Dear reviewers and editors,

We are submitting the responses to your valuable comments about our "**Changes in soil physicochemical properties and bacterial communities among different soil depths after long-term straw mulching under a no-till system**" (No.: soil-2021-25).

In this response, we have addressed the suggestions and advice from you. An item-by-item response to your comments is enclosed. We thank you for the helpful comments and suggestions, and hope that these revisions successfully address your concerns and requirements. We will ask one native English editor from the International Science Editing, one English language editing services company, to check the whole manuscript and avoid any grammar or syntax error when we are allowed to submit the revised manuscript. Hope the paper could be accepted to publish in SOIL.

We do appreciate the great efforts made by you and valuable comments from reviewers to improve the quality of this manuscript.

Thank you for kind considerations!

Looking forward to hearing from you soon.

Best regards!

Zijun Zhou (Ph. D, Professor)

On behalf of the co-authors

Institute of Agricultural Resources and Environment, Sichuan Academy of Agricultural Sciences. Shizishan Road 4#, Chengdu 610066, China

E-mail: zhouzijun1007@163.com

Itemized responses to reviewers' comments are provided below.

Responses to comments:

This manuscript is a long-term experiment (started in 2005) and includes a detailed study on the impact of straw removal (control treatment) and straw mulching on soil parameters physicochemical and microbial community assembly at different soil depths. This paper contains very good data and it is an interesting field study. In general, the article is well written and provides relevant information on the management of mulch in no-till system. Unfortunately, the results have been not been described or explained in a clear or specific manner. Moreover, the discussion of the results is greatly lacking in clearly explaining the effects which have been observed. There is a reasonable connection with previous studies, but often the results from the present study are poorly explained in context to and in comparison to the published studies.

As a result, the abstract is written in a very general / vague manner with little given on the results. What is presented is not specific at all.

Response: Thanks for your comments, and we revised a lot in this section in red as following. Please look through it.

"Conservation tillage has attracted increasing attention over recent decades, mainly due to its benefits in improving soil organic matter content and reducing soil erosion. However, long-term straw mulching effects on soil physicochemical properties and bacterial communities among different soil depths under a no-till system are still obscure. One twelve-year experiment included straw removal (CK) and straw mulching (SM) treatments was used to collect soil samples at 0–5, 5–10, 10–20, and 20–30 cm soil depths. The results showed that the contents of organic carbon (C), nitrogen (N) and phosphorus (P) fractions, and bacterial abundance significantly decreased, while pH significantly increased with soil depth. Compared with CK treatment, SM treatment significantly increased total N and inorganic N, available P and potassium, and soil water content at 0–5 cm depth, total organic C at 0–10 cm, and dissolved organic C and N contents at 0–20 cm depth. Regarding bacterial community, SM treatment increased relative abundances of Proteobacteria, Bacteroidetes, and Acidobacteria but reduced those of Actinobacteria, Chloroflexi, and Cyanobacteria. Bacterial Shannon and Shannon's evenness index at 0–5 cm was significantly reduced in SM treatment compared to CK treatment. Furthermore, SM increased the relative abundances of some C-cycling genera (such as *Terracidiphilus*, and *Acidibacter*) and N-cycling genera (such as *Rhodanobacter*, *Rhizomicrobium*, *Dokdonella, Reyranella*, and *Luteimonas*) at 0–5 cm depth. Principal coordinate analysis showed the largest difference about the composition of soil bacterial communities between CK and SM treatments occurred at 0–5 cm depth. Soil pH, and nitrogen and organic carbon fractions were the major drivers shaping soil bacterial community. Overall, straw mulch is highly recommended for use under a no-till system because of its benefits to soil fertility and bacterial abundance."

Specific comments

1. Introduction

Probably too long and needs to be more focused. I suggest that the authors substantially reduce the text size, replacing long sentences with more objective ones. The connection between paragraphs should also be improved.

Response: Thanks for your comments. We did a lot efforts to rewrote this section, and deleted some too specific parts in the section. We have modified the whole part of this section. Given many sentences were deleted and revised, we list the whole section as following, and the revised part were in red. Please look through it.

"The global demand for food largely depends on agriculture production to feed a growing population in the future (Karthikeyan et al., 2020). Conventional intensive agriculture puts unprecedented stress on soils and results in their unsustainable degradation, such as soil organic matter loss, erosion, and genetic diversity loss (Hou et al., 2020; Kopittke et al., 2019; Lupwayi et al., 2012). By contrast, conservation agriculture centered on conservation tillage has been widely recommended for sustaining and improving agriculture production in recent decades because it could increase soil organic matter content, improve soil structure, reduce soil erosion, and decrease the need for farm labor (Jena, 2019; 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). Generally, conservation tillage practice is composed of two key principles, minimal soil disturbance (no or reduced tillage) and soil cover (mainly straw mulch) (Pittelkow et al., 2014). Some researchers have compared the differences between conventional tillage and conservation tillage in crop yield and soil properties (Bu et al., 2020; Gao et al., 2020; Hao et al., 2019; Hu et al., 2021). However, straw mulching was not always combined with no-till in many countries due to the poor productivity, the prioritization of livestock feeding, or the insufficient time to apply straw mulching (Giller et al., 2009; Jin, 2007; Pittelkow et al., 2014; Zhao et al., 2018). Therefore, separation of straw mulching effects could refine the understanding of straw function on soil properties with increasing the area of conservation tillage in the world.

Soil physicochemical properties are important contributors to soil fertility, which is a critical factor determining crop productivity and agriculture sustainability (Liu et al., 2019). Since straw contains large amounts of carbon (C), nitrogen (N), phosphorus (P), and potassium (K), straw mulching is reported to increase soil total organic C and its fractions, soil enzymes (invertase, phosphatase, urease, and catalase), and other physicochemical properties (Akhtar et al., 2018; Dai et al., 2019; Duval et al., 2016; Wang et al., 2019b; Zhou et al., 2019a and b). Many studies have focused on these properties changes in the topsoil since the topsoil provides large amounts of nutrients to plants (Dai et al., 2019; Wang et al., 2019b; Zhou et al., 2019a). However, soil physicochemical properties in the subsoil should also be considered since some nutrients could move from topsoil to deeper soil during irrigation and rainfall (Blanco-

Canqui and Lal, 2007; Stowe et al., 2010). Inconsistent results on the physicochemical properties distribution along soil depth were reported in cultivated agriculture soils or grassland (Li et al., 2017b; Peng and Wang, 2016). The variation in physicochemical properties among different soil depths under a no-till system is still unclear after long-term straw mulching, since the no-till practice did little disturbance to soil, and it was quite different from the heavy tillage in conventional agriculture.

Soil bacterial communities have been used as sensitive indicators of soil quality in agricultural systems (Ashworth et al., 2017), and play a vital role in soil ecological processes such as soil carbon, nutrient cycling, and greenhouse gas release (Hobara et al., 2014; Tellez-Rio et al., 2015; Thompson et al., 2017). The responses of soil bacterial abundance and community to straw mulching were inconsistent in the topsoil (Bu et al., 2020; Chen et al., 2017; Hao et al., 2019; Qiu et al., 2020). Chen et al. (2017) proposed that straw return significantly increased bacterial biomass in one region but had no significant effects in other regions. Regarding the relative abundances of bacterial phyla, Actinobacteria were enriched in straw mulch soils in the Loess Plateau of China (Qiu et al., 2020), while it was reduced under wheat-maize rotation in Hao et al. (2019). Moreover, soil microorganisms at deep soil layer have attracted the attention of researchers because they demonstrated important effects on soil formation, ecosystem biochemistry processes, and maintaining groundwater quality (Li et al., 2014). Several studies have showed the bacterial abundances and community composition changed with soil depths (Fierer et al., 2003; van Leeuwen et al., 2017). Unfortunately, no detailed information has been obtained on the soil bacterial community changes in response to straw mulching among different soil depths under no-till systems.

Rice-wheat rotation is a major cropping system in China, and approximately 80 million tons of crop straw are produced annually in southwestern China (Li et al., 2016; Zhou et al., 2019b). This area has a humid mid-subtropical monsoon climate with an average annual precipitation of 1200 mm. The abundant precipitation could promote the leaching of water-soluble organic matter and nutrients derived from straw to the deep soil, which may result in the significant differences in soil properties at deep soil profiles. We hypothesized that (1) compared with straw removal, straw mulching will significantly change soil properties, which will decline with increasing soil depth; and (2) the key soil physicochemical properties shaping bacterial communities will be different at different depths. In this study, a field experiment subjected to two straw management programs under a 12-year no-till regime in the Chengdu Plain was used to (1) determine the effects of straw mulching on the soil physicochemical parameters, bacterial abundance and community composition at different depths, and (2) clarify the differences in the key soil physicochemical properties shaping bacterial communities with increasing soil depths."

2 - Material and Methods

Line 176: it is necessary to present more details about the fertilization used for the crops. Source, dose and frequency of application must be added.

Response: We added the details about fertilization in the revised manuscript as following:

"During the experiment, the amounts of inorganic fertilizer added were equal in both treatments, and they were manually broadcast over soil surface without tillage. The doses of N, P_2O_5, and K_2O fertilizers were at 180, 90, and 90 kg ha^{-1}, respectively, in wheat season, while the doses were at 165, 60, and 90 kg ha^{-1}, respectively, in rice season. Nitrogen fertilization as urea was applied at sowing and tillering stage at rates of 30% and 70% during wheat season, respectively, while it was applied at rates of 70% and 30% during rice season. Potassium fertilizer as potassium chloride was applied at sowing and tillering stage at the rates of 50% and 50% during both wheat and rice seasons. Phosphorus fertilizer as calcium superphosphate was applied once at sowing both during wheat and rice growing seasons."

Section 2.2 Soil sampling

Have soil collections at different depths been randomized? that is, were they sampled at the same sampling point? If so, the comparison between depths is not statistically correct, and the results are obvious.

Response: The experiment included two treatments with three replications at a randomized design. Soil columns of 0–30 cm depth was collected at five points in each plot using a stainless-steel auger (40 mm interior diameter). Each soil column was divided into four samples at soil depths of 0–5, 5–10, 10–20, and 20–30 cm. The same soil depth from five points were pooled to make one composite sample of 0–5, 5–10, 10–20, and 20–30 cm respectively for each plot.

We think the composite sample from five points in each plot was enough to represent the soil in the plot. The similar method of collecting different soil depths were also found in other studies (Coonan et al., 2019; Li et al., 2017; Hou et al., 2019; Qiao et al., 2020; Schlatter et al., 2020; Zuo et al., 2021). Please consider it. Thanks!

References:

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China, Geoderma, 290, 69–74, https://doi.org/10.1016/j.geoderma.2016.11.006, 2017.
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Qiao, Y., Wang, J., Liu, H., Huang, K., Yang, Qi., Lu, R., Yan, L., Wang, X., and Xia, J.: Depth-dependent soil C-N-P stoichiometry in a mature subtropical broadleaf forest, Geoderma, 370: 114357. https://doi.org/10.1016/j.geoderma.2020.114357, 2020.
Schlatter, D. C., Kahl, K., Carlson, B., Huggins, D. R., and Paulitz, T.: Soil acidification modifies soil depth-microbiome relationships in a no-till wheat cropping system, Soil Biol. Biochem., 149, 107939. https://doi.org/10.1016/j.soilbio.2020.107939, 2020.
Zuo, Y., Zhang, H., Li, J., Yao, X., Chen, X., Zeng, H., and Wang, W.: The effect of soil depth on temperature sensitivity of extracellular enzyme activity decreased with elevation: Evidence from mountain grassland belts, Sci. Total Environ., 777: 146136. https://doi.org/10.1016/j.scitotenv.2021.146136, 2021.

Section 2.3 Soil physicochemical properties

Details of extractor must be included.

Response: We added the brief descriptions of the methods for soil physicochemical parameters in the manuscript as following:

"Soil DOC and DON were extracted from the soil by shaking fresh soil samples with distilled water (1:5 soil: solution ratio), and the extracts were then filtered to determine by a Multi N/C 3100 analyzer (Analytik Jena AG, Jena, Germany) (Zhou et al., 2019b). Soil water content was determined using the gravimetric method after drying the soil to a constant weight at 105 °C (Akhtar et al., 2018). Soil inorganic N, pH, total organic C, total N, total P, total K, available P, and available K were determined according to Lu (2000). Briefly, concentrations of NH_4^+–N and NO_3^-–N in filtered 2 M KCl extracts from fresh soil were measured by a continuous-flow auto-analyzer (AA3, Seal Analytical Inc., Southampton, UK). Inorganic N concentration was the sum of the NH_4^+–N and NO_3^-–N. Soil pH was determined in a 1:2.5 soil: water aqueous suspension using an Orion 3-star benchtop pH meter (Thermo Scientific, Waltham, MA). Soil total organic C was determined using the dichromate oxidation and ferrous sulfate titration method, and soil total N was determined with the continuous-flow auto-analyzer after digestion based on the Kjeldahl method. For measurement of soil total P and total K, soils were first digested by a mixed acid solution of H_2SO_4 and $HClO_4$, and total P was then analyzed by the determined using the continuous-flow auto-analyzer, and total K was determined by atomic absorption photometry. Soil available P was extracted by 0.025 M HCl–0.03 M NH_4F and determined by ammonium molybdate colorimetry, and available K was extracted by 2 M HNO_3 and determined by atomic

absorption photometry."

The soil used to determine ammonium and nitrate was stored under what conditions? This information is missing.

Response: We rewrote this section in the revised manuscript.

"The soil was kept at 4 ℃ (<1 week) for soil NH_4^+–N, NO_3^-–N, dissolved organic C (DOC), and dissolved organic N (DON) analysis".

3 - Results

Here is the biggest problem with this study. I do not agree, at all, to compare the different layers of the soil. It is almost logical that the effects of soil fertility are described. Additionally, for this type of comparison to take place, soil collection at different depths must also be randomized and not all from the same collection point. Comparisons between treatments must occur in each layer of the soil and not between layers. I suggest that the authors opt for this approach. The same is true for the relative abundances of bacterial phyla in Table 3.

Response: Thanks for your suggestions. After thorough consideration, some points we are not totally agree with some points and our reasons were followed. Please consider it.

First comments on the method of soil samples collection. Both us admitted that one composite sample at different depths in each plot should minimize the differences in physical and chemical properties of soil samples, and could represent the soil at each depth in the plot. As we mentioned above, soil columns of 0–30 cm depth was collected at five points in each plot using a stainless-steel auger (40 mm interior diameter). Each soil column was divided into four samples at soil depths of 0–5, 5–10, 10–20, and 20–30 cm. Actually, it means that four soil depths were collected at the same point. One composite sample at each depth was mixed from five points at same depth in each plot. We think the composition soil could represent the soil at each depth in the plot and the method of collecting soil samples are acceptable. The similar method of collecting different soil depths were also found in other studies (Coonan et al., 2019; Li et al., 2017; Hou et al., 2019; Qiao et al., 2020; Schlatter et al., 2020; Zuo et al., 2021).

Second comments on the comparation among different soil layers. I agreed with you about the importance of comparisons between two treatments in each soil depth and it could give us understandings about the long-term straw mulching effects on soil properties at each depth. However, the comparations among different depths may give

us some information about changes of soil properties along soil depth gradient under a no-till system. Therefore, we replaced the original Table 1 by new Table 1 and Table 2, and replaced original Table 2 and Table 3 by new Table 3 and Table 4. These tables gave us information about not only differences between two treatments at each depth, but also soil property changes among four soil depths. New tables in the revised manuscript are as following.

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

Physicochemical properties	Straw		Depth		Straw × Depth	
	F	*P*	*F*	*P*	*F*	*P*
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**

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

Physicochemical properties	Treatments	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
		5.09 ± 0.27A	5.90 ± 0.35B	6.56 ± 0.29C	7.17 ± 0.29D
Total organic C	CK	23.01 ± 0.15*	19.42 ± 1.23*	14.22 ± 2.23	6.90 ± 1.19
(g kg^{-1})	SM	33.24 ± 1.47	22.26 ± 0.25	15.76 ± 1.41	7.15 ± 0.43
		28.13 ± 5.73A	20.84 ± 1.75B	14.99 ± 1.87C	7.03 ± 0.81D
Total N	CK	2.84 ± 0.10*	2.13 ± 0.34	1.54 ± 0.27	0.62 ± 0.10
(g kg^{-1})	SM	3.50 ± 0.18	2.39 ± 0.17	1.54 ± 0.25	0.66 ± 0.11
		3.17 ± 0.38A	2.26 ± 0.28B	1.54 ± 0.23C	0.64 ± 0.10D
Total P	CK	0.88 ± 0.13	0.67 ± 0.02	0.43 ± 0.11	0.22 ± 0.04
(g kg^{-1})	SM	0.86 ± 0.02	0.74 ± 0.09	0.53 ± 0.10	0.20 ± 0.04
		0.87 ± 0.08A	0.70 ± 0.07B	0.48 ± 0.11C	0.21 ± 0.04D

Total K	CK	12.42 ± 0.38	12.40 ± 0.42	11.75 ± 0.30	11.81 ± 0.62
(g kg^{-1})	SM	12.44 ± 0.34	12.55 ± 0.58	12.80 ± 1.00	12.07 ± 0.27
		12.43 ± 0.33A	12.48 ± 0.46A	12.28 ± 0.88A	11.94 ± 0.45A
Inorganic N	CK	21.43 ± 1.02*	18.33 ± 2.25	14.21 ± 2.53	11.31 ± 1.06
(mg kg^{-1})	SM	29.05 ± 0.83	16.64 ± 2.42	14.45 ± 1.52	11.89 ± 0.41
		25.24 ± 4.25A	17.49 ± 2.29B	14.33 ± 1.87C	11.60 ± 0.79D
Available P	CK	94.49 ± 7.59*	39.30 ± 4.11	14.74 ± 3.70	2.43 ± 2.48
(mg kg^{-1})	SM	126.63 ± 17.52	53.74 ± 14.21	17.06 ± 0.81	1.60 ± 0.87
		110.55 ± 21.34A	46.52 ± 12.25B	15.90 ± 2.71C	2.01 ± 1.73D
Available K	CK	152.33 ± 15.93*	107.85 ± 3.08	103.37 ± 1.55	103.70 ± 5.25
(mg kg^{-1})	SM	183.72 ± 13.09	115.88 ± 13.95	100.31 ± 3.93	100.84 ± 9.81
		168.02 ± 21.58A	111.86 ± 10.05B	101.83 ± 3.16B	102.26 ± 7.21B
DOC	CK	41.42 ± 5.74*	35.05 ± 4.38*	20.59 ± 1.24*	12.69 ± 6.23
(mg kg^{-1})	SM	73.01 ± 9.22	55.41 ± 1.99	36.31 ± 8.04	8.48 ± 2.88
		57.21 ± 18.62A	45.23 ± 11.54B	28.45 ± 10.03C	10.58 ± 4.92D
DON	CK	16.11 ± 1.89*	17.29 ± 3.69	12.33 ± 0.85*	4.97 ± 1.21
(mg kg^{-1})	SM	26.22 ± 2.51	18.08 ± 2.24	18.36 ± 1.21	5.98 ± 0.94
		21.16 ± 5.89A	17.68 ± 2.77B	15.34 ± 3.43B	5.48 ± 1.12C
Soil water content	CK	16.99 ± 0.69*	17.46 ± 0.77	15.21 ± 0.66	12.68 ± 0.81
(%)	SM	19.03 ± 0.89	16.71 ± 0.73	16.20 ± 0.68	13.81 ± 1.18
		18.01 ± 1.32A	17.09 ± 0.79A	15.71 ± 0.80B	13.25 ± 1.10C

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

Bacterial properties	Straw		Depth		Straw × Depth	
	F	*P*	*F*	*P*	*F*	*P*
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**
Chao 1	**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**

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

Bacterial properties	Treatments	Soil depth gradient			
		0–5 cm	5–10 cm	10–20 cm	20–30 cm
Copy number of 16S	CK	14.77 ± 2.69*	7.18 ± 2.59	6.30 ± 1.75	2.10 ± 0.54
rRNA gene	SM	24.65 ± 3.93	13.59 ± 4.98	6.12 ± 2.65	1.97 ± 1.34
		19.71 ± 6.19A	10.38 ± 4.99B	6.22 ± 2.01C	2.03 ± 0.92D
Shannon	CK	6.53 ± 0.03*	6.38 ± 0.08	6.34 ± 0.05	6.07 ± 0.16
	SM	6.40 ± 0.08	6.42 ± 0.09	6.40 ±0.06	6.27 ± 0.12
		6.46 ± 0.09A	6.40 ± 0.08A	6.37 ± 0.06A	6.17 ± 0.17B
Shannon's evenness	CK	0.864 ± 0.002*	0.844 ± 0.006	0.843 ± 0.007	0.816 ± 0.016
	SM	0.852 ± 0.007	0.846 ± 0.008	0.842 ± 0.004	0.832 ± 0.009
		0.858 ± 0.008A	0.845 ± 0.006B	0.843 ± 0.005B	0.824 ± 0.015C
Chao 1	CK	2417 ± 64	2563 ± 198	2506 ± 166	2437 ± 18
	SM	2421 ± 46	2714 ± 74	2689 ± 146	2472 ± 185
		2419 ± 50A	2639 ± 156C	2597 ± 172BC	2455 ± 119AB
Proteobacteria	CK	32.11 ± 0.82*	29.51 ± 2.16	29.08 ± 1.78	26.69 ± 3.70
	SM	38.87 ± 2.57	31.31 ± 0.71	30.93 ± 0.32	28.06 ± 1.36
		35.49 ± 4.08A	30.41 ± 1.75B	30.00 ± 1.53B	27.37 ± 2.60C
Actinobacteria	CK	17.02 ± 2.99	12.57 ± 2.44	12.15 ± 0.66*	10.32 ± 1.62
	SM	12.66 ± 1.82	11.30 ± 2.52	8.83 ± 0.56	9.76 ± 0.73
		14.84 ± 3.26A	11.94 ± 2.32B	10.49 ± 1.90B	10.04 ± 1.16B
Acidobacteria	CK	17.17 ± 1.96	19.56 ± 0.56	20.14 ± 0.70*	14.32 ± 1.30*
	SM	21.23 ± 2.25	20.16 ± 0.97	22.52 ± 0.28	16.44 ± 0.01
		19.20 ± 2.92B	19.86 ± 0.78BC	21.33 ± 1.39C	15.38 ± 1.42A
Chloroflexi	CK	13.82 ± 1.37*	13.33 ± 2.03	14.63 ± 1.84*	20.46 ± 2.96
	SM	10.03 ± 1.30	12.02 ± 1.25	11.56 ± 0.20	18.10 ± 0.99
		11.92 ± 2.40A	12.67 ± 1.67A	13.10 ± 2.05A	19.28 ± 2.36B
Planctomycetes	CK	4.29 ± 0.50	3.68 ±0.22	4.16 ± 0.28	2.56 ± 1.04
	SM	3.95 ± 0.51	3.76 ± 0.07	4.23 ± 0.16	2.93 ± 0.40
		4.12 ± 0.49A	3.72 ± 0.15A	4.20 ± 0.21A	2.74 ± 0.73B
Nitrospirae	CK	5.25 ± 1.17	10.39 ± 1.39	8.50 ± 1.40	13.18 ± 2.54
	SM	4.66 ± 0.23	10.26 ± 0.93	10.40 ± 1.35	12.29 ± 0.66
		4.96 ± 0.82A	10.33 ± 1.06B	9.45 ± 1.61B	12.74 ± 1.73C
Bacteroidetes	CK	1.74 ± 0.21*	1.37 ± 0.36	0.78 ± 0.16*	0.62 ± 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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5 - The discussion is very detailed and consistent with the results;

The discussion is very long. It needs to be more focused. In addition, many results are repeated in the discussion.

This section should be improved.

Response: Thanks for your suggestion. Actually, we did our best to improve this section. We removed many sentences repeated results, and rewrote the whole discussion. Given many sentences were deleted and revised, we list the whole section as following, and the revised part were in red. Please look through it.

"**4 Discussion**

4.1 Straw mulching changed soil physicochemical properties with soil depth

Our study demonstrated that compared to straw removal, straw mulching increased contents of total N, inorganic N, available P, available K at 0–5 cm, water content at 0–5 cm, and total organic C at 0–10 cm depths. The results possibly because straw was mulched at soil surface, rather than incorporated into soil, and large C and nutrients were released to surface soil from straw decomposition (Blanco-Canqui and Lal, 2007; Akhtar et al, 2018). Furthermore, the decrease in gaseous N loss through ammonia volatilization and denitrification caused by straw mulching may also contribute to the accumulation of soil nitrogen fractions (Cao et al., 2018). During straw decomposition, large amounts of soluble organic matter, such as starch, protein, and monosaccharides, could be leached and accumulated in the subsoil (Blanco-Canqui and Lal, 2007), which increased soil DOC and DON at 0–20 cm depth. For soil water content, mulched straw can reduce water evaporation and increase water retention (Palm et al., 2014; Wang et al, 2019c). However, there was no significant difference in pH, total P, and total K levels between CK and SM treatments. Similar pH result after straw mulching was consistent with Wang et al. (2020). The unchanged soil total P and total K results possibly because of their high levels in the soil (Dong et al., 2012; Zhang et al., 2016).

The results of the present study indicated that soil total organic C, total N, total P, inorganic N, available P and K, DOC, DON and water content decreased with increasing soil depth, which was partly consistent with our hypothesis. One reason for this was that most crop roots distributed in 0–10 cm or 0–20 cm soil layers (Li et al., 2020), and root exudates and C release after root decomposition led to higher soil total and DOC contents in the topsoil than in the subsoil. Except the effects of roots, inorganic N, P, and K fertilizers were applied to soil surface without tillage, and these elements were firstly enriched in the topsoil and decreased with soil depth. Large

amounts of N fertilizer over a long period of time could result in soil acidification (Guo et al., 2010), which resulted in a lower pH value in the topsoil than in subsoil. The total K content did not change with soil depth, mainly because of its high levels in the studied soil.

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

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

Soil bacteria can be divided into copiotrophic and oligotrophic groups based on their performances on different substrates (Fierer et al., 2007, 2012). Straw mulching produced a nutrient-rich soil environment, which would benefit copiotroph bacterial growth and lead to a shift in the predominant bacterial community (Fierer et al., 2012). In addition, high soil inorganic N content decreased bacterial diversity (Yu et al., 2019; Zhao et al., 2019). These factors contributed to the reduced value of Shannon diversity and Shannon's evenness index at 0–5 cm soil depth after straw mulching. Soil biodiversity was important for maintain ecosystem function (Wagg et al., 2014), and sustainable agriculture should adopt management practices that preserve or increase microbial diversity rather than destroy or threaten it (Pastorelli et al., 2013). Consequently, inorganic N fertilizer could be reduced under straw mulching and may thus be more beneficial for maintaining or improving bacterial diversity.

Regarding on bacterial phyla, they demonstrated different strategies to straw managements and soil depth. The relative abundances of the copiotrophic groups, such as Proteobacteria, Actinobacteria, and Bacteroidetes, were decreased with soil depth due to their preference to abundant soil resources in topsoil (Fierer et al., 2007, 2012; Liang et al., 2018; Ling et al., 2017). As a result, compared with CK, straw mulching increased soil C and nutrients and then increased the relative abundances of Proteobacteria and Bacteroidetes (Fierer et al., 2007, 2012; Liang et al., 2018; Ling et al., 2017). Bacteroidetes are additionally involved in hemicellulose breakdown and mulched straw stimulated it proliferation during straw decomposition (Wegner and Liesack, 2016). Chloroflexi was classified as oligotrophic groups, and enriched soil nutrients restricted it growth at topsoil or after straw mulching, which agreed with the result of Liang et al. (2018). Notably, soil nutrient condition was not the only one factor

influencing bacterial phyla proliferation, such as Actinobacteria and Acidobacteria. Actinobacteria was classified as copiotrophs by Fierer et al. (2012), but straw mulching decreased the Actinobacteria in our study, which was also observed in other studies (Calleja-Cervantes et al., 2015; Hao et al., 2019; Liang et al., 2018). One possible reason is that straw mulching increased soil water content and reduced soil oxygen content, but most Actinobacteria favor aerobic environments (Hamamura et al., 2006). Though Acidobacteria was classified as oligotrophic groups, it was involved in hemicellulose breakdown (Wegner and Liesack, 2016), leading increased its relative abundance after straw mulching.

Our results confirmed that straw return could change soil special bacterial genera associated with C and N cycles (Shang et al., 2011; Xu et al., 2017; Wang et al., 2012). For example, straw mulching favored *Rhodanobacter* growth, which was the dominant bacterial genus containing denitrifying species and positively associated in N_2O emissions (Huang et al., 2019). Similarly, the relative abundances of the *Rhizomicrobium*, *Dokdonella, Reyranella*, and *Luteimonas* genera are N-cycling-related bacterial taxa containing denitrifiers and they were increased in straw mulching soil (Chen et al., 2020a; Nie et al., 2018; Wang et al., 2019a; Wolff et al., 2018). *Terracidiphilus*, *Acidibacter*, *Flavobacterium*, and *Lysobacter* was respectively involved in the degradation of plant-derived biopolymers (Garcia-Fraile et al., 2015), organic substrates (Ai et al., 2018), labile carbon (Nan et al., 2020), and macromolecules (Maarastawi et al., 2018), and large C materials from mulched straw increased their relative abundances. Although little is known about the ecology of *Pseudolabrys*, its relative abundance was increased in soil after compost application (Joa et al., 2014). Wang et al. (2019a) found that organic carbon can inhibit the growth of chemolithotrophic bacteria and favor *Dokdonella*. According to Foesel et al. (2013), *Blastocatella fastidiosa* was the only known isolate from *RB41*, and the former preferred protein-containing substrates. Straw mulching might possibly increase the contents of these substrates and, therefore, *RB41* relative abundance.

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

Lines 465 -467: This sentence is obvious for the physicochemical parameters of the soil. I believe it is more appropriate for the microbial community.

Response: We have rewritten these sentences and added some description for soil community in the Discussion section as following.

“The results of the present study indicated that soil total organic C, total N, total P, inorganic N, available P and K, DOC, DON and water content decreased, but pH increased with increasing soil depth, which was partly consistent with our hypothesis.”

“Regarding on bacterial phyla, they demonstrated different strategies to straw managements and soil depth.”

6 - Conclusions

It is very well written and answers the questions raised by the hypothesis

Response: Thanks for your kindness.