Effects of biochar on bacterial genetic diversity in soil contaminated with cadmium

Zhang Qiu | Zhang Yinghua | Zhang Xiu | Shi Jing

Abstract
Biochar has various ecological effects on heavy metal-contaminated soils. Biochar can be used to passivate the activity of heavy metals and improve environmental conditions for microbial growth. A greenhouse pot experiment was conducted to explore the diversity of microbes in red soil under cadmium (Cd) stress following the application of wheat straw biochar. The contaminant Cd was prepared at 2.5 mg kg⁻¹ with deionized water to simulate the heavy metal pollution of red soil under natural conditions, and the proportions of wheat straw biochar used were 2.5% and 10%. The complete genetic diversity of red soil bacteria in this study was evaluated using high-throughput sequencing. The results showed that the bacterial genetic diversity of Cd-contaminated red soils was restored with biochar treatment, and recovery with 2.5% biochar was significant. Biochar significantly affected the richness of soil bacteria by 6.79%–21.04%. Forty-three phyla of bacteria, including Proteobacteria, Acidobacteria and Gemmatimonadetes, constituted the bacterial community in the red soils. Further principal component analysis showed that Cd pollution and biochar application collectively affected the bacterial genetic diversity. Hydrogenophaga, Rubrivivax, Haliscomenobacter, Citrobacter, Methylibium and Azospirillum were indicator strains for Cd-contaminated red soils, while Steroidobacter, Bradyrhizobium, Anaerolinea, Chloronema Dubinina and Gorlenko were key strains for the biochar remediation of Cd contamination. In conclusion, for soil polluted with 2.5 mg kg Cd⁻¹, the application of 2.5% wheat straw biochar significantly increases soil microbial abundance and genetic diversity and plays an active role in improving the soil micro-ecosystem.

KEYWORDS
heavy metal pollution, micro-ecosystem, red soils, wheat straw
1 | INTRODUCTION

The heavy metal cadmium (Cd) has a toxicity that threatens the health of the environment and endangers the lives of humans and animals. Cd pollution can result in a decline in cultivated land quality, poor crop growth and development, and reduces large-scale grain yield. Heavy metal pollution in China is typified by Cd, but a wide range of heavy metals pollute large areas of the country and have resulted in huge economic losses. A national survey of soil pollution found that a large amount of cultivated land, forest land, grassland and unused land has Cd concentrations above natural levels, and it is urgent that we explore methods for remediating Cd pollution (Shi et al., 2011). Because of ecological development drives, the remediation of heavy metal pollution has become a focus of interest in the academic field (Van, Ainsworth, & Maeseleele, 2018). Previous studies into the remediation of pollution have used various mitigating substances, such as lime, zeolite, sepiolite and phosphate, which were successful in reducing the effects and migration of heavy metals (Xie, Liang, & Meng, 2014; Cheng, Li, & Gao, 2018). Currently, biochar, which is made from organic biological materials, such as crop straw, animal manure and biological waste, is attracting scientific attention because of its toxic-mediation characteristics. Studies have shown that biochar can adsorb and immobilize Cd, reducing the risk of Cd migration through soil and even reducing Cd bioavailability (Wang, Hu, et al., 2020). Biochar has been highly beneficial for improving soil fertility, reducing atmospheric emissions, changing soil microbial activity and recycling energy (Palansooriya et al., 2019). Therefore, the use of biochar as an in situ passivation agent for Cd could lead to additional ecological and economic gains.

Soil microbes are one of the most sensitive indicators of soil health and fertility, as various soil characteristics are important for the growth and reproduction of these organisms. When the heavy metal pollution of soil reaches a certain threshold, there are changes to the soil properties, which are reflected in the physiology and ecology of soil microorganisms. A study found that Cd pollution inhibited the growth of soil bacteria and reduced the diversity of soil microbes, and there was a highly significant positive correlation between the degree of inhibition and the concentration of pollutant (Zhao, Zhao, & Yin, 2014). The addition of biochar to soil can relieve the stress effect of Cd pollution and increase the activity of soil microorganisms (Zeng et al., 2020). However, there have been few studies into changes in soil microbiological diversity during the interactions between biochar and heavy metals. The results of a Biolog ECO plate study showed that biochar can improve the diversity of microbial carbon metabolism in the rhizomes of the tea plant (Saqib, Qaiserl, & Muhammad, 2018). PCR-denaturing gradient gel electrophoresis experiments showed the distribution of a large number of bacterial populations after the application of biochar to soil, and the abundance of bacteria increased (He, Yang, & Zhong, 2014). However, there are limitations to these two methods for analysing soil microorganisms: a limited amount of data can be measured, and they cannot reflect large-scale changes in the soil microbial community and diversity. In contrast, high-throughput sequencing methods can provide a more complete picture of the composition and distribution of soil microbial communities because of their speed and the large amounts of data obtained. High-throughput sequencing (Chen, Wang, & Chen, 2016) has been used to show that biochar improves the diversity of soil microbes: the abundance of the phyla Actinomycetes and Microphyllum verruca increased, while Acidobacteria and Microphyllum verruca decreased. Currently, high-flux sequencing methods are being used to study soil microbial abundance after biochar decontamination of heavy metals, such as Cd, and soil microbe diversity is decreasing in polluted soils. We hypothesized that adding biochar can increase the abundance and genetic diversity of bacteria in the Cd-contaminated soil.

Therefore, the objective of this study was to reveal changes in microbial diversity and abundance in soil under Cd stress following the application of different proportions of biochar (2.5% and 10%).

2 | MATERIALS AND METHODS

2.1 | Soil samples

The red soil used for the pot experiment was collected from a mountain at Yunnan Agricultural University (25°08′11.81″N, 102°45′3.27″E) in June 2018. There are no sources of heavy metal pollution near the sampling site, and the soil is not contaminated with heavy metals. The basic physical and chemical properties of the soil are detailed in Table 1.

2.2 | Biochar

The study material was bought from the Henan Sanli Biochar Production Company. The basic physical and chemical properties of the material are provided in Table 2.

2.3 | Experimental design

The experiment was conducted in a plastic bucket (height of 19 cm, diameter of 10.5 cm) containing 8 kg of soil. A CdCl2·2.5H2O solution was prepared as described in a
The straw biochar was added to the above mixed soil to mass ratios of 0, 2.5 and 10%. In a previous study, the Cd form and soil enzyme activity did not change significantly after the application of 5% biochar (Shang, Wang, & Shi, 2015); thus, it is possible that this proportion of biochar has no significant effect on soil microorganisms under the test conditions. On this basis, only 2.5% and 10% biochar were used in this experiment. The experiment was divided into two groups: for the blank control group, we used common soil without biochar, and the other group received one of four treatments: 0 mg kg⁻¹ Cd and 0% biochar (CK), 2.5 mg kg⁻¹ Cd and 0% biochar (B0), 2.5 mg kg⁻¹ Cd and 2.5% biochar (B2.5), and 2.5 mg kg⁻¹ Cd and 10% biochar (B10). Each treatment was run in triplicate.

N (0.25 g kg⁻¹), P₂O₅ (0.5 g kg⁻¹) and K₂O (0.5 g kg⁻¹) were applied simultaneously as base fertilizers. The soil samples were kept in a greenhouse in a natural state, and Cd solution was added to the undisturbed soil for 7 days. Then, the biochar was added to the undisturbed soil for 30 days so that the pot experiment mimicked the conditions of contaminated soil in the environment. Three to four rice seedlings (Yunjing 37) were cultivated per bucket of soil during this time, and the soil moisture was stabilized at 70% of the field water holding capacity. Routine management was performed during rice plant growth, and when the rice grew to maturity, the plants were subjected to drainage and aeration. The plants were aerated by adjusting the ventilation of the facility and increasing the spaces between the potted plants to 0.5 metres per row. The five-point method was used to collect soil samples using the following steps: for the regular land sample, the midpoint of the diagonal was determined first, which was used as the central sampling point. We then selected four additional sample points equidistant on the diagonal, resulting in a total of five sample points. The five-point sampling method was only used for collecting soil samples. Each treatment was repeated three times according to the standard operation and statistical methods for this experiment. The data variability indicated the probability of a significant difference (p < .05). The treated soil samples were taken from a vertical depth of 0–15 cm; rice roots and stones were removed from the samples; and the samples were stored for further extraction, detection, PCR amplification and sequencing of the total DNA of soil microorganisms.

2.4 Extraction and detection of total DNA from soil microbes

Total soil microbial DNA was extracted using the Power Soil TM DNA Isolation Kit (Mo Bio). A 0.3 g soil sample was weighed, and DNA was extracted according to the kit
instruction manual. Then, 0.8% agarose gel electrophoresis was used to confirm the presence of clear and complete DNA bands.

2.5 | PCR amplification and sequencing

The universal primers, 341F/806R, were used to amplify the V3–V4 region of the bacterial 16 Sr DNA gene sequence. The sequences of the primer were 341F (forward): 5′-CCTAYGGGGBGCASCAG-3′ and 806R (reverse): 5′-GGACTACNNGGGTATCTAAT-3′. The 50 µl PCR amplification mix included 10 µl of Fly Buffer, 5 µl of dNTPS, 1 µl of upstream primer, 1 µl of downstream primer, 1 µl of DNA template, 1 µl of Premix Taq Polymerase (Takara Bio, USA) and 31 µl RNase-free water; some of the reagents used were from Qiagen kits. The following PCR amplification conditions were used: 1 min at 98℃, 10 s at 98℃, 30 s at 50℃ and 60 s at 72℃ for a total of 30 cycles, followed by 5 min at 72℃. DNA fragments were recovered from the gel by 2% agarose electrophoresis of PCR amplification products. The DNA was stored at −20℃, and an aliquot was sent for DNA sequencing by the NOA Source Bio Information Company using the Illumina MiSeq platform.

2.6 | Sequencing data analysis

After sequencing the downstream data and removing truncated barcode and primer sequences, we merged the sequencing data using FLASH (V1.2.7). QIIME (V1.7.0) software filtering was used to obtaining high-quality clean tags. The clean tags were compared with two GOLD databases, and the UCHIME algorithm was used to detect chimera sequences to obtain the effective tags. UPARSE software (V7.0.1001) was used to create clusters, or operational taxonomic units (OTUs), from the effective tags with more than 97% similarity. The Ribosomal Database Project (RDP) classifier (V 2.2) software was used with the Green Gene database for systematic sequence classification and taxonomy information for the soil bacteria. A rarefaction curve was drawn based on QIIME software. The sequence data were analysed for alpha diversity, including the Chao1 and ACE richness indexes, the Shannon and Simpson diversity indexes, and Good's coverage index.

3 | RESULTS

3.1 | Bacterial sequencing information

The sequencing information and the number of OTUs for the V3 to V4 region of the bacteria in the four soil samples are shown in Table 3. According to the analysis, 191,129 original sequences were sequenced. After filtering the low-quality sequences and chimeras, the remaining 146,345 effective sequences were clustered at 97% similarity to generate 7,528 OTUs.

The rarefaction curve directly reflects the amount of sequencing data and indirectly reflects the abundance of species in the samples. The data shown in Figure 1 (data represent the medians), the rarefaction curve for the four soils increased slightly, but generally tended to stabilize; therefore, most of the soil bacterial groups were detected. Since the sequencing data covered a large number of species in the sample (OTUs), further sequencing could only produce a small number of new species; therefore, high-throughput sequencing reflected the whole population structure and components of the soil bacteria.

### Table 3

| Sequence | Sums of Original data | Sums of valid sequential data | Sums of OTU amount |
|----------|-----------------------|-------------------------------|-------------------|
| CK       | 49,617                | 37,122                        | 1,820             |
| BO       | 42,044                | 32,149                        | 1,794             |
| B2.5     | 55,124                | 43,411                        | 2,067             |
| B10      | 44,344                | 33,663                        | 1,847             |
groups, whereas the Shannon and Simpson indexes evaluated the soil bacterial community diversity, as shown in Table 4. The ACE and Chao1 and the Shannon index all showed a trend of B2.5 > B10 > CK > B0 for the four treatments. The B0 group, treated with Cd only, had the lowest abundance and diversity compared with the CK blank control and experimental groups, indicating that Cd pollution decreased both the abundance and diversity of the soil bacteria. The changes in the Simpson index were not significant, but all four indexes peaked for the B2.5 treatment. Compared with the B0 treatment group, the B2.5 treatment group’s ACE, Chao1, Shannon and Simpson indexes increased by 21.04, 20.76, 2.37 and 0.01%, respectively, whereas the four indexes of the B10 treatment group increased by 6.79%, 8.22%, 0.54% and 0.00%, respectively. Therefore, the biochar had a recovery effect on the soil bacteria strains stressed by Cd. The recovery effect of the B2.5 treatment was significant compared with the two biochar treatments; however, the recovery effect of the B10 treatment group was not obvious. According to the four indexes, biochar had a substantial influence on the abundance of bacterial populations in the Cd-contaminated soil, but the effect on bacterial diversity was negligible. The coverage index of each treatment was over 99%, indicating that the sequencing coverage rate was high; therefore, the data provided an accurate reflection of the soil bacterial diversity. This was consistent with the results of the dilution curve.

3.3 | Analysis of soil bacterial community structure characteristics under different treatments

3.3.1 | Distribution of OTUs of bacterial communities under different treatments

The correlation and overlap of the OTU distribution of the four treated samples are presented in the Venn diagram in Figure 2. The total amount of OTUs for the four treatments follows a trend of B2.5 > B10 > CK > B0. Of the treatments, 75 OTUs were specific to the CK and B10-treated groups, and 94 and 174 OTUs were specific to the B0 and B2.5 treatment groups, respectively. The amount of specific OTUs in the B0 group (Cd only) was greater than those of the CK blank control, implying that Cd may promote the growth of some Cd-resistant bacterial groups in the contaminated soil. The B2.5 group had the most specific OTUs, which suggests that the biochar concentration boosted the growth of some biochar-producing bacterial groups. The four processes had a total of 1,154 OTUs, accounting for 15.33% of the total OTUs, indicating that these OTUs were related to bacterial populations in the soil that were not stressed by Cd heavy metal pollution and the biochar did not play a protective role.

3.3.2 | Distribution of phylum and genus components of the bacterial community under different treatments

Forty-three bacterial phyla were detected from four soil samples. The 10 most common species were selected to analyse abundances at the phylum classification level, and a relative abundance column accumulation map of species was generated. The distribution and proportion of soil bacterial phyla at the classification level are shown in Figure 3. The 10 strains with the highest relative abundance accounted for 93.53%, 91.14%, 95.01% and 93.92% of the total bacteria in the CK, B0, B2.5 and B10 treatments, respectively. This demonstrated that the genetic diversity of soil bacteria was reduced under Cd toxicity, and there was a mitigating effect after addition of the biochar. The remission of the B10 group was not obvious, while remission for the B2.5 group was highly significant. The dominant bacteria in the four soil treatments were of the phyla of Proteobacteria, Acidobacteria and Gemmatimonadetes and accounted for more than 71% of the total strains. In particular, the Proteobacteria represented over 50% of strains. In contrast, the Bacteroidetes (3.29%–7.79%), Actinobacteria (4.42%–7.23%), Chloroflexi (3.64%–4.44%) and Cyanobacteria (0.79%–3.55%) were suboptimal strains in the bacterial community. According to the analysis of the proportion of bacteria in each group, the trend for the four treatments with regard to the phyla Pleurotus and Chloroflexi was B2.5 > B10 > CK > B0; for the anhydride bacteria, it was B0 > CK > B10 > B2.5, while for the actinomyces, it was B2.5 > B10 > B0 > CK. The results show that the structures of the bacterial communities were similar in each treatment, but the group proportions were distinct.

A total of 215 bacterial genera were detected in the four soil samples. To further explore the evolution of the bacterial community during the processes of heavy metal pollution and biochar mitigation, the top 40 most abundant genera were selected. A heat map was constructed in which the difference between each sample was represented by different colours, clearly outlining which species were distributed in the four treatment samples (Figure 4). The dominant genera under the four treatments were Geobacter (1.08%–2.23%), Acinetobacter (0.23%–1.96%), Nitrospira (1.03%–1.82%), Anaeromyxobacter (1.21%–1.82%), Rhodoplanes (0.66%–1.76%), Kastobacter (0.55%–1.38%) and Steroidobacter (0.59%–1.20%). There were obvious differences in the community structure and distribution of soil bacteria under the different treatments, and some patterns emerged. Compared with the other the three treatment groups, B0 had the highest abundance of Hydrogenophaga, Rubrivivax, Haliscomenobacter, Citrobacter, Methylibium and Azospirillum, indicating that these six genera could
adapt to the Cd-polluted environment and may be Cd-resistant. Compared with the blank group, Steroidobacter, Bradyrhizobium, Anaerolinea, and Chloronema abundance decreased in group B0 (Cd-only treatment), but abundance increased for the two biochar groups. The results show that the four bacteria were significantly affected by biochar remediation of the Cd-contaminated soil. Changes in the four bacteria suggest they might be the major strains controlling the biochar remediation of Cd-contaminated soil.

In the principal component analysis (PCA) of bacterial community structure and composition, the data for the four treatments, CK, B0, B2.5 and B10, were processed by Analyze–Dimension Reduction–Factor in SPSS software, which was used for comparing the similarities among the soil bacterial community structure and composition under the four treatments. The smaller the distance between the sample coordinates, the greater the similarity of the community structure. As shown in Figure 5, the PC1 contribution rate was 36.41%, the PC2 contribution rate was 33.50%, and the cumulative contribution rate reached 69.91%. The four treatments clearly differentiated on the PCA axis. Both the addition of Cd and the application of biochar affected the bacterial community structures. The CK, B10 and B2.5 treatment groups were located on the positive axis of PC2, while B0 was located on the negative axis and had the greatest distance and most discrete data compared with the other three treatment groups. The difference between the Cd-only treatment and the other treatments was remarkable. CK was located on the positive axis of PC1, B2.5 was on the negative axis, and B10 was placed between the two. The discrete distance between the B10 and B2.5 groups was relatively large; in comparison, the CK group had a smaller discrete distance, and there was a high degree of similarity in community structure.
and composition between B10 and the CK control group. In summary, Cd pollution changed the soil bacterial community structure and diversity, and a biochar content of 2.5% had a more significant effect than that of 10% on the structure of the bacterial community under Cd stress.

4 | DISCUSSION

Bacteria are the most abundant and widely distributed soil microorganisms; they play important roles in the formation of the soil, the material cycle and the evolution of fertility, and bacteria abundances are constrained by subtle factors within the ecological environment (Wang, Hu, et al., 2020). There have been few studies on the trends in the variability and regularity of soil bacterial genetic diversity during the remediation of Cd heavy metal pollution with biochar. The results of this study show the effects of four Cd/biochar treatments on the abundance and diversity indexes of bacteria. The number of OTUs and the abundance of community polygenes in the soil increased in the following manner: 2.5 mg kg Cd⁻¹ and 2.5% biochar > 2.5 mg kg Cd⁻¹ and 10% biochar > 0 mg kg Cd⁻¹ and 0% biochar > 2.5 mg kg Cd⁻¹ and 0% biochar. Previous studies by this research group into carbon metabolism also showed that, when biochar was applied to Cd, the soil microbial diversity for the four treatments showed the same trend as described herein (Zhang, Shang, & Shi, 2016). The results show that Cd leads to a change in the bacterial community structure, and the genetic diversity of the bacteria significantly reduced under Cd heavy metal stress. Different mass fractions of biochar had different effects on community recovery. The most significant recovery effect was observed at a 2.5% low-mass fraction of biochar. Changes in the bacterial community structure and diversity are closely related to

**FIGURE 4** Heat map of the bacterial community of red soil at genus level under different treatments [Colour figure can be viewed at wileyonlinelibrary.com]
the physical and chemical properties of the soil, such as pH, organic matter, nitrogen, phosphorus and potassium levels, and urease and oxidoreductase activities (Zhou et al., 2020). Heavy metals and other soil pollutants are also influencing factors (Liu et al., 2018). The application of biochar effectively improved the physical and chemical properties of the red soil, and the effect increased with increasing biochar proportions. However, high levels of biochar lead to a decrease in soil microbes (Yang et al., 2017). Therefore, the structure and diversity of the soil microbial community are not directly proportional to the improvement of the physical and chemical properties of soil via biochar (Shang, Wang, & Shi, 2015). Levels of urease in Cd-contaminated soil are sensitive to biochar, and the highest amount of oxidoreductase was observed for 2.5% biochar. Some studies have shown that the amount of biochar is not directly proportional to other passivation effects on Cd. Under 1% low-mass fraction, the effect of Cd passivation is remarkable and bioavailability is clearly reduced (Wang, Cang, & Yu, 2015). In Wang et al.’s study, there was no linear relationship between the amount of biochar and the recovery effect on soil microbial genetic diversity, which may be because of the improvement in the physical and chemical properties of red soil and the promotion of some enzyme activities. The 2.5% mass fraction of Cd was more suitable for microbial growth and reproduction than the 10% mass fraction of biochar in the three aspects of Cd fixation. The high similarity in the community structure and composition between the 2.5 mg kg Cd⁻¹ and 10% biochar group and the blank control according to the PCA was also attributed to the above reasons.

Analysis indicated that the community structure of red soil was similar under the four different treatments. The dominant phyla were strains of Proteobacteria, Anaeromonas, Buomonas, Bacteroides, Actinomycetes, green bacteria and Cyanobacteria, of which Proteobacteria were present in the highest abundance. An studied the distribution of microorganisms in irrigated grey desert soil and reported that the main bacterial groups were Proteobacteria, Acidobacteria, Bacillus and Actinomycetes (An et al., 2019), which is similar to the pattern found in this study. However, there are differences in the dominant groups, which may be due to the addition of Cd and biochar to the soil. Wei conducted high-throughput sequencing analysis of three representative desert vegetation soils from the Junggar Basin, and Actinomycetes, Proteobacteria, Campylobactercoli, Acidobacteria, Bacillus and Bacillus Bacteroides were the main constituents of the soil bacterial community (Wei et al., 2020). The abundance of Anhydride, a kind of acido-philic bacterium that thrives in poor nutrient environments, negatively correlates with soil pH (Tian et al., 2013), while the abundance of actinomycetes positively correlates with soil pH (Liu, Wang, & Liu, 2015). The red soils tested were acidic and nutrient-deficient, whereas biochar is alkaline. Hence, the pH and nutrient content of the soil increased after the biochar was added, and the abundance of anhydride decreased while that of actinomycetes increased. A study has shown that, as one of the components of activated sludge, green bacteria can promote the degradation of toxic substances in soil (Chen, Chen, & Liang, 2015). The abundance of green bacteria decreased in Cd-contaminated soil but increased after the application of biochar, possibly because of an increase in the Cd-degrading bacterial community under Cd passivation. Analyses of the influence of community structure and composition on the level of soil bacteria showed that the abundance of Hydrogenophaga, Rubrivivax, Haliscomenobacter, Citrobacter, Methylibium and Azospirillium was highest in Cd-contaminated soil. Previous studies have shown that soil microbes adapt to polluted environments by changing the microbial community structure among the aggregates (Zhu et al., 2020). As a result, soil microbial diversity does not decrease with an increase in heavy metal pollution. Moderate pollution levels were found to stimulate the growth of some heavy metal-tolerant microbes and increased the diversity of soil microorganisms (Zhu et al., 2020). The amount of Cd added to the current experiment was 2.5 mg kg⁻¹, which simulated low-to-medium concentrations of heavy metal pollution. This level of toxicity stimulated an increase in the abundance of the above six Cd-resistant dominant species, and these species can be regarded as indicator strains for Cd heavy metal soil pollution. Different doses of radiation resulted in differences between the dominant soil strains (Zhang, Gu, & Wang, 2016), and these dominant bacteria could be used as indicators for the corresponding contaminated areas, similar to the strains in this study. Compared with the blank control group, the abundances of Steroidobacter, Bradyrhizium,
Anaerolinea and Chloronema initially increased and then decreased in the B0 group and two biochar groups. It is thought that these four bacteria were involved in the biochar repair of Cd-contaminated soil. Gemmatimonas strains of Gemmatimonadaceae play a crucial role in soybean and corn remediation of Cd-contaminated soil (Ding, Zhen, & Ren, 2016), which is in accordance with the conclusion of this study. However, the different reagents used resulted in different strains. Analyses indicated that biochar in Cd-contaminated soil has a significant influence on the abundances of the bacterial populations; however, there was no obvious effect on bacterial diversity. The mechanisms behind this phenomenon are not clear, as the response mechanisms of soil microorganisms to biochar under conditions of exogenous Cd are complex. In addition, this research only focused on the 16 Sr DNA genetic diversity of soil bacteria, which is insufficient to determine the complete population structure and diversity of soil microorganisms. The soil microbial complex also includes fungi and actinomycetes; therefore, future research should focus on these microbes using the 18 Sr DNA internal transcribed spacer high-throughput sequencing method. This will facilitate further comprehensive analysis of changes in the soil microbial gene diversity under heavy metal Cd inactivation with biochar. Finally, whether or not the application of 2.5% wheat straw biochar in the pot experiment could be used to restore soil under the same degree of Cd soil pollution in mining areas is uncertain; further field trials are needed.

5 | CONCLUSION

The application of biochar to Cd-contaminated soil can improve microbial abundance and genetic diversity as well as significantly alleviating stress. The effect of adding biochar at a mass fraction 2.5% was remarkable in all treatments, especially on the four strains Steroidobacter, Bradyrhizobium, Anaerolinea and Chloronema, the statuses of which substantially changed; variations in the suggested indicator microbe strains can be used to monitor restoration.

DATA AVAILABILITY STATEMENT

Data available on request from the authors. The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Zhang Yinghua [1] https://orcid.org/0000-0002-1045-0123

REFERENCES

An, M. J., Wang, K. Y., Wang, H. J. & Yulian E., & Meng, C. M. (2019). The effect of remediation agents on the soil microbial diversity in lead and cadmium polluted cotton fields [J]. Soil, 51(3):541–548.

Chen, D. M., Chen, X. M. & Liang, Y. J. (2015). Influence of crop rotation on soil nutrients, microbial activities and bacterial community structures [J]. Acta Prataculturae Sinica (in Chinese), 24(12):56–65.

Chen, Q. R., Wang, C. J. & Chen, X. (2016). Effect of tobacco stalk-derived biochar on microbes in rhizosphere soil at red paddy fields [J]. Fu Jian Journal of Agricultural Sciences (in Chinese), 31(2):184–188.

Cheng, J. Z., Li, Y. L. & Gao, W. C. (2018). Effects of biochar on Cd and Pb mobility and microbial community composition in a calcareous soil planted with tobacco [J]. Biology and Fertility of Soils, 54(3):373–383.

Ding, C. Y., Zhen, Y. & Ren, X. M. (2016). Changes in bacterial composition during the remediation of Cd contaminated soils of bioenergy crops [J]. Acta Scientiae Circumstantiae (in Chinese), 36(8):3009–3016.

He, L. L., Yang, H. M. & Zhong, Z. K. (2014). PCR-DGGE analysis of soil bacterium community diversity in farmland influenced by biochar [J]. Acta Ecologica Sinica (in Chinese), 34(15):4288–4294.

Liu, S. S., Fu, J. P., Cai, X. D., Zhou, J. M., Dang, Z. & Zhu, R. L. (2018). Research progress of the influence of heavy metal pollution on the ecological characteristics of soil microbes[J]. Journal of Eco-Environment, 27(6):1173–1178.

Liu, X., Wang, S. J. & Liu, X. M. 2015. (2015) Compositional characteristics and variations of soil microbial community in karst area of puding county, Guizhou province, China [J]. Earth and Environment (in Chinese), 43(5), 490–497.

Palansooriya, K. N., Wong, J., Tsz, F. & Hashimoto, Y. (2019). Response of microbial communities to biochar-amended soils: A critical review[J]. Biochar, 1(1):3–22.

Saqib, B., Qaiser, H. & Muhammad, A. (2018). Sugarcane bagasse-derived biochar reduces the cadmium and chromium bioavailability to mash bean and enhances the microbial activity in contaminated soil[J]. Journal of Soils and Sediments, 18(3), 874–886.

Shang, Y. J., Wang, H. B., & Shi, J. (2015). Effects of straw biochar on enzyme activity in Cd contaminated soil. Journal of Agricultural Resources and Environment (in Chinese), 32(1), 20–25.

Shi, Y., Cheng, C. W., Zhu, Y., Lei, P., Zhou, H. D. & Wen, T. J. 2011. National survey of soil pollution bulletin[J]. Agricultural Economics and Management, 2, 27–37.

Tian, D., Ma, X. & Li, Y. E. 2013. (2013) Research on soil bacteria under the impact of sealed CO₂ leakage by high-throughput sequencing technology[J]. Environmental Science (in Chinese), 34(10), 4096–4104.

Van, P. R., Ainsworth, J. & Maeseele, M. 2018. Chemical stabilization of Cd-contaminated soil using biochar[J]. Applied Geochemistry, 2018(88), 122–130.

Wang, W., Cang, L. & Yu, Y. C. (2015). Effects of the biochar application and water management on the rice growth and Cd accumulation [J]. Journal of Safety and Environment (in Chinese), 15(5), 310–314.

Wang, X. D., Hu, J., Li, W. J., Shang, K. L., Huang, C. H., Ji, W. H. & Chen, H. S. 2020. Effects of different biochar on soil Cd forms and plant absorption of Cd[J]. Energy Conservation and Environmental Protection, 2020(03), 50–52.

Wei, P., An, S. Z., Dong, Y. Q., Sun, Z. J., Berda, W. S. & Li, C. 2020. (2020). A high-throughput sequencing evaluation of bacterial diversity and community structure of desert soil in Junggar Basin [J]. Grass Industry Journal, 29(5), 182–190.
Xie, F., Liang, C. H. & Meng, Q. H. (2014). Effects of natural zeolite and lime on form transformation of cadmium in soil[J]. Chinese Journal of Environmental Engineering (in Chinese), 8(8), 3505–3510.

Yang, D. Y., Wang, X. M., Feng, H. P. & Xie, H. (2017). Effect of biomass carbon on rhizosphere microorganisms and soil enzyme activities of celery in facilities[J]. Guang dong Agricultural Sciences, 44(1), 82–87.

Zeng, X. J., Huang, X. P., Cheng, K., He, G. Q., Fu, Z. Q. & Zhao, X. Y. (2020). Effects of Lime Group with Organic Amendment on Microbial Activity of Farmland Lead and Cadmium Polluted Soil[J]. Environmental Science Research, 33(10), 2361–2369.

Zhang, X., Shang, Y. J. & Shi, J. (2016). Effects of biochar on carbon metabolic capacity and functional diversity of soil microbial communities under Cd contamination[J]. Journal of Agro-Environment Science (in Chinese), 35(7), 1308–1313.

Zhang, X., Xia, Y. S. & Shi, J. (2017). Effect of biochar (BC) on microbial diversity of cadmium (Cd) contaminated soil [J]. China Environmental Science (in Chinese), 37(1), 252–262.

Zhang, Z. D., Gu, M. Y. & Wang, W. (2016). Analysis of bacterial community in radiation polluted soils by high-throughput sequencing[J]. Microbiology China (in Chinese), 43(6), 1218–1226.

Zhao, Y. S., Zhao, Y. X. & Yin, K. (2014). Impact of Cd2+ in construction waste on microflora in soil [J]. Journal of Huaihai Institute of Technology (in Chinese) (Natural Sciences Edition), 23(4), 41–43.

Zhou, L. J., Lin, X. B., Wu, L., Huang, Q. R., Yu, Y., Zhang, H. Y., Guo, N. J., Zhang, Y. & Liu, H. (2020). Differences In physicochemical properties, microbial and enzyme activities of cadmium contaminated paddy [J]. Environmental Engineering, 1–8. http://kns.cnki.net/kcms/detail/11.2097.X.20200609.1133.018.html

Zhu, M. T., Liu, X. X., Wang, J. M., Liu, Z. W., Zheng, J. F., Bian, R. J., Wang, G. M., Zhang, X. H., Li, L. Q. & Pan, G. X. (2020). Effects of biomass carbon on microbial diversity of aggregates in paddy soil[J]. Acta Ecologica Sinica, 40(5), 1505–1516.

How to cite this article: Qiu Z, Yinghua Z, Xiu Z, Jing S. Effects of biochar on bacterial genetic diversity in soil contaminated with cadmium. Soil Use Manage. 2021;37:289–298. https://doi.org/10.1111/sum.12678