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Effects of biochar additions on the soil chemical properties, bacterial community structure and rape growth in an acid purple soil

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Abstract: Biochar is considered as a universal conditioner to improve soil quality, but its effects of different addition rates on soil properties, bacterial community structure and plant growth are still unclear, particularly in the typical acid purple soil in the southwest of China. In this study, 110 days of rape growth pot experiment under the application rate of 0.0% rice husk biochar (CK), 0.8% (CT1), 2.0% (CT2) and 4.0% (CT3) to the acid purple soil. Results showed that all biochar additions improved soil pH, soil organic carbon (SOC), total phosphorus, available phosphorus, available potassium concentrations in the acid purple soil. The activity of both invertase and catalase, not urease, was significantly increased with the increasing of biochar addition rates. The 16s-gene sequencing results showed that the Chao1 index was increased only under CT3, and the Shannon index was increased after all biochar applications. Furthermore, biochar increased the relative abundance of bacteria that play important roles in soil carbon and nitrogen cycles, SOC decomposition, plant diseases control and growth. The plant height and biomass production of rapes were increased under the low biochar level (CT1), but not under the higher rates of CT2 and CT3. These results demonstrated that biochar, as a soil conditioner to the acid purple soil, could increase soil pH value, SOC, available phosphorus and potassium and affect carbon and nitrogen cycles related to bacterial communities for promoting plant performance under low application rate.

Keywords: biochar; acidification; bacterial abundance; nutrient availability; plant biomass

Acid purple soil, a unique soil, is weathered from the purplish sandstone and mudstone of the Jurassic Period in the subtropical areas (Han et al. 2014). This type of purple soil is mainly distributed in the hilly land in the Sichuan Basin (26×10^4 km^2), southwest China, for providing a variety of agricultural products to ~120 million population along the upper reaches of the Yangtze River (Zhu and Bo 2015). However, the increase of soil acidification, while the decrease of soil organic carbon (SOC), has become increasingly serious under the recently rapid urbanisation in the acid purple soil areas (Han et al. 2014). These changes in fertility of purple soil have severely hindered agriculture production and thus food security in the Sichuan Basin (Lin et al. 2009). As a result, it is critical to sustaining soil fertility while promoting crop productivity in such an important purple soil area.

Biochar is produced by the pyrolysis of crop stalks, wood material, livestock manure and other waste biomass under high temperature, hypoxia or complete hypoxia (Lehmann 2007). As a conditioner, biochar has been applied to soil to improve soil fertility and C sequestration (Lehmann 2007, Warnock et al.

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Table 1. Chemical properties of the experimental biochar

	SOC	TK	TP	ACa	AMg	SiO_2	Cu	Mn	Zn	Fe	pH
	(g/kg)						(mg/kg)				
Biochar	239.70	42.64	1.33	2.63	0.38	470	10	27	40	18	10.4

SOC – soil organic carbon; TK – total potassium; TP – total phosphorus; ACa – available calcium; AMg – available magnesium; SiO_2 – silicon dioxide

2007, Laird 2008, Meier et al. 2019). For instance, biochar significantly improved soil nutrient availability to plants and plant productivity in highly weathered soils (Glaser et al. 2002). The application of rice and maize straw biochar in red paddy soil reduced soil acidity while increased the activities of soil enzymes and microorganisms and changed soil microbial community structure (Gul et al. 2015). The application of biochar in the Mediterranean region had been considered as a promising way to improve soil quality and health (Teutscherova et al. 2018). The biochar addition in a metal-contaminated soil increased soil pH and SOC, improved habitats for microorganisms, and enhanced plant growth and biomass production (Meier et al. 2019). Biochar also increased SOC, available phosphorus and potassium, and alkaline hydrolysed nitrogen in red acid soil, though the effects of biochar on soil properties varied with biochar and soil characteristics (Zhang et al. 2013).

Information on the interactive mechanisms of biochar additions on soil property and microbial community composition is still limited, particularly in the unique acid purple soil in southwest China. With greenhouse pot experiments to explore the effects of different biochar dosages or addition rates on soil chemical properties, enzyme activity, bacterial community structure and plant growth in the acid purple soil, this present study focuses on establishing the relationships between soil bacterial community structure and soil chemical properties and/or crop biomass, and on further assessing the practical potential that the biochar addition can improve the acid purple soil fertility.

MATERIAL AND METHODS

Seeds of rape, biochar, and soil sampling. Seeds of rape (*Brassica campestris* L. cv. Zheyouza 108) were purchased commercially from the Mianyang Seed Company, Sichuan, China. The biochar from Chongqing Lihong Technology Company, China, was made from a rice husk pyrolysis under 500 °C for 6 h. The basic properties of this biochar were shown in Table 1.

The cultivated layer (5 ~ 10 cm) of the acid purple soil (Eutric Regosol, FAO Soil Classification System), from the field of the National Monitoring Station of Soil Fertility and Fertiliser Efficiency on Purple Soils, located in the Southwest University campus (106°24'37''E; 29°48'32''N) Beibei, Chongqing, China, was collected and air-dried. The soils were thoroughly mixed after removing debris and sieved to 2 mm, then divided into 1.9 kg every pot. The soil had low pH of 5.52, and the other basic chemical properties were shown in Table 2.

Experimental design. The pot experiment was conducted in the South Zone of the No. 1 greenhouse, locating in the Southwest University campus. The four biochar treatments were: (1) no or 0.0% biochar (biochar:soil = weight:weight) added as the control (CK); (2) 0.8% (CT1); (3) 2.0% (CT2) and (4) 4.0% (CT3). The biochar was mixed into 1.9 kg soil in the plastic pot, and the moisture of this mixed soil growth medium was adjusted to 50%. After one-month (assuming soil bacteria could well respond to biochar), and then six rape seeds were sown, and three 3-leaf seedlings were remained in each plot when soil moisture was monitored under no fertili-

Table 2. Chemical properties of the experimental soil

	SOC	TN	TP	TK	AN	AP	AK	pH
	(g/kg)				(mg/kg)			
Purple soil	6.16	0.84	0.63	21.33	70.43	31.21	117.22	5.52

SOC – soil organic carbon; TN – total nitrogen; AN – available nitrogen; AP – available phosphorus; AK – available potassium

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sation. The greenhouse temperature was controlled at 20 °C and the air humidity 60%. Each treatment 30 pots and the test were repeated 3 times.

Sample collection and pretreatment. The rhizosphere soils attached to the root surface (within 0.4 ~ 4 mm) of the rape were sampled with a bristle brush when the rape was 80-days old (110 days after biochar added). The samples were divided into two portions. One portion was stored at –80 °C for the analysis of the microbiology population. Another portion was air-dried and then passed through a 0.2 mm sieve for the determination of soil enzyme activities and chemical analyses.

The determination of soil physicochemical properties. Soil organic carbon (SOC) and total nitrogen (TN) were respectively measured by the potassium dichromate volumetric, and half trace Kjeldahl methods; soil total phosphorus (TP) and available phosphorus (AP) were digested with H_2SO_4-$HClO_4$ and then analysed by the sodium bicarbonate extraction-spectrophotometry; the ammonium nitrogen (AN) was extracted with 0.5 mol/L $NaHCO_3$ and measured with the diffusion method; the total potassium (TK) and available potassium (AK) were digested or extracted with HNO_3-$HClO_4$ or 1.0 mol/L NH_4Ac respectively, and then analysed with a sodium hydroxide melting-flame photometer; soil pH was determined from soil water suspension (1:5 *w*/*v*) with a pH meter. All these analyses on the soil mentioned above variables were accorded to Cai et al. (2019).

The determination of soil enzyme activity. Invertase was analysed based on the product of glucose, which was determined colorimetrically at 508 nm with a spectrophotometer; urease was assayed by the determination of ammonium released from a solution of urea (10%) and citrate buffer (pH 7) after incubated at 37 °C for 24 h; catalase was measured by the determination of hydrogen peroxide complex in the buffer (pH 7) with potassium permanganate after 10 min incubation. All analyses mentioned above were accorded to Wang et al. (2017).

Soil DNA extraction. Soil total DNA was extracted from 0.5 g fresh soils with a Power Soil ® DNAIsolation Kit according to the Kit instructions (Soliman et al. 2017). The DNA quality was estimated with the NanoDrop 2000 Spectrophotometer (Thermo Scientific, Massachusetts, USA). The extracted DNA was stored at –80 °C for the sequencing of 16S rDNA genes.

The sequencing of 16S rRNA genes. The PCR product library was created by the two-step PCR amplification method (White et al. 1990). The first step: the V4 regions of the 16S rRNA gene sequence were amplified in prokaryotes with the primer 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Caporaso et al. 2012). The second step: the specific primers were designed to increase the base diversity of the sample library. The Miseq platform (Illumina, San Diego, USA) was used for sample library sequencing (Kong 2011).

The raw sequencing data generated from MiSeq were processed to combine paired-end reads, and poorly overlapped and unqualified sequences were filtered out by using a Galaxy pipeline at http://zhoulab5.rccc.ou.edu:8080. After demultiplexing of raw fastq data (barcode error is set as zero) and primer trim, the reads with an average quality score less than 20 were removed by Btrim (Kong 2011) and the paired-end reads were combined by Flash (Magoč and Salzberg 2011). Then, sequences containing N (unidentified base) or out the range of length (240–260 without primers) were removed. Chimeras were detected by UCHIME (Edgar et al. 2011), and OTUs (operational taxonomic units) were generated by UCLUST (Edgar 2010) with a 97% similarity threshold. The reference databases of 16S were Greengenes (http://greengenes.lbl.gov) (DeSantis et al. 2006). OTUs were taxonomically identified using the Ribosomal Database Project (RDP) classifier. The number of samples sequences was homogenised with the minimum sequence number before further analyses. The Chao1 and Shannon diversity index was calculated in QIIME with the following formula:

$$H_{Shannon} = -\sum_{i=1}^{R} P_i \ln P_i \quad (1)$$

$$S_{Chao1} = R + \frac{n_1(n_1 - 1)}{2(n_2 + 1)} \quad (2)$$

Where: R – actual number of operational taxonomic units (OTUs) measured in a single sample; P_i – ratio between the number of sequences contained in OTU i and all sequence numbers; n_1 – number of OTUs containing only one sequence; n_2 – number of OTUs containing only two sequences.

Determination of plant height and biomass. The plant height: The distance from the base of the root of the harvested rape plants to the highest point of the stem was measured.

The plant biomass: When the rape was harvested, it was gently pulled out of the potted soil (be careful not to break the root). The soil attached to the root

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was cleaned, then the whole rape plant was put into the sample collection bag. After dried in the oven at 72 °C, the total biomass of the underground and aboveground part of the rape was weighed.

Data analyses. Using the statistical software SPSS 21.0 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, USA), significant differences between treatments were compared with Duncan's multiple range test at $P < 0.05$. The Pearson correlation of soil bacterial community structure with soil chemical properties and crop biomass was analysed by R3. The figures and tables were generated with Excel 2017, Origin 9.0 and R3 (Califonia, USA).

RESULTS AND DISCUSSIONS

Effects of biochar on nutrient concentrations and pH. It has been reported that the application of biochar to soil increases soil pH and improves nutrient availability, though the effects vary with biochar types/doses and soil types (Khodadad et al. 2011, Gul et al. 2015). The effects of biochar on soil nutrients can be summarised in two aspects. One is that biochar is rich in mineral nutrients, and some nutrients can be returned to the soil after biochar application (Glaser et al. 2002). On the other hand, the high adsorption capacity of biochar can reduce nutrient loss and increase soil fertility (Gul et al. 2015). The composition and properties of biochars are dependent on the material types and pyrolysis temperatures. For example, with the increasing of pyrolysis temperature, both carbon and nitrogen concentrations in the generated biochar are increased, the aromatisation is enhanced, and the properties of surface adsorption and pore of the biochar are also changed (Yuan et al. 2011). In this study, the tested biochar was formed from the pyrolysis of rice husk under 500 °C for 6 h. The carbon and other properties were different from the biochar formed with other materials under different conditions. The results in our research showed that SOC, total and available phosphorus and available potassium and pH all were increased under biochar additions, but the total and available nitrogen was decreased (Table 3). The decreasing of soil total and available nitrogen may be due to the application of biochar that is able to improve the utilisation of soil nitrogen by plants. In addition, the decline of soil available nitrogen may also be caused by the increase in soil pH, which can promote the transformation of ammonium nitrogen into nitrate nitrogen, leading to the reduction of available soil nitrogen (Chen et al. 2013). Although the biochar added rates were relatively low, the increase in soil pH may be the high pH values (10.4) of the added biochar that can increase the pH of an acidic soil by increasing soil base saturation, decreasing the level of exchangeable aluminum, and consuming soil protons (Smider and Singh 2014, Zhao et al. 2015).

Effects of biochar on soil enzyme activity. Soil enzyme activity is one of the important factors to measure soil fertility and biological activity (Nelissen et al. 2015), and the increasing of invertase, catalase and urease activity is beneficial to soil carbon and nitrogen cycle. Studies have shown that biochar can increase soil enzyme activity (Khodadad et al. 2011, Gul et al. 2015), and the effects vary with biochar and soil types, the dose of biochar and soil enzyme types. In this study, invertase activity was decreased in the acid purple soil under

Table 3. Effects of biochar addition on soil chemical properties

Treatment	SOC	TN	TP	TK	AN	AP	AK	pH
	(g/kg)				(mg/kg)			
CK	6.75 ± 0.40^{c}	0.80 ± 0.03^{a}	0.67 ± 0.03^{D}	19.66 ± 0.76^{a}	70.98 ± 1.89^{a}	30.88 ± 2.42^{D}	95.00 ± 2.89^{D}	5.41 ± 0.03^{d}
CT1	7.90 ± 0.88^{b}	0.77 ± 0.04^{a}	0.74 ± 0.01^{C}	20.37 ± 0.16^{a}	69.62 ± 1.16^{a}	45.23 ± 0.58^{C}	208.33 ± 0.00^{C}	5.49 ± 0.02^{c}
CT2	7.96 ± 1.18^{b}	0.70 ± 0.08^{a}	0.84 ± 0.01^{B}	20.38 ± 0.22^{a}	61.70 ± 4.12^{b}	73.27 ± 1.16^{B}	333.33 ± 0.00^{B}	5.70 ± 0.06^{b}
CT3	9.30 ± 0.79^{a}	0.75 ± 0.03^{a}	0.99 ± 0.01^{A}	20.63 ± 0.18^{a}	63.88 ± 1.64^{b}	115.50 ± 12.51^{A}	569.44 ± 24.06^{A}	6.32 ± 0.03^{a}

CK – 0.0%; CT1 – 0.8%; CT2 – 2.0%; CT3 – 4% of biochar addition in the purple soil. Values are means ± standard error; different lowercase or uppercase letters indicate significant differences among treatments at $P < 0.05$ or $P < 0.01$ by Duncan's multiple range test. SOC – soil organic carbon; TN – total nitrogen; TP – total phosphorus; ; TK – total pottasium; AN – available nitrogen; AP – available phosphorus; AK – available potassium

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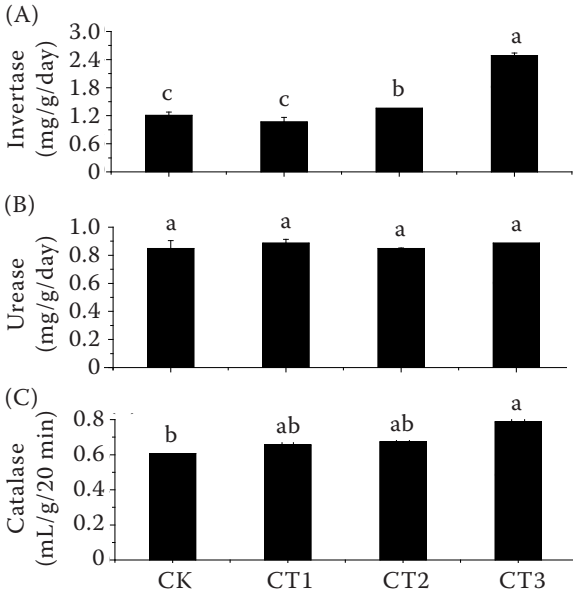

Figure 1. The effects of biochar on soil enzyme activity. CK – 0.0%; CT1 – 0.8%; CT2 – 2.0%; CT3 – 4% of biochar addition in the purple soil. Values are means ± standard error; different letters indicate significant differences among treatments at $P < 0.05$ by Duncan's multiple range test

CT1 but increased under CT2 and CT3 (Figure 1A), there was no significant ($P < 0.05$) difference in urease activity between different treatments (Figure 1B), and catalase activity gradually was increased with the increasing in doses of biochar (Figure 1C). The special structure and adsorption properties of biochar determine the complexity of effects of biochar on soil enzymes (Teutscherova et al. 2018); on the one hand, the adsorption of biochar to the substrate of reaction is conducive to promote the enzymatic reaction and increase soil enzyme activity, on the other hand, the adsorption of biochar to enzyme molecule protects the binding site of the enzymatic reaction, thus, which may inhibit the enzymatic reaction.

Effects of biochar on soil bacterial community structure. Soil bacteria ecological system can be directly or indirectly involved in the degradation, migration and transformation process of biochar added in soil (Khodadad et al. 2011). Biochar can also influence the community structure and abundance of soil bacteria and regulate the interaction of soil environmental factors and microorganisms, and improve the soil microbial ecosystem (Ameur et al. 2018). Kolton et al. (2011) found that the addition of 3% citrus biochar to sandy soil increased the abundance of *Bacteroidetes* and reduced the abundance of *Proteobacteria*. Nielsen et al. (2014) showed that the abundance of *Acidobacteria*, *Actinobacteria* and *Verrucomicrobia* was increased in agricultural soil added biochar. In this study, the OTUs index presented the trend as CT2 > CK > CT1 > CT3 in acid purple soil (Table 4), the Chao1 index was gradually decreased under CT1 and CT2 but increased under CT3, and the Shannon index gradually was increased with the increasing in doses of biochar, and the results showed that the application of biochar could influence the richness and diversity of soil bacteria, but the degree of effects varied with the doses of biochar; biochar increased the relative abundance of some bacteria in the acid purple soil, such as *Bacteroidetes*, *Firmicutes* and *Gemmatimonadetes* (Figure 2), *Rhizomicrobium*, *Burkholderia* and *Bacillus* within *Proteobacteria*, *Gp1* within *Acidobacteria*, *Gemmatimonas* within *Gemmatimonadetes* (Table 5) and so on. These bacteria have positive effects on soil nutrient cycle (Takachi et al. 2010), organic matter decomposition (Jiang et al. 2007, Kodama and Watanabe 2011), diseases control and plant growth (Hussain et al. 2013, Myers and King 2016).

Effects of biochar on the plant height and biomass of rapes. This study found that the plant height (Figure 3A) and biomass (Figure 3B) of rapes were significantly ($P < 0.05$) increased in the acid purple soil under the low biochar level (CT1) while decreased under the high level (CT3). The effects of biochar on crops also will be changed by soil properties, the characteristics and dosage of biochar, crop types, climate, and the proportion with fertilisers and so on various comprehensive factors (Xi et al. 2015).

Table 4. Effects of biochar additions on the diversity of soil bacterial community

Treatment	High quantity reads	Re-sample	OTUs	Chao1	Shannon
CK	53 954	24 957	3 911	6 079	6.65
CT1	57 685	24 957	3 470	5 456	6.69
CT2	37 356	24 957	3 972	4 799	6.75
CT3	24 957	24 957	3 101	6 078	6.95

CK – 0.0%; CT1 – 0.8%; CT2 – 2.0%; CT3 – 4% of biochar addition in the purple soil; OTUs – operational taxonomic units

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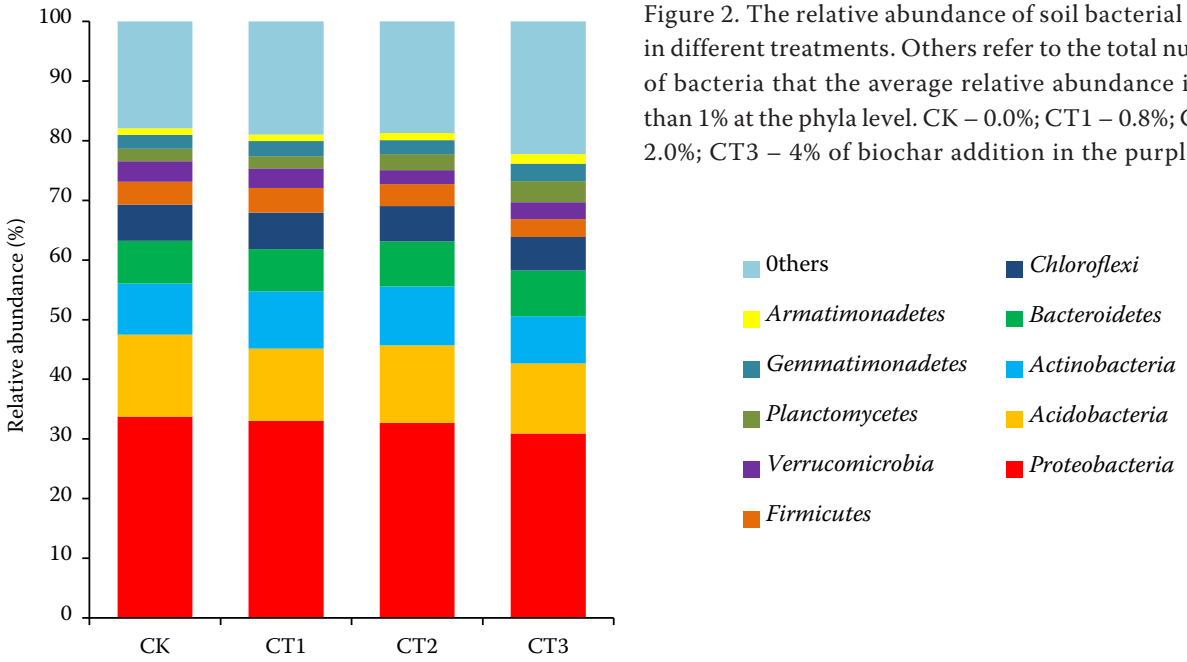

Figure 2. The relative abundance of soil bacterial phyla in different treatments. Others refer to the total number of bacteria that the average relative abundance is less than 1% at the phyla level. CK – 0.0%; CT1 – 0.8%; CT2 – 2.0%; CT3 – 4% of biochar addition in the purple soil

Table 5. The relative abundance (%) of bacterial at the genera level (the average relative abundance > 0.5%)

Phyla	Genera	CK	CT1	CT2	CT3
	Gp1	3.85	3.59	3.96	3.37
	Gp2	2.48	1.65	1.26	1.21
	Gp3	2.56	2.35	2.40	2.01
Acidobacteria	*Gp4*	0.81	0.77	1.37	1.41
	Granulicella	1.03	1.01	1.07	0.79
	Gp6	0.48	0.52	0.52	0.79
	Gp13	0.77	0.51	0.42	0.40
Gemmatimonadetes	*Gemmatimonas*	2.32	2.50	2.38	2.93
Chloroflexi	*Ktedonobacter*	1.53	1.51	1.65	1.89
Actinobacteria	*Actinoallomurus*	1.73	2.29	1.70	1.11
	Conexibacter	1.31	1.19	1.08	0.85
Firmicutes	*Clostridium*	1.31	0.94	1.10	0.48
Bacteroidetes	*Mucilaginibacter*	1.15	1.20	0.68	0.69
Planctomycetes	*Gemmata*	0.48	0.46	0.75	1.56
	Rhizomicrobium	3.62	3.33	4.10	2.26
	Sphingomonas	1.60	1.25	1.51	1.01
	Skermanella	1.14	0.94	0.65	0.43
	Burkholderia	0.77	0.71	0.99	0.67
Proteobacteria	*Limnobacter*	0.70	1.00	0.79	0.65
	Acidisoma	0.60	0.89	0.70	0.80
	Stella	0.59	0.92	0.63	0.57
	Aquicella	0.56	0.69	0.66	0.61
	Acidovorax	0.73	0.83	0.27	0.22
	Chondromyces	0.27	0.30	0.61	0.83
Chlamydiae	*Simkania*	0.54	0.54	0.54	0.98
Armatimonadetes	*Armatimonadetes_gp5*	0.50	0.60	0.60	0.73

CK – 0.0%; CT1 – 0.8%; CT2 – 2.0%; CT3 – 4% of biochar addition in the purple soil

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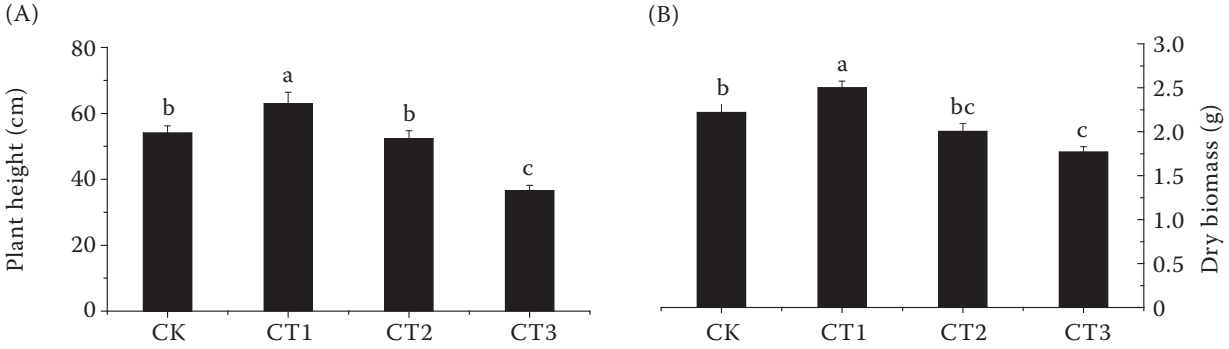

Figure 3. The effects of biochar on the plant height (A) and biomass (B) of rapes. CK – 0.0%; CT1 – 0.8%; CT2 – 2.0%; CT3 – 4% of biochar addition in the purple soil. Values are means ± standard error; different letters indicate significant differences among treatments at $P < 0.05$ by Duncan's multiple range test

On the one hand, biochar itself contains certain nutrients that can be directly utilised by crops and improve the growth of crops (Fox et al. 2016). On the other hand, biochar can indirectly promote the growth of crops by improving soil chemical properties, enzyme activity, microbiology ecosystems and other environmental conditions (Liu et al. 2014). However, the application of high doses of biochar in soil may inhibit the growth of crops due to the effects on the utilisation of soil nitrogen, the soil pH and the effectiveness of some trace elements (Laird et al. 2010, Van Zwieten et al. 2010).

Pearson correlation of the top phyla with soil chemical properties and crop biomass. The application of biochar has changed soil chemical properties, such as soil organic matter, available phosphorus,

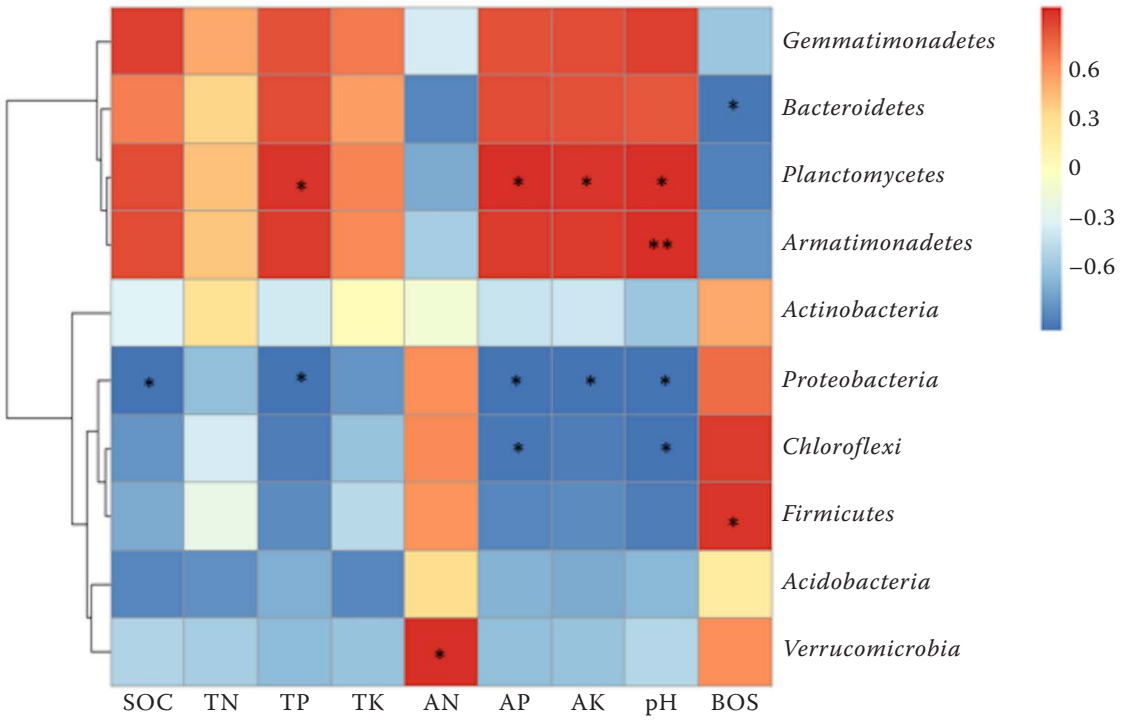

Figure 4. The heat map of correlation of the top phyla with soil chemical properties and crop biomass. BOS: Crop dry biomass. *X* and *Y* axes are environmental factors and genera (the average relative abundance is higher than 0.5%). *R* is shown with different colors, and the right side of the legend is the color range of different *R* values. $^{*}P < 0.05$; $^{**}P < 0.01$; SOC – soil organic carbon; TN – total nitrogen; TP – total phosphorus; TK – total pottasium; AN – available nitrogen; AP – available phosphorus; AK – available potassium

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available potassium, etc., which are closely related to bacterial community structure (Ameur et al. 2018). Ding et al. (2013) showed that available soil phosphorus and available potassium had a great influence on the soil bacterial community. Liu et al. (2014) found that the composition and diversity of soil bacterial communities were influenced by soil pH and total carbon. Zhou et al. (2017) indicated that plant biomass was significantly positively correlated with *Firmicutes* while significantly negatively correlated with *Thermotogae*, *Latescibacteria* and *Parcubacteria*. In this study, biochar changed soil chemical properties, plant biomass production, and soil bacterial community structure in the acid purple soil, and the results of Pearson correlation analysis of soil bacterial community structure were closely related to SOC, TP, AN, AP, AK and pH, as well as crop biomass (Figure 4). Nevertheless, further studies on the interactive effects of biochar additions on soil chemical properties and microbiology community structure at different crop growth stages and soil profiles are timely needed.

To sum up, biochar can improve soil pH and other chemical properties, enhance invertase and catalase activity, increase bacterial alpha diversity and the relative abundance of bacteria that associated with soil carbon and nitrogen cycles, SOC decomposition, disease control, and promote crops growth (under the low biochar level) in the acid purple soil. So we concluded that biochar could be used as a soil conditioner in acid purple soil.

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