Dear reviewers and editors,

We are submitting a response 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).

We have studied reviewer's comments and suggestions carefully and have made revision which are marked in red in the manuscript. After that, we have asked one native English editor from the International Science Editing, one English language editing services company, to check the whole manuscript and avoid grammar errors in the revised manuscript. In addition, 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. 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

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Itemized responses to reviewers' comments are provided below.

Responses to comments

Referee #1:

The paper soil-2021-25 entitled "Changes in soil physicochemical properties and bacterial communities among different soil depths after long-term straw mulching under a no-till system" presented interesting results about soil fertility and bacterial community related to straw management in an important rice and wheat production region in China. With just two mulch treatments, the authors collected adequate data and tried to tell a good story. However, some questions should be addressed before considering for publication.

1. There were some syntax errors through the manuscript. The language should be improved.

Response: Thanks for your suggestion. We have asked one native English editor from the International Science Editing, one English language editing services company, to check the whole manuscript and avoid grammar errors in the revised manuscript. The certificate of language editing is as following.

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DATE: July 28, 2021

To whom it may concern,

The paper "Changes in soil physicochemical properties and bacterial communities at different soil depths after long-term straw mulching under a no-till system" by Zijun Zhou was edited by International Science Editing. We were asked not to check the references. Please contact us if you would like to view the edited paper.

Kindest regards,

David Cushley

2. Introduction:

In this section, the authors enumerated numbers of findings and literatures and gave too much general information on conservation tillage/no tillage as well as microbial ecology. The introduction is long (with long paragraphs), with subjects dispersed in paragraphs. This section should be rewritten more concisely. Suggesting delete some unrelated description and readjust this section.

Response: Actually, all three reviewers gave the similar evaluation about the Introduction section. We did a lot of efforts to rewrote this section, and deleted some too specific parts in the section. Given too many sentences were deleted and revised, we marked the revised part in red in the resubmitted manuscript.

3. Materials and methods:

P6, L175: Fertilization details should be added, such as fertilization rate and time.

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

"During the experiment, equal amounts of inorganic fertilizer were added in both treatments by manual broadcast over the 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 the wheat season and 165, 60, and 90 kg ha^{-1}, respectively, in the rice season. Nitrogen as urea was applied as fertilizer in the sowing and tillering stages at rates of 30 % and 70 %, respectively, during the wheat season and 70 % and 30 %, respectively, during the rice season. Potassium as potassium chloride was applied as fertilizer in the sowing and tillering stages at rates of 50 % each during both the wheat and rice seasons. Phosphorus as calcium superphosphate was applied as fertilizer once at sowing during both the wheat and rice growing seasons."

4. P6, L181: Did these depths cross over soil horizons, or were they all still disturbed from previous tillage before the experiment started?

Response: These depths did not cross over soil horizons. The local agricultural soil was seldom tilled due to the shortage of tillage machines before the experiment. We collected four soil depths at 0–5, 5–10, 10–20, and 20–30 cm for several reasons. Firstly, fertilizers were applied at soil surface for both treatments, and straw was mulched over the soil surface in straw mulching treatment, which led to more N, P_2O_5, K_2O, and C materials being accumulated in topsoil than those in subsoil layers. Secondly, crop roots were mainly distributed in the 0–10 or 0–20 cm soil layers, and root exudates affected the soil properties at topsoil much more largely than that at 20–30 cm subsoil. Our previous study demonstrated soil organic C and labile fractions mainly changed at surface soil. However, this area has a humid, mid-subtropical monsoon climate with an average annual precipitation of 1200 mm. The abundant precipitation could promote leaching of water-soluble organic matter and nutrients derived from straw deep into the soil, which may result in significant differences in soil properties at deeper depths. The aim of the study was to (1) determine the effects of straw mulching on 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 at increasing soil depths. As a result, we just collected four soil depths from 0–5 cm to 20–30 cm, rather than all soil horizons. All soil horizons may give more information, but soil samples from the four depths were enough to gain our objectives.

5. P7, L196-L197: "The air-dried soil samples were analyzed for soil pH, TOC, TN, TP, TK,

AP, and AK as described by Lu". Even though a reference is given for the procedures, mentioning the extractants used will be very useful to readers.

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

"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 for analysis using 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 as described by Lu (2000). Briefly, concentrations of NH_4^+–N and NO_3^-–N in filtered 2 M KCl extracts from fresh soil were measured using a continuous-flow auto-analyzer (AA3, Seal Analytical Inc., Southampton, UK). Inorganic N concentrations were calculated as the sum of 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, USA). Soil total organic C was determined using the dichromate oxidation and ferrous sulfate titration method, and soil total N was determined using the continuous-flow auto-analyzer after digestion based on the Kjeldahl method. For measurements of soil total P and total K, soils were first digested using a mixed acid solution of H_2SO_4 and $HClO_4$; total P was then analyzed using the continuous-flow auto-analyzer, and total K was determined by atomic absorption photometry. Soil available P was extracted using 0.025 M HCl–0.03 M NH_4F and measured by ammonium molybdate colorimetry, and available K was extracted using 2 M HNO_3 and measured by atomic absorption photometry."

6. P7: Please add the citation the DOC and TOC results, since they were published in your previous study (the reference on p33, lines 982-985) though you used different presentations and statistical methods.

Response: We added the reference in the revised manuscript.

7. Lines 243-252 should be moved to part 2.6.

Response: We moved these sentences to part 2.6.

8. Results:

Some statistical methods were repeated in this part, which should be removed, such as line 332 and line 364.

Response: We carefully checked the manuscript, and removed those repeated description about the statistical methods in the revised manuscript.

9. P19, L504-508: Rewrite the first sentence "Proteobacteria and Bacteroidetes, often classified as copiotrophic groups, preferentially consume labile soil organic pools and have higher growth rates under conditions with abundant resources, while oligotrophic groups, such as Acidobacteria and Chloroflexi, are highly abundant in low-nutrient environments (Fierer et al., 2007, 2012; Liang et al., 2018; Ling et al., 2017)", as the definition of the copiotrophic groups was mentioned in the P18. It is repeated.

Response: We revised this sentence in the revised manuscript.

10. Discussion:

The discussion is too long and covered everything. The repeat of the results should be removed.

Response: Thanks for your suggestions. We did our best to revise the Discussion section. Given too many sentences were deleted and revised, we marked the revised part in red in the resubmitted manuscript. Please look through it.

Referee #2:

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 in the revised manuscript. 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, the effects of long-term straw mulching under a no-till system on soil physicochemical properties and bacterial communities at different soil depths are still unclear. In this 12-year experiment of straw removal (CK) and straw mulching (SM) treatments, soil samples were collected at 0–5, 5–10, 10–20, and 20–30 cm soil depths. The results showed that the contents of organic carbon (C), nitrogen (N) and phosphorus (P) fractions, and bacterial abundance significantly decreased, whereas pH significantly increased with soil depth. Compared with CK, SM significantly increased total N, inorganic N, available P, available potassium, and soil water content at 0–5 cm, total organic C content at 0–10 cm, and dissolved organic C and N contents at 0–20 cm. Regarding bacterial communities, SM increased the relative abundances of Proteobacteria, Bacteroidetes, and Acidobacteria but reduced those of Actinobacteria, Chloroflexi, and Cyanobacteria. Bacterial Shannon diversity and Shannon's evenness at 0–5 cm were reduced by 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. Principal coordinate analysis showed that the largest difference in the composition of soil bacterial communities between CK and SM occurred at 0–5 cm. Soil pH and N and organic C fractions were the major drivers shaping soil bacterial communities. Overall, SM treatment is highly recommended 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 of efforts to rewrote this section, and deleted some too specific parts in the section. Given too many sentences were deleted and revised, we marked the revised part in red in the revised manuscript. Please look through it.

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, equal amounts of inorganic fertilizer were added in both treatments by manual broadcast over the 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 the wheat season and 165, 60, and 90 kg ha^{-1}, respectively, in the rice season. Nitrogen as urea was applied as fertilizer in the sowing and tillering stages at rates of 30 % and 70 %, respectively, during the wheat season and 70 % and 30 %, respectively, during the rice season. Potassium as potassium chloride was applied as fertilizer in the sowing and tillering stages at rates of 50 % each during both the wheat and rice seasons. Phosphorus as calcium superphosphate was applied as fertilizer once at sowing during both the 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 and used a randomized design. Soil columns of 0–30 cm were collected from five points in each plot using a stainless steel auger (40 mm interior diameter). Each soil column was divided into four samples from soil depths of 0–5, 5–10, 10–20, and 20–30 cm. Samples from the same soil depth at five different sampling points were pooled to make one composite sample for each depth of 0–5, 5–10, 10–20, and 20–30 cm for each plot.

In our opinion, the composite sample from five points in each plot could be used 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:

Coonan, E. C., Richardson, A. E., Kirkby, C. A., Kirkegaard, J. A., Amidy, M. R., Simpson, R. J., and Strong, C. L.: Soil carbon sequestration to depth in response to long-term phosphorus fertilization of grazed pasture, Geoderma, 338: 226–235. https://doi.org/10.1016/j.geoderma.2018.11.052, 2019.

Li, X., Sun, J., Wang, H., Li, X., Wang, J., and Zhang, H.: Changes in the soil microbial phospholipid fatty acid profile with depth in three soil types of paddy fields in 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:

"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 for analysis using 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 as described by Lu (2000). Briefly, concentrations of NH_4^+–N and NO_3^-–N in filtered 2 M KCl extracts from fresh soil were measured using a continuous-flow auto-analyzer (AA3, Seal Analytical Inc., Southampton, UK). Inorganic N concentrations were calculated as the sum of 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, USA). Soil total organic C was determined using the dichromate oxidation and ferrous sulfate titration method, and soil total N was determined using the continuous-flow auto-analyzer after digestion based on the Kjeldahl method. For measurements of soil total P and total K, soils were first digested using a mixed acid solution of H_2SO_4 and $HClO_4$; total P was then analyzed using the continuous-flow auto-analyzer, and total K was determined by atomic absorption photometry. Soil available P was extracted using 0.025 M HCl–0.03 M NH_4F and measured by ammonium molybdate colorimetry, and available K was extracted using 2 M HNO_3 and measured 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 °C (<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, we do not agree with some points and our reasons were followed. Please consider it.

First comments on the method of soil samples collection. Both we 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 were collected from five points in each plot using a stainless steel auger (40 mm interior diameter). Each soil column was divided into four samples from soil depths of 0–5, 5–10, 10–20, and 20–30 cm. Samples from the same soil depth at five different sampling points were pooled to make one composite sample for each depth of 0–5, 5–10, 10–20, and 20–30 cm for 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	Treatment	Soil depth gradient			
		0–5 cm	5–10 cm	10–20 cm	20–30 cm
pH	CK	5.27 ± 0.19	6.04 ± 0.30	6.63 ± 0.36	7.11 ± 0.36
	SM	4.90 ± 0.21	5.76 ± 0.40	6.48 ± 0.26	7.23 ± 0.26
		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	Treatment	Soil depth gradient			
		0–5 cm	5–10 cm	10–20 cm	20–30 cm
Copy number of 16S rRNA gene	CK	14.77 ± 2.69*	7.18 ± 2.59	6.30 ± 1.75	2.10 ± 0.54
	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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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.

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 too many sentences were deleted and revised, we marked the revised part in red in the resubmitted manuscript. Please look through it.

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, available K, DOC, DON and water content decreased, but pH increased with increasing soil depth, which was partly consistent with our hypothesis."

"Bacterial phyla demonstrated different responses to straw management strategies and soil depths."

6. Conclusions

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

Response: Thanks for your kindness. We did slight revision in the revised manuscript.

Referee #3:

The manuscript "Changes in soil physicochemical properties and bacterial communities among different soil depths after long term straw mulching under a no-till system" presents an interesting experiment looking at an important aspect of agricultural sciences. The authors have collected a useful and impressive dataset to give a detailed analysis of the mulching treatments they have used here. Some aspects can be clarified and improved.

1. Introduction

The Introduction covers the important points but is perhaps too specific in parts when mentioning cited literature, so the reader may struggle to stay with the bigger picture and context of this study. Suggest removing some of the more specific sections and move these to the discussion section where they are relevant to the reported results from this work, rather than the study background in general. Otherwise, these parts could be removed from the manuscript.

Response: Thanks for your suggestions. We did a lot of efforts to rewrote this section, and deleted some too specific parts in the section. Given too many sentences were deleted and revised, we marked the revised part in red in the revised manuscript. Please look through it.

2. Hypotheses are generally sound, although perhaps a little vague. It is not clear what is meant by saying that mulching will "increase most soil physicochemical parameters". I assume this means measurable quantities such as total carbon, dissolved organic carbon, organic nitrogen and others will increase in the mulch treatment, but it could be phrased differently so that this is clearer. The same applies in the discussion section where similar phrasing is used, for example on L464, L574.

Response: We rewrote the sentence in the sections of Introduction, Discussion and Conclusions as following.

In Introduction section: "We hypothesized that (1) compared with straw removal, straw mulching would significantly change soil properties, which would decline with increasing soil depth; and (2) the key soil physicochemical properties shaping bacterial communities would be different at different depths."

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

In Conclusions section: "The results showed that soil total organic C, total N, total P, inorganic N, available P, available K, DOC, DON, water content, and bacterial abundance decreased but soil pH increased with soil depth."

3. Methods:

Methods section is generally good although could be clearer in places and some important details are missing. In the first paragraph it is not currently obvious that the mulch addition/removal treatment was carried out annually for entire duration of the experiment, or if it was done once, or periodically, etc.

Response: we rewrote the description about mulch management in CK and SM treatments. We have revised the sentences in the 2.1 section as following:

“The straw was removed in the CK treatment, whereas rice and wheat straw were distributed over the soil surface without being chopped after harvest each year in the SM treatment. The mulch consisted of approximately 8.5 t ha^{-1} rice straw and 6.0 t ha^{-1} wheat straw each year.”

4. What size were the experimental plots and how were they spatially arranged? Were plots randomly arranged to minimise risk of field effects? The authors state that soil heterogeneity is assumed to be minimal, but this is not sufficient, and a randomised design for a trial is necessary. Acknowledgment/detail should be given regarding the number of technical replicates per plot that were taken, or if one sample per plot was used. Often there can be substantial variation within a field trial plot, and this justifies pooling multiple samples per plot to give a plot average, then multiple plots are compared to give treatment means (again, stating the size of plots will be important to allow the reader to gauge the rigour of the sampling methods).

Response: The experiment included two treatments with three replicates and used a randomized design. Each plot measured 12 m^2 (3 × 4 m). Five soil points were collected and then pooled to make one composite sample in each plot to minimize the sampling variation. Many studies employed the similar method of soil sampling (Akhtar et al., 2018; Bu et a., 2020; Cao et al., 2018). We have revised this in the manuscript.

References:

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

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

5. More detail is needed L175-178 about fertiliser addition, the reader should not have to find another paper to find these important details for the study.

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

“During the experiment, equal amounts of inorganic fertilizer were added in both treatments by manual broadcast over the 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 the wheat season and 165, 60, and 90 kg ha^{-1}, respectively, in the rice season. Nitrogen as urea was applied as fertilizer in the sowing and tillering stages at rates of 30 % and 70 %, respectively, during the wheat season and 70 % and 30 %, respectively, during the rice season. Potassium as potassium chloride was applied as fertilizer in the sowing and tillering stages at rates of 50 % each during both the wheat and rice seasons. Phosphorus as calcium superphosphate was applied as fertilizer once at sowing during both the wheat and rice growing seasons."

6. Section 2.3 – more detail/definitions are needed here for the soil physicochemical characteristics of the soils for readers who might not already be familiar with these terms. The authors should add brief descriptions of the methods for these parameters.

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

"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 for analysis using 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 as described by Lu (2000). Briefly, concentrations of NH_4^+–N and NO_3^-–N in filtered 2 M KCl extracts from fresh soil were measured using a continuous-flow auto-analyzer (AA3, Seal Analytical Inc., Southampton, UK). Inorganic N concentrations were calculated as the sum of 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, USA). Soil total organic C was determined using the dichromate oxidation and ferrous sulfate titration method, and soil total N was determined using the continuous-flow auto-analyzer after digestion based on the Kjeldahl method. For measurements of soil total P and total K, soils were first digested using a mixed acid solution of H_2SO_4 and $HClO_4$; total P was then analyzed using the continuous-flow auto-analyzer, and total K was determined by atomic absorption photometry. Soil available P was extracted using 0.025 M HCl–0.03 M NH_4F and measured by ammonium molybdate colorimetry, and available K was extracted using 2 M HNO_3 and measured by atomic absorption photometry."

7. Statistical analysis – did data meet the assumptions for ANOVA? The authors say data were tested for homogeneity of variance but don't specify what these tests indicated. Data often will not meet assumptions for tests of normality and homogeneity of variance where there are small replicate numbers. Where data do not meet the assumptions of the statistical tests, non-parametric tests should be used instead.

Response: We did Levene and Shapiro Wilk tests to determine the homogeneity of variance and normality using before analysis of variance (ANOVA). In our study, only several parameters data were not at normal distribution. Data normalization was achieved by transforming soil available P content by log(x), and relative abundances of Acidobacteria and Planctomycetes $1/(x)^{0.5}$. We revised the description as following:

"The homogeneity of variance and normality were assessed using Levene and Shapiro-Wilk tests before analysis of variance (ANOVA). Data normalization was achieved by transforming soil available P content by log(x) and relative abundances of Acidobacteria and Planctomycetes by $1/(x)^{0.5}$."

8. Results: Through the section, statistics outputs need to show the effect size. The F-value (or equivalent for ANOVA) must be reported in addition to the p-value. This applies to the tables as well as in the text. Statements of data variability (for example standard deviation, standard error) must also be included. Without these, it is not clear what kind of data distribution lies behind the mean values reported.

Response: We added *F*-value in the new Table 1 and Table 3, and some descriptions. We also added the standard deviation to describe data variability 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 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**

"Soil DOC (*F* = 4.1, *P* = 0.001), total organic C (*F* = 3.5, *P* = 0.049), and pH (*F* = 2.3, *P* = 0.027) had significant effects on bacterial communities between the two treatments at 0–5 cm, whereas only soil pH (*F* = 4.4, *P* = 0.015) had a significant effect at 5–10 cm. At 10–20 cm, soil pH (*F* = 3.1, *P* = 0.022) and total organic C (*F* = 2.6, *P* = 0.038) had the most significant effects, and at 20–30 cm, soil inorganic N (*F* = 4.3, *P* = 0.003), pH (*F* = 3, *P* = 0.027), DON (*F* = 2.7, *P* = 0.032), and total N (*F* = 2.7, *P* = 0.030) most influenced soil bacterial communities."

9. The layout of table 1 is confusing. It is not clear why the CK vs SM data for pH are spread across one row with separate columns for CK and SM, while for TOC, there are two rows. This should be explained, and it would be better if the table were sorted by data presentation mode.

Response: We replaced Table 1 by the new Table 1 and Table 2 as following to made the data more readable.

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 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, straw was removed from the plot; SM, straw was mulched into the plot soil. 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	Treatment	Soil depth gradient			
		0–5 cm	5–10 cm	10–20 cm	20–30 cm
pH	CK	5.27 ± 0.19	6.04 ± 0.30	6.63 ± 0.36	7.11 ± 0.36
	SM	4.90 ± 0.21	5.76 ± 0.40	6.48 ± 0.26	7.23 ± 0.26
		5.09 ± 0.27A	5.90 ± 0.35B	6.56 ± 0.29C	7.17 ± 0.29D
Total C (g kg^{-1})	CK	23.01 ± 0.15*	19.42 ± 1.23*	14.22 ± 2.23	6.90 ± 1.19
	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 (g kg^{-1})	CK	2.84 ± 0.10*	2.13 ± 0.34	1.54 ± 0.27	0.62 ± 0.10
	SM	3.50 ± 0.18	2.39 ± 0.17	1.54 ± 0.25	0.66 ± 0.11
		3.17 ± 0.38A	2.26 ± 0.28B	1.54 ± 0.23C	0.64 ± 0.10D
Total P (g kg^{-1})	CK	0.88 ± 0.13	0.67 ± 0.02	0.43 ± 0.11	0.22 ± 0.04
	SM	0.86 ± 0.02	0.74 ± 0.09	0.53 ± 0.10	0.20 ± 0.04
		0.87 ± 0.08A	0.70 ± 0.07B	0.48 ± 0.11C	0.21 ± 0.04D
Total K (g kg^{-1})	CK	12.42 ± 0.38	12.40 ± 0.42	11.75 ± 0.30	11.81 ± 0.62
	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

10. Discussion. The discussion section is good but could be more concise and avoid unnecessary repetition of the results. Conclusions section may be better used to provide wider context, give suggestions for future work. As written, it seems like too much of a repeat of a list of results of microbial community patterns.

Response: We did our best to revise the sections of Discussion and Conclusions, and the revised sections were in red in the resubmitted manuscript. Please look through it.

11. Specific comments

L164: Strongly suggest avoiding the use of the word “cultivated" here. To some readers, cultivated is another way of saying “tillage”, and this is likely to cause confusion as the treatments are both no-till. “Managed” may be a better alternative.

Response: Thanks for your suggestion. We replaced it by “managed” in the revised manuscript.

12. Use of multiple acronyms for soil physicochemical properties is confusing when there are this many being studied. It may even be better to have them (TOC, TN, TP, IN and others) written out in full so that the reader can more easily follow what the authors are discussing.

Response: We replaced almost multiple acronyms by their full name in the whole manuscript.

13. L468: What is meant by “Apart from roots” here? This is not clear and should be amended.

Response: We firstly wanted to say that both inorganic fertilizer and crop roots could affect on some soil nutrients distribution along soil depth. We rewrote this sentence in the revised manuscript.