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The impacts of a biochar application on selected soil properties and bacterial communities in an Albic Clayic Luvisol

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Abstract: In this four-year study, we focused on the impacts of a biochar application on physicochemical soil properties (soil total carbon, total nitrogen, total potassium, total phosphorus, available nitrogen, available potassium, available phosphorus, pH, bulk density and moisture) and bacterial communities in an Albic Clayic Luvisol. The biochar was applied to plots only once with rates of 0, 10, 20 and 30 t/ha at the beginning of the experiment. The soil samples were collected from the surface (0–10 cm) and second depth (10–20 cm) soil layers after four years. The results showed that that the soil total carbon (TC) and pH increased, but the soil bulk density (BD) decreased with the biochar application. The soil bacterial sequences determined by the Illumina MiSeq method resulted in a decrease in the relative abundance of *Acidobacteria*, but an increase in the *Actinobacteria* with the biochar application. The bacterial diversity was significantly influenced by the biochar application. The nonmetric multidimensional scaling (NMDS) and canonical correspondence analysis (CCA) indicated that the soil bacterial community structure was affected by both the biochar addition and the soil depth. The Mantel test analysis indicated that the bacterial community structure significantly correlated to a soil with a pH (r = 0.525, P = 0.001), bulk density (r = 0.539, P = 0.001) and TC (r = 0.519, P = 0.002) only. In addition, most of the differences in the soil properties, bacterial relative abundance and community composition in the second depth soil layer were greater than those in the surface soil layer.

Keywords: bacterial relative abundance; bacterial community structure; bacterial community diversity; soil properties; soil depth; Illumina MiSeq

Biochar is often produced via the pyrolysis of an agricultural biomass (plant residues and animal manure, etc.) under limited oxygen and specific temperature (400–700°C) conditions (Lehmann 2009; Cantrell et al. 2012). In general, a biochar is a carbon-rich material with outstanding properties, such as a small bulk density, a large surface area, a strong adsorption capacity, strong stability, and a high pH (Van Zwieten et al. 2010; Lehmann et al. 2011). Many studies have shown that a biochar application to the soil can change the physicochemical soil properties, such as increasing the soil pH (Lehmann 2009), increasing the contents of the soil organic carbon (Jones et al. 2012), improving the soil fertility and soil structure (Acosta-Martínez et al. 2010). In addition, Gomez et al. (2014) indicated that a biochar application can change the soil microbial activity. Xu et al. (2014) used high-throughput sequencing to show that a biochar addition significantly influences the relative abundance and diversity of the microorganisms associated with carbon and nitrogen cycles.

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An Albic Clayic Luvisol (IUSS Working Group WRB 2015) has a typical albic horizon that is an eluvial, 1 cm or thicker and contains approximately 85% albic materials (Soil Survey Staff 2014). Albic materials are soil materials with a colour that is largely determined by the colour of the primary sand and silt particles rather than by the colour of their coatings (IUSS Working Group WRB 2015). Albic Clayic Luvisols have the characteristics of poor water and air permeability, low fertility, slight acidity, low organic matter and few microorganisms, which limit the growth of crop roots and the use of fertilisers (Liu et al. 2014). The area of the Albic Clayic Luvisol is 3.3 million ha, accounting for 10.07% of the total cultivated land area in the Heilongjiang Province (Xu et al. 2014). Albic Clayic Luvisols are an important main cultivated land resource for the Heilongjiang Province. Therefore, it is very necessary to study the improvement of Albic Clayic Luvisols.

A biochar application is one of the measures for improving Albic Clayic Luvisols. However, few studies have reported the effects of a biochar addition on the soil's microbial communities in an Albic Clayic Luvisol. In this study, we focused on analysing the relationship between the soil's bacterial communities and the physicochemical properties four years after the biochar addition in the surface and the second depth soil layer.

MATERIAL AND METHODS

Soil and biochar. The experimental plot, located on the Shuguang Farm (Jiamusi Heilongjiang Province, China), was treated on 5 May 2014. The albic horizon is usually 3–5 cm thick, and its depth ranges from 3 to 15 cm. The basic properties of the Albic Clayic Luvisol at the beginning of the experiment were as follows: a total C of 19.31g/kg, a total N of 1.64 g/kg, a total P of 0.71 g/kg, a total K of 20.06 g/kg, an available P of 76.20mg/kg, an available K of 145.53mg/kg, and a pH of 6.03 (water/soil = 2.5/1, volume of the water/weight of the soil(v/w)). The biochar was produced from corn stalks under oxygen-limited conditions at 550°C (Runnong Ltd., China). The characteristics of the biochar were as follows: a total C of 803.42 g/kg, a total N of 1.38 g/kg, an available P of 80.95 mg/kg, a total K of 23.53 g/kg, and a pH of 9.81 (water/biochar = 10/1 v/w).

Experimental design and sample collocation. Each treatment area was 25 m^2 (5 × 5 m) with three replicates. The biochar was applied to the plots only once at the rates of 0, 10, 20 and 30 t/ha. The biochar was mixed with the 20 cm deep soil by ploughing. Corn was the crop grown throughout the four-year cultivation period. Equal amounts of chemical fertilisers, 160 kg of N/ha, 50 kg of P_2O_5/ha and 50 kg of K_2O/ha, were supplied for each treatment. The corn stalks were removed out of the plots each year after the corn harvest. Soil samples were collected on 25 September 2018 after the corn harvest. Two soil samples were taken from each treatment area: one was a mix of five individual soil cores (one each from the four quadrat corners and one centre point) collected from the surface (0–10 cm) layer, and the other was from the second depth (10–20 cm) layer. The labels –10 and –20 represent the soil samples collected from the 0–10 cm soil layer and the 10–20 cm soil layer. B0, B1, B2 and B3 represent the 0, 10, 20 and 30 t/ha doses of the biochar addition, respectively.

Basic soil property analysis. The soil pH was determined by a soil liquid extraction process (water/soil = 2.5/1 v/w) using a pH meter (Lu 2000). The soil's total carbon (TC) and nitrogen (TN) were determined by an elemental analyser (Vario EL III, Elementar, Germany) (Jones & Willett 2006). The soil's total potassium (TK) and available potassium (AK) were determined by a flame photometer (FP6410, Shanghai Jingke, China) (Lu 2000). The soil's total phosphorus (TP) and available phosphorus (AP) were determined by an ultraviolet spectrophotometer (UV2600, Shimadzu, Japan) (Lu 2000). The soil's bulk density (BD) and moisture were determined gravimetrically (Lu 2000). The soil's available nitrogen (AN) was determined by the alkaline hydrolysis diffusion method (Lu 2000).

16S rDNA amplification, Illumina MiSeq sequencing and data analysis. The genomic DNA was extracted from the soil samples by using a MiSeq Reagent Kit v3 (Illumina, USA) for the soil according to the instructions. The V4-V5 hypervariable regions of the bacterial 16S rRNA genes were amplified by the Polymerase Chain Reaction (PCR) from the extracted DNA for each sample by using the universal primers 515F and 907R. The cycling conditions were 98°C for 30 s; 25 cycles of 98°C for 15 s, 55°C for 30 s and 72°C for 30 s; and then an extension at 72°C for 5 min. Equal dosages of the PCR products were sequenced on an Illumina MiSeq at Genesky Biotechnologies Inc. (Shanghai, China) (Jiang et al. 2013). Three replicates of each treatment were repeatedly amplified independently. The original sequences were analysed by the National Center for Biotechnology

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Information (NCBI). The taxonomy per phylotype was acquired by the Basic Local Alignment Search Tool (BLAST) and compared against the GenBank (Quast et al. 2013). An operational taxonomic unit (OTU) cluster analysis was carried out with 97% similarity using the USEARCH software (Ver. 10.0.240, 2017), and the chimera sequences were removed by the de novo template of USEARCH (Ver. 10.0.240, 2017) (Price et al. 2010).

Statistical analysis. The α-diversity indices were calculated using mothur (Ver. 1.35.1, 2015). The differences in the soil properties, bacterial relative abundances, and diversity indices were calculated using a one-way analysis of variance (ANOVA) followed by the least significant difference through SPSS (Ver. 17.0, 2009) at a $P < 0.05$ significance level. The Spearman correlation analysis was used to analyse the correlations between the basic soil properties and the amount of the biochar using SPSS (Ver. 17.0, 2009). The differences in the bacterial community compositions were analysed by Nonmetric multidimensional scaling (NMDS) ordination plots. The relationship between the soil properties and the bacterial communities was analysed by the canonical correspondence analysis (CCA). The NMDS analysis and the CCA were performed using the "vegan" package in the R environment (Ver. 4.3.2, 2017). The Figures and Tables were made using Excel 2016 and SigmaPlot (Ver. 12.5, 2013).

RESULTS AND DISCUSSION

The impacts of the biochar application on the soil's physicochemical properties. The Albic Clayic Luvisol physicochemical properties after the biochar application are shown in Table 1. Overall, the soil pH and TC significantly increased with the biochar application in the surface and the second depth soil layer (Table 1). The pairwise correlation analysis showed that the soil TC ($k = 0.492$, $R^2 = 0.985$) and pH ($k = 0.025$, $R^2 = 0.846$) had an obvious positive correlation with the amount of the biochar in the soil (Figure 1A, B). The increases in the soil TC and pH were attributed to the biochar properties, high pH and rich carbon. The biochar was a highly stabile carbonaceous material with hardly any degradation (Prayogo et al. 2014), its application promoted the increase of the soil's TC in this study. However, the soil moisture, TN, TP, TK, AN, AP and AK were not significantly affected by the biochar application. We speculated that the contents of these indices from the biochar had already been

Table 1. The effects of the different amounts of the biochar on the soil's properties in the Albic Clayic Luvisol

Treatments	TC	TN	TP	TK	AN	AP	AK	Moisture (%)	BD (g/cm^3)	pH
	(g/kg)				(mg/kg)					
B0-10	19.30 ± 0.98^{d}	1.61 ± 0.08^{a}	0.73 ± 0.05^{a}	20.42 ± 2.22^{a}	111.23 ± 6.63^{a}	77.19 ± 3.40^{a}	147.77 ± 3.07^{ab}	39.44 ± 2.91^{a}	1.36 ± 0.03^{a}	6.03 ± 0.04^{d}
B1-10	24.85 ± 1.91^{c}	1.63 ± 0.10^{a}	0.74 ± 0.04^{a}	20.85 ± 1.82^{a}	111.55 ± 6.58^{a}	76.49 ± 2.79^{a}	147.57 ± 4.84^{ab}	40.50 ± 1.61^{a}	1.33 ± 0.03^{b}	6.20 ± 0.06^{d}
B2-10	30.46 ± 2.31^{b}	1.68 ± 0.14^{a}	0.72 ± 0.03^{a}	21.02 ± 2.29^{a}	113.27 ± 8.24^{a}	77.37 ± 0.59^{a}	145.27 ± 8.20^{ab}	40.02 ± 3.02a	1.27 ± 0.02^{c}	6.35 ± 0.06^{c}
B3-10	35.47 ± 1.93^{a}	1.72 ± 0.10^{a}	0.72 ± 0.02^{a}	21.32 ± 2.78^{a}	110.69 ± 7.28^{a}	77.01 ± 1.87^{a}	143.23 ± 1.69^{b}	39.43 ± 1.43^{a}	1.25 ± 0.02^{d}	7.02 ± 0.08^{a}
B0-20	19.28 ± 1.07^{d}	1.60 ± 0.090^{a}	0.73 ± 0.02^{a}	20.40 ± 2.39^{a}	108.90 ± 9.60^{a}	75.76 ± 3.17^{a}	143.96 ± 7.01^{ab}	40.34 ± 2.30^{a}	1.37 ± 0.03^{a}	6.01 ± 0.05^{d}
B1-20	24.69 ± 1.32^{c}	1.58 ± 0.12^{a}	0.73 ± 0.05^{a}	20.86 ± 0.35^{a}	109.23 ± 11.63^{a}	76.78 ± 1.17^{a}	148.40 ± 6.13^{a}	40.23 ± 1.95^{a}	1.33 ± 0.02^{b}	6.19 ± 0.02^{d}
B2-20	30.42 ± 1.15^{b}	1.67 ± 0.11^{a}	0.74 ± 0.03^{a}	20.20 ± 1.74^{a}	109.47 ± 4.25^{a}	77.02 ± 2.02^{a}	146.74 ± 2.21^{ab}	40.34 ± 3.48^{a}	1.27 ± 0.01^{c}	6.34 ± 0.03^{c}
B3-20	35.20 ± 2.11^{a}	1.73 ± 0.12^{a}	0.72 ± 0.03^{a}	21.28 ± 1.94^{a}	110.20 ± 6.69^{a}	76.23 ± 1.04^{a}	147.27 ± 6.03^{ab}	40.73 ± 2.13^{a}	1.27 ± 0.02^{d}	6.73 ± 0.13^{b}

TC – the soil's total carbon; TN – the total nitrogen; TP – the total phosphorus; TK – the total potassium; AN – the available nitrogen; AP – the available phosphorus; AK – the available potassium; BD – the bulk density; the different letters indicated significant differences between the treatments using the one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) at the $P < 0.05$ significance level

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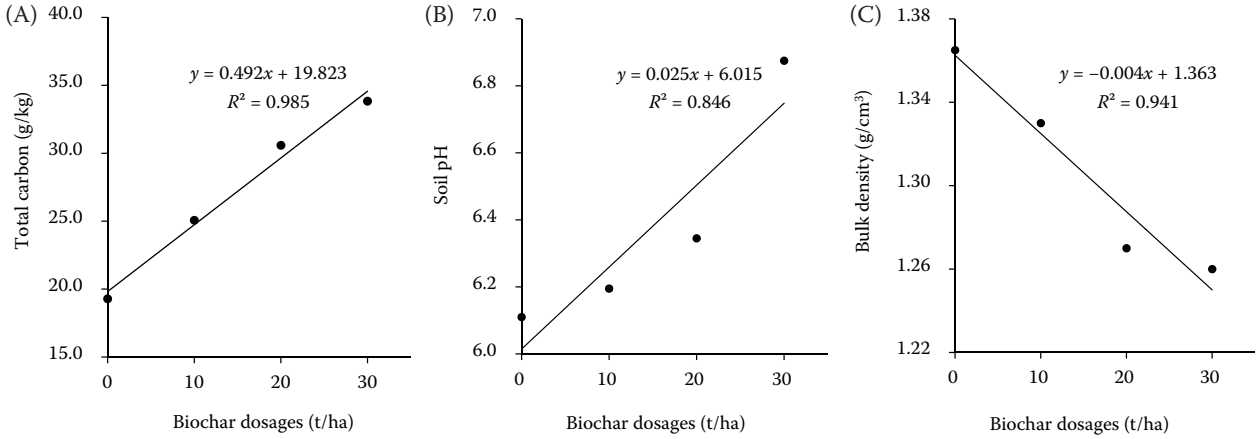

Figure 1. The relationship between the dosages of the biochar addition and the soil's total carbon (A), soil (B) and bulk density (C); the data are the means of the values in the 0–10 cm soil layer and the values in the 10–20 cm soil layer; R^2 – multiple correlation of determination

depleted earlier, and the influence of the biochar on the soil's chemical properties was negligible in the four-year experiment. Burrell *et al.* (2016) reported that the soil nutrients were nearly unchanged when the dosage of the biochar addition was as low as 5% of the weight of the soil. In addition, the soil bulk density was significantly decreased by the biochar addition, especially in the second depth soil layer (Table 1), and the soil bulk density (k = –0.004, R^2 = 0.941) had an obvious negative correlation with the amount of the biochar (Figure 1C).

The impacts of the biochar application on the relative abundance and taxonomic classification of the soil's bacterial community. In total, 1 816 864 bacterial sequences were measured through MiSeq from all of the soil samples. Of all the sample sequences, 96.34% were identified by BLAST when compared against the NCBI database. An equal analysis level of 60 432 sequences (the minimum amount of the bacterial sequences) per soil sample was randomly selected to exclude the differences caused by the different sequencing amounts.

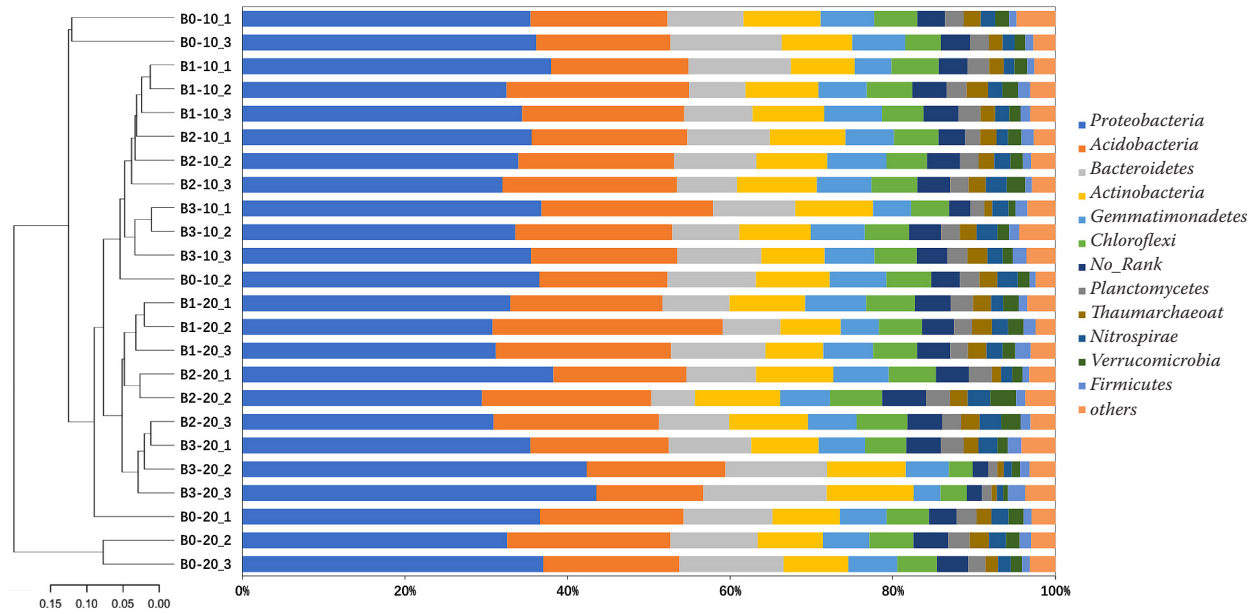

Figure 2. The phylogenetic relationships and the relative abundances of the bacterial communities across all the soil samples at the phylum level; others – the sum of the bacterial phyla of the low relative abundances (means ≤ 0.01%)

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The dominant bacterial phyla (relative abundance > 5%) were *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, *Gemmatimonadetes* and *Chloroflexi,* which accounted for 82.42% of the total sequences, their relative abundances ranged from 28.71% to 43.18%, 12.83% to 27.56%, 5.24% to 15.10%, 6.94% to 10.51%, 3.19% to 7.05% and 2.78% to 6.25%, respectively, across all the soil samples (Figure 2). Those dominant bacterial phyla were similar with those observed by Matsushita et al. (2019). Overall, the relative abundance of *Actinobacteria* increased, but *Acidobacteria* decreased with the biochar application, especially in the second depth soil layer (Figure 3A, B). Hengst & Buttner (2008) indicated that most *Actinobacteria* are obligate aerobes. Therefore, the biochar application improved the air permeability of the soil and it resulted in the increase of the *Actinobacteria* in this study.

Further taxonomic classification showed more than 60 bacterial classes were identified in most of the soil samples. Among them, the relative abundance of *Actinobacteria* increased with the biochar addition, whereas the abundance of *α-Proteobacteria*, *Acidobacteria_Gp4* and *Acidobacteria_Gp3* decreased, especially in the second depth soil layer (Figure 3C, D). Among them, *α-Proteobacteria* and *Actinobacteria* belonged to the *Proteobacteria* and *Actinobacteria* phyla, respectively. *Acidobacteria_Gp4* and *Acidobacteria_Gp3* belonged to the *Acidobacteria* phylum (Table S2 in the Electronic Supplementary Material (ESM)). Lauber et al. (2009) indicated that the soil's pH was one of the dominant factors influencing *Acidobacteria*. Therefore, the increase of the soil pH was one of the reasons for the decrease in the *Acidobacteria* in this study.

Many studies have also indicated that the soil's moisture played an important role in the changes in the bacterial abundance and community (Brockett et al. 2012). However, there was no significant difference between all the treatments in the soil moisture due to the frequent precipitation in this study. The effects

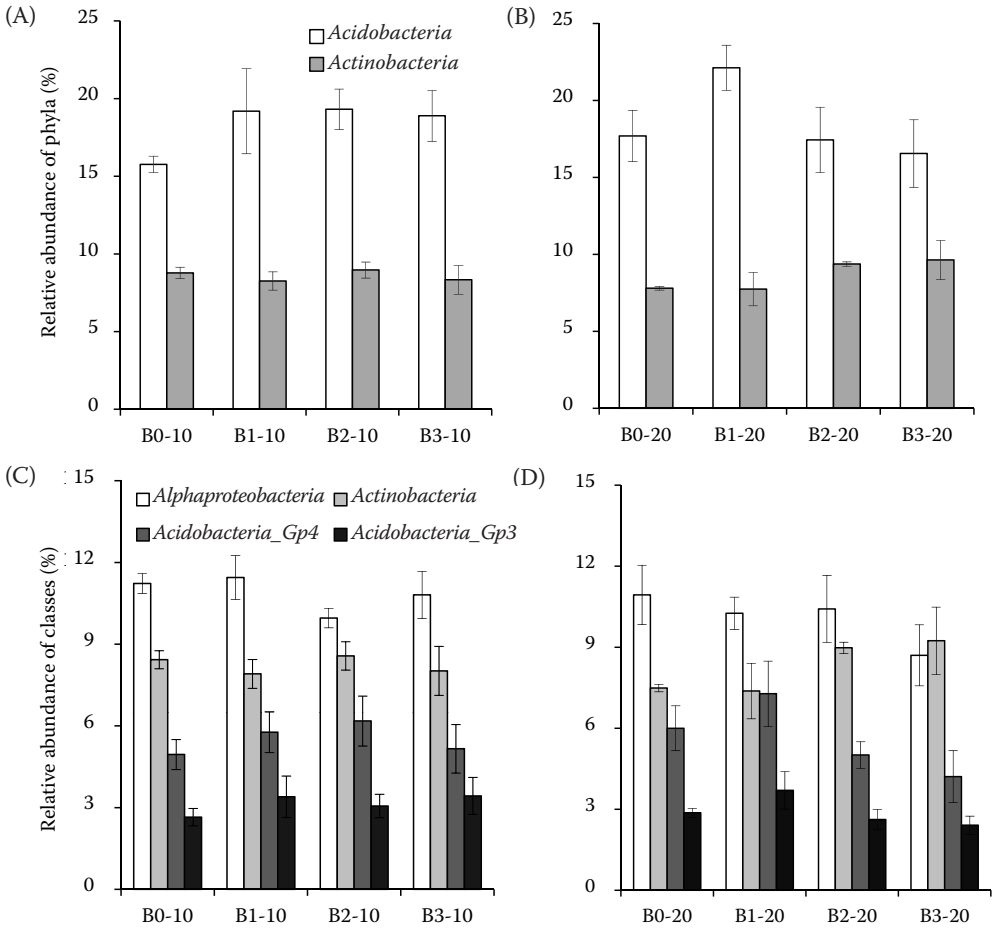

Figure 3. The changes in the relative abundances of the bacterial phyla (A, B) and classes (C, D) with the different amounts of biochar in the 0–10 cm soil layer and the 10–20 cm soil layer

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Table 2. The effect of the biochar application on the bacterial community diversity based on the 16S rRNA gene at 97% sequence similarity

Samples	Bacterial sequences	Chao1 richness	Shannon's diversity	Simpson's diversity	Coverage (%)
B0-10	2773 ± 420	3759 ± 440^{ab}	6.06 ± 0.48^{ab}	0.0091 ± 0.0068^{a}	98.50
B1-10	3076 ± 46	3996 ± 68^{ab}	6.43 ± 0.07^{a}	0.0045 ± 0.0003^{a}	98.54
B2-10	3178 ± 86	4137 ± 117^{a}	6.50 ± 0.07^{a}	0.0042 ± 0.0006^{a}	98.47
B3-10	3205 ± 115	4194 ± 189^{a}	6.46 ± 0.08^{a}	0.0043 ± 0.0005^{a}	98.46
B0-20	2669 ± 357	3603 ± 393^{b}	5.88 ± 0.63b	0.0113 ± 0.0097^{a}	98.46
B1-20	3198 ± 164	4114 ± 224^{a}	6.49 ± 0.05^{a}	0.0045 ± 0.0007^{a}	98.52
B2-20	3213 ± 87	4159 ± 86^{a}	6.52 ± 0.08^{a}	0.0042 ± 0.0005^{a}	98.44
B3-20	3258 ± 122	4201 ± 233^{a}	6.56 ± 0.03^{a}	0.0039 ± 0.0004^{a}	98.47

Coverage – Good's nonparametric coverage estimator; the data has been calculated from 60 432 bacterial sequences per soil sample; the different letters indicate significant differences between the treatments using the one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) at the $P < 0.05$ significance level

of the soil moisture on the soil bacterial abundance has not been shown in this study.

The impacts of the biochar application on the soil's bacterial community diversity. In this study, α-diversity was used to illustrate the differences in the soil's bacterial community diversity. The results are shown in Table 2. The coverage was greater than 98%, demonstrating that the depth of the sequencing is enough to evaluate the whole diversity of the bacterial communities. The one-way ANOVA showed that the Chao1 richness and Shannon diversity of the biochar addition treatments were significantly higher than those of the control treatment (B0), but there was no significant difference between the different dosages of the biochar treatment (B1, B2, B3) (Table 2). Some studies showed that the application of a biochar could influence the agricultural soil's bacterial diversity and change the community structure of the soil's bacteria (Gomez et al. 2014; Jiang et al. 2017). In contrast, Liu et al. (2016) reported that a biochar addition had no effect on the microbial community structure in paddy soil. However, in this study, our findings indicated that the community diversity of the soil's bacteria in the Albic Clayic Luvisol was affected by the biochar application.

The impacts of the biochar application on the soil's bacterial community structure. In this study, an NMDS analysis was used to examine the soil's bacterial community structure (Figure 4A). The three replicates per treatment were very similar, proving the stability and reliability of the soil's bacterial communities across all the samples. The results

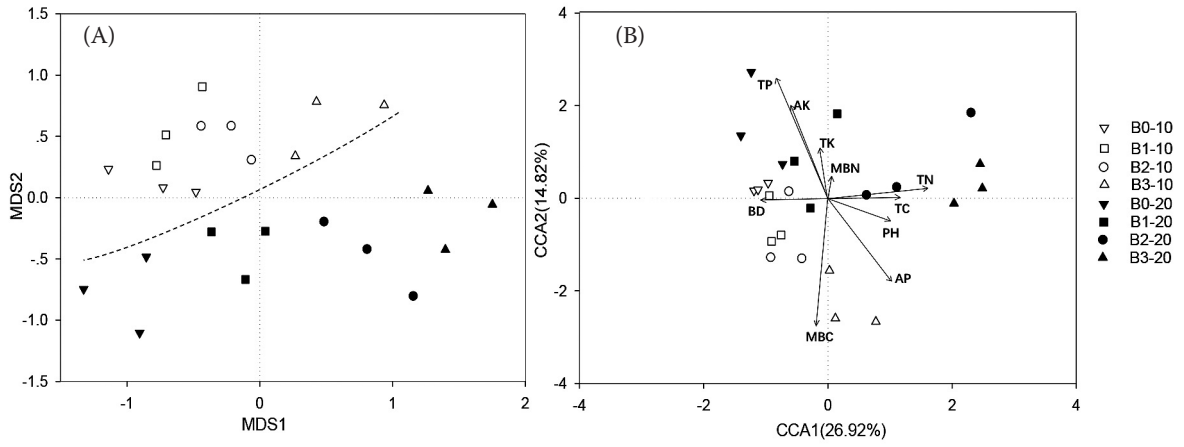

Figure 4. The nonmetric multidimensional scaling analysis (NMDS) of the soil's bacterial communities for the different biochar treatments (A) and the canonical correspondence analysis (CCA) of the bacterial community changes with the soil properties (B)

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showed that the bacterial communities for the different dosages of the biochar treatments (B1, B2, B3) were separated from each other according to the NMDS1 axis. This finding indicated that the effects of the biochar on the bacterial community structure were similar with those observed by Yao et al. (2017) in a Chernic Phaeozem (IUSS Working Group WRB 2015). The soil bacterial communities of the surface layer and the second depth layer soil treatments (0–10, 10–20 cm) were divided into two distinctly different parts according to the NMDS2 axis. These findings showed that the structure of the soil's bacterial communities was affected by the biochar application and the soil depth.

The CCA was used to estimate the correlation among all the soil samples to illustrate the relationship between the structure of the bacterial communities and the soil's physicochemical properties (Figure 4B). The CCA showed that the distribution of the bacterial communities was affected by the biochar dosages along the CCA1 axis and by soil depth along the CCA2 axis, similar to the NMDS. Among the soil properties, the soil pH, bulk density, TN and TC were near the CCA1 axis, and the soil moisture and TK were near the CCA2 axis. The Mantel test analysis indicated that the bacterial community structure significantly correlated with the soil pH (r = 0.525, P = 0.001), bulk density (r = 0.539, P = 0.001) and TC (r = 0.519, P = 0.002) only (Table S1 in ESM). Yao *et al.* (2017) reported that the soil's total C, total N, total P, available P, available K and pH were all closely associated with the distribution of the bacterial communities in the soil. Lauber et al. (2008) reported that bacterial community compositions were positively correlated with the soil's total N, pH and available K. However, our findings indicated that the soil's TC, pH and bulk density played an important role in the differences of the soil's bacterial community structure in the Albic Clayic Luvisol.

CONCLUSION

Our study demonstrated that a biochar application still had the function of improving the Albic Clayic Luvisol, the improvement effect of the B3 treatment (30 t biochar/ha) was better than that of the other treatments in this study. Four years after the biochar application, the soil bulk density had decreased, the soil had become more porous, and the soil TC and pH had increased and positively correlated with the biochar dosages. The biochar application still influenced the soil's bacterial relative abundance, and the differences came mainly from the *Acidobacteria* and *Actinobacteria* phyla. The Chao1 richness and Shannon diversity of the bacterial community showed significant positive correlations with the biochar dosages. The structure of the soil's bacterial communities was affected by the soil depth and biochar application. In short, biochar applications could improve parts of the soil properties and affect the soil's bacterial communities in Albic Clayic Luvisols.

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