Biogas residue biochar shifted bacterial community, mineralization, and molecular structure of organic carbon in a sandy loam Alfisol

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Funding information
Guangdong Basic and Applied Basic Research Foundation, Grant/Award Number: 2019A15151110777; Natural Science Foundation of Shandong Province, Grant/Award Number: ZR2019BD062; National Natural Science Foundation of China, Grant/Award Number: 31901195; Chinese Academy of Agricultural Sciences, Grant/Award Number: ASTIP-TRIC, - and ZD01

ABSTRACT
Pyrolysis into biochar as a soil amendment has been treated as an eco-friendly and environmentally sustainable method to recycle biogas residue (BR). However, the effect of BR biochar on soil bacterial community, mineralization, and structure of organic carbon (OC) remains unrevealed, which limits the soil application of BR biochar. This study performed a microcosm incubation experiment for a sandy soil with BR and BR-derived biochar produced at relatively low (300°C) and high (600°C) temperature (BC300 and BC600), and explored the shift in bacterial community, mineralization, and structure of OC. Results showed that BR decreased the richness and diversity of bacterial community by 19.0%–28.0%, while BR biochar caused lower reduction (4.0%–7.0%), suggesting the potential of pyrolysis in mitigating the harmful effect of BR in bacterial community. Fourier-transform ion cyclotron resonance mass spectrometry and 13C nuclear magnetic resonance demonstrated that BR and BR biochar shifted dissolved OC toward components with 12.0%–26.0% higher molecular weight and 18.0%–21.0% more aromatics but 10.0%–22.0% lower polarity and less protein-, carbohydrate-, and tannin-like species, with the shift extent being stronger for BC600. Furthermore, BC600 increased the aromaticity of bulk OC but reduced the carbohydrate, possibly due to more Actinobacteria genera. Additionally, BR significantly elevated the amount and rate of soil carbon mineralization, while the potentially mineralizable C was the lowest in the BC600-treated soil. These findings suggested that the pyrolysis into biochar at high temperature would be a promising
1 | INTRODUCTION

Biochar, the product of pyrolysis of organic biomass, is characterized by abundant recalcitrant organic carbon (OC) and highly porous structure (Huang et al., 2018; Liu, Zheng, et al., 2020; Liu, Wang, et al., 2020). It has been demonstrated as a more cost-effective relative to activated carbon and eco-friendly soil amendment to improve soil carbon storage (Anyaoha et al., 2018; Kumar et al., 2017; Lian et al., 2020), ameliorate soil properties (Dunnigan et al., 2018), and enhance crop growth and productivity (Hale et al., 2020). Simultaneously, biochar can positively or negatively alter the composition and structure (Atkinson et al., 2010) as well as the network connectivity of soil bacteria (Huang et al., 2019), which mediates vast ecological processes (e.g., nutrient cycling [Balser & Firestone, 2005] and litter decomposition [Prescott, 2010]). The positive response of soil bacteria community to biochar amendment has been found to link with the following mechanisms: additional habitat provided by biochar's porous structure protected soil bacteria from predators (Quilliam et al., 2013); new supplement of consumption source from the labile carbon of biochar is favorable to bacteria metabolism (Gomez et al., 2014); adsorption of organic pollutants by biochar alleviates their toxic effect on the bacteria (Liang et al., 2017); the improvement of soil properties such as nutrient availability and pH buffering capacity is beneficial to bacteria growth (Liu et al., 2015). Meanwhile, possibly due to the introduction of toxic compounds (e.g., polycyclic aromatic hydrocarbons, polar pyrolysis condensates) that inhibited bacteria activity, biochar could also negatively change soil bacteria (Hale et al., 2012). Obviously, the shift direction and intensity of bacterial community is highly dependent upon the physicochemical properties of biochar which were primarily affected by feedstock type and pyrolysis temperature (Elkhalifa et al., 2019; Lian & Xing, 2017; Ronsse et al., 2013). Thus, a case-by-case evaluation of how soil bacteria responded to specific biochars is needed prior to large-scale soil application.

Biogas residue (BR), the final remnant of the original waste left in the digester, is constantly increasing due to the worldwide interest in disposing organic waste by anaerobic digestion to mitigate the fossil energy crisis (Ragauskas et al., 2006). Owing to the ability of BR to provide nutrients and improve soil structure, the most frequently disposal way is to use it as an organic soil amendment, to date (Arthurson, 2009). However, the foul odors, toxic organic compounds, pathogens and phytoxins of BR are potentially harmful to soil bacteria (Nkoa, 2014). In addition, BR amendment could result in more NH3 and N2O emission as well as higher NO3– leaching loss (Vallejo et al., 2006). Therefore, pretreatment of BR is essential before it is used as a soil amendment. The pyrolysis of BR into biochar has been treated as one of the most promising and eco-friendly treatments (Stefaniuk et al., 2016).

Many studies have revealed the application potential of BR-derived biochar, for example, efficiently sorbing pollutants (Sun et al., 2013) and NH4+ (Wang et al., 2017). However, the impact of BR biochar on the soil bacteria community remains unclear, which limits the field-scale application of BR-derived biochar. Bacterial communities play critical roles in regulating the decomposition and composition of soil organic carbon (SOC; Mitchell et al., 2015), which in turn affects the global carbon cycle (Schlesinger & Andrews, 2000) and soil fertility (Srinivasarao et al., 2012). For instance, Sun et al. (2020) stated that a statistical reduction in Sphingobacterium and an increase in Flavobacterium and Anerolinea induced by hydrochar application could increase aromatic compounds but decrease carbohydrates of native SOC. Anderson et al. (2011) observed that biochar shifted soil bacterial community to species of bacteria which preferred to degrade more recalcitrant SOC constituents. Besides the microbial driver, two other processes would also contribute to the structural alternation of SOC after biochar application. First, the “contamination” of native SOC with biochar-derived OC, which generally provides a higher amount of recalcitrant carbon and lower labile carbon than native SOC (Han et al., 2020). Second, the porous structure of biochar would help to retain some SOC fractions through pore-filling, thereby protecting these fractions being mineralized or lost (Pignatello et al., 2006). Obviously, all the above mechanisms were mediated by the characteristics of biochar which were strongly influenced by the pyrolysis temperature and feedstock type as aforementioned. By setting BR as the feedstock, it could be speculated that the change direction and intensity of SOC structure induced by biochar amendment as well as the dominant underlying mechanism would vary with the pyrolysis temperature of biochar. As reviewed in our previous study (Han et al., 2020), native SOC was different from the OC extracted from biochar, especially the high temperature biochar; the OC of high temperature biochar had lower atomic

KEYWORDS

bacterial community, biochar, biogas residue, carbon mineralization, waste recycling
H/C ratio and more abundant aromatic C than native SOC. In addition, high temperature biochar generally had higher porosity than the low temperature one, and thus would retain more native SOC (Zhang et al., 2019). Based on these analyses, we hypothesized that the high temperature biochar would more strongly shift the structure of native SOC.

To fill existing knowledge gaps and verify the hypothesis, we conducted a laboratory incubation experiment, in which biochars pyrolyzed from BR under 300°C and 600°C were applied to a sandy loam Alfisol. The main goals were to (1) explore the shift in bacteria community after BR biochar application; (2) reveal the changes in SOC molecular structure after BR biochar application; and (3) determine the dominant mechanism controlling SOC structure alternation after BR biochar application. Herein, solid-state 13C nuclear magnetic resonance (NMR) spectroscopy and high-resolution mass spectrum techniques of electrospray ionization coupled with Fourier-transform ion cyclotron resonance mass spectrometry (ESI-FT-ICR-MS) were adopted to explore the structure change of bulk SOC and DOC fraction, respectively. The findings will help to understand the role of BR biochar in carbon biogeochemical cycle in the soil and provide the basis for its application as soil amendments.

2 | MATERIALS AND METHODS

2.1 | Soil sampling

Soil sample was collected from a family farm located in Qingdao Municipality of Shandong Province, North China (36.73°N, 119.95°E). Wheat–maize rotation is the dominant cropping regime. The local climate is warm temperature monsoon with average annual precipitation and temperature of 680 mm and 11.9°C, respectively. Five sampling points were selected randomly before the collection of top 20 cm of soil using a shovel. Visible plant residue and rocks were hand-picked from the soil. The samples were air-dried, crushed, and passed through a 2-mm sieve. Then, a composited and homogeneous soil sample was obtained by manual mixing evenly and fully using hand. The soil texture was sandy loam and classified as an Alfisol under the USDA Soil Taxonomy System. The soil properties are summarized in Table 1.

2.2 | Biochar production

Details on BR preparation have been reported previously (Zheng et al., 2018). For biochar production, oven-dried BR samples were placed inside capped porcelain crucibles and loaded into a muffle furnace (KSL-1200X, Kejing Materials Tec.) under N2 (99.999%) saturated atmosphere (0.5 dm3 min−1 of flowing rate). The final furnace temperature was set at 300°C and 600°C, with heating rate and final residence time of 5°C min−1 and 60 min, respectively. Subsequently, after the reactor temperature cooled to below 100°C under N2 atmosphere, the biochar sample was collected. The biochars were crushed using a ceramic mortar and pestle, filtered through a 2-mm sieve. The biochars produced from 300°C and 600°C were denoted as BC300 and BC600, respectively. The selected properties of BR, BC300, and BC600 are presented in Table 1. Other basic properties have been detailed in the study of Zheng et al. (2018).

2.3 | Incubation experiment

For the incubation experiment, each microcosm was established by weighing 60 g air-dried soil into a 125-ml wide neck glass bottle. Four treatments were set up: soil only (CK), soil with BR (SBR), BC300 (SBC300), and BC600 (SBC600), respectively. Each treatment was conducted in triplicate. The rate of 4% (w/w, 0–20 cm) equivalent to 2.40 g BR or biochar per microcosm was applied. This rate was sufficient to alter the bacterial community and within the range of rates (0.05–8 wt%) adopted in the biochar incubation (Gao et al., 2019; He et al., 2016; Lin et al., 2017). Soil was thoroughly mixed with the BR or biochar. After mixing, 60% water-holding capacity was constantly maintained during the whole incubation by weighing and adding deionized water. Prior to the incubation, all the mixtures were pre-incubated at 22°C for 24 h to re-establish soil humidity equilibrium and recover the microbial activity. Thus, the soil bottles were put in an incubation cabinet at 22°C for 60 days. Previous studies have demonstrated that within 60 days, biochar addition significantly increased the richness and diversity of bacterial community, and reduced the relative abundance of carboxyl, alkyl, O-alkyl, and methoxyl groups by 20%–31% (Huang et al., 2019). The CO2 concentration of bottles was monitored at 1, 2, 3, 5, 8,
The bottles were covered by an airtight rubber stopper with a glass tube once the experiment began. The glass tube was sealed using a sealing cap. Subsequently, the headspace gas in each bottle was sampled at regular intervals using a 1.0-ml gas-tight locking plastic syringe through the sealing cap. After each sampling, the bottles were left open to maintain aerobic condition before sealing to prepare the next measurement. The start and end time for each interval were recorded. The CO₂ concentration was measured by a GC-2014 gas chromatograph (Shimadzu).

To assess the OC mineralization kinetics of soils, the cumulative amount of C mineralization (\(C_m\), mg CO₂-C kg⁻¹ soil) at time \(t\) was plotted against incubation time (\(t\), day) and a first-order kinetic model was fitted to the data using the Levenberg–Marquardt algorithm to obtain the kinetic parameters in Origin Pro 9.1 (Sarma et al., 2017): \(C_m = C_0 (1 - e^{-kt})\), (1)

where \(C_0\) is the potentially available C at time zero (mg C kg⁻¹ soil) and \(k\) (day⁻¹) is the mineralization rate constant.

### 2.4 Measurements of physicochemical properties of soil and biochar

Soil pH was analyzed in a soil to water ratio of 1:10 (w/v) using a pH meter (PB-10, Sartorous). The content of OC and total nitrogen of soil was determined by dry combustion via a vario EL III CN analyzer (Elementar). The specific surface area (SSA) of soil and biochar was determined by N₂ adsorption isotherms at 77 K after vacuum degassing for 4 h at 200°C using an ASAP 2020 M+C instrument (Micromeritics). The ash content of biochar was measured by heating samples at 760°C for 6 h under air atmosphere. To obtain the dissolved organic matter (DOM), about 3 g soil was weighed in a 50-ml centrifuge bottle, and then 15 ml ultrapure water was added. After shaking at 10 rpm for 48 h, the soil–water mixture was centrifuged at 4000 rpm for 20 min. The supernatant was collected, and filtrated using a 0.45-μm nylon membrane filter (Chen et al., 2016). Then the concentration of OC in the DOM (DOC) was obtained by a TOC analyzer (TOC-L, Shimadzu) with an ASI-L auto-sampler.

### 2.5 ESI-FT-ICR-MS measurement of DOC

ESI-FT-ICR-MS analysis was conducted with a Bruker Solari X FT-ICR-MS equipped with a 15.0 T superconducting magnet and a dual-mode ESI/MALDI ion source in order to get the information of molecular composition of DOC. Prior to analysis, solid phase extraction (SPE) was performed using Varian Bond Elute PPL cartridges to desalinate and concentrate the samples. More details on SPE treatment are provided in Data S1. Samples were then dissolved in the mixture of 0.5 ml methanol and 0.5 ml ultrapure water, and vortexed for 30 s. Subsequently, samples with same OC concentrations were continuously infused into the ESI unit by syringe infusion at a flow rate of 120 μL/h. Further details on ESI-FT-ICR-MS analysis and data processing are available in Data S1.

### 2.6 DNA extraction, 16S rRNA sequencing, and bioinformatic analyses

Total genomic DNA was extracted from the homogenized soil samples (0.5 g fresh soil per sample) by using the Fast® DNA SPIN Kit (MP Biomedicals) according to its protocols. The resulting DNA concentration was detected with a NanoDrop™ 2000 spectrophotometer (Thermo Scientific). The V4 region universal primers were selected to amplify the bacterial 16S rRNA gene (Caporaso et al., 2012). The PCR reaction system (25 μl) included 10x PCR buffer II (2.5 μl), AccuPrime DNA Polymerase (Invitrogen; 0.5 units), primers (0.5 mM) and DNA template (1 μl, approximately 10–20 ng of extracted soil DNA). The PCR reactions were conducted using the following thermocycling steps: initial denaturation at 94°C for 1 min, then 25 cycles at 94°C for 20 s, 53°C for 25 s and 68°C for 45 s, and a final extension at 68°C for 10 min (Liang et al., 2015). The PCR products were purified by using the Agarose Gel DNA Purification Kit (TaKaRa), and equal amounts of PCR products were mixed for each sample. MiSeq high-throughput sequencing platform was performed at Majorbio Bio Technology Co. Ltd., Shanghai, China.

The raw data were analyzed by QIIME software (v 1.9.0). After quality control, the resulting sequences were clustered into operational taxonomic units (OTUs) using the Closereg reference OTU selection method and the Greengene database for reference in QIIME (gg_13_5 version). The Chao 1, Shannon, and Simpson indices were chosen to describe alpha-diversity of different bacterial community. Principal coordinates analysis (PCoA) was used to calculate beta-diversity based on the Bray–Curtis distance for different treatments, which was performed in ape library of the R software package (version 3.1.2).

### 2.7 Solid-state \(^{13}\)C NMR analyses of SOC

The bulk OC chemistry of soil, BR, and biochar was determined by obtaining solid-state cross-polarization magic-angle-spinning \(^{13}\)C nuclear magnetic resonance (\(^{13}\)C CP-MAS NMR) with a Bruker AVANCE III HD 400 MHz wide bore
NMR spectrometer. Before doing the NMR analysis, the bulk soils were demineralized with 10% (v/v) of hydrofluoric acid (HF) to concentrate OC and remove paramagnetic ions (Rossi et al., 2016). Details of the demineralization are provided in Data S1. The demineralized soil was operated at a C frequency of 100.63 MHz and at a magic-angle-spinning (MAS) rate of 5.0 kHz. The instrument was run under the following conditions: contact time, 4 ms; acquisition delay, 2 s; number of scans, 2000–5000. The spectra were integrated as follows: 0–45 ppm (alkyl C), 45–63 ppm (methoxyl C), 63–93 ppm (carbohydrate), 93–148 ppm (aryl C), 165–190 ppm (carboxyl C) and 190–220 ppm (carbonyl C; Han et al., 2014). In addition, based on mass balance and assuming no cross-effect between soil and each amendment, the percentage of functional groups of the mixtures was also predicted as follows:

\[ A_{\text{amended soil}} = \frac{(C_{\text{ck}}A_{\text{ck}} + 0.04C_{\text{amendment}}A_{\text{amendment}})}{(C_{\text{ck}} + 0.04C_{\text{amendment}})} \]

where \( A_{\text{amended soil}} \) is the predicted value of functional group percentage of amended soils; \( A_{\text{ck}} \) and \( A_{\text{amendment}} \) are the functional group percentages of CK soil and each amendment, respectively; \( C_{\text{ck}} \) and \( C_{\text{amendment}} \) are the OC content of CK soil and each amendment, respectively; 0.04 is the application rate of each amendment in the soil.

### 2.8 Statistical analysis

Statistical analysis, including one-way analysis of variance, was performed with SPSS for Windows 20.0. Duncan’s multiple range test at the 5% level of probability was used to test the significance of differences between treatment means. Graphics were created using Origin Pro 9.1.

### 3 RESULTS

#### 3.1 Soil and biochar properties

As shown in Table 1, the soil under investigation was neutral with a pH of 7.2. Its OC content, TN, and C/N ratio were 9.2 g kg\(^{-1}\), 1.3 g kg\(^{-1}\), and 6.9, respectively. The basic properties of the three amendments were detailed in a previous study (Zheng et al., 2018). In brief, the OC, TN, SSA, and ash content were higher for BR biochar than BR, and were higher for 600°C biochar than 300°C biochar. The C/N ratio of BC300 (24.4) showed a higher value than that of BR (23.5) and BC600 (16.8). After addition to soils, all the three amendments increased TOC, TN, and C/N of soil, and it was in the order of SBC600 > SBC300 > SBR. However, the pH and SSA of soil remained almost unchanged.

#### 3.2 Shifts in the diversity, composition, and structure of bacterial community

After quality filtration, a total of 503,398 high-quality reads, ranging from 27,281 to 59,537 per sample, were identified from the soil samples. Based on 97% sequence similarity, the bacterial sequences were classified into 2735–3273 OTUs which belonged to 36 phyla, 131 classes, 379 orders, 696 families, and 1273 genera. In order to compare the difference of bacterial community among the treatments, an equal amount of OTUs were subsampled from each sample. Three diversity indices (Chao 1, Shannon, and Simpson index) and the number of observed OTUs were used to reveal the shift in bacterial community. As presented in Figure 1, both the number of OTUs and
Chao 1, as the richness index (Lin et al., 2014), were significantly lower in the SBR by 19.0%–28.0%, SBC300 and SBC600 compared with the CK ($p < 0.05$), and was higher in SBC300 and SBC600 compared to SBR ($p < 0.05$). However, there was no significant difference between SBC300 and SBC600 ($p > 0.05$). As the indicator of microbial community diversity, Shannon and Simpson index exhibited a similar variation pattern by the amendment of BR and BR biochar as the OTUs and Chao 1. These results suggested that BR and BR biochars reduced the richness and diversity of bacterial community, while BR biochar produced less reduction than BR, which was further verified by the PCoA analysis on the basis of the Bray–Curtis similarity distance. As seen from Figure 1, the horizontal and vertical axes explained 12.4% and 68.1% of the total community variation, respectively. The horizontal axes separated the bacterial communities of CK with that of SBR, SBC300, and SBC600, and the vertical axes separated the bacterial community of SBR with that of SBC300, SBC600, and CK.

BR and BR biochars also altered the relative abundance of microbial species assessed based on the numbers of 16S rRNA gene copies (Figure S1). Bacterial abundance of SBR was significantly higher than that of CK ($p < 0.05$), while no significant difference was observed among CK, SBC300, and SBC600 ($p > 0.05$). The bacterial community mainly comprised *Actinobacteria* (16.6%–30.4%), *Proteobacteria* (27.8%–33.0%), *Bacteroidetes* (12.1%–30.7%), *Chloroflexi* (3.9%–9.9%), *Planctomycetes* (5.5%–7.4%), and *Acidobacteria* (3.4%–5.5%) at the phylum level (Figure 1; Table S1). The relative abundance of most bacterial phyla in soil was shifted after the amendment of BR and BR biochars, to varying extents. For the biochar treatments, *Actinobacteria* was significantly higher in SBC300 and SBC600 ($p < 0.05$), with relative abundance increasing from 24.8% for CK to 29.6% for SBC300 and 30.4% for SBC600. However, the slight decrease in *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Planctomycetes*, *Acidobacteria*, and *Gemmatimonadetes* was seen, with the difference being insignificant ($p > 0.05$; except for *Chloroflexi* and *Gemmatimonadetes*). Regarding BR, the relative abundance of *Proteobacteria* and *Bacteroidetes* were significantly increased relative to the CK ($p < 0.05$), whereas that of *Actinobacteria*, *Chloroflexi*, *Planctomycetes*, *Acidobacteria*, and *Gemmatimonadetes* decreased ($p < 0.05$).

### 3.3 Shifts in the concentration and chemical composition of DOC

Compared to the CK, BR addition had no significant effect on the DOC content ($p > 0.05$), while BC300 and BC600 reduced the DOC content from 543.3 mg C/kg to 488.9 mg C/kg and 413.2 mg C/kg, respectively (Figure S2). Relative to BC300, BC600 produced a stronger reduction ($p < 0.05$), which might be partly related to the fact that BC600 had a particularly high SSA (131.4 m²/g) so that DOC fraction could be prevented through pore-filling mechanism from leaching or being used by organisms (Han et al., 2020; Pignatello et al., 2006). In addition to the DOC concentration, the analysis of ESI-FT-ICR-MS demonstrated the change in the molecular structure of DOC. As shown in Figure S3, the ESI-FT-ICR-MS negative-ion mass spectra of DOC in CK were mostly distributed in the mass range of 200–550 Da (>90%), and the MW$_w$ value was 329.5 (Table 2). After the amendment of BR, BC300, and BC600, the DOC shifted toward higher MW$_w$ with the shift extent being much stronger for SBC300 (407.5) and SBC600 (414.8). These data clearly revealed the MW$_w$ enhancement of DOC caused by biochar amendment. In addition, as seen from Table 2, the H/C$_w$ reduced from 1.10 to 0.94–0.95, while magnitude-weighted aromaticity index (AI$_w$) increased from 0.39 to 0.46–0.47 after the amendment of BR, BC300, and BC600, suggesting the enhancement of aromaticity of DOC. This result was also supported by the higher DBE$_w$ in treated soil, particularly SBC600. Besides aromaticity, the polarity of DOC was also altered. The O/C$_w$, as the index of polarity, declined from 0.51 for CK to 0.46 for SBR to 0.42 for SBC300 and to 0.40 for SBC600. The Van Krevelen diagram in Figure 2a–d clearly shows the alteration of molecular components of DOC. By using the element ratios of H/C and O/C, all the detected molecules displayed in the Van Krevelen diagram were divided into seven discrete groups (Hockaday et al., 2009), namely lipids, proteins, carbohydrates, unsaturated hydrocarbons, lignins, tannins, and condensed aromatic structures. Obviously, all the DOC samples contained molecular formulas predominantly characteristic of lignin-type species (58.4%–65.9%) and they

### TABLE 2  Magnitude-weighted (indicated by w as a subscript) averaged characteristics of the dissolved organic carbon (DOC) of different soil treatments

| Treatments | MW$_w$ | C$_w$ | H$_w$ | O$_w$ | N$_w$ | O/C$_w$ | H/C$_w$ | DBE$_w$ | AI$_w$ |
|------------|--------|------|------|------|------|--------|--------|--------|--------|
| CK         | 329.5  | 19.5 | 19.8 | 8.7  | 1.0  | 0.51   | 1.10   | 11.6   | 0.39   |
| SBR        | 370.2  | 19.3 | 18.0 | 8.0  | 1.5  | 0.46   | 0.95   | 12.0   | 0.46   |
| SBC300     | 407.5  | 19.7 | 17.3 | 6.2  | 1.2  | 0.42   | 0.94   | 12.6   | 0.47   |
| SBC600     | 414.8  | 20.5 | 17.9 | 7.0  | 1.2  | 0.40   | 0.94   | 13.1   | 0.47   |

**Notes:** CK, soil only; SBR, soil amended with biogas residue; SBC300, soil amended with biochar pyrolyzed from biogas residue under 300°C; SBC600, soil amended with biochar pyrolyzed from biogas residue under 600°C.
had differences as well (Figure 2e). The DOC extracted from CK was composed of 1.3% lipid, 6.3% protein, 0.1% carbohydrate, 4.6% unsaturated hydrocarbon, 58.4% lignin, 24.0% tannin, and 5.3% condensed aromatics. By contrast, the DOC extracted from SBR, SBC300, and SBC600 had less molecules in the region of protein, carbohydrate, and tannin, but contained more molecules in the region of lignin. In addition, SBC300 and SBC600 contained more condensed aromatic-like components (8.1% and 8.0%, respectively).

### 3.4 | Shifts in the molecular structure of bulk SOC

Solid-state $^{13}$C NMR spectra of bulk OC of the biochar and soil as well as its integration results are illustrated in Figure 3a and Table S3. Obvious differences between CK and treatments soils can be seen. As for the CK, the NMR spectra were mainly composed of alkyl C (25.7%) and carbohydrate (22.6%), followed by aryl C (16.7%) and carboxyl group (15.9%). After BR and BC300 addition, the carbohydrate percentage slightly increased to 25.9% and 28.2%, respectively. In contrast, BC600 caused carbohydrate to strongly reduce to 12.0%. Also, the BC600 application induced the alkyl and carboxyl C to decrease to 19.8% and 8.6%, respectively. In addition, the percentage of aryl C and the aromaticity of SOC in SBC600 increased significantly to 45.1% and 57.2% ($p < 0.05$), which were both roughly three and twofold higher than that of other treatments, respectively.

### 3.5 | Shifts in the mineralization of bulk SOC

Figure 3b,c illustrates the rate and cumulative amount of CO$_2$ emission in the CK and treated soils. It was seen that the evolution of CO$_2$ emission rate with the incubation time of most soils (except for SBR) could be generally categorized into two stages: declined rapidly in the first 8 days, and then tended to be stable during 8–60 days. For the SBR, emission rate sharply increased during 43–51 days. Additionally, it was apparent that during the whole incubation period, the CO$_2$ release rate was higher in SBR and SBC300 ($p < 0.05$) than in CK (Figure 3b), with the increase percentage of average emission rate of 211.1% and 106.8%, respectively (Figure S4). By contrast, there was no
significant difference between CK and SBC600 ($p > 0.05$). Similar to the shift in emission rate, the cumulative CO$_2$ emission (Table S4; Figure S5) was significantly higher in SBR (1740 mg kg$^{-1}$) and SBC300 (942 mg kg$^{-1}$) in comparison with CK (541 mg kg$^{-1}$) and SBC600 (549 mg kg$^{-1}$; $p < 0.05$). Moreover, by fitting the kinetic of cumulative amount of CO$_2$ emission to a first-order kinetic model ($R^2 > 0.99$ for all the soils in this study; Figure S5; Table S4), it was further found that the potentially mineralizable C ($C_0$) was significantly higher in the SBR than in the other treatments (Table S4; $p < 0.05$). By comparison, potentially mineralizable C was the lowest in SBC600 ($p < 0.05$).

4 | DISCUSSION

The results of high-throughput sequencing showed that BR and BR biochars reduced the richness and diversity of the bacterial community, while BR biochar produced less reduction than BR. The strong decrease in bacterial richness and diversity in SBR was consistent with the study of Nkoa (2014) which highlighted that odor emission, toxic organic compounds, pathogens, and phytotoxicity of BR might have a high potential to harm soil bacteria. Herein, the identified decrease in bacterial richness and diversity might be partly attributable to the high contents of volatile matter of BR (65.6%; Table 1). Many studies have shown the toxicity of volatile substances to the microorganism (Sun et al., 2015; Thies & Rillig, 2009). Lower contents of volatile matters of BR biochar (10.3%–48.7%) than BR corresponded to the significantly less decrease in the richness and diversity of the bacterial community in BR biochar-amended soil. These results indicate the potential of pyrolysis in mitigating the harmful effects of BR in the bacterial community.

ESI-FT-ICR-MS and $^{13}$C-NMR clearly demonstrated that BR and BR biochar shifted SOC toward components with higher molecular weight and aromaticity but lower polarity and less carbohydrate, with the shift extent being much stronger for BC600. These modifications in SOC structure might result from the physical mixture of biochar with CK soil, given that BR and BR biochar are carbon-rich materials. If the physical mixture was a mere mechanism, the OC structure of treatment soil could be predicted from the structure of the CK and amendments. Thus, to determine whether physical mixture was the dominant mechanism, we used aryl C as the example to compare its predicted and experimentally measured value. According to Equation (2), the predicted percentages of aryl C of SBR, SBC300, and SBC600 were 17.4%, 1.2%, and 22.3%, respectively (Table S3), which differed from their experimentally measured value. Specifically, as for SBC300 and SBC600, their predicted values were lower than the measured values, while the predicted value of SBR was slightly higher than the measured value. These observations suggest that the shift in the structure of SOC was not
merely caused by the physical mixture of amendments with soil, but resulted from other factors. We have previously identified the crucial role of microbial community in regulating the SOC molecular structure (Sun et al., 2020). As discussed before, biochar-treated soil, particularly SBC600, contained significantly more Actinobacteria, while BR-amended soil had more Proteobacteria and Bacteroidetes. The active involvement of Actinobacteria in the degradation of polysaccharide compounds in soils has been generally accepted (McCarthy, 1987; Warren, 1996). By contrast, several Proteobacteria and Bacteroidetes (e.g., γ-Proteobacteria) were found as efficient decomposers in degrading aromatics (Eriksson et al., 2003; Mao et al., 2012). In this sense, more abundant Actinobacteria in SBC300 and SBC600 would induce decomposition of more polysaccharides in soils, thereby causing the increase in the relative percentage of aromatic components. Likewise, more Proteobacteria and Bacteroidetes resulted in less aromatics, thus explaining why the predicted percentage of aryl C of SBR was higher than the measured percentage.

Alterations in the bacterial community and SOC structure were partly translated into the change in mineralization of SOC. Relative to the CK soil, the average rate of CO₂ emission and amount of potentially mineralizable C were higher for SBR and SBC300, but lower for SBC600, which could be explained by the result that SBR and SBC300 contained relatively more carbohydrates which were comparatively readily mineralizable (Laffely et al., 2020), while SBC600 had high aromatics as mentioned above. The aromatics of OC have been proven to relatively resist mineralization (Lehmann et al., 2011; Li et al., 2014). These results suggest that the transformation of BR into biochar at high temperature would have the potential to improve the soil carbon sequestration potential.

5 | CONCLUSION

Relative to BR, the addition of BR biochar led to an obviously lower reduction in the richness and diversity of the soil bacterial community. After the addition of BR and BR biochar (especially BC600), DOC in soil contained higher molecular weight and aromaticity but lower polarity and less protein-, carbohydrate-, and tannin-like species. Furthermore, BC600 increased the aromaticity of bulk OC but reduced the carbohydrate, possibly owing to more Actinobacteria genera in the amended soil. These results, along with the lowest content of potentially mineralizable C in the BC600-treated soil, indicate that OC in the BC600-treated soil more resist mineralization, to a greater extent. This study suggested that conversion of BR into biochar at high temperature would not only alleviate the harmful effect of BR on the richness/diversity of the bacterial community but also improve the soil carbon sequestration potential. However, this study was conducted under laboratory conditions for a relatively short time, and only the bacterial community was considered. Therefore, the results of this study need to be confirmed by long-term field studies (perhaps in combination with other more advanced techniques, e.g., isotope tracing). Also, further studies are necessary to assess the robustness of our results by considering more diverse soil organisms.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (31901195), Shandong Provincial Natural Science Foundation (ZR2019BD062), Agricultural Science and Technology Innovation Program of the Chinese Academy of Agricultural Sciences (ASTIP-TRIC-ZD01), and the Guangdong Basic and Applied Basic Research Foundation (2019A1515110777).

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

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

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.