(3):512–523. https://doi.org/10.1111/gcbb.12266
Wang LL, Cao YG, Wang F et al (2021a) Effect of biochar on reconstructed soil chemical properties and drought resistance of Medicago sativa. Res Soiland Water Conserv 28(06):105–114. https://doi.org/10.13869/j.cnki.rswc.2021.06.009
Wang XH, Guo GX, Zheng RL et al (2013) Effect of biochar on abundance of N-related functional microbial communities in degraded greenhouse soil. Acta Pedolog Sinica 50(03):624–631
Wang Y, Sun CC, Zhou JH et al (2019) Effects of biochar addition on soil bacterial community in semi-arid region. China Environ Sci 39(05):2170–2179. https://doi.org/10.19674/j.cnki.issn1000-6923.2019.0259
Wang ZQ, Zhang JX, Yang XL et al (2021b) Characteristics of soil microbial diversity in different patches of alpine meadow. Acta Agrestia Sin 29(09):1916–1926. https://doi.org/10.11733/j.issn.1007-0435.2021.09.007
Warnock DD, Lehmann J, Kuyper TW, Rillig MC (2007) Mycorrhizal responses to biochar in soil concepts and mechanisms. Plant and Soil 300:9–20. https://doi.org/10.1007/s11104-007-9391-5
Wildman J, Derbyshire F (1991) Origins and functions of macroporosity in activated carbons from coal and wood precursors. Fuel 70(5):655–661. https://doi.org/10.1016/0016-2361(91)90181-9
Wu C, Shi LZ, Xue SG et al (2019) Effect of sulfur-iron modified biochar on the available cadmium and bacterial community structure in contaminated soils. Sci Total Environ 647:1158–1168. https://doi.org/10.1016/j.scitotenv.2018.08.087
Wu S, He H, Inthapanya X et al (2017) Role of biochar on composting of organic wastes and remediation of contaminated soils-a review.

Environ Sci Pollut Res 24(20):16560–16577. https://doi.org/10.1007/s11356-017-9168-1
Wu YG, Zhang GL, Lai X et al (2014) Effects of biochar applications on bacterial diversity in fluvor-aquic soil of North China. J Agro-Environ Sci 33(05):965–971
Wüst PK, Nacke H, Kaiser K et al (2016) Estimates of Soil Bacterial Ribosome Content and Diversity Are Significantly Affected by the Nucleic Acid Extraction Method Employed. Applied and Environmental Microbiology 82(9) 2595–2607. https://doi.org/10.1128/AEM.00019-16
Yao Q, Liu J, Yu Z et al (2017) Changes of bacterial community compositions after three years of biochar application in a black soil of northeast China. Appl Soil Ecol 113:11–21. https://doi.org/10.1016/j.apsoil.2017.01.007
Yin QY, Li X, Wang D et al (2021) Effects of continuous application of biochar for 4 years on soil bacterial diversity and community structure. J Henan Agric Univ 55(04):752–760+775. https://doi.org/10.16445/j.cnki.1000-2340.20210512.001
Yuan JJ, Tong YA, Shao-Hui LU et al (2018) Combined application of biochar and inorganic nitrogen influnces the microbial properties in soils of jujube orchard. J Plant Nutr Fertil 24(04):1039–1046
Zackrisson O, Wardle N (1996) Key ecological function of charcoal from wildfire in the Boreal forest. Oikos 77(1):10–19. https://doi.org/10.2307/3545580
Zeng J, Liu X, Song L, Lin X, Chu H (2016) Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. SBB 92:41–49. https://doi.org/10.1016/j.soilbio.2015.09.018
Zhang G (2014) Effects of biochar applications on bacterial diversity in fluvor-aquic soil of North China. J Agro-Environ Sci 33(5):965–971
Zhang JN, Zhou S, Guang-Nan Ll (2018a) Improving the coastal mudflat soil chemical properties and rice growth using straw biochar. J Agric Resourc Environ 35(06):492–499. https://doi.org/10.13254/j.jare.2017.0332
Zhang Q, Liu BJ, Lu YU, Wang RR, Li FM (2019) Effects of biochar amendment on carbon and nitrogen cycling in coastal saline soils: a review. J Nat Resourc 34(12):2529–2543. https://doi.org/10.31497/zrzyxb.20191204
Zhang Y, Drigo B, Bai SH, Menke C, Zhang M, Xu Z (2018b) Biochar addition induced the same plant responses as elevated CO2 in mine spoil. Environ Sci Pollut Res 25:1460–1469. https://doi.org/10.1007/s11356-017-0574-1
Zhang YJ, Wu T, Zhao J et al (2017) Effect of biochar amendment on bacterial community structure and diversity in straw-amended soils. Acta Sci Circumstantiae 37(2):712–720. https://doi.org/10.13671/j.hjkxxb.2016.0333
Zhang YL, Chen H, Bai SH, Menke C, Xu ZH (2017a) Interactive effects of biochar addition and elevated carbon dioxide concentration on soil carbon and nitrogen pools in mine spoil. J Soil Sediment 17(3):1–10. https://doi.org/10.1007/s11368-017-1757-6
Zou Q, An WH, Wu C et al (2017) Red mud-modified biochar reduces soil arsenic availability and changes bacterial composition. Environm Chem Lett 16:615–622. https://doi.org/10.1007/s10311-017-0688-1
Zwieten LV et al (2010) Influence of biochars on flux of N2O and CO2 from ferrosol. Soil Res 48(11):1043–1046

Publisher's Note