

1 **Changes in soil physicochemical properties and bacterial communities**
2 **at different soil depths after long-term straw mulching under a no-till**
3 **system**

4 Zijun Zhou^{1,4,*}, Zengqiang Li^{2,*}, Kun Chen^{1,4}, Zhaoming Chen³, Xiangzhong Zeng^{1,4}, Hua Yu^{1,4}, Song
5 Guo^{1,4}, Yuxian Shangguan^{1,4}, Qingrui Chen^{1,4,*}, Hongzhu Fan^{1,4}, Shihua Tu^{1,4}, Mingjing He^{1,4}, Yusheng
6 Qin^{1,4,*}

7 ¹ Institute of Agricultural Resources and Environment, Sichuan Academy of Agricultural Sciences, Chengdu, China

8 ² College of Resources and Environment, Qingdao Agricultural University, Qingdao, China

9 ³ Institute of Environmental Resources and Soil Fertilizer, Zhejiang Academy of Agricultural Sciences, Hangzhou, China

10 ⁴ Monitoring and Experimental Station of Plant Nutrition and Agro-Environment for Sloping Land in South Region, Ministry
11 of Agriculture and Rural Affairs, Chengdu, China

12 * These authors contributed equally to this work.

13 *Correspondence to:* Yusheng Qin (shengyuq@126.com), Qingrui Chen (qingruichen@163.com)

14

15 **Abstract.** Conservation tillage has attracted increasing attention over recent decades, mainly due to its benefits in improving
16 soil organic matter content and reducing soil erosion. However, the effects of long-term straw mulching under a no-till system
17 on soil physicochemical properties and bacterial communities at different soil depths are still unclear. In this 12-year
18 experiment of straw removal (CK) and straw mulching (SM) treatments, soil samples were collected at 0–5, 5–10, 10–20, and
19 20–30 cm soil depths. The results showed that the contents of organic carbon (C), nitrogen (N) and phosphorus (P) fractions,
20 and bacterial abundance significantly decreased, whereas pH significantly increased with soil depth. Compared with CK, SM
21 significantly increased total N, inorganic N, available P, available potassium, and soil water content at 0–5 cm, total organic
22 C content at 0–10 cm, and dissolved organic C and N contents at 0–20 cm. Regarding bacterial communities, SM increased
23 the relative abundances of Proteobacteria, Bacteroidetes, and Acidobacteria but reduced those of Actinobacteria, Chloroflexi,
24 and Cyanobacteria. Bacterial Shannon diversity and Shannon’s evenness at 0–5 cm were reduced by SM treatment compared
25 to CK treatment. Furthermore, SM increased the relative abundances of some C-cycling genera (such as *Terracidiphilus* and
26 *Acidibacter*) and N-cycling genera (such as *Rhodanobacter*, *Rhizomicrobium*, *Dokdonella*, *Reyranella*, and *Luteimonas*) at 0–
27 5 cm. Principal coordinate analysis showed that the largest difference in the composition of soil bacterial communities between
28 CK and SM occurred at 0–5 cm. Soil pH and N and organic C fractions were the major drivers shaping soil bacterial
29 communities. Overall, SM treatment is highly recommended under a no-till system because of its benefits to soil fertility and
30 bacterial abundance.

31 **Keywords:** bacterial community composition, conservation tillage, Illumina sequencing, physicochemical properties, soil
32 depth, straw mulching

33

34 **1 Introduction**

35 The global demand for food depends largely on agriculture production to feed growing populations (Karthikeyan et al., 2020).
36 Conventional intensive agriculture puts unprecedented stress on soils and results in their degradation through soil organic
37 matter loss, erosion, and genetic diversity loss (Hou et al., 2020; Kopittke et al., 2019; Lupwayi et al., 2012). In contrast,
38 conservation agriculture centered on conservation tillage has been widely recommended for sustaining and improving
39 agriculture production in recent decades because it can increase soil organic matter content, improve soil structure, reduce soil
40 erosion, and decrease the need for farm labor (Jena, 2019; Singh et al., 2020). In 2013, the global conservation tillage area was
41 approximately 155 Mha, corresponding to approximately 11 % of crop land worldwide (Kassam et al., 2014). Generally,
42 conservation tillage practices follow two key principles, minimal soil disturbance (no or reduced tillage) and soil cover (mainly
43 straw mulch) (Pittelkow et al., 2014). Researchers have assessed the differences between conventional tillage and conservation
44 tillage in terms of crop yield and soil properties (Bu et al., 2020; Gao et al., 2020; Hao et al., 2019; Hu et al., 2021). However,
45 straw mulching is not always combined with no-till practices in many countries due to poor productivity, the prioritization of
46 livestock feeding, or insufficient time available to apply straw mulch (Giller et al., 2009; Jin, 2007; Pittelkow et al., 2014;
47 Zhao et al., 2018). Therefore, separation of straw mulching effects could refine our understanding of the function of straw in
48 soil properties as the area of conservation tillage in the world increases.

49 Soil physicochemical properties are important contributors to soil fertility, which is a critical factor determining crop
50 productivity and agriculture sustainability (Liu et al., 2019). Because straw contains large amounts of carbon (C), nitrogen (N),
51 phosphorus (P), and potassium (K), straw mulching is reported to increase soil total organic C and its fractions, soil enzymes
52 (invertase, phosphatase, urease, and catalase), and other physicochemical properties (Akhtar et al., 2018; Dai et al., 2019;
53 Duval et al., 2016; Wang et al., 2019b; Zhou et al., 2019a, 2019b). Many studies have focused on changes in these properties
54 in topsoil, as topsoil provides large amounts of nutrients to plants (Dai et al., 2019; Wang et al., 2019b; Zhou et al., 2019a).
55 However, soil physicochemical properties in the subsoil should also be considered, as some nutrients may move from topsoil
56 to deeper soil depths during irrigation and rainfall (Blanco-Canqui and Lal, 2007; Stowe et al., 2010). Inconsistent results on
57 the distribution of physicochemical properties along soil depths have been reported in cultivated agricultural soils or grasslands
58 (Li et al., 2017; Peng and Wang, 2016). Variations in physicochemical properties among different soil depths after long-term
59 straw mulching under a no-till system are still unclear, as no-till practices cause few disturbances to the soil and are quite
60 different from the heavy tillage practiced in conventional agriculture.

61 Soil bacterial communities have been used as sensitive indicators of soil quality in agricultural systems (Ashworth et
62 al., 2017) and play a vital role in soil ecological processes such as soil carbon, nutrient cycling, and greenhouse gas release
63 (Hobara et al., 2014; Tellez-Rio et al., 2015; Thompson et al., 2017). Reports of the responses of soil bacterial abundance and
64 communities to straw mulching in the topsoil have been inconsistent (Bu et al., 2020; Chen et al., 2017; Hao et al., 2019; Qiu
65 et al., 2020). Chen et al. (2017) proposed that straw return significantly increased bacterial biomass in one region but had no
66 significant effect in other regions. Regarding bacterial phyla, the relative abundance of Actinobacteria was enriched in straw

67 mulch soils in the Loess Plateau of China (Qiu et al., 2020) but was reduced under a wheat-maize rotation system (Hao et al.,
68 2019). Moreover, soil microorganisms in deep soil layers have attracted the attention of researchers because they have
69 important effects on soil formation, ecosystem biochemistry processes, and maintaining groundwater quality (Li et al., 2014).
70 Several studies have shown that bacterial abundances and community composition change with soil depth (Fierer et al., 2003;
71 van Leeuwen et al., 2017). Unfortunately, no detailed information has been obtained on the soil bacterial community changes
72 that occur in response to straw mulching at different soil depths under no-till systems.

73 Rice-wheat rotation is a major cropping system in China, and approximately 80 million tons of crop straw are
74 produced annually in southwestern China (Li et al., 2016; Zhou et al., 2019b). This area has a humid, mid-subtropical monsoon
75 climate with an average annual precipitation of 1200 mm. The abundant precipitation could promote leaching of water-soluble
76 organic matter and nutrients derived from straw deep into the soil, which may result in significant differences in soil properties
77 at deeper depths. Although we assessed some soil organic C fractions under a no-till system in our previous study (Zhou et al.,
78 2019b), little is known about how other soil physicochemical parameters vary with soil depth. We hypothesized that (1)
79 compared with straw removal, straw mulching would significantly change soil properties, which would decline with increasing
80 soil depth; and (2) the key soil physicochemical properties shaping bacterial communities would be different at different depths.
81 In this study, a field was subjected to two straw management programs under a 12 year no-till system in the Chengdu Plain to
82 (1) determine the effects of straw mulching on soil physicochemical parameters, bacterial abundance, and community
83 composition at different depths, and (2) clarify the differences in the key soil physicochemical properties shaping bacterial
84 communities at increasing soil depths.

85

86 **2 Materials and methods**

87 **2.1 Experimental site and design**

88 A long-term field experiment was begun in 2005 in Guanghan, Sichuan Province, China (31°08'38" N, 104°29'45" E). Before
89 the experiment, the local agricultural soil was seldom tilled due to a shortage of tillage machines. The soil had been managed
90 for a long period of time under the same agricultural cropping system, and consequently the fertility heterogeneity of the soil
91 was considered minimal. The soil is a fluvo-aquic soil with loamy clay. The soil pH in 2005 was 5.54, and the total organic C,
92 total N, available N, available P, and available K levels were 18.1 g kg⁻¹, 2.03 g kg⁻¹, 189.76 mg kg⁻¹, 12.61 mg kg⁻¹, and
93 258.2 mg kg⁻¹, respectively.

94 The experiment included two treatments with three replicates and used a randomized design. Each plot measured 12
95 m² (3 × 4 m). Two treatments, i.e., a control (CK, straw removal) and straw mulching (SM), were applied using a no-till rice-
96 wheat rotation system. The straw was removed in the CK treatment, whereas rice and wheat straw were distributed over the
97 soil surface without being chopped after harvest each year in the SM treatment. The mulch consisted of approximately 8.5 t
98 ha⁻¹ rice straw and 6.0 t ha⁻¹ wheat straw each year. During the experiment, equal amounts of inorganic fertilizer were added

99 in both treatments by manual broadcast over the soil surface without tillage. The doses of N, P₂O₅, and K₂O fertilizers were at
100 180, 90, and 90 kg ha⁻¹, respectively, in the wheat season and 165, 60, and 90 kg ha⁻¹, respectively, in the rice season. Nitrogen
101 as urea was applied as fertilizer in the sowing and tillering stages at rates of 30 % and 70 %, respectively, during the wheat
102 season and 70 % and 30 %, respectively, during the rice season. Potassium as potassium chloride was applied as fertilizer in
103 the sowing and tillering stages at rates of 50 % each during both the wheat and rice seasons. Phosphorus as calcium
104 superphosphate was applied as fertilizer once at sowing during both the wheat and rice growing seasons. Other detailed
105 information about the experimental design is provided in our previous study (Zhou et al., 2019b).

106

107 **2.2 Soil sampling**

108 Immediately after the wheat harvest in 2018, soil columns of 0–30 cm were collected from five points in each plot using a
109 stainless steel auger (40 mm interior diameter). Each soil column was divided into four samples from soil depths of 0–5, 5–10,
110 10–20, and 20–30 cm. Samples from the same soil depth at five different sampling points were pooled to make one composite
111 sample for each depth of 0–5, 5–10, 10–20, and 20–30 cm for each plot. The mixed soil was passed through a 2 mm mesh and
112 divided into three parts: one was air-dried and used to measure soil pH, total organic C, total N, total P, total K, available P,
113 and available K; one was kept at 4 °C (< 1 week) for soil NH₄⁺-N, NO₃⁻-N, dissolved organic C (DOC), and dissolved organic
114 N (DON) analysis; and the third was stored at -80 °C for soil bacterial community analysis.

115

116 **2.3 Soil physicochemical properties**

117 DOC and DON were extracted from the soil by shaking fresh soil samples with distilled water (1:5 soil:solution ratio), and the
118 extracts were then filtered for analysis using a Multi N/C 3100 analyzer (Analytik Jena AG, Jena, Germany) (Zhou et al.,
119 2019b). Soil water content was determined using the gravimetric method after drying the soil to a constant weight at 105 °C
120 (Akhtar et al., 2018). Soil inorganic N, pH, total organic C, total N, total P, total K, available P, and available K were
121 determined as described by Lu (2000). Briefly, concentrations of NH₄⁺-N and NO₃⁻-N in filtered 2 M KCl extracts from fresh
122 soil were measured using a continuous-flow auto-analyzer (AA3, Seal Analytical Inc., Southampton, UK). Inorganic N
123 concentrations were calculated as the sum of NH₄⁺-N and NO₃⁻-N. Soil pH was determined in a 1:2.5 soil:water aqueous
124 suspension using an Orion 3-star benchtop pH meter (Thermo Scientific, Waltham, MA, USA). Soil total organic C was
125 determined using the dichromate oxidation and ferrous sulfate titration method, and soil total N was determined using the
126 continuous-flow auto-analyzer after digestion based on the Kjeldahl method. For measurements of soil total P and total K,
127 soils were first digested using a mixed acid solution of H₂SO₄ and HClO₄; total P was then analyzed using the continuous-flow
128 auto-analyzer, and total K was determined by atomic absorption photometry. Soil available P was extracted using 0.025 M
129 HCl-0.03 M NH₄F and measured by ammonium molybdate colorimetry, and available K was extracted using 2 M HNO₃ and

130 measured by atomic absorption photometry. Results of soil total organic C and DOC were reported in our previous study (Zhou
131 et al., 2019b).

132

133 **2.4 DNA extraction and qPCR amplification**

134 DNA was extracted from 0.5 g of fresh soil using the Fast[®] DNA SPIN Kit (MP Biomedicals, Santa Ana, CA, USA) according
135 to the manufacturer's instructions (Zhou et al., 2017). The extracted DNA was dissolved in 50 μ L of double-distilled water,
136 and its quality and concentration were checked using a NanoDrop 2000 spectrophotometer (Calleja-Cervantes et al., 2015).
137 The DNA samples were then stored at -80 $^{\circ}$ C until further use. qPCR was used to quantify bacterial abundances based on the
138 16S rRNA gene using the primers 338F (5'-ACTCCT ACGGGAGGCAGCAG-3') and 518R (5'-
139 ATTACCGCGGCTGCTGG-3') (Fierer et al., 2005). The qPCR procedure was carried out according to Chen et al. (2019)
140 with some modifications. PCR was performed using a Bio-Rad CFX 96-well Thermocycler (Bio-Rad, Hercules, CA, USA).
141 The reactions were performed in a 20 μ L mixture containing 16.5 μ L 2 \times SYBR Color qPCR Master Mix, 0.5 μ M (0.8 μ L)
142 each primer, and 2 μ L DNA template. The PCR conditions were as follows: 95 $^{\circ}$ C for 5 min; 40 cycles of 30 s at 95 $^{\circ}$ C, 30 s
143 at 58 $^{\circ}$ C, and 40 s at 72 $^{\circ}$ C; and finally, 10 min at 72 $^{\circ}$ C. All samples were evaluated in triplicate. Standard curves were
144 obtained using 10-fold serial dilutions of linearized recombinant plasmids containing cloned 16S rDNA with known copy
145 numbers. Melting curve analysis was performed at the end of each qPCR run to check the specificity of PCR products. PCR
146 amplification efficiencies were between 96 % and 105 %, with R^2 values > 0.99 .

147

148 **2.5 16S rRNA amplification for Illumina sequencing and data processing**

149 The primers 515F (5'-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTC AATTCMTTTRAGTTT-3') were used to
150 amplify the V4–V5 regions of bacterial DNA (Caporaso et al., 2012). Detailed operational information can be found in Zhang
151 et al. (2019). The 16S rRNA sequences were analyzed on the I–Sanger Cloud Platform (<https://cloud.majorbio.com/>). Raw
152 sequences were merged using FLASH version 1.2.7 (Magoc and Salzberg, 2011) and processed using Quantitative Insights
153 Into Microbial Ecology (QIIME v.1.9.0; <http://www.qiime.org/>) (Quast et al., 2013). Poor-quality sequences (average quality
154 score < 25) and short sequences (< 200 bp) were removed. Primers were matched exactly, allowing two mismatched
155 nucleotides, and reads with ambiguous bases were removed. Sequences with overlaps longer than 10 bp were merged according
156 to their overlap sequence. After this step, 945,665 clean reads were obtained, with 30,241 to 58,191 reads per sample.
157 Operational taxonomic units (OTUs) were clustered at a similarity threshold of 97 % using the ribosomal database project
158 (RDP) classifier with the Bayesian algorithm. The number of sequences per soil sample was rarefied to an equal abundance as
159 the sample with the lowest number of sequences (Menéndez-Serra et al., 2019; Ye et al., 2017), and 4101 OTUs were identified
160 across all samples. The taxonomy of each 16S rRNA gene sequence was analyzed using RDP Classifier against the SILVA
161 database version 132 with a confidence threshold of 70 % (Quast et al., 2013). Good's coverage was used to investigate the

162 sequence coverage of the bacterial communities. The α -diversity parameters, including Shannon index, Shannon's evenness,
163 and Chao1, were estimated using the Mothur program (<http://www.mothur.org>). Shannon index and Shannon's evenness were
164 used to investigate soil bacterial community diversity and evenness, respectively. Chao1 was used to describe soil bacterial
165 community richness.

166

167 **2.6 Statistical analysis**

168 The homogeneity of variance and normality were assessed using Levene and Shapiro-Wilk tests before analysis of variance
169 (ANOVA). Data normalization was achieved by transforming soil available P content by $\log(x)$ and relative abundances of
170 Acidobacteria and Planctomycetes by $1/(x)^{0.5}$. Two-way ANOVA was used to determine the main effects of soil depth and
171 straw management strategy and their interactions on soil physicochemical parameters, bacterial abundance, bacterial α -
172 diversity indices, and relative abundances of bacterial phyla. Welch's t-tests within STAMP (Parks et al., 2014) were used to
173 identify genera with significant differences in relative abundance between CK and SM at each depth. Pearson's correlation
174 analysis was used to assess the relationships between bacterial communities and soil physicochemical parameters. These
175 statistical analyses were performed using SPSS 19.0 (SPSS, Inc., Chicago, IL, USA). Principal coordinate analysis (PCoA)
176 was then used to demonstrate patterns of similarity in bacterial community structures between CK and SM based on weighted
177 UniFrac distances. Environmental factors were selected using Monte Carlo permutations (calculated based on 999), and
178 environmental factors with $P > 0.05$ were removed from a redundancy analysis (RDA) (Fan and Xing, 2016). Analysis of
179 similarity (Adonis) analysis was performed based on OTU data using the vegan package of the R project ([http://www.r-](http://www.r-project.org)
180 [project.org](http://www.r-project.org)). The Monte Carlo Mantel test and RDA were performed using Canoco 5.0 (CANOCO, Microcomputer Power
181 Inc., Ithaca, NY, USA) to identify the soil environmental factors that were significantly correlated with soil bacterial
182 communities. PCoA plots were drawn using the I-Sanger Cloud Platform (<https://cloud.majorbio.com/>), and other graphs were
183 prepared using SigmaPlot ver. 12.5 (Systat, Software, Inc., San Jose, California, USA).

184

185 **3 Results**

186 **3.1 Soil physicochemical properties**

187 Data shown are expressed as means \pm standard deviations of three replicates. Two-way ANOVA showed that straw
188 management, soil depth, and their interaction had significant effects on soil total organic C, total N, inorganic N, available P,
189 available K, DOC, and DON, and both the main effects of straw management and soil depth had significant effects on soil
190 water content (Table 1). All soil physicochemical parameters, except total K, were changed significantly with soil depth.
191 Specifically, soil pH values were lowest at 0–5 cm and increased with soil depth; total K was unchanged among the four depths,
192 and other physicochemical properties decreased with soil depth (Table 1). Soil total organic C, total N, inorganic N, available

193 P, available K, DOC, DON, and water content were generally significantly higher under SM treatment than CK treatment
 194 (Table 1), especially soil total organic C at 0–5 and 5–10 cm, soil total N, inorganic N, available P, available K, and water
 195 content at 0–5 cm, soil DOC at 0–5, 5–10, and 10–20 cm, and soil DON at 0–5 and 10–20 cm (Table 2).

196

197 **Table 1:** Two-way ANOVA analysis of soil physicochemical properties at four depths under two straw management strategies, each with
 198 three replicates. The data in bold indicate soil physicochemical properties that were not affected by straw management, soil depth, or their
 199 interaction ($P > 0.05$). DOC, dissolved organic carbon; DON, dissolved organic nitrogen.

Physicochemical properties	Straw		Depth		Straw × Depth	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
pH	1.91	0.186	52.93	<0.0001	0.75	0.537
Total organic C	48.47	<0.0001	281.08	<0.0001	17.58	<0.0001
Total N	7.99	0.012	160.85	<0.0001	3.13	0.050
Total P	0.99	0.334	74.60	<0.0001	0.88	0.473
Total K	2.79	0.114	1.21	0.339	1.09	0.381
Inorganic N	6.01	0.026	73.66	<0.0001	8.80	0.001
Available P	11.45	0.004	184.96	<0.0001	4.429	0.019
Available K	4.37	0.049	62.53	<0.0001	4.08	0.025
DOC	47.75	<0.0001	78.20	<0.0001	10.60	0.0004
DON	29.23	0.0001	65.80	<0.0001	7.23	0.003
Soil water content	6.55	0.021	38.72	<0.0001	3.07	0.058

200

201 **Table 2:** Soil physicochemical properties at different soil depths under SM and CK treatment. CK, no-till with straw removal; SM, no-till
 202 with straw mulching. Data are means ± standard deviations, n = 3. Different capital letters indicate significant differences ($P < 0.05$) among
 203 the four depths; * indicates significant differences ($P < 0.05$) among the two straw managements within each depth (Duncan's test). DOC,
 204 dissolved organic carbon; DON, dissolved organic nitrogen.

Physicochemical properties	Treatment	Soil depth gradient			
		0–5 cm	5–10 cm	10–20 cm	20–30 cm
pH	CK	5.27 ± 0.19	6.04 ± 0.30	6.63 ± 0.36	7.11 ± 0.36
	SM	4.90 ± 0.21	5.76 ± 0.40	6.48 ± 0.26	7.23 ± 0.26
Total organic C (g kg ⁻¹)	CK	5.09 ± 0.27A	5.90 ± 0.35B	6.56 ± 0.29C	7.17 ± 0.29D
	SM	23.01 ± 0.15*	19.42 ± 1.23*	14.22 ± 2.23	6.90 ± 1.19
Total N (g kg ⁻¹)	CK	33.24 ± 1.47	22.26 ± 0.25	15.76 ± 1.41	7.15 ± 0.43
	SM	28.13 ± 5.73A	20.84 ± 1.75B	14.99 ± 1.87C	7.03 ± 0.81D
Total P (g kg ⁻¹)	CK	2.84 ± 0.10*	2.13 ± 0.34	1.54 ± 0.27	0.62 ± 0.10
	SM	3.50 ± 0.18	2.39 ± 0.17	1.54 ± 0.25	0.66 ± 0.11
Total K (g kg ⁻¹)	CK	3.17 ± 0.38A	2.26 ± 0.28B	1.54 ± 0.23C	0.64 ± 0.10D
	SM	0.88 ± 0.13	0.67 ± 0.02	0.43 ± 0.11	0.22 ± 0.04
Inorganic N (mg kg ⁻¹)	CK	0.87 ± 0.08A	0.70 ± 0.07B	0.48 ± 0.11C	0.21 ± 0.04D
	SM	12.42 ± 0.38	12.40 ± 0.42	11.75 ± 0.30	11.81 ± 0.62
Available P (mg kg ⁻¹)	CK	12.44 ± 0.34	12.55 ± 0.58	12.80 ± 1.00	12.07 ± 0.27
	SM	12.43 ± 0.33A	12.48 ± 0.46A	12.28 ± 0.88A	11.94 ± 0.45A
Available K (mg kg ⁻¹)	CK	21.43 ± 1.02*	18.33 ± 2.25	14.21 ± 2.53	11.31 ± 1.06
	SM	29.05 ± 0.83	16.64 ± 2.42	14.45 ± 1.52	11.89 ± 0.41
Available P (mg kg ⁻¹)	CK	25.24 ± 4.25A	17.49 ± 2.29B	14.33 ± 1.87C	11.60 ± 0.79D
	SM	94.49 ± 7.59*	39.30 ± 4.11	14.74 ± 3.70	2.43 ± 2.48
Available K (mg kg ⁻¹)	CK	126.63 ± 17.52	53.74 ± 14.21	17.06 ± 0.81	1.60 ± 0.87
	SM	110.55 ± 21.34A	46.52 ± 12.25B	15.90 ± 2.71C	2.01 ± 1.73D
Available K (mg kg ⁻¹)	CK	152.33 ± 15.93*	107.85 ± 3.08	103.37 ± 1.55	103.70 ± 5.25
	SM	183.72 ± 13.09	115.88 ± 13.95	100.31 ± 3.93	100.84 ± 9.81

		168.02 ± 21.58A	111.86 ± 10.05B	101.83 ± 3.16B	102.26 ± 7.21B
DOC (mg kg ⁻¹)	CK	41.42 ± 5.74*	35.05 ± 4.38*	20.59 ± 1.24*	12.69 ± 6.23
	SM	73.01 ± 9.22	55.41 ± 1.99	36.31 ± 8.04	8.48 ± 2.88
		57.21 ± 18.62A	45.23 ± 11.54B	28.45 ± 10.03C	10.58 ± 4.92D
DON (mg kg ⁻¹)	CK	16.11 ± 1.89*	17.29 ± 3.69	12.33 ± 0.85*	4.97 ± 1.21
	SM	26.22 ± 2.51	18.08 ± 2.24	18.36 ± 1.21	5.98 ± 0.94
		21.16 ± 5.89A	17.68 ± 2.77B	15.34 ± 3.43B	5.48 ± 1.12C
Soil water content (%)	CK	16.99 ± 0.69*	17.46 ± 0.77	15.21 ± 0.66	12.68 ± 0.81
	SM	19.03 ± 0.89	16.71 ± 0.73	16.20 ± 0.68	13.81 ± 1.18
		18.01 ± 1.32A	17.09 ± 0.79A	15.71 ± 0.80B	13.25 ± 1.10C

205

206 **3.2 Bacterial abundance**

207 Straw management, soil depth, and their interaction significantly affected soil bacterial abundance as measured by 16S rRNA
 208 gene copy number (Table 3). Soil bacterial abundance declined significantly as soil depth increased in both treatment groups
 209 ($P < 0.0001$), and bacterial abundance under SM treatment was 52.69 % higher than that under CK treatment ($P < 0.05$).
 210 Compared with CK treatment, SM treatment significantly increased bacterial abundance at 0–5 cm ($P < 0.05$), but there was
 211 no significant difference between the two treatments at the other three depths (Table 4).

212

213 **Table 3:** Two-way ANOVA analysis of soil bacterial properties at four depths under two straw management strategies, each with three
 214 replicates. The data in bold indicate soil bacterial properties that were not affected by straw management strategy, soil depth, or their
 215 interaction ($P > 0.05$).

Bacterial properties	Straw		Depth		Straw × Depth	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Copy number of 16S rRNA gene	11.59	0.004	41.38	<0.0001	4.51	0.018
Shannon	1.15	0.299	11.37	0.0003	3.21	0.050
Shannon's evenness	0.14	0.712	17.04	<0.0001	3.11	0.056
Chao1	3.11	0.097	4.09	0.025	0.68	0.577
Proteobacteria	13.32	0.002	17.69	<0.0001	2.50	0.096
Actinobacteria	9.53	0.007	7.90	0.0019	1.32	0.302
Acidobacteria	20.27	0.0004	24.85	<0.0001	1.94	0.165
Chloroflexi	14.87	0.001	24.68	<0.0001	0.60	0.626
Planctomycetes	0.05	0.833	11.22	0.0003	0.54	0.664
Nitrospirae	0.02	0.894	34.12	<0.0001	1.27	0.317
Bacteroidetes	20.28	0.0004	30.74	<0.0001	1.86	0.177
Firmicutes	3.15	0.095	2.27	0.120	1.91	0.169
Gemmatimonadetes	0.17	0.686	14.09	0.0001	0.04	0.990
Cyanobacteria	22.41	0.0002	69.95	<0.0001	18.48	<0.0001
Unclassified	0.37	0.553	35.70	<0.0001	2.31	0.115
Verrucomicrobia	1.43	0.249	1.40	0.278	1.32	0.304
Latescibacteria	4.73	0.045	33.21	<0.0001	2.08	0.143
Others	0.71	0.412	58.55	<0.0001	0.83	0.497

216

217 3.3 Bacterial α -diversity

218 The Good's coverage value for all samples was greater than 96 % in our study, which indicated that the number of sequence
 219 reads adequately represented the bacteria. Table 3 shows that soil depth had a significant effect on three α -diversity indices
 220 (Shannon diversity, Shannon's evenness, and Chao1) ($P < 0.05$). Shannon diversity was higher at 0–20 cm than at 20–30 cm,
 221 whereas Shannon's evenness was highest at 0–5 cm and lowest value at 20–30 cm. Chao1 first increased, reaching the highest
 222 value at 5–10 cm, then decreased with soil depth (Table 4). Compared to CK treatment, SM treatment reduced Shannon
 223 diversity and Shannon's evenness at 0–5 cm, but there was no difference at the other three depths. Chao1 did not differ between
 224 CK and SM at any depth.

225

226 **Table 4:** Soil bacterial properties at different soil depths under SM and CK treatment. CK, no-till with straw removal; SM, no-till with straw
 227 mulching. Data are means \pm standard deviations, $n = 3$. Different capital letters indicate significant differences ($P < 0.05$) among the four
 228 depths; * indicates significant differences ($P < 0.05$) among the two straw management strategies within each depth (Duncan's test).

Bacterial properties	Treatment	Soil depth gradient			
		0–5 cm	5–10 cm	10–20 cm	20–30 cm
Copy number of 16S rRNA gene	CK	14.77 \pm 2.69*	7.18 \pm 2.59	6.30 \pm 1.75	2.10 \pm 0.54
	SM	24.65 \pm 3.93	13.59 \pm 4.98	6.12 \pm 2.65	1.97 \pm 1.34
Shannon	CK	19.71 \pm 6.19A	10.38 \pm 4.99B	6.22 \pm 2.01C	2.03 \pm 0.92D
	SM	6.53 \pm 0.03*	6.38 \pm 0.08	6.34 \pm 0.05	6.07 \pm 0.16
Shannon's evenness	CK	6.40 \pm 0.08	6.42 \pm 0.09	6.40 \pm 0.06	6.27 \pm 0.12
	SM	6.46 \pm 0.09A	6.40 \pm 0.08A	6.37 \pm 0.06A	6.17 \pm 0.17B
Chao1	CK	0.864 \pm 0.002*	0.844 \pm 0.006	0.843 \pm 0.007	0.816 \pm 0.016
	SM	0.852 \pm 0.007	0.846 \pm 0.008	0.842 \pm 0.004	0.832 \pm 0.009
Proteobacteria	CK	0.858 \pm 0.008A	0.845 \pm 0.006B	0.843 \pm 0.005B	0.824 \pm 0.015C
	SM	2417 \pm 64	2563 \pm 198	2506 \pm 166	2437 \pm 18
Actinobacteria	CK	2421 \pm 46	2714 \pm 74	2689 \pm 146	2472 \pm 185
	SM	2419 \pm 50A	2639 \pm 156C	2597 \pm 172BC	2455 \pm 119AB
Acidobacteria	CK	32.11 \pm 0.82*	29.51 \pm 2.16	29.08 \pm 1.78	26.69 \pm 3.70
	SM	38.87 \pm 2.57	31.31 \pm 0.71	30.93 \pm 0.32	28.06 \pm 1.36
Chloroflexi	CK	35.49 \pm 4.08A	30.41 \pm 1.75B	30.00 \pm 1.53B	27.37 \pm 2.60C
	SM	17.02 \pm 2.99	12.57 \pm 2.44	12.15 \pm 0.66*	10.32 \pm 1.62
Planctomycetes	CK	12.66 \pm 1.82	11.30 \pm 2.52	8.83 \pm 0.56	9.76 \pm 0.73
	SM	14.84 \pm 3.26A	11.94 \pm 2.32B	10.49 \pm 1.90B	10.04 \pm 1.16B
Nitrospirae	CK	17.17 \pm 1.96	19.56 \pm 0.56	20.14 \pm 0.70*	14.32 \pm 1.30*
	SM	21.23 \pm 2.25	20.16 \pm 0.97	22.52 \pm 0.28	16.44 \pm 0.01
Bacteroidetes	CK	19.20 \pm 2.92B	19.86 \pm 0.78BC	21.33 \pm 1.39C	15.38 \pm 1.42A
	SM	13.82 \pm 1.37*	13.33 \pm 2.03	14.63 \pm 1.84*	20.46 \pm 2.96
Firmicutes	CK	10.03 \pm 1.30	12.02 \pm 1.25	11.56 \pm 0.20	18.10 \pm 0.99
	SM	11.92 \pm 2.40A	12.67 \pm 1.67A	13.10 \pm 2.05A	19.28 \pm 2.36B
Nitrospirae	CK	4.29 \pm 0.50	3.68 \pm 0.22	4.16 \pm 0.28	2.56 \pm 1.04
	SM	3.95 \pm 0.51	3.76 \pm 0.07	4.23 \pm 0.16	2.93 \pm 0.40
Bacteroidetes	CK	4.12 \pm 0.49A	3.72 \pm 0.15A	4.20 \pm 0.21A	2.74 \pm 0.73B
	SM	5.25 \pm 1.17	10.39 \pm 1.39	8.50 \pm 1.40	13.18 \pm 2.54
Firmicutes	CK	4.66 \pm 0.23	10.26 \pm 0.93	10.40 \pm 1.35	12.29 \pm 0.66
	SM	4.96 \pm 0.82A	10.33 \pm 1.06B	9.45 \pm 1.61B	12.74 \pm 1.73C
Bacteroidetes	CK	1.74 \pm 0.21*	1.37 \pm 0.36	0.78 \pm 0.16*	0.62 \pm 0.29
	SM	2.45 \pm 0.21	1.67 \pm 0.39	1.52 \pm 0.15	0.78 \pm 0.22
Firmicutes	CK	2.09 \pm 0.43A	1.52 \pm 0.37B	1.15 \pm 0.43C	0.70 \pm 0.25D
	SM	1.16 \pm 0.35	1.48 \pm 0.31	2.29 \pm 0.73	1.35 \pm 0.59

	SM	1.12 ± 0.34	1.47 ± 0.45	1.23 ± 0.31	1.18 ± 0.16
		1.14 ± 0.31A	1.48 ± 0.35AB	1.76 ± 0.77B	1.26 ± 0.40AB
Gemmatimonadetes	CK	1.40 ± 0.21	2.42 ± 0.31	2.31 ± 0.32	1.98 ± 0.52
	SM	1.42 ± 0.19	2.42 ± 0.32	2.42 ± 0.14	2.05 ± 0.24
		1.41 ± 0.18A	2.42 ± 0.28C	2.37 ± 0.23BC	2.01 ± 0.37B
Cyanobacteria	CK	1.25 ± 0.29*	0.20 ± 0.02	0.10 ± 0.05	0.12 ± 0.02*
	SM	0.48 ± 0.04	0.15 ± 0.03	0.14 ± 0.06	0.06 ± 0.02
		0.87 ± 0.46A	0.17 ± 0.03B	0.12 ± 0.05B	0.09 ± 0.04B
Unclassified	CK	1.27 ± 0.30*	2.19 ± 0.14	2.08 ± 0.18	2.41 ± 0.26
	SM	0.76 ± 0.11	2.05 ± 0.20	2.23 ± 0.36	2.63 ± 0.42
		1.01 ± 0.34A	2.12 ± 0.17B	2.15 ± 0.27B	2.52 ± 0.33C
Verrucomicrobia	CK	1.51 ± 1.63	0.42 ± 0.23	0.58 ± 0.72	0.13 ± 0.07
	SM	0.34 ± 0.02	0.59 ± 0.42	0.21 ± 0.03	0.22 ± 0.08
		0.93 ± 1.21A	0.50 ± 0.31A	0.40 ± 0.50A	0.17 ± 0.08A
Latescibacteria	CK	0.46 ± 0.13	1.32 ± 0.24	1.31 ± 0.37	1.38 ± 0.19
	SM	0.56 ± 0.03	1.25 ± 0.09	1.81 ± 0.11	1.58 ± 0.25
		0.51 ± 0.10A	1.29 ± 0.17B	1.56 ± 0.37C	1.48 ± 0.23BC
Others	CK	1.55 ± 0.24	1.55 ± 0.16	1.89 ± 0.09	4.49 ± 1.05
	SM	1.47 ± 0.19	1.59 ± 0.10	1.96 ± 0.24	3.91 ± 0.22
		1.51 ± 0.20A	1.57 ± 0.12A	1.92 ± 0.17A	4.20 ± 0.75B

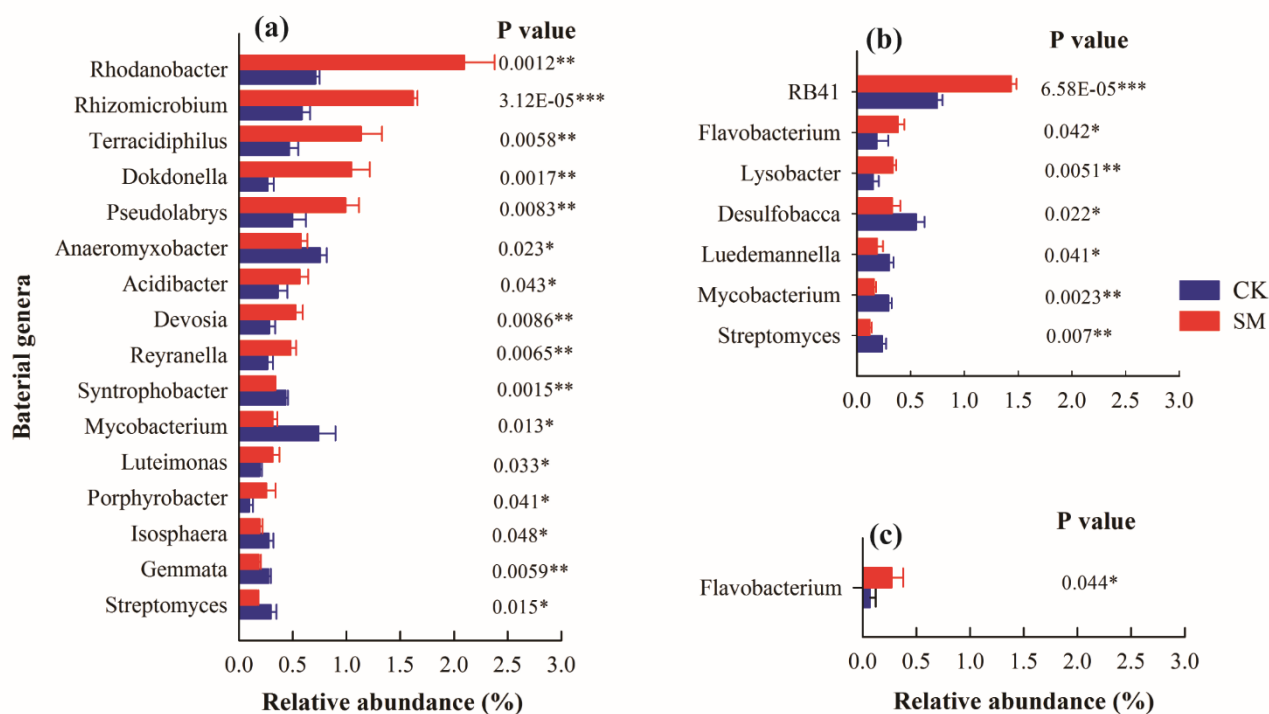
229

230 3.4 Bacterial community composition

231 Phyla whose relative abundances accounted for less than 1 % of all soil samples were merged into the “Others” category. As
232 a result, 14 phyla were identified in the study. From highest to lowest relative abundance these were Proteobacteria,
233 Acidobacteria, Chloroflexi, Actinobacteria, Planctomycetes, Nitrospirae, Others, Gemmatimonadetes, Unclassified,
234 Firmicutes, Bacteroidetes, Latescibacteria, Verrucomicrobia, and Cyanobacteria (Fig. S1). Two-way ANOVA showed that
235 soil depth significantly altered the relative abundances of almost all phyla, except Firmicutes and Verrucomicrobia (Table 3).
236 Specially, the relative abundances of Proteobacteria, Actinobacteria, Bacteroidetes, and Cyanobacteria decreased, whereas
237 those of Chloroflexi, Nitrospirae, and Latescibacteria increased as soil depth increased ($P < 0.05$) under both treatments. The
238 relative abundance of Acidobacteria increased from 0–5 to 10–20 cm, then decreased at 20–30 cm. The relative abundance of
239 Planctomycetes did not change among the 0–5, 5–10, and 10–20 cm depths but significantly decreased at 20–30 cm. The
240 relative abundance of Gemmatimonadetes first increased and then decreased with soil depth, and its highest abundance was at
241 5–10 cm. Meanwhile, two-way ANOVA showed that compared to CK treatment, SM treatment significantly increased the
242 relative abundances of Proteobacteria, Acidobacteria, Bacteroidetes, and Latescibacteria, but decreased those of Actinobacteria,
243 Chloroflexi, and Cyanobacteria (Tables 3 and 4). Table 4 shows that SM treatment significantly increased relative abundances
244 of Proteobacteria at 0–5 cm, Acidobacteria at 10–20 and 20–30 cm, and Bacteroidetes at 0–5 and 10–20 cm compared with
245 CK treatment, whereas SM treatment significantly reduced the relative abundances of Actinobacteria at 10–20 cm, Chloroflexi
246 at 0–5 and 10–20 cm, and Cyanobacteria at 0–5 and 20–30 cm compared with CK treatment ($P < 0.05$).

247 After taxonomic assignment, 297, 290, 286, and 288 classified genera were obtained from the 0–5, 5–10, 10–20, and
248 20–30 cm soil layers, respectively, across the two treatments. In this study, we focused on the genera that accounted for more
249 than 0.25 % of the relative abundance of the bacterial community in any soil sample (Fig. 1). Compared to CK treatment, SM

250 treatment increased the relative abundances of the genera *Rhodanobacter*, *Rhizomicrobium*, *Dokdonella*, *Pseudolabrys*,
 251 *Acidibacter*, *Devosia*, *Reyranella*, *Luteimonas*, and *Porphyrobacter* in the phylum Proteobacteria and the genus
 252 *Terracidiphilus* in the phylum Acidobacteria but decreased those of the genera *Anaeromyxobacter* and *Syntrophobacter* in the
 253 phylum Proteobacteria, the genera *Mycobacterium* and *Streptomyces* in the phylum Actinobacteria, and the genera *Gemmata*
 254 and *Isosphaera* in the phylum Planctomycetes at 0–5 cm ($P < 0.05$). There were no significantly different genera with an
 255 abundance greater than 0.25 % between CK and SM at 5–10 cm ($P > 0.05$). At 10–20 cm, the relative abundances of the genus
 256 *RB41* in the phylum Acidobacteria, the genus *Flavobacterium* in the phylum Bacteroidetes, and the genus *Lysobacter* in the
 257 phylum Proteobacteria were increased, whereas those of the genus *Desulfobacca* in the phylum Proteobacteria and the genera
 258 *Luedemannella*, *Mycobacterium*, and *Streptomyces* in the phylum Actinobacteria were decreased under SM treatment ($P <$
 259 0.05). Compared to CK treatment, SM treatment significantly increased the relative abundance of *Flavobacterium* at 20–30
 260 cm ($P < 0.05$).

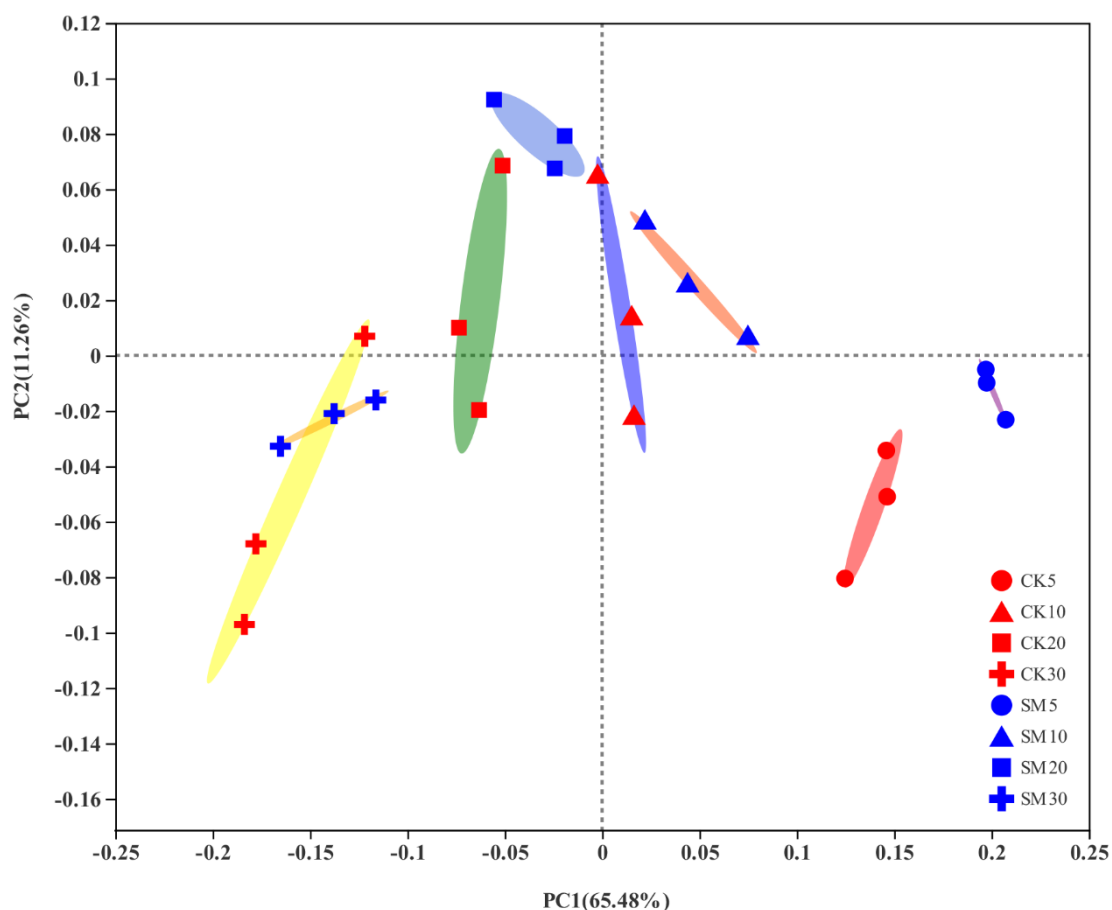


261 **Fig. 1:** Bacterial genera that had significantly different relative abundances under CK and SM treatments at 0–5 cm (a), 10–20 cm (b), and
 262 20–30 cm (c) determined using *t*-tests with 95 % confidence intervals. CK, no-till with straw removal; SM, no-till with straw mulching.

263

264 3.5 Bacterial community structure

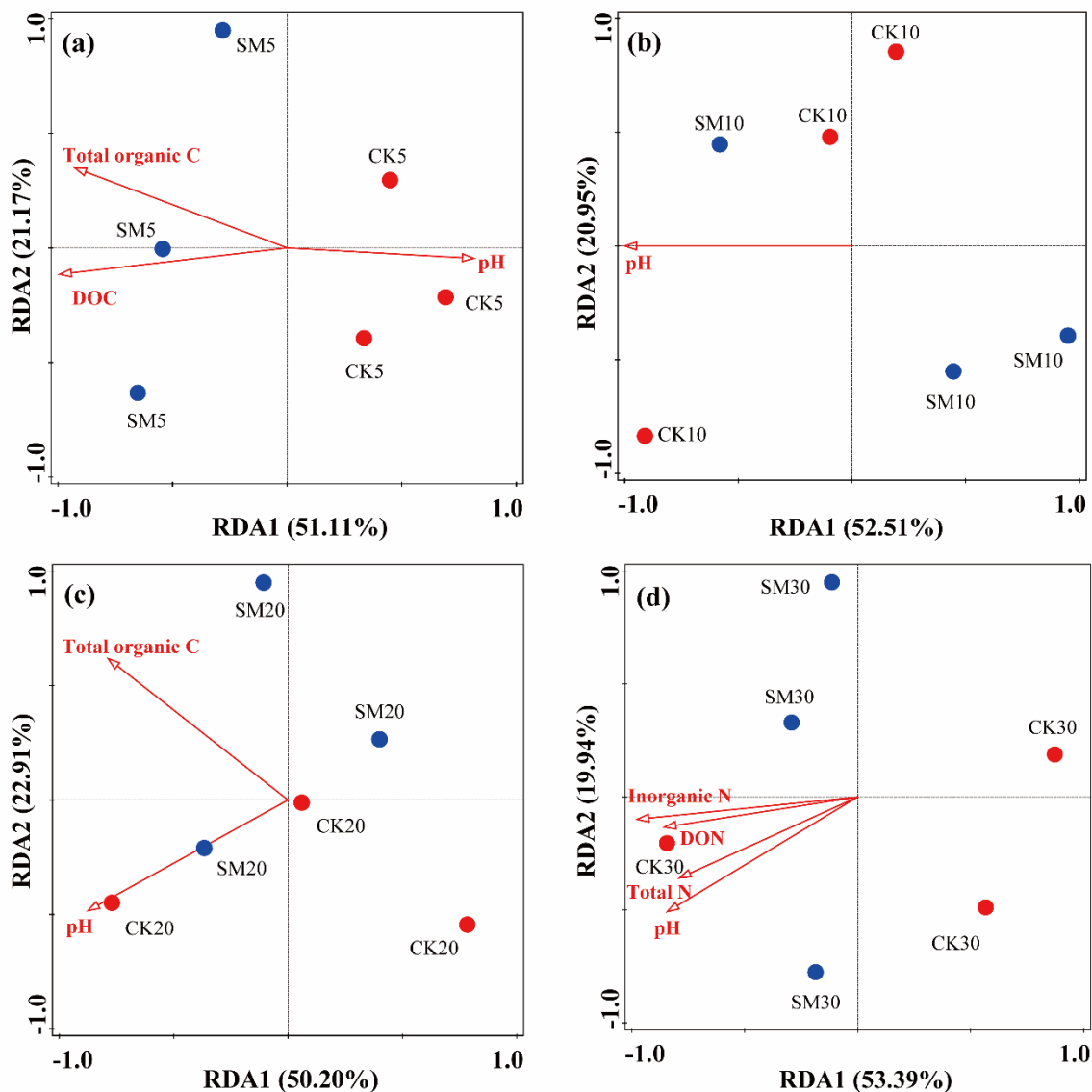
265 PCoA showed differences among bacterial community structures in the 24 samples (Fig. 2). The first two principal coordinates,
266 PC1 and PC2, accounted for 65.48 % and 11.26 % of the total variation, respectively. The PC1 coordinate separated the soil
267 samples into four groups along the soil depth gradient, regardless of straw treatment. Furthermore, the largest difference in the
268 composition of soil bacterial communities between CK and SM occurred at 0–5 cm from the PCoA plot. The results of Adonis
269 analyses showed that bacterial communities under SM treatment were marginally but significantly different (Adonis $R^2 = 0.61$,
270 $P = 0.10$) from those under CK treatment at 0–5 cm. A similar difference was observed between the two treatments at 10–20
271 cm (Adonis $R^2 = 0.44$, $P = 0.10$). There was no significant difference between SM and CK bacterial communities at 5–10 cm
272 (Adonis $R^2 = 0.11$, $P = 0.60$) or 20–30 cm (Adonis $R^2 = 0.19$, $P = 0.30$). In addition, soil bacterial communities were
273 significantly different among the four soil depths under both the CK (Adonis $R^2 = 0.76$, $P = 0.0003$) and SM (Adonis $R^2 =$
274 0.88 , $P = 0.0002$) treatments.



275 **Fig. 2:** Principal coordinate analysis (PCoA) plot of soil bacterial communities based on OTUs from 24 samples. CK5, CK10, CK20, and
276 CK30 represent soils sampled at 0–5, 5–10, 10–20, and 20–30 cm, respectively, from the straw removal group. SM5, SM10, SM20, and
277 SM30 represent soil sampled at 0–5, 5–10, 10–20, and 20–30 cm, respectively, from the straw mulching group. The ellipses serve as visual
278 aids to distinguish between different straw treatments at different soil depths and have no statistical meaning.

280 **3.6 Relationships between soil bacterial characteristics and physicochemical properties**

281 Pearson's correlation analysis demonstrated that bacterial abundance, as determined by qPCR, was significantly correlated
 282 with soil total organic C, total N, DOC, DON, total and available P, available K, and water content (Table S1).



283 **Fig. 3:** Redundancy analysis (RDA) of soil bacterial community changes at the OTU level and soil physicochemical property differences
 284 between CK and SM plots at 0–5 cm (a), 5–10 cm (b), 10–20 cm (c), and 20–30 cm (d). CK5, CK10, CK20, and CK30 represent soil sampled
 285 at 0–5, 5–10, 10–20, and 20–30 cm, respectively, from the straw removal group. SM5, SM10, SM20, and SM30 represent soil sampled at
 286 0–5, 5–10, 10–20, and 20–30 cm, respectively, from the straw mulching group. DOC, dissolved organic carbon; DON, dissolved organic
 287 nitrogen.

288 To explore possible relationships between soil physicochemical properties and the structure of microbial communities,
289 an RDA was conducted using all OTU and environmental variables (Fig. 3). Figures 3a, 3b, 3c, and 3d show that the first two
290 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
291 variation in the bacterial communities between CK and SM at the four soil depths, respectively. The contributions made by
292 specific soil environmental factors varied with soil depth. Soil DOC ($F = 4.1$, $P = 0.001$), total organic C ($F = 3.5$, $P = 0.049$),
293 and pH ($F = 2.3$, $P = 0.027$) had significant effects on bacterial communities between the two treatments at 0–5 cm, whereas
294 only soil pH ($F = 4.4$, $P = 0.015$) had a significant effect at 5–10 cm. At 10–20 cm, soil pH ($F = 3.1$, $P = 0.022$) and total
295 organic C ($F = 2.6$, $P = 0.038$) had the most significant effects, and at 20–30 cm, soil inorganic N ($F = 4.3$, $P = 0.003$), pH (F
296 $= 3$, $P = 0.027$), DON ($F = 2.7$, $P = 0.032$), and total N ($F = 2.7$, $P = 0.030$) most influenced soil bacterial communities.
297

298 4 Discussion

299 4.1 Straw mulching changed soil physicochemical properties with soil depth

300 Our study demonstrated that compared to straw removal, long-term straw mulching increased contents of total N, inorganic N,
301 available P, and available K at 0–5 cm, water content at 0–5 cm, and total organic C at 0–5 and 5–10 cm. These results may
302 be explained by the fact that the straw was mulched at the soil surface rather than being incorporated into the soil, leading to
303 large amounts of C and nutrients being released at the soil surface as the straw decomposed (Akhtar et al, 2018; Blanco-Canqui
304 and Lal, 2007). Furthermore, the decrease in gaseous N loss through ammonia volatilization and denitrification caused by
305 straw mulching may have also contributed to the accumulation of soil N fractions (Cao et al., 2018). During straw
306 decomposition, large amounts of soluble organic matter, such as starch, protein, and monosaccharides, can be leached and
307 accumulate in the subsoil (Blanco-Canqui and Lal, 2007), which may have increased soil DOC and DON at 0–20 cm. For soil
308 water content, mulched straw can reduce water evaporation and increase water retention (Palm et al., 2014; Wang et al, 2019c).
309 However, there was no significant difference in pH, total P, or total K levels between CK and SM. Similarities in pH values
310 after straw mulching are consistent with reports by Wang et al. (2020). Unchanged soil total P and total K may be explained
311 by the high levels of these elements in the soil (Dong et al., 2012; Zhang et al., 2016).

312 The results of the present study showed that soil total organic C, total N, total P, inorganic N, available P, available
313 K, DOC, DON, and water content decreased but pH increased with increasing soil depth, which was partly consistent with our
314 hypothesis. One reason for this was that most crop roots are distributed at depths of 0–10 cm or 0–20 cm (Li et al., 2020), and
315 root exudates and C released after root decomposition lead to higher total organic C and DOC contents in the topsoil than in
316 the subsoil. Beyond the effects of roots, inorganic N, P, and K fertilizers were applied to the soil surface without tillage, and
317 these elements were initially enriched in the topsoil but decreased with soil depth. Large amounts of N fertilizer over a long
318 period of time could result in soil acidification (Guo et al., 2010), which results in a lower pH value in the topsoil than in the
319 subsoil. The total K content did not change with soil depth, mainly because of its high levels in the studied soil.

321 **4.2 Straw mulching altered soil bacterial abundance and communities with soil depth**

322 Soil bacterial communities play an important role in regulating soil processes, and the biomass and composition of soil bacteria
323 determine the sustainability of agricultural soils (Segal et al., 2017). Our results provide strong support for the view of Bai et
324 al. (2018), who showed that straw can provide energy and nutrients for soil bacterial growth. Compared to CK treatment, straw
325 mulching increased soil organic C, soil nutrients, and water moisture, which favored soil bacterial abundance, especially in
326 topsoil (Table S1, Table 3). Similar results were also reported by Ji et al. (2018). Previous studies reported that soil moisture
327 (Brockett et al., 2012), C and/or N availability (van Leeuwen et al., 2017), and total P (Song et al., 2020) were significantly
328 and positively correlated with soil bacterial abundance. Meanwhile, most soil bacterial abundance-related physicochemical
329 parameters were reduced in deeper soil layers, which largely contributed to the decreasing soil bacterial abundance with soil
330 depth (Table 3 and 4). This was consistent with the results of van Leeuwen et al. (2017).

331 Soil bacteria can be divided into copiotrophic and oligotrophic groups based on their performance in different
332 substrates (Fierer et al., 2007, 2012). Straw mulching produced a nutrient-rich soil environment, which benefits copiotroph
333 bacterial growth and leads to a shift in the predominant bacterial community (Fierer et al., 2012). In addition, high soil
334 inorganic N content decreases bacterial diversity (Yu et al., 2019; Zhao et al., 2019). These factors contributed to the reduced
335 Shannon diversity and Shannon's evenness index values at 0–5 cm after straw mulching. Soil biodiversity is important for
336 maintaining ecosystem functioning (Wagg et al., 2014), and sustainable agriculture requires adoption of management practices
337 that preserve or increase microbial diversity rather than destroy or threaten it (Pastorelli et al., 2013). Consequently, inorganic
338 N fertilizer should be reduced under straw mulching, which may further contribute to maintaining or improving bacterial
339 diversity.

340 Bacterial phyla demonstrated different responses to straw management strategies and soil depths. The relative
341 abundances of copiotrophic bacteria, such as Proteobacteria, Actinobacteria, and Bacteroidetes, decreased with soil depth due
342 to their preference for the abundant soil resources in topsoil (Fierer et al., 2007, 2012; Liang et al., 2018; Ling et al., 2017). As
343 a result, compared with CK, straw mulching increased soil C and nutrients, thereby increasing the relative abundances of
344 Proteobacteria and Bacteroidetes (Fierer et al., 2007, 2012; Liang et al., 2018; Ling et al., 2017). Bacteroidetes are involved in
345 hemicellulose breakdown, and mulched straw stimulated Bacteroidetes proliferation during straw decomposition (Wegner and
346 Liesack, 2016). Chloroflexi is classified as an oligotrophic group, and enriched soil nutrients restricted Chloroflexi growth in
347 topsoil or after straw mulching, which is in agreement with the results of Liang et al. (2018). Notably, soil nutrient condition
348 was not the only factor influencing the proliferation of bacterial phyla such as Actinobacteria and Acidobacteria. The phylum
349 Actinobacteria was classified as copiotrophic by Fierer et al. (2012), but straw mulching decreased Actinobacteria in our study,
350 similar to the observations of other studies (Calleja-Cervantes et al., 2015; Hao et al., 2019; Liang et al., 2018). One possible
351 reason is that straw mulching increased soil water content and reduced soil oxygen content, whereas most Actinobacteria favor

352 aerobic environments (Hamamura et al., 2006). Although Acidobacteria is classified as oligotrophic, it is involved in
353 hemicellulose breakdown (Wegner and Liesack, 2016), leading to increases in its relative abundance after straw mulching.

354 Our results confirmed that straw return changed certain soil bacteria genera associated with C and N cycles (Shang
355 et al., 2011; Wang et al., 2012; Xu et al., 2017). For example, straw mulching favored *Rhodanobacter* growth, which is the
356 dominant bacterial genus containing denitrifying species and is positively associated in N₂O emissions (Huang et al., 2019).
357 Similarly, the relative abundances of the genera *Rhizomicrobium*, *Dokdonella*, *Reyranella*, and *Luteimonas*, N-cycling-related
358 bacterial taxa containing denitrifiers, were increased in straw mulched soil (Chen et al., 2020a; Nie et al., 2018; Wang et al.,
359 2019a; Wolff et al., 2018). *Terracidiphilus*, *Acidibacter*, *Flavobacterium*, and *Lysobacter* are involved in the degradation of
360 plant-derived biopolymers (Garcia-Fraile et al., 2015), organic substrates (Ai et al., 2018), labile carbon (Nan et al., 2020), and
361 macromolecules (Maarastawi et al., 2018), and large C-based materials from mulched straw increased their relative abundances.
362 Although little is known about the ecology of *Pseudolabrys*, its relative abundance was increased in soil after compost
363 application (Joa et al., 2014). Wang et al. (2019a) found that organic carbon can inhibit the growth of chemolithotrophic
364 bacteria and favor *Dokdonella*. According to Foesel et al. (2013), *Blastocatella fastidiosa* is the only known isolate from *RB41*
365 and prefers protein-containing substrates. Straw mulching may increase the contents of these substrates and, therefore, the
366 relative abundance of *RB41*.

367 RDA results suggested that the key soil physicochemical parameters distinguishing soil bacteria between SM and CK
368 changed with soil depth, which was consistent with our hypothesis. However, the main parameters were soil pH and different
369 N and organic C fractions. A similar relationship was found in other studies (Schreiter et al., 2014; Sun et al., 2015). Schreiter
370 et al. (2014) demonstrated that soil total organic C, pH, and some available nutrients were closely related to soil bacterial
371 communities. Sun et al. (2015) proposed that soil pH was the driving factor in shaping bacterial community structure after
372 straw addition.

373

374 **5 Conclusions**

375 In this study, we investigated the effects of long-term straw mulching on soil properties along a soil depth gradient under a no-
376 till rice-wheat rotation system. The results showed that soil total organic C, total N, total P, inorganic N, available P, available
377 K, DOC, DON, water content, and bacterial abundance decreased but soil pH increased with soil depth. Compared with CK,
378 straw mulching increased soil total organic C at 0–10 cm, soil total and inorganic N, available P and K, and water content at
379 0–5 cm, DOC and DON at 0–20 cm, and bacterial abundance 0–5 cm but reduced Shannon diversity and Shannon's evenness
380 of the bacterial community at 0–5 cm. Regarding bacterial communities, straw mulching increased the relative abundances of
381 Proteobacteria, Bacteroidetes, and Acidobacteria, but reduced those of Actinobacteria, Chloroflexi, and Cyanobacteria.
382 Additionally, straw mulching increased some C- and N-cycling genera, such as *Rhodanobacter*, *Rhizomicrobium*,
383 *Terracidiphilus*, *Dokdonella*, *Pseudolabrys*, *Acidibacter*, *Devosia*, *Reyranella*, *Luteimonas*, and *Porphyrobacter*. PCoA

384 showed that the largest difference in the composition of soil bacterial communities between CK and SM occurred at 0–5 cm.
385 Soil pH and N and organic C fractions were the major drivers shaping soil bacterial communities. Overall, straw mulching is
386 highly recommended under a no-till system in southwestern China because of its benefits for soil fertility and bacterial
387 abundance. However, to maintain or increase soil bacterial Shannon diversity, the amount of inorganic N fertilizer could be
388 reduced after straw mulching in future studies.

389

390 **Data availability**

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

393

394 **Author contributions**

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

398

399 **Competing interests**

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

402

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