Biochar induces mineralization of soil recalcitrant components by activation of biochar responsive bacteria groups

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Abstract:

Amendment of soil with biochar induces a shift in microbial community structure and promotes faster mineralization of soil organic carbon (SOC), thus offsetting C sequestration effects. Whether biochar induces losses of labile or persistent SOC pools remains largely unknown, and the responsible decomposers await identification. Towards addressing these ends, a C3 soil was amended with $Biochar_{500}$ or $Biochar_{600}$ (pyrolyzed at 500 ℃ and 600 ℃, respectively) produced from a C4-maize feedstock and incubated for 28 days. Combination of stable isotope ^{13}C techniques, high-throughput sequencing and Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS) allowed changes in soil chemodiversity and biodiversity, as well as their interactive effects on biochar induced SOC mineralization to be elucidated. Results indicated that: i) biochar addition shifted the bacterial community towards dominance of Gemmatimonadetes, Bacteroidia, Alphaproteobacteria and Gammaproteobacteria classes, and coincidence with recalcitrant C components and neutral pH soil; ii) the persistent DOM components (such as condensed aromatics and tannin) were depleted in biochar amended soils, while labile DOM components (such as unsaturated hydrocarbons, lipids, carbohydrates and proteins/amino sugar) were relatively enriched, and; iii) $Biochar_{600}$ promoted additional soil derived CO_2 carbon loss over 28 days (93 mg C kg^{-1} soil). Collectively, these results suggested that the majority of soil derived CO_2 efflux in biochar amended soils originated from recalcitrant components that were mineralized by the persistent organic matter decomposers. This research highlights the significance of biochar responsive taxa in changes of DOM chemodiversity and potential loss of SOC via mineralization.

Key words**:** *Organic matter decomposition; dissolved organic matter; chemodiversity and biodiversity; microbial communities; Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS).*

1. Introduction

On account of their high carbon (C) content and resistance to degradation, biochars, the product of pyrolyzed plant residues or other organic materials, have drawn attention as promising materials to enhance C sequestration (Lehmann, 2007). Although biochar *per se* increase soil organic C (SOC) content, it induces contradictory effects via interactions with non-biochar C. For example, biochar causes lower decomposition of plant-derived C, e.g., rhizodeposits (Weng et al., 2017); while some studies reported larger loss of rhizodeposits in biochar amended soils (Chen et al., 2021; Fu et al., 2022). Aside from the inconsistent results of plant-derived C decomposition in biochar amended soil, biochar addition to soil has been reported to result in greater and lower SOC mineralization, depending on the types of soil and biochar (Luo et al., 2011; Luo et al., 2016; Zimmerman et al., 2011). Soil priming effects wherein more (positive priming) and less (negative priming) CO_2 is released from SOC following substrate amendment, including glucose, rhizodeposits, straw, and biochar, have been widely reported (Fontaine, 2003; Kuzyakov et al., 2010; Blagodatskaya 2010; Ling et al., 2021). Biochar induced positive priming, in particular, is of concern as it offsets the C sequestration effects of biochar *per se* (Wardle et al., 2008; Lehmann et al., 2008).

The mechanisms involved in biochar induced soil priming effects can be attributed to changes in the soil microbiome (Chen et al., 2019). Microbial biomass, activity, community and enzyme production shifts in the charsphere, the zone where biochar meets soil (Lehmann et al., 2011). These biological property changes following biochar addition can be largely regulated by the altered microbial habitats including: i) increase in soil pH (Luo et al., 2017b), which relieves acid stress of acidic soil and consequently benefits the microbial community (Lehmann et al., 2011; Luo et al., 2013), e.g., Xu et al. (2014) reported that biochar increased soil pH from 4.48 to 6.03, which increased the relative abundances of microbial groups that adapted to neutral pH and consequently affected C and nitrogen (N) cycling; ii) large porosity and surface area of biochar (Lehmann et al., 2011; Luo et al., 2013; Yu et al., 2019), which consequently creates better conditions for fungi and bacteria growth,

and; iii) increased supply of C, nutrients and energy contained within biochar (Sheng and Zhu, 2018; Liao et al., 2019).

The intensity of CO_2 efflux is closely related to the characteristics of biochar, such as the content of labile component, which largely depends on feedstock type and pyrolysis conditions (Yu et al., 2018; Luo et al., 2011). Pyrolysis drives chemical transformation of plant biomass via heating in an oxygen limited environment, causing volatile compounds released, and aliphatic components to be depleted. This leads to lower hydrogen (H) and oxygen (O) contents but enriched C-content (thus lower H/C and O/C ratios) within the biochar product (Novak et al., 2009). Increase of pyrolyzed temperature from 400 °C to 700 °C tends to increase the fixed C and inorganic mineral content, but decrease biochar yield, volatile matter, cation exchange capacity (CEC), surface area and porous structure (Lee et al., 2019). When pyrolysis temperatures are increased from 500 to 600 ℃, biochar morpho-physiochemical characteristics changes dramatically, e.g., high-temperature pyrolysis (>550 ℃) produces biochars that generally have high surface areas, pore space and pyrogenic amorphous C (Keiluweit et al. 2010). Also, pyrolysis above 550 °C causes sharply decrease of biochar yields (Bruun et al., 2010), as well as the shift of chemical composition, e.g., less C=O and C-H functional groups but higher content of persistent C (mostly poly-condensed aromatic moieties and tannin) under higher temperatures (Singh et al., 2012; Leng et al., 2018). Pyrolysis above 550 ℃ cause sharp decrease of microbial degradability of biochar (Bruun et al., 2010). Comparably, labile resources are more likely to be retained if feedstock is pyrolyzed under low temperatures, e.g., with the decrease of temperature from 575 to 475 °C, the contents of cellulose and hemicellulose increases from 5.5% to 30%. Also, pyrolysis temperature lower than 550 °C favors greater recovery of nutrients such as nitrogen, potassium and sulphate; that are increasingly lost at higher temperatures (Keiluweit et al. 2010). By offering easily consumed C and nutrients, biochars produced under relative low temperature (< 550 ℃) are more likely to activate microbial biomass, particularly those of fast-growing microbes (Liu et al., 2019; Zhang et al., 2020).

The initial flush of CO_2 efflux from soil immediately following biochar amendment is often found to be short-lived and linked to the mineralization of labile organic matter components delivered to soil with the biochar (Cross and Sohi, 2011; Zimmerman et al., 2011). Therefore, most biochar induced positive priming diminishes after a short period of time as these available resources are exhausted (Luo et al., 2011; Zimmerman et al., 2011). Since the proportions of labile resources, e.g., dissolved organic matter (DOM) decline, the microbial activity decreases. However, the decline of activity does not occur for all microbial taxa. When the content of labile components decreases, some microbial groups, e.g., Gemmatimonadetes, respond to refractory OM components, such as polyaromatic C (Whitman et al., 2019; Campos et al., 2020). Ling et al. (2021) also found the enriched refractory components, i.e., phenolic substances, favored oligotrophs in the post-fire soils (3 months). To obtain knowledge about i) the shift in the bacterial community (e.g., strategy/metabolisms) with respect to C/nutrient conditions (oligotrophic or eutrophic), and; ii) the proportions of labile/recalcitrant components decomposed as a dominant outcome of changed community structure, it is critical to improve the understanding of biochar related C dynamics.

Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS) can be used to characterize DOM chemodiversity and has been applied in a range of environmental media, including, ocean water (Osterholz et al., 2016), lakes (Yuan et al., 2017), sediments (Butturini et al., 2020), rhizodeposits (Roth et al., 2019) and soils (Zhang et al., 2016; Li et al., 2018). Through the alignment of FT-ICR MS chemodiversity profiling with molecular ecology profiling of microbial community composition, co-occurrence network analysis has been applied to gain insights into chemodiversity and biodiversity linkages (Zhao et al., 2019). Li et al. (2018) investigated the manifold associations between the diversity of microbiota and the heterogeneity of soil DOM under long-term organic and inorganic fertilization practices. They showed that the continuous addition of organic C and nutrients (especially the high dose treatments) maintained not only high diversity of DOM, but also a more complex network between microorganisms and DOM molecules, i.e., the number of active hubs was more pronounced in the organic fertilizer regime. This

result indicates that sustained organic C addition to soil shaped the bacterial community toward a more eutrophic and diversified population that had more interactions with more diversified DOM molecules. Also, Wu et al. (2021) identified specific linkages between bacterial community and DOM traits by correlating top 1000 most abundant DOM formulas and top 1000 most abundant bacterial genus. Exploring the associations between DOM chemodiversity (i.e., chemical moieties) and bacterial community composition offers opportunity to clarify the molecular mechanisms underlying microbially driven soil C processes.

While a large number of studies have investigated biochar induced SOC loss and the associated shift in microbial community structure (Luo et al., 2011; Chen et al., 2018), only a few studies have provided deep insights at the molecular level to explore C-microbial interactions underpinning SOC mineralization. Biochar induced SOC losses require mechanistic understanding, for example, who (decomposers) is responsible for the decomposition of which (moieties) SOC components. Aiming to decipher the underlying mechanisms of biochar induced SOC mineralization, we combined FT-ICR MS with high-throughput sequencing and stable ^{13}C isotope technique to characterize DOM chemical composition, bacterial community composition and SOC decomposition. We hypothesized that biochar induced changes in soil abiotic conditions, such as pH and DOM components, would shift the bacterial community and the abundance. More specifically, neutralization of acidic conditions and the introduction of recalcitrant compounds following biochar amendment would increase the relative abundance of persistent carbon mineralizers, and consequently break down of complex DOM components. In biochar-free soil, acid-tolerant bacteria would persist in relatively more stressed environments (e.g., pH < 5), as this microbial group possesses stress tolerance at the expense of other traits.

2. Materials and methods

2.1 Soils and biochar preparation

The soil samples were collected from Wenling region (28°170' N, 121°126' E), Zhejiang Province, China. The soil texture was a loamy clay (43% clay, 37% silt and 20% sand) and classified as an Alfisol according to the U.S. Department of

Agriculture (USDA) classification. The soil was sieved (2 mm) and plant residues removed. The maize biochar was produced at 500 ($Biochar_{500}$) and 600 °C ($Biochar_{600}$). The rate of heating was 1 °C per minute from 40 °C, followed by 30 minutes of continuous heating at the final temperature. The biochars were sieved (2 mm). Basic properties of biochars, including dissolved organic carbon (DOC), dissolved organic nitrogen (DON), total carbon (TC), total nitrogen (TN), pH and $\delta^{13}C$ (‰) and these properties (TC, TN, DOC, DON, pH and $\delta^{13}C$) of soils, including the control soil, $Biochar_{500}$ amended soil (BC_{500}), $Biochar_{600}$ amended soil (BC_{600}), were measured. Water content was adjusted to 40% water holding capacity (WHC) using ultra-pure water and pre-incubated at 25 °C for 7 days to allow initial sampling and sieving effects to subside.

2.2 Experimental design

The BC_{500} and BC_{600} biochars (< 2 mm) were added to soil at a dose of 20 g kg^{-1} soil (n = 3). Samples of biochar augmented and biochar-free soil (50 g; n = 3) were hydrated to 40% WHC and subsequently incubated in 100 mL beakers placed inside a 1 L glass jar. All jars were sealed with a rubber bung and incubated (25 °C) for 28 days (jars were randomized and moved periodically). All jars contained a vial of 1.0 M NaOH (20 mL). Vials were changed after 1, 3, 7, 14 and 28 days and used to quantify evolved CO_2 and $^{13}CO_2$ (‰). Deionized water (10 mL) was put in the bottom of each glass jar to maintain humidity during the incubation. Control soil was prepared in the same way (but no biochar was added) (n = 3). Following the 28-day incubation period, soil samples were taken to investigate: i) changes in bacterial community structure by using high throughput sequencing (0.5 g) and ii) DOM chemical compositions (5 g) by Fourier transform ion cyclotron resonance mass spectrometry.

2.3 Chemical analysis

Soil pH was measured in a 1:2.5 (w/w) soil solution (ultra-pure H_2O) using a pH electrode. Total C and N contents were measured with a CNS-2000 dry combustion instrument (LECO CNS 2000, LECO Corporation, Michigan, USA). Dissolved organic C and N (DOC/DON) contents were extracted from fresh soil (5 g) with Milli-Q water (25 mL) in centrifuge tubes. The tubes were shaken (220 rpm) for 30 min, then the supernatant was filtered through a 0.45 µm quantitative filter paper, and the resultant solution analyzed using a TOC/TN Analyzer (Shimadzu, Analytical Sciences, Kyoto, Japan). The natural ^{13}C abundance ($\delta^{13}C$) was measured with an elemental analyzer-coupled-isotope ratio mass spectrometer (EA-IRMS) (Sercon Ltd, Crewe, UK).

2.4 Soil respiration and $^{13}CO_2$

CO_2 evolved was measured by titration using a TIM840 auto titrator (Radiometer Analytical, Villeurbanne Cedex, France) with standard HCl (0.0501 M L^{-1}). To determine the $\delta^{13}C$ (‰) of the trapped CO_2, 4 ml aliquots of sample were added to 1 M $BaCl_2$ (8 ml) in centrifuge tube. The precipitated $BaCO_3$ was carefully rinsed with pure H_2O (3 times), then dried overnight (60 °C) in the centrifuge tube. The precipitate was scraped off the tube, weighed (1 mg) into a tin capsule and analyzed for $\delta^{13}C$ using an elemental analyzer-coupled-isotope ratio mass spectrometer (EA-IRMS) (Sercon Ltd, Crewe, UK). For the calculation of CO_2 derived sources refer to the SI material 1.

2.5 Chemical composition of dissolved organic matter

Solid phase extraction of dissolved organic matter (SPE-DOM) was performed as described in Li et al, (2018). DOM extracts (see above) were analyzed at the State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences. The detailed sample testing and analysis procedures are given in SI material 2 and 3. The aromatic index (AI) value was calculated using the equation: AI = (1 + C - 0.5*O - S - 0.5*H) / (C- O.5*O - S -

N -P) (Li et al., 2018). DOM was delineated into seven molecular groups based on H/C and O/C value: aromatic structures (0.3 - 0.7, 0.1 - 0.7), tannin (0.3 - 1.5, 0.7 - 0.9), lignin (0.7 - 1.5, 0.2 - 0.7), unsaturated hydrocarbons (0.7 - 1.5, 0.1 - 0.3), aliphatic/proteins (1.5 - 2.0, 0.2 - 0.7), lipids (0.1 - 0.3, 1.5 - 2.0) and carbohydrate (1.5 - 1.7, 0.7 - 0.8). According to AI, H/C value and the relationship with bacteria, condensed aromatic and tannin-like compounds were ascribed as persistent components, while unsaturated hydrocarbons, aliphatic/proteins, lipids and carbohydrate were ascribed as labile components (Li et al., 2018).

2.6 Bacterial community composition analysis

2.6.1 PCR amplification and high throughput Illumina sequencing

DNA was extracted from fresh soil samples (0.5 g) obtained using a FastDNA Spin Kit (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's instructions. The isolated DNA was eluted in 100 μL of TE buffer. The extracted DNA quality and quantity were checked using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Finally, the DNA in the samples was preserved at -80 °C until further sequencing.

To discriminate each sample, a unique 5 base pair (bp) sequence was inserted into the reverse primer. The PCR amplification of each sample was performed in triplicate; 50 μL reaction mixtures contained 0.5 μL (125 pmol) of each forward/reverse primer, 1 μL (approximately 50 ng) of genomic DNA, 23 μL of double distilled water, and 25 μL of Premix Taq (Takara, Shiga, Japan). Thirty-five thermal cycles (30 s at 94 °C, 30 s at 54 °C, and 45 s at 72 °C) were carried out with a final extension for 10 min at 72 °C. The PCR products were purified, mixed, and sent to Novogene, Inc. (HiSeq2500 platform; PE150, Beijing, China) for sequencing. The V3 - V4 region of the bacterial 16S rRNA gene was amplified using the primer pair 341F (5'-CCTAYGGGRBGCASCAG-3') / 806R (5'-GGACTACNNGGGTATCTAAT-3') (Yuan et al., 2018).

2.6.2 Taxonomic assignments and clustering of 16S rRNA gene fragments

The raw sequencing data were quality screened and trimmed using the Quantitative Insights into Microbial Ecology (QIIME package version 1.8.0) pipeline as previously described (Caporaso et al., 2010). QIIME quality trimming was performed in accordance with the following criteria: (1) truncated before three consecutive low-quality bases and re-evaluated for length, (2) no ambiguous bases, and (3) the minimum sequence length of 469 bp (16S rRNA) after trimming. Operational taxonomic units (OTUs) were clustered from the assembled high-quality sequences and classified with Basic Local Alignment Search Tool (BLAST) in the Silva Release 119 database and UNITE version 6.0 database (Quast et al., 2012). OTUs with abundances of < 0.001% were discarded.

The 16S rRNA sequence data were deposited to the National Center for Biotechnology Information under accession number PRJNA698294 (releasing Jan 31st, 2021). Alpha diversity (Shannon and Simpson diversity indices) and Bray-Curtis distances for a principal component analysis of soil bacteria community were calculated using the OTU table after rarefying all samples to the same sequencing depth.

2.7 Data analysis

The effects of the biochar on soil were analyzed by one-way ANOVA at $p < 0.05$, following the Tukey *post-hoc* test. A principal coordinate analysis (PCoA) based on Bray-Curtis distance from all samples was used to differentiate bacterial community structure in the three treatments. PERMANOVA (Adonis function in vegan of R) was used to quantify these effects. The characterization of bacteria community features in the biochar amended soils using the linear discriminant analysis (LDA) effect size (LEfSe) method (http://huttenhower.sph.harvard.edu/lefse/) for biomarker discovery, which emphasizes statistical significance and biological relevance (Segata et al., 2011). Distance-based linear model multivariate analysis (DISTLM) was used to understand the relative effects of DOM compositions on the soil bacteria community (Mcardle and Anderson, 2001).

For further details regarding constructing networks refer to SI material 4.

Network visualization was conducted using Cytoscape 3.6.1 (Smoot et al., 2010). Genera (belong to phyla) with the highest betweenness centrality scores were considered as core bacteria species (González et al., 2010); data is shown in the SI Table 9. The calculated topological characteristics of the bacterial and environmental factors network included the following: positive and negative correlations, nodes, edges, graph density and modularity in the SI Table 8.

Abiotic factors (e.g., pH and C components) and biotic properties (e.g., the relative abundance of class level bacteria) were conducted using random forest modeling to quantitatively assess their contributions to SOC and biochar derived CO_2. The significance of the model was determined by rfPermute and rfUtilities packages in R.

3. Results

3.1 Soil properties and $^{13}CO_2$ evolution

Following incorporation of biochar into soil, TC was quantified as follows: BC_{500} (3.52%), BC_{600} (3.74%) and the biochar-free soil (3.08%, Table 1). Thus, soil amended with BC_{500} had its TC content increased by 4.4 mg g^{-1}, and soil amended with BC_{600} by 6.6 mg g^{-1} (Table 1). The DOC content in BC_{500}, BC_{600} and Control soils were 272, 262 and 282 µg g^{-1}, respectively (Table 1). Total CO_2 emissions from soil amended with biochar over the 28 days incubation period were larger than those from unamended soil samples and ranked as: BC_{600} (604 µg C g^{-1}) > BC_{500} (548 µg C g^{-1}) > Control (381 µg C g^{-1}) ($p < 0.05$: Control *vs* BC_{500}, Control *vs* BC_{600}, BC_{500} *vs* BC_{600}) (Fig. 1a, SI Table 1). The SOC derived CO_2 increased in biochar amended soil to 384 (BC_{500}) and 474 µg C g^{-1} soil (BC_{600}) compared to Control (381 µg C g^{-1}) (Fig. 1b, SI Table 1). Mineralized biochar in BC_{500} and BC_{600} after 28 days of incubation were 164 and 130 µg C g^{-1} soil ($p < 0.05$), respectively. The mineralization of BC_{500} and BC_{600} derived from primed SOC were, respectively, 3 and 93 µg C g^{-1} ($p < 0.05$) (SI Table 1).

3.2 Characterization of dissolved organic matter

The DOC content (water extracted) was not significantly different ($p > 0.05$) across BC_{500}, BC_{600} and the biochar-free soil after 28 days (Table 1), while the general characteristics of DOM revealed unique molecular compositions across these regimes (Fig. 1c, 1d, SI Table 3). The proportion of DOM chemical components were significantly different among control and biochars (ANOSIM: R = 0.67, $p = 0.02$) (SI Fig. 1, SI Table 5). Lignin-like DOM compounds were dominant in all soil samples, accounting for 60 - 72% of all assigned molecules. The proportions of persistent components, such as condensed aromatics and tannin, were lower than the proportions of labile components (H/C $\geq$ 1.5) in BC_{500} and BC_{600} (SI Fig. 1, SI Table 3). The application of biochar changed (decreased) the chemodiversity of the DOM molecules compared to control, the substances that decreased were mostly condensed aromatics and tannin moieties, while those that increased were mainly labile moieties, such as protein/amino sugar (Fig. 1c and 1d). The molecular composition profile in the BC_{600} treatment covered much lower H/C and wider O/C ratios, indicating greater recalcitrance. Similarly, persistent molecules decreased, and labile components increased after BC_{600} addition (SI Fig. 1d, SI Table 3).

3.3 Characterization of soil bacterial community composition

The bacterial community composition showed little difference in α-diversity (Chao1) across control and the biochars (Fig. 2a). The principal co-ordinates analysis (PCoA) plot suggested that bacterial community compositions were altered by biochar addition (Fig. 2b). Further comparison of the bacterial community composition at the class level revealed the major bacterial classes (Fig. 2c). Compared to control soil, the relative abundance of classes, including Alphaproteobacteria, Gammaproteobacteria, Gemmatimonadetes and Bacteroidia increased following biochar addition, while Bacilli, Acidobacteriia, Thermoleophilia, Clostridia and Acidimicrobiia (class level) were decreased (Fig. 2c). LEfSe also showed that Gemmatimonadetes ($\log_{10}4.4$) have higher abundance in biochar amended treatments, while Acidobacteriia ($\log_{10}4.2$) and Acidimicrobiia ($\log_{10}3.6$) in control soil were higher than BC_{500} and BC_{600} (Fig. 2d, SI Fig. 3). Through best multivariate model analysis (DISTLM), lignin (80.6%), DON (5%), DOC (1.5%), pH (0.7%) and condensed aromatics (0.4%) were the top 5 abiotic variables that modulated bacterial community composition (SI Table 6). Bacteria

(y-axis), at class level, were associated with chemical components variables (x-axis) using Spearman's rank correlation (Fig. 3a). Biochar increased the bacterial classes of Alphaproteobacteria, Gammaproteobacteria (belong to Proteobacteria) and Gemmatimonadetes (belongs to Gemmatimonadetes); these were negatively correlated with condensed aromatics and tannin. While biochar decreased the bacterial classes Bacilli and Clostridia (belonging to the phylum Firmicutes) (Fig. 2c), these may be influenced by pH and labile components, such as lipids and unsaturated hydrocarbons (SI Fig. 5, SI Table 7). The Acidobacteriia (belonging to Acidobacteria) was affected by pH significantly ($r = -0.89^{**}$, $p < 0.05$).

3.4 Linkages between DOM molecular compositions and soil bacterial communities

The increased bacterial classes, including Alphaproteobacteria, Gammaproteobacteria and Gemmatimonadetes were negatively correlated with recalcitrant components, such as condensed aromatics and tannin. While labile components (lignin, unsaturated hydrocarbons, lipids, carbohydrates and proteins/amino sugar) were negative correlated with Bacilli, Acidobacteriia, Thermoleophilia, Clostridia and Acidimicrobiia (Fig. 2c, 3a), which were observed to decrease in their relative abundance.

To explore the interconnections between bacterial species and DOM molecules at the OTU and molecular levels, respectively, co-occurrence network analysis was used to visualize the interactions (Fig. 3b). There were 98 nodes and 256 edges in the network of the combined biochars. In addition, the DOM nodes (51.02%) and copresence edges (73.00%) were more often detected compared to bacterial nodes (48.98%) and mutual-exclusion (26.95%) edges in the network, respectively (SI Table 8). 256 edges were established among the DOM molecules and OTUs, including 192 correlations between DOM and DOM molecules, 31 OTU to OTU connections and 33 edges between DOM molecules (four categories) and OTUs (four phyla), of which 33 linked 23 DOM molecules and 14 OTUs (SI Table 9). The 14 OTUs were distributed among the Proteobacteria (10: there were 3 Alphaproteobacteria, 4 Gammaproteobacteria and 3 Deltaproteobacteria), Acidobacteria (1), Gemmatimonadetes (1), Verrucomicrobia (1) and unidentified Bacteria (1), respectively (SI Table 8).

The 23 DOM molecules were comprised of tannin (2), lignin (19) and other molecules ($C_{18}H_{30}O_3S_1$: unsaturated hydrocarbons and $C_{12}H_{26}O_4S_1$: lipids). *Ramlibacter* (Gammaproteobacteria) was negatively related to $C_{12}H_{15}N_1O_7$ (lignin). *Burkholderiaceae* (Gammaproteobacteria), including OTU981 and OTU111, had negative correlations (blue lines) with $C_{20}H_{28}O_{12}$, $C_{20}H_{26}O_{12}$ and $C_{17}H_{24}O_{11}$ (all of which belong to lignin). $C_{20}H_{28}O_{12}$ was also negatively associated with *Vulgatibacter* (Deltaproteobacteria) (Fig. 3b, SI Table 9). Whereas $C_{18}H_{30}O_3S_1$ (unsaturated hydrocarbons) and $C_{12}H_{26}O_4S_1$ (lipids) showed positive correlations (orange lines) with *Candidatus_Solibacter* (Acidobacteria) and *Verrucomicrobiae* (Verrucomicrobia), respectively (Fig. 3b, SI Table 9). The network pattern indicated that the same classes, or even same genus, had diverse associations with DOM molecules of contrasting chemical characteristics, i.e., having opposite correlations to the same category of DOM compounds or having the same correlations to different categories of molecules. For example, Proteobacteria showed positive and negative correlations with lignin or tannin, respectively (Fig. 3b, SI Table 9). In particular, *Burkholderiaceae* (Gammaproteobacteria) showed negative and positive correlations to lignin. Further relations between OTUs (Proteobacteria) and DOM (lignin and tannin) are shown in SI Table 9. These results improve the understanding of the connections between C molecules and bacterial community compositions at the molecular level. This may contribute to the defining of a mechanism to translate current knowledge, regarding soil C status, bacterial community composition and their correlations, into actionable pathways.

3.5 DOM composition and bacterial community in relation to C dynamics

Random Forest analysis revealed the contributions of major class level microorganisms, DOM composition, environment factors to SOC and biochar derived CO_2 (Fig. 4). The main contributions to SOC derived CO_2 were abiotic factors (lignin, condensed aromatics and pH) and biotic producers (Gemmatimonadetes, Acidobacteriia, Acidimicrobiia and Bacteroidia) (Fig. 4a).

Bacterial taxa, such as Clostridia, Thermoleophilia, Gemmatimonadetes, Bacilli,

Bacteroidia, Gammaproteobacteria, and Acidimicrobiia, influenced biochar derived CO_2 during the 28-day incubation (Fig. 4b). Compared to biotic factors, abiotic factors, including condensed aromatics, lignin, tannin, lipids and pH, had a stronger correlation with biochar-derived CO_2 (Fig. 4b). These linkages suggest those species utilized soluble lignin and condensed aromatics introduced into the soil by biochar. SOC derived CO_2 was influenced by lignin, pH, condensed aromatics and unsaturated hydrocarbons, while biochar derived CO_2 was mainly contributed by condensed aromatics and lignin from the biochar over the short-term (28 days).

4. *Discussion*

4.1 Direction of correlation between DOM composition and bacterial community composition

To date, some studies have attempted to reveal the link between bacterial community biodiversity and DOM chemical diversity (Osterholz et al., 2015; Underwood et al., 2019). Several studies have noted the close molecular-level association between the relative abundance of DOM components and bacterial groups, and that chemodiversity-biodiversity interactive effects can be bidirectional, wherein: i) DOM chemical composition shapes microbial community composition, and in general more complex DOM profiles maintain relative high diversity and abundance of microbiome (e.g., Zhao et al. (2019) reported that the increased bacterial diversity are caused by the diverse DOM molecules, and particularly, the abundance of Gammaproteobacteria, Betaproteobacteria, and Flavobacteria were enhanced by the enrichment of complex DOM substrates); ii) specific microbial taxa selectively decompose specific DOM components (Lladó et al., 2016), e.g., Genus *Nitrospira* have been negatively correlated to DOM recalcitrant compounds, suggesting *Nitrospira* specialized on this DOM category (Li et al., 2018). The latter has been explained by the affinities of microorganisms for individual C compounds.

Considering the consumption of DOM compounds by microorganisms, higher microbial biomass has been reported to lead to fewer C compounds remaining after utilization, particularly in a closed system where substrates supplement is precluded. We, therefore, suggest that i) a negative correlation indicates affinity of

microorganism to compounds over short time periods or in a closed system (no continuous substrates supplied); while ii) a positive correlation implies an environmental niche that has selected for a soil microbiome assemblage over longer time scales or in an open system (Fig. 3c). Because of the closed system of this study, negative correlation between soil microbes and individual DOM molecules suggested the utilization of chemical compounds by microorganisms.

4.2 Bacterial groups that associated with DOM components

High yield bacteria closely associated with labile DOM components

Labile components (such as unsaturated hydrocarbons, lipids, carbohydrates and proteins/amino sugar) were negatively correlated with Bacilli and Clostridia (both belonging to the phylum Firmicutes) (Fig. 3a), indicating their utilization capacity for these molecules (Li et al., 2018). Firmicutes has been reported to be a dominant assimilator of labile compounds (Ramirez et al., 2012; Malik et al., 2019). DNA-SIP revealed Firmicutes to be the dominant glucose consumer within the bacterial community during the early decomposition stage (Wang et al., 2021). The relative abundances and the proportions of Bacilli and Clostridia were much lower in biochar amended soil, after 28 days of incubation, compared to control (Fig. 2c, SI Table 4). This might be due to i) the adsorption of labile DOM components on biochar surface or their entrapment in small pores, which reduced the C and nutrients contents (Table 1), as well as their accessibility to fast-growing microorganisms (Lehmann et al., 2011; Luo et al., 2013), and; ii) labile C components (delivered by the biochar) may have been consumed early in the incubation period (Luo et al., 2011). In support, Yu et al. (2018) reported microbial community succession from the dominance of Firmicute to non-fast growth microorganisms occurred at day 8 of incubation. Future research to explore dynamics of abundance of high yield bacteria and their consumption of labile DOM fractions (evaluated by using labeled DOM extracts) during the first few days after biochar addition, would help create a deeper understanding of the microbial mechanisms underlying mineralization of more soluble DOM components.

Acidity-resistant microbial group correlated to labile components

Acid-tollerant microorganisms (e.g., Acidobacteriia) were observed to be more abundant in control soil (this soil noted to have a lower pH value of 4.86 (Table 1; Fig.

2c, SI Table 4). These microorganisms possess specific traits to tolerate low pH conditions. For example, *Candidatus Solibacter* (phylum of Acidobacteria) produces a polysaccharide biofilm to confer stress resilience (Eichorst et al., 2018). Class of Acidobacteriia are able to change the structure and composition of their cell envelopes to maintain cellular integrity in acidic environments (Rousk et al., 2010; Lipson, 2015). Acidobacteria can encode enzymes for the transport and utilization of carbohydrates (mainly for energy production to maintain cell integrity) and thereby resist low pH (Rawat et al., 2012).Through such adaptations, they can outcompete other bacteria, and have a high relative abundance under acidity stress conditions (Fig. 2c, SI Table 4).

Biochar addition increased soil pH from 4.86 to 6.15 (Table 1), and thus weakened the competitive ability of acid tolerant bacteria with respect to other microorganisms (Sheng and Zhu, 2018), decreasing the relative abundance of this organism type, e.g., the Acidobacteriia class (Fig. 2c, 3a, Table 2).

Acidobacteriia was negatively correlated with DOM-lignin and the labile components such as carbohydrates (Fig. 3a), suggests affinity of Acidobacteriia for both recalcitrant and easily degradable C sources. Although Acidobacteria members are usually characterized by an ecological K-strategy, they can make use of labile substances when most other bacteria are not active in acidic environments. Acidobacteriia were observed to be dominant in the lower pH control soil. These observations are consistent with Kielak et al. (2016) and Pankratov et al. (2008), who reported Acidobacteria are dominant in utilization of labile components, such as D-glucose, D-xylose, lactose and most of the tested oligosaccharides as C sources under acidic conditions.

Class within phylum Actinobacteria associated with DOM components

Classes of Acidimicrobiia and Thermoleophilia (belonging to phylum Actinobacteria) were negatively correlated to labile DOM components (particularly carbohydrates) and DOM-lignin (Fig. 3a). This suggests these two classes caused decomposition of both labile carbohydrates and recalcitrant DOM-lignin compounds. Due to its wide range of niches for C sources and

environmental conditions, phylum Actinobacteria (class Acidimicrobiia and Thermoleophilia as proxy in this study) have been reported to be present in a diversity of habitats (particularly in the harsh condition). For example, strains belonging to class Acidimicrobiia have been isolated from various harsh habitats including the deep ocean, desert and acid mine (pH > 3) environments (Hu et al., 2018). Similar to class Acidobacteriia, the increased soil pH in biochar amended soil, might have decreased the competition of Acidimicrobiia and thus decrease its abundance relative to other microbial groups (Fig. 3a).

In contrast to classes Acidimicrobiia and Thermoleophilia, Actinobacteria was observed to have a weak negative correlation with persistent DOM, including as tannin and condensed aromatics (Fig. 3a). The conflict results between these classes within the same phylum of Actinobacteria might be due to inconsistent response of microorganisms at different class (even at the same phylum) to biochar. Woolet and Whitman. (2020) reported the lack of phylum-level microbial community response to biochar to be consistent across 16 studies. The weak correlation between class Actinobacteria and DOM tannin and condensed aromatics indicated that this class played a less significant role in utilization of DOM compounds. It is possible that Actinobacteria, with hyphae spatially extend their acquisition of C/nutrients distantly, and are therefore less sensitive to changes in DOM profile.

Bacterial groups related to persistent components

Class of Alphaproteobacteria, Gammaproteobacteria, Gemmatimonadetes and Bacteroidia were more prevalent in biochar amended soils and were negatively correlated with the abundance of persistent components in DOM, such as tannin and condensed aromatics (Fig. 2c, 3a, SI Table 4). Gemmatimonadetes can respond to refractory OM components, such as polyaromatic C (Whitman et al., 2019). Additionally, Ling et al. (2021) found that Proteobacteria were negatively correlated with recalcitrant substrates, such as phenolic-C. Woolet and Whitman (2020) collected data across 16 studies and reported Proteobacteria to be the main microbial groups that adapted biochar amended soils. Zhu et al. (2019) reported that Proteobacteria was the primary bacterial phylum observed in biochar amended soils, and some taxa

within this phylum could be efficient decomposers of persistent organic matter such as polycyclic aromatic hydrocarbons.

The network pattern from this study revealed the genus within Alphaproteobacteria and Gammaproteobacteria to be involved in the utilization of DOM-lignin molecules (Fig. 3b). Detailed, negative correlations between some persistent DOM-lignin molecules, for instance $C_{20}H_{28}O_{12}$ and $C_{20}H_{26}O_{12}$ (lignin-like compound group), and OTU981 and OTU111 (both belong to Genus *Burkholderiaceae*, class Gammaproteobacteria) were established (Fig. 3b, SI Table 9). Also, a negative correlation between *Burkholderiaceae* (Gammaproteobacteria) and the moiety with formula $C_{17}H_{24}O_{11}$ (a lignin-like compound: possibly, gardenoside, secoxyloganin (CHEBI: 132712, according to ChEBI library, https://www.ebi.ac.uk/chebi/) or scandoside methyl ester) were observed. These results are consistent with other reports, wherein *Burkholderiaceae* have been reported to dominate in solid lignin enriched environments (Bugg et al., 2011) and utilizing this recalcitrant C resource by secreting enzymes such as laccase and peroxidase (Morya et al., 2019). In other instances, *Ramlibacter* (Gammaproteobacteria) was negatively correlated with $C_{12}H_{15}N_1O_7$ (DOM-lignin compounds) (Fig. 3b, SI Table 9). Similarly, *Ramlibacter* had been reported to be an effective aromatic compound degrading genus of bacteria (Sun et al., 2019).

In the present study, positive correlations between DOM-lignin and Alphaproteobacteria, Gammaproteobacteria, Gemmatimonadetes and Bacteroidia were observed (Fig. 3a), suggesting these persistent organic matter decomposers did not utilize lignin. *Burkholderiaceae* (Gammaproteobacteria) was positive correlated to $C_{11}H_{16}N_2O_6$, $C_{10}H_{12}O_5$, $C_{11}H_{16}O_6$ and $C_{11}H_{13}N_1O_5$ (DOM-lignin like compounds) (Fig. 3b, SI Table 9), suggesting the possibility of these moieties being utilized by *Burkholderiaceae* was unlikely.

In contextualizing these conflict observations it is important to appreciate that DOM-lignin is distinct from insoluble lignin noted. Lignin is a complex, highly aromatic/condensed substance (Tarasov et al., 2018), and is conventional considered recalcitrant and difficult for microorganism to utilize (Brown and Chang, 2014). Lignin has been reported to have a very large molecular weight, such as Tolbert et al. (2014) found that the maximum molecule weight of lignin isolated form wheat straw

was 6100.. In contrast DOM-lignin characterized in this research had a much smaller molecular weight (minimum molecule weight: 188, $C_9H_{16}O_4$). Therefore, the apparent incongruence of molecular recalcitrance but utilization might be explained by the fact that DOM moieties of relatively low molecular weight will have been available and biodegradable microorganisms. It is highlighted that high-resolution FT-ICR MS is able to separate DOM-lignin molecules into labile and persistent parts (as ascribed by H/C and O/C ratios). Yet, such Van Krevelen plots provide only a partial view on putative reactance/degradability, as Van Krevelen plots do not convey molecular weight. Arguably, Van Krevelen DOM plots require an additional Z-axis to define the molecular mass of the DOM moiety (SI Fig. 2). Such plots provide a deeper insight into the physical (molecular size) and chemical (atomic ratios) underpinnings that, together, influence how recalcitrant/degradable a molecule might be.

4.3 The bacteria related to SOC mineralization

SOM mineralization is governed by the substrate recalcitrance, soil matrix and the microbial community (Fontaine et al., 2003; Cotrufo et al., 2013). DOM is the most active part of SOM and provides soluble organic substrates to heterotrophic microorganisms, and thus the DOM composition determines microbial community and its activity. The association between DOM composition and soil microbiome composition lies at the heart of C cycling, i.e., SOM mineralization. Here, biochar augmentation altered bacteria communities via changes in soil physiochemical properties such as DOM chemical composition profiles and soil pH (SI Fig. 4). Consequently, these changes influenced the amount of SOM mineralization (Fig. 4a). Different bacterial taxa with distinguishable traits, i.e., metabolisms, gave variate contributions to SOC mineralization in biochar amended soil.

Less contribution to SOC mineralization by Firmicutes

Firmicutes, e.g., class Bacilli and Clostridia, with lower relative abundance in biochar amended soils, likely had relatively a small influence on SOC mineralization (Fig. 2c, 4a). Anthony et al. (2020) attributed microbiotas involved in C fluxes to microbial trade-offs among characteristics related to growth yield, stress tolerance and resource acquisition. Firmicutes maximize the uptake of C resource to biosynthesis by investing in associated assimilatory pathways such as synthesis of amino acid, fatty

acid, and nucleotide (Malik et al., 2019). By using these indispensable substrates to establish cells and increase yield, the microbial biomass and necromass of Firmicutes might contribute to C transformation rather than mineralization to CO_2 (Malik et al., 2018). This large biomass of Bacilli and Clostridia (both belong to Firmicutes) can use labile components released from biochar (Fig. 4b), rather than the persistent compounds of SOC (Fig. 4a).

Firmicutes would likely flourish in the early stage of incubation by assimilating labile C sources, nutrients and basic cations delivered with the biochar (Liao et al., 2019; Wang et al., 2021). On the basis that available C presence would be short-term, i.e., up to 7 days (Luo et al., 2011), these bacteria potentially decreased between 7 and 28 days as labile C sources and nutrients from biochar were exhausted (Fig. 2c, Table 1). Decline of both labile biochar delivered chemical components and the low abundance of Firmicutes likely underpinned a limited contribution to SOC mineralization (Fig. 4a). Confirmation of this, however, awaits future work to obtain data of dynamic changes of bacterial communities following biochar amendment.

The role of Actinobacteria in SOC mineralization of biochar amended soils

Class of Acidimicrobiia and Thermoleophilia correlated to both labile DOM components (particularly carbohydrates) and DOM-lignin (Fig. 3a. These two classes might cause decomposition of chemically divergent DOM moieties via co-metabolism. Actinobacteria are important saprophytes that are able to decompose plant derived rhizodeposits and straw (Kabuyah et al., 2012), via a range of enzymes (β-glucosidase, xylanase, protease, cellobiohydrolase, cellulases, hemicelluloses, and other ligninolytic enzymes) that can act upon polysaccharide, amino sugar, cellulose, lignocellulose and lignin (Manivasagan et al., 2013; Chen et al., 2014; Lladó et al., 2016). Through the action of these enzymes both small molecular substances and also complex compounds from soil can be degraded (Zhang et al., 2017). Actinobacteria has been reported as able to degrade recalcitrant polycyclic aromatic hydrocarbon (Zhu et al., 2019).

Unlike class Acidimicrobiia and Thermoleophilia, class Actinobacteria was not predicted by random forest analysis as one of the key contributors to C mineralization (Fig. 4a). Many studies, however, have reported Actinobacteria is one of the major responders of microbial community to biochar addition, and widely acknowledged its

great contributions to SOC mineralization in biochar enriched soils (Chen et al., 2021; Jeewani et al., 2020; Woolet and Whitman 2020). We, therefore, group Actinobacteria to one of the taxa involved in biochar induced mineralization (Table 2), due to: i) the hyphal-like morphology of Actinobacteria that aids their contact with SOC and facilitates its decomposition (Luo et al., 2013; Jeewani et al., 2020; Kabuyah et al. (2012) reported that Actinomycetes (belonging to the phylum Actinobacteria) to utilize biochar-C and organic matter in remote area, and thus out-compete other microorganisms in an oligotrophic environment); and ii) large surface and pore size provided to promote microbial colonization (Luo et al., 2013). For instance, genus *Nocardioides* (belong to order Actinomycetales) are widely found to be dominant in biochar amended soils (Woolet and Whitman 2020) and play a key role in rhizodeposits and SOC mineralizaiton in a planted soil (Fu et al., 2022). Genus *Streptomyces* is another filamentous bacterium (belong to order Actinomycetales) and inoculation of *Streptomyces* strains can promote the mineralization of pine wood biochar.

Possible key players in SOC mineralization in biochar amended soils

Class Gemmatimonadetes, Bacteroidia, Alphaproteobacteria and Gammaproteobacteria were the responders of biochar addition (particularly DOM-R, i.e. tannin, condensed aromatics) and most likely played the key role in mineralization of persistent components of SOC in biochar amended soils (Fig. 2; Fig. 4; Table 2). These DOM-R shaped class can utilize recalcitrant SOC components. Aromatic compounds of DOM resulted in higher abundance of K-strategy bacteria that could in turn degrade persistent components (Zhang et al., 2020). Additional C can increase the activity of soil microorganisms that prime the mineralization of native SOC as a result of co-metabolism via enzyme production (Blagodatskaya and Kuzyakov, 2008). Biochar induced SOC mineralization via releasing extracellular enzyme has been widely reported (Li et al., 2019; Anthony et al., 2020; Campos et al., 2020).

Studies suggest Gemmatimonadetes can adapt and survive in arid environments (Aanderud et al., 2011), but are sensitive to acidic environments (Luo et al., 2003). The isolated strain (*Gemmatimonas aurantiaca T-27*) has been reported to grow better under pH within the range 6.5-9.0 (Luo et al., 2003). The increase of soil pH by biochar promoted the growth and doubled the abundance of class Gemmatimonadetes

(Fig. 2c), and consequently caused SOC mineralization (Fig. 4c). Xu et al. (2014) reported that the relative abundance of Gemmatimonadetes increases with the addition of rice straw biochar to a farmed Acrisol, and this class is adapted to a lifestyle associated with recalcitrant C sources and able to mineralize them. Similarly, others reported Gemmatimonadetes can respond quickly to the refractory organic matter, such as polyaromatic components in soils (Whitman et al., 2019; Campos et al., 2020). Members in Gemmatimonadetes are found to accumulate phosphorus and might exploit SOM for nutrients via production of catalase and oxidase (Luo et al., 2003).

As the predominant class in biochar amended soils (Fig. 2c), Alphaproteobacteria contributed to SOC mineralization, and the mechanisms underlying include: i) nutrients mining, e.g, Razanamalala et al. (2018) reported that Alphaproteobacteria could use straw-derived energy to produce extracellular enzymes to mine humified SOM for nutrients; and iii) biochar responders/adaptors, Alphaproteobacteria that adapted to biochar aromatic compounds might cause the decomposition of similar components of SOM, e.g., some members of class Alphaproteobacteria, such as *Phenylobacterium*, that dominant in biochar amended soils are often identified as being fire-responders, and might use SOM as C sources when plants are absent (Woolet and Whitman 2020). Additionally, Bacteroidia has been reported to be one of the main responsive taxa to biochar (Woolet and Whitman. 2020) and might also contribute to the observed SOC mineralizaiton. Yet, these key SOC decomposers were identified/predicted based on correlation analysis. To confirm their role in biochar induced SOC mineralization requires further validation by cultivation and inoculation approaches.

4.4 Implications and directions

Biochar has drawn a great deal of attention as a biologically inert and slowly decomposing material with the potential to increase SOC stocks (Lehmann, 2007). However, the interactions between biochar and non-biochar C pools of soil have largely been ignored as the input of biochar C *per se* and output of CO_2 (i.e., positive priming) is disproportionate. Recent results have indicated biochar induced C loss to be mainly via mineralization of labile components originating from both biochar and SOC (Luo et al., 2011; Zimmerman and Ouyang, 2019). There is a gap of knowledge regarding the composition of SOC loss during the processes of biochar induced SOC

mineralization. This study revealed that the molecules that were lost via priming were persistent components, such as condensed aromatics and tannin (Fig. 1, Fig 4).

It was observed after 28 days, that most of the decreased molecules in biochar amended soil (vs control) belonged to DOM-R (recalcitrant components), and a small increase in DOM-L (labile components) was observed (Fig. 1c, d). These outcomes were most pronounced and accompanied by higher SOC mineralization in the $biochar_{700}$ amended soil (Fig. 1b). These results suggested most of the SOC derived CO_2 was likely derived from mineralization of DOM-R. The implication of the finding is significant. Loss of refractory SOC is detrimental, as i) soil persistent components are built up over hundreds to thousands of years; and ii) loss of a fraction of the persistent SOC pool cannot simply be compensated for by biochar incorporation (biochar lacks biological traits and is therefore not comparable to SOM).

While counter intuitive, the outcome of persistent C loss might be linked to i) higher incorporation of persistent DOM into non-extractable insoluble SOC following biochar augmentation, which might cause mineralization of this persistent fraction via activation of putative microbial groups, and ii) biochar addition priming persistent SOC mineralization through a continuum of progressively decomposed organic compounds (Lehmann and Kleber, 2015). Considering the contentious nature of SOC, and the observed shift in DOM chemical composition (low ratio of persistent to labile) following biochar addition, further researcher is justified in this area. Additionally, future study needs to consider long-term (up to years) effects, as the shift of the microbial community, particularly their eco-physiology traits (e.g., towards more oligotrophs), might promote the continuous decomposition of persistent SOC in biochar amended soils (Kuzyakov et al., 2009; Luo et al., 2017a). The observations of dynamic changes of microbial community, coupled with SOC mineralization, are required, as a succession of microbial community, depending on C recalcitrance in biochar amended soil, would be responsible for the magnitude of soil CO_2 emission and compositional loss of SOC during the respective stages.

5. Conclusions

This study revealed biochar particularly $biochar_{700}$ (produced at a higher pyrolysis temperature: 700^{o}C) induced larger SOC mineralization (an additional CO_2 loss of 93 mg C kg^{-1} soil) after 28 days of incubation. Class Gemmatimonadetes, Bacteroidia, Alphaproteobacteria and Gammaproteobacteria were dominant in biochar amended soils and most likely gave the largest contributions to the mineralization of SOM, particularly via mineralization of recalcitrant DOM-R components, i.e., tannin, condensed aromatics. In contrast, fast growth classes, i.e., Bacilli and Clostridia (both belonging to Firmicute), likely had minimal influence on SOC loss. By adopting analysis of sequencing and FT-ICR MS, this research indicated that primed SOC losses from biochar amended soils were mostly derived from the more persistent DOM-R fractions being utilized by putative biochar responsive taxa.

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