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Long-term effects of biochar amendment on rhizosphere and bulk soil microbial communities in a karst region, southwest China

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ABSTRACT

Biochar (BC) addition is widely used in agriculture to condition soils. However, the effects of BC addition on soil microbial community diversity and composition in karstic regions are unclear, especially after long-term application. To address this knowledge-gap, a field experiment was established to examine changes in soil physicochemical properties and microbial communities following six years of BC amendment. BC was applied to calcareous soils in a karstic region of southwestern China at four levels (w/w): 0%, 1.0%, 5.0%, and 10%. Bacterial community composition was then investigated in both the rhizosphere and bulk soils by 16S rRNA gene sequencing on the Illumina MiSeq Platform. BC addition increased soil pH, total carbon (TC), total nitrogen (TN), and total hydrogen (TH) contents in the rhizosphere and bulk soils. In addition, BC amendment was associated with changes in soil bacterial community compositions and diversities, especially at higher BC application levels. The relative abundances of *Gemmatimonadetes* increased in rhizosphere soils with increasing BC amendment, while those of the *Bacteroidetes*, *Firmicutes*, and *Cyanobacteria* decreased. The relative abundances of *Proteobacteria* and *Chloroflexi* increased in bulk soils with increasing BC application levels, while those of the *Bacteroidetes* and *Verrucomicrobia* decreased. Canonical correspondence analysis indicated that bacterial community composition was related to soil characteristics including pH, TC, TN, and TH contents in both rhizosphere and bulk soils. Importantly, variations in these soil parameters were closely associated with BC application rates. These results indicate that long-term BC application significantly impacts soil bacterial community characteristics in karstic regions via modulation of soil physiochemical properties.

1. Introduction

Biochar (BC) is a carbon-rich material that is obtained via the anaerobic pyrolysis of biomass materials such as manure, industrial organic wastes, and agricultural wastes. The pyrolysis process results in BC with high C content, high porosity, high thermal stability, large surface areas, and high sorption characteristics (Cheng et al., 2017). Due to its unique properties, BC has been suggested to help in mitigate the process of climate change via the immediate enhancement of soil C sequestration, because BC will remain sequestered for hundreds of years (Al-Wabel et al., 2018; El-Naggar et al., 2018). Moreover, lower levels of soil nitrous oxide (N_2O) and methane (CH_4) emissions are associated with BC-amended soils (Brassard et al., 2016). In addition to its C sequestration, BC amendment can also enhance soil quality and improve crop productivity (Lehmann, 2007; Trifunovic et al., 2018). BC principally improves soil quality due to its effects on soil physicochemical properties, including increases in water and nutrient holding capacities in addition to cation exchange capacity (Chan et al., 2007; Pandit et al., 2018). Moreover, BC can improve ecosystem health by absorbing pollutants, including organic pesticides, polycyclic aromatic hydrocarbons, and heavy metals, thereby reducing their mobility and bioavailability (Chen and Yuan, 2011; Cheng et al., 2017; Cheng et al., 2018).

BC amendments also influence soil microbial communities (Anderson et al., 2011; Luo et al., 2017). For example, BC application to soils increases the relative abundances of *Bradyrhizobiaceae* and *Hyphomicrobiaceae* bacterial populations that C and N cycling activities (Anderson et al., 2011). Further, Cheng et al. (2018) demonstrated that the relative abundances of *Adhaeribacter*, *Rhodoplanes*, and *Pseudoxanthomonas* bacteria increased within soils after BC addition, while

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those of *Kaistobacter*, *Pirellula*, and *Lacibacter* decreased following amendment. However, most investigations of the effects of BC on microbial communities have been conducted only over 1–2 year time spans (Anderson et al., 2011, 2014; Khodadad et al., 2011). Recent longer-term investigations have been conducted to evaluate the effects of BC on soil microbial communities over 3–3.5 years (Luo et al., 2017; Yao et al., 2017). Long-term application of BC to soils is likely to lead to different physical, chemical, and biological effects than shorter-term applications (Yao et al., 2017). In addition, some research has indicated that longer-term BC applications are associated with higher microbial diversities and abundances (O'Neill et al., 2009). Nevertheless, the effects of long-term BC amendments on soil microbial community compositions are still poorly understood, and especially six years of application in the calcareous soil of regions with karst bedrock. Moreover, most investigations have focused on evaluating the effects of BC on microbial communities in bulk soils (Anderson et al., 2011; Yao et al., 2017). In contrast, little research attention has been directed at understanding the effects of BC application on root-associated bacterial communities, despite that they are important components of soil ecosystems (Liu et al., 2018). Such impacts could potentially influence the roles of root-associated populations in plant growth and resistance to pests and diseases (Kolton et al., 2011).

Based on the Chinese Soil Classification System, calcareous soils are Calcaric Cambisols under the FAO-UNESCO soil classification scheme. Calcareous soils are mainly distributed in areas with karst bedrock, which are prevalent in southwestern China. These soils are characterized by high organic matter contents, high pH, and high concentrations of Ca^{2+} and Mg^{2+} ions (Yang et al., 2016; Zhang et al., 2016). However, much of the calcareous soils in these karstic regions have been degraded over the past several decades from long-term intensive agricultural activities. Degrade soils are characterized by a loss of soil organic carbon (SOC) and other nutrients, including nitrogen (N), phosphorus (P), and potassium (K), in addition to increased soil erosion (Yang et al., 2016). The unique physical and chemical characteristics of BC may allow it to improve nutrient retention when applied to these degraded calcareous soils (Al-Wabel et al., 2018; Sadegh-Zadeh et al., 2018).

The aim of the present study was to investigate the effects six years of BC application on soil physicochemical characteristics and microbial community compositions in calcareous soils. Specifically, the impacts of long-term BC application on changes in bacterial community compositions were investigated for both bulk and rhizosphere soils, in addition to the associations among bacterial communities and physicochemical properties.

2. Materials and methods

2.1. Soil and BC characterizations

Field experiments were conducted at the Tiankan Village (27° 00′ N, 107° 02′ E, 1118 m) in Kaiyang County within Guizhou Province of China. The region represents a typical karstic area in southwestern China and is characterized by a subtropical monsoon climate with an annual average temperature of 10.6–15.3 °C and an annual average precipitation of 926–1419 mm. The soils of the region are calcareous, as determined by the FAO-UNESCO classification system. Soil TC and TN contents were 61.23 g kg^{-1} and 1.49 g kg^{-1}, respectively, and the C/N ratio of the soil was 41.23.

The BC used in these experiments was generated from crop straws of maize and rapeseed. In particular, air-dried stalks were pyrolyzed in a ring kiln under oxygen-limiting conditions. The materials were subjected to pyrolysis over 30 min at a temperature of 550 °C. The BC was characterized by 753.0 g kg^{-1}C, 12.3 g kg^{-1} N, 1.59 mg kg^{-1} NH_4^+-N, 4.58 mg kg^{-1} NO_3^--N, and a pH (H_2O) of 10.46. The BC was sieved through a 2 mm mesh screen and homogenized prior to application in soils.

2.2. Field experiments

The BC was applied manually and thoroughly mixed into the first 10 cm of topsoil in June 2010. BC was applied to soils at rates of 0%, 1%, 5%, and 10% (i.e., 0, 12.8, 64, and 128 t ha^{-1}). These treatments are hereafter referred to as B_0, B_1, B_5, and B_{10} for bulk soils, and R_0, R_1, R_5, and R_{10} for rhizosphere soils, respectively. Each treatment was conducted in a 2 m × 4 m plot that was separated from others by boundary rows (0.4 m in width). The annual rotation system in all of the BC treatment plots alternated between rapeseed and maize. Multi-element compound fertilizers were applied as base fertilizers to the plots at rates of 1250 kg ha^{-1} and 370 kg ha^{-1} for rapeseed and maize, respectively.

2.3. Sample collection and analyses

Soil samples were taken after harvesting on August 27, 2016, when the current crop was maize. After removing the maize plants, soil that was tightly attached to the fine roots was considered the rhizosphere soil, while the remaining soil was considered the bulk soil. Soil samples from five different locations within each plot were thoroughly mixed to generate a composite sample for each plot. Field-fresh soil samples were sieved through a 2 mm mesh to remove visible non-soil residues, including roots and stones. Sieved samples were partitioned into two subsamples where the first was used for community DNA extraction and the other was used to analyze physicochemical characteristics.

Soil pH was measured on a pH solution of soil deionized water that was mixed at a ratio of 1:2.5 (w/v). TC, TN, total hydrogen (TH) contents and the C:N ratios (C/N) were determined using an elemental analyzer (vario MACRO Analyzer, Elementar, Analysensysteme, GmbH, Germany) (Cheng et al., 2018).

2.4. Soil DNA extraction and purification

Total DNA was extracted from the bulk and rhizosphere soils using a PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. DNA concentrations and purities were evaluated using 1% gel electrophoresis. Diluted DNA was then immediately stored at −20 °C until further analysis.

2.5. Illumina sequencing and sequence data processing

The V3–V4 hypervariable regions of bacterial 16S rRNA genes were amplified using the bacterial-specific universal primers 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′). PCRs were conducted in total reaction volumes of 30 μl that contained Phusion® High-Fidelity PCR Master Mix (15 μl), each primer (0.2 μM final concentration), and DNA templates (~10 ng). Thermal cycling conditions were as follows: 1 min of denaturation at 98 °C followed by 30 cycles at 98 °C for 10 s, annealing at 50 °C for 30 s, elongation at 72 °C for 1 min, and final extension for 5 min at 72 °C. PCR product was evaluated by agarose gel electrophoresis with 2% agarose gels. Successful PCR reactions for each sample were then mixed in equimolar ratios. The pooled PCR products were then purified using a Qiagen Gel Extraction Kit (Qiagen, Hilden, Germany). Illumina sequencing libraries were prepared from the purified PCR products using the TruSeq® DNA PCR-Free Sample Preparation Kit using the manufacturer's protocols, followed by the addition of barcoded index adapters. Library quality was checked using a Qubit®2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and an Agilent Bioanalyzer 2100 system (Agilent, Santa Clara CA, USA). The sequence library was then were sequenced on an Illumina MiSeq system (Illumina, San Diego, CA, USA). Paired-end sequence reads were assigned to samples using the unique barcodes and then joined using FLASH (v. 1.2.7). The joined contig sequences were considered the raw sequence tags.

Quality filtering of raw tags was used to obtain high-quality clean tags using the QIIME pipeline (v.1.7.0). Chimeric sequences were detected and using the UCHIME algorithm and removed from the dataset. The Uparse software package (v.7.0.1001) was then used to cluster sequences into operational taxonomic units (OTUs) at the 97% sequence similarity threshold. Representatives from each OTU were then taxonomically annotated using the Greengenes reference database and the RDP classifier algorithm (v.2.2). The phylogenetic relationships among OTUs were evaluated by subjecting the OTU representatives to multiple sequence alignment using the MUSCLE program (v.3.8.31) (Edgar, 2004). Lastly, sequences within each sample library were subsampled to normalize for sequencing effects and use in alpha and beta diversity analysis. Sequence data were deposited into the European Nucleotide Archive (ENA) (http://www.ebi.ac.uk/ena) and are accessible under the accession number PRJEB28279.

2.6. Data analysis

Relationship among soil bacterial diversity, taxonomic abundance and chemical properties were evaluated using the SPSS 13.0 software program. The distributions of all parameters were compared using a one-way analysis of variance (ANOVA) with a least significant difference (LSD) test. Alpha and beta diversity were measured in QIIME and visualized using the R software package (v.2.15.3). Several alpha diversity metrics including Chao1, ACE, Shannon, and Simpson indices were used to determine the species diversity within samples. Beta diversity among samples was calculated using both weighted and unweighted UniFrac distances. Non-metric multidimensional scaling (NMDS) was subsequently used to evaluate the differences in OTU compositions among samples. In addition, canonical correspondence analysis (CCA) was conducted to explore the relationships between bacterial community compositions and environmental factors. Lastly, correlational and regression analyses were used to investigate the relationships between soil physicochemical parameters and individual taxonomic phyla, community diversity indices, or community compositional indices. Statistical significance was considered at the $p \leq 0.05$ level.

3. Results

3.1. Chemical properties of bulk and rhizosphere soils

Among the different treatments, the B_{10} group exhibited the highest average TN, TC, and TH contents. Specifically, the TN, TC, and TH contents in the B_{10} treatment were approximately 106%, 83%, and 60% higher than those of the B_0 treatment, respectively. Likewise, the R_{10} exhibited the highest average TN, TC, and TH contents, which were approximately 82%, 85%, and 53% higher than those of the R_0 treatment, respectively (Table 1). No statistically significant differences were observed in C/N ratios between BC treatments either in bulk or rhizosphere soils ($p > 0.05$). In contrast, soil pH, TC, TH, and C/N ratios were significantly different between bulk soil and rhizosphere soils ($p \leq 0.05$). Specifically, pH, TC, and C/N values were all significantly higher in the bulk soils than in the rhizosphere soils. Further, BC application rates and soil type interaction effects also significantly affected soil chemical properties, with the exception of soil TH.

3.2. Richness and diversity of bacterial communities

Subsampling of bacterial community 16S rRNA gene sequence reads resulted in 18, 970 sequences within each sample. Good's coverage estimates exceeded 95% for all samples indicating that the sequencing depth used in these analyses was sufficient to capture the bacterial diversity present within the soils (Table 2). Microbial community diversity was higher in the 5% and 10% BC amendment treatments, as measured by comparison of observed OTUs and phylogenetic diversity (PD) against the communities from the 1% BC treatment. In contrast, no statistically significant differences were observed for these indices when comparing the 0% and 1% BC amendments. Soil type was significantly associated with bacterial community diversity differences based on Shannon and Simpson diversity indices. However, BC addition did not significantly affect the bacterial diversity indices in either the bulk or rhizosphere soils. In addition, BC-soil interactions were significantly associated with variation in the Simpson diversity index.

3.3. Predominant bacterial taxa

The dominant bacterial phyla (> 1% relative abundance) of the soil samples were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Bacteroidetes*, *Firmicutes*, *Nitrospirae*, *Chloroflexi*, *Latescibacteria* and *Verrucomicrobia*. These phyla comprised > 98% of the bacterial communities in both the bulk and rhizosphere soils (Fig. 1). Among these, the *Proteobacteria* were particularly dominant among all treatments, varying from 40.83% to 49.74% relative abundance in the bulk soils and from 45.49% to 56.70% in rhizosphere soils (Fig. 1). *Proteobacteria* and *Chloroflexi* were especially abundant in bulk soils of BC treatments, and particularly in the B_{10} treatment. In contrast, the community of the B_0 treatment had significantly higher relative abundances of *Bacteroidetes* and *Verrucomicrobia* ($p \leq 0.05$). However, the relative abundances of *Acidobacteria*, *Actinobacteria*, and *Latescibacteria* were not significantly different among BC application treatments (Fig. 1a). Increasing application rates of BC resulted in increased relative abundances of *Gemmatimonadetes* and *JL-ETNP-Z39* in the rhizosphere soils, while those of *Bacteroidetes*, *Firmicutes*, and *Cyanobacteria* decreased (Fig. 1b; Table S1). The *Acidobacteria* and *Gemmatimonadetes* had significantly higher relative abundances in the R_{10} treatment compared to the R_0 treatment ($p \leq 0.05$), while the *Bacteroidetes*, *Nitrospirae*, and *Firmicutes* had significantly lower abundances ($p \leq 0.05$). No significant differences were observed in the relative abundances of *Actinobacteria*, *Chloroflexi*, and *Verrucomicrobia* among treatments (Fig. 1b).

3.4. Genus-level taxonomic compositions

The most abundant (> 1%) bacterial genera in BC-treated bulk soils were *Sphingomonas*, *Gaiella*, *Bacillus*, *Haliangium*, *Candidatus Alysiosphaera*, *Alysiosphaera*, unidentified *Nitrospiraceae*, *Bryobacter*, and *Blastocatella* (Fig. 2a; Table S2). Of these, the relative abundances of *Sphingomonas* increased with increasing BC application levels, wherein *Sphingomonas* were significantly ($p \leq 0.05$) more abundant in soils within the B_{10} treatment relative to those of the B_0 and B_1 treatments. In contrast, *Haliangium* abundances were higher in the B_1 treatment compared to those of the B_0 treatment, but thereafter decreased in abundance with higher BC amendments. Specifically, *Haliangium* was significantly ($p \leq 0.05$) less abundant in the B_5 and B_{10} treatments compared to B_0 and B_1 treatments (Fig. 2a).

Bacterial communities in the rhizosphere soils was primarily composed of *Sphingomonas* (9.00% average relative abundance), unidentified *Nitrospiraceae* (1.32%), *Gaiella* (1.22%), *Blastocatella* (1.19%), *Lysobacter* (1.15%), *Bacillus* (1.02%), *Haliangium* (0.92%), and *Candidatus Alysiosphaera* (0.89%) (Fig. 2b). Among these, *Sphingomonas* (within the *Proteobacteria* phylum) was highly prevalent with relative abundances ranging from 5.65% to 12.21% across all soil samples. *Blastocatella* relative abundances decreased in R_5 treatment compared to R_0 treatment, but they then increased in the R_{10} treatment. In contrast, *Gaiella* relative abundances increased in the R_5 treatment relative to those of the R_0 treatment, and then decreased in the R_{10} treatment (Fig. 2b). In addition, the relative abundances of *Bacillus*, *Haliangium*, and *Candidatus Alysiosphaera* increased in the R_1 treatment compared to the R_0 treatment, but then decreased in the R_{10} treatment. In particular, *Haliangium* and *Candidatus Alysiosphaera* were significantly ($p \leq 0.05$) more abundant in the R_1 treatment relative to those of the R_0 treatment.

Table 1
Chemical properties of bulk and rhizosphere soils amended with different BC application rates.

Types	BC	pH	TN ($g\,kg^{-1}$)	TC ($g\,kg^{-1}$)	TH ($g\,kg^{-1}$)	C/N
Bulk soil	B_0	7.90 ± 0.04	1.49 ± 0.08	61.23 ± 3.27	3.06 ± 0.12	41.23 ± 2.81
	B_1	7.93 ± 0.01	1.72 ± 0.09	68.99 ± 1.43	3.27 ± 0.15	40.17 ± 2.81
	B_5	7.99 ± 0.02	2.09 ± 0.08	90.75 ± 1.37	3.51 ± 0.09	43.55 ± 2.17
	B_{10}	8.01 ± 0.03	3.07 ± 0.14	112.29 ± 2.00	4.90 ± 0.23	36.61 ± 1.56
Rhizosphere soil	R_0	7.59 ± 0.05	1.58 ± 0.12	60.63 ± 1.76	3.69 ± 0.10	38.47 ± 3.47
	R_1	7.68 ± 0.06	1.74 ± 0.04	67.59 ± 3.42	3.95 ± 0.20	38.86 ± 2.47
	R_5	7.83 ± 0.02	2.27 ± 0.11	84.76 ± 1.25	4.47 ± 0.24	37.37 ± 2.37
	R_{10}	7.85 ± 0.02	2.87 ± 0.20	112.41 ± 1.29	5.65 ± 0.41	39.28 ± 2.57
			BC effects (B)			
0%		7.75^{c}	1.54^{d}	60.93^{d}	3.37^{d}	39.85^{ns}
1%		7.80^{b}	1.73^{c}	68.29^{c}	3.61^{c}	39.51^{ns}
5%		7.91^{a}	2.18^{b}	87.85^{b}	3.99^{b}	40.46^{ns}
10%		7.93^{a}	2.97^{a}	112.35^{a}	5.28^{a}	37.95^{ns}
			Soil types (S)			
Bulk soil		7.96^{a}	2.09^{ns}	83.31^{a}	3.68^{b}	40.39^{a}
Rhizosphere soil		7.74^{b}	2.12^{ns}	81.34^{b}	4.44^{a}	38.50^{b}
			Significance			
BC effects (B)		**	**	**	**	ns
Soil types (S)		**	ns	*	**	*
B × S		**	*	*	ns	*

Values are mean ± standard deviation. The abbreviations B_0, B_1, B_5, and B_{10} represent bulk soils without BC amendment, with 1%, 5%, and 10% BC amendment, respectively. R_0, R_1, R_5, and R_{10} represent rhizosphere soils without BC amendment, with 1%, 5%, and 10% BC amendment, respectively. Columns with different lower case letters are significantly different. *$p \le 0.05$; **$p \le 0.01$; ns, not significant.

Lastly, the relative abundances of *Lysobacter* (within the *Proteobacteria* phylum) decreased with increasing BC application levels, as evinced by significantly higher abundances in the R_0 treatment than in those of the R_{10} treatment ($p \le 0.05$; Fig. 2b).

3.5. Soil physicochemical factors affecting bacterial communities

Soil pH was positively related to variation in observed OTUs, Good's coverage estimates, and α-diversity metrics (i.e., Shannon, Simpson, Chao1, and ACE indices). TC, TN, TH contents in addition to the C/N ratio were positively correlated to variation in observed OTUs, Chao1, and ACE diversity estimators, and negatively correlated with Good's coverage estimates. Observed OTUs were significantly and positively correlated with soil pH and TC content ($p \le 0.05$). In addition, the Shannon and Simpson diversity indices were significantly and positively correlated with soil pH and significantly and negatively correlated with soil TH content. No significant correlations were observed between Chao1 and ACE richness indices and soil physicochemical parameters (Table 3).

The abundances of bacterial phyla exhibited variable relationships with physicochemical factors (Fig. 3). For example, the abundances of *Proteobacteria* and *Saccharibacteria* were significantly and positively correlated with soil TH content and extremely significantly and negatively correlated with soil pH ($p \le 0.01$). In contrast, the abundances of *Actinobacteria* and *Latescibacteria* were significantly and positively correlated with soil pH, but significantly and negatively correlated with

Table 2
Effect of BC applications on the richness and diversity of bacterial community in bulk and rhizosphere soils.

Types	BC	Observed OTUs	Shannon	Simpson	Chao1	ACE	Goods coverage	PD whole tree
Bulk soil	B_0	2376 ± 36	9.86 ± 0.06	0.998 ± 0.001	3037 ± 162	3089 ± 154	0.9612 ± 0.0039	198 ± 3
	B_1	2227 ± 191	9.74 ± 0.10	0.997 ± 0.001	2754 ± 489	2775 ± 464	0.9682 ± 0.0121	188 ± 14
	B_5	2470 ± 36	9.88 ± 0.01	0.998 ± 0.000	3223 ± 181	3267 ± 180	0.9578 ± 0.0038	209 ± 4
	B_{10}	2398 ± 147	9.86 ± 0.09	0.998 ± 0.001	2948 ± 297	3015 ± 321	0.9638 ± 0.0067	204 ± 15
Rhizosphere soil	R_0	2324 ± 92	9.51 ± 0.07	0.995 ± 0.001	3074 ± 198	3078 ± 166	0.9600 ± 0.0032	196 ± 8
	R_1	2263 ± 76	9.52 ± 0.11	0.996 ± 0.001	2932 ± 268	2977 ± 234	0.9622 ± 0.0051	189 ± 5
	R_5	2353 ± 103	9.46 ± 0.12	0.994 ± 0.001	3034 ± 343	3088 ± 284	0.9602 ± 0.0060	196 ± 6
	R_{10}	2415 ± 23	9.60 ± 0.04	0.995 ± 0.001	3252 ± 104	3289 ± 76	0.9562 ± 0.0017	202 ± 3
				BC effects (B)				
0%		2350^{ab}	9.69^{ab}	0.9964^{ns}	3056^{ns}	3083^{ns}	0.9606^{ns}	197^{ab}
1%		2245^{b}	9.63^{b}	0.9964^{ns}	2843^{ns}	2876^{ns}	0.9652^{ns}	189^{b}
5%		2411^{a}	9.67^{ab}	0.9958^{ns}	3128^{ns}	3177^{ns}	0.9590^{ns}	203^{a}
10%		2406^{a}	9.73^{a}	0.9964^{ns}	3100^{ns}	3152^{ns}	0.9600^{ns}	203^{a}
				Soil types (S)				
Bulk soil		2367^{ns}	9.84^{a}	0.9976^{a}	2990^{ns}	3036^{ns}	0.9627^{ns}	200^{ns}
Rhizosphere soil		2338^{ns}	9.52^{b}	0.9948^{b}	3073^{ns}	3108^{ns}	0.9597^{ns}	196^{ns}
				Significance				
BC effects (B)		*	ns	ns	ns	ns	ns	**
Soil types (S)		ns	**	**	ns	ns	ns	ns
B × S		ns	ns	*	ns	ns	ns	ns

Values are means ± standard deviation. Values in the same column followed by different lowercase letters indicate a significant difference. *$p \le 0.05$; **$p \le 0.01$; ns, not significant.

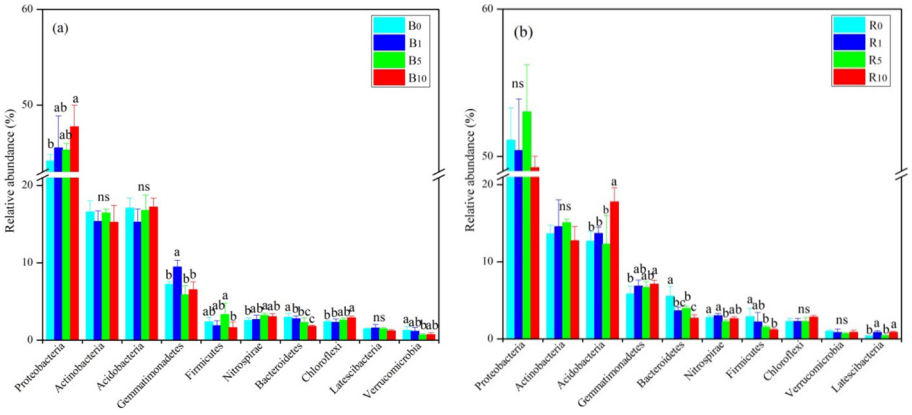

Fig. 1. Relative abundances of main bacterial communities at the phyla levels in (a) bulk and (b) rhizosphere soils following different BC application rates. Values are mean ± standard deviation. Different letters indicate significant differences at $p \leq 0.05$.

soil TH content ($p \leq 0.05$). The relative abundances of *Firmicutes*, *Verrucomicrobia*, and *Cyanobacteria* were also significantly and negatively related to soil TC, TN, and TH contents. *Chlamydiae* abundances were significantly and negatively correlated with soil TH content, and extremely significantly and positively correlated with soil pH and C/N ratios ($p \leq 0.01$). *Bacteroidetes* abundances were significantly and negatively correlated with soil pH and TC and TN contents, while the abundances of *GOUTA4* were significantly and positively correlated with these variables. In addition, the abundances of *Chloroflexi* and *JL.ETNP.Z239* were significantly and positively correlated with soil pH, TC and TN contents. *Hydrogenedentes* and *Deinococcus Thermus* abundances were also significantly and positively correlated with soil TC and TN contents ($p \leq 0.05$).

3.6. Bacterial community composition

NMDS analysis indicated that the replicate samples for each treatment, exhibited generally similar community compositions, suggesting a high level of reproducibility (Fig. 4). The bacterial communities from bulk soils were clearly distinguished from those in the rhizosphere soils, as indicated by separation along the first NMDS component. Likewise, the bacterial communities from soils with different BC application rates were clearly separated from each other along the second NMDS component. Thus, these results indicated that the soil bacterial community compositions were influenced by both soil types and BC amendment levels.

Table 3
Correlation analysis of diversity indices and soil properties.

Factors	Observed OTUs	Shannon	Simpson	Chao1	ACE	Goods coverage
pH	0.365*	0.799**	0.730**	0.063	0.095	0.073
C	0.418*	0.172	0.027	0.259	0.326	−0.253
N	0.312	0.017	−0.096	0.209	0.247	−0.197
H	0.127	−0.354*	−0.481**	0.130	0.159	−0.188
C/N	0.262	0.302	0.283	0.113	0.168	−0.127

Significant values are shown as *$p \leq 0.05$; ** $p \leq 0.01$.

To evaluate the influence of major environmental variables on bacterial community compositions, the data were subjected to canonical correlation analysis (CCA) (Fig. 5). The first two CCA axes explained 62.85% and 19.78% of the total variation in community composition, respectively. The bacterial communities varied according to soil types and BC amendment levels along the CCA1 and CCA2 axes, respectively, which was consistent with the NMDS analyses. Soil pH and C/N ratio were highly associated with the CCA1 axis, indicating that these two factors were associated with soil type differences and significantly affected bacterial community compositions. In addition, TC, TN, and TH were highly associated with the CCA2 axis and influenced bacterial community compositions.

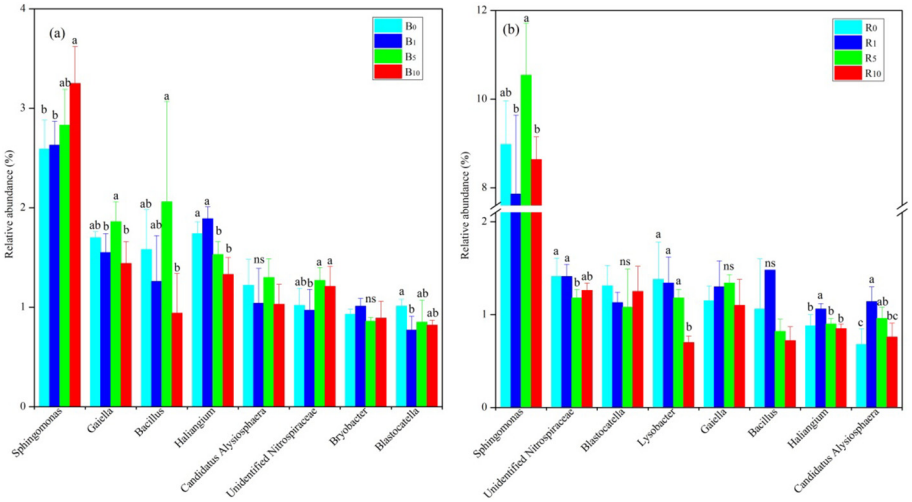

Fig. 2. Relative abundances of main bacterial communities at the genus levels in (a) bulk and (b) rhizosphere soils following different BC application rates. Different letters show significant differences at $p \leq 0.05$.

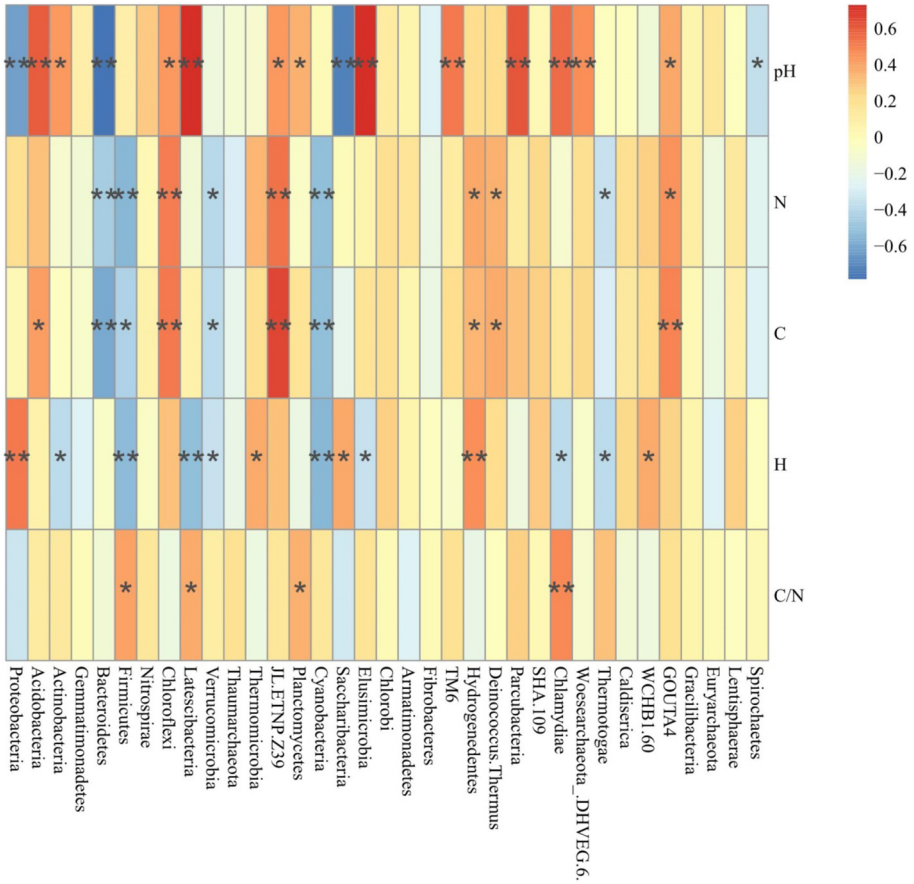

Fig. 3. Correlation heat map of the phyla and soil properties. X- and Y-axes are phyla and environmental factors, respectively. Colors are used to show the correlation coefficient, and significant values are shown as: *p ≤ 0.05; **p ≤ 0.01.

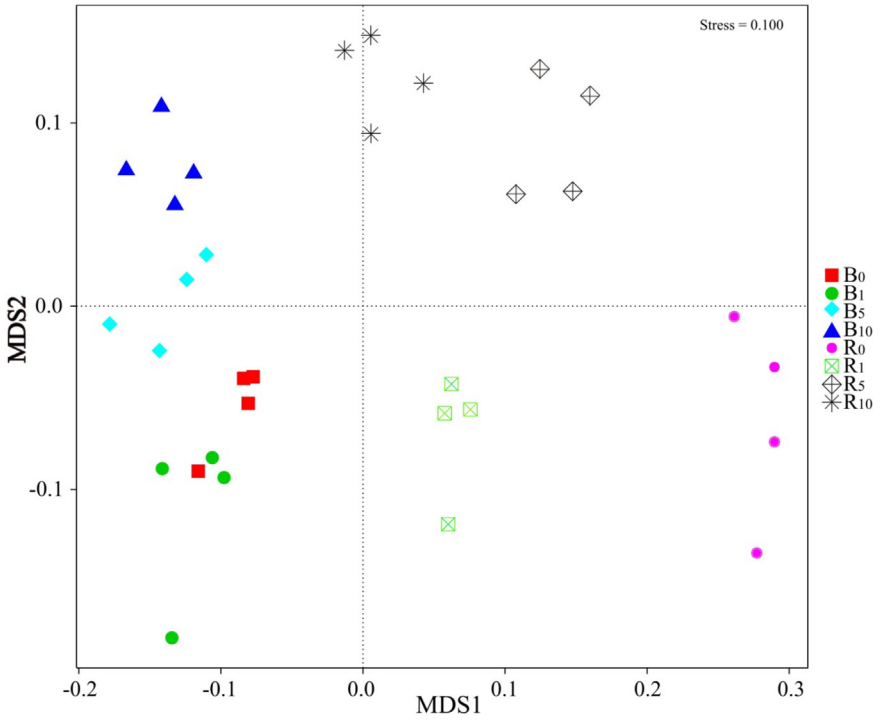

Fig. 4. Nonmetric multidimensional scaling of bacterial communities in bulk and rhizosphere soils amended with different BC application rates.

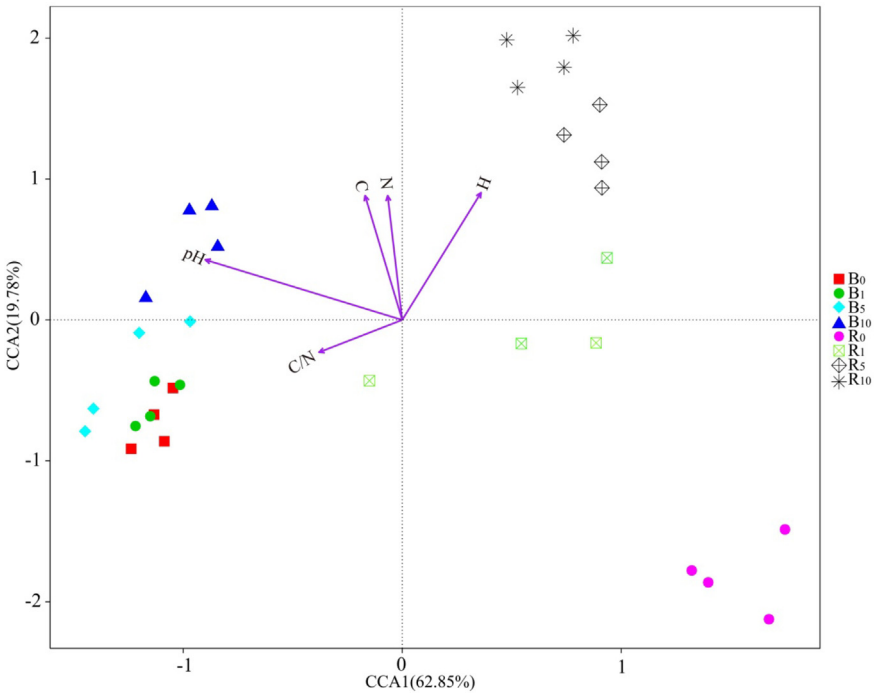

Fig. 5. Canonical correspondence analysis of bacterial community changes at the genus level with environmental factors under different BC amendment levels in both bulk and rhizosphere soils.

4. Discussion

4.1. Effects of BC amendment on soil properties and bacterial communities

The amendment of BC to soils influenced several soil parameters, including pH, and the TC and TN contents (Table 1). Most of the soil physicochemical parameters that varied due to BC amendment were also significantly correlated to variation in bacterial community composition. Among these, soil pH explained the largest fraction of variation in soil bacterial community composition and diversity (Fig. 5). These results are consistent with those of Cao et al. (2016), who also observed that variation in soil pH, SOC, and TN contents were significantly and positively correlated with microbial community variation (Cao et al., 2016). Moreover, others have observed that BC-induced alteration of soil physicochemical properties favored bacterial growth (Anderson et al., 2011; Lehmann et al., 2011). Taken together, these results suggest that BC amendment introduces soil nutrients, improves soil physicochemical properties, and stimulates soil bacteria activities. These trends ultimately lead to shifts in the bacterial community composition as a consequence of BC amendment.

Soil bacteria can generally be considered to exhibit either oligotrophs or copiotrophs functions, based on their activities with respect to nutrient availabilities (Fierer et al., 2007). Oligotrophs like *Acidobacteria* exhibit high abundances in low-nutrient environments, while copiotrophs like *Bacteroidetes* have high nutritional requirements and exhibit higher growth rates in nutrient-rich conditions (Sun et al., 2013). Therefore, BC addition could result in decreases of *Acidobacteria* relative abundances and concomitant increases in *Bacteroidetes* abundances in acidic and nutrient-poor environments. These effects can be further attributed directly to BC amendment, since BC is alkaline and has considerable abundances of mineralizable nutrients (Dai et al., 2016). Significant changes in the relative abundances of *Acidobacteria* were not observed in the calcareous soils of this study following long-term BC amendments. However, *Bacteroidetes* relative abundances significantly decreased. Similar bacterial community compositional changes were documented in other BC-amended soils (Ding et al., 2013; Jenkins et al., 2017). Thus, these effects could be due to initially high soil pH values and high nutrient levels in calcareous soils of karstic regions. Long-term BC additions did not significantly affect the C/N rations either the bulk or rhizosphere soils of these environments (Table 1). Future studies should investigate the impact of BC amendment in soils exhibiting gradients of nutrients and pH values.

4.2. Effect of root activities on bacterial community composition and function

Rhizospheric soil ecosystems are generally strongly influenced by composition of root exudates (Broeckling et al., 2008), wherein variation of root exudates can significantly alter soil microbial communities. Several rhizosphere-associated genera (e.g., *Sphingomonas*, *Blastocatella*, *Lysobacter*, and *Chitinophaga*) were in much higher abundances in rhizosphere communities than in bulk soils in this study, regardless of BC application levels (Table S2). *Sphingomonas*, *Pseudoxanthomonas*, and *Rhodanobacter* have all been shown to degrade aromatic hydrocarbons or pesticides in soil environments (Kim et al., 2004; Ding et al., 2012). In addition, *Flavobacterium* were more prevalent in root-associated soils, which could be possibly due to their ability to consume root metabolites and their fast growth rates in copiotrophic environments (Li et al., 2014). *Burkholderia* can also consume root metabolites, degrade aromatic substances, and produce antimicrobial compounds (Coenye and Vandamme, 2003; Goldfarb et al., 2011). Thus, the prevalence of *Burkholderia* in rhizosphere soils analyzed here agrees with previous reports (Li et al., 2014; Yang et al., 2017). Plant root exudates can stimulate soil microbial communities (Bais et al., 2006; Shi et al., 2012), but also alter soil physicochemical characteristics, including soil pH, water content, nutrient availability, and particle aggregation (Hinsinger et al., 2009). All of these changes could alter the soil habitats used by microorganisms, thereby modulating community compositions.

4.3. Effect of BC amendment on bacterial community composition and function

Numerous studies have suggested that BC amendment alters soil physicochemical properties and these changes subsequently alter

bacterial community compositions (Lehmann et al., 2011; Kelly et al., 2015). For example, some studies have indicated that BC application reduces the relative abundances of *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, and *Firmicutes* (Kolton et al., 2011; Ding et al., 2013; Hu et al., 2014; Wu et al., 2016). In contrast, others have shown that BC amendment enhances the relative abundances of *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Nitrospiraceae*, *Gemmatimonadetes*, *Chloroflexi*, and *Firmicutes* (Anderson et al., 2011; Khodadad et al., 2011; Kolton et al., 2011; Ding et al., 2013; Hu et al., 2014). In the present study, BC amendment resulted in increases the relative abundances of *Proteobacteria* and *Chloroflexi*, but decreased *Bacteroidetes* and *Verrucomicrobia* in bulk soils. Increasing BC application levels also lead to increases in the abundances of *Gemmatimonadetes* and *JL-ETNP-Z39* in rhizosphere soils, while those of *Bacteroidetes*, *Firmicutes*, and *Cyanobacteria* decreased (Fig. 2). Despite the number of studies that have investigated the impact of BC on soil bacterial community composition, universal or unidirectional patterns of BC influences on soil conditions have not been observed, for instance due to application rates, soil types, or other environmental conditions (Muhammad et al., 2014; Sun et al., 2016).

Proteobacteria were significantly enriched in the high BC amendment-soil communities and especially in bulk soils compared to their abundances in non- amended soils (Fig. 1). These results coincide with those of Xu et al. (2017). Members of *Proteobacteria* play important roles in the metabolism of chemically diverse C compounds (Makhalanyane et al., 2015), and are thus important contributors to C cycling. *Pedomicrobium* also exhibit critical C cycling functions in soils due to their metabolism of hydrocarbon pollutants (Orcutt et al., 2011). BC amendments resulted in significantly higher *Pedomicrobium* abundances in both bulk and rhizosphere soils analyzed here. Thus, BC amendment could significantly enhance the prevalence of some microorganisms that are important in C cycling and that are beneficial for plant growth. In contrast, *Firmicutes* abundances significantly decreased in the 10% BC-treated soils when compared to untreated soils. These results are potentially consistent with those of Mueller et al. (2015), wherein *Firmicutes* were observed to produce abundant spores and exhibit relatively rapid growth rates in oligotrophic environments. In addition, *Lysobacter* relative abundances significantly decreased in BC-amended soils, which could be due to their higher efficiency of nutrient use in nutrient-poor environments (Hayward et al., 2010). Likewise, *Bradyrhizobium* relative abundances decreased significantly in BC-amend soils, and especially in the rhizosphere soils (Table S2), which is consistent with the results of other studies (Khan et al., 2014; Yao et al., 2017). *Bradyrhizobium* are considered the predominant microbial taxa that fix N_2 into bioavailable forms and perform denitrification (Anderson et al., 2011). The lower abundance of *Bradyrhizobium* in BC-amended soils could be associated with the lowered utilization of NH_4^+ or NO_3^- as N sources in these soils (Khan et al., 2014). Thus, BC addition also significantly effects on N cycling functions in soil bacterial communities.

5. Conclusions

The composition and diversity of rhizosphere and bulk soil microbial communities differed in our analysis of calcareous soils from a karstic region in southwestern China. However, BC application also significantly influenced bacterial community diversity and composition in both soil types. BC application over six years resulted in the significantly increased relative abundances of *Gemmatimonadetes* in rhizosphere soils, while significantly decreasing the relative abundances of *Bacteroidetes*, *Firmicutes*, and *Cyanobacteria* in those soils. In addition, *Proteobacteria* and *Chloroflexi* relative abundances increased in bulk soils, while *Bacteroidetes* and *Verrucomicrobia* relative abundances decreased with increasing BC addition. Importantly, BC application influenced the relative abundances of some important C and N cycling bacterial taxa within the *Firmicutes*, *Pedomicrobium*, and *Bradyrhizobium* groups. Consequently, BC addition may lead to important long-term effects in soil C and N cycling. CCA analysis indicated that alteration of soil bacterial community composition was closely associated with changes in soil physicochemical properties like pH, TC, TN, and TH. The changes in all of these parameters were highly associated with BC application. Overall, the present study provides evidence that long-term BC application can indirectly affect soil bacterial community composition and diversity in karst regions by altering soil physical and chemical characteristics.

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References

Al-Wabel, M.I., Hussain, Q., Usman, A.R.A., Ahmad, M., Abduljabbar, A., Sallam, A.S., Ok, Y.S., 2018. Impact of biochar properties on soil conditions and agricultural sustainability: a review. Land Degrad. Dev. 29, 2124–2161.
Anderson, C.R., Condron, L.M., Clough, T.J., Fiers, M., Stewart, A., Hill, R.A., Sherlock, R.R., 2011. Biochar induced soil microbial community change: implications for biogeochemical cycling of carbon, nitrogen and phosphorus. Pedobiologia 54, 309–320.
Anderson, C.R., Hamonts, K., Clough, T.J., Condron, L.M., 2014. Biochar does not affect soil N-transformations or microbial community structure under ruminant urine patches but does alter relative proportions of nitrogen cycling bacteria. Agric. Ecosyst. Environ. 191, 63–72.
Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M., 2006. The role of root exudates in rhizosphere interations with plants and other organisms. Annu. Rev. Plant Biol. 57, 233–266.
Brassard, P., Godbout, S., Raghavan, V., 2016. Soil biochar amendment as a climate change mitigation tool: key parameters and mechanisms involved. J. Environ. Manag. 181, 484–497.
Broeckling, C.D., Broz, A.K., Bergelson, J., Manter, D.K., Vivanco, J.M., 2008. Root exudates regulate soil fungal community composition and diversty. Appl. Environ. Microbiol. 74, 738–744.
Cao, H.C., Chen, R.R., Wang, L.B., Jiang, L.L., Yang, F., Zheng, S.X., Wang, G.J., Lin, X.G., 2016. Soil pH, total phosphorus, climate and distance are the major factors influencing microbial activity at a regional spatial scale. Sci. Rep. 6, 25815.
Chan, K.Y., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2007. Agronomic values of greenwaste biochar as a soil amendment. Aust. J. Soil Res. 45, 629–634.
Chen, B.L., Yuan, M.X., 2011. Enhanced sorption of polycyclic aromatic hydrocarbons by soil amended with biochar. J. Soils Sediments 11, 62–71.
Cheng, J.Z., Lee, X.Q., Gao, W.C., Chen, Y., Pan, W.J., Tang, Y., 2017. Effect of biochar on the bioavailability of difenoconazole and microbial community composition in a pesticide-contaminated soil. Appl. Soil Ecol. 121, 185–192.
Cheng, J.Z., Li, Y.L., Gao, W.C., Chen, Y., Pan, W.J., Lee, X.Q., Tang, Y., 2018. Effects of biochar on Cd and Pb mobility and microbial community composition in a calcareous soil planted with tobacco. Biol. Fertil. Soils 54, 373–383.
Coenye, T., Vandamme, P., 2003. Diversity and significance of Burkholderia species occupying diverse ecological niches. Environ. Microbiol. 5, 719–729.
Dai, Z.M., Hu, J.J., Xu, X.K., Zhang, L.J., Brookes, P.C., He, Y., Xu, J.M., 2016. Sensitive responders among bacterial and fungal microbiome to pyrogenic organic matter (biochar) addition differed greatly between rhizosphere and bulk soils. Sci. Rep. 6, 36101.
Ding, G.C., Heuer, H., Smalla, K., 2012. Dynamics of bacterial cornmunities in two unpolluted soils after spiking with phenanthrene: soil type specific and common responders. Front. Microbiol. 3, 290.
Ding, G.C., Pronk, G.J., Babin, D., Heuer, H., Heister, K., Kogel-Knabner, I., Smalla, K., 2013. Mineral composition and charcoal determine the bacterial community structure in artificial soils. FEMS Microbiol. Ecol. 86, 15–25.
Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.
El-Naggar, A., Awad, Y.M., Tang, X.Y., Liu, C., Niazi, N.K., Jien, S.H., Tsang, D.C.W., Song, H., Ok, Y.S., Lee, S.S., 2018. Biochar influences soil carbon pools and facilitates interactions with soil: a field investigation. Land Degrad. Dev. 29, 2162–2171.
Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364.

Goldfarb, K.C., Karaoz, U., Hanson, C.A., Santee, C.A., Bradford, M.A., Treseder, K.K., Wallenstein, M.D., Brodie, E.L., 2011. Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. Front. Microbiol. 2, 94.
Hayward, A.C., Fegan, N., Fegan, M., Stirling, G.R., 2010. Stenotrophomonas and Lysobacter: ubiquitous plant-associated gamma-proteobacteria of developing significance in applied microbiology. J. Appl. Microbiol. 108, 756–770.
Hinsinger, P., Bengough, A.G., Vetterlein, D., Young, I.M., 2009. Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321, 117–152.
Hu, L., Cao, L.X., Zhang, R.D., 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, 1085–1092.
Jenkins, J.R., Viger, M., Arnold, E.C., Harris, Z.M., Ventura, M., Miglietta, F., Girardin, C., Edwards, R.J., Rumpel, C., Fornasier, F., Zavalloni, C., Tonon, G., Alberti, G., Taylor, G., 2017. Biochar alters the soil microbiome and soil function: results of next-generation amplicon sequencing across Europe. Glob. Change Biol. Bioenergy 9, 591–612.
Kelly, C.N., Calderon, F.C., Acosta-Martinez, V., Mikha, M.M., Benjamin, J., Rutherford, D.W., Rostad, C.E., 2015. Switchgrass biochar effects on plant biomass and microbial dynamics in two soils from different regions. Pedosphere 25, 329–342.
Khan, T.F., Ahmed, M.M., Huq, S.M.I., 2014. Effects of biochar on the abundance of three agriculturally important soil bacteria. J. Agric. Chem. Environ. 3, 31–39.
Khodadad, C.L.M., Zimmerman, A.R., Green, S.J., Uthandi, S., Foster, J.S., 2011. Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. Soil Biol. Biochem. 43, 385–392.
Kim, S.J., Jones, R.C., Cha, C.J., Kweon, O., Edmondson, R.D., Cerniglia, C.E., 2004. Identification of proteins induced by polycyclic aromatic hydrocarbon in mycobacterium vanbaalenii PYR-1 using two-dimensional polyacrylamide gel electrophoresis and de novo sequencing methods. Proteomics 4, 3899–3908.
Kolton, M., Harel, Y.M., Pasternak, Z., Graber, E.R., Elad, Y., Cytryn, E., 2011. Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. Appl. Environ. Microbiol. 77, 4924–4930.
Lehmann, J., 2007. A handful of carbon. Nature 447, 143–144.
Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota - a review. Soil Biol. Biochem. 43, 1812–1836.
Li, X.Z., Rui, J.P., Mao, Y.J., Yannarell, A., Mackie, R., 2014. Dynamics of the bacterial community structure in the rhizosphere of a maize cultivar. Soil Biol. Biochem. 68, 392–401.
Liu, X.R., Li, J., Yu, L., Pan, H., Liu, H.Y., Liu, Y.M., Di, H.J., Li, Y., Xu, J.M., 2018. Simultaneous measurement of bacterial abundance and composition in response to biochar in soybean field soil using 16S rRNA gene sequencing. Land Degrad. Dev. 29, 2172–2182.
Luo, S.S., Wang, S.J., Tian, L., Li, S.Q., Li, X.J., Shen, Y.F., Tian, C.J., 2017. Long-term biochar application influences soil microbial community and its potential roles in semiarid farmland. Appl. Soil Ecol. 117, 10–15.
Makhalanyane, T.P., Valverde, A., Gunnigle, E., Frossard, A., Ramond, J.B., Cowan, D.A., 2015. Microbial ecology of hot desert edaphic systems. FEMS Microbiol. Rev. 39, 203–221.
Mueller, R.C., Belnap, J., Kuske, C.R., 2015. Soil bacterial and fungal community responses to nitrogen addition across soil depth and microhabitat in an arid shrubland. Front. Microbiol. 6, 891.
Muhammad, N., Dai, Z.M., Xiao, K.C., Meng, J., Brookes, P.C., Liu, X.M., Wang, H.Z., Wu, J.J., Xu, J.M., 2014. Changes in microbial community structure due to biochars generated from different feedstocks and their relationships with soil chemical properties. Geoderma 226, 270–278.
O'Neill, B., Grossman, J., Tsai, M.T., Gomes, J.E., Lehmann, J., Peterson, J., Neves, E., Thies, J.E., 2009. Bacterial community composition in Brazilian anthrosols and adjacent soils characterized using culturing and molecular identification. Microb. Ecol. 58, 23–35.
Orcutt, B.N., Sylvan, J.B., Knab, N.J., Edwards, K.J., 2011. Microbial ecology of the dark ocean above, at, and below the seafloor. Microbiol. Mol. Biol. Rev. 75, 361–422.
Pandit, N.R., Mulder, J., Hale, S.E., Martinsen, V., Schmidt, H.P., Cornelissen, G., 2018. Biochar improves maize growth by alleviation of nutrient stress in a moderately acidic low-input Nepalese soil. Sci. Total Environ. 625, 1380–1389.
Sadegh-Zadeh, F., Parichehreh, M., Jalili, B., Bahmanyar, M.A., 2018. Rehabilitation of calcareous saline-sodic soil by means of biochars and acidified biochars. Land Degrad. Dev. 1–10.
Shi, S.J., O'Callaghan, M., Jones, E.E., Richardson, A.E., Walter, C., Stewart, A., Condron, L., 2012. Investigation of organic anions in tree root exudates and rhizosphere microbial communities using in situ and destructive sampling techniques. Plant Soil 359, 149–163.
Sun, B., Wang, X.Y., Wang, F., Jiang, Y.J., Zhang, X.X., 2013. Assessing the relative effects of geographic location and soil type on microbial communities associated with straw decomposition. Appl. Environ. Microbiol. 79, 3327–3335.
Sun, D.Q., Hale, L., Crowley, D., 2016. Nutrient supplementation of pinewood biochar for use as a bacterial inoculum carrier. Biol. Fertil. Soils 52, 515–522.
Trifunovic, B., Gonzales, H.B., Ravi, S., Sharratt, B.S., Mohanty, S.K., 2018. Dynamic effects of biochar concentration and particle size on hydraulic properties of sand. Land Degrad. Dev. 29, 884–893.
Wu, H.P., Zeng, G.M., Liang, J., Chen, J., Xu, J.J., Dai, J., Li, X.D., Chen, M., Xu, P.A., Zhou, Y.Y., Li, F., Hu, L., Wan, J., 2016. Responses of bacterial community and functional marker genes of nitrogen cycling to biochar, compost and combined amendments in soil. Appl. Microbiol. Biotechnol. 100, 8583–8591.
Xu, M., Xia, H.X., Wu, J., Yang, G., Zhang, X.H., Peng, H., Yu, X.Y., Li, L., Xiao, H., Qi, H., 2017. Shifts in the relative abundance of bacteria after wine-lees-derived biochar intervention in multi metal-contaminated paddy soil. Sci. Total Environ. 599, 1297–1307.
Yang, L.Q., Luo, P., Wen, L., Li, D.J., 2016. Soil organic carbon accumulation during post-agricultural succession in a karst area, Southwest China. Sci. Rep. 6, 37118.
Yang, Y., Wang, N., Guo, X.Y., Zhang, Y., Ye, B.P., 2017. Comparative analysis of bacterial community structure in the rhizosphere of maize by high-throughput pyrosequencing. PLoS One 12, e0178425.
Yao, Q., Liu, J.J., Yu, Z.H., Li, Y.S., Jin, J., Liu, X.B., Wang, G.H., 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.
Zhang, Y.Y., Zhang, S.Q., Wang, R.Z., Cai, J.P., Zhang, Y.G., Li, H., Huang, S.M., Jiang, Y., 2016. Impacts of fertilization practices on pH and the pH buffering capacity of calcareous soil. Soil Sci. Plant Nutr. 62, 432–439.