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Reply on RC3

Zijun Zhou et al.

Author comment on "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 et al., SOIL Discuss., https://doi.org/10.5194/soil-2021-25-AC1, 2021

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).

In this response, we have addressed the suggestions and advices of you and reviewers. An item-by-item response to your comments is enclosed. We thank you for the helpful comments and suggestion, 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!

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Itemized responses to reviewers' comments are provided below.

Responses to comments:

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.

- 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: Actually, all three reviewers gave the similar evaluation about the Introduction section. We did a lot effort 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.

“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). Bu et al. (2020) reported that straw return significantly increased the relative abundance of Proteobacteria, but it did not change in the study of 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. Although we determined some soil organic carbon fractions under a no tillage regime in our previous study (Zhou et al., 2019b), little is known about how other soil physicochemical parameters vary with soil depth. 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.”

- 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 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 Discussion section: "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."

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

- 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 respectively 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 during annually."

- 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 size of each plot was 12 m^2 (3 m × 4 m), and the plots were at a randomized design. Five soil points were collected and then pooled to make one composite sample in each plot to reduce the sampling variation. Many studies employed this sampling method (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.

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

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.

- 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, 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 2.3 – 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:

"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.”

- 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 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 $1/(x)^{0.5}$.”

- 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*	

pH	**1.91**	**0.186**	52.93	<0.0001
Total organic C	48.47	<0.0001	281.08	<0.0001
Total N	7.99	0.012	160.85	<0.0001
Total P	**0.99**	**0.334**	74.60	<0.0001
Total K	**2.79**	**0.114**	**1.21**	**0.339**
Inorganic N	6.01	0.026	73.66	<0.0001
Available P	11.45	0.004	184.96	<0.0001
Available K	4.37	0.049	62.53	<0.0001
DOC	47.75	<0.0001	78.20	<0.0001
DON	29.23	0.0001	65.80	<0.0001

Soil water content	6.55	0.021	38.72	<0.0001

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*		
Copy number of 16S rRNA gene	11.59	0.004	41.38	<0.0001		
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 the bacterial community in the two treatments at 0–5 cm soil depth, whereas only soil pH (*F* = 4.4, *P* = 0.015) had a significant effect at 5–10 cm. At 10–20 cm soil depth, 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) were the drivers that most influenced the soil bacterial community.”

- 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*		
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	Treatments	Soil depth gradient
0–5 cm	5–10 cm	10–20 cm
pH	CK	5.27 ± 0.19
SM	4.90 ± 0.21	5.76 ± 0.40
	5.09 ± 0.27A	5.90 ± 0.35B

Total C (g kg^{-1})	CK	23.01 ± 0.15*
SM	33.24 ± 1.47	22.26 ± 0.25
	28.13 ± 5.73A	20.84 ± 1.75B
Total N (g kg^{-1})	CK	2.84 ± 0.10*
SM	3.50 ± 0.18	2.39 ± 0.17
	3.17 ± 0.38A	2.26 ± 0.28B
Total P (g kg^{-1})	CK	0.88 ± 0.13
SM	0.86 ± 0.02	0.74 ± 0.09
	0.87 ± 0.08A	0.70 ± 0.07B

Total K (g kg^{-1})	CK	12.42 ± 0.38
SM	12.44 ± 0.34	12.55 ± 0.58
	12.43 ± 0.33A	12.48 ± 0.46A
Inorganic N (mg kg^{-1})	CK	21.43 ± 1.02*
SM	29.05 ± 0.83	16.64 ± 2.42
	25.24 ± 4.25A	17.49 ± 2.29B
Available P (mg kg^{-1})	CK	94.49 ± 7.59*
SM	126.63 ± 17.52	53.74 ± 14.21
	110.55 ± 21.34A	46.52 ± 12.25B

Available K (mg kg^{-1})	CK	152.33 ± 15.93*
SM	183.72 ± 13.09	115.88 ± 13.95
	168.02 ± 21.58A	111.86 ± 10.05B
DOC (mg kg^{-1})	CK	41.42 ± 5.74*
SM	73.01 ± 9.22	55.41 ± 1.99
	57.21 ± 18.62A	45.23 ± 11.54B
DON (mg kg^{-1})	CK	16.11 ± 1.89*
SM	26.22 ± 2.51	18.08 ± 2.24
	21.16 ± 5.89A	17.68 ± 2.77B
Soil water content	CK	16.99 ± 0.69*

(%)		
SM	19.03 ± 0.89	16.71 ± 0.73
	18.01 ± 1.32A	17.09 ± 0.79A

- 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 as following, and the revised sections were in red.

“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, which was largely associated with soil background properties and straw composition (Table 1 and Table 2). On the one hand, straw mulching increased contents of total N, inorganic N, available P, and available K at 0–5 cm, water content at 0–5 cm, and total organic C at 0–5 and 5–10 cm 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. The pH result in the study was inconsistent with Ok et al. (2011) and Sun et al. (2015), which may be due to different soil types, sampling times, crop rotations, and tillage management. 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 total organic C, total N, DOC, DON, available P levels, and water moisture, which favored soil bacterial abundance, especially in topsoil (Table S1, Table 3). Similar results after straw addition 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 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 should be reduced under straw mulching and may thus be more beneficial for maintaining or improving bacterial diversity.

Proteobacteria and Bacteroidetes are often classified as copiotrophic groups and have higher growth rates under conditions with abundant resources (Fierer et al., 2007, 2012; Liang et al., 2018; Ling et al., 2017). Long-term straw mulching increased soil nutrient levels, and then increased the relative abundances of Proteobacteria and Bacteroidetes. Additionally, Bacteroidetes are involved in hemicellulose breakdown and mulched straw stimulated it proliferation during straw decomposition (Wegner and Liesack, 2016). Chloroflexi is classified as oligotrophic groups, and enriched soil nutrients restricted it growth 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. Though Actinobacteria were classified as copiotrophs by Fierer et al. (2012), 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 is classified as oligotrophic groups, it is 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.

5 Conclusions

In this study, we investigated the effects of long-term straw mulching on soil properties along a soil depth gradient under a no-till rice-wheat rotation system. The results showed that soil total organic C, total N, total P, inorganic N, available P and K, DOC, DON, water content, and bacterial abundance decreased, but soil pH increased with soil depth. Compared with CK, straw mulching increased soil total organic C at 0–10 cm soil depth, soil total and inorganic N, available P and K, and water content at 0–5 cm, DOC and DON at 0–20 cm, and bacterial abundance 0–5 cm, but reduced the Shannon diversity and Shannon's evenness of the bacterial community at 0–5 cm. Regarding bacterial community, straw mulching increased the relative abundances of Proteobacteria, Bacteroidetes, and Acidobacteria, but reduced those of Actinobacteria, Chloroflexi, and Cyanobacteria. Additionally, straw mulching increased some C- and N-cycling genera, such as *Rhodanobacter*, *Rhizomicrobium*, *Terracidiphilus*, *Dokdonella*, *Pseudolabrys*, *Acidibacter*, *Devosia*, *Reyranella*, *Luteimonas*, and *Porphyrobacter*. The PCoA showed that the largest difference about the composition of soil bacterial communities between CK and SM treatments occurred at 0–5 cm depth. Soil pH, and N and organic C fractions were the major drivers shaping soil bacterial community. Overall, straw mulching is highly recommended under a no-till system in southwestern China because of its benefits in soil fertility and bacterial abundance. However, to maintain or increase soil bacterial Shannon diversity, the amount of inorganic N fertilizer can be reduced after straw mulching in future studies."

- 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 the word reminding. We replaced it by “managed” in the revised manuscript.

- 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.

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

Response: We firstly wanted to say that inorganic fertilizer, other than crop roots, also demonstrated effects on some soil nutrients distribution along soil depth. We rewrote this sentence in the revised manuscript.

Please also note the supplement to this comment:
https://soil.copernicus.org/preprints/soil-2021-25/soil-2021-25-AC1-supplement.pdf