

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**

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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°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 ATTACCGCGCTGCTGG-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 $^{\circ}\text{C}$ for 5 min; 40 cycles of 30 s at 95 $^{\circ}\text{C}$, 30 s
143 at 58 $^{\circ}\text{C}$, and 40 s at 72 $^{\circ}\text{C}$; and finally, 10 min at 72 $^{\circ}\text{C}$. 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'-CCGTCAATTCMTTTRAGTTT-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	3.17 ± 0.38A	2.26 ± 0.28B	1.54 ± 0.23C	0.64 ± 0.10D
Total K (g kg ⁻¹)	CK	0.88 ± 0.13	0.67 ± 0.02	0.43 ± 0.11	0.22 ± 0.04
	SM	0.86 ± 0.02	0.74 ± 0.09	0.53 ± 0.10	0.20 ± 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

DOC (mg kg ⁻¹)	CK	168.02 ± 21.58A	111.86 ± 10.05B	101.83 ± 3.16B	102.26 ± 7.21B
	SM	41.42 ± 5.74*	35.05 ± 4.38*	20.59 ± 1.24*	12.69 ± 6.23
DON (mg kg ⁻¹)	CK	73.01 ± 9.22	55.41 ± 1.99	36.31 ± 8.04	8.48 ± 2.88
	SM	57.21 ± 18.62A	45.23 ± 11.54B	28.45 ± 10.03C	10.58 ± 4.92D
Soil water content (%)	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
		16.99 ± 0.69*	17.46 ± 0.77	15.21 ± 0.66	12.68 ± 0.81
		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.**

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
Nitrospirae	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
Bacteroidetes	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
Bacteroidetes	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 ± 0.21	1.67 ± 0.39	1.52 ± 0.15	0.78 ± 0.22
		2.09 ± 0.43A	1.52 ± 0.37B	1.15 ± 0.43C	0.70 ± 0.25D
Firmicutes	CK	1.16 ± 0.35	1.48 ± 0.31	2.29 ± 0.73	1.35 ± 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

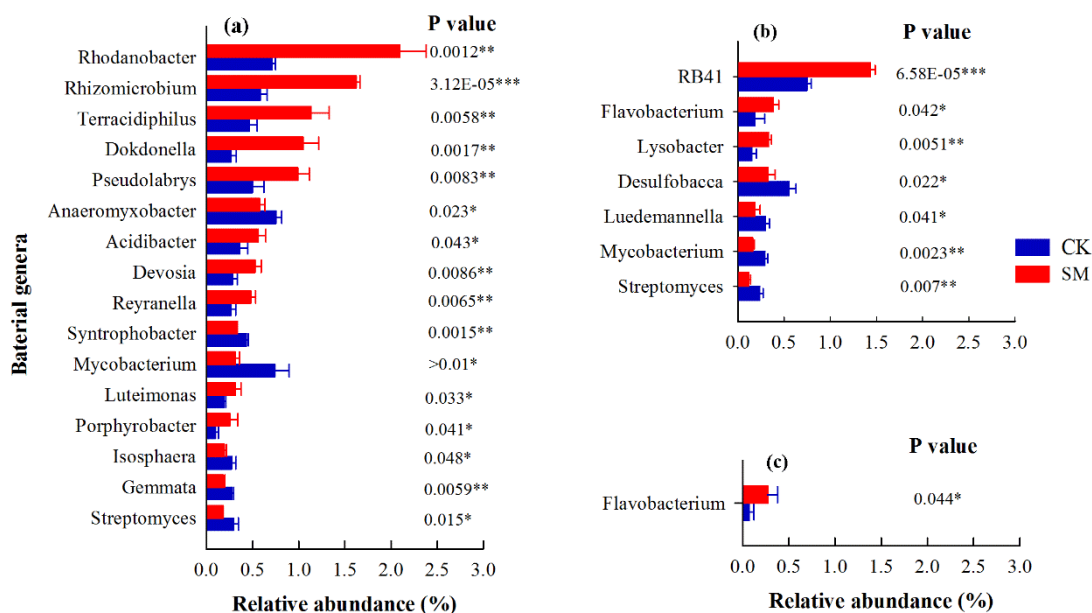
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230 3.4 Bacterial community composition

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



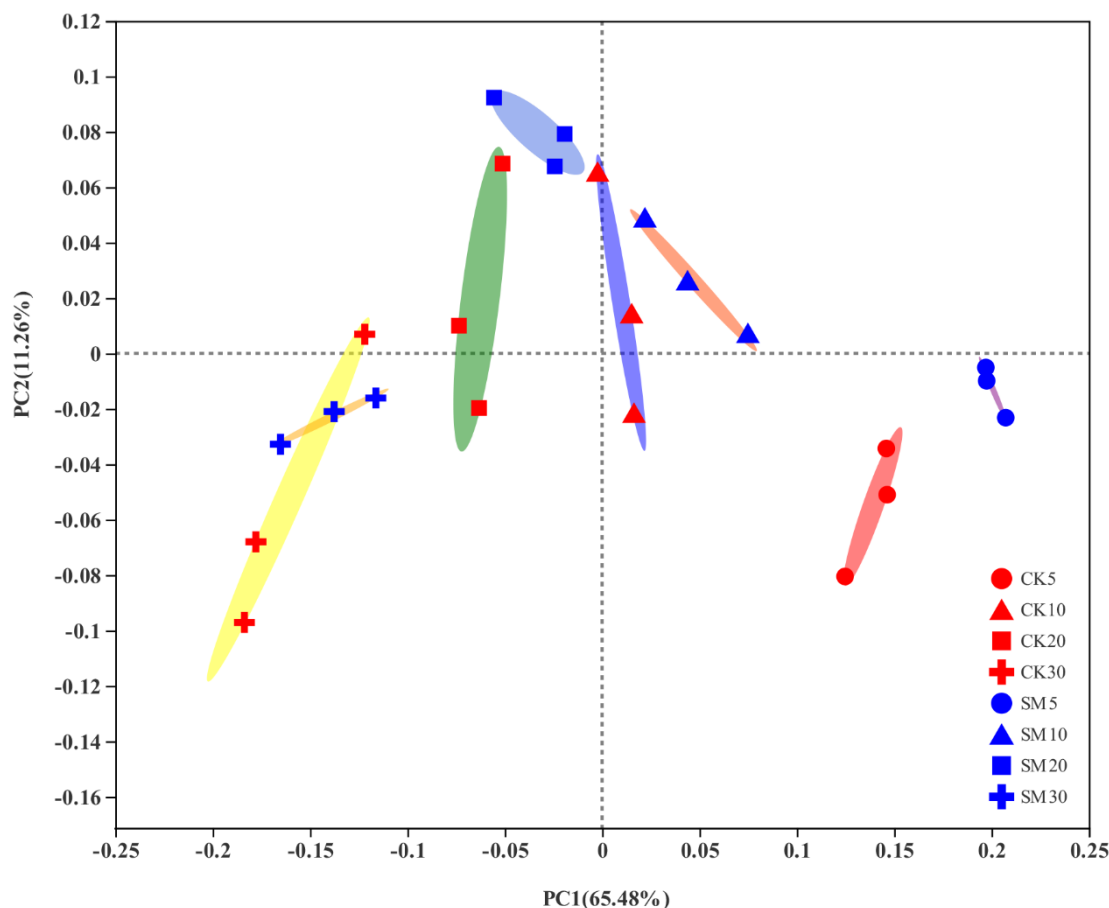
262

263 **Fig. 2:** Bacterial genera that had significantly different relative abundances under CK and SM treatments at 0–5 cm (a), 10–20 cm (b), and
 264 20–30 cm (c) determined using *t*-tests with 95 % confidence intervals. CK, no-till with straw removal; SM, no-till with straw mulching.

265

266 3.5 Bacterial community structure

267 PCoA showed differences among bacterial community structures in the 24 samples (Fig. 2). The first two principal coordinates,
268 PC1 and PC2, accounted for 65.48 % and 11.26 % of the total variation, respectively. The PC1 coordinate separated the soil
269 samples into four groups along the soil depth gradient, regardless of straw treatment. Furthermore, the largest difference in the
270 composition of soil bacterial communities between CK and SM occurred at 0–5 cm from the PCoA plot. The results of Adonis
271 analyses showed that bacterial communities under SM treatment were marginally but significantly different (Adonis $R^2 = 0.61$,
272 $P = 0.10$) from those under CK treatment at 0–5 cm. A similar difference was observed between the two treatments at 10–20
273 cm (Adonis $R^2 = 0.44$, $P = 0.10$). There was no significant difference between SM and CK bacterial communities at 5–10 cm
274 (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
275 significantly different among the four soil depths under both the CK (Adonis $R^2 = 0.76$, $P = 0.0003$) and SM (Adonis $R^2 =$
276 0.88 , $P = 0.0002$) treatments.

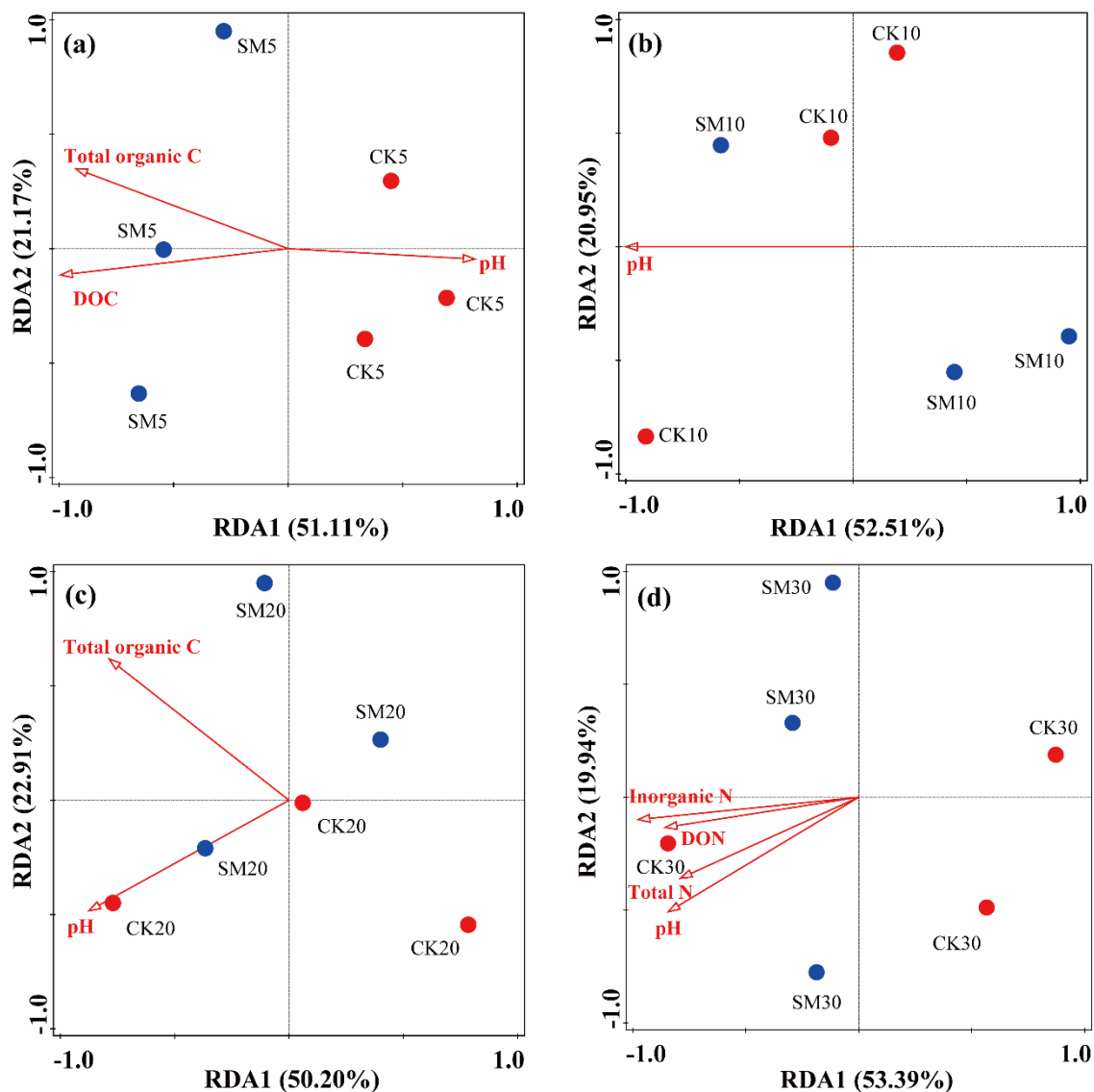


277

278 **Fig. 2:** Principal coordinate analysis (PCoA) plot of soil bacterial communities based on OTUs from 24 samples. CK5, CK10, CK20, and
279 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
280 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
281 aids to distinguish between different straw treatments at different soil depths and have no statistical meaning.

283 **3.6 Relationships between soil bacterial characteristics and physicochemical properties**

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



286 **Fig. 3:** Redundancy analysis (RDA) of soil bacterial community changes at the OTU level and soil physicochemical property differences
 287 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
 288 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

289 0–5, 5–10, 10–20, and 20–30 cm, respectively, from the straw mulching group. DOC, dissolved organic carbon; DON, dissolved organic
290 nitrogen.

291 To explore possible relationships between soil physicochemical properties and the structure of microbial communities,
292 an RDA was conducted using all OTU and environmental variables (Fig. 3). Figures 3a, 3b, 3c, and 3d show that the first two
293 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
294 variation in the bacterial communities between CK and SM at the four soil depths, respectively. The contributions made by
295 specific soil environmental factors varied with soil depth. Soil DOC ($F = 4.1, P = 0.001$), total organic C ($F = 3.5, P = 0.049$),
296 and pH ($F = 2.3, P = 0.027$) had significant effects on bacterial communities between the two treatments at 0–5 cm, whereas
297 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
298 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
299 $= 3, P = 0.027$), DON ($F = 2.7, P = 0.032$), and total N ($F = 2.7, P = 0.030$) most influenced soil bacterial communities.

300

301 4 Discussion

302 4.1 Straw mulching changed soil physicochemical properties with soil depth

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

315 The results of the present study showed that soil total organic C, total N, total P, inorganic N, available P, available
316 K, DOC, DON, and water content decreased but pH increased with increasing soil depth, which was partly consistent with our
317 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
318 root exudates and C released after root decomposition lead to higher total organic C and DOC contents in the topsoil than in
319 the subsoil. Beyond the effects of roots, inorganic N, P, and K fertilizers were applied to the soil surface without tillage, and
320 these elements were initially enriched in the topsoil but decreased with soil depth. Large amounts of N fertilizer over a long

321 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
322 subsoil. The total K content did not change with soil depth, mainly because of its high levels in the studied soil.

323

324 4.2 Straw mulching altered soil bacterial abundance and communities with soil depth

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

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

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

354 reason is that straw mulching increased soil water content and reduced soil oxygen content, whereas most Actinobacteria favor
355 aerobic environments (Hamamura et al., 2006). Although Acidobacteria is classified as oligotrophic, it is involved in
356 hemicellulose breakdown (Wegner and Liesack, 2016), leading to increases in its relative abundance after straw mulching.

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

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

376

377 **5 Conclusions**

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

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

392

393 **Author contributions**

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

397

398 **Competing interests**

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

401

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408 **References**

- 409 Ai, C., Zhang, S., Zhang, X., Guo, D., Zhou, W., and Huang, S.: Distinct responses of soil bacterial and fungal communities
410 to changes in fertilization regime and crop rotation, *Geoderma* 319, 156–166,
411 <https://doi.org/10.1016/j.geoderma.2018.01.010>, 2018.
- 412 Akhtar, K., Wang, W., Ren, G., Khan, A., Feng, Y., and Yang, G.: Changes in soil enzymes, soil properties, and maize crop
413 productivity under wheat straw mulching in Guanzhong, China, *Soil Tillage Res.*, 182, 94–102,
414 <https://doi.org/10.1016/j.still.2018.05.007>, 2018.
- 415 Ashworth, A. J., DeBruyn, J. M., Allen, F. L., Radosevich, M., and Owens, P. R.: Microbial community structure is affected
416 by cropping sequences and poultry litter under long-term no-tillage, *Soil Biol. Biochem.*, 114, 210–219,
417 <https://doi.org/10.1016/j.soilbio.2017.07.019>, 2017.

- 418 Bai, Z. G., Thomas, C., Ruiperez, G. M., Batjes, N. H., Mäder, P., Bünemann, E. K., de Goede, R., Brussaard, L., Xu, M.,
419 Ferreira, C. S. S., Reintam, E., Fan, H., Mihelič, R., Glavan, M., and Tóth, Z.: Effects of agricultural management
420 practices on soil quality: a review of long-term experiments for Europe and China, *Agric., Ecosyst. Environ.*, 265, 1–7,
421 <https://doi.org/10.1016/j.agee.2018.05.028>, 2018.
- 422 Blanco-Canqui, H., and Lal, R.: Soil structure and organic carbon relationships following 10 years of wheat straw management
423 in no-till, *Soil Tillage Res.*, 95, 240–254, <https://doi.org/10.1016/j.still.2007.01.004>, 2007.
- 424 Brockett, B. F., Prescott, C. E., and Grayston, S. J.: Soil moisture is the major factor influencing microbial community structure
425 and enzyme activities across seven biogeoclimatic zones in western Canada, *Soil Biol. Biochem.*, 44, 9–20,
426 <https://doi.org/10.1016/j.soilbio.2011.09.003>, 2012.
- 427 Bu, R., Ren, T., Lei, M., Liu, B., Li, X., Cong, R., and Lu, J.: Tillage and straw-returning practices effect on soil dissolved
428 organic matter, aggregate fraction and bacteria community under rice-rice-rapeseed rotation system, *Agric., Ecosyst.*
429 *Environ.*, 287, 106681, <https://doi.org/10.1016/j.agee.2019.106681>, 2020.
- 430 Calleja-Cervantes, M. E., Fernández-González, A. J., Irigoyen, I., Fernández-López, M., Aparicio-Tejo, P. M., and Menéndez,
431 S.: Thirteen years of continued application of composted organic wastes in a vineyard modify soil quality characteristics,
432 *Soil Biol. Biochem.*, 90, 241–254, <https://doi.org/10.1016/j.soilbio.2015.07.002>, 2015.
- 433 Cao, Y., Sun, H., Zhang, J., Chen, G., Zhu, H., Zhou, S., and Xiao, H.: Effects of wheat straw addition on dynamics and fate
434 of nitrogen applied to paddy soils, *Soil Tillage Res.*, 178, 92–98, <https://doi.org/10.1016/j.still.2017.12.023>, 2018.
- 435 Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L.,
436 Bauer, M., Gormley, N., Gilbert, J. A., Smith, G., and Knight, R.: Ultra-high-throughput microbial community analysis
437 on the Illumina HiSeq and MiSeq platforms, *ISME J.*, 6, 1621–1624, <https://doi.org/10.1038/ismej.2012.8>, 2012.
- 438 Chen, J., Wu, Q., Li, S., Ge, J., Liang, C., Qin, H., Xu, Q., and Fuhrmann, J. J.: Diversity and function of soil bacterial
439 communities in response to long-term intensive management in a subtropical bamboo forest, *Geoderma*, 354, 113894,
440 <https://doi.org/10.1016/j.geoderma.2019.113894>, 2019.
- 441 Chen, S., Qi, G., Ma, G., and Zhao, X.: Biochar amendment controlled bacterial wilt through changing soil chemical properties
442 and microbial community, *Microbiol. Res.*, 231, 126373, <https://doi.org/10.1016/j.micres.2019.126373>, 2020a.
- 443 Chen, Z., Wang, H., Liu, X., Zhao, X., Lu, D., Zhou, J., and Li, C.: Changes in soil microbial community and organic carbon
444 fractions under short-term straw return in a rice-wheat cropping system, *Soil Tillage Res.*, 165, 121–127,
445 <https://doi.org/10.1016/j.still.2016.07.018>, 2017.
- 446 Dai, X., Zhou, W., Liu, G., Liang, G., He, P., and Liu, Z.: Soil C/N and pH together as a comprehensive indicator for evaluating
447 the effects of organic substitution management in subtropical paddy fields after application of high-quality amendments,
448 *Geoderma*, 337, 1116–1125, <https://doi.org/10.1016/j.geoderma.2018.11.023>, 2019.
- 449 Dong, W., Zhang, X., Wang, H., Dai, X., Sun, X., Qiu, W., and Yang, F.: Effect of different fertilizer application on the soil
450 fertility of paddy soils in red soil region of southern China, *PLoS One*, 7, 44504,
451 <https://doi.org/10.1371/journal.pone.0044504>, 2012.

452 Duval, M. E., Galantini, J. A., Capurro, J. E., and Martinez, J. M.: Winter cover crops in soybean monoculture: Effects on soil
453 organic carbon and its fractions, *Soil Tillage Res.*, 161, 95–105, <https://doi.org/10.1016/j.still.2016.04.006>, 2016.

454 Fan, X., and Xing, P.: The vertical distribution of sediment archaeal community in the “black bloom” disturbing Zhushan bay
455 of lake Taihu, *Archaea*, 8232135, <http://doi.org/10.1155/2016/8232135>, 2016.

456 Fierer, N., Schimel, J. P., and Holden, P. A.: Variations in microbial community composition through two soil depth profiles,
457 *Soil Biol. Biochem.*, 35, 167–176, [https://doi.org/10.1016/S0038-0717\(02\)00251-1](https://doi.org/10.1016/S0038-0717(02)00251-1), 2003.

458 Fierer, N., Jackson, J. A., Vilgalys, R., and Jackson, R. B.: Assessment of soil microbial community structure by use of taxon-
459 specific quantitative PCR assays, *Appl. Environ. Microbiol.*, 71, 4117–4120, [https://doi.org/10.1128/AEM.71.7.4117-](https://doi.org/10.1128/AEM.71.7.4117-4120.2005)
460 4120.2005, 2005.

461 Fierer, N., Bradford, M. A., and Jackson, R. B.: Toward an ecological classification of soil bacteria, *Ecology*, 88, 1354–1364,
462 <https://doi.org/10.1890/05-1839>, 2007.

463 Fierer, N., Lauber, C. L., Ramirez, K. S., Zaneveld, J., Bradford, M. A., and Knight, R.: Comparative metagenomic,
464 phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients, *ISME J.*, 6, 1007–1017,
465 <https://doi.org/10.1038/ismej.2011.159>, 2012.

466 Fierer, N., and Jackson, R. B.: The diversity and biogeography of soil bacterial communities, *Proc. Natl. Acad. Sci. USA*, 103,
467 626–631, <https://doi.org/10.1073/pnas.0507535103>, 2006.

468 Foesel, B.U., Rohde, M., and Overmann, J.: *Blastocatella fastidiosa* gen. nov., sp. nov., isolated from semiarid savanna soil–
469 the first described species of Acidobacteria subdivision 4, *Syst. Appl. Microbiol.*, 36, 82–89,
470 <https://doi.org/10.1016/j.syapm.2012.11.002>, 2013.

471 Gao, Q., Ma, L., Fang, Y., Zhang, A., Li, G., Wang, J., Wu, D., Wu, W., and Du, Z.: Conservation tillage for 17 years alters
472 the molecular composition of organic matter in soil profile, *Sci. Total Environ.*, 762, 143116,
473 <https://doi.org/10.1016/j.scitotenv.2020.143116>, 2021.

474 Garcia-Fraile, P., Benada, O., Cajthaml, T., Baldrian, P., and Llado, S.: *Terracidiphilus gabretensis* gen. nov., sp nov., an
475 abundant and active forest soil Acidobacteria important in organic matter transformation, *Appl. Environ. Microbiol.*, 82,
476 560–569, <https://doi.org/10.1128/AEM.03353-15>, 2016.

477 Giller, K. E., Witter, E., Corbeels, M., and Tittonell, P.: Conservation agriculture and smallholder farming in Africa: The
478 heretics’ view, *Field Crop. Res.*, 114, 23–34, <https://doi.org/10.1016/j.fcr.2009.06.017>, 2009.

479 Guo, J. H., Liu, X. J., Zhang, Y., Shen, J. L., Han, W. X., Zhang, W. F., Christie, P., Goulding, K. W. T., Vitousek, P. M., and
480 Zhang, F. S.: Significant acidification in major Chinese croplands, *Science*, 327, 1008–1010,
481 <https://doi.org/10.1126/science.1182570>, 2010.

482 Hamamura, N., Olson, S. H., Ward, D. M., and Inskeep, W. P.: Microbial population dynamics associated with crude-oil
483 biodegradation in diverse soils, *Appl. Environ. Microbiol.*, 72, 6316–6324, <https://doi.org/10.1128/AEM.01015-06>, 2006.

484 Hao, M., Hu, H., Liu, Z., Dong, Q., Sun, K., Feng, Y., Li, G., and Ning, T.: Shifts in microbial community and carbon
485 sequestration in farmland soil under long-term conservation tillage and straw returning, *Appl. Soil Ecol.*, 136, 43–54,
486 <https://doi.org/10.1016/j.apsoil.2018.12.016>, 2019.

487 Hobara, S., Osono, T., Hirose, D., Noro, K., Hirota, M., and Benner, R.: The roles of microorganisms in litter decomposition
488 and soil formation, *Biogeochemistry*, 118, 471–486, <https://doi.org/10.1007/s10533-014-9956-3>, 2014.

489 Hou, D., Bolan, N. S., Tsang, D. C. W., Kirkham, M. B., and O'Connor, D.: Sustainable soil use and management: an
490 interdisciplinary and systematic approach, *Sci. Total Environ.*, 729, 138961,
491 <https://doi.org/10.1016/j.scitotenv.2020.138961>, 2020.

492 Hu, X., Liu, J., Liang, A., Li, L., Yao, Q., Yu, Z., Li, Y., Jin, J., Liu, X., Wang, G.: Conventional and conservation tillage
493 practices affect soil microbial co-occurrence patterns and are associated with crop yields.

494 Huang, R., Wang, Y., Liu, J., Li, J., Xu, G., Luo, M., Xu, C., Ci, E., and Gao, M.: Variation in N₂O emission and N₂O related
495 microbial functional genes in straw- and biochar-amended and non-amended soils, *Appl. Soil Ecol.*, 137, 57–68,
496 <https://doi.org/10.1016/j.apsoil.2019.01.010>, 2019.

497 Jena, P. R.: Can minimum tillage enhance productivity? Evidence from smallholder farmers in Kenya, *J. Cleaner Prod.*, 218,
498 465–475, <https://doi.org/10.1016/j.jclepro.2019.01.278>, 2019.

499 Ji, Y., Liu, P., and Conrad, R.: Response of fermenting bacterial and methanogenic archaeal communities in paddy soil to
500 progressing rice straw degradation, *Soil Biol. Biochem.*, 124, 70–80, <https://doi.org/10.1016/j.soilbio.2018.05.029>, 2018.

501 Jin, J.: Effects of different management practices on the soil–water balance and crop yield for improved dryland farming in
502 the Chinese Loess Plateau, *Soil Tillage Res.*, 96, 131–144, <https://doi.org/10.1016/j.still.2007.05.002>, 2007.

503 Joa, J. H., Weon, H. Y., Hyun, H. N., Jeun, Y. C., and Koh, S. W.: Effect of long-term different fertilization on bacterial
504 community structures and diversity in citrus orchard soil of volcanic ash, *J. Microbiol.*, 52, 995–1001,
505 <https://doi.org/10.1007/s12275-014-4129-6>, 2014.

506 Karthikeyan, L., Chawla, I., and Mishra, A. K.: A review of remote sensing applications in agriculture for food security: Crop
507 growth and yield, irrigation, and crop losses, *J. Hydrol.*, 586, 124905, <https://doi.org/10.1016/j.jhydrol.2020.124905>,
508 2020.

509 Kassam, A., Li, H., Niino, Y., Friedrich, T., Jin, H., and Wang, X.: Current status, prospect and policy and institutional support
510 for Conservation Agriculture in the Asia-Pacific region, *Int. J. Agric. Biol. Eng.*, 7, 1–13,
511 <https://doi.org/10.3965/j.ijabe.20140705.001>, 2014.

512 Kopittke, P. M., Menzies, N. W., Wang, P., McKenna, B. A., and Lombi, E.: Soil and the intensification of agriculture for
513 global food security, *Environ. Int.*, 132, 105078, <https://doi.org/10.1016/j.envint.2019.105078>, 2019.

514 Lauber, C. L., Hamady, M., Knight, R., and Fierer, N.: Pyrosequencing-based assessment of soil pH as a predictor of soil
515 bacterial community structure at the continental scale, *Appl. Environ. Microbiol.*, 75, 5111–5120,
516 <https://doi.org/10.1128/AEM.00335-09>, 2009.

- 517 Li, C., Yan, K., Tang, L., Jia, Z., and Li, Y.: Change in deep soil microbial communities due to long-term fertilization, *Soil*
518 *Biol. Biochem.*, 75, 264–272, <https://doi.org/10.1016/j.soilbio.2014.04.023>, 2014.
- 519 Li, Q., Li, A., Dai, T., Fan, Z., Luo, Y., Li, S., Yuan, D., Zhao, B., Tao, Q., Wang, C., Li, B., Gao, X., Li, Y., Li, H., and
520 Wilson, J. P.: Depth-dependent soil organic carbon dynamics of croplands across the Chengdu Plain of China from the
521 1980s to the 2010s, *Global Change Biol.*, 26, 4134–4146, <https://doi.org/10.1111/gcb.15110>, 2020.
- 522 Li, S., Zhang, S., Pu, Y., Li, T., Xu, X., Jia, Y., Deng, O., and Gong, G.: Dynamics of soil labile organic carbon fractions and
523 C-cycle enzyme activities under straw mulch in Chengdu Plain, *Soil Tillage Res.*, 155, 289–297,
524 <https://doi.org/10.1016/j.still.2015.07.019>, 2016.
- 525 Li, X., Sun, J., Wang, H., Li, X., Wang, J., and Zhang, H.: Changes in the soil microbial phospholipid fatty acid profile with
526 depth in three soil types of paddy fields in China, *Geoderma*, 290, 69–74, <https://doi.org/10.1016/j.geoderma.2016.11.006>,
527 2017.
- 528 Li, Y., Li, Y., Chang, S.X., Yang, Y., Fu, S., and Jiang, P.: Biochar reduces soil heterotrophic respiration in a subtropical
529 plantation through increasing soil organic carbon recalcitrancy and decreasing carbon-degrading microbial activity, *Soil*
530 *Biol. Biochem.*, 122, 173–185, <https://doi.org/10.1016/j.soilbio.2018.04.019>, 2018.
- 531 Liang, B., Ma, C., Fan, L., Wang, Y., and Yuan, Y.: Soil amendment alters soil physicochemical properties and bacterial
532 community structure of a replanted apple orchard, *Microbiol. Res.*, 216, 1–11,
533 <https://doi.org/10.1016/j.micres.2018.07.010>, 2018.
- 534 Ling, N., Chen, D., Guo, H., Wei, J., Bai, Y., Shen, Q., and Hu, S.: Differential responses of soil bacterial communities to
535 long-term N and P inputs in a semi-arid steppe, *Geoderma*, 292, 25–33, <https://doi.org/10.1016/j.geoderma.2017.01.013>,
536 2017.
- 537 Liu, Q., Liu, G., Huang, C., and Li, H.: Soil physicochemical properties associated with quasi-circular vegetation patches in
538 the Yellow River Delta, China, *Geoderma*, 337, 202–214, <https://doi.org/10.1016/j.geoderma.2018.09.021>, 2019.
- 539 Lu, R. (Eds): *Methods of soil and agro-chemistry analysis*, China Agricultural Science and Technology Press, Beijing, China,
540 2000. (in Chinese).
- 541 Lupwayi, N. Z., Lafond, G. P., Ziadi, N., and Grant, C. A.: Soil microbial response to nitrogen fertilizer and tillage in barley
542 and corn, *Soil Tillage Res.*, 118, 139–146, <https://doi.org/10.1016/j.still.2011.11.006>, 2012.
- 543 Lupwayi, N. Z., Larney, F. J., Blackshaw, R. E., Kanashiro, D. A., Pearson, D. C., and Petri, R. M.: Pyrosequencing reveals
544 profiles of soil bacterial communities after 12 years of conservation management on irrigated crop rotations, *Appl. Soil*
545 *Ecol.*, 121, 65–73, <https://doi.org/10.1016/j.apsoil.2017.09.031>, 2017.
- 546 Maarastawi, S. A., Frindte, K., Geer, R., Kröber, E., and Knief, C.: Temporal dynamics and compartment specific rice straw
547 degradation in bulk soil and the rhizosphere of maize, *Soil Biol. Biochem.*, 127, 200–212,
548 <https://doi.org/10.1016/j.soilbio.2018.09.028>, 2018.
- 549 Magoc, T., and Salzberg, S. L.: FLASH: fast length adjustment of short reads to improve genome assemblies, *Bioinformatics*,
550 27, 2957–2963, <https://doi.org/10.1093/bioinformatics/btr507>, 2011.

551 Menéndez-Serra, M., Triadó-Margarit, X., Castañeda, C., Herrero, J., and Casamayor, E. O.: Microbial composition, potential
552 functional roles and genetic novelty in gypsum-rich and hypersaline soils of Monegros and Gallocanta (Spain), *Sci. Total*
553 *Environ.*, 650, 343–353, <https://doi.org/10.1016/j.scitotenv.2018.09.050>, 2019.

554 Nan, Q., Wang, C., Wang, H., Yi, Q., Liang, B., Xu, J., and Wu, W.: Biochar drives microbially-mediated rice production by
555 increasing soil carbon, *J. Hazard. Mater.*, 387, 121680, <https://doi.org/10.1016/j.jhazmat.2019.121680>, 2020.

556 Nie, Y., Wang, M., Zhang, W., Ni, Z., Hashidoko, Y., and Shen, W.: Ammonium nitrogen content is a dominant predictor of
557 bacterial community composition in an acidic forest soil with exogenous nitrogen enrichment, *Sci. Total Environ.*, 624,
558 407–415, <https://doi.org/10.1016/j.scitotenv.2017.12.142>, 2018.

559 Palm, C., Blanco-Canqui, H., Declerck, F., Gatere, L., and Grace, P.: Conservation agriculture and ecosystem services: An
560 overview, *Agric., Ecosyst. Environ.*, 187, 87–105, <https://doi.org/10.1016/j.agee.2013.10.010>, 2014.

561 Parks, D. H., Tyson, G. W., Hugenholtz, P., and Beiko, R. G.: STAMP: statistical analysis of taxonomic and functional profiles,
562 *Bioinformatics*, 30, 3123–3124, <https://doi.org/10.1093/bioinformatics/btu494>, 2014.

563 Pastorelli, R., Vignozzi, N., Landi, S., Piccolo, R., Orsini, R., Seddaiu, G., Roggero, P. P., Pagliari, M.: Consequences on
564 macroporosity and bacterial diversity of adopting a no-tillage farming system in a clayish soil of Central Italy, *Soil Biol.*
565 *Biochem.*, 66, 78–93, <https://doi.org/10.1016/j.soilbio.2013.06.015>, 2013.

566 Peng, X., and Wang, W.: Stoichiometry of soil extracellular enzyme activity along a climatic transect in temperate grasslands
567 of northern China, *Soil Biol. Biochem.*, 98, 74–84, <https://doi.org/10.1016/j.soilbio.2016.04.008>, 2016.

568 Pittelkow, C. M., Liang, X., Linquist, B. A., van Groenigen, K. J., Lee, J., Lund, M. E., Gestel, N., Six, J., Venterea, R. T.,
569 van Kessel, C.: Productivity limits and potentials of the principles of conservation agriculture, *Nature*, 517, 368–368,
570 <https://doi.org/10.1038/nature13809>, 2015.

571 Qiu, Y., Lv, W., Wang, X., Xie, Z., and Wang, Y.: Long-term effects of gravel mulching and straw mulching on soil
572 physicochemical properties and bacterial and fungal community composition in the Loess Plateau of China, *Eur. J. Soil*
573 *Biol.*, 98, 103188, <https://doi.org/10.1016/j.ejsobi.2020.103188>, 2020.

574 Quast, C., Priesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glockner, F. O.: The SILVA ribosomal
575 RNA gene database project: improved data processing and web-based tools, *Nucleic Acids Res.*, 41, D590–D596,
576 <https://doi.org/10.1093/nar/gks1219>, 2013.

577 Segal, L. M., Miller, D. N., Mcghee, R. P., Loecke, T. D., Cook, K. L., and Shapiro, C. A.: Bacterial and archaeal ammonia
578 oxidizers respond differently to long-term tillage and fertilizer management at a continuous maize site, *Soil Tillage*
579 *Res.*, 168, 110–117, <https://doi.org/10.1016/j.still.2016.12.014>, 2017.

580 Shang, Q., Yang, X., Gao, C., Wu, P., Liu, J., Xu, Y., Shen, Q., Zou, J., and Guo, S.: Net annual global warming potential and
581 greenhouse gas intensity in Chinese double rice-cropping systems: a 3-year field measurement in long-term fertilizer
582 experiments, *Global Change Biol.*, 17, 2196–2210, <https://doi.org/10.1111/j.1365-2486.2010.02374.x>, 2011.

583 Singh, U., Choudhary, A. K., and Sharma, S.: Comparative performance of conservation agriculture vis-a-vis organic and
584 conventional farming, in enhancing plant attributes and rhizospheric bacterial diversity in *Cajanus cajan*: A field study,
585 *Eur. J. Soil Biol.*, 99, 103197, <https://doi.org/10.1016/j.ejsobi.2020.103197>, 2020.

586 Song, Y., Liu, C., Wang, X., Ma, X., Jiang, L., Zhu, J., Gao, J., and Song, C.: Microbial abundance as an indicator of soil
587 carbon and nitrogen nutrient in permafrost peatlands, *Ecol. Indic.*, 115, 106362,
588 <https://doi.org/10.1016/j.ecolind.2020.106362>, 2020.

589 Stowe, D. C., Lamhamedi, M. S., Carles, S., Fecteau, B., Margolis, H. A., Renaud, M., and Bernier, P. Y.: Managing irrigation
590 to reduce nutrient leaching in containerized white spruce seedling production, *New For.*, 40, 185–204,
591 <https://doi.org/10.1007/s11056-010-9193-0>, 2010.

592 Sun, R., Zhang, X., Guo, X., Wang, D., and Chu, H.: Bacterial diversity in soils subjected to long-term chemical fertilization
593 can be more stably maintained with the addition of livestock manure than wheat straw, *Soil Biol. Biochem.*, 88, 9–18,
594 <https://doi.org/10.1016/j.soilbio.2015.05.007>, 2015.

595 Tellez-Rio, A., García-Marco, S., Navas, M., López-Solanilla, E., Tenorio, J. L., and Vallejo, A.: N₂O and CH₄ emissions from
596 a fallow–wheat rotation with low N input in conservation and conventional tillage under a Mediterranean agroecosystem,
597 *Sci. Total Environ.*, 1, 85–94, <https://doi.org/10.1016/j.scitotenv.2014.11.041>, 2015.

598 Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., and Locey, K. J.: A communal catalogue reveals earth's
599 multiscale microbial diversity, *Nature*, 551, 457–463, <https://doi.org/10.1038/nature24621>, 2017.

600 van Leeuwen, J. P., Djukic, I., Bloem, J., Lehtinen, T., Hemerik, L., de Ruiter, P. C., and Lair, G. J.: Effects of land use on
601 soil microbial biomass, activity and community structure at different soil depths in the Danube floodplain, *Eur. J. Soil
602 Biol.*, 79, 14–20, <https://doi.org/10.1016/j.ejsobi.2017.02.001>, 2017.

603 Wagg, C., Bender, S. F., Widmer, F., and van der Heijden, M. G. A.: Soil biodiversity and soil community composition
604 determine ecosystem multifunctionality, *Proc. Natl. Acad. Sci. USA*, 111, 5266–5270,
605 <https://doi.org/10.1073/pnas.1320054111>, 2014.

606 Wang, D., Li, T., Huang, K., He, X., and Zhang, X.: Roles and correlations of functional bacteria and genes in the start-up of
607 simultaneous anammox and denitrification system for enhanced nitrogen removal, *Sci. Total Environ.*, 655, 1355–1363,
608 <https://doi.org/10.1016/j.scitotenv.2018.11.321>, 2019a.

609 Wang, H., Guo, Q., Li, X., Li, X., Yu, Z., Li, X., Yang, T., Su, Z., Zhang, H., and Zhang, C.: Effects of long-term no-tillage
610 with different straw mulching frequencies on soil microbial community and the abundances of two soil-borne pathogens,
611 *Appl. Soil Ecol.*, 148, 103488, <https://doi.org/10.1016/j.apsoil.2019.103488>, 2020.

612 Wang, J., Wang, D., Zhang, G., and Wang, C.: Effect of wheat straw application on ammonia volatilization from urea applied
613 to a paddy field, *Nutr. Cycling Agroecosyst.*, 94, 73–84, <https://doi.org/10.1007/s10705-012-9527-8>, 2012.

614 Wang, L., Yuan, X., Liu, C., Li, Z., Chen, F., Li, S., Wu, L., and Liu, Y.: Soil C and N dynamics and hydrological processes
615 in a maize-wheat rotation field subjected to different tillage and straw management practices, *Agric., Ecosyst. Environ.*,
616 285, 106616, <https://doi.org/10.1016/j.agee.2019.106616>, 2019b.

617 Wang, W., Akhtar, K., Ren, G., Yang, G., Feng, Y., and Yuan, L.: Impact of straw management on seasonal soil carbon dioxide
618 emissions, soil water content, and temperature in a semi-arid region of China, *Sci. Total Environ.*, 652, 1–482,
619 <https://doi.org/10.1016/j.scitotenv.2018.10.207>, 2019c.

620 Wegner, C.E., and Liesack, W.: Microbial community dynamics during the early stages of plant polymer breakdown in paddy
621 soil, *Environ. Microbiol.*, 18, 2825–2842, <https://doi.org/10.1111/1462-2920.12815>, 2016.

622 Wolff, D., Krah, D., Dötsch, A., Ghattas, A. K., Wick, A., and Ternes, T. A.: Insights into the variability of microbial
623 community composition and micropollutant degradation in diverse biological wastewater treatment systems, *Water Res.*,
624 143, 313–324, <https://doi.org/10.1016/j.watres.2018.06.033>, 2018.

625 Xu, S., Hou, P., Xue, L., Wang, S., and Yang, L.: Treated domestic sewage irrigation significantly decreased the CH₄, N₂O
626 and NH₃ emissions from paddy fields with straw incorporation, *Atmos. Environ.*, 169, 1–10,
627 <https://doi.org/10.1016/j.atmosenv.2017.09.009>, 2017.

628 Ye, J., Joseph, S. D., Ji, M., Nielsen, S., Mitchell, D. R. G., Donne, S., Horvat, J., Wang, J., Munroe, P., and Thomas, T.:
629 Chemolithotrophic processes in the bacterial communities on the surface of mineral-enriched biochars, *ISME J.*, 11,
630 1087–1011, <https://doi.org/10.1038/ismej.2016.187>, 2017.

631 Yu, H., Ling, N., Wang, T., Zhu, C., Wang, Y., Wang, S., and Gao, Q.: Responses of soil biological traits and bacterial
632 communities to nitrogen fertilization mediate maize yields across three soil types, *Soil Tillage Res.*, 185, 61–69,
633 <https://doi.org/10.1016/j.still.2018.08.017>, 2019.

634 Zhang, D., Ji, X., Meng, Z., Qi, W., and Qiao, K.: Effects of fumigation with 1,3-dichloropropene on soil enzyme activities
635 and microbial communities in continuous-cropping soil, *Ecotoxicol. Environ. Saf.*, 169, 730–736,
636 <https://doi.org/10.1016/j.ecoenv.2018.11.071>, 2019.

637 Zhang, P., Chen, X., Wei, T., Yang, Z., Jia, Z., Yang, B., Han, Q., and Ren, X.: Effects of straw incorporation on the soil
638 nutrient contents, enzyme activities, and crop yield in a semiarid region of China, *Soil Tillage Res.*, 160, 65–72,
639 <https://doi.org/10.1016/j.still.2016.02.006>, 2016.

640 Zhao, C., Song, Z. L., Zhuang, D. H., Wang, J., Xie, S., and Liu, G. B.: Urea fertilization decreases soil bacterial diversity, but
641 improves microbial biomass, respiration, and N-cycling potential in a semiarid grassland, *Biol. Fertil. Soils*, 55, 229–242,
642 <https://doi.org/10.1007/s00374-019-01344-z>, 2019.

643 Zhao, Y., Wang, M., Hu, S., Zhang, X., Ouyang, Z., Zhang, G., Huang, B., Zhao, S., Wu, J., Xie, D., Zhu, B., Yu, D., Pan, X.,
644 Xu, S., and Shi, X.: Economics- and policy-driven organic carbon input enhancement dominates soil organic carbon
645 accumulation in Chinese croplands, *Proc. Natl. Acad. Sci. USA*, 115, 4045–4050,
646 <https://doi.org/10.1073/pnas.1700292114>, 2018.

647 Zhou, Z., Shen, Y., Du, C., Zhou, J., Qin, Y., and Wu, Y.: Economic and soil environmental benefits of using controlled-
648 release bulk blending urea in the North China Plain, *Land Degrad. Dev.*, 28, 2370–2379, <https://doi.org/10.1002/ldr.2767>,
649 2017.

650 Zhou, Z., Chen, K., Yu, H., Chen, Q., Wu, F., Zeng, X., Tu, S., Qin, Y., Meakin, R., and Fan, X.: Changes in tea performance
651 and soil properties after three years of polyhalite application, *Agron. J.*, 111, 1967–1976,
652 <https://doi.org/10.2134/agronj2018.06.0393>, 2019a.

653 Zhou, Z., Zeng, X., Chen, K., Li, Z., Guo, S., Shangguan, Y., Tu, S., and Qin, Y.: Long-term straw mulch effects on crop yields
654 and soil organic carbon fractions at different depths under a no-till system on the Chengdu Plain, China, *J. Soils Sediments*,
655 19, 2143–2152, <https://doi.org/10.1007/s11368-018-02234-x>, 2019b.