Long-term biochar application governs the molecular compositions and decomposition of organic matter in paddy soil

Jiali Sun | Hongbo Li | Deshan Zhang | Ruliang Liu | Aiping Zhang | Zed Rengel

Abstract

Biochar addition can enhance soil quality and sequester carbon. However, changes in soil organic matter (SOM) molecular compositions in response to long-term biochar addition have rarely been studied. Therefore, we quantified soil organic carbon fractions, carbon-cycling enzyme activities, and a range of organic compounds and lignin-derived phenols in the rhizosphere and bulk soils in different rice growth stages in the 8-year field trial with biochar application rates of 0 (BC-0), 4.5 (BC-L), and 13.5 t ha⁻¹ year⁻¹ (BC-H). We found that higher amounts of biochar addition (BC-H) increased labile organic carbon (LOC), dissolved organic carbon (DOC) and particulate organic carbon (POC), and promoted activities of α-1,4-glucosidase, β-D-cellobiohydrolase and β-1,4-xylosidase; in contrast, BC-L treatment reduced activities of these enzymes. The concentrations of dichloromethane/methanol-extractable plant- and microbial-derived organic compounds in the rhizosphere and bulk soils at tillering decreased significantly in the treatment with low amount of biochar addition. BC-L also significantly altered the concentrations of extracted compounds in the rhizosphere soil at tillering and harvest. Concentration of lignin in the bulk soil was significantly reduced in BC-L at tillering (by 19%) and harvest (by 28%). The concentrations of extracted compounds (e.g., n-alkanols, n-alkanoic acids, steroids, and carbohydrates) and lignin were generally significantly higher in the bulk than the rhizosphere soil at tillering and harvest. Long-term biochar application (BC-H) promoted lignin decomposition in the bulk soil (at tillering) and the rhizosphere soil (at harvest). Hence, biochar decreased stability of lignin in paddy soil. Our study provided evidence that long-term biochar application changed the molecular composition and dynamics of degradation of SOM. These results deepen our understanding of the mechanisms governing SOM stability in agricultural ecosystems.

KEYWORDS

biomarkers, enzyme activity, growth stage, lignin, lipids, rhizosphere soil, SOC fraction
Biochar application has been seen as a potential effective measure to sequester carbon in soil and to improve soil quality (Li, Wen, et al., 2018; Yargicoglu & Reddy, 2017). Biochar contains a large amount of aromatic carbon, which can affect soil organic carbon (SOC; Hu et al., 2019) and alter soil organic matter (SOM) molecular composition (Mitchell, Simpson, Soong, Schurman, et al., 2016). Biochar application can stimulate bacterial and fungal activities (Chen et al., 2013; Liu et al., 2019), thus enhancing the decomposition of organic matter and recycling of nutrients via extracellular enzymes (Elzobair et al., 2016). In addition, the molecular structure of SOM is closely related to the rhizosphere community (Huang et al., 2019). Most studies conducted so far on the effect of biochar on SOM composition in forest and grassland were only short-term studies (Li, Hu, et al., 2018; Liu et al., 2021; Mitchell et al., 2016); hence, the effects of long-term, repeated biochar applications on the plant versus microbial origin and molecular composition of SOM in farmland rhizosphere soil remain unclear.

Biochar may release dissolved organic carbon (DOC) differing in structure from organic carbon in soil, resulting in the structural change of SOM (Li et al., 2015; Majumder et al., 2019; Tian et al., 2016). SOM is a heterogeneous mixture of plant and microbial residues, containing organic compounds with a huge range of molecular weights (Jackson et al., 2019; Ma et al., 2018). The important SOM component is SOC (including labile organic carbon [LOC], DOC, particulate organic carbon [POC], and other fractions; Wang et al., 2020). However, SOC fractions differ from SOM in molecular structure, whereby molecular structure of SOM includes long-chain aliphatics (n-alkanes, n-alkanols, and n-alkanolic acids) and steroids from higher plants, as well as short-chain aliphatics and trehalose derived from microbes (Gao et al., 2020; Otto et al., 2005). Biomarkers (such as free and bound lipids, lignin-derived phenols) are widely used to identify molecular composition of SOM (Gao et al., 2020; Otto & Simpson, 2007; Wang et al., 2019). These biomarkers are extracted in various ways (including solvent extraction, alkaline hydrolysis, CuO oxidation, etc.) and determined by gas chromatography-mass spectrometry (GC-MS), greatly promoting the understanding of the origin of SOM and its stability at the molecular level (Otto & Simpson, 2006). At present, although biochar-enhanced preservation of plant-derived molecules in paddy soils, mainly in lipids, was reported in a 2-year study (Chen, Ding, et al., 2021), the effects of biochar addition on SOM also depend on biochar application time and soil types (Dong et al., 2019b; Du et al., 2019; Ventura et al., 2015). Therefore, it is not clear how long-term biochar addition changes the molecular composition and source of SOM as well as dynamics of its decomposition in farmland.

In addition to biochar, crop root growth can also influence SOM by absorbing and releasing various compounds. Carbon supplied to the rhizosphere by plant roots can directly promote SOM decomposition (Finzi et al., 2015). In addition to litter input into the soil, plants also release active organic compounds to the soil in the form of rhizosphere deposition, including root exudates and sloughed off root cells (Pausch & Kuzyakov, 2018). Rhizodeposition plays a key role in stabilizing the SOC pool and can be used as an energy source for microorganisms, thereby increasing microbial activity (Dijkstra et al., 2021; Hütsch et al., 2015). Additionally, biochar application can regulate root growth and change rhizosphere organic matter (Huang et al., 2019). However, there is a lack of systematic research on how the interaction between long-term biochar application and root growth influences SOM molecular composition.

The molecular composition of SOM differs depending on the growth stage. For example, the concentration of sugars and sugar alcohols in root exudates was lower in the late stages (heading and flowering) of Arabidopsis thaliana than in the early (vegetative) stages (Chaparro et al., 2013). Biochar has important effects on root nutrient acquisition (Huang et al., 2019). Previous studies showed that long-term application of biochar promoted root growth at tillering, and delayed root senescence in the late growth stage, thus changing root activity at various growth stages (Liu et al., 2021). Altered root activity may have significant effects on the composition and quantity of root exudates (Tayier et al., 2014). Carbon supplied by plant roots can directly drive SOM decomposition, but it is not clear how the effect of carbon on composition of SOM in the rhizosphere soil changes at the molecular level (Huang et al., 2020). Therefore, with the long-term, repeated biochar applications, the characterization of the quantity and molecular composition of SOM in the crop rhizosphere and bulk soils during different growth stages will underpin a more comprehensive understanding of the SOM sources and its dynamics.

As one of the important indicators of soil quality (Zhu et al., 2017), extracellular microbial enzymes are involved in decomposition of SOM and nutrient recycling (Elzobair et al., 2016). However, many studies with short-term field and laboratory experiments have investigated the effects of biochar amendments on soil enzyme activities, as well as different biochar characteristics and soil types (Liu et al., 2017; Tian et al., 2016; Zhang et al., 2019). However, given the long-term kinetics of biochar decomposition, the relatively short-term experiments are unsuitable for determining how the addition of biochar would influence soil enzyme activities several years after incorporation,
with the long-term effects on the sources and composition of SOM in root environment.

The Yellow River irrigation area in Ningxia is one of the ancient irrigation areas in China, with a special soil type of anthropogenic alluvial soil (Liu, Li, Li, et al., 2021; Wang et al., 2017). Rice is one of the most important crops in the region. Soil capacity to retain nutrients is poor, and the organic matter content is low (Wang et al., 2017). At present, although some short-term studies have contributed important information about soil carbon dynamics after biochar addition (Mitchell, Simpson, Soong, Schurman, et al., 2016), the methods used do not provide any molecular-level detail with respect to shifts in the SOM composition, and there is a paucity of knowledge about whether long-term application of biochar may influence SOM molecular composition in different rice growth stages and alter soil carbon-cycling enzyme activities and SOC fractions.

To address these issues, we added three amounts of biochar (0, 4.5 t, and 13.5 t ha⁻¹ annually for 8 years) to paddy soil to elicit changes in soil enzyme activities and chemical composition and source of organic matter. We aimed to answer the following questions: (1) whether long-term biochar application and rice growth stage would affect the dichloromethane (DCM)/methanol (MeOH)-extractable compounds (n-alkanols, n-alkanoic acids, steroids, and carbohydrates), lignin-derived phenols, and decomposition of SOM in the rhizosphere and bulk soils; and (2) how long-term biochar addition effect on the carbon fractions (POC, DOC, and LOC) and activities of soil carbon-cycling and oxidative enzymes in anthropogenic alluvial soil.

2 | MATERIALS AND METHODS

2.1 | Site description and experimental design

The experimental station is located in Yesheng Town, Qingtongxia City, China (106°11′35″E, 38°07′26″N). The region has a mean altitude of 1100 m, and is dominated by temperate continental monsoon climate, with an average temperature of 9.4°C and an annual average precipitation of 192.9 mm. The soil is classified as anthropogenic alluvial soil, with soil texture comprising 18.25% clay, 53.76% silt, and 27.99% sand. The organic matter in topsoil (0–20 cm) was 16.1 g kg⁻¹, the total nitrogen was 1.08 g kg⁻¹, and the soil bulk density was 1.33 g cm⁻³.

The biochar experiment was established in 2012. There were three treatments: 0 (BC-0), 4.5 (BC-L), and 13.5 t ha⁻¹ year⁻¹ (BC-H). The rice variety selected for the experiment was Ningjing 43, which was raised on April 28, transplanted on May 29, and harvested on September 28. The treatments were set out in a randomized complete block design with three replicates. Distance between blocks was 1.5 m, and the plot size was 13 m × 5 m. Urea (N, 46%), and double superphosphate (P, 20%) and potassium chloride (K, 50%) were applied as basal fertilizers at the rates of 150 kg N ha⁻¹, 39.3 kg P ha⁻¹, and 74.5 kg K ha⁻¹ along with biochar before transplanting rice. Later, 90 and 60 kg N ha⁻¹ were applied in the seedling stage and jointing stage, respectively (Table S1). Biochar and fertilizers were broadcast on the soil surface and incorporated into the soil by plowing to a depth of approximately 20 cm. The biochar was produced by pyrolysis of wheat straw at 350°C by the Sanli New Energy Company, Shandong Province, China. Biochar had C and N contents of 65.7% and 0.5%, respectively, with a pH (H₂O) of 7.78 (Liu, Li, Li, et al., 2021). To maintain consistency, plowing was also performed for the plots without biochar addition. Crop management was consistent across plots and years. In the growing season, rice was irrigated by about 900–1200 mm from June to October. During tillering (11 June), jointing (23 June) and flowering (18 September) the field were flooded.

2.2 | Soil sampling

Soil samples (0–20 cm) were collected at tillering, jointing, flowering, and harvest in 2019. During the sampling process, we used shovels to excavate root systems of three randomly chosen rice plants in each plot; the roots were shaken to remove loosely adhered soil (bulk soil). The soil that adhered tightly to the roots was regarded as rhizosphere soil, and was removed by washing the roots with sterile water and then centrifuging to collect the sediment (Zhang et al., 2017). The soil sample was divided into three parts. A portion of each soil sample was freeze-dried and passed through a 2 mm sieve for determination of biomarkers. Another portion of each soil sample was air-dried and passed through a 2 mm sieve for determination of SOC and total nitrogen. The third portion of each soil sample was stored at −20°C for less than 1 week until enzyme analysis could be performed. The concentrations of SOC and TN were determined by an elemental analyzer (Vario Max CNS; Elementar). SOM biomarkers were extracted and analyzed by GC-MS in the samples of rhizosphere and bulk soils collected at tillering and harvest (Otto et al., 2005). Concentrations of soil POC, DOC, and LOC were measured in samples collected at tillering, jointing, flowering, and harvest.

2.3 | Laboratory measurements

We analyzed the concentrations of biomarkers (i.e., n-alkanols, n-alkanoic acids, carbohydrates, and steroids)
using DCM/MeOH extracts and CuO oxidation products (lignin-derived phenols: vanillyls, syringyls, and cinnamyls (Otto et al., 2005). Briefly, in a Teflon tube, 5 g of freeze-dried soil was mixed with 30 ml of DCM, sonicated for 15 min, followed by the addition of 30 ml DCM:MeOH (1:1, v/v), and 30 ml of MeOH in sequence. The extraction solutions were centrifuged (2880 g for 5 min), and then dried under nitrogen gas in 2 ml GC/HPLC vials. The dried extracts in the vials were stored in a freezer until derivatization (Otto et al., 2005). The remaining residues after DCM/MeOH extraction were air-dried and then oxidized with CuO to obtain lignin-derived phenols (Otto et al., 2005; Otto & Simpson, 2006). The detailed process was described by Gao et al. (2020).

We also measured the activities of four soil hydrolytic enzymes (α-1,4-glucosidase [AG], β-1,4-glucosidase [BG], β-D-celllobiohydrolase [CB], and β-1,4-xyllosidase [BX]), and two oxidative enzymes (polyphenol oxidase [PPO] and peroxidase [PER]). For all enzymes, we used six analytical replicates for each soil sample and control. We measured enzyme activities following a method modified from Jing et al. (2016) using fluorometric techniques. In brief, we homogenized 2.75 g soil in 100 ml of 50 mM Tris buffer (pH 8.0) in a blender for 2 min. For hydrolytic (i.e., AG, BG, CB, and BX) enzymes, soil slurries (200 μl) were added to 96-well microplates along with 50 μl of 200 mM fluorometric substrate (saturating concentration) in each well. The microplates were incubated in the dark at 25°C for 4 h. The amount of fluorescence was determined using a fluorescence spectrometer set to 365 nm for excitation and 450 nm for emission. Enzyme activity was expressed as nmol g⁻¹ dry weight h⁻¹. For oxidative (i.e., POX and PER) enzymes, soil slurries (150 μl) were added to 96-well microplates along with 50 μl of 25 mM 3,4-Dihydroxy-L-phenylalanine and 50 μl of 5 mM ethylenediaminetetraacetic acid disodium salt in each well. The sample control contained 150 μl of slurries and 100 μl of Tris buffer in each well. The microplates were incubated in the dark at 25°C for 3 h. Activity was quantified using a fluorescence spectrometer by measuring the absorbance at 450 nm. Enzyme activity was expressed as μmol g⁻¹ dry weight h⁻¹.

### 2.4 Biomarker parameters and calculations

The concentrations of individual DCM/MeOH-extractable compounds were calculated by comparison of their peak areas and those of the standards in the total ion current, then normalized to the mass of soil extracted. The DCM/MeOH-extractable plant-derived free lipids in SOM include the long-chain aliphatic lipids (≥C₂₀) and steroids (campesterol, stigmasterol, and sitosterol), whereas the DCM/MeOH-extractable microbial-derived SOM compounds include the short-chain aliphatic lipids (<C₂₀) and trehalose (Otto et al., 2005).

The aldehyde compounds of vanillyls and syringyls were oxidized to corresponding acids in the process of lign degradation. The ratios of acid to aldehyde in vanillyls and syringyls ([Ad/Al]v and [Ad/Al]s) were used to infer a degree of lignin decomposition (Clemente et al., 2012). The higher ratios of [Ad/Al]v and [Ad/Al]s represent higher decomposition of lignin. Moreover, because vanillyls are more difficult to break down than cinnamyls and syringyls, decomposition of cinnamyls and syringyls represents lignin degradation; hence, the monomeric decomposition rate (S/V and C/V) of lignin was calculated to reflect a degree of lignin decomposition (Pausch & Kuzyakov, 2018). The lower S/V and C/V ratios represent higher decomposition of lignin.

### 2.5 Statistical analyses

We used two-way ANOVA to test the effects of long-term biochar application, type of soil sample (rhizosphere and bulk soils), and their interaction on the DCM/MeOH-extractable compounds and lignin-derived phenols. To determine how the activities of C-cycling and oxidative enzymes responded to biochar rates and rice growth stage, we used two-way ANOVA to test the effects of biochar, growth stage, and their interaction. One-way ANOVA was used to test the main effect of biochar on the concentrations of SOC, TN, POC, DOC, and LOC. When the difference was significant (p < 0.05), the means were compared using a Fisher’s protected least significant difference (Duncan) test.

The Pearson correlation analysis was performed to derive the correlation coefficients between measured parameters, that is, DCM/MeOH-extractable organic matter fractions, lignin-derived phenols, enzyme activities, and organic carbon fractions. The statistical analyses were performed using SPSS 25.0 (SPSS).

### 3 RESULTS

#### 3.1 General soil physiochemical properties

Biochar addition had a significant effect on SOC and C/N, but not TN (Figure 1). The SOC content generally increased with an increase in the biochar rates (Figure 1). SOC content was increased by 38% (non-significantly) under BC-L and by 71% (p < 0.05) under BC-H (Figure 1).
Biochar addition significantly increased C/N by 30% and 44% under BC-L and BC-H, respectively (Figure 1).

3.2 | POC, DOC, and LOC

Biochar application influenced SOC fractions (POC, LOC, and DOC) differently in various rice growth stages application (Figure 2). The POC increased significantly with BC-H addition by 16%, 21%, and 13% at tillering, flowering, and harvest, respectively, but BC-L significantly decreased POC content at flowering (Figure 2a). With biochar application, BC-H significantly increased LOC at tillering (by 16%), flowering (by 25%), and harvest (by 21%), whereas BC-L significantly increased LOC at tillering and jointing, and significantly (by 6.5%) decreased LOC at harvest (Figure 2b). BC-L significantly (by 7.8%) decreased DOC at harvest, but BC-H significantly (by 8.4–13%) increased DOC at different growth stages (except jointing; Figure 2c).

3.3 | Composition and origin of DCM/MeOH-extractable compounds

The composition of DCM/MeOH-extractable compounds (i.e., n-alkanols, n-alkanoic acids, steroids, and carbohydrates) was significantly influenced by the interaction of biochar and the type of soil samples (rhizosphere and bulk soils; Table S2). At rice tillering, BC-L and BC-H significantly reduced the concentrations of n-alkanols, n-alkanoic acids, steroids, and total extracted compounds in the bulk soil (by 63%–71%, 42%–52%, 65%–66%, and 43%–53%, respectively; Figure 3a–c,e). The BC-L significantly (by 80%) increased the concentrations of carbohydrates in the bulk soil, whereas BC-H significantly (by 64%) decreased these concentrations relative to BC-0 (Figure 3d). Biochar significantly decreased the concentrations of n-alkanols, carbohydrates, and total extracted compounds in the rhizosphere soil (by 60%–63%, 68%–88%, and 30%–39%, respectively; Figure 3a,d,e). The BC-H significantly (by 53%) reduced the concentration of n-alkanoic acids in the rhizosphere soil (Figure 3b). In all treatments, the concentrations of n-alkanols, n-alkanoic acids, steroids, carbohydrates, and total extracted compounds were significantly higher in the bulk soil than the rhizosphere soil (except steroids in BC-H; Figure 3a–e).

At rice harvest, BC-H significantly reduced the concentrations of n-alkanols, n-alkanoic acids, carbohydrates, steroids, and total extracted compounds in the bulk soil (by 55%, 51%, 55%, 59%, and 53%, respectively; Figure 3f–j). The BC-L increased the concentrations of n-alkanoic acids by 42% (p < 0.05), carbohydrates by 7.6% (non-significantly), and total extracted compounds by 11% (p < 0.05) (Figure 3g,i,j), and reduced the concentrations of n-alkanols by 41% (p < 0.05) and steroids by 30% (p < 0.05) in the bulk soil relative to BC-0 (Figure 3f,h). Compared with BC-0, biochar significantly reduced the concentrations of steroids and total extracted compounds by 50%–93% and 46%–86%, respectively, in the rhizosphere soil (Figure 3h,i,j); biochar applied at the higher rate (BC-H) significantly reduced n-alkanols (by 77%), n-alkanoic acids (by 87%), and carbohydrates (by 63%) in the rhizosphere soil (Figure 3f,g,i). In the bulk soil, the concentrations of n-alkanols in BC-L and BC-H, n-alkanoic acids in all treatments, steroids in BC-0 and BC-H, carbohydrates in BC-0 and BC-L, and total extracted compounds in all treatments were significantly higher than those in the rhizosphere soil (Figure 3f–j). In comparison, in all the treatments, the concentration of total extracted compounds in

**FIGURE 1** Concentrations of soil organic carbon (SOC) and total nitrogen (TN) and the C/N ratio in soil organic matter in the treatments with biochar rates of 0 t ha$^{-1}$ (BC-0), 4.5 t ha$^{-1}$ (BC-L), and 13.5 t ha$^{-1}$ (BC-H). Data are means ± SE (n = 3). In each graph, different letters denote significant difference among biochar treatments (p < 0.05).
the rhizosphere soil was higher at tillering than harvest (Figure 3e).

At rice tillering, biochar significantly (by 62%–63%) decreased the DCM/MeOH-extractable plant-derived SOM in the bulk soil (Figure 4a). The DCM/MeOH-extractable plant-derived SOM decreased by 57% ($p < 0.05$) in the BC-L and increased by 65% ($p < 0.05$) in the BC-H treatment in the rhizosphere soil at tillering (Figure 4a). The DCM/MeOH-extractable microbial-derived SOM significantly decreased by 40%–52% and 36%–59% in the bulk and rhizosphere soils, respectively, with long-term biochar addition (Figure 4b). At rice harvest, compared with BC-0, biochar significantly decreased the DCM/MeOH-extractable plant-derived SOM in the bulk and rhizosphere soils by 56–90% and 33–57%, respectively (Figure 4c). The DCM/MeOH-extractable microbial-derived SOM increased by 43% ($p < 0.05$) in the BC-L and decreased by 51% ($p < 0.05$) in the BC-H treatment in the bulk soil (Figure 4d). The BC-H significantly (by 81%) decreased the DCM/MeOH-extractable microbial-derived SOM in the rhizosphere soil (Figure 4d). In all the treatments at tillering, the DCM/MeOH-extractable plant-derived SOM and microbial-derived SOM were significantly higher in the bulk soil than the rhizosphere soil (Figures 4a, b, and 6). In all the treatments at harvest, the DCM/MeOH-extractable microbial-derived SOM was significantly higher in the bulk soil than the rhizosphere soil (Figure 4d). In the BC-0 and BC-H treatments, the DCM/MeOH-extractable microbial-derived SOM was higher than plant-derived SOM in the bulk soil at tillering and harvest (Figure 4).

### 3.4 Lignin-derived phenols

The lignin-derived phenols (VSC: including vanillyls [V], syringyls [S], and cinnamyls [C]) were significantly influenced by the interaction of biochar and the type of soil samples (rhizosphere and bulk soils; Table S2). At rice tillering, BC-L significantly (by 64%) increased the concentrations of total vanillyls, whereas BC-H significantly (by 67%) decreased the concentrations of total syringyls in the bulk soil (Figure 5a, b). Compared with BC-0, biochar significantly decreased total cinnamyls (by 62%–86%) and VSC (by 19%–66%) in the bulk soil (Figure 5d). The BC-L significantly decreased the concentrations of total cinnamyls (by 37%) and VSC (by 21%) in the rhizosphere soil relative to BC-0 (Figure 5d). In the BC-0 and BC-L treatments, the concentration of VSC in the bulk soil was higher than that in the rhizosphere soil, but in the BC-H treatment, the concentration of VSC was lower in the bulk than the rhizosphere soil ($p < 0.01$; Figure 5d).

At rice tillering, biochar significantly decreased the S/V (by 59%–64%) and C/V ratios (by 76%–82%), but significantly
increased the values of (Ad/Al)_v (by 210%–262%) and (Ad/Al)_s (by 27%–63%) in the bulk soil (Figure 5e–h). The values of (Ad/Al)_s decreased by 12% (non-significantly) in the BC-L and by 128% (p < 0.05) in the BC-H treatment in the rhizosphere soil at tillering (Figure 5h).

Biochar significantly decreased the ratios of S/V, C/V, (Ad/Al)_v, and (Ad/Al)_s in the bulk soil (by 36%–53%, 65%–83%, 71%–88%, and 37%–39%, respectively; Figure 5m–p). In the rhizosphere soil, compared with BC-0, biochar significantly increased the values of S/V (by 156%–171%) and C/V (by 260%–295%); Figure 5m,n). In the rhizosphere soil, compared with the control, the values of (Ad/Al)_v decreased by 61% (p < 0.05) in the BC-L and increased by 42% (p < 0.05) in the BC-H (Figure 5o); in addition, (Ad/Al)_s increased significantly (by 68%) in the BC-H (Figure 5p). In the BC-H treatment, the concentrations of vanillyls, syringyls, and VSC were significantly higher in the bulk soil than the rhizosphere soil (Figure 5i,j,l). The ratios of S/V, (Ad/Al)_v, and (Ad/Al)_s were significantly higher in the bulk soil at tillering than harvest; in the rhizosphere soil, the same was true in the BC-0 and BC-H treatments (Figure 5d,l).

3.5 | Soil enzymes

The soil enzyme activities were significantly influenced by the biochar and/or growth stage, but not by the interaction
TABLE 1. PPO activity increased by 46% \((p < 0.05)\) in the BC-L at tillering. BC-L and BC-H significantly reduced the BG activity by 17% and 11%, respectively, at tillering; moreover, the BG activity was positively correlated with carbohydrates in the rhizosphere soil (Figure S1). CB activity increased by 87% \((p < 0.05)\) in the BC-H at tillering. At tillering, the AG activity decreased by 22% \((p < 0.05)\) in the BC-L and increased by 33.68% (non-significantly) in the BC-H relative to BC-0. Compared with BC-0, the BX activity significantly decreased in the BC-L treatment at tillering and harvest (by 34% in both cases). The PPO, PER, BG, and CB activities were higher at tillering than at harvest, but the AG and BX activities showed an opposite trend.

4 | DISCUSSION

4.1 | Effects of biochar addition on soil SOC, POC, DOC, and LOC

Biochar application was capable of enhancing SOC sequestration (Hu et al., 2021; Wang et al., 2016). Many previous studies have shown that biochar addition increased SOC (Blanco-Canqui et al., 2020; Wang et al., 2020; Zheng et al., 2016), which is confirmed in our study (Table 1). This can be explained by the high carbon content of biochar, which is mainly recalcitrant aromatic C, increasing the SOC stock (Zheng et al., 2016).
Many studies suggested that the incorporation of biochar was capable of changing POC, LOC, and DOC (Huang et al., 2018; Yang et al., 2017, 2018). The addition of biochar (BC-H) to soil increased the LOC at tillering and harvest in this study (Figure 2b). A potential explanation was that the biochar used in the experiment was anaerobically prepared at a relatively low temperature of about 350°C, suggesting it was incompletely oxidized and still contained highly oxidizable organic carbon components (Huang et al., 2018). In addition, our results showed that BC-L treatment decreased DOC at flowering and harvest (Figure 2c), mainly due to adsorption of DOC on biochar (Lu et al., 2014). After biochar was applied to the soil, soil-derived DOC may enter the biochar pores or be adsorbed on the external surface of biochar (Zheng et al., 2016). Once the adsorbed organic matter covered the surface of...
Table 1: Activity of PPO, PER, AG, BG, CB, and BX as influenced by the treatment with rates of biochar 0 t ha\(^{-1}\) (BC-0), 4.5 t ha\(^{-1}\) (BC-L), and 13.5 t ha\(^{-1}\) (BC-H) at tillering and harvest (mean ± SE, n = 3)

| Growth stages | Treatments | Enzyme activity |
|---------------|------------|-----------------|
|               | PER (μmol g\(^{-1}\) dry weight h\(^{-1}\)) | PPO (μmol g\(^{-1}\) dry weight h\(^{-1}\)) | AG (nmol g\(^{-1}\) dry weight h\(^{-1}\)) | BG (nmol g\(^{-1}\) dry weight h\(^{-1}\)) | CB (nmol g\(^{-1}\) dry weight h\(^{-1}\)) | BX (nmol g\(^{-1}\) dry weight h\(^{-1}\)) |
| Tillering     | BC-0       | 8.78 ± 0.68a    | 6.37 ± 0.27b    | 0.74 ± 0.02b | 16.77 ± 0.88a | 0.20 ± 0.00b | 0.20 ± 0.01b |
|               | BC-L       | 7.67 ± 0.44a    | 9.31 ± 0.32a    | 0.57 ± 0.03c | 13.85 ± 0.20b | 0.25 ± 0.03b | 0.13 ± 0.00c |
|               | BC-H       | 7.33 ± 0.10a    | 6.46 ± 0.99b    | 0.98 ± 0.02a | 14.88 ± 0.29ab | 0.38 ± 0.21a | 0.27 ± 0.15a |
| Harvest       | BC-0       | 8.09 ± 0.57a    | 5.34 ± 0.27a    | 0.89 ± 0.05ab | 14.01 ± 0.75a | 0.18 ± 0.03ab | 0.23 ± 0.02a |
|               | BC-L       | 5.99 ± 0.58b    | 6.60 ± 1.34a    | 0.65 ± 0.06b | 12.34 ± 0.13a | 0.13 ± 0.01b | 0.15 ± 0.00b |
|               | BC-H       | 6.49 ± 0.14b    | 6.46 ± 0.99a    | 1.12 ± 0.19a | 13.14 ± 0.69a | 0.34 ± 0.08a | 0.26 ± 1.28a |
| Biochar       | **         | *               | **              | ***           | ns            | ns             |
| Growth stage  | **         | *               | **              | ***           | ns            | ns             |
| Biochar × Growth stage | ns | ns | ns | ns | ns | ns |

Note: Lower case letters show statistically significant differences between biochar treatments at each growth stage (p < 0.05).

Abbreviations: AG, α-1,4-glucosidase; BG, β-1,4-glucosidase; BX, β-1,4-xylosidase; CB, β-D-cellobiohydrolase; PER, peroxidase; PPO, polyphenol oxidase.

4.2 Composition and source of DCM/MeOH-extractable compounds in soil as influenced by biochar addition

Regardless of biochar application, the concentrations of DCM/MeOH-extractable compounds (alcohols, aldehydes, ketones, fatty acids, carbohydrates, proteins, lipids, and total compounds extracted) in the bulk soil were significantly higher than in the rhizosphere soil, indicating that DCM/MeOH-extractable compounds represent a significant fraction of the SOM (Sanchez-Garcia et al., 2015). In the present study, the addition of biochar (BC-L) significantly reduced α-glucosidase activity in the bulk soil, whereas the activity of α-glucosidase was negatively correlated with carboxylate concentration (r = −0.71, P < 0.001, Figure S1). In the BC-H treatment, the concentrations of carbohydrates, total compounds extracted, and α-glucosidase activity were lower than in the BC-L treatment, indicating that biochar addition may have accelerated the decomposition of these components of SOM (Sanchez-Garcia et al., 2015). In this study, the rhizosphere priming effect on SOM decomposition may be positive or negative, depending on the biochar treatment (Chen et al., 2013; Hall & Silver, 2015). In the BC-H treatment, the concentrations of carbohydrates, total compounds extracted, and α-glucosidase activity were lower than in the BC-L treatment, indicating that biochar addition may have accelerated the decomposition of these components of SOM (Sanchez-Garcia et al., 2015). In this study, the rhizosphere priming effect on SOM decomposition may be positive or negative, depending on the biochar treatment (Chen et al., 2013; Hall & Silver, 2015).
carbohydrates (Figure 2d,i), which was consistent with the results of Wang et al. (2020), indicating that biochar inhibits the degradation of carbohydrates.

Short-chain aliphatic compounds were derived mainly from microbial communities, whereas long-chain aliphatic compounds and steroids were mainly of plant origin (Otto et al., 2005). It is increasingly recognized that microbially derived C may be more stable and persistent than plant-derived C, with microbially derived compounds easily interacting with soil minerals to form stable C, being the primary constituents of stable, long-term SOM stores (Angst et al., 2021; Chen, Hu, et al., 2021). In this study, the DCM/MeOH-extractable microbial-derived SOM was higher than plant-derived SOM in the bulk soil in the BC-0 and BC-H treatments regardless of growth stage (Figure 4). This might have been due to (i) microbially derived C can be physically protected in organo-mineral associations because of its proximity to, and interactions with, the soil mineral matrix, and therefore it tends to be more stable than the unaltered plant-derived C (Sokol et al., 2019), (ii) an addition of labile material in biochar that was available for microbial consumption (Farrell et al., 2013), (iii) enhanced availability of nutrients through biochar surface sorption (Sarkhod et al., 2013), or (iv) the presence of suitable microbial habitats and protection from predators in biochar micropores (Pietikäinen et al., 2000), with enhanced growth of microbes in suitable habitats contributing to increased organic matter decomposition.

4.3 Effects of biochar amendment on lignin-derived phenols

In this study, biochar addition changed the concentration of lignin-derived phenols (Figure 5). During rice tillering and harvest, the BC-H decreased the concentrations of VSC in the rhizosphere and bulk soils (Figure 5d,l). Gram-positive bacteria typically have the capacity to excrete several groups of lignin-degrading enzymes necessary for decomposition of complex biochar-derived carbon (Farrell et al., 2013; Lu et al., 2014; Wang et al., 2020). Indeed, previous study showed that biochar addition increased the abundance of Gram-positive bacteria in the bulk soil (Wang et al., 2015), which might lead to an increase in lignin-degrading enzymes, and a reduction in lignin-derived phenols concentration after biochar addition (i.e., decreased stability of lignin in paddy soil; Wang et al., 2020).

In the BC-0 and BC-H treatments, the concentration of VSC in the rhizosphere soil was higher at tillering than harvest (Figure 5d,l). Root exudates contain many organic compounds (including organic acid anions, sugars,
phenols), providing nutrients and energy to rhizosphere microbes to alter their activity (Chaparro et al., 2013). Zhang et al. (2018) found that the root microbiome of rice is highly dynamic at the vegetative stage, but less so in the reproductive stage, with little change until rice maturity. Therefore, the change in lignin concentration in the rhizosphere soil in various growth stages in the present study (Figure 5d, l) might have been caused indirectly by the changes in the root microbiome.

Biochar addition significantly increased the (Ad/Al) v and (Ad/Al)s ratios in the bulk soil at tillering (Figure 5g, h), indicating that biochar promoted the degradation of vanillyls and syringyls. However, biochar addition decreased these ratios at harvest (Figure 5o, p), indicating that a degree of lignin degradation decreased with the progression of growth stages. Previous studies showed that biochar addition affect microbes (Wang et al., 2020). Because microorganisms are the primary decomposers of organic matter in soil, biochar-induced changes in microbial activity and community composition may alter lignin degradation (Mitchell, Simpson, Soong, Schurman, et al., 2016). Lignin is bound to hemicellulose and cellulose to form the ligno-cellulose complex, and the degradation of ligno-cellulose in soil is believed to be initiated primarily by fungi, but also some species of bacteria (Ferreira et al., 2010). Wang et al. (2020) showed that the bacterial and fungal biomasses were enriched under biochar amendments and at the vigorous stages of the crop growing season. Therefore, biochar may stimulate the enrichment of microbial activity during the vigorous stages (tillering stage), accelerating lignin degradation, while the microbial activity decreases during the harvest stage, resulting lignin degradation decreased. In addition, the (Ad/Al)v and (Ad/Al)s ratios increased with higher amounts of biochar addition (indicating degradation of lignin), but decreased with lower amounts of biochar addition in the rhizosphere soil (Figure 5). The uncertainties regarding biochar-stimulated priming effect on lignin degradation are dependent on the application rate (Wang et al., 2020), soil type, and biochar application time (Wang et al., 2020), requiring further study. Our results also suggest that, although the presence of long-term biochar influenced the molecular-level composition of SOM, the SOM was closely related to microbial activity. Thus, the effect of long-term biochar addition on microbial activity in the rhizosphere may be necessary to clarify a role of root–SOM–microbe interactions, which may advance our understanding of SOM stability.

5 | CONCLUSIONS

This study clearly demonstrated the soil carbon fraction, enzyme activity, and the molecular compositions of organic matter change in responses to varying amounts of biochar addition. Relative to BC-0, after the addition of biochar, the bulk soil contained less lignin-derived phenols (in the BC-L treatment) and total DCM/MeOH-extractable compounds (in the BC-H treatment) at tillering and harvest, and the rhizosphere and bulk soils contained less DCM/MeOH-extractable plant-derived and microbial-derived SOM (in the BC-L treatment) at tillering. The concentrations of n-alkanols, n-alkanolic acids, carbohydrates, and total DCM/MeOH-extractable compounds were higher in the bulk than the rhizosphere soil in all the biochar treatments. The lignin degradation degree varied during the rice growth stages. These results indicated that different biochar addition rates could change the molecular composition, source, and decomposition of SOM, but the plant effect (differences between the rhizosphere and bulk soils) was also significant.

ACKNOWLEDGEMENTS

This study was supported by Ningxia Key R&D Program (2019BBF02026), National Natural Science Foundation of China (31601834 and 31801946), Ningxia Agricultural High-quality Development and Ecological Protection Technology Innovation Demonstration Project (NGSB-2021-11-04), and Ningxia Youth Talent Project (2018) RL.

DECLARATION OF INTERESTS

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.

ORCID

Jiali Sun © https://orcid.org/0000-0001-7459-4065
Hongbo Li © https://orcid.org/0000-0001-5273-1989
Deshan Zhang © https://orcid.org/0000-0002-7937-1503
Aiping Zhang © https://orcid.org/0000-0003-3074-5495
Zed Rengel © https://orcid.org/0000-0003-3433-161X

REFERENCES

Angst, G., Mueller, K. E., Nierop, K. G. J., & Simpson, M. J. (2021). Plant-or microbial-derived? A review on the molecular composition of stabilized soil organic matter. Soil Biology and Biochemistry, 156, 108189. https://doi.org/10.1016/j.soilbio.2021.108189
Blanco-Canqui, H., Laird, D. A., Heaton, E. A., Rathke, S., & Acharya, B. S. (2020). Soil carbon increased by twice the amount of biochar carbon applied after 6 years: Field evidence of negative
an alpine grassland ecosystem. Applied Soil Ecology, 107, 205–213. https://doi.org/10.1016/j.apsoil.2016.06.004

Li, J., Wen, Y. C., Li, X. H., Li, Y. T., Yang, X. D., Lin, Z., Song, Z. Z., Cooper, J. M., & Zhao, B. Q. (2018). Soil labile organic carbon fractions and soil organic carbon stocks as affected by long-term organic and mineral fertilization regimes in the North China Plain. Soil and Tillage Research, 175, 281–290. https://doi.org/10.1016/j.still.2017.08.008

Li, Y., Hu, S., Chen, J., Müller, K., Li, Y., Fu, W., Lin, Z., & Wang, H. (2018). Effects of biochar application in forest ecosystems on soil properties and greenhouse gas emissions: A review. Journal of Soils and Sediments, 18(2), 546–563. https://doi.org/10.1007/s11368-017-1906-y

Liu, B. T., Li, H. L., Li, H. B., Zhang, A. P., & Zed, R. (2021). Long-term biochar application promotes rice productivity by regulating root dynamic development and reducing nitrogen leaching. Global Change Biology Bioenergy, 13, 257–268. https://doi.org/10.1111/gcbb.12766

Liu, M., Li, P., Liu, M., Wang, J., & Chang, Q. (2021). The trend of soil organic carbon fractions related to the successions of different vegetation types on the tabletop of the Loess Plateau of China. Journal of Soils and Sediments, 21, 203–214. https://doi.org/10.1007/s11368-020-02710-3

Liu, P., Ptacek, C. J., Blowes, D. W., Berti, W. R., & Landis, R. C. (2015). Aqueous leaching of organic acids and dissolved organic carbon from various biochars prepared at different temperatures. Journal of Environmental Quality, 44(2), 684–695. https://doi.org/10.2134/jeq2014.08.0341

Liu, S. N., Meng, J., Jiang, L. L., Yang, X., Lan, Y., Cheng, X. Y., & Chen, W. F. (2017). Rice husk biochar impacts soil phosphorous availability, phosphatase activities and bacterial community characteristics in three different soil types. Applied Soil Ecology, 116, 12–22. https://doi.org/10.1016/j.apsoil.2017.03.020

Liu, Y., Zhu, J., Gao, W., Guo, Z., Xue, C., Pang, J., & Shu, L. (2019). Effects of biochar amendment on bacterial and fungal communities in the reclaimed soil from a mining subsidence area. Environmental Science and Pollution Research, 26(33), 34368–34376. https://doi.org/10.1007/s11356-019-06567-7

Lu, W. W., Ding, W. X., Zhang, J. H., Li, Y., Luo, J. F., Bolan, N., & Xie, Z. B. (2014). Biochar suppressed the decomposition of organic carbon in a cultivated sandy loam soil: A negative priming effect. Soil Biology and Biochemistry, 76, 12–21. https://doi.org/10.1016/j.soilbio.2014.04.029

Ma, L., Lv, X., Cao, N., Wang, Z., Zhou, Z., & Meng, Y. (2020). Alterations of soil labile organic carbon fractions and biological properties under different residue-management methods with equivalent carbon input. Applied Soil Ecology, 161, 103821. https://doi.org/10.1016/j.apsoil.2020.103821

Ma, T., Zhu, S., Wang, Z., Chen, D., Dai, G., Feng, B., Su, X., Hu, H., Li, K., Han, W., Liang, C., Bai, Y., & Feng, X. (2018). Divergent accumulation of microbial necromass and plant lignin components in grassland soils. Nature Communications, 9, 3480. https://doi.org/10.1038/s41467-018-05891-1

Majumder, S., Neogi, S., Dutta, T., Powel, M. A., & Banik, P. (2019). The impact of biochar on soil carbon sequestration: Meta-analytical approach to evaluating environmental and economic advantages. Journal of Environmental Management, 250, 109466. https://doi.org/10.1016/j.jenvman.2019.109466

Mitchell, P. J., Simpson, A. J., Soong, R., Schurman, J. S., Thomas, S. C., & Simpson, M. J. (2016). Biochar amendment and phosphorus fertilization altered forest soil microbial community and native soil organic matter molecular composition. Biogeochemistry, 130(3), 227–245. https://doi.org/10.1007/s10533-016-0254-0

Mitchell, P. J., Simpson, A. J., Soong, R., & Simpson, M. J. (2016). Biochar amendment altered the molecular-level composition of native soil organic matter in a temperate forest soil. Environmental Chemistry, 13(5), 854–866. https://doi.org/10.1071/EN16001

Otto, A., Shunthirasingham, C., & Simpson, M. J. (2005). A comparison of plant and microbial biomarkers in grassland soils from the Prairie Ecozone of Canada. Organic Geochemistry, 36, 425–448. https://doi.org/10.1016/j.orggeochem.2004.09.008

Otto, A., & Simpson, M. J. (2006). Evaluation of CuO oxidation parameters for determining the source and stage of lignin degradation in soil. Biogeochemistry, 80, 121–142. https://doi.org/10.1007/s10533-006-9014-x

Otto, A., & Simpson, M. J. (2007). Analysis of soil organic matter biomarkers by sequential chemical degradation and gas chromatography–mass spectrometry. Journal of Separation Science, 30, 272–282. https://doi.org/10.1002/jssc.200600243

Pausch, J., & Kuzyakova, Y. (2018). Carbon input by roots into the soil: Quantification of rhizodeposition from root to ecosystem scale. Global Change Biology, 24, 1–12. https://doi.org/10.1111/gcb.13850

Pietiküinen, J., Kiiikkiä, O., & Fritze, H. (2000). Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. Oikos, 89, 231–242. https://doi.org/10.1034/j.1600-0706.2000.890203.x

Sánchez-García, M., Alburquerque, J. A., Sánchez-Monedero, M. A., Roig, A., & Cayuela, M. L. (2015). Biochar accelerates organic matter degradation and enhances N mineralisation during composting of poultry manure without a relevant impact on gas emissions. Bioresource Technology, 192, 272–279. https://doi.org/10.1016/j.biortech.2015.05.003

Sarkhot, D. V., Ghezzehei, T. A., & Berhe, A. A. (2013). Effectiveness of biochar for sorption of ammonium and phosphate from dairy effluent. Journal of Environmental Quality, 42, 1545–1554. https://doi.org/10.2134/jeq2012.0482

Sokol, N. W., Sanderman, J., & Bradford, M. A. (2019). Pathways of mineral-associated soil organic matter formation: Integrating the role of plant carbon source, chemistry, and point of entry. Global Change Biology, 25(1), 12–24. https://doi.org/10.1111/gcb.14482

Taylor, M., Zhao, Y., Duan, D., Zou, R., Wang, Y., Ruan, R., & Liu, Y. (2014). Rhizosphere microbiome assembly is affected by plant development. The ISME Journal, 8, 790–803. https://doi.org/10.1038/ismej.2013.196

Tian, J., Wang, J., Dippold, M., Gao, Y., Blagodatskaya, E., & Kuzyakov, Y. (2016). Biochar affects soil organic matter cycling and microbial functions but does not alter microbial community structure in a paddy soil. Science of the Total Environment, 556, 89–97. https://doi.org/10.1016/j.scitotenv.2016.03.010

Ventura, M., Alberti, G., Viger, M., Jenkins, J. R., Girardin, C., Baronti, S., Zaldei, A., Taylor, G., Rumpel, C., Miglietta, F., & Tonon, G. (2015). Biochar mineralization and priming effect on SOM decomposition in two European short rotation coppices. Global Change Biology Bioenergy, 7(5), 1150–1160. https://doi.org/10.1111/gcbb.12219

Wang, C., Xue, L., Dong, Y., & Jiao, R. (2020). Soil organic carbon fractions, C-cycling hydrolytic enzymes, and microbial carbon metabolism in Chinese fir plantations. Science of the Total Environment, 758, 143695. https://doi.org/10.1016/j.scitotenv.2020.143695
Wang, J., Bowden, R. D., Lajtha, K., Washko, S. E., Wurzbacher, S. J., & Simpson, M. J. (2019). Long-term nitrogen addition suppresses microbial degradation, enhances soil carbon storage, and alters the molecular composition of soil organic matter. Biogeochemistry, 142, 299–313. https://doi.org/10.1007/s10533-018-00535-4

Wang, J., Xiong, Z., & Kuzyakov, Y. (2016). Biochar stability in soil: Meta-analysis of decomposition and priming effects. Global Change Biology Bioenergy, 8(3), 512–523. https://doi.org/10.1111/gcbb.12266

Wang, X. B., Song, D. L., Liang, G. Q., Zhang, Q., Ai, C., & Zhou, W. (2015). Maize biochar addition rate influences soil enzyme activity and microbial community composition in a fluvo-aquic soil. Applied Soil Ecology, 96, 265–272. https://doi.org/10.1016/j.apsoil.2015.08.018

Zhang, L., Xiang, Y., Jing, Y., & Zhang, R. (2019). Biochar amendment effects on the activities of soil carbon, nitrogen, and phosphorus hydrolytic enzymes: A meta-analysis. Environmental Science and Pollution Research, 26, 22990–23001. https://doi.org/10.1007/s11356-019-05604-1

Zhang, X. X., Zhang, R. J., Gao, J. S., Wang, X. C., Fan, F. L., Ma, X. T., Yin, H. Q., Zhang, C. W., Feng, K., & Deng, Y. (2017). Thirty-one years of rice-rice-green manure rotations shape the rhizosphere microbial community and enrich beneficial bacteria. Soil Biology and Biochemistry, 104, 208–217. https://doi.org/10.1016/j.soilbio.2016.10.023

Zhang, J., Chen, J., Pan, G., Liu, X., Zhang, X., Li, L., Bian, R., Cheng, K., & Jinwei, Z. (2016). Biochar decreased microbial metabolic quotient and shifted community composition four years after a single incorporation in a slightly acid rice paddy from southwest China. Science of the Total Environment, 571, 206–217. https://doi.org/10.1016/j.scitotenv.2016.07.135

Zhu, X., Chen, B., Zhu, L., & Xing, B. (2017). Effects and mechanisms of biochar-microbe interactions in soil improvement and pollution remediation: A review. Environmental Pollution, 227, 98–115. https://doi.org/10.1016/j.envpol.2017.04.032

SUPPORTING INFORMATION
Additional supporting information may be found in the online version of the article at the publisher’s website.

How to cite this article: Sun, J., Li, H., Zhang, D., Liu, R., Zhang, A., & Rengel, Z. (2021). Long-term biochar application governs the molecular compositions and decomposition of organic matter in paddy soil. GCB Bioenergy, 13, 1939–1953. https://doi.org/10.1111/gcbb.12896