SOIL
Discussions

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1 **Changes in soil physicochemical properties and bacterial**

2 **communities among different soil depths after long-term straw**

3 **mulching under a no-till system**

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21 **Abstract**

22 Conservation tillage has attracted increasing attention over recent decades, mainly due

23 to its benefits in improving soil organic matter content and reducing soil erosion. Under

24 intensive conventional tillage systems, some studies have focused on the responses of

25 soil properties in the topsoil to straw retention. However, long-term straw mulching

26 effects on soil physicochemical properties and bacterial communities among different

27 soil depths under a no-till system are still obscure. One twelve-year experiment was

28 conducted that included straw removal (CK) and straw mulching (SM) treatments. Soil

29 samples were collected at 0–5, 5–10, 10–20, and 20–30 cm soil depths. Most soil

30 physicochemical properties and the relative abundances of bacterial phyla were varied

31 with soil depth. Compared with CK, SM increased soil total nitrogen and organic

32 carbon, available phosphorus and potassium, dissolved organic carbon and nitrogen,

33 and water content. SM increased soil bacterial abundance but reduced the Shannon

34 diversity of the bacterial community at 0–5 cm depth. SM increased the relative

35 abundances of Proteobacteria, Bacteroidetes, and Acidobacteria but reduced those of

36 Actinobacteria, Chloroflexi, and Cyanobacteria. SM had different effects on the relative

37 abundances of some C- and N-cycling genera, for instance, increasing *Rhodanobacter*,

38 *Rhizomicrobium*, and *Terracidiphilus*, and reducing *Anaeromyxobacter*,

39 *Mycobacterium*, and *Syntrophobacter*. A principal coordinate analysis indicated that

40 SM largely affected soil bacterial communities at topsoil depth. Soil pH and different

41 nitrogen and organic carbon fractions were the major drivers shaping soil bacterial

42 community. Overall, straw mulch is highly recommended for use under a no-till system

43 because of its benefits to soil fertility and bacterial abundance. However, inorganic

44 nitrogen fertilizer levels may be reduced under straw mulching to maintain or increase

45 soil bacterial Shannon diversity in future studies.

46 **Keywords:** bacterial community composition, conservation tillage, Illumina

47 sequencing, physicochemical properties, soil depth, straw mulching

48

1 Introduction

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

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

82   al., 2019b). Besides its effects on crop yield, understanding soil physicochemical

83   properties and bacterial community changes is also an important aspect of assessing the

84   environmental effects of straw mulching.

85   Soil physicochemical properties are important contributors to soil fertility, and the

86   latter is a critical factor determining crop productivity and agriculture sustainability

87   (Liu et al., 2019). Since straw contains large amounts of carbon and several mineral

88   elements, previous studies have shown that straw mulching increased soil total organic

89   carbon and its fractions, several soil enzymes, and other physicochemical properties in

90   the soil surface layer (Akhtar et al., 2018; Duval et al., 2016; Zhou et al., 2019b). Many

91   studies have focused on these physicochemical properties in the topsoil under

92   conventional tillage system since the topsoil provides large amounts of nutrients to

93   plants (Dai et al., 2019; Wang et al., 2019b; Zhou et al., 2019a). However, soil

94   physicochemical properties in the subsurface should also be considered since some

95   nutrients could move from topsoil to deeper soil during irrigation and rainfall (Blanco-

96   Canqui and Lal, 2007; Stowe et al., 2010). The responses of soil physicochemical

97   properties to soil depth varied across different regions (Li et al., 2017b; Peng and Wang,

98   2016). Li et al. (2017b) found that soil total organic carbon (TOC), total nitrogen (TN),

99   dissolved organic carbon (DOC), pH, and water content (WC) differed significantly

100   among six depths, while NH_4^+-N concentration and the C/N ratio did not change

101   significantly with soil depth. Similarly, TOC, TN and total phosphorus (TP) decreased

102   significantly with soil depth, but pH did not change consistently across the three steppes

103   in Peng and Wang (2016). These studies focused only on heavy tillage or grassland, but

104   the variation in physicochemical properties among different soil depths after long-term

105   straw mulching under a no-till system is still unclear, since the no-till practice did little

106   disturbance to soil, and it was quite different from the heavy tillage in conventional

107   agriculture.

108   Changes in nutrition distribution along soil depth would affect not only soil

109   fertility but also soil bacterial communities. Previous reports have suggested that soil

110   bacterial communities are highly associated with soil environmental factors (Bowles et

111   al., 2014; Li et al., 2017a; Schreiter et al., 2014; Sun et al., 2016). Recently, soil bacterial

112   communities have attracted great interest, and they are often used as sensitive indicators

113   of soil quality, especially in agricultural systems (Ashworth et al., 2017). They

114   participate in soil ecological processes and play a vital role in soil carbon and nutrient

115   cycling (Hobara et al., 2014; Thompson et al., 2017), crop growth, and greenhouse gas

116   release (Tellez-Rio et al., 2015). The responses of soil bacterial abundance to straw

117   mulching were inconsistent in different studies in the topsoil. Zhang et al. (2017) found

118   that general, gram-positive, and gram-negative bacteria increased under straw mulching

119   in one paddy soil in northeast China. Chen et al. (2017) proposed that straw return

120   significantly increased bacterial biomass in one region but had no significant effects in

121   the other two regions. Straw mulching can also change bacterial community

122   composition (Bu et al., 2020; Qiu et al., 2020). Actinobacteria were enriched in straw

123   mulch (SM) soils, and pH and soil WC are key factors driving soil bacterial community

124   structure changes in the Loess Plateau of China (Qiu et al., 2020). Bu et al. (2020)

125   reported that straw return significantly increased the relative abundance of

126   Proteobacteria and decreased the relative abundance of Acidobacteria, and the positive

127   effect of straw mulching on the soil bacterial community structure probably resulted

128   from the increased soil organic carbon fractions. However, soil microbial communities,

129   including bacterial communities, varied with soil depth (Fierer et al., 2003; van

130   Leeuwen et al., 2017), and soil microbes in subsoil demonstrated important effects on

131   soil formation, ecosystem biochemistry and maintaining groundwater quality (Li et al.,

132   2014). For instance, the abundances of gram-positive bacteria increased with depth,

133   while the abundances of gram-negative bacteria generally declined with soil depth

134   (Fierer et al., 2003). Bacterial biomass significantly decreased with soil depth in forest

135   and grassland but tended to only decrease in arable land as assessed using the

136   phospholipid method (van Leeuwen et al., 2017). Apparently, straw management and

137   soil depth are two key factors influencing the soil bacterial community. Unfortunately,

138   no detailed information, especially by using high-throughput sequencing analysis,

139   about soil bacterial community changes in response to straw mulching among different

140   soil depths under no-till systems has been obtained. Moreover, little is known about the

141   relationship between these communities and the soil physicochemical properties in

142   deeper soils after long-term straw mulching.

143   Rice-wheat rotation is a major cropping system in China, and approximately 80

144   million tons of crop straw are produced annually in southwestern China (Li et al., 2016;

145   Zhou et al., 2019b). Although we determined some soil organic carbon fractions under

146   a no tillage regime in this system (Zhou et al., 2019b), little is known about how other

147   soil physicochemical parameters vary with soil depth. In addition, how soil bacteria

148   responded to long-term straw mulching and which soil physicochemical factors had the

149   greatest effect on shaping bacterial communities among different depths remain poorly

150   understood. In this study, we hypothesized that (1) compared with straw removal, straw

151   mulching will increase most soil physicochemical parameters, which will decline with

152   increasing soil depth; (2) straw mulching and depth will have significant effects on the

153   soil bacterial community; and (3) the key soil physicochemical properties shaping

154   bacterial communities will be different at different depths. To answer these questions,

155   soil samples were collected from four soil depths, which had been subjected to two

156   straw management programs under a 12-year no-till regime in southwestern China.

157   Then, soil physicochemical properties, bacterial abundances (based on quantitative

158   PCR, qPCR), and bacterial community compositions (using Illumina high-throughput

159   sequencing) were determined.

160   **2 Materials and methods**

161   **2.1 Experimental site and design**

162   A long-term field experiment was installed in 2005 in Guanghan, Sichuan Province,

163   China (31°08′38″ N, 104°29′45″ E). Before the experiment, the local agricultural soil

164   was seldom tilled due to the shortage of tillage machines. The soil had been cultivated

165   for a long period of time under the same agricultural cropping system, and consequently

166   the soil fertility heterogeneity was considered minimal. The soil is a fluvo-aquic soil

167   with loamy clay. The soil pH in 2005 was 5.54, and the TOC, TN, available nitrogen,

168   AP, and available potassium (AK) levels were 18.1 g kg^{-1}, 2.03 g kg^{-1}, 189.76 mg kg^{-1},

169   12.61 mg kg^{-1}, and 258.2 mg kg^{-1}, respectively.

170   No-till practice was conducted in both the rice and wheat seasons. Two treatments,

171   i.e., a control (CK, straw removal) and SM, with three replications under a no-till

172   regime were selected in this study. The straw was removed in the CK treatment, whereas

173   it was distributed over the soil surface without being chopped in the SM treatment. The

174   mulch consisted of approximately 8.5 t ha^{-1} rice straw and 6.0 t ha^{-1} wheat straw during

175   each year. The amounts of inorganic fertilizer added were equal in both treatments, and

176   they were manually broadcast over the plot soil surface without tillage. Other detailed

177   information about the experimental design was in our previous study (Zhou et al.,

178   2019b).

179   **2.2 Soil sampling**

180   Immediately after the wheat harvest in 2018, soil samples were collected at five points

181   in each plot. The samples were taken at soil depths of 0–5, 5–10, 10–20, and 20–30 cm.

182 Five subcores taken from the same depth were pooled to make one composite sample

183 for each plot. The mixed soil was passed through a 2-mm mesh and divided into three

184 parts: one was air-dried and used to measure some of the soil physicochemical

185 parameters; one was kept at 4 °C for soil NH_4^+–N, NO_3^-–N, DOC, and DON analysis;

186 and the third was stored at −80 °C until it was needed for soil bacterial community

187 analysis.

2.3 Soil physicochemical properties

189 Ten grams of fresh soil was extracted with 50 mL of 2 M KCl. The NH_4^+–N and NO_3^-–

190 N concentrations in the extracts were determined using a SAN++ Continuous Flow

191 Analyzer (Skalar, Breda, The Netherlands) (Lu, 2000). Inorganic nitrogen (IN) was the

192 sum of the NH_4^+–N and NO_3^-–N concentrations. The DOC and DON were extracted

193 with 0.5 M K_2SO_4. Then, a TOC analyzer (Multi N/C 2100; Analytic, Jena, Germany)

194 was used to determine their concentrations in centrifuged supernatant that had been

195 filtered through sterile 0.45 μm syringe filters (Zhou et al., 2019b). The soil WC was

196 measured using the oven-drying method (Akhtar et al., 2018). The air-dried soil samples

197 were analyzed for soil pH, TOC, TN, TP, TK, AP, and AK as described by Lu (2000).

2.4 DNA extraction and qPCR amplification

199 The soil DNA from 0.5 g of fresh soil was extracted using the Fast® DNA SPIN Kit

200 (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions

201 (Zhou et al., 2017). The extracted DNA was dissolved in 50 μL of double-distilled water,

202 and its quality and concentration were checked by a NanoDrop 2000 spectrophotometer

203 (Calleja-Cervantes et al., 2015). Then, the DNA samples were stored at –80°C until

204 further use. The qPCR was used to quantify bacterial abundances based on the 16S

205 rRNA gene, and the primers were 338F (5′-ACTCCT ACGGGAGGCAGCAG-3′) and

206 518R (5′-ATTACCGCGGCTGCTGG-3′) (Fierer et al., 2005). The qPCR procedure

207 was carried out according to Chen et al. (2019) with some modifications. PCR was

208 performed using a Bio-Rad CFX 96-well Thermocycler (Bio-Rad, Hercules, CA,

209 America). The reactions were performed in a 20 μL mixture containing 16.5 μL of 2 ×

210 SYBR Color qPCR Master Mix, 0.5 μM (0.8 μL) each primer, and 2 μL of DNA

211 template. The PCR conditions were as follows: 95 °C for 5 min; 40 cycles of 30 at

212 95 °C, 30 s at 58 °C and 40 s at 72 °C; and finally 10 min at 72 °C. All samples were

213 evaluated in triplicate. Standard curves were obtained using 10-fold serial dilutions of

214 linearized recombinant plasmids containing cloned 16S rDNA with known copy
215 numbers. Melting curve analysis was performed at the end of each qPCR run to check
216 the specificity of PCR products. PCR amplification efficiencies were between 96 % and
217 105 %, with R^2 values > 0.99.

2.5 16S rRNA amplification for Illumina sequencing and data processing

219 The primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-
220 CCGTCAATTCMTTTRAGTTT-3′) were used to amplify the V4–V5 regions of the
221 bacterial DNA (Caporaso et al., 2012). Detailed operational information can be found
222 in Zhang et al. (2019). The 16S rRNA sequences were analyzed on the I–Sanger Cloud
223 Platform (https://cloud.majorbio.com/). Raw sequences were merged using FLASH
224 version 1.2.7 (Magoc and Salzberg, 2011) and then processed using quantitative
225 insights into microbial ecology (QIIME v.1.9.0; http://www.qiime.org/) (Quast et al.,
226 2013). Poor-quality sequences (below an average quality score of 25) and short
227 sequences (< 200 bp) were removed. Primers were matched exactly, allowing 2
228 mismatch nucleotides, and reads with ambiguous bases were removed. Sequences with
229 overlaps longer than 10 bp were merged according to their overlap sequence. After this
230 step, 945,665 clean reads were obtained, with 30,241 to 58,191 reads per sample.
231 Operational taxonomic units (OTUs) were clustered at a similarity threshold of 97 %
232 by the ribosomal database project (RDP) classifier with the Bayesian algorithm. The
233 number of sequences per soil sample was rarefied to an equal abundance as the sample
234 with the lowest number of sequences (Menéndez-Serra et al., 2019; Ye et al., 2017), and
235 4101 OTUs were identified across all samples. The taxonomy of each 16S rRNA gene
236 sequence was analyzed by RDP Classifier against the SILVA database version 132 using
237 a confidence threshold of 70 % (Quast et al., 2013). Good's coverage was used to
238 investigate the sequence coverage of the bacterial communities. The α-diversity
239 parameters, including the Shannon index, Shannon's evenness, and Chao1, were
240 estimated with the mothur program (http://www.mothur.org). The Shannon index and
241 Shannon's evenness were used to investigate soil bacterial community diversity and
242 evenness, respectively. Chao1 was used to describe soil bacterial community richness.
243 Principal coordinate analysis (PCoA) was then used to demonstrate patterns of
244 similarity in bacterial community structures between CK and SM treatments based on
245 weighted UniFrac distances. Environmental factors were selected using Monte Carlo
246 permutations (calculated based on 999), and environmental factors with a $P > 0.05$ were

247   removed from a redundancy analysis (RDA) (Fan and Xing, 2016). Analysis of

248   similarity (Adonis) analysis was performed with the vegan package of the R project

249   (http://www.r-project.org). The Monte Carlo Mantel test and RDA were performed by

250   Canoco 5.0 (CANOCO, Microcomputer Power Inc., Ithaca, NY, USA) to identify the

251   soil environmental factors that were significantly correlated with soil bacterial

252   communities.

2.6 Statistical analysis

254   Prior to analysis, the data were tested for homogeneity of variance using Levene's test.

255   Two-way analysis of variance (ANOVA) was used to determine the main effects of

256   depth, straw management and their interactions on soil physicochemical parameters,

257   soil bacterial abundance, soil bacterial α-diversity indices, and soil phylum relative

258   abundances. If the depth and straw management interactions were significantly

259   different, a one-way ANOVA was used to analyze the differences between the four

260   depths in the CK and SM plots, and Welch's t-test within STAMP (Parks et al., 2014)

261   was used to identify genera with significant differences in relative abundance between

262   CK and SM plots at each depth. The differences in soil genus relative abundances at

263   each depth among the straw management treatments were tested by an independent-

264   samples *t*-test. Pearson's correlation analysis was used to show the connections between

265   bacterial communities and soil physicochemical parameters. These statistical analyses

266   were performed by SPSS 19.0 (SPSS, Inc., Chicago, IL, USA). RDA plots were

267   prepared using Canoco 5.0 (CANOCO, Microcomputer Power Inc., Ithaca, NY, USA).

268   PCoA plots were drawn on the I–Sanger Cloud Platform (https://cloud.majorbio.com/),

269   and other graphs were prepared using SigmaPlot ver. 12.5 (Systat, Software, Inc., San

270   Jose, California, USA).

3 Results

3.1 Straw mulch effects on soil physicochemical properties

273   As shown in Table 1, the ANOVA suggested that soil pH significantly increased with

274   soil depth, whereas soil TOC, TN, TP, IN, AP, AK, DOC, DON, and WC significantly

275   decreased with soil depth ($P < 0.05$). Soil TK did not vary among the soil depths. Straw

276   mulching increased TOC by 22.09 %, TN by 13.48 %, IN by 10.32 %, AP by 9.02 %,

277   AK by 7.17 %, DOC by 69.98 %, DON by 41.98 %, and WC by 5.13 % compared to

278   their CK values, while pH, TP, and TK did not change between SM and CK treatments.

279   The interactions between straw management and soil depth were significant for TOC,

280   IN, AK, DOC, and DON. The independent-samples t-test demonstrated that TOC at 0–

281   10 cm, IN at 0–5 cm, AK at 0–10 cm, and DON at 0–5 and 10–20 cm was significantly

282   higher under SM than for the CK treatment.

283

284   **Table 1.** Soil physicochemical properties at different soil depths under the SM and CK

285   treatments. Means of three replicates per treatment are shown. CK, straw was removed from

286   the plot; SM, straw was mulched into the plot soil. TOC, total organic carbon; TN, total nitrogen;

287   TP, total phosphorus; TK, total potassium; IN, inorganic nitrogen; AP, available phosphorus;

288   AK, available potassium; DOC, dissolved organic carbon; DON, dissolved organic nitrogen;

289   WC, water content. Different lowercase letters within a column indicate significant differences

290   between the four soil depths; different capital letters within a column indicate significant

291   differences between the two straw management treatments across the four soil depths; and *

292   indicates differences between the two straw management treatments at the same soil depth at P

293   = 0.05. ns represents no statistical significance at the P = 0.05 level.

Variable		0–5 cm	5–10 cm	10–20 cm	20–30 cm	CK	SM	ANOVA		
								Depth (d)	Straw (s)	d × s
pH		5.09d	5.90c	6.56b	7.17a	6.26A	6.09A	$p < 0.001$	ns	ns
TOC (g kg⁻¹)	CK	23.0a	19.4b	14.2c	6.90d	–	–	$p < 0.001$	$p < 0.001$	$p < 0.001$
	SM	33.2a*	22.3b*	15.8c	7.14d					
TN (g kg⁻¹)		3.17a	2.26b	1.54c	0.64d	1.78B	2.02A	$p < 0.001$	$p < 0.05$	ns
TP (g kg⁻¹)		0.87a	0.70b	0.48c	0.21d	0.55A	0.58A	$p < 0.001$	ns	ns
TK (g kg⁻¹)		12.4a	12.5a	12.3a	11.9a	12.1A	12.5A	ns	ns	ns
IN (mg kg⁻¹)	CK	21.4a	18.3ab	14.2bc	11.3c	–	–	$p < 0.001$	$p < 0.05$	$p < 0.01$
	SM	29.1a*	16.6b	14.5bc	11.9c					
AP (mg kg⁻¹)		107.2a	46.5b	15.9c	2.01d	37.7B	48.1A	$p < 0.001$	$p < 0.05$	ns
AK (mg kg⁻¹)	CK	152a	108b	103b	104b	–	–	$p < 0.001$	$p < 0.05$	$p < 0.05$
	SM	183a*	116b	100b	101b					
DOC (mg kg⁻¹)	CK	41.4a	35.1a	20.6b	12.7b	–	–	$p < 0.001$	$p < 0.001$	$p < 0.001$
	SM	73.0a*	55.4b*	36.3c*	8.47d					
DON (mg kg⁻¹)	CK	16.1a	17.3a	12.3a	4.97b	–	–	$p < 0.001$	$p < 0.001$	$p < 0.01$
	SM	26.2a*	18.1b	18.4b*	5.98c					
WC (%)		18.0a	17.1a	15.7b	13.2c	15.6B	16.4A	$p < 0.001$	$p < 0.05$	ns

294   **3.2 Straw mulch effects on bacterial abundance**

295   Straw management, soil depth and their combined effects significantly affected soil

296   bacterial abundance in terms of the 16S rRNA gene copy number (Fig. 1). Soil bacterial

297   abundance significantly declined as the soil depth increased for the two treatments (P

298   < 0.001), and the SM bacterial abundance was 52.69% higher than CK (P < 0.05). An

299   independent-samples t-test demonstrated that compared with the CK treatment, the SM

300   treatment significantly increased bacterial abundance in the 0–5 cm soil layer (P < 0.05),

301   but there was no significant difference in other three layers between the two treatments

302    $(P > 0.05)$.
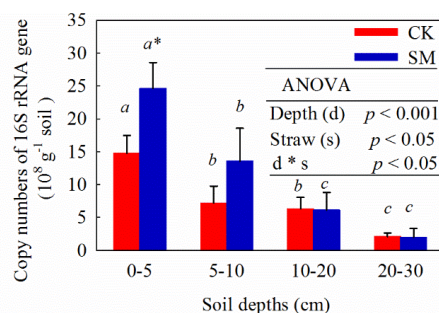
Figure 1. Straw mulching effects on soil bacterial abundance assessed using qPCR. Data are the means and standard deviations of three repeats. CK, no-till with straw removal; SM, no-till with straw mulching. Different lowercase letters indicate significant differences between the four soil depths at the $P = 0.05$ level. * indicates differences between the CK and SM treatments for the same soil depth at the $P = 0.05$ level.

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

Variable	Shannon		Shannon's evenness	Chao1
	CK	SM		
0–5 cm	6.53a	6.40a*	0.858a	2419a
5–10 cm	6.38ab	6.42a	0.845b	2639b
10–20 cm	6.34b	6.40a	0.843b	2597ab
20–30 cm	6.07c	6.27a	0.824c	2455ab
CK	–		0.841A	2481A
SM	–		0.843A	2573A
ANOVA				
Depth (d)	$p < 0.001$		$p < 0.001$	$p < 0.05$
Straw (s)	ns		ns	ns
d × s	$p < 0.05$		ns	ns

3.3 Straw mulch effects on bacterial α-diversity

317   The Good's coverage value for all samples was more than 96% in our study, which

318   indicated that the number of sequence reads adequately represented the bacteria. Table

319   2 shows that the three α-diversity indices (Shannon, Shannon's evenness, and Chao1)

320 significantly decreased with soil depth under CK and SM treatments, and the soil

321 sampled at 0–5 cm had the highest values for Shannon and Shannon's evenness, except

322 for the case that the Shannon diversity did not change under SM treatment. The lowest

323 value for Chao1 was observed at the 0–5 cm soil depth among the four soil depths.

324 Compared to the CK treatment, straw mulching did not change Shannon's evenness and

325 Chao1 indices, but it decreased the Shannon index at 0–5 cm depth.

3.4 Straw mulch effects on bacterial community composition

327 The phyla whose relative abundances accounted for less 1% in all soil samples were

328 merged into the "Others" category. As a result, 14 phyla were observed in the study.

329 From highest to lowest in relative abundance, these were Proteobacteria, Acidobacteria,

330 Chloroflexi, Actinobacteria, Planctomycetes, Nitrospirae, Others, Gemmatimonadetes,

331 Unclassified, Firmicutes, Bacteroidetes, Latescibacteria, Verrucomicrobia, and

332 Cyanobacteria (Fig. S1). A two-way ANOVA (Table 3) demonstrated that compared to

333 the CK treatment, straw mulching significantly increased the relative abundances of

334 Proteobacteria, Acidobacteria, Bacteroidetes and Latescibacteria but significantly

335 decreased Actinobacteria and Chloroflexi ($P < 0.05$). There was no significant

336 difference in the relative abundances of Planctomycetes, Nitrospirae, Firmicutes,

337 Gemmatimonadetes, and Verrucomicrobia between the two treatments. The relative

338 abundances of Proteobacteria, Actinobacteria, Bacteroidetes, and Cyanobacteria

339 decreased, but those of Chloroflexi, Nitrospirae, and Latescibacteria increased as soil

340 depth increased ($P < 0.05$) for the two treatments. The relative abundance of

341 Acidobacteria increased from 0–5 to 10–20 cm depth and then decreased at 20–30 cm

342 depth. The relative abundance of Planctomycetes did not change among the 0–5, 5–10,

343 and 10–20 cm depths but then significantly decreased at the 20–30 cm depth. The

344 relative abundance of Gemmatimonadetes first increased and then decreased with soil

345 depth, and its highest value was at 5–10 cm. The relative abundances of Firmicutes and

346 Verrucomicrobia did not change with soil depth. The combined effects of straw

347 management and depth were significant for the phyla Proteobacteria and Cyanobacteria.

348 Straw mulching led to a higher Proteobacteria relative abundance at the 0–5 cm depth

349 but lower values for Cyanobacteria at the 0–5 and 20–30 cm depths compared to their

350 CK values.

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Table 3. Relative abundances of the 14 most abundant bacterial phyla at different soil depths under the two straw management treatments. Prot, Proteobacteria; Acid, Acidobacteria; Acti, Actinobacteria; Chlo, Chloroflexi; Plan, Planctomycetes; Nitr, Nitrospirae; Bact, Bacteroidetes; Firm, Firmicutes; Gemm, Gemmatimonadetes; Cyan, Cyanobacteria; Uncl, Unclassified; Verr, Verrucomicrobia; Late, Latescibacteria; Othe, Others. CK, straw was removed from the plot; SM, straw was mulched into the plot soil. Different lowercase letters within a column indicate significant differences between the four soil depths; different capital letters within a column indicate significant differences between the two straw management treatments across the four soil depths; and * indicates differences between the two straw managements at the same soil depth at $P = 0.05$. ns represents no statistical significance at the $P = 0.05$ level.

| Variable		0–5 cm	5–10 cm	10–20 cm	20–30 cm	CK	SM	ANOVA		
								Depth (d)	Straw (s)	d × s
Prot	CK	32.11a	29.51ab	29.08ab	26.69b	–	–	$p < 0.001$	$p < 0.01$	$p < 0.05$
	SM	38.87a*	31.31b	30.93b	28.06c					
Acid		19.20b	19.86ab	21.33a	15.38c	17.80B	20.09A	$p < 0.001$	$p < 0.001$	ns
Acti		14.84a	11.94b	10.49b	10.01b	13.02A	10.64B	$p < 0.01$	$p < 0.01$	ns
Chlo		11.92b	12.67b	13.10b	19.28a	15.56A	12.93B	$p < 0.001$	$p < 0.01$	ns
Plan		4.12a	3.72a	4.20a	2.75b	3.68A	3.72A	$p < 0.01$	ns	ns
Nitr		4.96c	10.33b	9.45b	12.74a	9.33A	9.40A	$p < 0.001$	ns	ns
Bact		2.09a	1.52b	1.15c	0.70d	1.12B	1.60A	$p < 0.001$	$p < 0.001$	ns
Firm		1.14a	1.47a	1.76a	1.26a	1.57A	1.25A	ns	ns	ns
Gemm		1.41c	2.42a	2.37ab	2.01b	2.03A	2.08A	$p < 0.001$	ns	ns
Cyan	CK	1.25a*	0.20b	0.10b	0.12b*	–	–	$p < 0.001$	$p < 0.001$	$p < 0.001$
	SM	0.48a	0.15b	0.15b	0.06c					
Uncl		1.01c	2.12b	2.15b	2.52a	1.98A	1.92A	$p < 0.001$	ns	ns
Verr		0.93a	0.50a	0.40a	0.17a	0.66A	0.34A	ns	ns	ns
Late		0.51b	1.29a	1.56a	1.48a	1.12B	1.30A	$p < 0.001$	$p < 0.05$	ns
Othe		1.51c	1.57c	1.92b	4.20a	2.37A	2.23A	$p < 0.001$	ns	ns

After taxonomic assignment using the SILVA database (Version 132), 297, 290, 286, and 288 classified genera were obtained from the 0–5, 5–10, 10–20, and 20–30 cm soil layers, respectively, across the two treatments. In this study, we paid more attention to the genera that accounted for more than 0.25% of the relative abundance in the bacterial community in any soil sample (Fig. 2). At 0–5 cm, compared to their values for the CK treatment, the relative abundances of *Rhodanobacter*, *Rhizomicrobium*, *Dokdonella*, *Pseudolabrys*, *Acidibacter*, *Devosia*, *Reyranella*, *Luteimonas*, and *Porphyrobacter* genera from the Proteobacteria phylum and *Terracidiphilus* genus from the Acidobacteria phylum increased, whereas those of *Anaeromyxobacter* and *Syntrophobacter* genera from the Proteobacteria phylum, *Mycobacterium* and *Streptomyces* genera from the Actinobacteria phylum, and *Gemmata* and *Isosphaera* genera from the Planctomycetes phylum decreased in the SM treatment ($P < 0.05$).

376 There were no significantly different genera of more than 0.25% relative abundance at

377 the 5–10 cm depth between CK and SM treatments ($P > 0.05$). At 10–20 cm, the relative

378 abundances of *RB41* genus from the Acidobacteria phylum, *Flavobacterium* genus from

379 the Bacteroidetes phylum, and *Lysobacter* genus from the Proteobacteria phylum were

380 increased, while those of *Desulfobacca* genus from the Proteobacteria phylum, and

381 *Luedemannella*, *Mycobacterium*, and *Streptomyces* genera from the Actinobacteria

382 phylum were decreased in the SM treatment ($P < 0.05$). At 20–30 cm, compared to that

383 in the CK treatment, the relative abundance of *Flavobacterium* was significantly

384 increased in the SM treatment ($P < 0.05$).
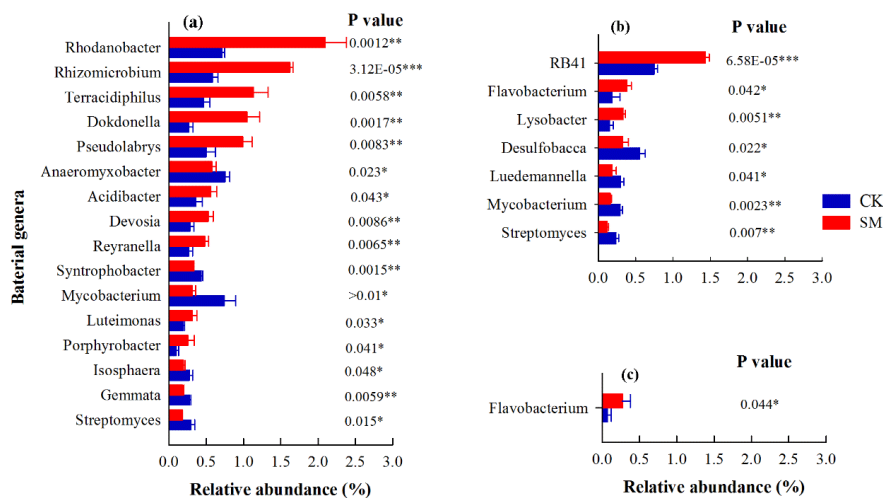
385

386 **Figure 2.** Bacterial genera that had significantly different relative abundances between CK and

387 SM plots at 0–5 cm (a), 10–20 cm (b), and 20–30 cm (c) depths determined using a *t*-test

388 analysis with 95 % confidence intervals. CK, no-till with straw removal; SM, no-till with straw

389 mulching.

390

3.5 Straw mulch effects on bacterial community structure

392 A PCoA showed the differences among the bacterial community structures in 24

393 samples (Fig. 3). The first two principal coordinates, PC1 and PC2, accounted for 65.79%

394 and 11.18% of the total variation, respectively. The PC1 coordinate separated the soil

395 samples into four groups along the soil depth gradient, regardless of straw treatment.

396 Furthermore, Adonis analyses were performed with the OTU data calculated using the

397 weighted UniFrac distances. The results showed that the bacterial communities in the

398 SM treatment were marginally but significantly different (Adonis $R^2 = 0.61$, $P = 0.10$)

399 from those in the CK treatment at 0–5 cm soil depth. A similar difference was observed

400 between the two treatments at 10–20 cm (Adonis $R^2 = 0.44$, $P = 0.10$). There was no

401 significant difference between SM and CK bacterial communities at 5–10 cm (Adonis

402 $R^2 = 0.11$, $P = 0.60$) and 20–30 cm (Adonis $R^2 = 0.19$, $P = 0.30$). In addition, the soil

403 bacterial communities were significantly different among the four soil depths under CK

404 (Adonis $R^2 = 0.76$, $P = 0.0003$) and SM (Adonis $R^2 = 0.88$, $P = 0.0002$) treatments.

405
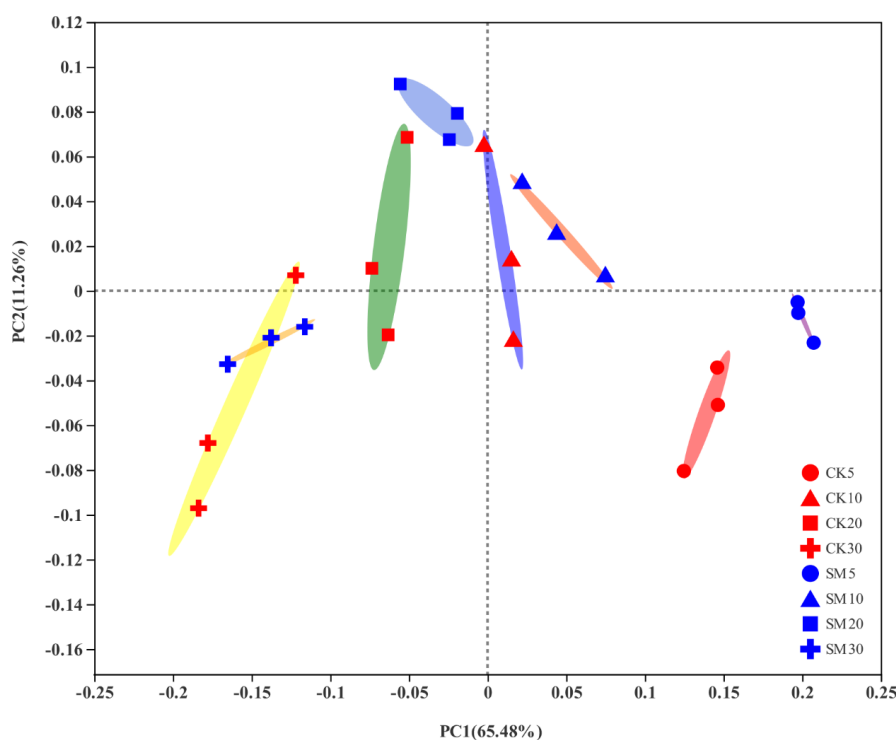
Figure 3. Principal coordinates analysis (PCoA) plot of the soil bacterial communities based on OTUs from 24 samples. CK5, CK10, CK20, and CK30 represent the soil sampled at 0–5, 5–10, 10–20, and 20–30 cm depths, respectively, under straw removal. SM5, SM10, SM20, and SM30 represent the soil sampled at 0–5, 5–10, 10–20, and 20–30 cm depths, respectively, under straw mulching. The ellipses serve as only visual aids to distinguish different straw treatments under different soil depths and have no statistical meaning.

414 The data were subjected to an RDA to demonstrate the influence of major soil physicochemical properties on soil bacterial community composition (Fig. 4). Figures 4a, 4b, 4c, and 4d showed that the first two axes explained 51.11% and 21.17%, 52.51%

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417 and 20.95%, 50.20% and 22.91%, and 53.39% and 19.94% of the total variation in the

418 bacterial communities between CK and SM at the four soil depths, respectively. The

419 contributions made by specified soil environmental factors varied with soil depth. Soil

420 DOC ($P = 0.001$), TOC ($P = 0.049$), and pH ($P = 0.027$) had significant effects on the

421 bacterial community in the two treatments at 0–5 cm soil depth, whereas only soil pH

422 ($P = 0.015$) had a significant effect at 5–10 cm. At 10–20 cm soil depth, soil pH ($P = $

423 0.022) and TOC ($P = 0.038$) had the most significant effects, and at 20–30 cm, soil IN

424 ($P = 0.003$), pH ($P = 0.027$), TN ($P = 0.03$), and DON ($P = 0.032$) were the drivers that

425 most influenced the soil bacterial community.

426
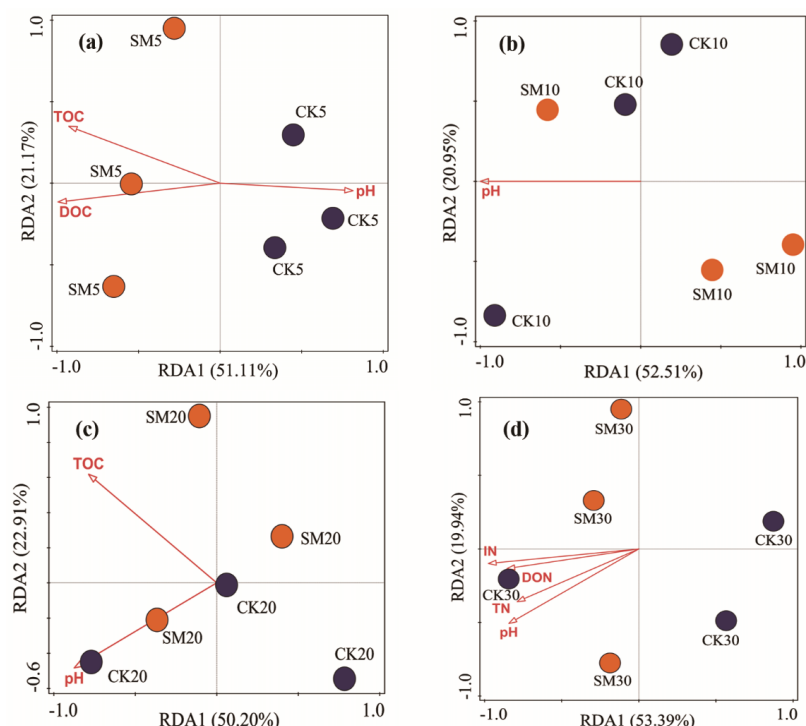
Figure 4. Redundancy analysis (RDA) of the soil bacterial community changes at the

OTU level and the soil physicochemical property differences between CK and SM plots

in the 0–5 cm (a), 5–10 cm (b), 10–20 cm (c), and 20–30 cm (d) layers. CK5, CK10,

CK20, and CK30 represent the soil sampled at 0–5, 5–10, 10–20, and 20–30 cm depths,

respectively, under straw removal. SM5, SM10, SM20, and SM30 represent the soil

sampled at 0–5, 5–10, 10–20, and 20–30 cm depths, respectively, under straw mulching.

TOC, total organic carbon; TN, total nitrogen; IN, inorganic nitrogen; DOC, dissolved

organic carbon; DON, dissolved organic nitrogen.

4 Discussion

4.1 Straw mulching changed soil physicochemical properties with soil depth

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

The results of the present study indicated that most soil physicochemical parameters decreased with increasing soil depth, which was partly consistent with our hypothesis. Crop roots were mainly distributed in the 0–10 or 0–20 cm soil layers, which meant that introducing a large carbon input to the surface layer led to lower TOC

468 and DOC contents in the subsoil than in the surface soil (Li et al., 2020). Apart from

469 the roots, inorganic fertilizers were applied to the no-till soil surface, and consequently

470 most soil nutrients (TN, TP, AP, AK, IN, DON) were enriched in the surface layer and

471 decreased with soil depth. Large amounts of N fertilizer over a long period of time could

472 result in soil acidification (Guo et al., 2010), which resulted in a lower pH value in the

473 soil surface layer than in lower layers in our study. The TK content did not change with

474 soil depth, mainly because of its high levels in the studied soil.

475 **4.2 Straw mulching altered soil bacterial abundance and community with soil**
476 **depth**

477 The Pearson's correlation analysis demonstrated that soil bacterial abundance, as

478 determined via qPCR, was significantly correlated with soil TOC, TN, TP, IN, AP, AK,

479 DOC, DON, and WC (Table S1). Similarly, soil moisture (Brockett et al., 2012), C

480 and/or N availability (van Leeuwen et al., 2017; Cai et al., 2020), and TP (Song et al.,

481 2020) were reported to be significantly and positively correlated with soil bacterial

482 abundance. Straw mulching increased soil C, N, P and WC in our study, which favored

483 soil bacterial abundance under the SM treatment. Ji et al. (2018) also found that the soil

484 bacterial abundance increased after straw addition. Most soil bacterial abundance-

485 related physicochemical parameters, such as C, N, P, and WC, were reduced in deeper

486 soil layers, which contributed to the decreasing soil bacterial abundance with increasing

487 soil depth (Fig. 1). This was consistent with the results of van Leeuwen et al. (2017).

488 Soil bacteria can be divided into copiotrophic and oligotrophic groups based on

489 their growth on different substrates (Fierer et al., 2007, 2012). Straw mulching produced

490 a nutrient-rich soil environment, especially in the 0–5 cm soil layer, which would

491 benefit copiotroph bacterial growth and lead to a shift in the predominant bacterial

492 community (Fierer et al., 2012). In addition, high soil inorganic nitrogen content

493 decreased bacterial diversity (Yu et al., 2019; Zhao et al., 2019). These factors may

494 explain the reduced value of Shannon diversity at the 0–5 cm soil depth in the SM plots

495 compared to the CK plots. Soil biodiversity loss impairs ecosystem function (Wagg et

496 al., 2014), and sustainable agriculture should adopt management practices that preserve

497 or increase microbial diversity rather than destroy or threaten it (Pastorelli et al., 2013).

498 Consequently, our results suggested that inorganic N fertilizer should be reduced under

499 straw mulching and may thus be more beneficial for maintaining or improving bacterial

500 diversity. Other soil microbial diversities, as measured by the Shannon evenness and

501 Chao1 indices, were not affected by straw mulching, possibly because these diversity

502 indices are limited and do not take into account subtle changes in the soil environment

503 (Hartmann and Widmer, 2006).

504 Proteobacteria and Bacteroidetes, often classified as copiotrophic groups,

505 preferentially consume labile soil organic pools and have higher growth rates under

506 conditions with abundant resources, while oligotrophic groups, such as Acidobacteria

507 and Chloroflexi, are highly abundant in low-nutrient environments (Fierer et al., 2007,

508 2012; Liang et al., 2018; Ling et al., 2017). Long-term straw mulching increased soil

509 nutrient levels, which was one reason for the higher relative abundances of

510 Proteobacteria and Bacteroidetes in SM plots than in CK plots. Additionally,

511 Bacteroidetes are involved in hemicellulose breakdown (Wegner and Liesack, 2016).

512 Large quantities of straw were mulched to SM, and this management promoted

513 Bacteroidetes proliferation. Larger available C and N pools reduced the relative

514 abundances of oligotrophic groups, for example, Chloroflexi in SM plots, which agreed

515 with the results of Liang et al. (2018). Since Acidobacteria are involved in

516 hemicellulose breakdown (Wegner and Liesack, 2016), straw mulching increased their

517 relative abundance. Although Actinobacteria were classified as copiotrophs by Fierer et

518 al. (2012), straw mulching decreased the Actinobacteria in our study, which was also

519 observed in other studies (Calleja-Cervantes et al., 2015; Hao et al., 2019; Liang et al.,

520 2018). One possible reason is that most Actinobacteria favor aerobic environments

521 (Hamamura et al., 2006), but straw mulching increased the WC and reduced the oxygen

522 content in the soil. Soil nutrient levels were low in the soil subsurface layers, which was

523 one reason for decreasing the relative abundances of Proteobacteria, Planctomycetes,

524 Actinobacteria, Bacteroidetes, and Cyanobacteria and increasing the relative

525 abundances of Chloroflexi and Nitrospirae with soil depth.

526 Our results confirmed that the different bacterial genera within each phylum had

527 different strategies (Fig. 2). At the 0–5 cm soil depth, the relative abundances of nine

528 genera from the Proteobacteria phylum increased under SM, while those of two genera

529 from Proteobacteria decreased. This may explain why a higher relative abundance of

530 Proteobacteria was detected in SM plots than in CK plots in the 0–5 cm soil layer.

531 Returned straw was largely decomposed by soil microbes, and the soil bacterial

532 community played important roles in increasing CH_4, N_2O and NH_3 emissions under

533 straw return in many studies (Shang et al., 2011; Xu et al., 2017; Wang et al., 2012).

534 Specifically, *Rhodanobacter* growth was favored and increased N_2O emissions in SM

535   soil, as it was the dominant bacterial genus containing denitrifying species (Huang et

536   al., 2019). Similarly, the relative abundances of the *Rhizomicrobium*, *Dokdonella*,

537   *Reyranella*, and *Luteimonas* genera were also increased in SM soil since they are N-

538   cycling-related bacterial taxa that contain denitrifiers (Chen et al., 2020a; Nie et al.,

539   2018; Wang et al., 2019a; Wolff et al., 2018). *Terracidiphilus* and *Acidibacter* was

540   involved in the degradation of plant-derived biopolymers (Garcia-Fraile et al., 2015)

541   and organic substrates (Ai et al., 2018), respectively, which may explain their increased

542   relative abundance in the SM treatment. Although little is known about the ecology of

543   *Pseudolabrys*, its relative abundance was increased in soil that had received compost

544   (Joa et al., 2014). Organic carbon can inhibit the growth of chemolithotrophic bacteria

545   and favor *Dokdonella* (Wang et al., 2019a). The genus *Devosia* increases in composted

546   soil because its species are plant growth-promoting bacteria (Liang et al., 2018).

547   *Mycobacterium*, one genus of Actinobacteria, decreased after straw mulching in the 0–

548   5 cm soil layer. One reason is that Actinobacteria was found to be dominant in soils

549   with low organic matter levels (Bell et al., 2013). In addition, Sellstedt and Richau

550   (2013) suggested that *Mycobacterium* is capable of nitrogen fixation by root nodulation.

551   Higher soil inorganic nitrogen concentration may depress *Mycobacterium* growth in

552   SM plots. *RB41* was enriched in SM plots at the 10–20 cm soil depth. Unfortunately,

553   there have been few reports on this relationship. According to Foesel et al. (2013),

554   *Blastocatella fastidiosa* was the only known isolate from *RB41*, and it preferred protein-

555   containing substrates. Straw mulching might possibly increase the contents of these

556   substrates and, therefore, *RB41* relative abundance. *Flavobacterium* was one genus

557   from the Bacteroidetes phylum, and its relative abundance was higher in SM plots than

558   CK plots. Nan et al. (2020) proposed that *Flavobacterium* possibly decomposes labile

559   carbon. The relative abundance of *Lysobacter* was increased in SM soil, and

560   Maarastawi et al. (2018) proposed that root exudates and additional resource carbon

561   with strong lytic abilities could be used to degrade macromolecules.

562   The RDA results suggest that the key soil physicochemical parameters affecting

563   soil bacteria partly changed with soil depth between straw mulching and straw removal,

564   which was consistent with our hypothesis. However, the main key parameters were soil

565   pH and different organic carbon and nitrogen fractions. A similar relationship was found

566   in other studies (Schreiter et al., 2014; Sun et al., 2015). Schreiter et al. (2014)

567   demonstrated that soil TOC, pH, and some available nutrients were closely related to

568   soil bacterial communities. Sun et al. (2015) proposed that soil pH was the driving

569   factor in shaping bacterial community structure after straw addition.

570   **5 Conclusions**

571   In this study, we investigated the effects of long-term straw mulching on soil

572   physicochemical properties and bacterial communities along a soil depth gradient under

573   a no-till rice-wheat rotation system. Our results showed that most soil physicochemical

574   parameters decreased, but soil pH increased with soil depth. Straw mulching increased

575   most physicochemical parameters and bacterial abundance, but reduced the Shannon

576   diversity of the bacterial community at 0–5 cm soil depth, with no difference in

577   Shannon's evenness and Chao1 indices. The reduced Shannon diversity in SM plots

578   was possibly attributed to the enriched soil nutrition environment, especially the

579   increased soil IN contents. The relative abundances of the bacterial phyla and genera

580   varied with soil depth. At the phylum level, straw mulching increased the relative

581   abundances of Proteobacteria, Bacteroidetes, and Acidobacteria, but reduced those of

582   Actinobacteria, Chloroflexi, and Cyanobacteria. At the genera level, straw mulching

583   had different effects on some C- and N-cycling genera, mainly increasing the relative

584   abundances of *Rhodanobacter*, *Rhizomicrobium*, *Terracidiphilus*, *Dokdonella*,

585   *Pseudolabrys*, *Acidibacter*, *Devosia*, *Reyranella*, *Luteimonas*, *Porphyrobacter*, *RB41*,

586   *Flavobacterium*, and *Lysobacter* and reducing those of *Anaeromyxobacter*,

587   *Mycobacterium*, *Syntrophobacter*, *Streptomyces*, *Gemmata*, *Isosphaera*, *Desulfobacca*,

588   *Luedemannella*, and *Mycobacterium*. An RDA showed that the significant correlations

589   between the environmental factors and the soil bacterial community varied with depth,

590   but soil pH and different organic carbon and nitrogen fractions were the major drivers.

591   Consequently, straw mulching is highly recommended under a no-till system in

592   southwestern China because of its benefits in soil fertility and bacterial abundance.

593   However, to maintain or increase soil bacterial Shannon diversity, the amount of

594   inorganic nitrogen fertilizer can be reduced after straw mulching in future studies.

595   **Data availability**

596   All data are available. The sequencing data have been submitted to the NCBI Sequence

597   Read Archive database (SRA accession PRJNA625832).

598   **Author contributions**

599   ZZ analyzed the data and wrote the manuscript. ZL and ZC helped to analyze the data

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600 and write the manuscript. ZZ, KC, and XZ collected the soil samples. ZZ, HY, SG, YS,
601 and HF determined the soil attributes. QC, ST, MH, and YQ installed the experiment
602 and reviewed the manuscript. All authors approved the final version of the manuscript.

603 **Competing interests**

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

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613 **References**

614 Ai, C., Zhang, S., Zhang, X., Guo, D., Zhou, W., and Huang, S.: Distinct responses of
615 soil bacterial and fungal communities to changes in fertilization regime and crop
616 rotation, Geoderma 319, 156–166,
617 https://doi.org/10.1016/j.geoderma.2018.01.010, 2018.

618 Akhtar, K., Wang, W., Ren, G., Khan, A., Feng, Y., and Yang, G.: Changes in soil
619 enzymes, soil properties, and maize crop productivity under wheat straw mulching
620 in Guanzhong, China, Soil Tillage Res., 182, 94–102,
621 https://doi.org/10.1016/j.still.2018.05.007, 2018.

622 Ashworth, A. J., DeBruyn, J. M., Allen, F. L., Radosevich, M., and Owens, P. R.:
623 Microbial community structure is affected by cropping sequences and poultry litter
624 under long-term no-tillage, Soil Biol. Biochem., 114, 210–219,
625 https://doi.org/10.1016/j.soilbio.2017.07.019, 2017.

626 Bell, T. H., Yergeau, E., Maynard, C., Juck, D., Whyte, L. G., and Greer, C. W.:
627 Predictable bacterial composition and hydrocarbon degradation in Arctic soils
628 following diesel and nutrient disturbance, ISME J., 7, 1200–1210,
629 https://doi.org/10.1038/ismej.2013.1, 2013.

630 Blanco-Canqui, H., and Lal, R.: Soil structure and organic carbon relationships

631   following 10 years of wheat straw management in no-till, Soil Tillage Res., 95,
632   240–254, https://doi.org/10.1016/j.still.2007.01.004, 2007.

633 Bowles, T. M., Acosta-Martínez, V., Calderón, F., and Jackson, L. E.: Soil enzyme
634   activities, microbial communities, and carbon and nitrogen availability in organic
635   agroecosystems across an intensively-managed agricultural landscape, Soil Biol.
636   Biochem., 68, 252–262, https://doi.org/10.1016/j.soilbio.2013.10.004, 2014.

637 Brockett, B. F., Prescott, C. E., and Grayston, S. J.: Soil moisture is the major factor
638   influencing microbial community structure and enzyme activities across seven
639   biogeoclimatic zones in western Canada, Soil Biol. Biochem., 44, 9–20,
640   https://doi.org/10.1016/j.soilbio.2011.09.003, 2012.

641 Bu, R., Ren, T., Lei, M., Liu, B., Li, X., Cong, R., and Lu, J.: Tillage and straw-returning
642   practices effect on soil dissolved organic matter, aggregate fraction and bacteria
643   community under rice-rice-rapeseed rotation system, Agric., Ecosyst. Environ.,
644   287, 106681, https://doi.org/10.1016/j.agee.2019.106681, 2020.

645 Cai, S., Wang, J., Lv, W., Xu, S., and Zhu, H.: Nitrogen fertilization alters the effects of
646   earthworms on soil physicochemical properties and bacterial community structure,
647   Appl. Soil Ecol., 150, 103478, https://doi.org/10.1016/j.apsoil.2019.103478, 2020.

648 Calleja-Cervantes, M. E., Fernández-González, A. J., Irigoyen, I., Fernández-López, M.,
649   Aparicio-Tejo, P. M., and Menéndez, S.: Thirteen years of continued application
650   of composted organic wastes in a vineyard modify soil quality characteristics, Soil
651   Biol. Biochem., 90, 241–254, https://doi.org/10.1016/j.soilbio.2015.07.002, 2015.

652 Cao, Y., Sun, H., Zhang, J., Chen, G., Zhu, H., Zhou, S., and Xiao, H.: Effects of wheat
653   straw addition on dynamics and fate of nitrogen applied to paddy soils, Soil Tillage
654   Res., 178, 92–98, https://doi.org/10.1016/j.still.2017.12.023, 2018.

655 Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N.,
656   Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith,
657   G., and Knight, R.: Ultra-high-throughput microbial community analysis on the
658   Illumina HiSeq and MiSeq platforms, ISME J., 6, 1621–1624,
659   https://doi.org/10.1038/ismej.2012.8, 2012.

660 Chen, J., Wu, Q., Li, S., Ge, J., Liang, C., Qin, H., Xu, Q., and Fuhrmann, J. J.: Diversity
661   and function of soil bacterial communities in response to long-term intensive
662   management in a subtropical bamboo forest, Geoderma, 354, 113894,
663   https://doi.org/10.1016/j.geoderma.2019.113894, 2019.

664 Chen, S., Qi, G., Ma, G., and Zhao, X.: Biochar amendment controlled bacterial wilt

665   through changing soil chemical properties and microbial community, Microbiol.
666   Res., 231, 126373, https://doi.org/10.1016/j.micres.2019.126373, 2020a.

667 Chen, Z., Wang, H., Liu, X., Zhao, X., Lu, D., Zhou, J., and Li, C.: Changes in soil
668   microbial community and organic carbon fractions under short-term straw return
669   in a rice–wheat cropping system, Soil Tillage Res., 165, 121–127,
670   https://doi.org/10.1016/j.still.2016.07.018, 2017.

671 Dai, X., Zhou, W., Liu, G., Liang, G., He, P., and Liu, Z.: Soil C/N and pH together as
672   a comprehensive indicator for evaluating the effects of organic substitution
673   management in subtropical paddy fields after application of high-quality
674   amendments, Geoderma, 337, 1116–1125,
675   https://doi.org/10.1016/j.geoderma.2018.11.023, 2019.

676 Dong, W., Zhang, X., Wang, H., Dai, X., Sun, X., Qiu, W., and Yang, F.: Effect of
677   different fertilizer application on the soil fertility of paddy soils in red soil region
678   of southern China, PLoS One, 7, 44504,
679   https://doi.org/10.1371/journal.pone.0044504, 2012.

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

683 Fan, X., and Xing, P.: The vertical distribution of sediment archaeal community in the
684   "black bloom" disturbing Zhushan bay of lake Taihu, Archaea, 8232135,
685   http://doi.org/10.1155/2016/8232135, 2016.

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

689 Fierer, N., Jackson, J. A., Vilgalys, R., and Jackson, R. B.: Assessment of soil microbial
690   community structure by use of taxon-specific quantitative PCR assays, Appl.
691   Environ. Microbiol., 71, 4117–4120, https://doi.org/10.1128/AEM.71.7.4117-
692   4120.2005, 2005.

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

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

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

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

706   Garcia-Fraile, P., Benada, O., Cajthaml, T., Baldrian, P., and Llado, S.: Terracidiphilus
707   gabretensis gen. nov., sp nov., an abundant and active forest soil Acidobacteria
708   important in organic matter transformation, Appl. Environ. Microbiol., 82, 560–
709   569, https://doi.org/10.1128/AEM.03353-15, 2016.

710   Giller, K. E., Witter, E., Corbeels, M., and Tittonell, P.: Conservation agriculture and
711   smallholder farming in Africa: The heretics' view, Field Crop. Res., 114, 23–34,
712   https://doi.org/10.1016/j.fcr.2009.06.017, 2009.

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

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

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

724   Hemwong, S., Cadisch, G., Toomsan, B., Limpinuntana, V., Vityakon, P., and
725   Patanothai, A.: Dynamics of residue decomposition and N_2 fixation of grain
726   legumes upon sugarcane residue retention as an alternative to burning, Soil Tillage
727   Res., 99, 84–97, https://doi.org/10.1016/j.still.2008.01.003, 2008.

728   Hobara, S., Osono, T., Hirose, D., Noro, K., Hirota, M., and Benner, R.: The roles of
729   microorganisms in litter decomposition and soil formation, Biogeochemistry, 118,
730   471–486, https://doi.org/10.1007/s10533-014-9956-3, 2014.

731   Hou, D., Bolan, N. S., Tsang, D. C. W., Kirkham, M. B., and O'Connor, D.: Sustainable
732   soil use and management: an interdisciplinary and systematic approach, Sci. Total

733   Environ., 729, 138961, https://doi.org/10.1016/j.scitotenv.2020.138961, 2020.

734 Huang, R., Wang, Y., Liu, J., Li, J., Xu, G., Luo, M., Xu, C., Ci, E., and Gao, M.:
735   Variation in N_2O emission and N_2O related microbial functional genes in straw-
736   and biochar-amended and non-amended soils, Appl. Soil Ecol., 137, 57–68,
737   https://doi.org/10.1016/j.apsoil.2019.01.010, 2019.

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

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

744 Jin, J.: Effects of different management practices on the soil–water balance and crop
745   yield for improved dryland farming in the Chinese Loess Plateau, Soil Tillage Res.,
746   96, 131–144, https://doi.org/10.1016/j.still.2007.05.002, 2007.

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

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

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

758 Kopittke, P. M., Menzies, N. W., Wang, P., McKenna, B. A., and Lombi, E.: Soil and
759   the intensification of agriculture for global food security, Environ. Int., 132,
760   105078, https://doi.org/10.1016/j.envint.2019.105078, 2019.

761 Lal, R.: Soil health and carbon management, Food Energy Secur., 5, 212–222,
762   https://doi.org/10.1002/fes3.96, 2016.

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

767 Li, C., Yan, K., Tang, L., Jia, Z., and Li, Y.: Change in deep soil microbial communities
768   due to long-term fertilization, Soil Biol. Biochem., 75, 264–272,
769   https://doi.org/10.1016/j.soilbio.2014.04.023, 2014.

770 Li, F., Chen, L., Zhang, J., Yin, J., and Huang, S.: Bacterial community structure after
771   long-term organic and inorganic fertilization reveals important associations
772   between soil nutrients and specific taxa involved in nutrient transformations, Front.
773   Microbiol., 8, 187, https://doi.org/10.3389/fmicb.2017.00187, 2017a.

774 Li, Q., Li, A., Dai, T., Fan, Z., Luo, Y., Li, S., Yuan, D., Zhao, B., Tao, Q., Wang, C., Li,
775   B., Gao, X., Li, Y., Li, H., and Wilson, J. P.: Depth-dependent soil organic carbon
776   dynamics of croplands across the Chengdu Plain of China from the 1980s to the
777   2010s, Global Change Biol., 26, 4134–4146, https://doi.org/10.1111/gcb.15110,
778   2020.

779 Li, S., Zhang, S., Pu, Y., Li, T., Xu, X., Jia, Y., Deng, O., and Gong, G.: Dynamics of
780   soil labile organic carbon fractions and C-cycle enzyme activities under straw
781   mulch in Chengdu Plain, Soil Tillage Res., 155, 289–297,
782   https://doi.org/10.1016/j.still.2015.07.019, 2016.

783 Li, X., Sun, J., Wang, H., Li, X., Wang, J., and Zhang, H.: Changes in the soil microbial
784   phospholipid fatty acid profile with depth in three soil types of paddy fields in
785   China, Geoderma, 290, 69–74, https://doi.org/10.1016/j.geoderma.2016.11.006,
786   2017b.

787 Li, Y., Li, Y., Chang, S.X., Yang, Y., Fu, S., and Jiang, P.: Biochar reduces soil
788   heterotrophic respiration in a subtropical plantation through increasing soil organic
789   carbon recalcitrancy and decreasing carbon-degrading microbial activity, Soil Biol.
790   Biochem., 122, 173–185, https://doi.org/10.1016/j.soilbio.2018.04.019, 2018.

791 Liang, B., Ma, C., Fan, L., Wang, Y., and Yuan, Y.: Soil amendment alters soil
792   physicochemical properties and bacterial community structure of a replanted apple
793   orchard, Microbiol. Res., 216, 1–11, https://doi.org/10.1016/j.micres.2018.07.010,
794   2018.

795 Ling, N., Chen, D., Guo, H., Wei, J., Bai, Y., Shen, Q., and Hu, S.: Differential responses
796   of soil bacterial communities to long-term N and P inputs in a semi-arid steppe,
797   Geoderma, 292, 25–33, https://doi.org/10.1016/j.geoderma.2017.01.013, 2017.

798 Liu, Q., Liu, G., Huang, C., and Li, H.: Soil physicochemical properties associated with
799   quasi-circular vegetation patches in the Yellow River Delta, China, Geoderma, 337,
800   202–214, https://doi.org/10.1016/j.geoderma.2018.09.021, 2019.

801 Lu, R. (Eds): Methods of soil and agro-chemistry analysis, China Agricultural Science
802   and Technology Press, Beijing, China, 2000. (in Chinese).

803 Lupwayi, N. Z., Lafond, G. P., Ziadi, N., and Grant, C. A.: Soil microbial response to
804   nitrogen fertilizer and tillage in barley and corn, Soil Tillage Res., 118, 139–146,
805   https://doi.org/10.1016/j.still.2011.11.006, 2012.

806 Lupwayi, N. Z., Larney, F. J., Blackshaw, R. E., Kanashiro, D. A., Pearson, D. C., and
807   Petri, R. M.: Pyrosequencing reveals profiles of soil bacterial communities after
808   12 years of conservation management on irrigated crop rotations, Appl. Soil Ecol.,
809   121, 65–73, https://doi.org/10.1016/j.apsoil.2017.09.031, 2017.

810 Maarastawi, S. A., Frindte, K., Geer, R., Kröber, E., and Knief, C.: Temporal dynamics
811   and compartment specific rice straw degradation in bulk soil and the rhizosphere
812   of   maize,   Soil   Biol.   Biochem.,   127,   200–212,
813   https://doi.org/10.1016/j.soilbio.2018.09.028, 2018.

814 Magoc, T., and Salzberg, S. L.: FLASH: fast length adjustment of short reads to improve
815   genome   assemblies,   Bioinformatics,   27,   2957–2963,
816   https://doi.org/10.1093/bioinformatics/btr507, 2011.

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

821 Nan, Q., Wang, C., Wang, H., Yi, Q., Liang, B., Xu, J., and Wu, W.: Biochar drives
822   microbially-mediated rice production by increasing soil carbon, J. Hazard. Mater.,
823   387, 121680, https://doi.org/10.1016/j.jhazmat.2019.121680, 2020.

824 Navarro-Noya, Y. E., Gómez-Acata, S. G., Montoya-Ciriaco, N., Rojas-Valdez, A.,
825   Suárez-Arriaga, M. C., Valenzuela-Encinas, C., Jiménez-Bueno, N., Verhulst, N.,
826   Govaerts, B., and Dendooven, L.: Relative impacts of tillage, residue management
827   and crop-rotation on soil bacterial communities in a semi-arid agroecosystem, Soil
828   Biol. Biochem., 65, 86–95, https://doi.org/10.1016/j.soilbio.2013.05.009, 2013.

829 Nie, Y., Wang, M., Zhang, W., Ni, Z., Hashidoko, Y., and Shen, W.: Ammonium
830   nitrogen content is a dominant predictor of bacterial community composition in an
831   acidic forest soil with exogenous nitrogen enrichment, Sci. Total Environ., 624,
832   407–415, https://doi.org/10.1016/j.scitotenv.2017.12.142, 2018.

833 Ok, Y. S., Usman, A. R. A., Lee, S. S., El-Azeem, S. A. M. A., Choi, B., Hashimoto, Y.,
834   and Yang, J. E.: Effects of rapeseed residue on lead and cadmium availability and

835   uptake by rice plants in heavy metal contaminated paddy soil, Chemosphere, 85,
836   677–682, https://doi.org/10.1016/j.chemosphere.2011.06.073, 2011.
837   Palm, C., Blanco-Canqui, H., Declerck, F., Gatere, L., and Grace, P.: Conservation
838   agriculture and ecosystem services: An overview, Agric., Ecosyst. Environ., 187,
839   87–105, https://doi.org/10.1016/j.agee.2013.10.010, 2014.
840   Parks, D. H., Tyson, G. W., Hugenholtz, P., and Beiko, R. G.: STAMP: statistical
841   analysis of taxonomic and functional profiles, Bioinformatics, 30, 3123–3124,
842   https://doi.org/10.1093/bioinformatics/btu494, 2014.
843   Pastorelli, R., Vignozzi, N., Landi, S., Piccolo, R., Orsini, R., Seddaiu, G., Roggero, P.
844   P., Pagliai, M.: Consequences on macroporosity and bacterial diversity of adopting
845   a no-tillage farming system in a clayish soil of Central Italy, Soil Biol. Biochem.,
846   66, 78–93, https://doi.org/10.1016/j.soilbio.2013.06.015, 2013.
847   Peng, X., and Wang, W.: Stoichiometry of soil extracellular enzyme activity along a
848   climatic transect in temperate grasslands of northern China, Soil Biol. Biochem.,
849   98, 74–84, https://doi.org/10.1016/j.soilbio.2016.04.008, 2016.
850   Pittelkow, C. M., Liang, X., Linquist, B. A., van Groenigen, K. J., Lee, J., Lund, M. E.,
851   Gestel, N., Six, J., Venterea, R. T., van Kessel, C.: Productivity limits and
852   potentials of the principles of conservation agriculture, Nature, 517, 368–368,
853   https://doi.org/10.1038/nature13809, 2015.
854   Postma-Blaauw, M. B., De Goede, R. G. M., Bloem, J., Faber, J. H., and Brussaard, L.:
855   Soil biota community structure and abundance under agricultural intensification
856   and extensification, Ecology, 91, 460–473, https://doi.org/10.1890/09-0666.1,
857   2010.
858   Qiu, Y., Lv, W., Wang, X., Xie, Z., and Wang, Y.: Long-term effects of gravel mulching
859   and straw mulching on soil physicochemical properties and bacterial and fungal
860   community composition in the Loess Plateau of China, Eur. J. Soil Biol., 98,
861   103188, https://doi.org/10.1016/j.ejsobi.2020.103188, 2020.
862   Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and
863   Glockner, F. O.: The SILVA ribosomal RNA gene database project: improved data
864   processing and web-based tools, Nucleic Acids Res., 41, D590–D596,
865   https://doi.org/10.1093/nar/gks1219, 2013.
866   Schreiter, S., Ding, G. C., Heuer, H., Neumann, G., Sandmann, M., Grosch, R., Kropf,
867   S., and Smalla, K.: Effects of the soil type on the microbiome in the rhizosphere
868   of   field-grown   lettuce,   Front.   Microbiol.,   5,   144,

869   https://doi.org/10.3389/fmicb.2014.00144, 2014.

870 Sellstedt, A., and Richau, K. H.: Aspects of nitrogen–fixing Actinobacteria, in particular
871   free–living and symbiotic Frankia, FEMS Microbiol. Lett., 342, 179–186,
872   https://doi.org/10.1111/1574-6968.12116, 2013.

873 Shang, Q., Yang, X., Gao, C., Wu, P., Liu, J., Xu, Y., Shen, Q., Zou, J., and Guo, S.: Net
874   annual global warming potential and greenhouse gas intensity in Chinese double
875   rice-cropping systems: a 3-year field measurement in long-term fertilizer
876   experiments, Global Change Biol., 17, 2196–2210, https://doi.org/10.1111/j.1365-
877   2486.2010.02374.x, 2011.

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

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

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

890 Sun, B., Jia, S., Zhang, S., McLaughlin, N. B., Zhang, X., Liang, A., Chen, X., Wei, S.,
891   and Liu, S.: Tillage, seasonal and depths effects on soil microbial properties in
892   black soil of Northeast China, Soil Tillage Res., 155, 421–428,
893   https://doi.org/10.1016/j.still.2015.09.014, 2016.

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

898 Tellez-Rio, A., García-Marco, S., Navas, M., López-Solanilla, E., Tenorio, J. L., and
899   Vallejo, A.: N_2O and CH_4 emissions from a fallow–wheat rotation with low N input
900   in conservation and conventional tillage under a Mediterranean agroecosystem,
901   Sci. Total Environ., 1, 85–94, https://doi.org/10.1016/j.scitotenv.2014.11.041,
902   2015.

903 Thompson, L. R., Sanders, J. G., Mcdonald, D., Amir, A., Ladau, J., and Locey, K. J.:
904   A communal catalogue reveals earth's multiscale microbial diversity, Nature, 551,
905   457–463, https://doi.org/10.1038/nature24621, 2017.

906 van Leeuwen, J. P., Djukic, I., Bloem, J., Lehtinen, T., Hemerik, L., de Ruiter, P. C., and
907   Lair, G. J.: Effects of land use on soil microbial biomass, activity and community
908   structure at different soil depths in the Danube floodplain, Eur. J. Soil Biol., 79,
909   14–20, https://doi.org/10.1016/j.ejsobi.2017.02.001, 2017.

910 Xu, S., Hou, P., Xue, L., Wang, S., and Yang, L.: Treated domestic sewage irrigation
911   significantly decreased the CH_4, N_2O and NH_3 emissions from paddy fields with
912   straw incorporation, Atmos. Environ., 169, 1–10,
913   https://doi.org/10.1016/j.atmosenv.2017.09.009, 2017.

914 Wagg, C., Bender, S. F., Widmer, F., and van der Heijden, M. G. A.: Soil biodiversity
915   and soil community composition determine ecosystem multifunctionality, Proc.
916   Natl. Acad. Sci. USA, 111, 5266–5270, https://doi.org/10.1073/pnas.1320054111,
917   2014.

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

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

925 Wang, L., Yuan, X., Liu, C., Li, Z., Chen, F., Li, S., Wu, L., and Liu, Y.: Soil C and N
926   dynamics and hydrological processes in a maize-wheat rotation field subjected to
927   different tillage and straw management practices, Agric., Ecosyst. Environ., 285,
928   106616, https://doi.org/10.1016/j.agee.2019.106616, 2019b.

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

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

936 Wolff, D., Krah, D., Dötsch, A., Ghattas, A. K., Wick, A., and Ternes, T. A.: Insights

937   into the variability of microbial community composition and micropollutant
938   degradation in diverse biological wastewater treatment systems, Water Res., 143,
939   313–324, https://doi.org/10.1016/j.watres.2018.06.033, 2018.

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

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

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

952   Zhang, H., Lal, R., Zhao, X., Xue, J., and Chen, F.: Opportunities and challenges of soil
953   carbon sequestration by conservation agriculture in China, Adv. Agron., 124, 1–
954   36, https://doi.org/10.1016/B978-0-12-800138-7.00001-2, 2014.

955   Zhang, H., Tang, J., and Liang, S.: Effects of snow cover plus straw mulching on
956   microorganisms in paddy soil during winter, Appl. Soil Ecol., 119, 339–344,
957   https://doi.org/10.1016/j.apsoil.2017.05.023, 2017.

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

962   Zhao, X., Liu, S., Pu, C., Zhang, X., Xue, J., Ren, Y., Zhao, X., Chen, F., Lal, R., and
963   Zhang, H.: Crops yields under no-till farming in China: A meta-analysis, Eur. J.
964   Agron., 84, 67–75, https://doi.org/10.1016/j.eja.2016.11.009, 2017.

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

969   Zhao, Y., Wang, M., Hu, S., Zhang, X., Ouyang, Z., Zhang, G., Huang, B., Zhao, S.,
970   Wu, J., Xie, D., Zhu, B., Yu, D., Pan, X., Xu, S., and Shi, X.: Economics- and

971   policy-driven organic carbon input enhancement dominates soil organic carbon
972   accumulation in Chinese croplands, Proc. Natl. Acad. Sci. USA, 115, 4045–4050,
973   https://doi.org/10.1073/pnas.1700292114, 2018.

974   Zhou, Z., Shen, Y., Du, C., Zhou, J., Qin, Y., and Wu, Y.: Economic and soil
975   environmental benefits of using controlled-release bulk blending urea in the North
976   China Plain, Land Degrad. Dev., 28, 2370–2379, https://doi.org/10.1002/ldr.2767,
977   2017.

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

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