Response of soil microbial community to application of biochar in cotton soils with different continuous cropping years

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The bacterial community in soils of cotton that have continuously been cropped for 2 years, 6 years, 11 years and 14 years and treated with biochar (B0, 0 t·ha−1; B1, 12.5 t·ha−1; and B2, 20 t·ha−1) was investigated using next-generation sequencing. Of the 45 bacterial genera (relative abundance ratio of genera greater than 0.3%), 21 genera were affected (p < 0.05) by the biochar treatment, whereas 20 genera were affected by the continuous cropping. Between the soils that have been continuously cropped for 2 years and 14 years, 12 different genera were significantly observed (p < 0.05), and 6 genera belonged to the phylum Acidobacteria. The relative abundance of Sphingomonas and Pseudomonas in the biochar-treated soils was significantly higher than that in the soil without biochar treatment (p < 0.05), and the relative abundance of Sphingomonas and Pseudomonas in soils that have been continuously cropped for 2 years and 6 years was significantly higher than that in the soils continuously cropped for 11 years and 14 years (p < 0.05). The results suggest that the biochar application has a significant impact on the soil bacterial community, which may improve the microbial diversity of continuous cropping systems in cotton soils.

Biochar has been recommended as a soil modification additive in several soil management regimes1,2, and biochar application has achieved a great deal of positive effects, including improvements in the soil fertility, increases in the size of the soil microbial community3–5, and decreases in nitrous oxide (N2O) and methane (CH4) emissions1,6,7. In particular, modification of soils with biochar has been shown to increase plant yield and modify the soil habitat of microbes, protecting microbes from predation by soil microarthropods8,9, thus altering the soil microbial activity and community structure10,11. Lehmann et al.12 reported that application of biochar increases microbial activity and biomass and changes the microbial community composition and abundance11. However, the beneficial effect of biochar on the soil environment depends on the type of biochar, application rate, soil type and plant response7. More recently, compounds inhibiting microbial activity have been found in the biochar13,14 as well as in the soil (released after biochar introduction)15. Regardless, these changes will likely spread unequally across different phylotypes or functional groups. In addition, little is known about how specific microorganisms are affected by such changes8.

Hubei Province is the second-largest production area of cotton in China. The practice of continuous cotton cropping is significant in this area. Continuous cropping has resulted in a decline in cotton production, in quality and in soil microbial diversity. At the same time, the diseases and insect pests of cotton, such as Verticillium wilt and Fusarium wilt, commonly occur and affect the income of farmers. Continuous cropping has been one of the key issues in need of solving in cotton production industry. Recently, organic alterations have provided a substrate for the burgeoning soil microbial community, resulting in the development of a self-sustaining, below-ground microbial community that will generally increase the success of reclamation efforts16. Biochar, as a new type of environmental friendly soil-improvement material, is widely used in agricultural production, especially for improving soil quality. For decades, research has focused on the physical and chemical soil variables, with less attention paid to those effects on microorganisms. In the present study, we determined the relative effects of biochar application on the composition of the soil microbial community in different cotton soils that have been...
Results

Sequence data and bacterial taxonomic richness. A total of 1,669,315 paired-end 250 bp reads were acquired, and the average read length per sample was 0.16 Gb, with 87,323 to 204,627 raw reads in cotton soils with different continuous cropping years and biochar treatments (Table S1). After the initial quality control process, 1,608,450 high-quality sequences were obtained. On average, 134,038 sequences were obtained per sample. Based on 97% species similarity, 6552 to 9420 operational taxonomic units (OTUs) were separately obtained from the samples of different continuously cropped cotton soils with biochar treatment (Table S1). The average length of the sequence reads was 440 bp, and they were classified into different taxonomic groups using Uclust. The bacterial diversity is reflected by the Chao1 index; the Chao1 index in the soils cropped continuously for 2 years and 6 years was higher than that in the soils continuously cropped for 11 years and 14 years (Fig. 1), with significant differences (except for the 6-B0 soil). This finding indicates that the bacterial diversity decreased during the continuous years.

Effect of biochar on the bacterial community composition. Results shown in Fig. S1 describe the distribution of the DNA sequences into phyla. A total of 34 phyla were shared by the 12 soil samples. The main phyla were as follows: Proteobacteria, Acidobacteria, Actinobacteria, Nitrospira, Bacteroidetes, Gemmatimonadetes, Planctomycetes, Firmicutes and Verrucomicrobia. Proteobacteria was the most dominant among the 34 phyla (p < 0.05) in the samples and comprised 39% to 54.5% of the total sequences. Acidobacteria was the second-largest phylum in all groups, comprising approximately 14.2% to 21.3% of the different groups (Fig. 2). Seven phyla (Proteobacteria, Acidobacteria, Nitrospira, Bacteroidetes, Planctomycetes, Firmicutes and Verrucomicrobia) differed (p < 0.05) between the continuous cropping year and biochar treatment (Table S2). The relative abundance of Proteobacteria, Nitrospira, Planctomycetes and Firmicutes was significantly reduced by the continuous year, while the relative abundance of Acidobacteria and Bacteroidetes increased by the continuous year (Fig. 2). Biochar treatment increased the relative abundance of Proteobacteria in the soils continuously cropped for 2 years and 6 years but not in the continuous 11-year and 14-year soils. Biochar treatment also increased the relative abundance of Planctomycetes and Firmicutes, especially for the B1 (12.5 t·ha⁻¹) treatment regarding Planctomycetes. However, biochar treatment reduced the relative abundance of Acidobacteria and Bacteroidetes in different continuous-year soils.

At the genus level, a total of 853 genera were identified from all the samples, regardless of the treatments administered. The 46 most abundant genera (with relative abundance of more than 0.3%), comprising more than 66.6% of the total sequences, are shown in Fig. S2. Of the 46 genera, 29 were affected by the continuous cropping year, biochar treatment and interactions between the continuous cropping year and biochar treatment (p < 0.05). Among these genera, 20 were affected by the continuous cropping year, 21 by the biochar treatment and 12 by the interactions between continuous cropping year and biochar treatment (Table 1). The relative abundance of Gemmatimonas, Nitrospira, Sphingomonas, Pseudomonas, Dongia, Phaselicystis, Kofleria, Nitrosospira, Geobacter, Ramlibacter, Novosphingobium and Ohtaekwangia was significantly reduced by the continuous year, while the relative abundance of Gp6, Gp4 and Gp10 was increased by the continuous year. Biochar treatment could increase the relative abundance of Gemmatimonas, Nitrospira, Sphingomonas, Pseudomonas and Anaeromyxobacter but reduce the relative abundance of Rhodoplanes, Ilumatobacter, Gp6, Gp4 and Gp10 (Table 2).

The five most abundant genera were Sphingomonas, Gemmatimonas, Nitrospira, Pseudomonas and Gp6 (Fig. S2). The relative abundance of Sphingomonas and Pseudomonas in the biochar-treated soils was significantly
higher than that the soils without biochar treatment (Fig. 3, p < 0.05), and the relative abundance in the cotton soils continuously cropped for 2 years and 6 years was significantly higher than that of the 11-year and 14-year continuously cropped soils (p < 0.05). However, the relative abundance of *Nitrospira* in the soils without biochar treatment in different continuous cropping years was significantly higher compared with that of the biochar-treated soils (Fig. 3, p < 0.05).

Eight genera significantly differed (p < 0.05) between soil continuously cropped for 2 years and 6 years, and 10 genera differed between 2-year and 11-year continuously cropped soils (Table 3). Most of the genera belonged to the phylum *Proteobacteria*. However, between soils continuously cropped for 2 years and 14 years, 12 significantly different genera (p < 0.05) were identified, with 6 of those genera belonging to the phylum *Acidobacteria*. The relative fold changes in *Pseudomonas* between soils continuously cropped for 11 years and 2 years and between 14-year and 2-year soils were −19.81 and −19.02, respectively, and the differences were significant (p < 0.05). The soil samples were separated into categories, which matched their different continuous cropping year (Fig. 4). The separation was clearer for the cotton soils continuously cropped for 11 years and 14 years than for that of 6-year and 2-year soils, suggesting that the number of years of continuous cropping influenced the bacterial community composition.

**Figure 2.** The abundance of *Acidobacteria*, *Proteobacteria*, *Nitrospira*, *Bacteroidetes*, *Planctomycetes* and *Firmicutes* in cotton soils that have been continuously cropped for 2 years, 6 years, 11 years and 14 years and treated with biochar (B0, 0 t·ha$^{-1}$; B1, 12.5 t·ha$^{-1}$; and B2, 20 t·ha$^{-1}$).
In the cotton soil that has been continuously cropped for 14 years was significantly lower than that in the 2-year continuously cropped soil. This result is in accordance with the findings of previous studies that also investigated the changes in the taxonomy of soil microbial communities. The soil samples were separated into categories, which matched the biochar treatments (Fig. 4). Principle component analysis separated the biochar treatments and the control soils, suggesting that biochar treatment influences the bacterial community composition.

### Discussion

Bacterial community composition and its relative proportion in soil microbial communities varied and was influenced by both the biochar treatment and number of continuous cropping years. This result is in accordance with the findings of previous studies that also investigated the changes in the taxonomy of soil microbial communities after biochar amendment. Possible causes of the ecological shifts in the relative abundance of the bacterial community observed may include effects of the root exudates on a portion of the microbial community as a result of continuous cropping; changes to the physiochemical state of the soil environment, including pH, mineral content, pore and particle size; and changes to the water and nutrient availability due to the application of biochar and to the continuous cropping system, all of which have been shown to influence the composition of the bacterial community in soils. Further work will be required to determine the relative importance of these factors in altering the composition of the microbial community due to biochar amendments to soils.

### Table 1. ANOVA for genus abundance.

| Phyla        | Genus             | p value (*p < 0.05, **p < 0.01) | Continuous cropping years | Biochar treatments | Continuous cropping years + biochar treatments |
|--------------|-------------------|---------------------------------|---------------------------|-------------------|-----------------------------------------------|
| Gemmatimonadetes | Gemmatimonas     | <0.001**                        | <0.001**                  | <0.001**          |                                               |
| Nitrospira   | Nitrospira        | 0.002**                         | <0.001**                  | <0.001**          |                                               |
|              | Sphingomonas      | <0.001**                        | <0.001**                  | 0.037*            |                                               |
|              | Pseudomonas       | <0.001**                        | <0.001**                  | <0.001**          |                                               |
|              | Steroidobacter    | <0.001**                        | 0.068                     | 0.057             |                                               |
|              | Dongia            | 0.042*                          | 0.094                     | 0.135             |                                               |
|              | Anaeromycobacter  | 0.054                           | 0.001**                   | 0.051             |                                               |
|              | Skermanella       | 0.068                           | 0.004**                   | 0.054             |                                               |
|              | Lyso bacter        | 0.009**                         | 0.003**                   | 0.001**           |                                               |
|              | Phaelecystis       | 0.012*                          | 0.024*                    | 0.078             |                                               |
|              | Kofleria           | 0.025*                          | 0.088                     | 0.020*            |                                               |
|              | Nitrosospira      | 0.032*                          | 0.026*                    | 0.023*            |                                               |
|              | Rhizobium          | 0.053                           | 0.028*                    | 0.051             |                                               |
|              | Geobacter          | 0.058                           | 0.028*                    | 0.035*            |                                               |
|              | Rhodoplanes       | 0.064                           | 0.041*                    | 0.056             |                                               |
|              | Humatobacter       | 0.072                           | 0.045*                    | 0.051             |                                               |
|              | Ramlibacter        | 0.082                           | 0.049*                    | 0.068             |                                               |
|              | Novosphingobium   | 0.075                           | 0.041*                    | 0.058             |                                               |
| Acidobacteria | Gp6               | 0.031                           | 0.023*                    | 0.013*            |                                               |
|              | Gp4               | 0.001**                         | <0.001**                  | 0.152             |                                               |
|              | Gp10              | 0.044*                          | 0.084                     | 0.067             |                                               |
|              | Gp3               | 0.025*                          | 0.068                     | 0.072             |                                               |
|              | Gp7               | 0.026*                          | 0.027*                    | 0.061             |                                               |
|              | Gp16              | 0.042*                          | 0.032*                    | 0.029*            |                                               |
|              | Gp5               | 0.041*                          | 0.052                     | 0.055*            |                                               |
|              | Gp17              | 0.042*                          | 0.055                     | 0.056             |                                               |
| Firmicutes   | Pasteuria          | <0.001**                        | <0.001**                  | 0.164             |                                               |
| Planctomycetes | Gemmata           | 0.001**                         | 0.134                     | <0.001**          |                                               |
| Bacteroidetes | Ohtaekwangia      | 0.020*                          | 0.010*                    | 0.052             |                                               |

### Biochar has a significant effect on soil bacterial composition.

Twenty-one genera were affected (p < 0.05) by biochar treatment (Table 1). Specifically, 10 different genera were observed (p < 0.05) between B1- and B0-treated soils, and 13 genera between B2- and B0-treated soils based on T-test results (Table 4). Among the 21 genera, the relative fold changes of Pseudomonas, Sphingomonas, Pasteuria and Nitrospira between B2- and B0-treated soils were 12.45, 19.40, −19.48 and −17.75, respectively, and the differences were significant (p < 0.05). Our data suggest that the genera in the biochar-treated soil were significantly different.
continuously cropped soil (Table S3). Soil pH has been recently documented in various soil samples as the major factor that determines soil bacterial diversity and composition. Previous studies 21–23 reported that soil pH influences bacterial communities in soils across North and South America, in Britain and on Changbai Mountain. The effects of soil pH on the relative abundance of some bacterial genera in this study are consistent with these studies, which indicate that the relative abundance of Acidobacteria tends to increase with lower pH values 22–26. Thus, our results further emphasize that soil pH plays an important role in shifting the composition of the bacterial community in the cotton soils with different continuous cropping years. However, Chan et al. 27 reported that the addition of biochar to soil can significantly increase the pH of the bulk soil, which potentially provides a more favorable habitat for microbial organisms, especially bacteria that are sensitive to pH. This result is in accordance with the findings of our study. The application of biochar increased the pH of the cotton soils that have been continuously cropped for a different number of years (Table S3).

Previous studies 4, 8, 28, 29 have reported that high porosity, cation exchange capacity and sorption capacity of biochar provide a suitable habitat for microorganisms, promoting their activity in soil and affecting different microbial processes involved in nutrient cycling and organic matter decomposition. In this study, the four most abundant genera that were significantly affected by the biochar treatments were Sphingomonas, Gemmatimonas, Nitrospira and Pseudomonas (Table S2). Biochar treatment improved the relative abundance of Sphingomonas and Pseudomonas (Fig. 2), possibly due to biochar providing a suitable habitat. At the same time, the soil microbial communities are mostly limited by organic carbon sources, and biochar could provide abundant carbon resources for microbial growth; thus, the greater quantity of a few dominant microorganisms, such as Sphingomonas and Pseudomonas, as a result of the biochar treatment may also be due to improved carbon sources.

In addition, the bacterial genera Sphingomonas and Pseudomonas have been detected in a variety of environments. These genera are thought to be beneficial to plants, and they have been reported as potential antagonists of plant pathogens 30, 31. Recently, these bacteria have been the focus of study due to their possible application in bioremediation 32. Therefore, biochar application could possibly enhance cotton growth by improving bacterial

Table 2. The relative abundance of genera (had significant differences) in cotton soils that have been continuously cropped for 2 years, 6 years, 11 years and 14 years treated with biochar (B0, 0 t·ha⁻¹; B1, 12.5 t·ha⁻¹; and B2, 20 t·ha⁻¹). The data are expressed as the means ± SD (n = 3). The superscript letters that differ within a column indicate significant differences between treatments (p < 0.05).
genera abundance\textsuperscript{1, 3}, which contributes to increased cotton productivity (Table S4). In addition, biochar is highly recalcitrant to microbial decomposition and thus guarantees a long-term benefit to soil fertility\textsuperscript{33}. The actual effects of biochar application depend on various factors, such as the soil type and the water balance at a given site and possibly even the cultivated genotype, which currently require further studies.

**Material and Methods**

**Site description.** The experimental site was established at the experimental farm of the Industrial Crops Institute, Hubei Academy of Agricultural Sciences in Hubei Province, China (30°35′N, 114°37′E, 50 m a.s.l.). This region has a typical subtropical monsoon climate, with an average annual precipitation of 1269 mm and an average temperature between 15.8 °C and 17.5 °C.

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**Figure 3.** The abundance of *Sphingomonas*, *Pseudomonas* and *Nitrospira* in cotton soils that have been continuously cropped for 2 years, 6 years, 11 years and 14 years and treated with biochar (B0, 0 t·ha\textsuperscript{−1}; B1, 12.5 t·ha\textsuperscript{−1}; and B2, 20 t·ha\textsuperscript{−1}). Error bars indicate standard deviation (SD) (n = 3). Different letters above the bars denote statistical significance at p < 0.05, according to the LSD test.
Soil and Biochar. The soil was collected from the surface layer of cotton soils (0–15 cm) that have been continuously cropped for 2, 6, 11 and 14 years at the cotton research station in Qianjiang City (Fig. 5). The collected soil was classified as acrisols according to the FAO, and the clay content was 66.3%. The original years of ‘2, 6 and 11-year soil’ was planted with corn, and the culturing and soil management were consistent. Corncob was used as the feedstock for biochar. The corncob was first air-dried and then pyrolyzed under controlled conditions to ensure uniform heating and treatment conditions. Biochar production was carried out using a traditional kiln reactor (Fengben Biological Technology Co., Ltd, Shandong, China) at a heating rate of 10 °C min−1 up to 550 °C.

The basic properties of the collected soil and biochar are presented in Table 5.

Pot experimental design. A pot trial was conducted at the experimental base of the Industrial Crops Institute, Hubei Academy of Agricultural Sciences in Hubei Province, China. This region has a typical subtropical monsoon climate, with an average annual precipitation of 1269 mm and average temperature between 15.8 °C and 17.5 °C.

After the removal of plant debris and stones, soils collected from different continuous cropping cotton fields (2, 6, 11 and 14 years) were mixed with biochar. The additive amount of collected soil in each experimental pot (the upper bore and pot height were 40.0 cm and 27.0 cm, respectively) was 7.5 kg of dry soil. The biochar application rates of each continuously cropped soil were 0 t·ha−1 (B0), 12.5 t·ha−1 (B1) and 20 t·ha−1 (B2), resulting in biochar weights of 0%, 1.538% and 3.077%, respectively, which accounted for the dry weights of the potted soil. In total, there were four continuous cropping soils, and each had three application rates of biochar; thus, there were twelve treatments in this experiment. Each treatment was replicated in ten pots; therefore, the twelve treatments employed 120 pots. Basal fertilizer was added to all pots. Equal amounts (10 g) of compound fertilizer (N:P:K = 15:15:15) were applied to all pots. The cotton seed was sown on 1 May 2014. One cotton (Gossypium

| Phyla          | Genus     | Relative fold change | p value (*p < 0.05, **p < 0.01) |
|----------------|-----------|----------------------|---------------------------------|
| **Planctomycetes** | Gemmata   | 19.19                | 0.003**                         |
| **Proteobacteria** | Steroidobacter | −2.17              | 0.01*                           |
|                | Sphingomonas | −19.07              | 0.010**                         |
|                | Skermanella  | 18.90                | 0.010**                         |
|                | Pseudomonas   | −2.26                | 0.021*                          |
|                | Kofleria      | −1.96                | 0.012*                          |
| **Nitrospira** | Nitrospira   | −18.61               | 0.020*                          |
| **Firmicutes** | Pasteuria    | −2.94                | 0.020*                          |
| **Phyla**      | Genus      | Relative fold change | p value (*p < 0.05, **p < 0.01) |
| **Gemmatimonadetes** | Gemmatimonas | −2.20                | 0.003**                         |
| **Proteobacteria** | Pseudomonas | −19.81               | <0.001**                        |
|                | Anaeromyxobacter | −4.57               | 0.005**                         |
|                | Lysobacter    | 1.33                 | 0.015*                          |
|                | Steroidobacter | 1.04                | 0.043*                          |
|                | Phaeicystis   | −1.99                | 0.003**                         |
| **Planctomycetes** | Gemmata   | 1.46                 | 0.007**                         |
| **Nitrospira** | Nitrospira   | −1.70                | 0.011*                          |
| **Acidobacteria** | Gp17       | 1.35                 | 0.011*                          |
|                | Gp6          | 10.33                | 0.031*                          |
| **Phyla**      | Genus      | Relative fold change | p value (*p < 0.05, **p < 0.01) |
| **Proteobacteria** | Steroidobacter | 3.58                | 0.008**                         |
|                | Nitrospira    | −2.75                | 0.010**                         |
|                | Pseudomonas   | −19.02               | 0.010**                         |
|                | Gemmata       | −1.31                | 0.032*                          |
|                | Lysobacter    | 1.53                 | 0.034*                          |
| **Acidobacteria** | Gp4         | 3.62                 | 0.001**                         |
|                | Gp10         | 1.74                 | 0.003**                         |
|                | Gp3          | −4.20                | 0.004**                         |
|                | Gp7          | −2.81                | 0.005**                         |
|                | Gp16         | 1.87                 | 0.012*                          |
|                | Gp17         | −1.75                | 0.012*                          |
| **Firmicutes** | Pasteuria    | 3.26                 | 0.007**                         |

Table 3. Comparison (t-tests and Metastats) of different continuous cropping years for genus abundance.
hirsutum L.) seedling was planted per pot on 12 May 2014. The experimental pots were laid out in a randomized complete block design. Grasses and weeds were detached manually and left in situ, and water was added when necessary.

**Sample collection and preparation.** The soil samplings were collected at the boll-opening stage (September 14th) in 2014 as follows: continuous cropping for 2 years with 0 t·ha\(^{-1}\); 12.5 t·ha\(^{-1}\); and 20 t·ha\(^{-1}\); and 6 years with 0 t·ha\(^{-1}\); 12.5 t·ha\(^{-1}\); and 20 t·ha\(^{-1}\).

**Figure 4.** PCoA of Bray-Curtis distances for the bacteria in cotton soils that have been continuously cropped for 2 years, 6 years, 11 years and 14 years and treated with biochar (B0, 0 t·ha\(^{-1}\); B1, 12.5 t·ha\(^{-1}\); and B2, 20 t·ha\(^{-1}\)).
(6-B2) biochar; continuous cropping for 11 years with 0 t·ha\(^{-1}\) (11-B0), 12.5 t·ha\(^{-1}\) (11-B1) and 20 t·ha\(^{-1}\) (11-B2) biochar. For each treatment, soil samples (5 cm from the cotton trunk at a depth of 0–15 cm) were randomly collected from six of the ten replicates and mixed. Thus, a total of 12 mixed samples were obtained for the 12 treatments. The samples were immediately transported to the lab on ice and measured within one week after collection. Part of each soil sample was stored at -80 °C for soil microbiological analysis, and another part was air-dried, ground and passed through 1- and 2-mm mesh sieves for chemical analysis.

DNA extraction and PCR amplification of 16S rRNA. The genomic DNA was directly extracted from the soil using an E.Z.N.A.® Soil DNA kit (Omega Bio-Tec, Inc., USA) according to the manufacturer’s instructions. The quality of the extracted DNA was preserved using 1% agarose gels. The V3–V4 hypervariable regions of 16 S rRNA were amplified via PCR from the microbial genomic DNA using barcoded fusion primers (forward primers: 341 F CCTACACGAGCTCTTCCGATCT (barcode) CCTACGGGNGGCWGGCAG, reverse primers: 805 R GACTGGAGTTCCTTGGCACCCGAGAATTCCAGACTACHVGGGTATCTAATCC). The reaction mixtures (50 µl) contained 5 µl of 10 × PCR reaction buffer (TakaRa, Japan), 10 ng of DNA template, 0.5 µl of each primer, 0.5 µl of dNTPs and 0.5 µl of Platinum Taq DNA polymerase (TakaRa, Japan). The PCR conditions were as follows: 94 °C for 3 min, 94 °C for 30 s, annealing at 45 °C for 20 s and 65 °C for 30 s, which was repeated for 5 cycles, followed by 94 °C for 20 s, 55 °C for 20 s and 72 °C for 30 s, which was repeated for 20 cycles, before a final elongation at 72 °C for 5 min. The PCR product was excised from the 1.5% agarose gel and purified using a QIAquick Gel Extraction Kit.

Amplicon sequence and sequence data processing. The barcoded V3 and V4 amplicons were sequenced using the paired-end method with an Illumina MiSeq (Illumina, San Diego, CA, USA) system with a 6-cycle index. Sequences with an average Phred score of less than 25 that contain ambiguous bases, a homopolymer run exceeding 6, mismatches in primers or a length of less than 100 bp were removed using Prinseq software (PRINSEQ-lite 0.19.5). For the V3 and V4 paired-end reads, only the sequences that overlapped by more than 10 bp and without any mismatch were assembled according to their overlapping sequences using Flash software (FLASH v1.2.7). Reads that could not be assembled were discarded. Barcode and sequencing primers were trimmed from the assembled sequence (V3 and V4).

| Chemical Properties | Biochar | Continuous cropping soil 2 Years | Continuous cropping soil 6 Years | Continuous cropping soil 11 Years | Continuous cropping soil 14 Years |
|---------------------|--------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Total N (g·kg\(^{-1}\)) | 2.7    | 1.31 ± 0.03\(^{a}\)          | 0.93 ± 0.02\(^{b}\)          | 0.61 ± 0.02\(^{a}\)          | 0.63 ± 0.05\(^{a}\)          |
| NH\(_4\)^+ (mg·kg\(^{-1}\)) | <0.1   | 15.2 ± 0.1\(^{a}\)          | 13.1 ± 0.1\(^{a}\)          | 11.2 ± 0.1\(^{a}\)          | 10.8 ± 0.1\(^{a}\)          |
| NO\(_3\)^− (mg·kg\(^{-1}\)) | <0.2   | 2.3 ± 0.1\(^{a}\)          | 2.3 ± 0.0\(^{a}\)          | 1.8 ± 0.0\(^{a}\)          | 1.70 ± 0.1\(^{a}\)          |
| Total C (g·kg\(^{-1}\)) | 680.0  | 10.5 ± 0.5\(^{a}\)          | 9.2 ± 0.4\(^{b}\)          | 9.1 ± 0.3\(^{b}\)          | 9.0 ± 0.2\(^{b}\)          |
| pH (CaCl\(_2\)) | 8.3    | 6.3 ± 0.3\(^{a}\)          | 6.1 ± 0.4\(^{a}\)          | 5.6 ± 0.1\(^{a}\)          | 5.3 ± 0.1\(^{a}\)          |
| EC (µS·cm\(^{-1}\)) | 526.3  | 42.3 ± 2.7\(^{a}\)          | 42.1 ± 1.6\(^{a}\)          | 39.8 ± 2.0\(^{a}\)          | 36.8 ± 3.0\(^{a}\)          |

Table 5. Basic chemical properties of biochar and soil. The data are expressed as mean ± SD (n = 3). Superscript letters that differ within rows indicate significant differences between treatments (p < 0.05).
Sequences were clustered and assigned to operational taxonomic units (OTUs) at a 3% dissimilarity level using Uclust software (Uclust v1.1.579). Taxonomic ranks were assigned to each sequence using the Ribosomal Database Project (RDP) Naïve Bayesian Classifier v.2.2 trained on the Greengenes database (October 2012 version) (Lan et al. 2012). The relative abundance count at the genus level was log2-transformed and then normalized, as described in the following. The arithmetic mean of all transformed values was subtracted from each log-transform measure, and the difference was divided by the standard deviation of all log-transformed values for a given sample. After this procedure, the relative abundance profiles for all the samples exhibited a mean of 0 and a standard deviation of 1. Principal coordinates analysis (PCoA) at the genus level was performed using Bray-Curtis distances with Mothur 1.29.2 software.

**Soil characteristics.** The total carbon and nitrogen were determined by combustion analysis (vario Macro CNS; Elementar, Germany). The ammonium (NH₄⁺) and nitrate (NO₃⁻) contents were determined through extraction with 0.5 M K₂SO₄ and colorimetric analysis of NH₄⁺ (Krom 1980; Searle 1984) and NO₃⁻ (Kamphake et al. 1967; Kempers and Luft 1988) extracts using an automated flow injection Skalar Auto-analyzer (Skalar San Plus). The carbonate equivalence of the biochar was assessed using the method of Raymont and Lyons (2011). The electrical conductivity (EC) and pH of the biochar were determined in a 1:5 (w/v; g cm⁻³) soil:water environment and in 0.01 M CaCl₂ mixtures, respectively.

**Statistical analysis.** The results were analyzed using the SPSS software program (v10.0 for Windows, Chicago, IL, USA). The differences in the relative abundance of individual genera and the treatment means among plant age were tested using one-way variance analysis (ANOVA), and significant differences among the means were determined using the LSD test. Normal distribution and homogeneity of variance were verified by the Bartlett and Dunnett tests. The differences were considered statistically significant when p < 0.05. T-tests and Metastats (http://metastats.cbcb.umd.edu/) in Mothur were used to compare the differences, and all p-values were adjusted with the false discovery rate (FDR) using the BH method with the mt.rawp2adjp function in R.

**Accession number of DNA sequence.** The raw data has been submitted to a public repository (NCBI) and the accession number was SRP099813.

**References**

1. Lehmann, J., Gaunt, J. & Rondon, M. Bio-char sequestration in terrestrial ecosystems – a review. *Mitig Adapt Strat Glob Chang* 11, 403–427 (2006).

2. Ogawa, M., Okimori, Y. & Takahashi, F. Carbon sequestration by carbonisation of biomass and forestation: three case studies. *Mitig Adapt Strat Glob Chang* 11, 429–444 (2006).

3. Glaser, R., Lehmann, J. & Zech, W. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—a review. *Biol Fertil Soils* 35, 219–230 (2002).

4. Lehmann, J. Bioenergy in the black. *Front Ecol Environ* 5, 381–387 (2007).

5. Zhou, Z. J. et al. Biodegradation of a biochar-modified waterborne polycrylate membrane coating for controlled-release fertilizer and its effects on soil bacterial community profiles. *Environ Sci Pollut R* 22, 8672–8682 (2015).

6. Atkinson, C. J., Fitzgerald, J. D. & Hipp, N. A. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant Soil* 337, 1–18 (2010).

7. Sohi, S. P., Krull, E., Lopez-Capel, E. & Bol, R. A review of biochar and its use and function in soil. *Adv Agron* 105, 47–82 (2010).

8. Graber, E. R. et al. Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. *Plant Soil* 337, 481–496 (2010).

9. Zackrisson, O., Nilsson, M. C. & Wardle, D. A. Key ecological function of charcoal from wildfire in the Boreal forest. *Oikos* 77, 10–19 (1996).

10. Pietikäinen, J., Käikkö, O. & Fritz, H. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. *Oikos* 89, 231–242 (2000).

11. Steinbeiss, S., Gleixner, G. & Antonietti, M. Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biol Biochem* 41, 1301–1310 (2009).

12. Lehmann, J. et al. Biochar effects on soil biota - a review. *Soil Biol Biochem* 43, 1812–1836 (2011).

13. Deenik, J. L., McClellan, T., Uehara, G., Antal, M. J. & Campbell, S. Charcoal volatilite matter content influence plant growth and soil nitrogen transformations. *Soil Sci Soc Am J* 74, 1259–1270 (2010).

14. Dempster, D. N., Gleeson, D. B., Solaiman, Z. M., Jones, D. L. & Murphy, D. V. Decreased soil microbial biomass and nitrogen mineralisation with Eucalyptus biochar addition to a coarse textured soil. *Plant Soil*. doi:10.1007/s11104-011-0987-5 (2011).

15. Spokas, K. A., Baker, J. M. & Reicosky, D. C. Ethylene: potential key for biochar amendment impacts. *Plant Soil* 333, 443–452 (2010).

16. Ussiri, D. A. N. & Lal, R. Carbon sequestration in reclaimed minesloos. *Critt Rev Plant Sci* 24, 151–165 (2005).

17. Edgar, R. C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26, 2460–2461 (2010).

18. Khodadad, C. L. M., Zimmerman, A. R., Green, S. J., Uthandi, S. & Foster, J. S. Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. *Soil Biol Biochem* 43, 385–392 (2011).

19. Su, P. et al. Taxon-specific responses of soil microbial communities to different soil priming effects induced by addition of plant residues and their biochars. *J Soil Sediment* 1–11, doi:10.1007/s11368-015-1238-8 (2015).

20. Carson, J. K., Campbell, L., Rooney, D., Clipson, N. & Gleeson, D. B. Minerals in soil select distinct bacterial communities in their mineralisation with Eucalyptus biochar addition to a coarse textured soil. *Plant Soil*. doi:10.1007/s11104-011-0987-5 (2011).

21. Griffiths, R. J. et al. The bacterial biogeography of British soils. *Environ Microbiol* 13, 1642–1654 (2011).

22. Lauber, C. L., Hamady, M., Knight, R. & Fierer, N. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microbiol* 75, 5111–5120 (2009).

23. Shen, C. C. et al. Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. *Soil Biol Biochem* 57, 204–211 (2013).

24. Chiu, H. Y. et al. Soil bacterial diversity in the Arctic is not fundamentally different from that found in other biomes. *Environ Microbiol* 12, 2998–3006 (2010).

25. Jones, R. T. et al. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. *ISME J* 3, 442–453 (2009).

26. Männisto, M. K., Tirola, M. & Häggblom, M. M. Bacterial communities in Arctic fields of Finnish Lapland are stable but highly pH dependent. *FEMS Microbiol Ecol* 59, 452–465 (2007).
27. Chan, K. Y., Van Zwieten, L., Meszaros, I., Downie, A. & Joseph, S. Using poultry litter biochars as soil amendments. Aust J Soil Res 46, 437–444 (2008).
28. Sun, D. Q. et al. Effect of volatile organic compounds absorbed to fresh biochar on survival of Bacillus mucilaginosus and structure of soil microbial communities. J Soil Sediment 15, 271–281 (2015).
29. Thies, J. E. & Rillig, M. C. Characteristics of biochar: biological properties. In: Lehmann, J., Joseph, S., editors. Biochar for Environmental Management. Science and Technology London: Earthscan, pp. 85–105 (2009).
30. Gopalakrishnan, S., Srinivas, V., Prakash, B., Satya, A. & Vijayaraghavan, R. Plant growth-promoting traits of Pseudomonas geniculata isolated from chickpea nodules. Biotech 5, 653–661 (2015).
31. Liu, H. et al. Characterization of a phenazine producing strain Pseudomonas chlororaphis GP72 with broad spectrum antifungal activity from green pepper rhizosphere. Curr Microbiol 54, 302–306 (2007).
32. Yu, F. B., Shan, S. D., Luo, L. P., Guan, L. B. & Qin, H. Isolation and characterization of a Sphingomonas sp. strain F-7 degrading fenvalerate and its use in bioremediation of contaminated soil. J Environ SCI Health B 48, 198–207 (2013).
33. Steiner, C., Das, K. C., Garcia, M., Forster, B. & Zech, W. Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol. Pedobiologia 51, 359–366 (2008).

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Conceived and designed the experiments: G.M.H., Performed the experiments: G.M.H., J.Y.L. and Q.Q.C. Analyzed the data: G.M.H., C.Y. and Q.Q.C. Contributed reagents/materials/analysis tools: G.M.H. and C.Y. Contributed to the writing of the manuscript: G.M.H. All of the authors reviewed the manuscript.

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