Title

Generalists and specialists decomposing labile and aromatic biochar compounds and sequestering carbon in soil

Authors

Liu, Huaiting
Wang, Xiu
Song, Xiaoqing
et al.

Publication Date

2022-12-01

DOI

10.1016/j.geoderma.2022.116176

Peer reviewed

Geoderma 428 (2022) 116176

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Generalists and specialists decomposing labile and aromatic biochar compounds and sequestering carbon in soil

Huaiting Liu [a,b], Xiu Wang [a,b], Xiaoqing Song [c], Peng Leng [d], Jihui Li [d], Jorge L. Mazza Rodrigues [e], Zhiqi Hong [f], Yakov Kuzyakov [g,h], Jianming Xu [a,i], Zhongmin Dai [a,b,i,*]

[a] Institute of Soil and Water Resources and Environmental Science, College of Environmental and Resource Sciences, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, China
[b] Shandong (Linyi) Institute of Modern Agriculture, Zhejiang University, Linyi 276000, China
[c] Yiwu Seed and Plant Quarantine Station, Jinhua, China
[d] Linyi City Academy of Agricultural Sciences, Linyi 276012, Shandong, China
[e] Department of Land, Air, and Water Resources, University of California, Davis, Davis, CA 95616, USA
[f] Agricultural Experiment Station, Zhejiang University, Hangzhou 310058, China
[g] Department of Agricultural Soil Science, University of Göttingen, 37077 Göttingen, Germany
[h] Peoples Friendship University of Russia (RUDN University), 117198 Moscow, Russian Federation
[i] The Rural Development Academy at Zhejiang University, Zhejiang University, Hangzhou 310058, China

ARTICLE INFO

Handling Editor: Mariluz Cayuela

Keywords:
Carbon sequestration
Absolute abundance
Diversity
Metabolic quotient
Generalist
Specialist

ABSTRACT

Biochar plays an important role in soil carbon (C) sequestration, while the effectiveness of sequestration is mostly determined by microbial preferences to utilize the different biochar components (i.e. labile substances or fused aromatic backbone). Thus, it is important to investigate the responses of the bacterial community and specific taxa, and associated C metabolic functions to biochar labile C (i.e. easily mineralizable C) or aromatic backbone (i.e. persistent C). Here, we separated the biochar components into labile C and aromatic backbone, and used the 16S sequencing in conjunction with the addition of an internal standard to quantify the absolute copy number of various bacterial taxa. The aromatic backbone decreased microbial metabolic quotient by 47 %, incorporating more C into biomass and contributing to C sequestration rather than releasing it into the atmosphere, while the labile C caused the opposite trends. The bacterial generalists utilizing aromatic backbone and labile C were mainly from *Oxalobacteraceae* (*Proteobacteria*) and *Sporosarcina* (*Firmicutes*). The bacterial specialists responsive for aromatic backbone were from *Myxococcaceae* and *Flavisolibacter*, while those responsive to labile C were from *Ktedonobacteraceae* and *Bradyrhizobiaceae*. Microbial abundance of specialists responsive to labile C was 3.3 times larger than those responsive to aromatic backbone, indicating the preferences of labile C specialists for energy sources and aromatic C specialists for slow utilization of persistent C in versatile habitats. Our work revealed microbial strategies for utilization of organic substances of very contrast availability, and their contributions to soil C sequestration depending on biochar components.

1. Introduction

Biochar is produced by the pyrolysis of organic materials under low oxygen conditions and is known to increase carbon sequestration in soil (Lehmann et al., 2011). Biochar mainly consists of fused aromatic backbone and to a lesser extent labile organic compounds such as easily mineralizable carbohydrate and *N*-containing compounds (e.g. from 21 to 49 %) (Pereira et al. 2011). Labile compounds provide available energy source for microbial metabolism, growth and reproduction, affecting the mineralization of soil organic matter and biochar itself (Kluepfel et al., 2014; Buss et al., 2018). An aromatic backbone, however, is very resistant to decomposition and provides a versatile environment (e.g. high surface area, electron shuttle function) for microbial colonization (Enders and Lehmann, 2012). Some bacteria such as

* Corresponding author at: Institute of Soil and Water Resources and Environmental Science, College of Environmental and Resource Sciences, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, China.
E-mail address: zmdai@zju.edu.cn (Z. Dai).

https://doi.org/10.1016/j.geoderma.2022.116176
Received 13 May 2022; Received in revised form 9 September 2022; Accepted 9 September 2022
Available online 18 September 2022

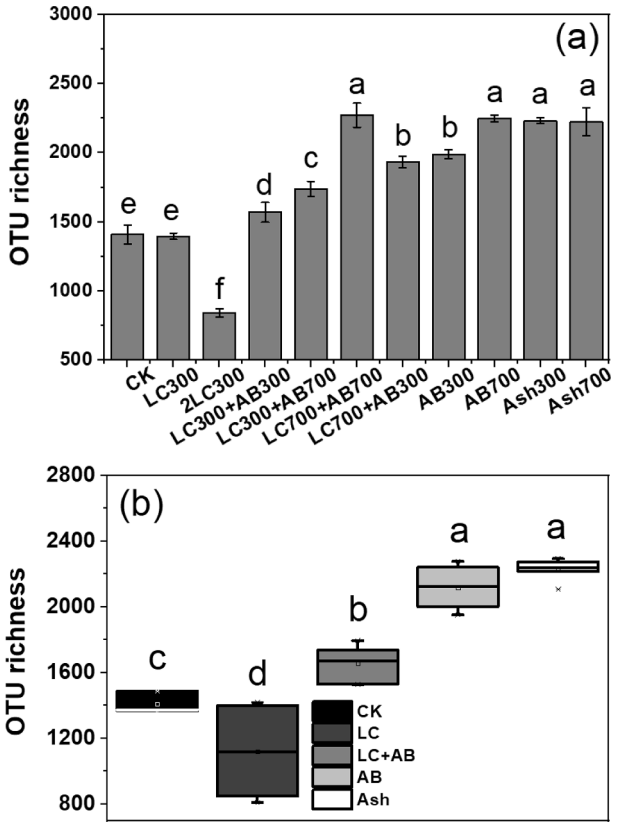

Fig. 1. Bacterial OTU richness in soils amended with labile C, aromatic backbone and ash (a) in individual treatments and (b) categories of similar treatments (left row: individual treatments; right row: categories of similar treatments). Error bars represent the standard deviation among replicates. Lowercase letters represent a significant difference ($p < 0.05$) between treatments and categories. Acetone extraction experiments showed that no LC700 components were extracted from LC700 + AB700.

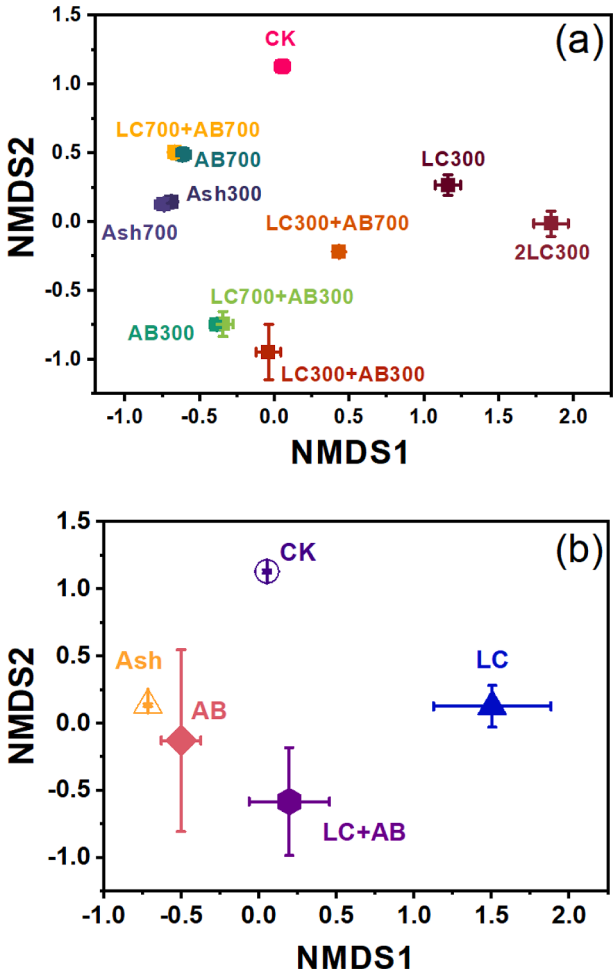

Fig. 2. Bacterial community patterns in the soils amended by labile C, aromatic backbone and ash compared to unamended soil. (a) non-metric multidimensional scaling plots (NMDS) based on Bray-Curtis metric using OTU abundances in different treatments, and (b) NMDS based on Bray-Curtis metric using OTU abundances in different categories. Error bars represent the standard deviation among replicates. PERMANOVA showed the significant differences ($p < 0.05$) between treatments in community composition as follows: LC300 versus CK, LC300 versus AB300, LC300 versus AB700, LC300 versus Ash300 and Ash700.

Chloroflexi and *Actinobacteria* can slowly utilize biochar-based aromatic C for co-metabolism (Dai et al., 2017a). Therefore, microbial preferences for utilizing labile C or aromatic backbone determine the effectiveness of C sequestration in biochar-treated soil.

The aliphatic: aromatic ratio of C in biochar decreases with increasing pyrolysis temperatures and when feedstocks are switched from manure to those that are lignocellulose-based (Baldock and Smernik, 2002). Thus, many researchers have used a wide range of feedstocks and pyrolysis temperatures to produce biochar with various aliphatic: aromatic ratios to examine the responses of soil microorganisms to the different C components (Jindo et al., 2012; Chen et al., 2013; Dai et al., 2014; Chen et al., 2018). For example, biochars pyrolyzed at high temperatures increase the bacterial richness and diversity, as well as the abundance of *Chloroflexi* (Dai et al., 2017a; Yu et al., 2018), while biochars produced at low temperatures increase the microbial biomass (Jenkins et al., 2010; Farrell et al., 2013). Moreover, lignocellulose-based biochar (oak feedstock) increases the *Actinobacteria* abundance more than grass-based biochar (Khodadad et al., 2011). Production conditions also result in variations of biochar properties such as pH and the ash and mineral contents. For example, biochar alkalinity favors the growth of bacteria that are sensitive to increases in pH (Warnock et al., 2007). The porous structure of biochar contains nutrients and water and serves as a refuge for soil bacteria, protecting them from predators. Alterations to the soil bacterial community may not, however, completely be attributed to biochar C components and may be influenced by other properties. Thus, separating the labile and aromatic compounds in biochar may more precisely reveal the underlying mechanisms involved in the responses of bacterial diversity, community and metabolic processes to biochar components.

Previous studies have reported the effects of biochar on microbial respiration and microbial biomass (Jenkins et al., 2010; Farrell et al., 2013; Khadem and Raiesi, 2017; Xu et al., 2018). The microbial metabolic quotient (i.e. respiration: biomass) reflects the microbial C use efficiency and determines how a C source is incorporated into the microbial biomass or released into the atmosphere as CO_2. As the microbial necromass is an important component of soil organic matter (Kallenbach et al., 2015; Liang et al., 2019; Wang et al., 2021), we believe the microbial metabolic quotient is also important in reflecting soil C sequestration. A higher microbial metabolic quotient leads to more CO_2 being emitted into the atmosphere than being sequestered in the soil. The way in which labile and aromatic C affect the microbial metabolic quotient and microbial diversity is not yet understood.

Researchers have recently started to consider microbial diversity and microbial C metabolic processes as important parameters that reflect ecosystem functions such as C sequestration and organic matter decomposition (Delgado-Baquerizo et al., 2020). Other studies have reported that microbial functions do not decrease with a loss of microbial diversity under, for example, mineral fertilization (Allison and Martiny, 2008) because of functional redundancy in microbial communities (Kuzyakov et al., 2009; Ling et al., 2021). This finding has

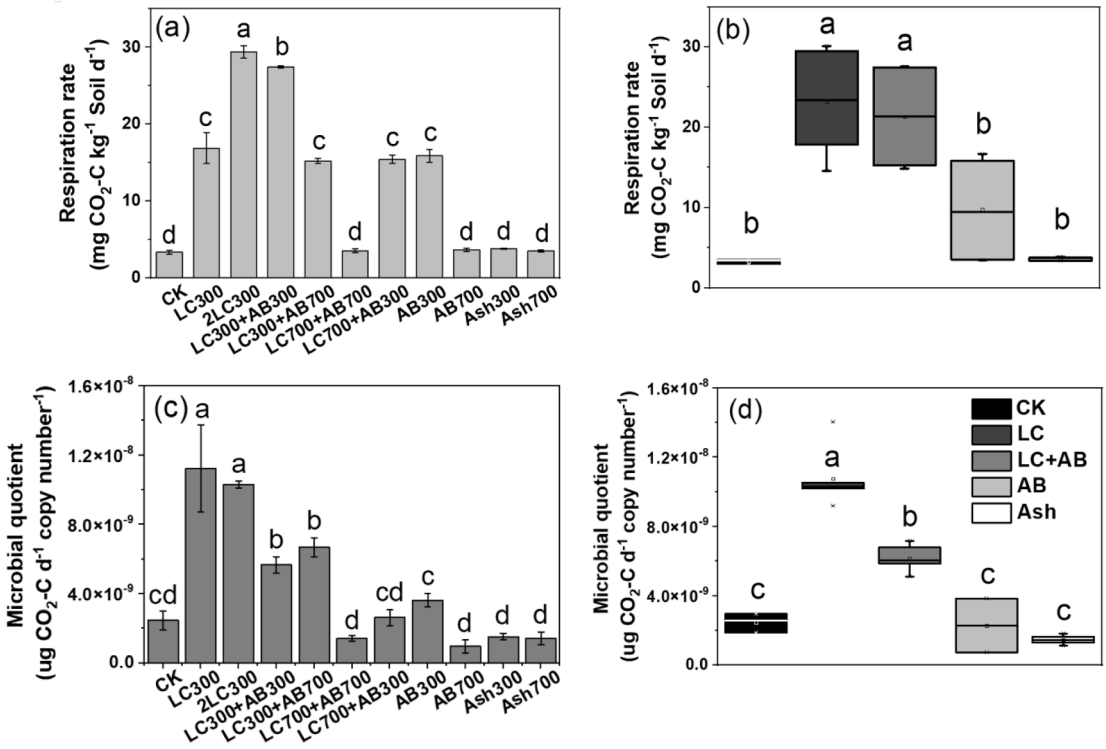

Fig. 3. Respiration rate (a, b) and microbial metabolic quotient (c, d) in soils amended with labile C, aromatic backbone and ash (left row: individual treatments; right row: categories of similar treatments). Error bars represent the standard deviation among replicates. Lowercase letters represent a significant difference ($p < 0.05$) between treatments and categories. Acetone extraction experiments showed that no LC700 components were extracted from LC700 + AB700.

important practical implications, because, if the microbial C metabolic quotient remains stable as microbial diversity decreases with the addition of labile or aromatic C, it can be argued that a focus on maintaining microbial diversity during soil C sequestration is unnecessary. Understanding the relationship between the microbial metabolic quotient and diversity that depend on the amounts of labile and aromatic C in the added biochar will reveal how competition for biochar components in diverse microbial communities affects their contributions to C sequestration.

Although the responses of bacterial taxa to biochar addition have been reported (Jindo et al., 2012; Kappler et al., 2014; Shin et al., 2021), their specific responses to the labile and aromatic components have not been identified. The generalists that feed on a broad range of biochar compounds, i.e., those that utilize both labile and aromatic C, contribute importantly to soil C sequestration. The specialists that utilize only the labile C in biochar can reduce the easily-mineralizable C content and have soil priming effects (Cross and Sohi, 2011). Moreover, the specialists that utilize aromatic C affect biochar structure in ways such as increasing its porosity (Enders and Lehmann, 2012). Hence, these specialized bacterial groups selectively affect biochar and soil organic matter decomposition and soil C sequestration. Notably, as absolute quantitative 16S sequencing develops, the absolute copy number of bacteria in soil at the genus or operational taxonomic unit (OTU) levels can be obtained directly (Smets et al., 2016). This will make it possible to compare the biomass of identified specialists and generalists in soils with added biochar-associated labile and aromatic C. Without the identification of these specialists, the lower relative abundances of bacterial taxa that are responsive to labile C compounds may be higher than those responsive to aromatic C, which would mask the contribution of specific taxa to soil C sequestration.

Here, we separated biochar components into labile compounds and those with an aromatic backbone using acetone extraction. Then, we used 16S rRNA gene sequencing in conjunction with the addition of an internal standard (*Aliivibriofischeri*) to quantify the absolute abundance of taxa that utilize labile C or aromatic backbone. Our objectives were to: (1) investigate the effects of labile and aromatic compounds on bacterial metabolic processes and determine their relationships with microbial diversity; and (2) identify the generalists and specialists at the OTU level that are associated with labile C or aromatic backbone. We hypothesized that the aromatic backbone would decrease the microbial metabolic quotient, increase diversity and favor the growth of differential bacterial groups in comparison to the labile C.

2. Methods and materials

2.1. Biochar preparation and characteristics

Two biochars (B300 and B700) were produced by pyrolyzing manure at temperatures of 300 and 700 °C under an O_2-limited atmosphere. The residence time and heating rate were 0.5 h and 2.5 °C min^{-1}, respectively. To extract labile C from B300 and B700, we added anhydrous acetone (100 %) to both (biochar: acetone = 1:10 g:ml). The biochar-acetone mixtures were then shaken end-over-end at room temperature for 24 h. The details of the extraction methods were described by Dai et al. (2017a, 2018). This extraction method was verified to be effective at separating labile compounds and those with an aromatic backbone from biochars. The compounds extracted with acetone were separated from biochar particles by filtration and identified as labile C compounds (hereafter named 'labile C', LC). Thus, the labile C extracted from B300 and B700 were identified as LC300 and LC700, respectively. The residual biochar particles from B300 and B700 (hereafter named 'aromatic backbone', AB) were named AB300 and AB700, respectively. The acetone extraction experiments indicated that no LC700 was extracted from B700, as B700 was highly pyrolyzed. Therefore, similar effects of B700 and AB700 on microbial diversity and function were expected. In addition, the LC300 and LC700 samples were collected and added back to AB700 and AB300, respectively, resulting in new samples: LC300 + AB700 and LC700 + AB300. Correspondingly, the B300 and B700 were

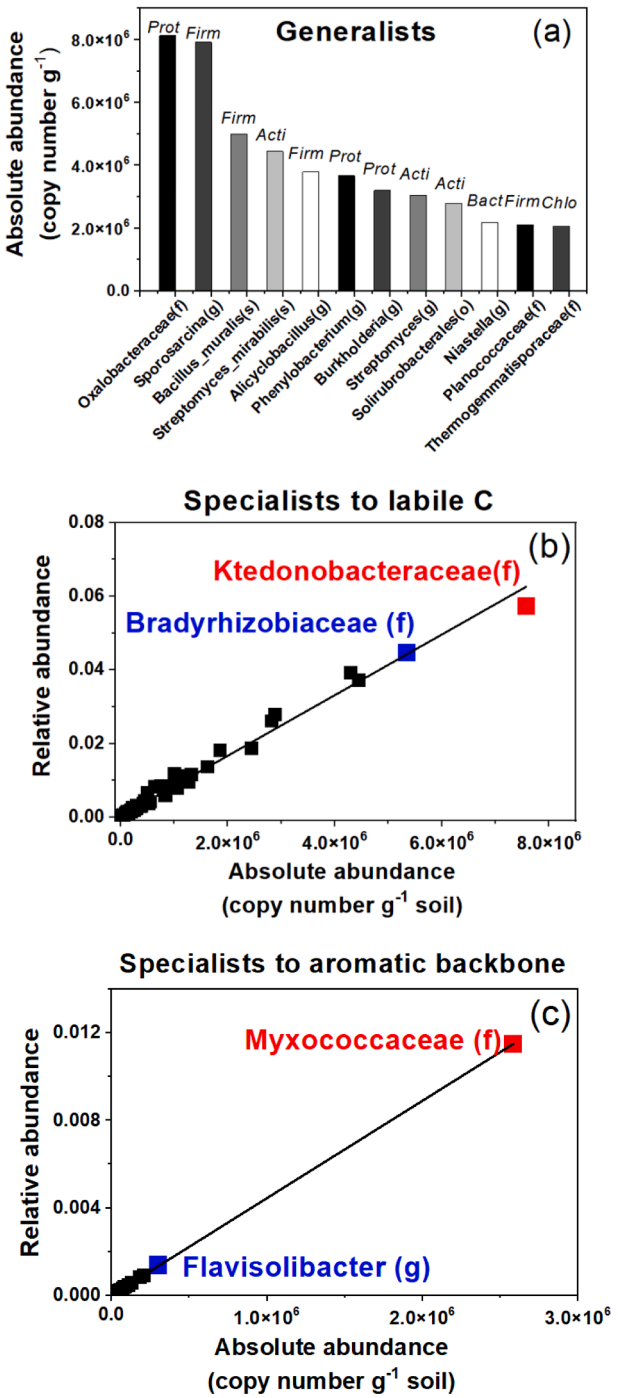

Fig. 4. Average absolute abundance (a) of bacterial generalists in biochar materials and the specialists in the groups of labile C (b) and aromatic backbone (c). The bacterial generalists that existed in all biochar C components were identified by setting up the occurrence frequency of generalist OTUs at 1.0 with the highest average abundance (i.e., $>2 \times 10^6$ copy number g^{-1}). The specialists that were only highly responsive to labile C or aromatic backbone were identified using the indicator value metric (Indval).

renamed as LC300 + AB300 and LC700 + AB700, respectively. All of the samples were dried at 40 °C in an oven at ambient pressure for 12 h and under vacuum for 1 h to thoroughly remove the excess acetone residues. The ash of B300 and B700 was obtained by the process of heating the biochar at 750 °C, which resulted in the production of ash samples Ash300 and Ash700. The C structures of LC300 and AB700 were characterized by Quantitative 13C solid-state nuclear magnetic resonance (NMR) spectroscopy (Bruker Avance III 400 MHz, Bruker Corporation, Germany). The properties of biochar materials are presented in Table S1.

2.2. Incubation experiments

Prior to incubation, we adjusted the pH of all biochar materials to 7.6 (equal to the initial pH of B300) to avoid any pH effects on soil microbial parameters by HCl and NaOH. Then, a soil sample collected from the upper layer (0–20 cm) of a forest hillside was air-dried, crushed, sieved < 1 mm and thoroughly homogenized. Biochar materials were added at 50 g kg^{-1} soil into the soil (5 % w/w) and mixed thoroughly, i.e., each 40 g soil received 2 g biochar. We also included two treatments in which the soil received a mass of ash equivalent to the maximum masses contained in B300 and B700. These treatments addressed the question "What is the effect of biochar ash on soil microorganisms if all the mineral nutrients have been removed from the biochar?" Thus, 0.63 g of Ash300 and 1.14 g Ash700 were added into 40 g soil based on the ash contents of B300 and B700. In addition, we included two treatments in which soils received an equivalent mass of LC300 and two times the amount of LC300 (i.e., 2LC300 for double LC300) so as to eliminate the influence of biochar structure. Thus, 0.03 g and 0.06 g of LC300 were added into 40 g soil based on the mass of LC300 that was extracted from B300. Overall, 11 treatments were used: (1) CK (no biochar addition); (2) LC300; (3) 2LC300; (4) LC300 + AB300; (5) LC300 + AB700; (6) LC700 + AB700; (7) LC700 + AB300; (8) AB300; (9) AB700; (10) Ash300 and (11) Ash700.

In addition to the treatments presented individually, we grouped them into five categories according to the similarities in their material properties: (1) CK; (2) soil to which only labile C was added at either a single or double rate ("LC"): LC300 and 2LC300; (3) soil to which both labile C and aromatic backbone were added ("LC + AB"): LC300 + AB300 and LC300 + AB700; (4) soil to which only aromatic backbone was added with no labile C ("AB"): AB300 and AB700; (5) soil to which only ash was added ("Ash"): Ash300 and Ash 700. Given that no LC700 was extracted from biochar pyrolyzed at 700℃, the LC700 + AB700 and LC700 + AB300 treatments were not grouped as they are similar to the AB700 and AB300 treatments.

The biochar-soil, ash-soil and soil-LC300 mixtures were incubated in 473 mL Mason jars, in which a 60 mL glass jar containing the soil-biochar mixture and a 20 mL glass vial containing 15 mL KOH in CO_2-free deionized water were established (Whitman et al., 2014). To create a stable humid atmosphere, 5 mL CO_2-free deionized water were added to the bottom of the Mason jar. All of the treatments were incubated in darkness at 30 °C for 150 days in three replicates, with the moisture held at 50 % water holding capacity. The treatment containing soil on its own was regarded as the control. The CO_2 respiration rate was measured cumulatively over 150 days of incubation by converting the electrical conductivity of the KOH traps to CO_2 volume (Strotmann et al., 2004).

2.3. Molecular analyses

After incubation, samples were collected for DNA extraction and then for Illumina Miseq sequencing. We used DNA from the marine species *Aliivibrio fischeri* as an internal standard to quantify the total abundance of bacterial 16S rRNA genes (herein called "biomass") across the samples (Smets et al., 2015). The *A. fischeri* strain (ATCC 7744 T) was purchased from ATCC (Manassas, VA, USA) and grown following the procedures detailed by Tavares et al. (2010). Briefly, the growing temperature was 25 °C and the culture medium was tryptic soy agar (BD, Franklin Lakes, NJ, USA) containing 3 % NaCl (Fisher Scientific, Pittsburgh, PA, USA). The *A. fischeri* DNA was extracted using a PowerSoil® DNA Isolation Kit, and the concentration was determined using Qubit 3.0 (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Prior to DNA extraction, genomic DNA (gDNA) from *A. fischeri* was added to each sample (0.5 g). The amount of *A. fischeri* DNA added was targeted to be approximately 0.5 % of the total DNA extracted from the

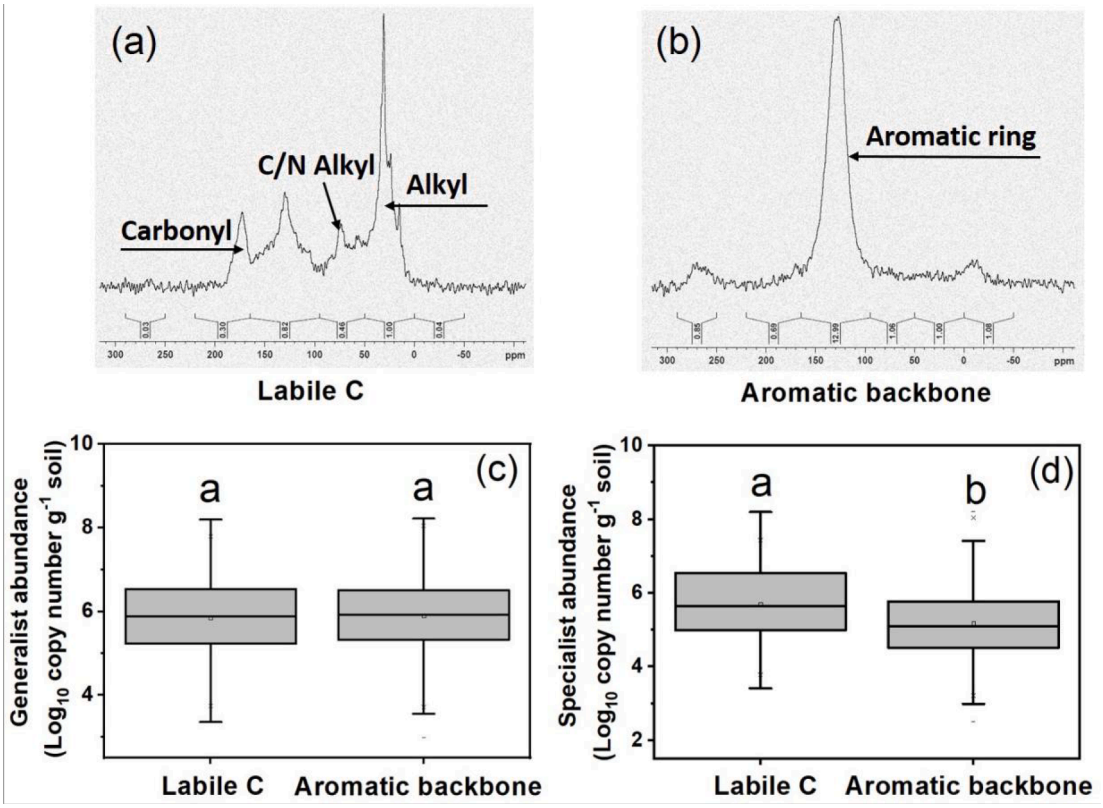

Fig. 5. Carbon structures of labile C (a) and aromatic backbone (b) by NMR, and the absolute abundance of bacterial generalists and specialists in the groups of labile C (c) and aromatic backbone (d). No differences in generalist biomasses were observed between the groups of labile C and aromatic backbone, while the biomasses of specialists in the group of labile C were significantly higher than those in the group of aromatic backbone.

test samples, based on a preliminary DNA extraction from which the total concentration of the DNA extracted from each sample was determined. Immediately afterwards, DNA was extracted following the manufacturer's instructions. The amplification of the V4 region of 16S rRNA genes in each sample was conducted at the Beijing Genomics Institute, China. The forward primer was 338F:5′- ACTCCTACGGGAGGCAGCA-3′ and the reverse primer was 806R:5′- GGACTACHVGGGTWTCTAAT-3′. The amplification procedures and conditions were as described previously (Caporaso et al., 2011). Each sample was amplified in triplicate, and the amplicons were then purified, pooled and sequenced on an Illumina MiSeq sequencing platform, generating 250 bp paired-end reads.

2.4. Sequencing data processing

Raw data were pre-processed to remove the low-quality reads by using the procedures described by Fadrosh et al. (2014). Then, the paired-end sequences were merged to tags by FLASH (Fast Length Adjustment of Short reads) (Magoč and Salzberg, 2011), and the tags were clustered into OTUs with USEARCH (Edgar, 2013). The taxonomy for each OTU was identified using the Ribosomal Database Project (RDP) Classifier (Cole et al., 2013) trained on the Greengenes database (DeSantis et al., 2006) using a 0.8 confidence cutoff value. To calculate the biomass (i.e. absolute abundance) of 16S rRNA genes in each bacterial taxa found in each sample, the following equation was used (Smets et al., 2015):

$$\frac{R_i}{R_S} = \frac{\frac{w_i}{g_i} * C_i}{X} \Rightarrow X = \frac{R_S * \left(\frac{w_i}{g_i} * C_i\right)}{R_i}$$

where R_i is the number of reads assigned to *A. fischeri*, R_s is the number of reads assigned to the targeted taxa, w_i is the weight of *A. fischeri* gDNA added to the samples, c_i is the 16SrRNA gene copy number of *A. fischeri* (i.e. 8), g_i is the weight of the genome of *A. fischeri* (i.e. 4.49×10^{-15} g per genome) (Hallin and Ussery, 2004) and X is the targeted taxa number of 16S rRNA genes per sample. Then, the OTU of *A. fischeri* was deleted from the OTU matrix table manually, to eliminate the effects of *A. fischeri* on the community composition and total abundance analyses. The biomass (i.e., absolute abundance of 16S rRNA genes) in each sample was calculated by the sum of the biomass of 16S rRNA genes in each taxonomic unit. OTU richness was calculated with the R package "vegan" (Oksanen et al., 2013) using the OTU table without *A. fischeri*. All sequences were deposited in the GenBank short-read archive SRS10719196. The microbial metabolic quotient was calculated by dividing the respiration rate by the microbial biomass (Wardle and Ghani, 1995).

2.5. Statistical analysis

Hierarchical clustering and non-metric multidimensional scaling plots (NMDS) of taxonomic similarity (Bray–Curtis) were conducted to observe similarities and differences in the bacterial community composition between treatments and between categories using the "vegan" R package (Oksanen et al., 2013). One-way analysis of variance (ANOVA) was performed to investigate the significant differences ($p < 0.05$) in the (1) alpha diversity (OTU richness and Shannon index), (2) soil respiration rate, (3) total bacterial biomass and (4) microbial metabolic quotient between treatments and between categories. We tested the relationship between the OTU richness and respiration rate, microbial biomass and microbial metabolic quotient using Spearman's rank correlations. We identified the bacterial generalists that existed in all biochar treatments by setting up the occurrence frequency of generalist OTUs at 1.0 with the highest average abundance (i.e., $>2 \times 10^6$ copy number g^{-1}). We identified the specialists that were only

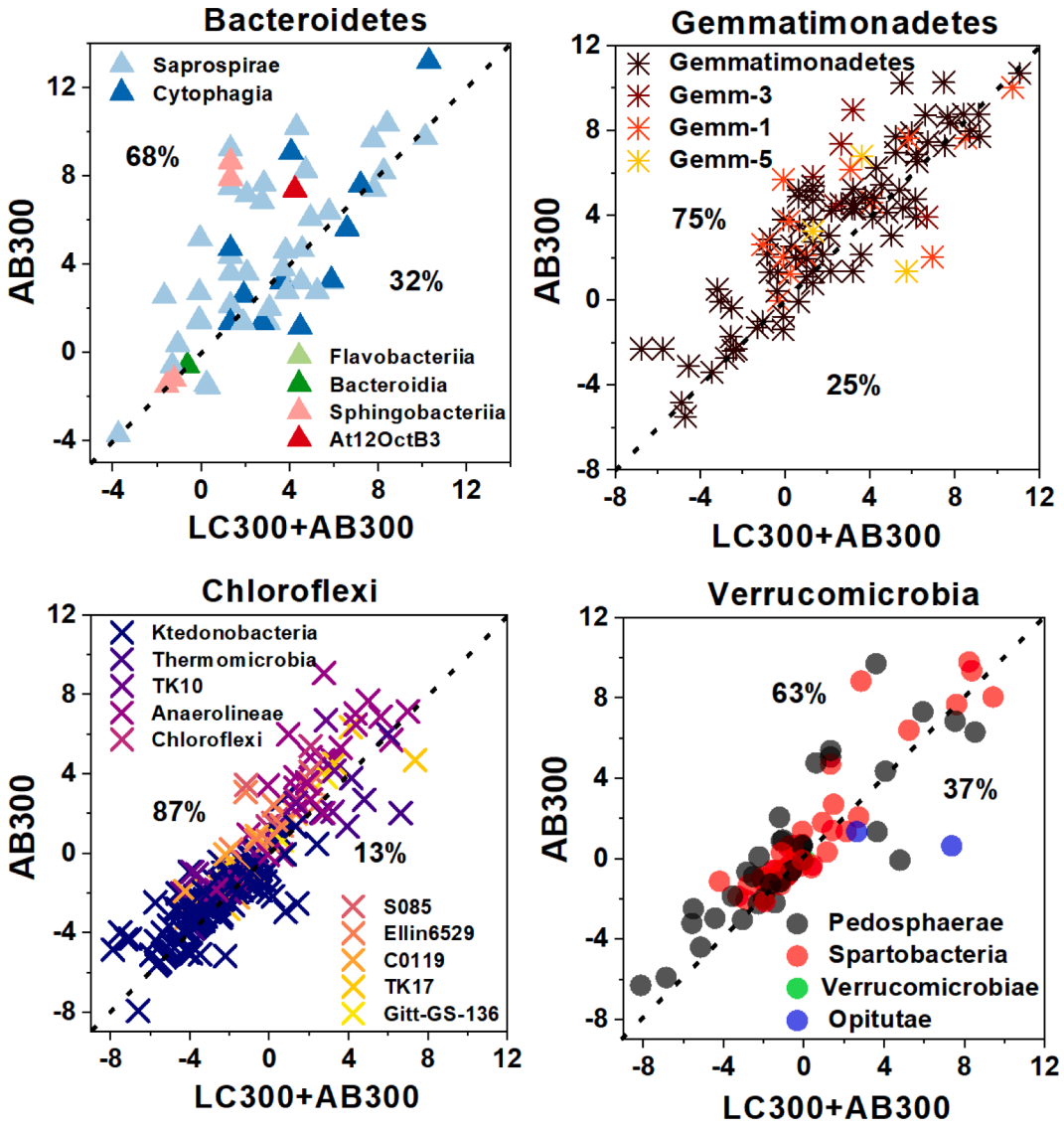

Fig. 6. The "Log2-fold change" in biomass of differentially abundant genera from *Bacteroidetes*, *Gemmatimonadetas*, *Verrucomicrobia* and *Chloroflexi* in LC300 + AB300 (Labile C + Aromatic backbone) and AB300 (Aromatic backbone) treatments. Each dot represents a single genus. Differentially abundant genera that were not significant (with log2-fold change < 1 or adjusted p values > 0.1) when compared with the soil control are not presented. The solid line indicates that the preference of genera for LC300 + AB300 was equivalent that for AB300. The genera below the solid line thrived preferentially on LC300 + AB300 than AB300, whereas the genera above the solid line showed the opposite lifestyle (the percentages of both groups are presented above and below the 1:1 line). The legends indicate the classes to which the OTUs belong.

highly responsive to labile C or the aromatic backbone using the indicator value metric (Indval) as implemented in the labdsv R package (Dufrêne and Legendre, 1997; Roberts, 2012).

3. Results

3.1. Microbial community composition and diversity

The incorporation of LC700 + AB700 and AB700 ($p < 0.05$) resulted in the largest increases in OTU richness and Shannon index, whereas the incorporation of 2LC300 ($p < 0.05$) decreased the soil OTU richness and Shannon index, (Fig. 1a and Fig. S1a). Consequently, the soil OTU richness and Shannon index in the AB group was larger than those in the LC group ($p < 0.05$) (Fig. 1b and Fig. S1b). The effects of Ash300 and Ash700 on bacterial alpha diversity were similar to those of LC700 + AB700 and AB700 (Fig. 1 and Fig. S1).

The bacterial community compositions after the additions of LC300 and 2LC300 clustered distantly with those found after the additions of AB300 and AB700. However, the community compositions following the additions of LC700 + AB700 and AB700 clustered closely together (Fig. 2a). The effects of Ash300 and Ash700 on the bacterial community composition were similar to those of LC700 + AB700 and AB700 (Fig. 2a). Consequently, the communities after the additions of AB clustered distantly with those after the additions of LC and LC + AB (Fig. 2b).

3.2. Microbial respiration activity, biomass and quotient

The addition of LC300 increased the microbial respiration rates ($p < 0.05$) (Fig. 3a and b). The respiration increase was proportional to the amount of LC300 used, as the addition of 2LC300 approximately doubled the respiration rate. The addition of LC700 + AB700 and AB700, however, had no effects on the respiration rates (Fig. 3a). When grouping the data into categories, the additions of LC and LC + AB increased the respiration rate (Fig. 3b). The incorporation of biochar materials containing AB (i.e. AB700 and AB300) increased the microbial

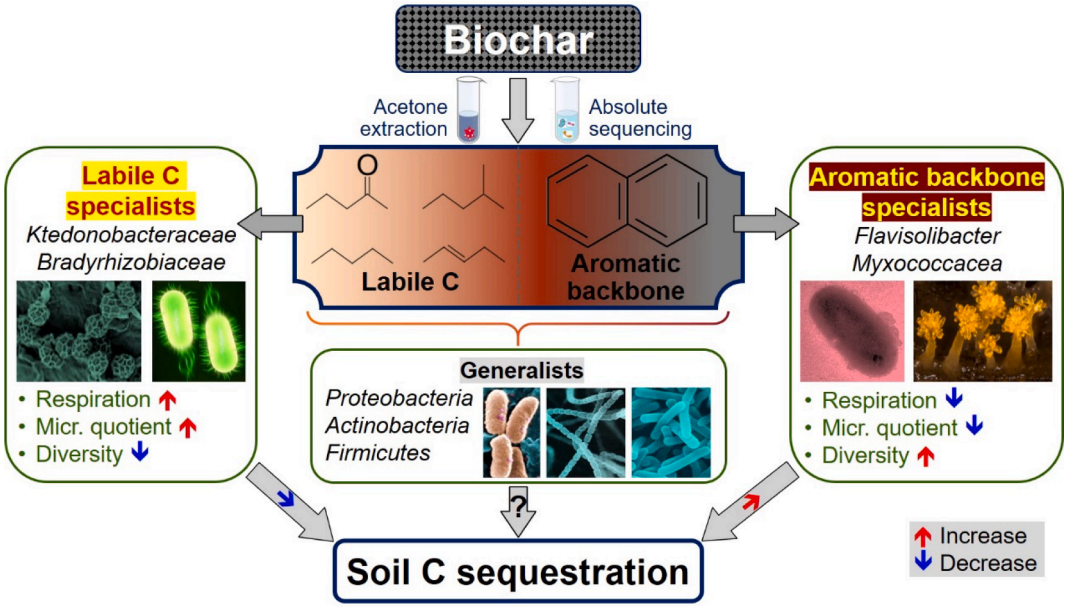

Fig. 7. Conceptual diagram revealing the impact of labile C and the fused aromatic backbone in biochar on bacterial diversity, biomass and microbial quotient, as well as bacterial generalists and specialists utilizing biochar compounds.

abundance (Fig. S2a), while no effects on abundance were seen when only the LC were added (Fig. S2b).

The microbial quotients were the highest when LC300 and 2LC300 were added, and the lowest following the additions of LC700 + AB700 and AB700, whereas combinations of LC300 and AB300/AB700 resulted in intermediate effects (Fig. 3c). Similarly, the highest microbial quotients were observed with the addition of LC (Fig. 3d). The effects of Ash300 and Ash700 on microbial quotients were similar to those of LC700 + AB700 and AB700 (Fig. 3). With the increase in OTU richness, the bacterial respiration rate decreased ($p < 0.05$) (Fig. S3a) and abundance did not change (Fig. S3b), while the microbial quotient decreased ($p < 0.05$) (Fig. S3c). The correlation coefficient between the microbial quotients and OTU richness ($r = -0.82$, $p < 0.05$) was greater than that between the respiration rate and OTU richness ($r = -0.56$, $p < 0.05$) (Fig. S3).

3.3. Generalists that were not responsive to C components

The bacteria observed in all of the biochar types (i.e., the occurrence rate = 1.0) were identified as generalists that can utilize a broad range of biochar C components. In total, 205 bacterial OTUs were identified as generalists, and 12 bacterial OTUs had an abundance $> 2 \times 10^6$ copy number g^{-1} soil (Fig. 4a and Fig. S4). Among those, four were classified in the phylum *Firmicutes*, six were identified as *Proteobacteria* and *Actinobacteria*, and two belonged to the phyla *Bacteroidetes* and *Chloroflexi* (Fig. 4a). The family *Oxalobacteraceae* from the *Proteobacteria* and the genus *Sporosarcina* from the phylum *Firmicutes* comprised the predominant biomass, with values $> 8 \times 10^6$ copy number g^{-1}. They were followed by *Bacillus muralis* and *Streptomyces mirabilis*, belonging to *Firmicutes* and *Actinobacteria*, respectively, with a biomass of $> 4.0 \times 10^6$ copy number g^{-1}.

3.4. Specialists responsive to labile or aromatic C

In total, 101 of bacteria were identified as specialists responsive to LC, and 23 of bacteria were identified as specialists responsive to AB backbone. The abundance of specialists according to absolute 16S sequencing was consistent with their relative abundance as calculated using general 16S sequencing (Fig. 4b and 4c). Bacterial taxa observed only in the LC group were identified as specialists for biochar labile C. The bacterial OTUs responsive to labile C with the highest abundances were *Ktedonobacteraceae* (7.6×10^6 copy number g^{-1}) from the phylum *Chloroflexi* and *Bradyrhizobiaceae* (5.6×10^6 copy number g^{-1}) from the *Proteobacteria* (Fig. 4b). The bacterial OTUs responsive to aromatic backbone with the two highest abundances were *Myxococcaceae* (2.6×10^6 copy number g^{-1}) from the phylum *Proteobacteria* and *Flavisolibacter* (3.0×10^5 copy number g^{-1}) from the *Bacteroidetes* (Fig. 4c). NMR showed that labile C was comprised by aliphatic C such as alkyl and carbonyl (Fig. 5a), while aromatic backbone was mainly comprised of aromatic rings (Fig. 5b). The average abundance of generalists from the LC group was the same as that of those in the AB group (Fig. 5c). The average abundance of specialists from the LC group was higher ($p < 0.05$) than that of those in the AB group (Fig. 5d).

We further analyzed the differences in the abundance of bacterial OTUs in the typical treatments of AB300 and LC300 + AB300, which revealed how the bacterial OTUs changed when the labile C (i.e. LC300) was removed from the biochar. Within the phyla *Bacteroidetes*, *Gemmatimonadetas*, *Chloroflexi* and *Verrucomicrobia*, the numbers of OTUs that thrived preferentially on AB300 than LC300 + AB300 were larger those that thrived preferentially on LC300 + AB300 than AB300 (Fig. 6). Within the phylum *Bacteroidetes,* the classes identified as *Saprospirae* and *Sphingobacteriia* were responsive to AB300, while the *Cytophagia* did not show any response. Within the *Gemmatimonadetas,* most bacteria such as *Gemmatimonadetes, Gemm-3, Gemm-5, etc.* were responsive to AB300. Similarly, two bacterial groups, *Pedosphaerae* and *Spartobacteria* from the phylum *Verrucomicrobia*, were responsive to AB300, while the *Opitutae* were more responsive to LC300 + AB300. Finally, the phylum *Chloroflexi* contained taxa from the groups *Anaerolineae* and *Ellin6529* that were responsive to AB300, while the *Thermomicrobia* were more responsive to LC300 + AB300.

4. Discussion

4.1. Bacterial diversity, biomass and metabolic quotient

Biochar is often used for C sequestration in soil, while microbial preferences for utilizing different biochar components determine the effectiveness of C sequestration. Previously, we confirmed that a higher content of aliphatic compounds was observed in LC300 and aromatic compounds in AB300 and AB700 (Dai et al., 2017b). The dramatically

larger increases in soil CO_2 respiration after LC300 addition compared with AB300 and AB700 addition (Fig. 3) supports the above premise. In our experiments, the compounds extracted from biochars represent the "labile C" that can be rapidly utilized by microorganisms, while the residual biochar particles represent the "aromatic backbone" that is resistant to microbial decomposition and provides a microbial habitat. The comparison between "labile C" and "aromatic backbone" are relative designations (Lehmann and Joseph, 2015).

The effect of biochar labile C on microbial respiration was consistent with previous reports that labile organic compounds are used as an energy source for microbial metabolism (Dai et al., 2019), and consistently the ash without C source did not change microbial metabolism (Lehmann and Joseph, 2015; Khadem and Raiesi, 2017). Notably, the larger increase in microbial quotients after labile C addition compared to aromatic backbone probably indicated that the labile C decreased microbial C-use efficiency, tending to cause more CO_2 emissions and lower incorporation of C into biomass. These CO_2 losses decrease microbial biomass formation and consequently necromass production, which is the main source of soil organic matter (Kallenbach et al., 2015; Liang et al., 2019; Wang et al., 2021). In contrast, the addition of aromatic backbone components decreases the microbial C metabolic quotient, maintaining more C in soil pools and releasing less into the atmosphere, contributing to C sequestration (Zheng et al., 2016). In addition, the labile C pool in biochar is usually smaller compared to aromatic backbone pool, regardless of feedstock and pyrolysis temperature. The labile C can be consumed by microorganisms in short-term (Budai et al. 2016). Thus, biochar aromatic backbone compounds contribute greatly to long-term soil carbon sequestration.

The increased bacterial diversity by aromatic backbone components was attributed to: 1) the versatility of the aromatic backbone attracted diverse bacteria to colonize, such as high porosity (Kinney et al., 2012), electron shuttle characteristic (Kluepfel et al., 2014) and aromatic compounds for microbial co-metabolism (Payne et al., 2013); and 2) a higher diversity of bacteria can encode a higher diverse of enzymes to degrade more complex C fractions. Despite this high diversity, the respiration rate in the biochar was low due to the low levels of available C in the backbone (Fig. 7).

The negative correlation between microbial respiration rates/metabolic quotients and diversity (Fig. S3) showed no metabolic redundancy with the addition of the biochar components. Increasing labile C, i.e. energy source, raised indigenous microbial competitivity and caused their self-assembly, i.e., building of colonies (Xue et al. 2018), leading to an overall decrease in diversity. Conversely, the aromatic backbone components provided a versatile habitat for the growth of various microbial groups (Lehmann and Joseph, 2015). Here, we point out the ashes also induced a very large increase in bacterial diversity, which was probably attributed to the pH effects, since increase of soil pH has been related to increases in bacterial diversity (Rousk et al. 2010). The combinations of biochar materials (e.g. LC300 + AB300 and LC300 + AB700) showed the intermediate effects between labile C and aromatic backbone, regardless of bacterial diversity, respiration rate and metabolic quotients, probably due to dilution effects. Therefore, we mainly focused on the specific bacterial taxa that are only responsible to labile C or aromatic backbones in the following discussion.

4.2. Generalists and specialists

Previous studies used 16S rRNA-based sequencing approaches to investigate the relative abundance of soil bacterial taxa under biochar addition. The relative abundances did not, however, provide precise information on the absolute abundances of the targeted bacterial taxa (Jindo et al., 2012). Microorganisms may have the same relative abundances in two or more samples, while their absolute abundances can differ by more than one order of magnitude (Pollock et al., 2018). Therefore, relative abundances were not applicable for the identification of the non-responsive generalists and responsive specialists in this study. Here, we found that the relative abundances of some phyla such as *Actinobacteria* and *Firmicutes* had strong positive correlations with the absolute abundances quantified by absolute 16S rRNA gene sequencing, but some such as those for *Acidobacteria* were not correlated (Fig. S5). In addition, most individual correlations did not fall on the correlative line, indicating that the relative abundances of these bacterial taxa were not comparable between treatments (Fig. S5). These findings emphasized the importance of identifying the generalists and specialists using their absolute abundance rather than relative abundances.

We quantified the absolute abundance of each generalist that utilized a broad range of biochar C components and the specialists that were either responsive to labile C or aromatic backbone (Props et al., 2016; Smets et al., 2016; Xu et al., 2020). Although the DNA extraction efficiencies in biochar-, ash-treated and control soils were different, the equivalent ratios of internal DNA to the total soil DNA between materials illuminated the bias and allow for the quantitative comparison of bacterial abundances across different samples (Smets et al., 2016). One of the generalist species with the high abundance were the *Sporosarcina* from the phylum *Firmicutes* (Fig. 4), which have a wider niche and adapt to adverse environmental stresses (e.g. drought and heavy metals) (Gomez-Montano et al., 2013; Tamez-Hidalgo et al., 2016). Thus, we speculate that *Sporosarcina* survives in both C-rich and C-limited environments. Similarly, the generalists *Streptomyces_mirabilis* and *Solirubrobacterales* from *Actinobacteria* have patterns of filamentous growth and have a strong survival capacity (Smith et al., 2008). These traits may have assisted bacteria in acquiring C sources from internal biochar pores when the C source was insufficient. Although the generalists have broad environmental tolerance, their lifestyles and physiological traits are not well known. For instance, the *Oxalobacteraceae* can promote plant growth and nitrogen acquisition by triggering root development (Yu et al., 2021). *Streptomyces* in the plant rhizosphere (e.g. tomato) plays an important role in plant nutrition and productivity (Liao et al., 2021). Given their high biomass as generalists, further studies should be focused on their behaviors to make full use of their potentials beyond soil C sequestration.

The identified specialists that were highly responsive to labile C, producing the highest abundance, were the *Ktedonobacteraceae* and *Bradyrhizobiaceae* (Fig. 4). *Ktedonobacteria* from the phylum *Chloroflexi* has a large genome size and may produce bioactive compounds (Zheng et al., 2019). *Bradyrhizobiaceae* (from *Rhizobiales*) are symbionts in plant roots and have a good N_2 fixing capacity, although they require a C source (e.g. biochar labile C) (Itakura et al., 2009). Regardless of their traits, these specialists are effective organic matter decomposers and decrease soil C sequestration. The higher abundance of specialists in the category of labile C than that in the aromatic backbone category also supports that the labile C-requiring specialists were highly dependent on their energy sources (Fig. 5).

The *Myxococaceae* are responsive to aromatic backbone, which is consistent with their trait to feed on more complex C compounds (Petters et al., 2021). Thus, some types of bacteria may utilize persistent forms of C found in biochars. However, the abundant specialist *Flavisolibacter* (from the *Chitinophagaceae*) cannot degrade the aromatic C (Lim et al., 2009). In addition, the abundances of specialists to aromatic backbone were 3.3 times lower than those responsive to labile C (Fig. 5). Thus, utilizing aromatic C was not the main source of energy for their growth. The aromatic backbone may provide an appropriate environment (e.g. porous structure) and act as electron shuttle, which can also promote the growth of specialists (Enders and Lehmann, 2012; Kluepfel et al., 2014).

We also investigated how the bacterial OTUs changed when the labile C was removed from the biochar. Members of the phyla *Bacteroidetes*, *Gemmatimonadetas*, *Verrucomicrobia* and *Chloroflexi* thrived on the biochar C backbone without the labile C supply. The thrive of Saprospirae and *Sphingobacteriia* from *Bacteroidetes* on aromatic backbone was consistent with previous finding of positive correlation between biochar aromatic compounds and their abundances (Kolton et al., 2011;

Xu et al., 2016). *Gemmatimonadetes, Gemm-3* and *Gemm-1* are Gram-negative bacteria that adapt to environments limited by available substrates (Naether et al., 2012) and with sufficient internal cores of micro-aggregates (Mummey et al., 2006). These habitats are similar to the aromatic backbone. *Verrucomicrobia* is abundant in the biochar-amended plant rhizosphere (Liao et al., 2021). The positive responses of *Pedosphaerae* and *Spartobacteria* (from *Verrucomicrobia)* to the aromatic backbone provided evidence that biochar can act as a bridge between *Verrucomicrobia* growth and plant root development. The aromatic backbone favored the growth of *Anaerolineae* and *TK10* from *Chloroflexi* (Fig. 6). The growth of these groups may be attributed to the properties of aromatic backbone such as providing aromatic energy sources, electron transport shuttle (Yu et al., 2021) or anaerobic sites for specific metabolism (Payne et al., 2013).

5. Conclusions

Overall, microbial groups have clear preferences for utilizing the labile and/or aromatic backbone components of biochar. The lifestyles and physiological traits of generalists such as *Oxalobacteraceae* and *Sporosarcina* require further investigation. Bacterial groups such as *Ktedonobacteraceae* and *Bradyrhizobiaceae*, which were responsive to labile C, are inefficient at soil carbon sequestration. Notably, biochar aromatic backbone contribute greatly to soil carbon sequestration. This was not only attributed to the larger persistent C pool in biochar compared to labile C pool, but also to the stimulated growth of bacteria such as *Myxococcaceae* and *Flavisolibacter* that incorporated more C into their biomass (i.e. more C remained in the soil pools) rather than releasing it into atmosphere as CO_2. Hence, designing biochars with high content of aromatic backbone compounds to inhibit microbial metabolic quotient greatly contribute to soil C sequestration. Further studies should be focused on the characterization of the specific chemical compounds in biochar C fractions, and the changes in the microbial genes/enzymes that are responsible for the degradation of these compounds.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgements

This study was supported by the National Key Research and Development Program of China (2019YFC1803704), the Serving Local Economic Development Project of Shandong (Linyi) Institute of Modern Agriculture, Zhejiang University (ZDNY-2020-FWLY01006) and Fundamental Research Funds for the Central Universities in China, and by the RUDN University Strategic Academic Leadership Program.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2022.116176.

References

Allison, S.D., Martiny, J.B.H., 2008. Resistance, resilience, and redundancy in microbial communities. Proc. Natl. Acad. Sci. 105 (supplement_1), 11512–11519.
Baldock, J.A., Smernik, R.J., 2002. Chemical composition and bioavailability of thermally altered Pinus resinosa (Red pine) wood. Org Geochem. 33 (9), 1093–1109.
Budai, A., Rasse, D.P., Lagomarsino, A., Lerch, T.Z., Paruch, L., 2016. Biochar persistence, priming and microbial responses to pyrolysis temperature series. Biol. Fertil. Soils 52 (6), 749–761.
Buss, W., Shepherd, J.G., Heal, K.V., Mašek, O., 2018. Spatial and temporal microscale pH change at the soil-biochar interface. Geoderma 331, 50–52.
Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. 108 (supplement_1), 4516–4522.
Chen, J., Liu, X., Zheng, J., Zhang, B., Lu, H., Chi, Z., Pan, G., Li, L., Zheng, J., Zhang, X., Wang, J., Yu, X., 2013. Biochar soil amendment increased bacterial but decreased fungal gene abundance with shifts in community structure in a slightly acid rice paddy from Southwest China. Appl. Soil Ecol. 71, 33–44.
Chen, J., Chen, D.e., Xu, Q., Fuhrmann, J.J., Li, L., Pan, G., Li, Y., Qin, H., Liang, C., Sun, X., 2019. Organic carbon quality, composition of main microbial groups, enzyme activities, and temperature sensitivity of soil respiration of an acid paddy soil treated with biochar. Biol. Fertil. Soils 55 (2), 185–197.
Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., Brown, C.T., Porras-Alfaro, A., Kuske, C.R., Tiedje, J.M., 2014. Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids Res. 42 (D1), D633–D642.
Cross, A., Sohi, S.P., 2011. The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. Soil Biol. Biochem. 43 (10), 2127–2134.
Dai, Z., Li, R., Muhammad, N., Brookes, P.C., Wang, H., Liu, X., Xu, J., 2014. Principle component and hierarchical cluster analysis of soil properties following biochar incorporation. Soil Sci. Soc. Am. J. 78 (1), 205–213.
Dai, Z., Barberán, A., Li, Y., Brookes, P.C., Xu, J., Kent, A.D., 2017a. Bacterial Community Composition Associated with Pyrogenic Organic Matter (Biochar) Varies with Pyrolysis Temperature and Colonization Environment. mSphere 2 (2).
Dai, Z., Webster, T.M., Enders, A., Hanley, K.L., Xu, J., Thies, J.E., Lehmann, J., 2017b. DNA extraction efficiency from soil as affected by pyrolysis temperature and extractable organic carbon of high-ash biochar. Soil Biol. Biochem. 115, 129–136.
Dai, Z., Enders, A., Rodrigues, J.L., Hanley, K.L., Brookes, P.C., Xu, J., Lehmann, J., 2018. Soil fungal taxonomic and functional community composition as affected by biochar properties. Soil Biol. Biochem. 126, 159–167.
Dai, Z., Li, Y., Zhang, X., Wu, J., Luo, Y.u., Kuzyakov, Y., Brookes, P.C., Xu, J., 2019. Easily mineralizable carbon in manure-based biochar added to a soil influences N_2O emissions and microbial-N cycling genes. Land Degrad. Dev. 30 (4), 406–416.
Delgado-Baquerizo, M., Reich, P.B., Trivedi, C., Eldridge, D.J., Abades, S., Alfaro, F.D., Bastida, F., Berhe, A.A., Cutler, N.A., Gallardo, A., García-Velázquez, L., Hart, S.C., Hayes, P.E., He, J.-Z., Hseu, Z.-Y., Hu, H.-W., Kirchmair, M., Neuhauser, S., Pérez, C. A., Reed, S.C., Santos, F., Sullivan, B.W., Trivedi, P., Wang, J.-T., Weber-Grullon, L., Williams, M.A., Singh, B.K., 2020. Multiple elements of soil biodiversity drive ecosystem functions across biomes. Nat. Ecol. Evol. 4 (2), 210–220.
DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72 (7), 5069–5072.
Dufrêne, M., Legendre, P., 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. 67, 345–366.
Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10 (10), 996–998.
Enders, A., Lehmann, J., 2012. Comparison of wet-digestion and dry-ashing methods for total elemental analysis of biochar. Commun. Soil Sci. Plant Anal. 43 (7), 1042–1052.
Fadrosh, D.W., Ma, B., Gajer, P., Sengamalay, N., Ott, S., Brotman, R.M., Ravel, J., 2014. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Microbiome 2, 1–7.
Farrell, M., Kuhn, T.K., Macdonald, L.M., Maddern, T.M., Murphy, D.V., Hall, P.A., Singh, B.P., Baumann, K., Krull, E.S., Baldock, J.A., 2013. Microbial utilisation of biochar-derived carbon. Sci. Total Environ. 465, 288–297.
Gomez-Montano, L., Jumpponen, A., Gonzales, M.A., Cusicanqui, J., Valdivia, C., Motavalli, P.P., Herman, M., Garrett, K.A., 2013. Do bacterial and fungal communities in soils of the Bolivian Altiplano change under shorter fallow periods? Soil Biol. Biochem. 65, 50–59.
Hallin, P.F., Ussery, D.W., 2004. CBS Genome Atlas Database: a dynamic storage for bioinformatic results and sequence data. Bioinformatics 20 (18), 3682–3686.
Itakura, M., Saeki, K., Omori, H., Yokoyama, T., Kaneko, T., Tabata, S., Ohwada, T., Tajima, S., Uchiumi, T., Honnma, K., Fujita, K., Iwata, H., Saeki, Y., Hara, Y., Ikeda, S., Eda, S., Mitsui, H., Minamisawa, K., 2009. Genomic comparison of Bradyrhizobium japonicum strains with different symbiotic nitrogen-fixing capabilities and other Bradyrhizobiaceae members. ISME J. 3 (3), 326–339.
Jenkins, S.N., Rushton, S.P., Lanyon, C.V., Whiteley, A.S., Waite, I.S., Brookes, P.C., Kemmitt, S., Evershed, R.P., O'Donnell, A.G., 2010. Taxon-specific responses of soil bacteria to the addition of low level C inputs. Soil Biol. Biochem. 42 (9), 1624–1631.
Jindo, K., Sánchez-Monedero, M.A., Hernández, T., García, C., Furukawa, T., Matsumoto, K., Sonoki, T., Bastida, F., 2012. Biochar influences the microbial community structure during manure composting with agricultural wastes. Sci. Total Environ. 416, 476–481.
Kallenbach, C., Grandy, A.S., Frey, S., Diefendorf, A., 2015. Microbial physiology and necromass regulate agricultural soil carbon accumulation. Soil Biol. Biochem. 91, 279–290.
Kappler, A., Wuestner, M.L., Ruecker, A., Harter, J., Halama, M., Behrens, S., 2014. Biochar as an electron shuttle between bacteria and Fe (III) minerals. Environ. Sci. Technol. Lett. 1 (8), 339–344.

Khadem, A., Raiesi, F., 2017. Responses of microbial performance and community to corn biochar in calcareous sandy and clayey soils. Appl. Soil Ecol. 114, 16–27.
Khodadad, C.L.M., Zimmerman, A.R., Green, S.J., Uthandi, S., Foster, J.S., 2011. Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. Soil Biol. Biochem. 43 (2), 385–392.
Kinney, T., Masiello, C., Dugan, B., Hockaday, W., Dean, M., Zygourakis, K., Barnes, R., 2012. Hydrologic properties of biochars produced at different temperatures. Biomass Bioenergy 41, 34–43.
Klüpfel, L., Keiluweit, M., Kleber, M., Sander, M., 2014. Redox properties of plant biomass-derived black carbon (biochar). Environ. Sci. Technol. 48 (10), 5601–5611.
Kolton, M., Meller Harel, Y., Pasternak, Z., Graber, E.R., Elad, Y., Cytryn, E., 2011. Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. Appl. Environ. Microbiol. 77 (14), 4924–4930.
Lehmann, J., Joseph, S., 2015. Biochar for environmental management: science, technology and implementation. Routledge.
Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota–a review. Soil Biol. Biochem. 43 (9), 1812–1836.
Liang, C., Amelung, W., Lehmann, J., Kästner, M., 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. Glob. Change Biol. 25 (11), 3578–3590.
Liao, H., Fan, H., Li, Y., Yao, H., 2021. Influence of reductive soil disinfestation or biochar amendment on bacterial communities and their utilization of plant-derived carbon in the rhizosphere of tomato. Appl. Microbiol. Biotechnol. 105 (2), 815–825.
Lim, J.H., Baek, S.-H., Lee, S.-T., 2009. Ferruginibacter alkalilentus gen. nov., sp. nov. and Ferruginibacter lapsinanis sp. nov., novel members of the family 'Chitinophagaceae' in the phylum Bacteroidetes, isolated from freshwater sediment. Int. J. Syst. Evol. Microbiol. 59 (10), 2394–2399.
Magoc, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27 (21), 2957–2963.
Mummey, D., Holben, W., Six, J., Stahl, P., 2006. Spatial stratification of soil bacterial populations in aggregates of diverse soils. Microb. Ecol. 51 (3), 404–411.
Naether, A., Foesel, B.U., Naegele, V., Wüst, P.K., Weinert, J., Bonkowski, M., Alt, F., Oelmann, Y., Polle, A., Lohaus, G., Gockel, S., Hemp, A., Kalko, E.K.V., Linsenmair, K.E., Pfeiffer, S., Renner, S., Schöning, I., Weisser, W.W., Wells, K., Fischer, M., Overmann, J., Friedrich, M.W., 2012. Environmental factors affect acidobacterial communities below the subgroup level in grassland and forest soils. Appl. Environ. Microbiol. 78 (20), 7398–7406.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R., et al., 2015. vegan: Community Ecology Package. R Package Version 2.2-1.
Payne, R.B., Fagervold, S.K., May, H.D., Sowers, K.R., 2013. Remediation of polychlorinated biphenyl impacted sediment by concurrent bioaugmentation with anaerobic halorespiring and aerobic degrading bacteria. Environ. Sci. Technol. 47 (8), 3807–3815.
Pereira, R.C., Kaal, J., Camps Arbestain, M., Pardo Lorenzo, R., Aitkenhead, W., Hedley, M., Macías, F., Hindmarsh, J., Maciá-Agulló, J.A., 2011. Contribution to characterisation of biochar to estimate the labile fraction of carbon. Org Geochem. 42 (11), 1331–1342.
Petters, S., Groß, V., Söllinger, A., Pichler, M., Reinhard, A., Bengtsson, M.M., Urich, T., 2021. The soil microbial food web revisited: Predatory myxobacteria as keystone taxa? The ISME journal 15 (9), 2665–2675.
Pollock, J., Glendinning, L., Wisedchanwet, T., Watson, M., Liu, S.-J., 2018. The madness of microbiome: attempting to find consensus "best practice" for 16S microbiome studies. Appl. Environ. Microbiol. 84 (7).
Props, R., Kerckhof, F.-M., Rubbens, P., De Vrieze, J.o., Hernandez Sanabria, E., Waegeman, W., Monsieurs, P., Hammes, F., Boon, N., 2017. Absolute quantification of microbial taxon abundances. ISME J. 11 (2), 584–587.
Roberts DW. (2012) Package "labdsv". https://cran.r-project.org/web/packages/labdsv/ .
Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME journal 4 (10), 1340–1351.
Shin, J., Bae, S., Chon, K., 2021. Fenton oxidation of synthetic food dyes by Fe-embedded coffee biochar catalysts prepared at different pyrolysis temperatures: A mechanism study. Chem. Eng. J. 421, 129943.
Smets, W., Leff, J.W., Bradford, M.A., McCulley, R.L., Lebeer, S., Fierer, N., 2016. A method for simultaneous measurement of soil bacterial abundances and community composition via 16S rRNA gene sequencing. Soil Biol. Biochem. 96, 145–151.
Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O., Howden, M., McAllister, T., Pan, G., Romanenkov, V., Schneider, U., Towprayoon, S., Wattenbach, M., Smith, J.o., 2008. Greenhouse gas mitigation in agriculture. Philos. Trans. R. Soc. B: Biol. Sci. 363 (1492), 789–813.
Strotmann, U., Reuschenbach, P., Schwarz, H., Pagga, U., 2004. Development and evaluation of an online CO_2 evolution test and a multicomponent biodegradation test system. Appl. Environ. Microbiol. 70 (8), 4621–4628.
Tamez-Hidalgo, P., Christensen, B.T., Lever, M.A., Elsgaard, L., Lomstein, B.A., 2016. Endospores, prokaryotes, and microbial indicators in arable soils from three long-term experiments. Biol. Fertil. Soils 52 (1), 101–112.
Tavares, A., Carvalho, C.M.B., Faustino, M.A., Neves, M.G.P.M.S., Tomé, J.P.C., Tomé, A. C., Cavaleiro, J.A.S., Cunha, Â., Gomes, N.C.M., Alves, E., Almeida, A., 2010. Antimicrobial photodynamic therapy: study of bacterial recovery viability and potential development of resistance after treatment. Mar. Drugs 8 (1), 91–105.
Wang, M., Yi, M., Lu, M., Zhu, X.i., Chen, G., Gao, F., Liu, Z., Cao, J., Zhang, D., 2019. Effect of in situ sediment remediation combining oyster shells and bottom microporous aeration on nitrogen removal and microbiota. Aquac. Res. 50 (1), 331–341.
Wardle, D.A., Ghani, A., 1995. A critique of the microbial metabolic quotient (qCO_2) as a bioindicator of disturbance and ecosystem development. Soil Biol. Biochem. 27 (12), 1601–1610.
Warnock, D.D., Lehmann, J., Kuyper, T.W., Rillig, M.C., 2007. Mycorrhizal responses to biochar in soil–concepts and mechanisms. Plant Soil 300 (1-2), 9–20.
Whitman, T., Zhu, Z., Lehmann, J., 2014. Carbon mineralizability determines interactive effects on mineralization of pyrogenic organic matter and soil organic carbon. Environ. Sci. Technol. 48 (23), 13727–13734.
Xu, Y., Seshadri, B., Sarkar, B., Wang, H., Rumpel, C., Sparks, D., Farrell, M., Hall, T., Yang, X., Bolan, N., 2018. Biochar modulates heavy metal toxicity and improves microbial carbon use efficiency in soil. Sci. Total Environ. 621, 148–159.
Xu, N., Tan, G., Wang, H., Gai, X., 2016. Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. Eur. J. Soil Biol. 74, 1–8.
Xu, W., Whitman, W.B., Gundale, M.J., Chien, C.-C., Chiu, C.-Y., 2021. Functional response of the soil microbial community to biochar applications. GCB Bioenergy 13 (1), 269–281.
Xue, C., Ryan Penton, C., Zhu, C., Chen, H., Duan, Y., Peng, C., Guo, S., Ling, N., Shen, Q., 2018. Alterations in soil fungal community composition and network assemblage structure by different long-term fertilization regimes are correlated to the soil ionome. Biol. Fertil. Soils 54 (1), 95–106.
Yu, Z., Chen, L., Pan, S., Li, Y., Kuzyakov, Y., Xu, J., Brookes, P.C., Luo, Y., 2018. Feedstock determines biochar-induced soil priming effects by stimulating the activity of specific microorganisms. Eur. J. Soil Sci. 69 (3), 521–534.
Yu, P., He, X., Baer, M., Beirinckx, S., Tian, T., Moya, Y.A.T., Zhang, X., Deichmann, M., Frey, F.P., Bresgen, V., Li, C., Razavi, B.S., Schaaf, G., von Wirén, N., Su, Z., Bucher, M., Tsuda, K., Goormachtig, S., Chen, X., Hochholdinger, F., 2021. Plant flavones enrich rhizosphere Oxalobacteraceae to improve maize performance under nitrogen deprivation. Nat. Plants 7 (4), 481–499.
Zheng, 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. Sci. Total Environ. 571, 206–217.
Zheng, Y., Saitou, A., Wang, C.-M., Toyoda, A., Minakuchi, Y., Sekiguchi, Y., Ueda, K., Takano, H., Sakai, Y., Abe, K., Yokota, A., Yabe, S., 2019. Genome features and secondary metabolites biosynthetic potential of the class Ktedonobacteria. Front. Microbiol. 10.