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ENVIRONMENTAL BIOTECHNOLOGY

Influence of reductive soil disinfestation or biochar amendment on bacterial communities and their utilization of plant-derived carbon in the rhizosphere of tomato

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Abstract

Root-associated microorganisms play an important role in plant nutrition and productivity. However, our understanding of how a plant-microbiome system responds to pre-planting soil management remains limited. Here, continuous labeling with $^{13}CO_2$ gas combined with stable isotope probing (SIP) was applied to explore bacterial utilization of plant-derived carbon (C) in the tomato rhizosphere as affected by biochar amendment or reductive soil disinfestation (RSD). Our results showed that RSD treatment strongly shaped the soil bacterial community composition, while biochar soil amendment had little impact on the community in the rhizosphere of tomato. We observed that the bacterial community in the RSD treatment, which actively utilized plant-derived C, belonged to various phyla (i.e., *Proteobacteria*, *Cyanobacteria*, *Verrucomicrobia*, and *Acidobacteria*), while the genus *Streptomyces* (phylum *Actinobacteria*) was the main bacterial taxa that actively utilized plant-derived C in the biochar and control treatments. This study provides evidence that biochar application or RSD pre-planting soil management practices induced distinct bacterial utilization of plant-derived C, which may in turn regulate plant productivity in agricultural systems.

Key points

- *Genus Streptomyces was the main bacterial group utilizing plant-derived carbon in both control and biochar treatments.*
- *Reductive soil disinfestation altered bacterial utilization of plant-derived carbon.*
- *Biochar did not alter the composition of the bacterial communities but had more labeled bacterial taxa utilizing plant-derived carbon.*

Keywords Soil management · Bacterial communities · Plant-derived C · Stable isotope probing

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Introduction

Tomato is a staple vegetable crop grown and consumed globally (Olaniyi et al. 2010). Driven by the great demand from the market, intensive continuous mono-cropping and fertilization are common management practices in tomato producing. Nevertheless, these practices decrease soil quality through soil acidification and soil-borne pathogen accumulation (Blok et al. 2000; Huang et al. 2016). In order to alleviate the soil degradation induced by continuous mono-cropping, pre-planting chemical soil fumigation has been used extensive across the globe (Liu et al. 2016). However, chemical soil fumigation has environmental and human health hazards that make it unsuitable in sustainable agricultural systems (Butler et al. 2012). In recent years, much progress has been made in seeking environmentally friendly alternatives (Jaiswal et al. 2017). As alternatives to traditional pre-planting soil

management regimes, biochar amendment and reductive soil disinfestation (RSD) are two popular soil treatments which have been increasingly used to improve soil quality (Messiha et al. 2007; Huang et al. 2015; Jaiswal et al. 2017; Kumar et al. 2018) due to their potential agronomic, environmental, and economic benefits.

Biochar is a C-rich product with a half-life ranging from hundreds to thousands of years (Zimmerman 2010). There is evidence that biochar soil amendment can improve soil quality (Glaser et al. 2002) and crop productivity (Graber et al. 2010). It has been shown that biochar application can increase soil pH, nutrient availability, and soil water-holding capacity (Lehmann et al. 2011; Abel et al. 2013; Whitman et al. 2016; Zheng et al. 2017). Additionally, it is known that biochar amendment is able to efficiently reduce soil pathogen density through pore adsorption (Gu et al. 2017) and alter microbial communities and their functions in soils (Kolton et al. 2017). Uzoma et al. (2011) found that the maize grain yield increased by 150% and 98% after the application of biochar at 15 and 20 t ha^{-1} in a degraded soil. Therefore, biochar is highly recommended as a soil amendment to remediate degraded soils (Kolton et al. 2011; Zheng et al. 2017).

RSD is another soil pre-planting treatment for improving soil quality. The RSD method, also called biological soil disinfestation, was simultaneously invented in the Netherlands (Blok et al. 2000) and Japan (Shinmura 2000). This method stimulates anaerobic processes by incubating organic wastes (e.g., corn stover, rice straw, or animal manures) in waterlogged soils covered with a polyethylene mulch (Butler et al. 2012; Huang et al. 2015). RSD can improve the quality of degraded soils by increasing soil nutrients and suppressing pathogens. Huang et al. (2016) reported that disease incidences in a RSD treatment were about six times lower than the control during two seasons of cultivation in a field experiment. Increasingly, studies have indicated that the RSD treatment can be effective against a wide range of pathogens, including harmful nematodes (Goud et al. 2004). For example, the genera *Bacillus* and *Pseudomonas* have been shown as the dominant microbial populations in RSD-treated soils, which have capabilities to suppress pathogens via antibiotics, lytic enzymes, and volatile organic compound production (Khabbaz et al. 2015; Shen et al. 2015). However, despite the fact that increasing number of studies have demonstrated the influence of both biochar amendment and RSD treatment on general soil microbial communities, few studies have sought to explore the effects on those rhizosphere microbial communities that specifically utilize plant-derived C.

The rhizosphere is a unique interface, at which microbes and plants carry out complex and diverse molecular interactions (Gkarmiri et al. 2017; Liu et al. 2019). On average, rhizodeposition accounts for about 17% of the total photoassimilated C (Nguyen 2003). Previous studies have shown that root exudate composition depends on multiple factors, e.g., developmental phase, root traits, soil types, plant species, nutrient availability, and environmental conditions (Jones 1998; Aulakh et al. 2001; Badri and Vivanco 2009; Mendes et al. 2017). Root exudates consumed by microorganisms as a substrate play a vital role in affecting the composition and activity of the soil microbial community (Ahmed et al. 2018; Sarr et al. 2020). Studies have suggested that plant root exudates can recruit the rhizosphere microbial community to build a suitable habitat for plant growth (Bulgarelli et al. 2013). For example, root exudates released by tomato can stimulate beneficial *Pseudomonad* abundance in the rhizosphere (Lemanceau et al. 1995). Clearly, the complex interactions and feedbacks among plant roots, microbes, and the soil environmental conditions shape the microbial community composition in the rhizosphere (Chapelle et al. 2016), and this may further influence on nutrient cycling and plant productivity (Hannula et al. 2017). The differences between biochar amendment and RSD treatment on soil rhizosphere microbial community composition have not been explored adequately (Huang et al. 2015; Whitman et al. 2016). Our previous study, focusing on application of biochar in a faba bean-maize intercropping system found that biochar amendment can stimulate soil bacterial utilization of plant-derived C (Liao et al. 2019). However, whether different pre-planting soil managements (i.e., biochar and RSD) can alter bacterial communities utilizing plant-derived C in continuous mono-cropping systems is still an open question.

The aims of this study were (i) to investigate the influence of two environmentally friendly soil management practices (i.e., biochar addition and RSD) on bacterial communities in the tomato rhizosphere; (ii) to identify the impacts of biochar and RSD managements on bacteria that actively utilize plant-derived C in the rhizosphere; and (iii) to assess roles of active bacteria that may potentially regulate plant development in the rhizosphere. We hypothesized that the biochar and RSD treatments could enhance utilization of root exudates by soil bacteria in the rhizosphere of tomato. To verify this hypothesis, continuous $^{13}CO_2$ labeling, stable isotope probing (SIP), and Illumina sequencing were performed to classify bacterial communities and their utilization of root exudates in the rhizosphere of tomato under different soil treatments.

Materials and methods

Soil sampling and biochar preparation

Soils used in this study were collected from a greenhouse in Changzhou (31°55′32″N, 119°51′39″E), Jiangsu Province, East China. The greenhouse soil is a clay loam texture, belonging to plinthosols according to the FAO/UNESCO classification. The greenhouse soil had received excessive fertilizer, was under continuous tomato cropping for 4 years, and

therefore suffered severe bacterial wilt disease. The disease incidence was about 40% with approximately 10^6 CFU (colony forming units) of *Ralstonia solanacearum* per gram soil. Soil samples were collected from 0 to 20-cm depth. Plants and root residues were removed from the soil before passing it through a 2-mm sieve.

Biochar was made from wood chip solid waste. First, chips were air-dried and then charred in the lab in a furnace for 5 h at 500 °C. The biochar was then milled (< 2-mm sieve) for further use. For the RSD treatment, maize straw was air-dried for 4 weeks and finely chopped to less than 2 mm. The basic properties of soil, biochar, and maize straw are shown in Table 1.

Experimental procedure

A pot experiment was conducted with three treatments: biochar amendment, RSD, and a control (CK). To each pot (6.8-cm diameter, 14.8-cm height), a dry weight basis of 300 g soil was added. Both biochar and maize straws were added and well mixed with the soil just immediately before filling the pots. Then Milli-Q (Merck, Darmstadt, Germany) water was added to reach 40% water-holding capacity (Liao et al. 2019). For the biochar treatment, biochar was incorporated into the soil at 2% (w/w). For both the biochar and CK treatments, Milli-Q water was added to maintain the soil water content during soil incubation. For the RSD treatment, air-dried maize straw was incorporated into the soil at 2% (w/w), and the soil irrigated to 5 cm above the upper surface to ensure flood conditions. All the three treatments were put into a step-in incubator at 28 °C for 21 days. After 21 days of flood incubation, soils in the RSD treatment were air-dried to achieve approximately 40% water-holding capacity. In addition, 3 replicate pots of each treatment were randomly destructively sampled to investigate the soil bacterial community composition before planting.

Tomato (*Lycopersicon esculentum* Mill.) seeds (Hezuo 903, Changfeng Co., Ltd., Shanghai, China) were planted in November 2017. Before planting, soil was amended with NH_4NO_3 and KH_2PO_4 at the rate of 100 mg total N kg^{-1} soil, 50 mg P_2O_5 kg^{-1} soil, and 50 mg K_2O kg^{-1} soil as base fertilizer. Three healthy seedlings were planted in each pot after the tomato plants had produced true leaves, and then, one healthy seedling per pot was kept after 7-day planting.

Table 1 Basic properties of soil and biochar

	pH	Total carbon	Total nitrogen	C:N
Soil	6.93	2.11%	0.18%	11.7
Biochar	10.9	73.6%	0.6%	122
Straw	–	45.6%	1.34%	33.7

The day and night periods and temperatures were set at 14 h and 10 h and at 25 °C and 18 °C, respectively.

The $^{13}CO_2$ continuous labeling process followed the descriptions of Yao et al. (2012) and Wang et al. (2016) after 14-day tomato growth. Briefly, 99.8 atom % of $^{13}CO_2$ gas (Sigma-Aldrich, Darmstadt, USA) at 350 ppm was applied to the labeled plant growth chamber. For the parallel unlabeled plant growth chamber, $^{12}CO_2$ was used to reach the same CO_2 concentration. Three pots for each treatment were put into both labeled and unlabeled plant growth chambers. Plants experienced similar temperature and light conditions in each growth chamber. Soil moisture was maintained by adding Milli-Q water every 3 days during the 35-day labeling. All soil in a given pot was considered as rhizosphere soil since root growth in each pot was extremely extensive. The collected soil was then separated into two parts, one was immediately freeze-dried for DNA extraction, and the other was stored at − 4 °C for soil property analysis. An overall conceptual diagram for the study design is shown in Fig. 1.

Soil property analysis

Soil pH (soil: water 1:2.5) was measured by a pH meter; total carbon and nitrogen contents were analyzed using an elemental analyzer (Vario MACRO cube, Hanau, Germany); soil NO_3^- and exchangeable NH_4^+ contents were extracted with 2 M KCl (1:10 w/v) for 30 min and then measured by a continuous flow analyzer (AutoAnalyzer3, Mequon, USA).

DNA extraction

Genomic DNA was extracted from 0.50 g of fresh soil samples using the Fast DNA SPIN Kit (MP Biomedicals, Santa Ana, USA). DNA content was determined by a NanoDrop™ 2000 (Thermo Scientific, Waltham, USA).

DNA-SIP

The DNA-SIP procedure was that by Liao et al. (2019). Briefly, for each sample, 3 μg DNA was added into a CsCl density fraction, then CsCl solutions were adjusted to 1.7102 g/ml with a refractometer (Reichert, Depew, USA). The solution was then centrifuged at 45,000 rpm (184,000*g*) at 20 °C for 44 h using a Vti 65.2 vertical rotor (Beckman Coulter, Brea, USA). Sixteen equal fractions of CsCl-DNA were then collected using a single-channel syringe pump. DNA samples were purified with PEG6000 and ethanol buffer (70%), then dissolved in 30 μL sterile water, and stored at − 20 °C for further use.

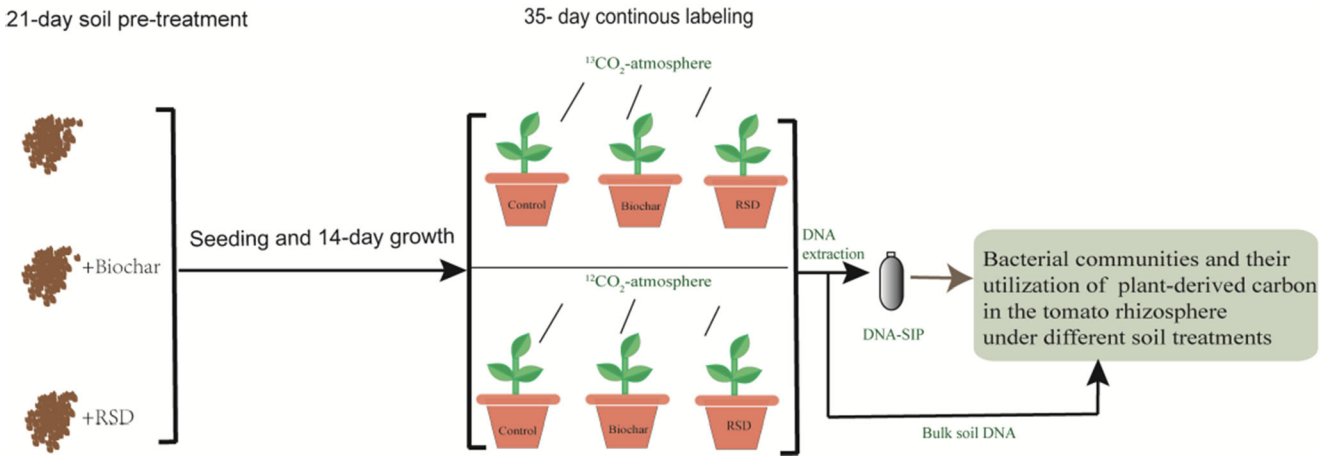

Fig. 1 A conceptual diagram for the study design, methods, and objective

PCR amplification and Illumina sequencing

Six bulk DNA samples for each treatment (three $^{13}CO_2$-labeled and three unlabeled soil samples) and three pre-planting soil DNA samples for each treatment were selected for sequencing.

qPCR results show that there were no clear differences in quantification of the16S rRNA gene between unlabeled and ^{13}C-labeled samples (Supplemental Note 1; Fig. 2). Because the bacteria utilizing the plant-derived C accounted for a small proportion of the whole bacterial communities, and the qPCR technique may not be sensitive enough (Wang et al. 2019), the high-resolution SIP approach was used to identify those ^{13}C-labeled bacteria that utilize plant rhizoexudates. Only buoyant densities ranging from 1.71 to 1.75 g/ml (7 fractions per sample) were analyzed. Bacterial 16S rRNA gene was amplified with barcoded primers 515f/907r (Hamady et al. 2008; Zhou et al. 2011). The PCR reaction was that used by Liao et al. (2019). Purified PCR samples were run on an Illumina HiSeq2500 platform.

Sequenced data was analyzed by the QIIME 1.9.1 pipeline (Caporaso et al. 2010). Briefly, chimeras were checked and filtered using the USEARCH tool (Edgar 2010). Subsequently, high-quality sequences were clustered into operational taxonomic units (OTUs) using the UCLUST algorithm with 0.97 similarity. Representative sequence clusters were then annotated with the Greengenes database (release v13.8) (McDonald et al. 2012). The SSU rRNA sequences have been deposited at the BioProject database under NCBI accession number PRJNA563242.

Statistical analysis

The statistical approach for identifying ^{13}C-labeled OTUs (high-resolution SIP) has been described previously (Pepe-Ranney et al. 2016; Angel et al. 2018; Youngblut et al. 2018; Koechli et al. 2019). In this study, the procedure for identifying ^{13}C-labeled OTUs was that by Liao et al. (2019). Briefly, we defined > 1.71 g/ml as the heavy category fraction and discarded the OTUs with low sequence counts to perform the statistical analyses using the Benjamini-Hochberg method of *P* value corrections (Benjamini and Hochberg 1995) using the DESeq2 package (Love et al. 2014). Statistically enriched OTUs from ^{13}C-labeled samples, compared to the unlabeled control, were expected to be the target $^{13}CO_2$-labeled microbiome (Youngblut and Buckley 2014; Pepe-Ranney et al. 2016).

SPSS 16.0 software (SPSS Inc., Chicago, USA) was used to for standard statistical tests. The statistical significance was determined by the least significant difference (LSD) test at the $P = 0.05$ level. PCoA and NMDS ordinations based on Bray-Curtis similarities were analyzed using the "Phyloseq" package (McMurdie and Holmes 2013) and the "ggplot2" package (Wickham 2010) in R (version 3.4.1) (R Core Team 2013).

Results

Soil properties and plant biomass

The biochar treatment significantly increases ($P < 0.05$) the tomato biomass (shoot + root) compared to RSD and CK, but no significant differences are found between the RSD and CK treatments (Table 2). Specifically, the RSD treatment had 34.2% lower biomass than the biochar treatment. Both biochar and RSD treatments significantly increase ($P < 0.05$) soil pH, total C and total N, and exchangeable NH_4^+ and C/N in the tomato rhizosphere compared with CK, while the NO_3^- concentration did not change among treatments (Table 2).

Changes in bacterial community composition as assessed by Illumina sequencing

Before planting, we measure the soil bacterial community composition among the different treatments (Supplemental

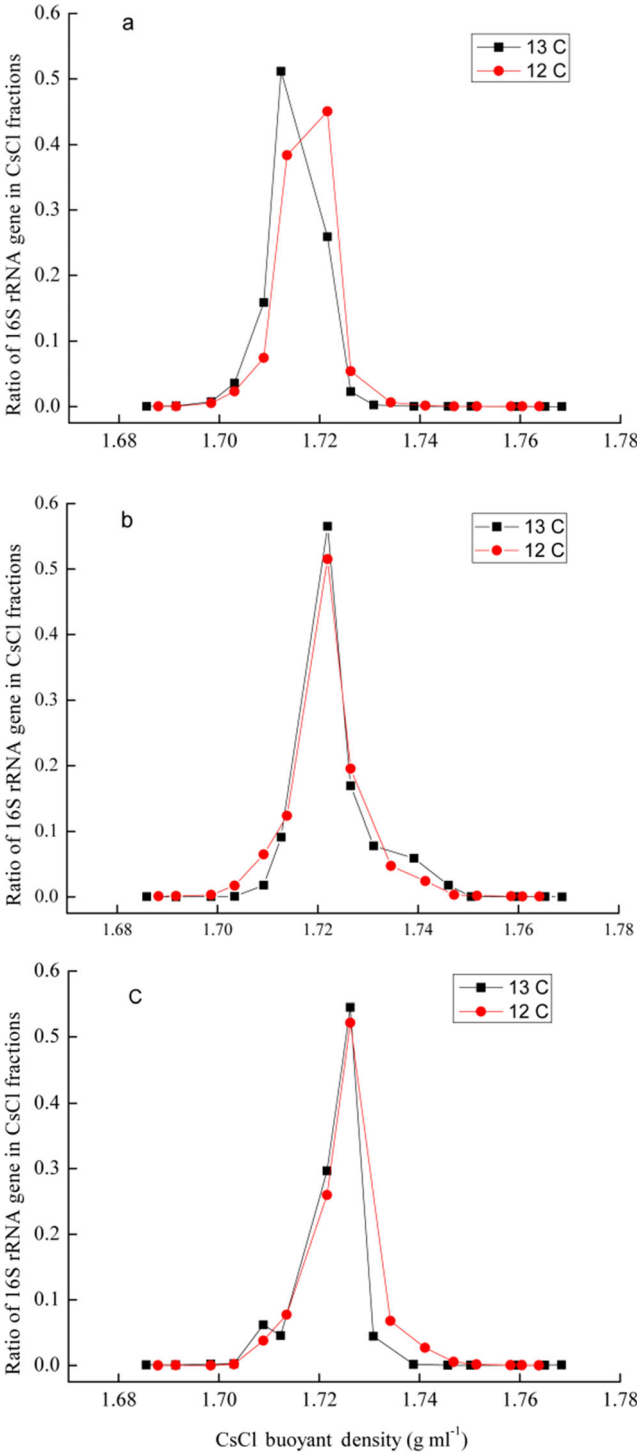

Fig. 2 Quantitative distribution of density-resolved bacterial 16S rRNA gene obtained from CK (**a**) and biochar (**b**) and RSD (**c**) 35-day labeling with either labeled ($^{13}CO_2$) or unlabeled ($^{12}CO_2$).The normalized data are the ratio of the copy number in each gradient fraction to the total quantities from each treatment

Fig. S1). The RSD treatment significantly changed the bacterial community composition (Adonis test, $P < 0.001$) compared with CK, whereas no significant differences were observed between CK and biochar treatments. We found that bacterial species from phylum *Firmicutes* (e.g., *Bacillales* and *Clostridiales*) were significantly enriched in the RSD treatment. After planting, the RSD treatment maintains a distinct bacterial community composition compared with the control and biochar treatments (Adonis test, $P < 0.001$; Supplemental Fig. S2). *Proteobacteria* and *Actinobacteria*, which accounted for more than 50% of sequences, are the two major phyla occurring in the tomato rhizosphere among treatments (Fig. 3). In contrast to our hypotheses, the bacterial community composition in the biochar and CK treatments is similar, and only two taxa, *Gemmatimonadetes* and *Planctomycetes*, are significantly ($P < 0.05$) altered in their relative abundances (Supplemental Fig. S3). The RSD treatment significantly decreases ($P < 0.05$) the relative abundance of *Actinobacteria* while increases the relative abundance of *Firmicutes* compared with CK (Supplemental Fig. S3).

^{13}C incorporation into DNA in the heavy density fractions

The CsCl fraction density plays an important role in composition of bacterial communities, in both the unlabeled and the ^{13}C-labeled samples (Fig. 4). This phenomenon was mainly attributed to the genome G + C concentrations of different bacteria in the CsCl buoyant density. We found that bacterial communities in the ^{13}C-labeled samples differed from unlabeled samples in the heavy gradient fraction, and significant differences were observed within the biochar treatment (Adonis test, $P < 0.001$; Fig. 4).

^{13}C-labeled rhizosphere bacterial communities

A total of 126 fractions (density > 1.71 g/ml) are chosen for 16S rRNA gene sequencing (Fig. 5). In total, there were 6923, 7651, and 8303 OTUs that passed the sparsity threshold in the CK, biochar, and RSD treatments, respectively. The OTUs that were significantly enriched in the heavy fraction relative to the unlabeled control were considered to have ^{13}C-labeled DNA. Among these, 91, 201, and 72 OTUs, which accounted for 1.3%, 2.6%, and 0.87% of the total detected OTUs in the CK, biochar, and RSD treatments, respectively, are enriched significantly in the ^{13}C-labeled samples (Fig. 5).

Various identified phyla (e.g., *Proteobacteria*, *Cyanobacteria*, *Verrucomicrobia*, and *Acidobacteria*) were more prominent among the ^{13}C-labeled OTUs in the RSD treatment. In contrast, *Actinobacteria* and *Proteobacteria* are identified as the major labeled taxa in both the CK and biochar treatments (Fig. 5). A Venn diagram shows that there were 33 labeled OTUs shared by the CK and biochar treatments, while there is only one labeled OTU shared by the CK and RSD treatments, and only two OTUs between the biochar and RSD treatments (Supplemental Fig. S4). Among the labeled OTUs,

Table 2 Rhizosphere soil chemical properties and tomato dry biomass (shoot + root)

Treatment	pH (soil:H_2O = 1:2.5)	Total C (g/kg)	Total N (g/kg)	NO_3^- (mg/kg)	NH_4^+ (mg/kg)	C/N	Dry biomass (g)
CK	6.79 (0.06)c	25.0 (0.73)c	1.94 (0.07)b	38.2 (7.2)a	6.27 (0.43)b	12.9 (0.17)c	3.21 (0.13)b
Biochar	7.12 (0.06)a	38.2 (0.55)a	2.26 (0.04)a	45.4 (7.3)a	6.65 (0.85)a	19.7 (0.41)a	4.88 (0.65)a
RSD	6.96 (0.07)b	33.3 (0.62)b	2.25 (0.09)a	59.7 (4.2)a	6.73 (0.66)a	14.8 (0.56)b	3.37 (0.22)b

CK, control; RSD, reductive soil disinfestation. Different letters in the same column indicate significant differences at $P < 0.05$. Values in parentheses are stand error

there are numerous shared ^{13}C-labeled OTUs between the CK and biochar treatments, while the RSD treatment had few shared ^{13}C-labeled OTUs with either biochar or CK treatment (Supplemental Fig. S5). After the absolute counts of OTUs were transformed into their relative abundances, we found relatively more *Actinobacteria* utilizing root exudates in the biochar treatment, while the phylum *Proteobacteria* that actively utilized root exudates was identified in the RSD treatment (Supplemental Fig. S6).

At the genus level, DNA-SIP reveals that *Streptomyces* was the dominant genus that utilized root exudates in both the CK and biochar treatment, which had 25 and 93 labeled OTUs, respectively (Fig. 6). In addition, two *Streptomyces* OTUs (OTU.113924 and OTU.599) are among the 10 most $^{13}CO_2$-labeled OTUs among the treatments (Supplemental Fig. S8). The relative abundances of *Streptomyces* (phylum *Actinobacteria*) are 15.9% and 12.0% in the rhizosphere of the CK and biochar treatments, respectively, which were significantly higher ($P < 0.05$) than that of the RSD treatment (Supplemental Fig. S7). In contrast, DNA-SIP reveals that the $^{13}CO_2$-labeled OTUs in the RSD treatment were mainly those of the phylum *Proteobacteria* (e.g., *Anaeromyxobacter*, *Bosea*, *Dechloromonas*, and *Geobacter*) (Fig. 6).

Discussion

Our results showed that the RSD treatment harbored a specific bacterial community either at pre-planting or after harvesting of the tomato plants. This phenomenon is likely due to the RSD treatment strongly influencing on soil pH, total C, total N, and the C/N ratio due to the flood incubation and amendment with maize straw. These changes likely shifted the soil bacterial community composition in the tomato rhizosphere and selected a distinct enriched bacterial community

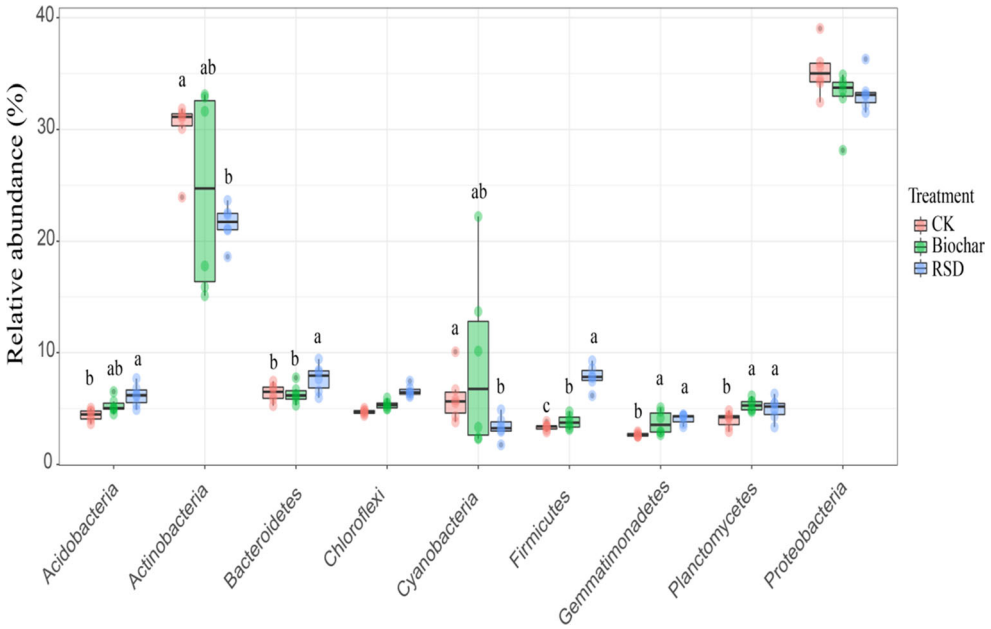

Fig. 3 Soil microbial community composition in the tomato rhizosphere at the phylum level

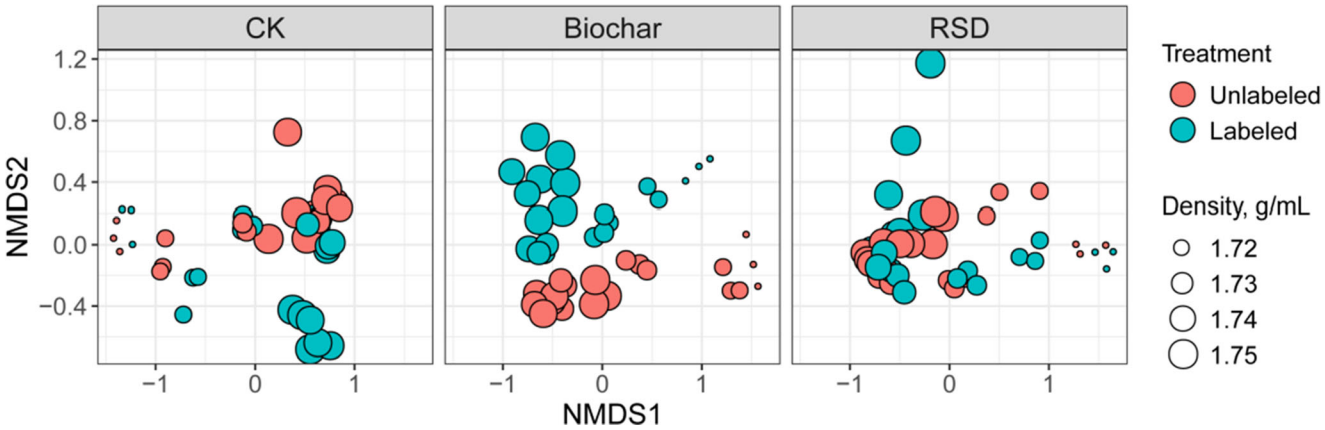

Fig. 4 Ordination of rhizosphere bacterial communities using the heavy (> 1.71 g ml^{-1}) fractions of different soil amendments and Bray-Curtis similarities based on their OTU contents. The size of a point indicates its fraction density, while the distance between points represents the similarity in bacterial community composition

compared with the control and biochar treatments. Specifically, *Clostridium* and *Coprococcus* (phylum *Firmicutes*) were the two major genera contributing to shifts in the rhizosphere microbial community composition. The *Clostridium* genus is known to be involved in the anaerobic degradation of rice straw (Huang et al. 2016). Weber et al. (2001) reported that the abundance of *Clostridium* spp. substantially increased in rice straw, colonizing and decomposing the straw within the first 2 weeks after flooding. Members of the genus *Coprococcus* can produce organic acids (Holdeman and Moore 1974), such as propionic, butyric, and acetic acids, which can decrease the abundance of pathogens (Huang et al. 2015).

We observed that the biochar application gave very similar bacterial communities to the control. *Streptomyces* accounted for about 10% of the sequences in the biochar and control samples. Members of the genus *Streptomyces* are ubiquitous in soils, where they play a significant role in global soil C cycling (Schrempf 2001). *Streptomyces* is the most abundant genus (> 600 species) producing antibiotics (Labeda et al. 2012). In addition, they have the capacity to produce a wide range of volatile organic compounds and increase soil nutrient levels, which directly and indirectly stimulate plant growth (Citron et al. 2015; Cordovez et al. 2015). Therefore, *Streptomyces* is recognized as a plant growth-promoting bacterial genus (Dias et al. 2017) and has been proven to stimulate

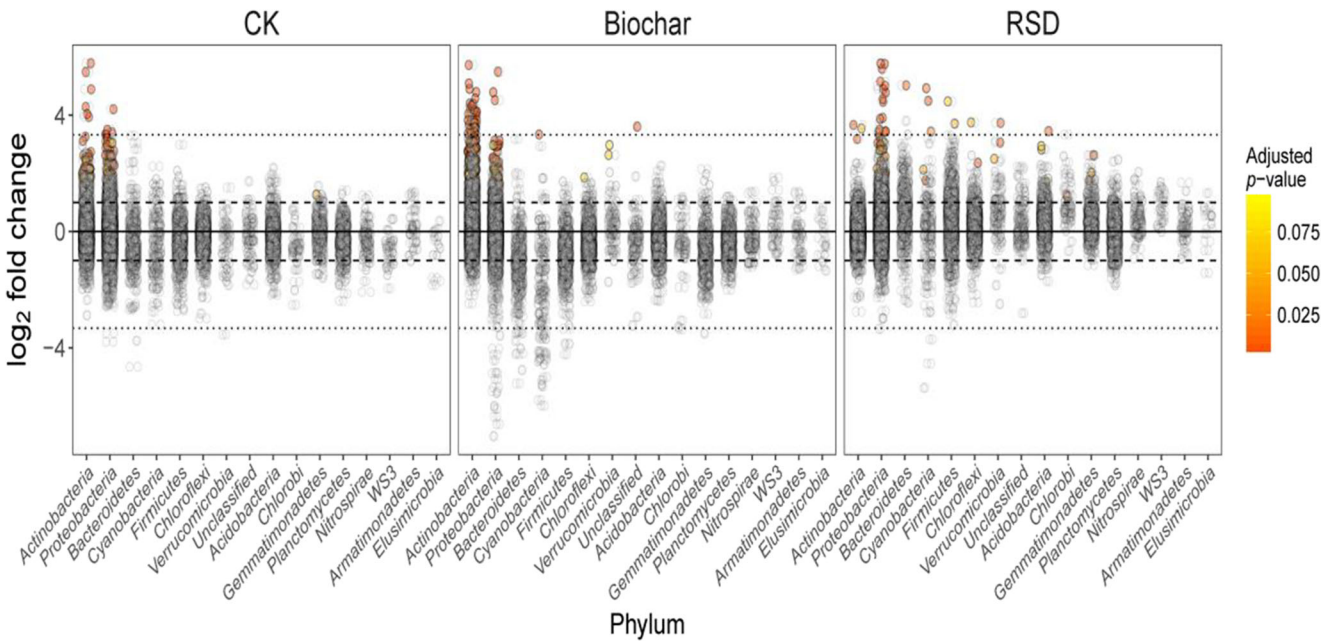

Fig. 5 Log_2-fold changes in the relative abundances of OTUs (^{13}C-labeled vs. ^{12}C-labeled) in the heavy (> 1.71 g ml^{-1}) fractions for three soil amendments. All the OTUs passed a 0.35 sparsity threshold for the heavy fractions. Each circle represents a single OTU (at 97% similarity level). The dashed and dotted lines denote the 2- and 10-fold changes, respectively (increases and decreases). Colored circles denote percentage fold-changes that had an adjusted *P* value below a false discovery rate of 10%

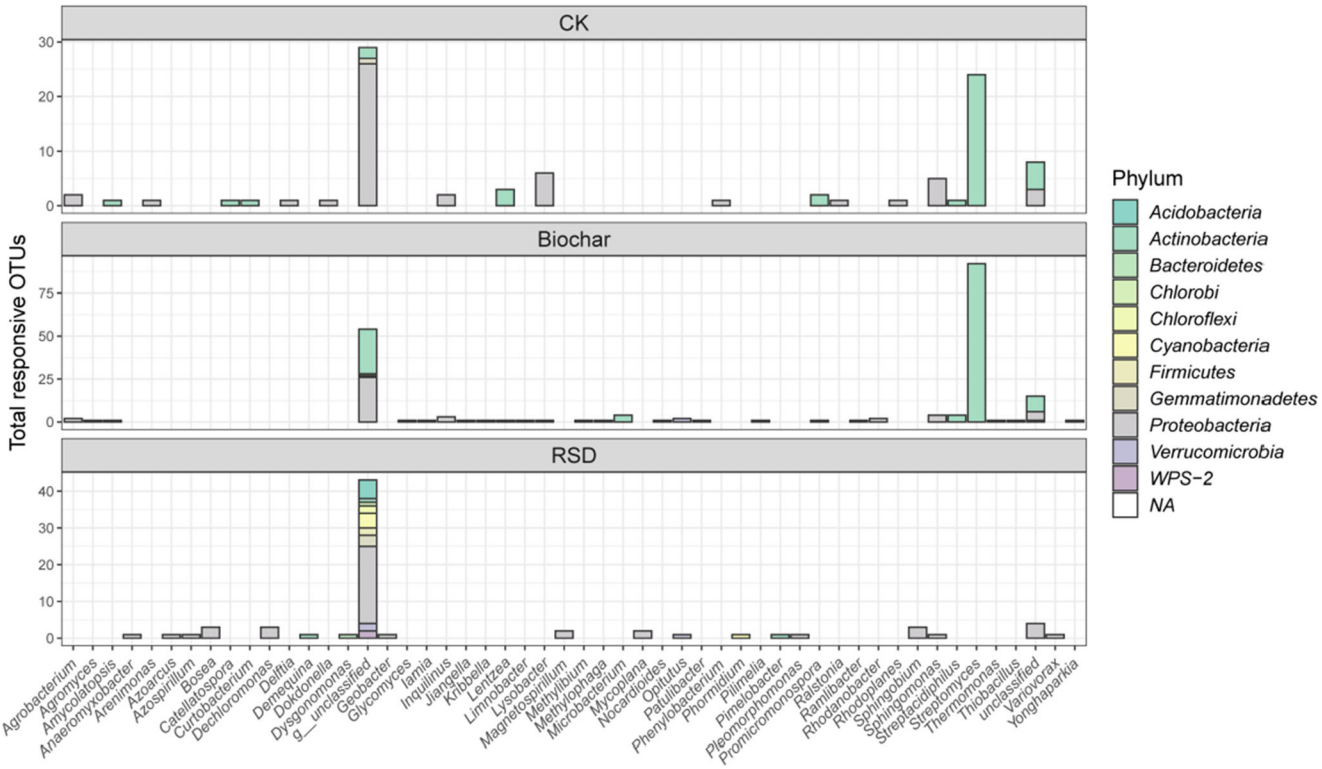

Fig. 6 OTUs that were identified as utilizing plant-derived C in the tomato rhizosphere at the genus level. (**a**) CK; (**b**) biochar; and (**c**) RSD

tomato growth (El-Tarabily 2008). Interestingly, we observed that the biochar treatment had the highest tomato biomass, while bacterial community composition was still similar to the control. The higher tomato biomass might be attributed to higher soil nutrients released by the biochar into the rhizosphere in comparison to the RSD and control treatments during the 49-day tomato growth (Enders et al. 2012). In general, our results suggest that the RSD treatment harbors distinct bacterial communities in the rhizosphere compared with the biochar treatment.

The $^{13}CO_2$-labeled plant-derived C released by tomato can participate in a series of microbial processes (Hernandez et al. 2015; Ge et al. 2019). A relatively short sampling time should be considered to avoid secondary metabolism of labeled C source between soil microbes. However, a short sampling time point that ensures high enough ^{13}C label in the soil is hard to obtain in practice during DNA-SIP studies (Yao et al. 2015; Liao et al. 2019). Despite the limitations, DNA-SIP coupled with continuous labeling is still the best technique for detecting those microbial communities that actively utilize root exudates (Haichar et al. 2008). DNA-SIP results revealed that different numbers of OTUs were detected under the three treatments. Only a low percentage (< 3%) of total OTUs was labeled, thus being involved in the use of root exudates and confirming previous findings (Ai et al. 2015; Gschwendtner et al. 2016).

Similar to what was observed for the total rhizosphere bacterial community, phylogenetic affiliation revealed that the RSD treatment had a distinct bacterial community utilizing plant-derived C compared with the biochar and control treatments. Studies have documented that soil type (Lian et al. 2017), plant host (Haichar et al. 2008), and N fertilization (Gschwendtner et al. 2016) have considerable effects on soil microbial utilization of root exudates. The biochar treatment had the highest numbers of ^{13}C-labeled OTUs among treatments. *Streptomyces* (phylum *Actinobacteria*) was the dominant genus consuming the ^{13}C root exudate in both control and biochar treatments. Biochar may have facilitated microbial activity due to root exudate absorption (Gu et al. 2017) and to its porous properties (Pietikäinen et al. 2000). In contrast, the phylum *Proteobacteria* (e.g., *Anaeromyxobacter*, *Bosea*, *Dechloromonas*, and *Geobacter*) was the main labeled bacterial taxa in the rhizosphere of the RSD treatment, favored by the incorporation of maize straw into the soil and the legacy of flood incubation (Huang et al. 2015). Generally, compared with the control and biochar treatments, the microbial communities of the RSD-treated soil had diverse abilities to decompose organic materials (Huang et al. 2019). More importantly, a relatively high diversity of various bacterial phyla (e.g., *Proteobacteria*, *Cyanobacteria*, *Verrucomicrobia*, and *Acidobacteria*) using plant-derived C in the rhizosphere soil might have altered more diversity and functions in the RSD treatment than in the biochar and the control treatments.

In general, the RSD had a stronger influence on rhizosphere bacterial communities and their utilization of root exudates relative to the biochar amendment. Biochar did not

alter the composition of the bacterial communities but had more labeled bacterial taxa utilizing plant-derived C in tomato rhizosphere soil. Our results provide experimental evidence that bacterial utilization of root exudates is strongly altered by soil management regimes. Further studies should be conducted to investigate the interactions among root exudate compounds on rhizosphere microbial community activity and function by using RNA-SIP or metagenomic techniques.

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Authors' contributions HL and HY conceived and designed the research. HL and YL conducted the experiments. HF contributed new reagents or analytical tools. HL and HY wrote the manuscript. All authors read and approved the manuscript.

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Data availability All datasets generated for this study are included in the article/Supplementary Material.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

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