Changes in soil physicochemical properties and bacterial communities among different soil depths after long-term straw mulching under a no-till system

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Abstract

Conservation tillage has attracted increasing attention over recent decades, mainly due to its benefits in improving soil organic matter content and reducing soil erosion. Under intensive conventional tillage systems, some studies have focused on the responses of soil properties in the topsoil to straw retention. However, long-term straw mulching effects on soil physicochemical properties and bacterial communities among different soil depths under a no-till system are still obscure. One twelve-year experiment was conducted that included straw removal (CK) and straw mulching (SM) treatments. Soil samples were collected at 0–5, 5–10, 10–20, and 20–30 cm soil depths. Most soil physicochemical properties and the relative abundances of bacterial phyla were varied with soil depth. Compared with CK, SM increased soil total nitrogen and organic carbon, available phosphorus and potassium, dissolved organic carbon and nitrogen, and water content. SM increased soil bacterial abundance but reduced the Shannon diversity of the bacterial community at 0–5 cm depth. SM increased the relative abundances of Proteobacteria, Bacteroidetes, and Acidobacteria but reduced those of Actinobacteria, Chloroflexi, and Cyanobacteria. SM had different effects on the relative abundances of some C- and N-cycling genera, for instance, increasing Rhodanobacter, Rhizomicrobium, and Terracidiphilus, and reducing Anaeromyxobacter, Mycobacterium, and Syntrophobacter. A principal coordinate analysis indicated that SM largely affected soil bacterial communities at topsoil depth. Soil pH and different nitrogen and organic carbon fractions were the major drivers shaping soil bacterial community. Overall, straw mulch is highly recommended for use under a no-till system because of its benefits to soil fertility and bacterial abundance. However, inorganic nitrogen fertilizer levels may be reduced under straw mulching to maintain or increase soil bacterial Shannon diversity in future studies.

Keywords: bacterial community composition, conservation tillage, Illumina sequencing, physicochemical properties, soil depth, straw mulching
1 Introduction

Greater quantities of food are needed to feed a growing population in the future, and producing them will largely depend on agriculture production (Karthikeyan et al., 2020). Currently, conventional agriculture is characterized by the intensification of farming by fertilizer and pesticide application, the use of high-yielding varieties of crops, heavy tillage, and damaging crop residue management (Postma-Blaauw et al., 2010), which puts unprecedented stress on soils and results in their unsustainable degradation (Kopittke et al., 2019). Loss of organic matter, erosion, contamination, acidification, salinization, and loss of genetic diversity are several typical aspects of soil degradation (Hou et al., 2020; Lupwayi et al., 2012), and they reduce soil quality, crop productivity and agricultural sustainability (Lal, 2016; Zhao et al., 2017). Compared to conventional agriculture, conservation agriculture practices centered on conservation tillage have been widely adopted in recent decades because they increase soil organic matter content, improve soil structure, reduce soil erosion, and decrease the need for farm labor (Jena, 2019; Navarro-Noya et al., 2013; Singh et al., 2020). In 2013, the global conservation tillage area was approximately 155 Mha, corresponding to approximately 11% of crop land worldwide (Kassam et al., 2014). According to the statistical data from China (https://data.cnki.net/trade/Yearbook/Single/N2019090050?z=Z032), the area of mechanized zero-tillage was increased by 38.57% from 10.19 Mha at 2009 to 14.12 Mha at 2017. The conservation tillage area will increase in the future because of the farm labor shortages in some countries, such as China (Zhang et al., 2014).

Minimal soil disturbance (no or reduced tillage) and soil cover (mainly straw mulch) are two key principles highly recommended in conservation tillage (Pittelkow et al., 2014). However, straw mulching was not always combined with no-till in many countries (Jin, 2007; Pittelkow et al., 2014). For instance, in some African countries, mulch is in short supply due to poor productivity and the prioritization of livestock feeding (Giller et al., 2009). Straw is burned to promote nutrient mineralization in the tropics and Europe (Hemwong et al., 2008). In some East Asian countries, mulch application was restricted by insufficient time before subsequent crop growth and some adverse effects of straw mulch on the next crop (Zhao et al., 2018). Straw mulching demonstrated varying effects on crop yields, depending on the mulching practices, climate, and soil conditions, which has been discussed in our previous study (Zhou et
Besides its effects on crop yield, understanding soil physicochemical properties and bacterial community changes is also an important aspect of assessing the environmental effects of straw mulching.

Soil physicochemical properties are important contributors to soil fertility, and the latter is a critical factor determining crop productivity and agriculture sustainability (Liu et al., 2019). Since straw contains large amounts of carbon and several mineral elements, previous studies have shown that straw mulching increased soil total organic carbon and its fractions, several soil enzymes, and other physicochemical properties in the soil surface layer (Akhtar et al., 2018; Duval et al., 2016; Zhou et al., 2019b). Many studies have focused on these physicochemical properties in the topsoil under conventional tillage system since the topsoil provides large amounts of nutrients to plants (Dai et al., 2019; Wang et al., 2019b; Zhou et al., 2019a). However, soil physicochemical properties in the subsurface should also be considered since some nutrients could move from topsoil to deeper soil during irrigation and rainfall (Blanco-Canqui and Lal, 2007; Stowe et al., 2010). The responses of soil physicochemical properties to soil depth varied across different regions (Li et al., 2017b; Peng and Wang, 2016). Li et al. (2017b) found that soil total organic carbon (TOC), total nitrogen (TN), dissolved organic carbon (DOC), pH, and water content (WC) differed significantly among six depths, while NH₄⁺-N concentration and the C/N ratio did not change significantly with soil depth. Similarly, TOC, TN and total phosphorus (TP) decreased significantly with soil depth, but pH did not change consistently across the three steppes in Peng and Wang (2016). These studies focused only on heavy tillage or grassland, but the variation in physicochemical properties among different soil depths after long-term straw mulching under a no-till system is still unclear, since the no-till practice did little disturbance to soil, and it was quite different from the heavy tillage in conventional agriculture.

Changes in nutrition distribution along soil depth would affect not only soil fertility but also soil bacterial communities. Previous reports have suggested that soil bacterial communities are highly associated with soil environmental factors (Bowles et al., 2014; Li et al., 2017a; Schreiter et al., 2014; Sun et al., 2016). Recently, soil bacterial communities have attracted great interest, and they are often used as sensitive indicators of soil quality, especially in agricultural systems (Ashworth et al., 2017). They participate in soil ecological processes and play a vital role in soil carbon and nutrient cycling (Hobara et al., 2014; Thompson et al., 2017), crop growth, and greenhouse gas
release (Tellez-Rio et al., 2015). The responses of soil bacterial abundance to straw mulching were inconsistent in different studies in the topsoil. Zhang et al. (2017) found that general, gram-positive, and gram-negative bacteria increased under straw mulching in one paddy soil in northeast China. Chen et al. (2017) proposed that straw return significantly increased bacterial biomass in one region but had no significant effects in the other two regions. Straw mulching can also change bacterial community composition (Bu et al., 2020; Qiu et al., 2020). Actinobacteria were enriched in straw mulch (SM) soils, and pH and soil WC are key factors driving soil bacterial community structure changes in the Loess Plateau of China (Qiu et al., 2020). Bu et al. (2020) reported that straw return significantly increased the relative abundance of Proteobacteria and decreased the relative abundance of Acidobacteria, and the positive effect of straw mulching on the soil bacterial community structure probably resulted from the increased soil organic carbon fractions. However, soil microbial communities, including bacterial communities, varied with soil depth (Fierer et al., 2003; van Leeuwen et al., 2017), and soil microbes in subsoil demonstrated important effects on soil formation, ecosystem biochemistry and maintaining groundwater quality (Li et al., 2014). For instance, the abundances of gram-positive bacteria increased with depth, while the abundances of gram-negative bacteria generally declined with soil depth (Fierer et al., 2003). Bacterial biomass significantly decreased with soil depth in forest and grassland but tended to only decrease in arable land as assessed using the phospholipid method (van Leeuwen et al., 2017). Apparently, straw management and soil depth are two key factors influencing the soil bacterial community. Unfortunately, no detailed information, especially by using high-throughput sequencing analysis, about soil bacterial community changes in response to straw mulching among different soil depths under no-till systems has been obtained. Moreover, little is known about the relationship between these communities and the soil physicochemical properties in deeper soils after long-term straw mulching.

Rice-wheat rotation is a major cropping system in China, and approximately 80 million tons of crop straw are produced annually in southwestern China (Li et al., 2016; Zhou et al., 2019b). Although we determined some soil organic carbon fractions under a no tillage regime in this system (Zhou et al., 2019b), little is known about how other soil physicochemical parameters vary with soil depth. In addition, how soil bacteria responded to long-term straw mulching and which soil physicochemical factors had the greatest effect on shaping bacterial communities among different depths remain poorly
understood. In this study, we hypothesized that (1) compared with straw removal, straw mulching will increase most soil physicochemical parameters, which will decline with increasing soil depth; (2) straw mulching and depth will have significant effects on the soil bacterial community; and (3) the key soil physicochemical properties shaping bacterial communities will be different at different depths. To answer these questions, soil samples were collected from four soil depths, which had been subjected to two straw management programs under a 12-year no-till regime in southwestern China. Then, soil physicochemical properties, bacterial abundances (based on quantitative PCR, qPCR), and bacterial community compositions (using Illumina high-throughput sequencing) were determined.

2 Materials and methods

2.1 Experimental site and design

A long-term field experiment was installed in 2005 in Guanghan, Sichuan Province, China (31°08′38″ N, 104°29′45″ E). Before the experiment, the local agricultural soil was seldom tilled due to the shortage of tillage machines. The soil had been cultivated for a long period of time under the same agricultural cropping system, and consequently the soil fertility heterogeneity was considered minimal. The soil is a fluvo-aquic soil with loamy clay. The soil pH in 2005 was 5.54, and the TOC, TN, available nitrogen, AP, and available potassium (AK) levels were 18.1 g kg⁻¹, 2.03 g kg⁻¹, 189.76 mg kg⁻¹, 12.61 mg kg⁻¹, and 258.2 mg kg⁻¹, respectively.

No-till practice was conducted in both the rice and wheat seasons. Two treatments, i.e., a control (CK, straw removal) and SM, with three replications under a no-till regime were selected in this study. The straw was removed in the CK treatment, whereas it was distributed over the soil surface without being chopped in the SM treatment. The mulch consisted of approximately 8.5 t ha⁻¹ rice straw and 6.0 t ha⁻¹ wheat straw during each year. The amounts of inorganic fertilizer added were equal in both treatments, and they were manually broadcast over the plot soil surface without tillage. Other detailed information about the experimental design was in our previous study (Zhou et al., 2019b).

2.2 Soil sampling

Immediately after the wheat harvest in 2018, soil samples were collected at five points in each plot. The samples were taken at soil depths of 0–5, 5–10, 10–20, and 20–30 cm.
Five subcores taken from the same depth were pooled to make one composite sample for each plot. The mixed soil was passed through a 2-mm mesh and divided into three parts: one was air-dried and used to measure some of the soil physicochemical parameters; one was kept at 4 °C for soil NH$_4^+$–N, NO$_3^-$–N, DOC, and DON analysis; and the third was stored at −80 °C until it was needed for soil bacterial community analysis.

### 2.3 Soil physicochemical properties

Ten grams of fresh soil was extracted with 50 mL of 2 M KCl. The NH$_4^+$–N and NO$_3^-$–N concentrations in the extracts were determined using a SAN++ Continuous Flow Analyzer (Skalar, Breda, The Netherlands) (Lu, 2000). Inorganic nitrogen (IN) was the sum of the NH$_4^+$–N and NO$_3^-$–N concentrations. The DOC and DON were extracted with 0.5 M K$_2$SO$_4$. Then, a TOC analyzer (Multi N/C 2100; Analytic, Jena, Germany) was used to determine their concentrations in centrifuged supernatant that had been filtered through sterile 0.45 μm syringe filters (Zhou et al., 2019b). The soil WC was measured using the oven-drying method (Akhtar et al., 2018). The air-dried soil samples were analyzed for soil pH, TOC, TN, TP, TK, AP, and AK as described by Lu (2000).

### 2.4 DNA extraction and qPCR amplification

The soil DNA from 0.5 g of fresh soil was extracted using the Fast® DNA SPIN Kit (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer’s instructions (Zhou et al., 2017). The extracted DNA was dissolved in 50 μL of double-distilled water, and its quality and concentration were checked by a NanoDrop 2000 spectrophotometer (Calleja-Cervantes et al., 2015). Then, the DNA samples were stored at −80°C until further use. The qPCR was used to quantify bacterial abundances based on the 16S rRNA gene, and the primers were 338F (5′-ACTCCT ACGGGAGGCAGCAG-3′) and 518R (5′-ATTACCGCGGCTGCTGG-3′) (Fierer et al., 2005). The qPCR procedure was carried out according to Chen et al. (2019) with some modifications. PCR was performed using a Bio-Rad CFX 96-well Thermocycler (Bio-Rad, Hercules, CA, America). The reactions were performed in a 20 μL mixture containing 16.5 μL of 2 × SYBR Color qPCR Master Mix, 0.5 μM (0.8 μL) each primer, and 2 μL of DNA template. The PCR conditions were as follows: 95 °C for 5 min; 40 cycles of 30 at 95 °C, 30 s at 58 °C and 40 s at 72 °C; and finally 10 min at 72 °C. All samples were evaluated in triplicate. Standard curves were obtained using 10-fold serial dilutions of
linearized recombinant plasmids containing cloned 16S rDNA with known copy numbers. Melting curve analysis was performed at the end of each qPCR run to check the specificity of PCR products. PCR amplification efficiencies were between 96% and 105%, with \( R^2 \) values > 0.99.

### 2.5 16S rRNA amplification for Illumina sequencing and data processing

The primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) were used to amplify the V4–V5 regions of the bacterial DNA (Caporaso et al., 2012). Detailed operational information can be found in Zhang et al. (2019). The 16S rRNA sequences were analyzed on the I–Sanger Cloud Platform (https://cloud.majorbio.com/). Raw sequences were merged using FLASH version 1.2.7 (Magoc and Salzberg, 2011) and then processed using quantitative insights into microbial ecology (QIIME v.1.9.0; http://www.qiime.org/) (Quast et al., 2013). Poor-quality sequences (below an average quality score of 25) and short sequences (< 200 bp) were removed. Primers were matched exactly, allowing 2 mismatch nucleotides, and reads with ambiguous bases were removed. Sequences with overlaps longer than 10 bp were merged according to their overlap sequence. After this step, 945,665 clean reads were obtained, with 30,241 to 58,191 reads per sample.

Operational taxonomic units (OTUs) were clustered at a similarity threshold of 97% by the ribosomal database project (RDP) classifier with the Bayesian algorithm. The number of sequences per soil sample was rarefied to an equal abundance as the sample with the lowest number of sequences (Menéndez-Serra et al., 2019; Ye et al., 2017), and 4101 OTUs were identified across all samples. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier against the SILVA database version 132 using a confidence threshold of 70% (Quast et al., 2013). Good's coverage was used to investigate the sequence coverage of the bacterial communities. The \( \alpha \)-diversity parameters, including the Shannon index, Shannon’s evenness, and Chao1, were estimated with the mothur program (http://www.mothur.org). The Shannon index and Shannon’s evenness were used to investigate soil bacterial community diversity and evenness, respectively. Chao1 was used to describe soil bacterial community richness.

Principal coordinate analysis (PCoA) was then used to demonstrate patterns of similarity in bacterial community structures between CK and SM treatments based on weighted UniFrac distances. Environmental factors were selected using Monte Carlo permutations (calculated based on 999), and environmental factors with a \( P > 0.05 \) were
removed from a redundancy analysis (RDA) (Fan and Xing, 2016). Analysis of similarity (Adonis) analysis was performed with the vegan package of the R project (http://www.r-project.org). The Monte Carlo Mantel test and RDA were performed by Canoco 5.0 (CANOCO, Microcomputer Power Inc., Ithaca, NY, USA) to identify the soil environmental factors that were significantly correlated with soil bacterial communities.

2.6 Statistical analysis

Prior to analysis, the data were tested for homogeneity of variance using Levene’s test. Two-way analysis of variance (ANOVA) was used to determine the main effects of depth, straw management and their interactions on soil physicochemical parameters, soil bacterial abundance, soil bacterial α-diversity indices, and soil phylum relative abundances. If the depth and straw management interactions were significantly different, a one-way ANOVA was used to analyze the differences between the four depths in the CK and SM plots, and Welch’s t-test within STAMP (Parks et al., 2014) was used to identify genera with significant differences in relative abundance between CK and SM plots at each depth. The differences in soil genus relative abundances at each depth among the straw management treatments were tested by an independent-samples t-test. Pearson’s correlation analysis was used to show the connections between bacterial communities and soil physicochemical parameters. These statistical analyses were performed by SPSS 19.0 (SPSS, Inc., Chicago, IL, USA). RDA plots were prepared using Canoco 5.0 (CANOCO, Microcomputer Power Inc., Ithaca, NY, USA). PCoA plots were drawn on the I–Sanger Cloud Platform (https://cloud.majorbio.com/), and other graphs were prepared using SigmaPlot ver. 12.5 (Systat, Software, Inc., San Jose, California, USA).

3 Results

3.1 Straw mulch effects on soil physicochemical properties

As shown in Table 1, the ANOVA suggested that soil pH significantly increased with soil depth, whereas soil TOC, TN, TP, IN, AP, AK, DOC, DON, and WC significantly decreased with soil depth (P < 0.05). Soil TK did not vary among the soil depths. Straw mulching increased TOC by 22.09 %, TN by 13.48 %, IN by 10.32 %, AP by 9.02 %, AK by 7.17 %, DOC by 69.98 %, DON by 41.98 %, and WC by 5.13 % compared to their CK values, while pH, TP, and TK did not change between SM and CK treatments.
The interactions between straw management and soil depth were significant for TOC, IN, AK, DOC, and DON. The independent-samples t-test demonstrated that TOC at 0–10 cm, IN at 0–5 cm, AK at 0–10 cm, and DON at 0–5 and 10–20 cm was significantly higher under SM than for the CK treatment.

Table 1. Soil physicochemical properties at different soil depths under the SM and CK treatments. Means of three replicates per treatment are shown. CK, straw was removed from the plot; SM, straw was mulched into the plot soil. TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; IN, inorganic nitrogen; AP, available phosphorus; AK, available potassium; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; WC, water content. Different lowercase letters within a column indicate significant differences between the four soil depths; different capital letters within a column indicate significant differences between the two straw management treatments across the four soil depths; and * indicates differences between the two straw management treatments at the same soil depth at \( P = 0.05 \) level.

<table>
<thead>
<tr>
<th>Variable</th>
<th>0–5 cm</th>
<th>5–10 cm</th>
<th>10–20 cm</th>
<th>20–30 cm</th>
<th>CK</th>
<th>SM</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Depth (d)</td>
<td>Straw (s)</td>
<td>d × s</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>pH</td>
<td>5.09d</td>
<td>5.90c</td>
<td>6.56b</td>
<td>7.17a</td>
<td>6.26A</td>
<td>6.09A</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td>TOC (g kg(^{-1}))</td>
<td>CK</td>
<td>23.0a</td>
<td>19.4b</td>
<td>14.2c</td>
<td>6.90d</td>
<td></td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td></td>
<td>SM</td>
<td>33.2a*</td>
<td>22.3b*</td>
<td>15.8c</td>
<td>7.14d</td>
<td></td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td>TN (g kg(^{-1}))</td>
<td></td>
<td>3.17a</td>
<td>2.26b</td>
<td>1.54c</td>
<td>0.64d</td>
<td>1.78B</td>
<td>2.02A</td>
</tr>
<tr>
<td>TP (g kg(^{-1}))</td>
<td>0.87a</td>
<td>0.70b</td>
<td>0.48c</td>
<td>0.21d</td>
<td>0.55A</td>
<td>0.58A</td>
<td>( p &lt; 0.001 )</td>
</tr>
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<td>TK (g kg(^{-1}))</td>
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<td>12.5a</td>
<td>12.3a</td>
<td>11.8a</td>
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<td>12.5A</td>
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<td>CK</td>
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<td>18.3ab</td>
<td>14.2bc</td>
<td>11.3c</td>
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<td></td>
<td>SM</td>
<td>29.1a*</td>
<td>16.6b</td>
<td>14.5bc</td>
<td>11.9c</td>
<td></td>
<td></td>
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<tr>
<td>AP (mg kg(^{-1}))</td>
<td>107.2a</td>
<td>46.5b</td>
<td>15.9c</td>
<td>2.01d</td>
<td>37.7B</td>
<td>48.1A</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td>AK (mg kg(^{-1}))</td>
<td></td>
<td>CK</td>
<td>152a</td>
<td>108b</td>
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<tr>
<td></td>
<td>SM</td>
<td>183a*</td>
<td>116b</td>
<td>100b</td>
<td>101b</td>
<td></td>
<td></td>
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<tr>
<td>DOC (mg kg(^{-1}))</td>
<td>41.4a</td>
<td>35.1a</td>
<td>20.6b</td>
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<td>36.3c*</td>
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<td>17.3a</td>
<td>12.3a</td>
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<td>18.1b</td>
<td>18.4b*</td>
<td>5.98c</td>
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<td></td>
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<tr>
<td>WC (%)</td>
<td>18.0a</td>
<td>17.1a</td>
<td>15.7b</td>
<td>13.2c</td>
<td>15.6B</td>
<td>16.4A</td>
<td>( p &lt; 0.001 )</td>
</tr>
</tbody>
</table>

3.2 Straw mulch effects on bacterial abundance

Straw management, soil depth and their combined effects significantly affected soil bacterial abundance in terms of the 16S rRNA gene copy number (Fig. 1). Soil bacterial abundance significantly declined as the soil depth increased for the two treatments (\( P < 0.001 \)), and the SM bacterial abundance was 52.69% higher than CK (\( P < 0.05 \)). An independent-samples t-test demonstrated that compared with the CK treatment, the SM treatment significantly increased bacterial abundance in the 0–5 cm soil layer (\( P < 0.05 \)), but there was no significant difference in other three layers between the two treatments.
Figure 1. Straw mulching effects on soil bacterial abundance assessed using qPCR. Data are the means and standard deviations of three repeats. CK, no-till with straw removal; SM, no-till with straw mulching. Different lowercase letters indicate significant differences between the four soil depths at the $P = 0.05$ level. * indicates differences between the CK and SM treatments for the same soil depth at the $P = 0.05$ level.

Table 2. Soil bacterial $\alpha$-diversity at different soil depths under the SM and CK treatments. Means of three replicates per treatment are shown. CK, straw was removed from the plot; SM, straw was mulched into the plot soil. Different lowercase letters within a column indicate significant differences between the four soil depths; different capital letters within a column indicate significant differences between the two straw management treatments across the four soil depths; and * indicates differences between the two straw management treatments at the same soil depth at $P = 0.05$. ns represents no statistical significance at the $P = 0.05$ level.

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Shannon’s evenness</th>
<th>Chao1</th>
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<tbody>
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<td></td>
<td>CK</td>
<td>SM</td>
<td></td>
</tr>
<tr>
<td>0–5 cm</td>
<td>6.53a</td>
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<td>0.858a</td>
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<td>6.42a</td>
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<td>10–20 cm</td>
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<td>20–30 cm</td>
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<tr>
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<td>$p &lt; 0.001$</td>
<td>$p &lt; 0.05$</td>
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<td>Straw (s)</td>
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<td>ns</td>
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<tr>
<td>d * s</td>
<td>$p &lt; 0.05$</td>
<td>ns</td>
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</tr>
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</table>

3.3 Straw mulch effects on bacterial $\alpha$-diversity

The Good’s coverage value for all samples was more than 96% in our study, which indicated that the number of sequence reads adequately represented the bacteria. Table 2 shows that the three $\alpha$-diversity indices (Shannon, Shannon’s evenness, and Chao1)
significantly decreased with soil depth under CK and SM treatments, and the soil
sampled at 0–5 cm had the highest values for Shannon and Shannon’s evenness, except
for the case that the Shannon diversity did not change under SM treatment. The lowest
value for Chao1 was observed at the 0–5 cm soil depth among the four soil depths.
Compared to the CK treatment, straw mulching did not change Shannon’s evenness and
Chao1 indices, but it decreased the Shannon index at 0–5 cm depth.

3.4 Straw mulch effects on bacterial community composition

The phyla whose relative abundances accounted for less 1% in all soil samples were
merged into the “Others” category. As a result, 14 phyla were observed in the study.
From highest to lowest in relative abundance, these were Proteobacteria, Acidobacteria,
Chloroflexi, Actinobacteria, Planctomycetes, Nitrospirae, Others, Gemmatimonadetes,
Unclassified, Firmicutes, Bacteroidetes, Latiscibacteria, Verrucomicrobia, and
Cyanobacteria (Fig. S1). A two-way ANOVA (Table 3) demonstrated that compared to
the CK treatment, straw mulching significantly increased the relative abundances of
Proteobacteria, Acidobacteria, Bacteroidetes and Latiscibacteria but significantly
decreased Actinobacteria and Chloroflexi ($P < 0.05$). There was no significant
difference in the relative abundances of Planctomycetes, Nitrospirae, Firmicutes,
Gemmatimonadetes, and Verrucomicrobia between the two treatments. The relative
abundances of Proteobacteria, Actinobacteria, Bacteroidetes, and Cyanobacteria
decreased, but those of Chloroflexi, Nitrospirae, and Latiscibacteria increased as soil
depth increased ($P < 0.05$) for the two treatments. The relative abundance of
Acidobacteria increased from 0–5 to 10–20 cm depth and then decreased at 20–30 cm
depth. The relative abundance of Planctomycetes did not change among the 0–5, 5–10,
and 10–20 cm depths but then significantly decreased at the 20–30 cm depth. The
relative abundance of Gemmatimonadetes first increased and then decreased with soil
depth, and its highest value was at 5–10 cm. The relative abundances of Firmicutes and
Verrucomicrobia did not change with soil depth. The combined effects of straw
management and depth were significant for the phyla Proteobacteria and Cyanobacteria.
Straw mulching led to a higher Proteobacteria relative abundance at the 0–5 cm depth
but lower values for Cyanobacteria at the 0–5 and 20–30 cm depths compared to their
CK values.
Table 3. Relative abundances of the 14 most abundant bacterial phyla at different soil depths under the two straw management treatments. Prot, Proteobacteria; Acid, Acidobacteria; Acti, Actinobacteria; Chlo, Chloroflexi; Plan, Planctomycetes; Nitr, Nitrospirae; Bact, Bacteroidetes; Firm, Firmicutes; Gemm, Gemmatimonadetes; Cyan, Cyanobacteria; Uncl, Unclassified; Verr, Verrucomicrobia; Late, Latescibacteria; Othe, Others. CK, straw was removed from the plot; SM, straw was mulched into the plot soil. Different lowercase letters within a column indicate significant differences between the two straw management treatments across the four soil depths; and * indicates differences between the two straw managements at the same soil depth at P = 0.05. ns represents no statistical significance at the P = 0.05 level.

<table>
<thead>
<tr>
<th>Variable</th>
<th>0–5 cm</th>
<th>5–10 cm</th>
<th>10–20 cm</th>
<th>20–30 cm</th>
<th>CK</th>
<th>SM</th>
<th>ANOVA Depth (d)</th>
<th>Straw (s)</th>
<th>d × s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prot</td>
<td>32.11a</td>
<td>29.51ab</td>
<td>29.08ab</td>
<td>26.69b</td>
<td>–</td>
<td>–</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.05</td>
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<tr>
<td>SM</td>
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<td>31.31b</td>
<td>30.93b</td>
<td>28.06c</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid</td>
<td>19.20b</td>
<td>19.86ab</td>
<td>21.33a</td>
<td>15.38c</td>
<td>17.80B</td>
<td>20.09A</td>
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<td>p &lt; 0.001</td>
<td>ns</td>
</tr>
<tr>
<td>Acti</td>
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<td>11.94b</td>
<td>10.49b</td>
<td>10.01b</td>
<td>13.02A</td>
<td>10.64B</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
<td>ns</td>
</tr>
<tr>
<td>Chlo</td>
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<td>12.67b</td>
<td>13.10b</td>
<td>19.28a</td>
<td>15.56A</td>
<td>12.93B</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.01</td>
<td>ns</td>
</tr>
<tr>
<td>Plan</td>
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<td>3.72a</td>
<td>4.20a</td>
<td>2.75b</td>
<td>3.68A</td>
<td>3.72A</td>
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<td>ns</td>
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<tr>
<td>Nitr</td>
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<td>10.33b</td>
<td>9.45b</td>
<td>12.74a</td>
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<td>ns</td>
</tr>
<tr>
<td>Bact</td>
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<td>1.52b</td>
<td>1.15c</td>
<td>0.70d</td>
<td>1.12B</td>
<td>1.60A</td>
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<tr>
<td>Firm</td>
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<td>1.47a</td>
<td>1.76a</td>
<td>1.26a</td>
<td>1.57A</td>
<td>1.25A</td>
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<td>ns</td>
<td>ns</td>
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<tr>
<td>Gemm</td>
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<td>ns</td>
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<tr>
<td>Cyan</td>
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<td>0.10b</td>
<td>0.12b*</td>
<td>–</td>
<td>–</td>
<td>p &lt; 0.001</td>
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<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>2.15b</td>
<td>2.52a</td>
<td>1.98A</td>
<td>1.92A</td>
<td>p &lt; 0.001</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
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<td>0.40a</td>
<td>0.17a</td>
<td>0.66A</td>
<td>0.34A</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Late</td>
<td>0.51b</td>
<td>1.29a</td>
<td>1.56a</td>
<td>1.48a</td>
<td>1.12B</td>
<td>1.30A</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.05</td>
<td>ns</td>
</tr>
<tr>
<td>Othe</td>
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<td>1.57c</td>
<td>1.92b</td>
<td>4.20a</td>
<td>2.37A</td>
<td>2.23A</td>
<td>p &lt; 0.001</td>
<td>ns</td>
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</tr>
</tbody>
</table>

After taxonomic assignment using the SILVA database (Version 132), 297, 290, 286, and 288 classified genera were obtained from the 0–5, 5–10, 10–20, and 20–30 cm soil layers, respectively, across the two treatments. In this study, we paid more attention to the genera that accounted for more than 0.25% of the relative abundance in the bacterial community in any soil sample (Fig. 2). At 0–5 cm, compared to their values for the CK treatment, the relative abundances of Rhodanobacter, Rhizomicrobium, Dokdonella, Pseudolabrys, Acidibacter, Devosia, Reyranella, Luteimonas, and Porphyrobacter genera from the Proteobacteria phylum and Terracidiphilus genus from the Acidobacteria phylum increased, whereas those of Anaeromyxobacter and Syntrophobacter genera from the Proteobacteria phylum, Mycobacterium and Streptomycyes genera from the Actinobacteria phylum, and Gemmata and Isosphaera genera from the Planctomycetes phylum decreased in the SM treatment (P < 0.05).
There were no significantly different genera of more than 0.25% relative abundance at the 5–10 cm depth between CK and SM treatments ($P > 0.05$). At 10–20 cm, the relative abundances of *RB41* genus from the Acidobacteria phylum, *Flavobacterium* genus from the Bacteroidetes phylum, and *Lysobacter* genus from the Proteobacteria phylum were increased, while those of *Desulfobaca* genus from the Proteobacteria phylum, and *Luedemannela*, *Mycobacterium*, and *Streptomyces* genera from the Actinobacteria phylum were decreased in the SM treatment ($P < 0.05$). At 20–30 cm, compared to that in the CK treatment, the relative abundance of *Flavobacterium* was significantly increased in the SM treatment ($P < 0.05$).

**Figure 2.** Bacterial genera that had significantly different relative abundances between CK and SM plots at 0–5 cm (a), 10–20 cm (b), and 20–30 cm (c) depths determined using a t-test analysis with 95% confidence intervals. CK, no-till with straw removal; SM, no-till with straw mulching.

### 3.5 Straw mulch effects on bacterial community structure

A PCoA showed the differences among the bacterial community structures in 24 samples (Fig. 3). The first two principal coordinates, PC1 and PC2, accounted for 65.79% and 11.18% of the total variation, respectively. The PC1 coordinate separated the soil samples into four groups along the soil depth gradient, regardless of straw treatment. Furthermore, Adonis analyses were performed with the OTU data calculated using the weighted UniFrac distances. The results showed that the bacterial communities in the SM treatment were marginally but significantly different (Adonis $R^2 = 0.61$, $P = 0.10$).
from those in the CK treatment at 0–5 cm soil depth. A similar difference was observed between the two treatments at 10–20 cm (Adonis $R^2 = 0.44$, $P = 0.10$). There was no significant difference between SM and CK bacterial communities at 5–10 cm (Adonis $R^2 = 0.11$, $P = 0.60$) and 20–30 cm (Adonis $R^2 = 0.19$, $P = 0.30$). In addition, the soil bacterial communities were significantly different among the four soil depths under CK (Adonis $R^2 = 0.76$, $P = 0.0003$) and SM (Adonis $R^2 = 0.88$, $P = 0.0002$) treatments.

**Figure 3.** Principal coordinates analysis (PCoA) plot of the soil bacterial communities based on OTUs from 24 samples. CK5, CK10, CK20, and CK30 represent the soil sampled at 0–5, 5–10, 10–20, and 20–30 cm depths, respectively, under straw removal. SM5, SM10, SM20, and SM30 represent the soil sampled at 0–5, 5–10, 10–20, and 20–30 cm depths, respectively, under straw mulching. The ellipses serve as only visual aids to distinguish different straw treatments under different soil depths and have no statistical meaning.

The data were subjected to an RDA to demonstrate the influence of major soil physicochemical properties on soil bacterial community composition (Fig. 4). Figures 4a, 4b, 4c, and 4d showed that the first two axes explained 51.11% and 21.17%, 52.51%
and 20.95%, 50.20% and 22.91%, and 53.39% and 19.94% of the total variation in the bacterial communities between CK and SM at the four soil depths, respectively. The contributions made by specified soil environmental factors varied with soil depth. Soil DOC \((P = 0.001)\), TOC \((P = 0.049)\), and pH \((P = 0.027)\) had significant effects on the bacterial community in the two treatments at 0–5 cm soil depth, whereas only soil pH \((P = 0.015)\) had a significant effect at 5–10 cm. At 10–20 cm soil depth, soil pH \((P = 0.022)\) and TOC \((P = 0.038)\) had the most significant effects, and at 20–30 cm, soil IN \((P = 0.003)\), pH \((P = 0.027)\), TN \((P = 0.03)\), and DON \((P = 0.032)\) were the drivers that most influenced the soil bacterial community.

**Figure 4.** Redundancy analysis (RDA) of the soil bacterial community changes at the OTU level and the soil physicochemical property differences between CK and SM plots in the 0–5 cm (a), 5–10 cm (b), 10–20 cm (c), and 20–30 cm (d) layers. CK5, CK10, CK20, and CK30 represent the soil sampled at 0–5, 5–10, 10–20, and 20–30 cm depths, respectively, under straw removal. SM5, SM10, SM20, and SM30 represent the soil sampled at 0–5, 5–10, 10–20, and 20–30 cm depths, respectively, under straw mulching. TOC, total organic carbon; TN, total nitrogen; IN, inorganic nitrogen; DOC, dissolved organic carbon; DON, dissolved organic nitrogen.
4 Discussion

4.1 Straw mulching changed soil physicochemical properties with soil depth

Our study demonstrated that compared to straw removal, long-term straw mulching had inconsistent effects on different soil physicochemical properties (Table 1). Since straw contained large amounts of carbon and some nutrients, straw mulching increased the carbon input to soil and consequently increased TOC in the 0–10 cm layer, which agreed with the results of Blanco-Canqui and Lal (2007) and Akhtar et al. (2018). Compared to the CK, TN was increased in the SM treatment, as straw mulching introduced large N to the soil. Straw mulching increased N fertilizer immobilization early in the crop season and subsequent N remineralization later (Cao et al., 2018), which would reduce gaseous N loss through ammonia volatilization and denitrification and increase N availability; for example, higher IN content was observed in SM soil in our study. The P and K contained in straw was one important reason for the significant increase in soil AP and AK in the SM treatment. However, there was no significant difference in TP and TK levels between CK and SM treatments, possibly because the amounts of P and K in the mulched straw were relatively lower than their total levels in the soil (Dong et al., 2012; Zhang et al., 2016). The DOC and DON levels were higher under SM than under CK treatments in the 0–20 cm layer. One reason for this was that some labile organic matter was derived from straw, leading to higher DOC and DON contents in SM than in CK plots at 0–5 cm. The labile organic matter can also be leached and accumulated in the subsurface soil layer (Blanco-Canqui and Lal, 2007), which led to higher contents in the 5–20 cm layer in the SM plots in the study. Mulched straw has also been reported to reduce water evaporation and increase water retention (Palm et al., 2014; Wang et al., 2019c), leading to a higher WC value under SM. There was no significant difference in pH between CK and SM plots in our study, which was inconsistent with the results of Ok et al. (2011) and Sun et al. (2015). They found that compared to straw removal treatment, straw return could decrease soil pH. Our pH results may be due to different soil types, sampling times, crop rotations, and tillage management.

The results of the present study indicated that most soil physicochemical parameters decreased with increasing soil depth, which was partly consistent with our hypothesis. Crop roots were mainly distributed in the 0–10 or 0–20 cm soil layers, which meant that introducing a large carbon input to the surface layer led to lower TOC.
and DOC contents in the subsoil than in the surface soil (Li et al., 2020). Apart from the roots, inorganic fertilizers were applied to the no-till soil surface, and consequently most soil nutrients (TN, TP, AP, AK, IN, DON) were enriched in the surface layer and decreased with soil depth. Large amounts of N fertilizer over a long period of time could result in soil acidification (Guo et al., 2010), which resulted in a lower pH value in the soil surface layer than in lower layers in our study. The TK content did not change with soil depth, mainly because of its high levels in the studied soil.

4.2 Straw mulching altered soil bacterial abundance and community with soil depth

The Pearson’s correlation analysis demonstrated that soil bacterial abundance, as determined via qPCR, was significantly correlated with soil TOC, TN, TP, IN, AP, AK, DOC, DON, and WC (Table S1). Similarly, soil moisture (Brockett et al., 2012), C and/or N availability (van Leeuwen et al., 2017; Cai et al., 2020), and TP (Song et al., 2020) were reported to be significantly and positively correlated with soil bacterial abundance. Straw mulching increased soil C, N, P and WC in our study, which favored soil bacterial abundance under the SM treatment. Ji et al. (2018) also found that the soil bacterial abundance increased after straw addition. Most soil bacterial abundance-related physicochemical parameters, such as C, N, P, and WC, were reduced in deeper soil layers, which contributed to the decreasing soil bacterial abundance with increasing soil depth (Fig. 1). This was consistent with the results of van Leeuwen et al. (2017).

Soil bacteria can be divided into copiotrophic and oligotrophic groups based on their growth on different substrates (Fierer et al., 2007, 2012). Straw mulching produced a nutrient-rich soil environment, especially in the 0–5 cm soil layer, which would benefit copiotroph bacterial growth and lead to a shift in the predominant bacterial community (Fierer et al., 2012). In addition, high soil inorganic nitrogen content decreased bacterial diversity (Yu et al., 2019; Zhao et al., 2019). These factors may explain the reduced value of Shannon diversity at the 0–5 cm soil depth in the SM plots compared to the CK plots. Soil biodiversity loss impairs ecosystem function (Wagg et al., 2014), and sustainable agriculture should adopt management practices that preserve or increase microbial diversity rather than destroy or threaten it (Pastorelli et al., 2013). Consequently, our results suggested that inorganic N fertilizer should be reduced under straw mulching and may thus be more beneficial for maintaining or improving bacterial diversity. Other soil microbial diversities, as measured by the Shannon evenness and
Chao1 indices, were not affected by straw mulching, possibly because these diversity indices are limited and do not take into account subtle changes in the soil environment (Hartmann and Widmer, 2006).

Proteobacteria and Bacteroidetes, often classified as copiotrophic groups, preferentially consume labile soil organic pools and have higher growth rates under conditions with abundant resources, while oligotrophic groups, such as Acidobacteria and Chloroflexi, are highly abundant in low-nutrient environments (Fierer et al., 2007, 2012; Liang et al., 2018; Ling et al., 2017). Long-term straw mulching increased soil nutrient levels, which was one reason for the higher relative abundances of Proteobacteria and Bacteroidetes in SM plots than in CK plots. Additionally, Bacteroidetes are involved in hemicellulose breakdown (Wegner and Liesack, 2016). Large quantities of straw were mulched to SM, and this management promoted Bacteroidetes proliferation. Larger available C and N pools reduced the relative abundances of oligotrophic groups, for example, Chloroflexi in SM plots, which agreed with the results of Liang et al. (2018). Since Acidobacteria are involved in hemicellulose breakdown (Wegner and Liesack, 2016), straw mulching increased their relative abundance. Although Actinobacteria were classified as copiotrophs by Fierer et al. (2012), straw mulching decreased the Actinobacteria in our study, which was also observed in other studies (Calleja-Cervantes et al., 2015; Hao et al., 2019; Liang et al., 2018). One possible reason is that most Actinobacteria favor aerobic environments (Hamamura et al., 2006), but straw mulching increased the WC and reduced the oxygen content in the soil. Soil nutrient levels were low in the soil subsurface layers, which was one reason for decreasing the relative abundances of Proteobacteria, Planctomycetes, Actinobacteria, Bacteroidetes, and Cyanobacteria and increasing the relative abundances of Chloroflexi and Nitrospirae with soil depth.

Our results confirmed that the different bacterial genera within each phylum had different strategies (Fig. 2). At the 0–5 cm soil depth, the relative abundances of nine genera from the Proteobacteria phylum increased under SM, while those of two genera from Proteobacteria decreased. This may explain why a higher relative abundance of Proteobacteria was detected in SM plots than in CK plots in the 0–5 cm soil layer. Returned straw was largely decomposed by soil microbes, and the soil bacterial community played important roles in increasing CH₄, N₂O and NH₃ emissions under straw return in many studies (Shang et al., 2011; Xu et al., 2017; Wang et al., 2012). Specifically, *Rhodanobacter* growth was favored and increased N₂O emissions in SM
soil, as it was the dominant bacterial genus containing denitrifying species (Huang et al., 2019). Similarly, the relative abundances of the *Rhizobium, Dokdonella, Reyranella, and Luteimonas* genera were also increased in SM soil since they are N-cycling-related bacterial taxa that contain denitrifiers (Chen et al., 2020a; Nie et al., 2018; Wang et al., 2019a; Wolff et al., 2018). *Terracidiphilus* and *Acidibacter* was involved in the degradation of plant-derived biopolymers (Garcia-Fraile et al., 2015) and organic substrates (Ai et al., 2018), respectively, which may explain their increased relative abundance in the SM treatment. Although little is known about the ecology of *Pseudolabrys*, its relative abundance was increased in soil that had received compost (Joa et al., 2014). Organic carbon can inhibit the growth of chemolithotrophic bacteria and favor *Dokdonella* (Wang et al., 2019a). The genus *Devisia* increases in composted soil because its species are plant growth-promoting bacteria (Liang et al., 2018). *Mycobacterium*, one genus of Actinobacteria, decreased after straw mulching in the 0–5 cm soil layer. One reason is that Actinobacteria was found to be dominant in soils with low organic matter levels (Bell et al., 2013). In addition, Sellstedt and Richau (2013) suggested that *Mycobacterium* is capable of nitrogen fixation by root nodulation. Higher soil inorganic nitrogen concentration may depress *Mycobacterium* growth in SM plots. *RB41* was enriched in SM plots at the 10–20 cm soil depth. Unfortunately, there have been few reports on this relationship. According to Foessel et al. (2013), *Blastocatella fastidiosa* was the only known isolate from *RB41*, and it preferred protein-containing substrates. Straw mulching might possibly increase the contents of these substrates and, therefore, *RB41* relative abundance. *Flavobacterium* was one genus from the Bacteroidetes phylum, and its relative abundance was higher in SM plots than CK plots. Nan et al. (2020) proposed that *Flavobacterium* possibly decomposes labile carbon. The relative abundance of *Lysobacter* was increased in SM soil, and Maarastawi et al. (2018) proposed that root exudates and additional resource carbon with strong lytic abilities could be used to degrade macromolecules.

The RDA results suggest that the key soil physicochemical parameters affecting soil bacteria partly changed with soil depth between straw mulching and straw removal, which was consistent with our hypothesis. However, the main key parameters were soil pH and different organic carbon and nitrogen fractions. A similar relationship was found in other studies (Schreiter et al., 2014; Sun et al., 2015). Schreiter et al. (2014) demonstrated that soil TOC, pH, and some available nutrients were closely related to soil bacterial communities. Sun et al. (2015) proposed that soil pH was the driving
factor in shaping bacterial community structure after straw addition.

5 Conclusions

In this study, we investigated the effects of long-term straw mulching on soil physicochemical properties and bacterial communities along a soil depth gradient under a no-till rice-wheat rotation system. Our results showed that most soil physicochemical parameters decreased, but soil pH increased with soil depth. Straw mulching increased most physicochemical parameters and bacterial abundance, but reduced the Shannon diversity of the bacterial community at 0–5 cm soil depth, with no difference in Shannon’s evenness and Chao1 indices. The reduced Shannon diversity in SM plots was possibly attributed to the enriched soil nutrition environment, especially the increased soil IN contents. The relative abundances of the bacterial phyla and genera varied with soil depth. At the phylum level, straw mulching increased the relative abundances of Proteobacteria, Bacteroidetes, and Acidobacteria, but reduced those of Actinobacteria, Chloroflexi, and Cyanobacteria. At the genera level, straw mulching had different effects on some C- and N-cycling genera, mainly increasing the relative abundances of Rhodanobacter, Rhizomicrobium, Terracidiphilus, Dokdonella, Pseudolabrys, Acidibacter, Devosia, Reyranella, Luteimonas, Porphyrobacter, RB41, Flavobacterium, and Lyso bacter and reducing those of Anaeromyxobacter, Mycobacterium, Syntrophobacter, Streptomyces, Gemmata, Isosphaera, Desulfobacca, Luedemannella, and Mycobacterium. An RDA showed that the significant correlations between the environmental factors and the soil bacterial community varied with depth, but soil pH and different organic carbon and nitrogen fractions were the major drivers. Consequently, straw mulching is highly recommended under a no-till system in southwestern China because of its benefits in soil fertility and bacterial abundance. However, to maintain or increase soil bacterial Shannon diversity, the amount of inorganic nitrogen fertilizer can be reduced after straw mulching in future studies.

Data availability

All data are available. The sequencing data have been submitted to the NCBI Sequence Read Archive database (SRA accession PRJNA625832).

Author contributions

ZZ analyzed the data and wrote the manuscript. ZL and ZC helped to analyze the data.
and write the manuscript. ZZ, KC, and XZ collected the soil samples. ZZ, HY, SG, YS, and HF determined the soil attributes. QC, ST, MH, and YQ installed the experiment and reviewed the manuscript. All authors approved the final version of the manuscript.

Competing interests
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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