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Reply on RC1

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-AC2, 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!

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:

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.

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

Response: Thanks for your suggestions. We will ask one native English editor from the International Science Editing, one English language editing services company, to check the whole manuscript carefully and avoid any grammar or syntax error when we are allowed to submit the revised manuscript.

- 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 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.”

- 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, 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.”

- 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. And 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 carbon and labile fractions mainly changed at surface soil. However, the abundant precipitation in the study site 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. The aim of the study was to show the differences on soil physicochemical properties and bacterial communities with soil depth between two straw managements. Consequently, 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 for us to gain our objectives.

- 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:

"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."

- 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.

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

Response: We moved these sentences to part 2.6.

- 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.

- 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 as following.

"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)."

- Discussion:

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

Response: We did our best to revise the Discussion section 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."

Please also note the supplement to this comment:
https://soil.copernicus.org/preprints/soil-2021-25/soil-2021-25-AC2-supplement.pdf